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Molecular Phylogenetics Unites Alternate Actinosporean and Myxosporean Stages of *Myxobolus cerebralis* the Causative Agent of Whirling Disease in Salmonid Fish

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Myxobolus cerebralis (Hofer 1903) is the myxozoan parasite responsible for whirling disease among several species of fish in the family salmonidae (11). The agent, believed to have originated in central Europe and Asia was dispersed with inter and intracontinental movement of trout so that currently its worldwide distribution includes 21 countries and 19 states of the U.S.A. (5,12). Myxozoans have long been known as parasites of fish but many of their characteristics, including multicellular developmental and sporogonic stages, and modes of transmission have only recently been described. Once considered as protozoans, recent studies on their small subunit ribosomal RNA (SSUrRNA) gene sequence show their greater affinities with the metazoa (8). In the early 1980s Wolf and Markiw published a series of papers describing an experimental approach to demonstrating that the transmission of *M. cerebralis* involved a life cycle including two hosts, the salmonid fish and an aquatic oligochaete worm (9,10,13,14,15). Certain taxonomic concerns were posed by this unification since the respective parasites were found in different classes within the phylum Myxozoa but due to precedent, *M. cerebralis* was accepted as the genus species for all

stages found in both hosts (1). The studies of Wolf and Markiw were criticized by others who were unable to demonstrate a similar mode of transmission or correspondence between the myxosporean and actinosporean forms of the parasite in their experimental trials (4). In contrast, similar stages with other myxosporean parasites have now been proven or highly suggested for 14 additional species, with over 1200 species of myxozoans known, the number of actinosporeans to be identified with their corresponding myxosporean remains a significant challenge (6).

Using rDNA as probes should facilitate matching myxosporeans with their corresponding actinosporean by comparisons of cloned 18S rDNA. Such an approach was also described by Lin et al (7) to compare sequences of three myxosporeans. In the study described here we demonstrate an approach to the positive identification of corresponding actinosporean and myxosporean stages. Using molecular tools, alternating stages obtained from the fish and oligochaete worm were compared for the myxozoan parasite *Myxobolus cerebralis*. This study confirms the identity of alternating myxosporean and actinosporean stages for *M. cerebralis* and demonstrates the utility of this approach for future recognition of unknown actinosporean stages for the more common myxosporean species parasitizing fish.

Material for DNA extraction was obtained from the collection M. Markiw as kindly provided by Dr. R. Herman. The triactinomyxon and myxosporean stages of *M. cerebralis* were collected from serial

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passages through the fish and oligochaete. This ensured that there would be little if any strain variation between life stages of the parasite. Due to the collection method for the oligochaete parasite this material was the least likely to carry contaminating host DNA. This, in combination with the difficulty associated with obtaining high quality DNA from the trout parasite, led us to begin cloning and sequencing with DNA from the triactinomyxon stage.

Conserved sequences were used to amplify the 18S rDNA of the triactinomyxon which was subsequently cloned into pCRII as a 1934 bp fragment. Sequencing was initiated on the cloned fragment and as sequencing progressed new primers were made to sequence farther into the gene. Ultimately both strands were sequenced. From this sequence data nested primers were made and used to amplify the corresponding gene from the myxosporean. The nested rDNA fragment from the myxosporean was 1552 bp in length. The two sequences were found to be 97.44% similar. This contrasts to only 65.7 and 3.1 % similarity in comparison of *M. cerebralis* to the two *Myxobolus* spp. described by Smothers et al. (8).

The near sequence identity between the triactinomyxon and *M. cerebralis* stages indicate convincingly, and for the first time at the molecular level, that they represent alternate stages of the same parasite. Minor differences observed between alternate stages could have resulted from microheterogeneity among multiple copies of the rDNA gene that exist in a single genome (2). Additionally, different life stages of the parasite *Plasmodium berghei* have been shown to express alternate transcripts of the 18S rDNA (3).

Given the recent increase in outbreaks of whirling disease in the western United States, this sequence data should be of particular interest. Antigen based assays such as ELISA and IFAT may not be easily applied to an organism which presents different antigens at different stages of the life cycle. In contrast, DNA based diagnostics, like the polymerase chain reaction, detect the presence of molecules that are constant throughout the life of the organism. Initial studies in our laboratory have shown the utility of this approach for the detection of the parasite in fish and worms. Sequencing of PCR products will also provide the basis for comparisons of the parasites in both fish and oligochaetes from different host species and geographic regions which are future objectives of our

laboratory studies

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BOOK REVIEW

Techniques in Fish Immunology - 4 Immunology and Pathology of Aquatic Invertebrates. 1995.

J.S. Stolen, T.C. Fletcher, S.A. Smith, J.T. Zelikoff, S.K. Kaatari, R.S. Anderson, K. Soderhall and B.A. Weeks-Perkins, eds. SOS Publications, 43 DeNormandie Ave., Fair Haven, NJ 07704-3303, USA, 289 pp. \$70.00 US

Tunicates, molluscs and crustaceans are the major species covered in this astounding collection of immunological techniques for this rapidly advancing field. Dr. Stolen and 7 eminent scientists have edited 29 papers on various aspects of dealing with the defense systems of aquatic invertebrates. The immune protection system of these of these animals are described in detail. Not only are detailed descriptions provided on how to collect samples and identify cells, but the techniques are given for dealing with the different physiological aspects of the species. For instance, hemolymph can be collected with a syringe containing anticoagulant, the hemocytes dispensed into density gradient solutions for cell separation and further studies on phagocytic function

or cytotoxic activity, or for such assays such as the release of lectins *in vitro*. Some procedures are similar to mammalian immunology, however important physiological differences exist that need modification of standard techniques.

Molluscan immunology has important economic aspects, as the aquaculture of oysters and mussels is expanding worldwide. Several chapters describe the morphology and basic functions of isolated hemopoietic cells and how the immunological techniques can be applied to investigate environmental modulators or toxicants. Heavy metals and other pollutants can affect the release of reactive oxygen metabolites in hemocytes. Monitoring the health of these animals by immunological techniques may become a standard procedure.

Shrimp and other crustaceans such as crayfish have active defense systems that include hemocytes that adhere to glass, degranulate, engulf foreign particles and have cytotoxic activity. Cytokines and other soluble blood proteins also exist as shown by the presence of recognition molecules that recognize and bind to lipopolysaccharides and beta-1,3 glucans, components of bacterial and fungal pathogens. A excellent and detailed summary chapter is included on the parasites and diseases of blue crabs; another applied chapter describes the use of shrimp phagocytes as indicators of pesticide poisoning.

A bonus to this manual is the addition of an appendix that gives helpful hints on the care, maintenance and handling of the invertebrate in the laboratory. This information is invaluable for keeping these animals healthy in preparation for immunological assays. Over 48 authors from the international community of scientists were involved in the production of this comprehensive text containing creative, helpful diagrams and flow charts. The spiral bound book will undoubtedly lie open on many laboratory benches throughout the world as researchers, graduate students and pathologists follow and adapt these techniques to experiment with a wide variety of invertebrate species. Dr. Stolen and her team are to be congratulated for bringing this specialized information under one cover.

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Ivermectin is not an Effective Anthelmintic Against Acanthocephalan Parasites in Adult Steelhead Trout Broodstock

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Recent surveys have indicated increasing rates of salmonid broodstock parasitism in the Great Lakes (personal communication, S. Marcquenski). The broodstock affected include steelhead trout (*Oncorhynchus mykiss*), coho (*O. kisutch*) and chinook salmon (*O. tshawytscha*). Adult steelhead broodstock with a history of sudden mortality and captured from Lake Michigan were previously diagnosed with hemorrhagic enteritis due to marked infestation with *Pomphorhynchus* sp. parasites. Additionally, severe acanthocephalan parasite infestation has been diagnosed in similar broodstock captured from the St. Joseph River and held at Bodine State Fish Hatchery (SFH) in Mishawaka, IN.

To determine the efficacy of ivermectin as an anthelmintic against acanthocephalan parasites in this study, the following procedures were performed: (1) acanthocephalan parasites from "immediate capture" adult steelhead broodstock were enumerated; (2) ivermectin injections at two different dose levels were given to similar broodstock; and (3) acanthocephalan parasite enumeration was performed after spawning (approximately 7 months following treatment), in surviving treated and untreated (control) broodstock.

A random sample of 10 adult steelhead broodstock (2 males and 8 females) were collected from the raceway at Bodine SFH on the same day they had been harvested from the fish ladder of the St. Joseph River in South Bend, IN. The entire gastrointestinal tract was removed from these fish and placed in 10% neutral-buffered formalin solution. The intestinal contents were flushed with chilled isotonic saline into a brass laboratory sieve (Scientific Products, Chicago, IL) and parasites were counted. The mucosal surface of the gastrointestinal tract was carefully inspected for parasites. The mean number of acanthocephalan parasites from this group of fish was 290, ranging from

117 to 512 parasites per fish.

Sixty-eight adult steelhead broodstock were given a single intramuscular injection of 0.27 ml (2.7 mg) of ivermectin (*Ivomec injection for porcine*®, 1% sterile solution, Merck and Co., Inc.). By day 3 post-treatment, 15 of these 68 fish were dead, and the remaining fish exhibited clinical signs of lethargy, disorientation, and loss of fright response. By day 14 post-treatment, 53 of the 68 fish were dead, with the remaining 15 dying by day 21 post-treatment. Post-mortem examination of a randomly selected sample of these fish failed to determine the cause of death, which was presumed to be due to ivermectin toxicosis.

Thirteen additional adult steelhead broodstock were given a single intramuscular injection of 0.09 ml (0.9 mg) of ivermectin. Clinical signs of illness were not detected in these fish. Following spawning, 9 of these 13 fish were examined and acanthocephalan parasite enumeration was performed on these 9 fish as well as 20 control fish. All 29 of these fish were females. The mean acanthocephalan count was 62 for the ivermectin treated group, ranging from 4 to 182 parasites per fish. The mean acanthocephalan count for the control group was 32, ranging from 10 to 83 parasites per fish.

Although the extra-label use of ivermectin-containing pharmaceutical agents has been limited in fish^{1,2,3,4,5}, the success rate of these agents as anthelmintics has been even more limited⁵, and toxicoses associated with these agents have been reported^{1,2,3,4}. In this study, the first sixty-eight fish were probably injected with too high a dosage, and ivermectin toxicosis was the most likely cause of death. In those cases where ivermectin products have been successfully used in fish species, the parasites were external, and the treatment protocols involved incorporation of ivermectin into

the diet^{1,4,5}

The rationale for the large decrease in the number of acanthocephalan parasites in the treated and control adult steelhead broodstock as compared to the broodstock immediately after capture is the disruption of the natural life cycle of the parasites. The life cycle of acanthocephalans⁶ requires an invertebrate intermediate host, which is infected by the parasite ova containing an acanthor larvae. This larval form develops into a cystacanth following ingestion by a smaller fish. The broodstock used in these studies were most likely infected with these parasites upon consumption of forage fish from the Great Lakes. Upon capture of the broodstock and placement into the holding raceways (seven months prior to spawning) the fish were anorexic and were not fed, thus the life cycle was broken.

The only conclusion which can be drawn from the higher acanthocephalan count in the treated versus the control fish is the lack of effectiveness of this drug against this parasite and the low sample numbers used. If larger sample sizes had been available, the mean number of acanthocephalan parasites may have been similar in both groups.

In conclusion, it is apparent that the *Ivermec injection for porcine*®, when given at a dosage of 0.9 mg intramuscularly, is ineffective against acanthocephalan parasites in adult steelhead broodstock. It is also obvious that at higher doses, this compound is lethal and can cause illness followed by high mortalities. More studies are needed to determine a more efficacious method for controlling acanthocephalans infesting adult steelhead broodstock.

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BOOK ENTITLED "PARASITES OF FISHES IN WYOMING" BY DOUG MITCHUM RECENTLY PUBLISHED

Doug Mitchum's monograph. "Parasites of Fishes in Wyoming" should be a practical reference for identification of fish parasites for those working in the field of fish diseases throughout much of the western U.S. The hard-bound, 344 page book contains 30 pages of color photos and 126 illustrations. It is available in an attractive format, with excellent quality paper. It is well indexed and it has keys for identification of parasites, a glossary of terms, a section on control and treatment of parasitic diseases, and a bibliography that includes many basic references pertaining to parasites of freshwater fishes in the region.

The book is available for the **unbelievable low price of \$30.00** from : Alternative Enterprises, Wyoming Game and Fish Department, 5400 Bishop Blvd., Cheyenne, WY. 82006; 1-800-548-9453 [Charlie E. Smith, Bozeman, MT]

FHS President's Annual Report - 1995

Theodore R. Meyers, President

1994-1995

This past year two issues continued to weigh prominently as concerns for the Fish Health Section membership; the lack of approved therapeutic drugs in aquaculture and the questioned recognition of our professional certification program by the American Veterinary Association (AVMA) and the future role of the non-DVM in fish health. I believe progress has been made to begin addressing these concerns but much more is needed in the near future. Concerning the first issue, the Investigational New Animal Drug (INAD) permitting system of the Food and Drug Administration (FDA), originally designed to accommodate unapproved drug use for small and large animals, has been better adapted to finfish through trial, error and sometimes frustration. Development of large regionally coordinated INAD programs such as that of the Fish and Wildlife Service and the Western Regional INAD Program contracted through the Columbia Basin Fish and Wildlife Foundation have helped with some of the paperwork and increased the efficiency of permitting drugs to nearly tolerable levels. Other programs are being pursued to investigate new therapeutants for fish diseases such as a nationwide five year project between the International Association of Fish and Wildlife Agencies (IAFWA) and the National Biological Service (NBS). About \$3.9 million would be contributed over five years from 39 state conservation agencies and about \$4.3 million from the NBS which would be conducting the research. However, the success of this project depends upon federal funding for the NBS which is still in question. Also of interest, is the current status of potassium permanganate and copper sulfate which, according to FDA, can be used in aquaculture without an INAD, although they are not yet LRP or otherwise formally approved. Studies with these compounds are currently underway to generate the necessary approval data for the FDA. Within about 18 months the formalin label may become more comprehensive to include approved uses for ectoparasites and fungus on all species of fish and fish eggs. This would eliminate the need for a formalin INAD. The FHS has recently contributed money towards funding of a National New Animal Drug Application (NADA) Coordinator along with monies contributed by several other organizations including the USDA, AVMA and FDA. The purpose of the NADA coordinator would be to facilitate aquatic animal drug approvals.

As for the second concern, the long and short of the issue is that there is plenty of room for both non-DVM and DVM practitioners in the field of fish and shellfish health. Ultimately, the FHS membership will be responsible for providing the expertise in future veterinary fish health programs. Hence, there

is continued opportunity for our leadership in this arena. The question is how will both parties work together as partners? This can only be determined by keeping attitudes and communication lines open between veterinarians and non-DVMs alike.

The certification program of the FHS is alive and well and continues to achieve higher standards with implementation of the new Fish Pathologist qualifications and continuing education for recertification of Fish Health Inspectors and Fish Pathologists through special training workshops on various fish health disciplines. The Section has provided funds to help the continuing education program be successful and should continue to do so whenever monies are needed. Membership participation has been outstanding. Hand-in-hand with the certification program is the new edition of the FHS Blue Book which has been available on the sales stand for the last several months. Although there has been some controversy within the FHS regarding whether the Blue Book is or is not all things to all people, it is nonetheless an exemplary accomplishment by the Blue Book Advisory and Technical Standards Committees worthy to be called our professional bible. Other issues this past year include the considerable growth of the FHS treasury thanks to the very successful International Symposium on Aquatic Animal Health in Seattle last September. The FHS is indebted to the shrewd organizers of this event, Ron Hedrick and Jim Winton. The FHS also agreed to cosponsor, with no funding commitment, an aquatic animal health symposium to be held at the 125th annual AFS meeting in Tampa, Florida during August 27-31. A draft procedural manual for FHS officers and committee members has been completed and is being reviewed by the Executive Committee for further revision. Many thanks to those committee chairs who contributed procedural information that made this job easier. The Newsletter continues to be the voice of the FHS featuring many research articles for rapid communication, many of which are later published as expanded contributions to the peer reviewed literature in the Section's equally successful Journal of Aquatic Animal Health. These media serve two very different and useful purposes and are true measures of the Section's prodigious professional activities.

These are exciting and interesting times to be a member of the FHS. Radical changes are in the wind, but we also have the opportunity to help shape these changes for the betterment of our professions and for aquatic animal health in general. I am very grateful for the help and support which I have received from the FHS members during my tenure as President. It has made a

difference. Thank you for the privilege to serve.

Finfish Diseases¹

Viruses

A **birnavirus** was detected in 17% of pooled samples from coho salmon broodstock returning to Puget Sound, Washington in the Nooksack Watershed. Epitope analysis indicated the isolate was identical to one isolated from Atlantic salmon in British Columbia in 1989, although the virus was found to be avirulent for chum, coho, chinook and Atlantic salmon, steelhead and brook trout, a distressing feature was its failure to produce CPE in EPC cells which are susceptible to IPNV viruses and widely used for the isolation of salmonid viruses. **Lymphocystis virus** has increased its prevalence in Chesapeake Bay striped bass. Although infected fish are unsightly, no associated mortality has been reported. White sturgeon **herpesvirus II** has become prevalent at sturgeon hatcheries in California in fish populations originating from wild stock. The virus infects skin and gills causing high mortality in juvenile fish (0.5 - 3.0 g) from gill hyperplasia and excessive mucus secretion and recurring skin ulceration in larger fish with much less mortality. This virus has recently been detected in wild fish from the lower Columbia River. The **North American** strain of **VHSV** continues to be prevalent in Pacific herring populations from Alaska to Puget Sound where new isolations of the virus were made in 1994 from hatchery coho salmon returning to the Columbia River. More recently the virus has been isolated from market sized pen-reared Atlantic salmon in British Columbia. North American VHSV can cause skin hemorrhaging and ulceration in herring but is relatively avirulent for most salmonids. However, mortality as high as 20% has been reported during waterborne exposure tests with rainbow trout. This first isolation of North American VHSV in the Columbia River has caused concern regarding management decisions and interstate policies for fish transport. The isolation of the virus in BC may result in required disinfection or contained disposal of water used for processing marketed fish. The marine environment overseas can apparently be a source for the **European** strain of **VHSV** as demonstrated by an outbreak at a turbot farm in Scotland resulting in clinical disease and high mortality. The virus had not been previously detected in that turbot broodstock in over 15 years of testing. **IHN** continues to produce sporadic outbreaks within its enzootic area in chinook, steelhead and sockeye in the Pacific Northwest and in sockeye salmon in Alaska. Isolation of an **aquareovirus** was made for the first time in Alaska from ovarian fluids of chinook salmon returning to Ship Creek, an urban stream in downtown Anchorage. The finding was incidental but the stock will be confined to its natal watershed and previous transplant sites. Aquareovirus isolations have also been reported from North Dakota and throughout the west coast of Washington State in asymptomatic adult salmonids. These viruses are the most frequently isolated "new" agent with at least 25 occurrences

since 1990. An **unknown viral agent** is suspected of causing a large mortality of marine catfish along a 1,700 km section of coastline in southern Brazil since February, 1994. Toxicants and parasites have been eliminated as possible causes but virus-like particles have been observed in the kidney tissues of affected fish. Virological studies are proceeding. Mass mortalities of **pilchard** have been reported at various sites in Australia, Tasmania and New Zealand. The cause is still unknown but stress associated with environmental changes coupled with either a **herpes-like** viral agent or an **amoeba** observed in affected fish is a suspected scenario.

Bacteria

Epizootics in striped bass were caused by *Edwardsiella tarda* isolated from internal organs and skin lesions of fish in poundnets in the Potomac and Eastern Bay regions of the Chesapeake Bay during the summer of 1994. The outbreak, likely due to warmer water temperatures and overwintering stress, declined by the fall. *Vibrio charchariae* was isolated from similar skin lesions of striped bass earlier in the summer which later shifted to *V. vulnificus*. Atypical *Aeromonas salmonicida* was apparently responsible for a hemorrhagic disease of skate held in captivity for several months. The fish originated from Vinyard Sound in Massachusetts. *Renibacterium salmoninarum*, causative agent for bacterial kidney disease, continues to frustrate fish disease managers regarding whether to manage the disease or the organism itself. This is not necessarily due to serious epizootics which occasionally do occur, but because of the development of more sensitive detection methods such as the ELISA. The ELISA indicates that the agent and/or its antigens, not necessarily the disease, are relatively widespread in wild and hatchery fish stocks. How should this be managed? The need to standardize the ELISA among different laboratories and make meaningful comparisons to potentially less sensitive but more widely used tests such as the FAT, are future concerns for certification purposes and fish disease policy management decisions. Isolates of *Flexibacter maritimes* were reported for the first time from the Pacific coast of North America at three separate netpen sites in southern California in white seabass and in captive Pacific sardines and northern anchovy. Clinical disease consisted of eroded gills and fins and the agent was similar to the pathogenic type strain isolated from Japan. A second *Cytophaga*-like bacterium isolated from skin lesions of netpen-reared chinook salmon could be synonymous with *F. maritimus*. Whether this is the same agent responsible for past outbreaks of mouth rot or yellow mouth disease in the British Columbia and Puget Sound netpen industry has some growers concerned. Vaccines have nearly eliminated **furunculosis** in Atlantic salmon in Scotland and Norway and in netpens on both coasts of the USA. Likewise, vaccines and good husbandry practices have significantly reduced Hitra Disease (*V. salmonicida*) in Atlantic salmon pen farming facilities in Maine. *Piscirickettsia*

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salmonis continues to be an important source of mortality in Chilean netpen-reared salmonids. However, this past year the pathogen was observed for the first time in fish held only in freshwater, in this case rainbow trout. However, experimental horizontal transmission of the pathogen has not been successful in freshwater as it has been in seawater. Additionally, an apparently different rickettsia-like organism has been isolated from Atlantic salmon in Chile.

Parasites

The most important problem that has galvanized fish health managers, the public and politicians alike over the past several months has been the discovery last December of the **Whirling Disease (WD)** agent *Myxobolus cerebralis* in wild rainbow trout of the famed Madison River in Montana. This finding was after surveys discovered a severe decline in the rainbow trout populations within a 50-mile stretch of the river. The question that will be investigated further is whether the parasite is responsible for this decrease in fish numbers. Nonetheless, this discovery in Montana has drawn attention to the possible effects of the disease on susceptible salmonid populations in 18 other states where the WD parasite has been found. The debate goes on which questions fish management and hatchery practices nationwide.

Ceratomyxa shasta was detected for the first time in Alaska from chum salmon adults returning to the Sushana River, a tributary in the Yukon River watershed. Typical spores were found in 9/150 hindguts examined with no clinical disease evident. Other myxosporean diseases causing poor flesh quality problems included *Kudoa thyrsites* in Atlantic salmon culture in the Pacific Northwest and *Henneguya salminicola* in commercially caught pink salmon in southeast Alaska.

The **rosette** disease agent (possible ciliophore) has produced severe systematic infections in 26% of winter run chinook salmon returning to the Sacramento River in California. In addition, 100% of these fish were infected with IHNV which made isolation of the rosette organism nearly impossible. Fish less than 18 months of age were apparently uninfected as were naturally exposed rainbow trout. Massive numbers of the metacercariae of the salmon poisoning fluke *Nanophyetus salminicola* have been found in adult chinook salmon returning to the Klamath River Basin in Oregon. Parasite intensities in fish have approached 6,000 per gram of kidney tissue with no gross signs of disease. Similar heavy infestations of up to 8,000 parasites per gram of tissue have been observed in adult chinook from the Trinity River of California. There, juvenile chinook have been found with up to 2,000 parasites per gram of tissue after only three weeks of exposure.

Sea lice have not been a problem in Maine but outbreaks in nearby New Brunswick, Canada have caused the concern of netpen farmers. The ciliate *Ichthyophthirius* sp. was responsible for extensive pre-spawning chinook salmon mortality in the lower

Non-Infectious or Idiopathic Conditions

Liver lesions in English sole, including megalocytic hepatitis, were found to decline in Eagle Harbor, Puget Sound when highly contaminated sediments were capped as part of an EPA Superfund project. This is additional evidence of the obvious conclusion, as suggested by other studies, that reducing toxic exposures of fish will reduce lesions as well as neoplasms suspected to be caused by these contaminant exposures.

Coho anemia disease (**CAD**) has been almost nonexistent this past year in Oregon and elsewhere. The etiology of CAD is still unknown but suspected to be of a noninfectious and possibly toxic nature.

Shellfish Diseases

Significant shellfish disease this past year continue to occur but mostly in crustaceans. **Taura syndrome (TSV)**, a necrotizing disease of cuticular epidermis, foregut, hindgut and connective tissues in cultured Pacific white shrimp, recently occurred in Hawaii and Texas. The disease was first discovered in Ecuador in 1992 and has spread to other countries in South and Central America and now may become a major threat to the shrimp industry in the US. TSV is caused by a small single-stranded RNA picorna-like virus and produced up to 95% losses in the two Hawaiian farms affected. The causative virus has been found in wild shrimp as well as in the salinity tolerant water boatman, which is a likely reservoir.

A picorna-like virus causing infectious hypodermal and hematopoietic necrosis (**IHHNV**) is established in wild penaeid shrimps in the Gulf of California including the three major species of white, blue and brown shrimps. Wild postlarvae are used for grow-out or adult shrimp are captured for broodstock which perpetuates the problem in the Mexican shrimp culture industry of this area. IHHNV virus has been widely disseminated worldwide by the transfer of latent to patently infected post larval and other life stages of shrimp. Hence, the disease also has become a worldwide problem in shrimp culture.

Bitter crab syndrome (BCS), caused by a parasitic dinoflagellate, continues to spread in populations of *Bairdi* Tanner crabs in southeast Alaska. Infected crabs are unmarketable due to poor meat quality and a bitter flavor causing economic losses to fishermen. BCS is fatal with prevalences of up to 100% in some crab populations. Although BCS also occurs in the Bering Sea *Opilio* Tanner crab populations, the prevalence is sporadic and has actually declined in certain areas during the last two years.

Texas shrimp farms continue to have a serious problem with necrotizing hepatopancreatitis (**NHP**) in white shrimp caused by one or two pleomorphic Gram-negative intracellular bacteria that cannot be cultured by

conventional methods. The disease apparently occurs at salinities greater than 20 ppt and is exacerbated by water temperatures of 30° C. Oxytetracycline-medicated feeds and good husbandry practices help to control NHP.

There is evidences to suggest that **shell disease** in impounded lobsters in Nova Scotia is due to a nutritional deficiency. Pelleted feed supplemented with vitamin C caused a reduction of shell disease as determined by a University of Maine study.

¹ An important source for this information was the USA Report to the ICES Working Group on Pathology and Diseases of Marine Organisms authored by Sharon A. MacLean, NOAA, NMFS, Naragansett, RI, USA.

Committee Reports

Archives

Photographs from the International Symposium on Aquatic Animal Health from Seattle, WA have been submitted to the Archives file. They include social events and awards presentation. As always, if anyone has photographs or information that could be included in the archives, please send them to me. Thank you.

Yolanda Brady, Chairperson

Awards

The Snieszko Distinguished Service Award was awarded to Dr. Tom Wellborn. Student Travel awards were given to Christine Densmore (NFHRL and VPI), Craig Shoemaker (Auburn), Ken Cain (U of Washington) and Deborah Siegal (U of Idaho). Each student made excellent oral presentations at the annual meeting in Syracuse. Nominations for the Special Achievement Award were not received.

Larisa Ford, Chairperson

Membership and Balloting

Ballots were mailed out to the membership in May, 1995. Election of officers for 1995-1996 was completed September, 1995. Election results are reported in this issue of the newsletter.

Jill Jenkins, Chairperson

Newsletter

Windows and Microsoft Publisher were purchased by the FHS to produce the newsletter. The improved software will help ensure consistent formats and quality. The April issue was mailed out late, but the other issues for the year were mailed out within the appropriate time periods. Members should contact the AFS office in Bethesda directly concerning any address changes.

Larisa Ford, Co-editor

Nominating

The committee, comprising Dave Tillinghast, Jim Bertolini and Pete Walker, submitted a slate of nominees to the membership. Ballots with brief qualifications statements were mailed to the membership on May 30, 1995.

Dave Tillinghast, Chairperson

Time and Place

The 1995 meeting of the Fish Health Section was held in Syracuse, New York on 19-22 July, at the Sheraton Inn Conference Center. The scientific program was a combination of presentations through the Fish Health Section and the Eastern Fish Disease Workshop. Local arrangements were made by John Schachte and Paul Bowser. Coordination of the scientific program was performed by Frank Hetrick. The 1996 meeting will be held on the campus of the University of Wisconsin in Madison, Wisconsin. The dates of the meeting are 7-9 August 1996. Sue Marcquenski will be coordinating the meeting which will be jointly sponsored by the Wisconsin Department of Natural Resources and the LaCrosse Fish Health Laboratory of the US Fish and Wildlife Service.

Paul Bowser, Chairperson

Committee Reports

Bylaws Review

It was decided at the FHS business meeting conducted at the Seattle International Symposium on Aquatic Animal Health last September, that action on amending the Section Bylaws would be suspended until a FHS committee/officer Procedural Manual was completed. The manual has been drafted and is being reviewed by FHS officers and committee chairs. The intent is to finalize the manual within the next few months. A vote by the Section membership would follow on any proposed amendments to the Bylaws as previously listed in the Newsletter (Vol 22, No 1), including extracation of much of the procedural information. The Bylaws would then be revised to include those amendments that passed.

Ted Meyers, Chairperson

Scientific Journal

During 1994 the Journal of Aquatic Animal Health received and processed 72 manuscripts. Of these, 18 were published, 28 were accepted and are in revision, 9 are still in review and 17 (24%) were rejected. The average time from receipt of the 18 papers to publication was 43 weeks. As of June 1, we have received 29 manuscripts for 1995. W.A. Rogers resigned as a Co-editor in 1994 and John Grizzle and John Plumb have served as Co-editors this year. John Grizzle has resigned as of September 30, 1995 and Margaret Ewing will assume the duties of Co-editor effective October 1, 1995. Associate Editors Kenneth Davis and Charlie Smith will go off the Editorial Board this year. We thank Ken and Charlie for their superb contributions to the Journal of Aquatic Animal Health since 1992. Manuscript processing through the American Fisheries Society Editorial Office and then to Associate Editors to arrange peer reviews has worked well. This has taken much of the "manuscript tracking" load off of the Co-editors, but more importantly this has expanded the reviewer pool and added a valuable layer of evaluation for the manuscripts. We feel that this has improved the quality of the papers that are accepted for publication.

John Plumb and John Grizzle, Co-editors

Continuing Education

The primary accomplishment this year was offering a class on histopathology in conjunction with the Western Fish Disease Workshop held in Twin Falls, ID on June 6. The full day class taught by John Morrison, Beth Mac Connell, and Charlie Smith consisted of three illustrated lectures and a lab period for examining example slides. Fifty-six people attended the course which they can use as credit toward recertification as Inspectors or Pathologists with the Fish Health Section. The most urgent task before the committee is to develop a credit value system for various kinds of credit and then establish credit requirements for recertification.

Craig Olson, Chairperson

Board of Certification

Committee members did an excellent job of returning evaluation packets in a very timely manner, and their professional handling of the work of the Board is very much appreciated. We have processed:

FHI Certifications completed: 7
FHI Recertifications completed: 7
FHI Denied: 1

FHI Certifications pending: 2
FHI Recertifications pending: 2

FP Certifications completed: 0
FP Recertifications completed: 20
FP Denied: 0

FP Certifications pending: 5
FP Recertifications pending: 6

8 additional requests to start the certification process were received and packets sent
2 people indicated their intention not to be recertified due to job changes/retirement.

The new standards for fish pathologist were published in the newsletter and several people have opted to apply under the new program. Forms have been revised, and scoring sheets developed for each certification type. One continuing frustration is the extreme delay in getting actual certificates from the parent society.

Phyllis Barney, Chairperson

Finance

As of July 1, 1995, we have a total of \$26, 056.09 in the General Account (West One Bank, Buhl, Idaho). A detailed accounting of this year's income and expenses are listed below.

	<u>Transactions</u>	<u>Subtotal</u>	<u>Total</u>
FHS General Account			
Beginning Balance			7091.66
Credits			
Section dues	7010 . 00		
Certificates	800. 00		
'94 WFDC	165. 98		
'94 Intl meeting	16646. 50		
'94 WFDC	1093. 44		
Interest	426. 35	26142.27	
Debits			
Newsletter	3937.71		
AFS Plaque/Cer/Seal	141.00		
Fish Path Forms	634.98		
Misc Postage	310.79		
'94 Ballot & Mailing	153.36		
NADA Contribution	1000.00		
Continuing Ed	1000.00	7177.84	
Ending Balance of General Account			26056.09

Since the section raised annual dues and the treasury appears to be growing, a yearly budget should be established. This would include setting dates for a fiscal year, budgets for each officer as needed, and forecasting income and expenses, including what monies will be available for continuing and new programs. Programs such as professional certifications, continuing education, and annual meetings should be looked upon as money making opportunities for the section. A short term goal may include hiring an executive secretary or an administrative assistant. Monies are also available for updating the FHS directory and developing a contribution / advertisement flyer that would aid in obtaining financial donations from corporate sponsors and increasing section membership.

Scott LaPatra, Chairperson

CONGRATULATIONS!!!!

Dr. Tom Wellborn was the 1995 recipient of the S.F. Snieszko Distinguished Service Award.

Dr. Pete Taylor presented the award to Dr. Wellborn, in his home, immediately following the 1995 meeting in Syracuse.



Toxicity of Hydrogen Peroxide to Different Life Stage and Species of Fish

J. J. Rach, T. M. Schreier, and G. E. Howe

National Biological Service, Upper Mississippi Science Center, P. O. Box 818, La Crosse, Wisconsin 54602, USA

Hydrogen peroxide has been identified as an effective fungicide (Marking et al. 1994) and has been granted low regulatory priority status by the U. S. Food and Drug Administration as a fungicide for fish and fish eggs; however, it has other potential applications in fish culture. Hydrogen peroxide has been used to treat fish for ectoparasites in freshwater since the 1930's (Schaperclaus et al. 1979) and is currently used to control sea lice in Norway (Thomassen 1993). Hydrogen peroxide is also used as a disinfectant in industry and has potential for the control of bacterial gill disease. However, limited toxicity data are available to guide fish culturists in administering hydrogen peroxide to diseased fish. Laboratory tests were conducted to determine: (1) the sensitivity of brown trout (*Salmo trutta*), lake trout (*Salvelinus namaycush*), fathead minnow (*Pimephales promelas*), walleye (*Stizostedion vitreum*), channel catfish (*Ictalurus punctatus*), and bluegill (*Lepomis machrochirus*) to hydrogen peroxide treatments; and (2) the toxicity of hydrogen peroxide treatments to various life stages of rainbow trout (*Oncorhynchus mykiss*). The purpose of these studies was to provide hatchery personnel with additional information on the toxicity of hydrogen peroxide to fish.

The test chemical was food grade hydrogen peroxide (35% active ingredient). All test concentrations were based on active ingredient. Hydrogen peroxide concentrations were verified analytically for each treatment by a titrimetric method (Jeffery et al. 1989). The tests were conducted in 12°C well water that had a mean alkalinity of 107 mg/L as CaCO₃ and mean hardness of 134 mg/L as CaCO₃.

The fish in the species sensitivity study were ~ 2 g in weight and in the life stage study weights ranged from 0.05 g to 171 g. The fish were acclimated to test conditions 24 h prior to the beginning of a test. Test fish were held in polypropylene mesh cages (105

mm diameter, 140 mm height) stainless steel mesh cages (15.4 cm x 15.4 cm x 25.0 cm, LWH), or 40 L glass aquaria (59.6 cm x 29.2 cm x 29.6 cm, LWH) depending on size. Static dip treatments were conducted on fish (0.05 - 7.7 g) held in the polypropylene and stainless steel cages. The cages were placed in a stainless steel tank (58 cm x 27 cm x 36 cm, LWH) containing 12 L of hydrogen peroxide treatment water. After a treatment, the cages were returned to a culture tank that was supplied with a continuous flow of 12°C well water. Fish (40-171 g) held in the aquaria had a continuous flow of 12°C well water except during the treatments, then the water was shut off and they received a static bath treatment. The water flow was restored after each treatment.

Fish were exposed to hydrogen peroxide concentrations ranging from 100 - 5000 µL/L for either 15 or 45 min. Fish were treated every-other day for a total of four treatments. Mortality readings were recorded on non-treatment days and prior to a chemical treatment and dead fish were removed daily from the cages. Dissolved oxygen, pH, and water temperature were monitored during treatments.

All the fish species, except walleyes, that were evaluated in the sensitivity study were healthy (based on fish culture records). The walleye were harvested from a pond and these fish exhibited signs of stress which was confirmed by a 20% mortality in the controls for the 15 min treatments (Table 1). All species of fish tested tolerated hydrogen peroxide treatments up to 500 µL/L except for walleye which demonstrated toxicity at 250 µL/L with mortalities of 85% (Table 1). The toxicity of the 45 min treatments was greater than that observed for 15 min exposures. Brown trout, fathead minnows, and walleye treated with 500 µL/L hydrogen peroxide had mortalities of 20, 35, and 100%, respectively. Hydrogen peroxide

(Continued on page 13)

Table 1. The mean percent mortality of six species of fish treated with hydrogen peroxide for 15 or 45 min every-other day for a total of four treatments.

Exposures and Fish Species	Nominal Treatment Concentration ($\mu\text{L/L}$)						
	Control	100	250	500	1000	3000	5000
15 min Exposures							
Brown trout	0.0	-- ^a	0.0	0.0	0.0	45.0	85.0
Lake trout	0.0	0.0	0.0	0.0	0.0	3.3	13.3
Channel catfish	0.0	-- ^a	0.0	0.0	5.0	5.0	30.0
Fathead minnow	0.0	-- ^a	5.0	10.0	10.0	90.0	100.0
Bluegill	0.0	-- ^a	0.0	0.0	0.0	80.0	100.0
Walleye	20.0	10.0	85.0	100.0	100.0	100.0	100.0
45 min Exposures							
Brown trout	0.0	-- ^a	5.0	20.0	0.0	100.0	100.0
Lake trout	0.0	0.0	0.0	0.0	0.0	100.0	-- ^a
Channel catfish	0.0	-- ^a	0.0	0.0	5.0	80.0	100.0
Fathead minnow	0.0	-- ^a	0.0	53.0	10.0	100.0	100.0
Bluegill	0.0	-- ^a	0.0	0.0	0.0	95.0	100.0
Walleye	0.0	45.0	100.0	100.0	100.0	100.0	100.0

^a These concentrations were not tested.

was not toxic up to concentrations of 1000 $\mu\text{L/L}$ to brown trout, lake trout, and bluegill; however, treatments of 1000 $\mu\text{L/L}$ or greater caused fish to exhibit visual signs of stress and damaged the gill epithelial tissue. The data indicated that toxicity varies among species and may also be dependent on the health of test fish as evidenced in the walleye tests.

All five life stages of rainbow trout evaluated were healthy (based on fish culture records) and there were no mortalities in the controls (Table 2). In the 15 min exposures, the percent mortality was < 11% for all sizes of fish for hydrogen peroxide treatments up to

500 $\mu\text{L/L}$. No mortality was observed for sac and swim-up fry at treatments up to 3000 $\mu\text{L/L}$; however, larger trout were more sensitive and exhibited a higher percent mortality. Mortality data for the 45 min exposures was similar to that of 15 min exposures with younger (smaller) fish being less sensitive to hydrogen peroxide treatments. Sac and swim-up fry had 0 and 3% mortality respectively, at the 500 $\mu\text{L/L}$ treatment concentration while the fingerling, small adult, and large adult fish had mortalities of 20, 67, and 100%, respectively.

(Continued on page 14)

Fish culturists must consider water chemistry, temperature, life stage, species, and health of the fish when determining treatment regimes. Based on our experience with hydrogen peroxide treatments on fish, a treatment range from 50 - 250 $\mu\text{L/L}$ for exposures up to 60 min is recommended. Any intended application of hydrogen peroxide should first be tested on a small subsample of fish. Additional research on hydrogen peroxide is needed to delineate effective treatment regimes and to verify the efficacy for treating other fish diseases.

Vogel's Textbook of Qualitative Chemical Analysis, 5th Ed., Longman Scientific and Technical, Essex, England.

Marking, L.L., J.J. Rach, and T.M. Schreire. 1994. Evaluation of antifungal agents for fish culture. *Progressive Fish-Culturist*, 56: 225-231.

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Jeffery, G.H., J. Bassett, J. Mendham, R.C. Denney. 1989. *Titrimetric Analysis* pp. 372-373 in

Thomassen, J.M. 1993. A new method for control of salmon lice. Report, Fish Farming Technology. Agricultural University of Norway.

Table 2. The mean percent mortality of rainbow trout treated with hydrogen peroxide for 15 or 45 min ever-other day for a total of four treatments.

Mean Exposures and Life Stages	Weight (g)	Nominal Treatment Concentration ($\mu\text{L/L}$)					
		Control	100	250	500	1000	3000
15 min Exposures							
Sac fry	0.05	0.0	0.0	1.3	1.3	0.0	-- ^a
Swim-up	0.12	0.0	0.0	0.0	0.0	0.0	0.0
Fingerling	7.71	0.0	0.0	6.7	0.0	26.7	100.0
Sm. adult	41.3	0.0	6.7	0.0	0.0	80.0	100.0
Lg. adult	171	0.0	11.1	0.0	0.0	11.1	88.9
45 min Exposures							
Sac fry	0.05	0.0	0.0	1.3	0.0	1.3	-- ^a
Swim-up	0.12	0.0	0.0	0.0	3.3	13.3	56.7
Fingerling	7.71	0.0	0.0	6.7	20.0	100.0	-- ^a
Sm. adult	41.3	0.0	6.7	20.0	66.7	100.0	-- ^a
Lg. adult	171	0.0	0.0	11.1	100.0	100.0	-- ^a

^aThese concentrations were not tested.

FHS 1995-1996 COMMITTEES

Executive Committee

Voting

Jim Winton, President
 JoAnn Leong, President-Elect
 Ted Meyers, Immediate Past President
 Scott La Patra, Secretary-Treasurer
 Jim Bertolini, Chair - Nominating Committee
 Paul Reno, Chair - Blue Book Advisory Committee

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 Yolanda Brady, Chair - Archives
 Larisa Ford, Chair - Awards, Co-editor - Newsletter
 Mike Kent, Chair - Professional Standards
 Rod Horner, Chair - Technical Procedures
 Ray Brunson, Chair - Board of Certification
 Beverly Dixon, Co-editor - Newsletter
 Paul Bowser, Chair - Time and Place
 John Plumb, Chair - Scientific Journal

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 Ron Hedrick
 Dave Groman

1995-1996 President's Message

I am honored to have been elected President of the Fish Health Section for the coming year and look forward to serving the membership. This is an important time for the Fish Health Section. Large-scale changes in fisheries management, the intergration of new diagnostic and certification technologies, a constraining fiscal climate, and the entry of new professionals and agencies into the aquatic animal health arena, make this a challenging and dynamic period. My job will be to assist you in insuring that the Fish Health Section retains its leadership role in the field of aquatic animal health and serves as a catalyst for integration of fish health professionals from several entities with a long-term goal of enhancing the health of aquatic animals in the private, state, tribal and federal areas of responsibility.

Ted Meyers has done a superb job as president in the past year. His example, and that of Ron Thune in the year before, are a source of inspiration. The completion of the Blue Book, establishment of a continuing education program, and updating the professional standards are important accomplishments. There are other tasks to be completed in the coming year. These include: updating the bylaws, creation of a new procedures manual, improvements to the awards process, and streamlining some committees. The Section is in excellent financial shape.

The Fish Health Section has been instrumental in improving the health of aquatic species by developing technical procedures for examination of fish and shellfish, assisting in the development of regulations to protect the health of aquatic animals, in defining standards of professional training and experience, and in sponsoring meetings, a journal, and a newsletter that serve as effective means for the exchange of scientific and technical information. These roles have been critical to the growth and professionalism of our field and we should take pride in these important accomplishments that are the result of substantial investment of time and effort by many dedicated members of the Section. I am extremely grateful to those who have worked so hard on behalf of the Section in the past and I will ask you to continue this level of commitment in the days ahead.

Jim Winton



Jim Winton presenting President's Award to Ted Meyers

Letter to the Editor

Is the Fish Farmer Becoming an Endangered Species?

From my travels throughout America and Canada since I left academia more than a year ago, I have come to the conclusion that the commercial fish farmer will soon be on the endangered species list. This conclusion is based largely on what are perceived as the hatchery bashing policies of many state, provincial, and federal conservation agencies. These policies have engendered some very negative perceptions of these agencies, particularly of those involved with fish health management. Now, it is not the issue at this point whether these perceptions are correct or not. The issue is that these perceptions are held and are being expressed publicly with increasing frequency and fervor throughout the commercial fish farming communities in America and Canada.

Specifically, these negative perceptions about the fish health profession and professional conduct of its members are largely aimed at those working in the public sector(s) under the guise of fish health management. Fish farmers have publicly expressed their concern(s) about the actions of state and federal fish pathologists and fish health inspectors. The major concern is that many, if not most, of these actions were perceived as capricious and arbitrary. In the opinion of the fish farmers, there was little legal basis for some of these actions.

In a few cases, where I knew the persons alleged to have caused the concern, I looked further into the situation and spoke with the person(s) involved. In more than one case I detected a definite feeling of anti-commercial fish farming. This attitude, on further looking about, seemed to be throughout the fishery section of the agency. The opinion that "these farms are the source of disease problems in our wild fish populations" was expressed by several agency personnel.

It is absolutely ridiculous, in my opinion, that professional conservation biologists and fish pathologists should publicly hold private fish farms accountable for episodes of whirling disease in free-living fish. It is even more nonsensical to depopulate hatcheries and fish farms because of having detected a heretofore undetected pathogen. This practice does not have a high success rate and the organism usually rears its ugly head later. In addition, if one were but to look, one would probably find that the heretofore undetected pathogen actually has quite a geographical range already established.

Quite frankly, I am very dismayed by all this. Has the fish

health profession become so enamored with the authority vested in it that they have forgotten their professional responsibilities? Those in state and federal agencies-including universities-are public servants. The primary professional responsibility is service. Whether it sits well or not, fish health professionals are members of a medical profession and should present themselves as such rather than as "pathogen vigilantes"

In closing, I shall not apologize if I have stepped upon someone's "professional toes". In fact, I think an apology would be due me from the person having placed his/her professional toes where I was to put my foot down. I might have done myself an injury had I been careless.

G. W. Klontz
 Moscow Idaho
 208-882-5812.

**IMPORTANT WORKSHOP
 MARK YOUR CALENDAR**

Whirling Disease - Where do we go from here?

- *Historic Overview and Biology of Whirling Disease
- *Whirling Disease Distribution in the USA
- *Experiences with Whirling Disease
- *Existing Policies and Regulations
- *Whirling Disease Management Strategies
- *Needs and Future Direction

**WHERE? Denver, Colorado
 WHEN? February 6-8, 1996**

PURPOSE:

To bring together concerned public resource agencies, coldwater angling organizations, interested members of the outdoor press, as well as interested individuals, to increase common understanding of the Whirling Disease problem and examine how best to address Whirling Disease, in a cooperative manner to ensure the long term protection, management, and use of public fishery resources.

SPONSORS:

Colorado Division of Wildlife, USFWS, Trout Unlimited, BLM, NBS, USFS, AFS, American Sportfishing Association, Colorado Aquaculture Association, Montana Fish Wildlife & Parks, Whirling Disease Foundation, Various state agencies.

CONTACT:

Eric Bergersen, Arrangements Chairman, immediately. 303-491-5396 or fax 491-1413. Attendance will be limited. Registration fee is \$75 through 12/31/95. Late registration \$100.

Fish Health Section Newsletter

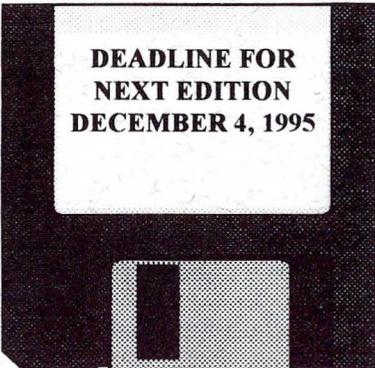
The editors of the FHS Newsletter thank the members for their support regarding their enthusiasm in submitting contributions for publication in the newsletter. The **prohibitive** cost of mailing more than a 20 page newsletter, however, imposes limits for the length of each article so we are implementing **new guidelines** for authors. Articles should not exceed 4 single spaced typed pages so that the maximum length would not exceed 6 newsletter columns. Also, please note that articles will continue to be accepted with the understanding that the material will be published without peer review. Articles should be submitted on disk in **Word perfect 5.1 or in generic form that can be read on WP5.1**. Disks will be returned if a SASE is included with your submitted article. Again, thank you all very much for your continued support, which allows for the publication of a high quality and informative newsletter. The Fish Health Section Newsletter is a quarterly publication of the Fish Health Section of the American Fisheries Society. Submissions should be addressed to the editors listed below:

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