FISH HEALTH SECTION
AFS
NEWS LETTER

Volume 13, Number 1 January 1985

WHIRLING DISEASE REPORTED AT TWO CALIFORNIA HATCHERIES

In early July of 1984, juvenile fish at the California Fish and Game, Mt. Whitney Hatchery were displaying the classic signs of whirling disease including tail-chasing and black peduncles. Although there was almost no mortality occurring, these fish were found to be infected with *Myxosoma cerebralis*. Infected stocks included rainbow, brook, brown and golden trout. Incidence studies showed that golden trout were most susceptible with 100% of the fish examined showing infection and harboring a high number of spores. Incidence in brook, rainbow and brown trout was 60%, 40% and 10-20%, respectively.

Because juvenile fish from Mt. Whitney are taken to Black Rock Hatchery for rearing to catchable size or for production of brood stock, the fish at this site were also examined and subsequently found to be infected with *M. cerebralis*. The yearling rainbow at Black Rock had lower incidence of infection and those infected had fewer spores than fish at the Mt. Whitney Hatchery.

Both Mt. Whitney and Black Rock Hatcheries are located on the eastern slope of the southern Sierra Mountains in Inyo County, California. They are in a watershed draining into the Owens River, which is eventually diverted to aqueducts and canals of the Los Angeles Water and Power Company for use as domestic water in southern California. No major salmonid fishery exists in the Owens River below the two affected hatcheries.

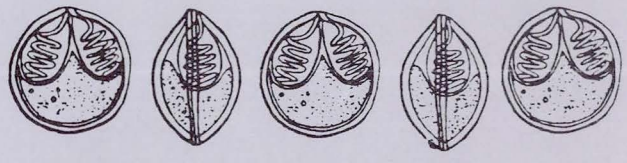
Unfortunately, some infected fish had been planted before *M. cerebralis* was diagnosed in the hatchery fish. The fish were planted in relatively close proximity to the Mt. Whitney and Black Rock Hatcheries and their distribution should not impact any other major watersheds. Fish which were planted in the Owens River above the hatcheries do have a potential of affecting a fishery in that area. Hatchery fish recovered from the wild were found to harbor *M. cerebralis*.

Following the diagnosis of whirling disease in the hatcheries, millions of fish were destroyed. These included fingerlings, catchable adults and brood fish. Some of the larger fish were used for human consumption after the heads had been removed and destroyed. Although numerous fish were sacrificed, 1000 juveniles of each species were monitored throughout the summer, and suffered no abnormal mortality until they were killed in November, 1984. A decision was made to maintain a small number of rainbow trout brood stock so that a seventeen year genetic development program would not be jeopardized. Sufficient numbers of these fish were kept to insure a future brood stock of these animals.

Pathologists believe that whirling disease probably first occurred, albeit undetected, in 1983 in the fish held at the Black Rock facility. When these fish were planted above the Mt. Whitney Hatchery, they became the source of infections which were detected in 1984. *M. cerebralis* was found in two and three year old fish at Black Rock, but not in four year old brood fish.

In addition to the depopulation of these hatcheries, a systematic chlorination of the facilities was implemented. An effort is also being made to remove all fish from the water supplies of both sites and to keep them free of fish for as long as possible.

Four commercial hatcheries in California also have had a history of whirling disease. In the early 1960's, two hatcheries located in the central coast (Santa Cruz/Monterey) area were affected and subsequently suspended operation. Recently, two hatcheries on the Mokelumne River on the western slope of the Sierra Nevada Mountains have become affected and are currently in quarantine and are being monitored. For additional information contact Don Manzer, California Department of Fish and Game, 2111 Nimbus Road, Rancho Cordova, CA 95670.



ADDENDUM TO FISH DISEASE LEAFLET 47

G.L. Hoffman, U.S.F.W.S., Fish Farming Experiment Station, Stuttgart, AK

When the revised Fish Disease Leaflet 47 on whirling disease (*Myxosoma cerebralis*) was written, omitted was the basic parasitological must of "this species differs from its closest relatives in--". Please clip this out and insert it in page 3 of FDL 47:

***Myxosoma cerebralis*--location, size, and morphology:** *Myxosoma cerebralis* is the only *Myxosoma* found in the cartilage of salmonids (the genus *Myxosoma* can be verified by the absence of an iodinophilous vacuole; it is present in *Myxobolus*.) *Myxosoma squamalis* occurs **in the scales** of Western USA salmonids, is about the same size as *M. cerebralis* (about 9 microns) but possesses a narrow, but obvious, ridge that parallels either side of the sutural ridge. *Myxobolus kisutchi*, another salmonid parasite, occurs **in the central nervous system,** is about the same size as *M. cerebralis,* but possesses an iodinophilous vacuole. *Myxobulus neurobius,* more widespread geographically, also is found **in the central nervous system**, but it is pear-shaped and larger--10-13 x 7.5-8 microns. *Myxobolus insidiosus* is found in the musculature with free spores in gills, kidney, liver and spleen of chinook and coho salmon, and cutthroat trout in Oregon and Washington, but it is pear-shaped and larger (13-17 x 6.4-9 microns). With this information any microscopist ought to be able to identify *M. cerebralis*. For further information contact Dr. G.L. Hoffman, U.S. Fish and Wildlife Service, Fish Farming Experimental Station, P.O. Box 860, Stuttgart, AK 72160.

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PASSAGES

Dave Groman has moved from his post in Iceland at the Institute for Experimental Pathology to a new research position at the Department of Agriculture and Fisheries (DAFS) Marine Laboratory, P.O. Box 101, Victoria Rd., in Aberdeen, Scotland.

Ron Hedrick has moved from the Bodega Bay Marine Laboratory to the main campus of the University of California-Davis. His new address is Department of Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616. Phone (916) 752-3411.

FROM THE EDITORS

During the year your editors have learned a lot about what it takes to put out a newsletter on time and of a quality that reflects well upon the Section. As we begin the new year we would like to share with you a few observations and request your help. The first observation is that the Section treasury will not support the current costs of the Newsletter much longer. The costs of publication now account for most of the expenses of the Section and exceed our revenues. The choices facing the Section seem to be: 1) raise FHS dues, 2) increase FHS membership, or 3) reduce newsletter expenses. Perhaps it reflects our bias as editors, but we feel that the Newsletter is the most visible arm of the Section and we have worked hard to produce a quality publication. The current costs for FHS are not high relative to the parent society. Perhaps the FHS is not receiving sufficient support from AFS to assist in maintaining a viable Section. However, the most obvious and distressing fact is that many fish health professionals are not FHS members. By increasing our membership we could easily retain our present dues structure and afford our Newsletter.

We have asked each other why more persons have chosen not to join FHS and can think of a few reasons. The most important must be a question of value. Many do not think the dues for AFS plus FHS equals a reasonable cost for what they are receiving. We suspect tha some additional members could be attracted if they could join only the Section without having to join the parent society. There is little doubt that such a proposal would meet with opposition in the AFS. It should be possible to create a foreign membership category for those interested in fish health whereby they could receive the Newsletter for the costs of production and mailing. This might add fifty or more members to the Section. A second problem is that many fish health workers (we all know several) are not members now but could be induced to join by peer pressure and a little arm twisting. Perhaps we could begin by publishing names of fish health workers who are not members along with their home phone numbers so you could call them at 2 A.M. to tell them what they are missing.

As for the Newsletter itself, we asked for your help and have been getting it. We sincerely appreciate the time and effort that many of you have taken to contribute. We have tried to provide space for submissions of any length and on any topic related to fish health. Among those that have been published are articles which begged for, no demanded, responses. It seems odd to us that we have received no letters to the editor, concerning these articles. The success of this Newsletter is directly in your hands. We need your continued support and pledge in the coming year to do our best. Please pledge to us that you will send at least one small article. Best wishes for the coming year.

J.S.R. *D.P.R.* *J.R.W.*

REPORTS FROM PAST MEETINGS

The First International Colloquium of Pathology in Marine Aquaculture was held September 11-14, in Monpellier, France. Topics included both fish and shellfish subjects. Subject sessions included: Protozoosis (with review by J. Lom); Molluscan Epidemiology; Pathology and the Environment (with review by H. Moller and K. Anders on high disease rates of estuarine fish); Fungal Diseases; Parasitology Round Table; Virus Diseases (with reviews on viral diseases of crustacea and marine fish by D. Lightner and B. Hill, respectively); Bacteriological Diseases (with review by H. Tubiash and R. Elston on bacteriological diseases in Molluscs); Tumor Round Table; Immunology and Hematology (with fish immunology and hematology review by M.J. Manning and M.S. Mughal) and Prophylaxy and Treatment. Mediterranean weather was mild and French hospitality, including ample oyster and wine tasting was superb. Details can be obtained from Ralph Elston, Center for Marine Disease Control, Battelle Marine Research Laboratory, 439 West Sequim Bay Road, Sequim, WA 98382.

FUTURE EVENTS

February 5-8, 1985. Second International Conference on Warm Water Aquaculture Fin Fish. To be held at Brigham Young University, Hawaii Campus, Laie, HI 96762. Contact T. Aaron Lim at the same location for additional information.

The first annual West Coast edition of the fish disease shortcourse entitled "Introduction to Fish Health", will be presented March 25-29, 1985. This shortcourse is co-sponsored by the Fish and Wildlife Service and the Aquaculture Program of Mt. Hood Community College, Gresham, OR. Resident instructors for this course will be Jim Warren and Steve Leek. The scope and content of the material presented is designed for hatchery personnel and is basd on the popular course held in LaCrosse, WI for the past 15 years. Twenty-four participants can be accommodated on a first-come, first-served basis. Additional information is available by calling Jim Warren at (206) 696-7605. If you want a space to be reserved in your name for the 1985 shortcourse, letters of application (including an approved form SF-182 for U.S. Government applicants) should be sent to Leetown Fisheries Academy, National Fisheries Center, Box 700, Kearneysville, WV 15430 no later than February 1, 1985.

A short course "Diagnosis and Treatment of Diseases of Warmwater Fish" (WL 4124/6124) will be taught at Mississippi State University May 13-24, 1985.

This short course is to provide instruction in the methodology of diagnosis and treatment of parasitic, bacterial, viral, nutritional and environmental diseases of warmwater fish. Undergraduate or graduate credit of four semester hours is given for successful completion of the course. Tuition for the course is $176.00, however, this is subject to change.

The course is limited to 24 persons, and applications must be received on or before March 1, 1985. Persons interested in taking the course should apply by writing Dr. Thomas L. Wellborn, Jr. at P.O. Box 5405, Mississippi State, MS 39762. All applicants will be advised whether or not they have been accepted to attend before April 1, 1985.

Students will be expected to provide their own compound microscope and dissecting kit for use in the laboratory. However, a limited number of microscopes will be available.

Instructors for the course will be Dr. Thomas L. Wellborn, Jr., Leader, Wildlife and Fisheries Department, Mississippi Cooperative Extension Service, Mississippi State University, and Dr. J. "Randy" MacMillan, Area Extension Fisheries Specialist, Stoneville, MS.

July 10-12, 1985. Fish Health Section 1985 Annual Meeting. The meeting will be held in Nanaimo, B.C. and additional information can be obtained from Trevor, P.T. Evelyn, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada.

July 9-11, 1985. Midwest Fish Disease Workshop. The 16th annual meeting will be held at the Continental Regency Hotel, Peoria, IL. A fish fry and a tour of the new Sand Ridge Hatchery will be part of the proceedings. For information contact Rodney Horner, Illinois Dept. of Conservation, R.R.3, Clearview Estates, Manito, IL 61546.

EDITORS' NOTE: The AFS is establishing a meeting and symposium clearing house. From the above, this would seem a good idea.

July 15-16, 1985. Western Fish Disease Workshop. The twenty-sixth annual meeting will be held in Seattle, WA. For information contact Kevin Amos, Washington Dept. of Fisheries, Olympia, WA 98502 or John Majnarich, Biomed Research Lab, Seattle, WA 98122.

POSITION ANNOUNCEMENT

A post-doctoral position (one year with possible renewal for additional year) is available at the Center for Marine Disease Control, Marine Research Laboratory, Battelle Northwest, Sequim, WA. The successful candidate will be expected to conduct research programs to develop methods to initiate and characterize cell, tissue and organ cultures (primary and potentially continuous cultures) from selected marine invertebrate species. Applicants should possess demonstrated technical background and research aptitude in biochemistry, invertebrate physiology and experience in cell and/or tissue culture. Applicants must possess U.S. citizenship.

Applicants should forward resume, technical reprints and statement of pertinent experience and objectives to: Dr. Ralph Elston, Center for Marine Disease Control, Battelle Marine Research Laboratory, 439 W. Sequim Bay Road, Sequim, WA 98382. The position is expected to be filled prior to July 1, 1985.

NOMINATIONS SOUGHT

We are now soliciting nominations for the S.F. Snieszko Distinguished Service Award. This is the highest award of the Fish Health Section and is presented for the purpose of honoring individuals for outstanding accomplishment in the field of fish health. Selection of a candidate for the Distinguished Service Award will be based on merit qualifications of the nominee and should be included with the nomination.

Nominations should be received no later than February 1, 1985, by the Awards Committee. Submit nomination to:

Emmett B. Shotts, Jr., Chairman
University of Georgia
Department of Medical Microbiology
Athens, GA 30602

John S. Rohovec
Department of Microbiology
Oregon State University
Corvallis, OR 97331

Dennis Anderson
Fish Disease Control Center
P.O. Box 917
Fort Morgan, CO 80701

FDA APPROVES USE OF FORMALIN

David F. Walsh, Registration Liaison Officer,
Division of Fishery Research, USFWS,
Washington, D.C., 20240

Formalin, probably the most widely used material in the treatment of fish diseases, can again be legally used in fish culture. The use of formalin was lawful until 1972, when the Food and Drug Administration (FDA) was given the Congressional mandate to assure that all drugs and chemicals used on food animals met certain standards. After nine years of intensive research at the Service's National Fishery Research Laboratory, La Crosse, Wisconsin, it was proven that the compound can be used without harm to the fish, the consumers, or the environment; and in April 1982, the FDA published an official notice that registration requirements had been satisfied. Now, a private firm, Natchez Animal Supply Company, Natchez, Mississippi, has agreed to sponsor formalin and apply for a New Animal Drug Application (NADA) permit. FDA officials have been notified that the Service's master file can be used to support this application. After meeting some minor but important requirements on labeling, information on manufacturing methods, facilities and controls, and preparing an environmental impact analysis report regarding the manufacture of formalin, the company will market a commercial product under the name "NAS-Formalin-F".

IMMUNOFLUORESCENT ANTIBODY PROCEDURES FOR DETECTION OF BACTERIAL KIDNEY DISEASE IN FISH TISSUE

D. P. Anderson, National Fish Health Research Laboratory, Box 700, Kearneysville, WV 25430

The use of fluorescein-conjugated bacterial kidney disease (BKD) antisera for the detection of this pathogen by immunofluorescent antibody techniques (FAT) in fish tissue has provided a rapid and sensitive serodiagnostic method.

In the mid-1970's antisera to BKD were prepared at the National Fish Health Research Laboratory (NFHRL)—Leetown and have continued to be used in precipitin, agglutinin, indirect and direct FAT, enzyme immunosorbent assays, and other developing immunological tests. A general procedure for the direct bacterial FAT is given in the 1979 **Fish Health Section Blue Book,** based on a procedure by G.L. Bullock and H.M. Stuckey (J. Fish. Res. Board Can. 32:2224, 1975). This method has continued to be used successfully; however, during the last 10 years, fish pathologists and researchers in other laboratories have adapted it to their own special needs. Feedback information to the Biologics Section NFHRL reveals some interesting data about the specificity and peculiarities of the antisera lots and variations of methods of use in field diagnostics.

Briefly, the general procedure entails the following

1. Prepare a smear (fish kidney tissue, for instance) on a glass slide and let it dry for 5 minutes at 60°C.
2. Overlay the specific antisera conjugate on the smear and allow to react for 5 minutes. A counterstain (like rhodamine) is added to make a background contrast. Rinse off the conjugate with phosphate buffered saline and examine the slide after adding pH 9.0 mounting fluid.

One particularly interesting alteration from the general procedure is that some laboratories are using fixatives or solvent washes to defat the slide after the first step of heat-fixing the tissue smear. For instance, John Cvitanich has flooded smears with methanol, ethanol, or xylene for 1 minute with one repeat. Evidently this harsh fixative procedure does not destroy the important serodiagnostic antigens of BKD. Tosh Yasutake has shown that Bouin's fixative can be used in preserving histological sections without destroying antigens functioning for BKD-FAT examination. Diane Elliott has observed good results with sections preserved in buffered formalin fixative.

In the second step, John Cvitanich, Eric Pelton, and Rod Horner have left conjugate on the slide for longer than 5 minutes--up to 1 hour--however, incubation should be done in a humid chamber to prevent drying. In some cases, the conjugate overlay can be repeated to heighten fluorescent intensity.

Another counter stain, 0.01% Evan's blue, can be used by flooding the slide for 2 to 5 minutes after the conjugation step. This gives a red (yes, red) background to contrast with the apple green fluorescent bacteria. Chuck Carlson and others do not use counterstains, yet report excellent results checking with control BKD cells preserved in formalin saline.

Particulate contamination of antisera has been reported in some cases. Antisera are vulnerable when samples are handled for dilution, refrozen, or held in the refrigerator for extended periods. The stock antisera held at the laboratory are filtered occasionally and rechecked for titer. However, clumps—possibly protein—and bacterial contamination do occur and antisera may have to be refiltered and restandardized.

Rod Horner reports fluorescence improving over time in reconstituted lyophilized conjugate stored at 4°C and wrapped in foil. Maximum fluorescence was obtained at 3 weeks after which it dropped off. A possible explanation for this is that the fluorescent tag had come off the antibody molecule and resident time in the refrigerator allowed it to recombine.

Undoubtedly, titers of the conjugate are reduced upon long-term storage, light exposure and repeated changes in temperature; therefore, some people report freezing small aliquots of diluted antisera for later use with little or no detectable loss of titer.

In the final step, pathologists stressed the removal of bubbles in the mounting fluid as important. In addition, large clumps of tissue may remain on the fixed slide, especially if the samples were prepared in the field. These should be removed before proceeding with the conjugate step.

The following individuals contributed to this work: J. Cvitanich, Anadromous, Inc., Corvallis, OR; R. Grischkowsky and J. Follett, Alaskan Fish and Game Dept., Anchorage, AK; D. Elliott, Univ. Washington, Seattle, WA; R. Horner, Illinois Fish and Game, Manito, IL; D. Anderson, R. Brunson, C. Carlson, D. Desens, O. Dixon, E. Pelton, C. Starliper, and G. Taylor, U.S. Fish and Wildlife Service.

ANOTHER METHOD FOR DETECTION OF BKD

Craig R. Banner, Oregon Department of Fish and Wildlife Department of Microbiology, Oregon State University Corvallis, Oregon 97331

Dr. M. Yoshimizu, from the Laboratory of Microbiology at Hokkaido University in Hakodate, Japan informed us of another method for detection of *Renibacterium salmoninarum* (BKD). For some time now Dr. Yoshimizu has been using the method, which utilizes a biotinylated antibody and fluorescein labeled avidin to produce a strong, specific fluorescence of BKD. Following is a brief explanation of the method. A tissue smear is incubated for 20 minutes with antibody directed against *R. salmoninarum* (in our laboratory we have used a purified preparation of rabbit IgG directed against BKD). After several washes with phosphate buffered saline, goat anti-rabbit IgG covalently linked to biotin is added and allowed to react for 20 minutes. The preparation is washed again and a solution containing fluorescein-labeled avidin is added. After final rinsing, the smear is mounted in glycerol buffered to pH 8.5 and observed by fluorescence microscopy. Although the method is time consuming, it seems to have several advantages over the direct fluorescent antibody technique currently used. Background fluorescence is minimal without the use of a counter stain and fluorescence does not "burn out" as fast as the fluorescence achieved through use of directly labeled antibody. Dr. Yoshimizu indicated the longer time required for staining is not as big a drawback as it seems because stained slides may be stored in the dark for several weeks without loss in the quality of the preparation. Reagents and more information are available from Vector Laboratories, Inc., 1429 Rollins Road, Burlingame, CA 94010. (415) 348-3737.

We have done some preliminary evaluation of this method for the detection of *R. salmoninarum, C. shasta, A. salmonicida,* as well as other fish pathogens, and feel it has potential as a diagnostic tool and an aid to research.

WATER HARDENING EGGS WITH ERYTHROMYCIN FAILS TO PREVENT BKD

T.P.T. Evelyn, L. Prosperi-Porta, and J. Ketcheson, Pacific Biological Station, Nanaimo, British Columbia, Canada V9R 5K6

In a carefully controlled study, coho salmon eggs infected internally by experimental means with *Renibacterium salmoninarum* gave rise to *R. salmoninarum* infected hatchlings despite treatment during water hardening with 50 ug/ml erythromycin phosphate, a concentration 25 times that normally used in hatcheries. The drug's lack of efficacy is apparently because of its inability to cross the egg's perivitelline membrane. It therefore fails to contact the intraovum pathogen which apparently occurs in the yolk.

ISOLATION OF A NEW FISH VIRUS FROM CHINOOK SALMON IN OREGON

J.R. Winton, C.N. Lannan and J.L. Fryer,
Department of Microbiology, Oregon State University,
Corvallis, OR 97331

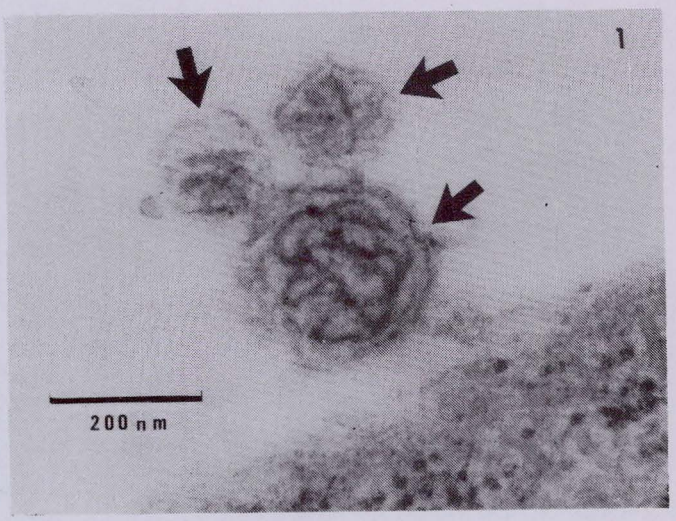
D.P. Ransom, Oregon Aqua-Foods,
88700 Marcola Rd., Springfield, OR 97478

A new fish virus has been isolated from returning adult chinook salmon (*Oncorhynchus tshawytscha*) at two locations in Oregon. The virus was recovered from pooled kidney and spleen tissues using CHSE-214 cells incubated at 18°C. In both cases, no evidence of virus was observed on the initial 14 day passage, and routine "blind passages" were conducted. At the end of the second 14-day incubation period, small foci of rounded cells began to develop in the monolayers.

The virus replicates in cell lines derived from Pacific salmon (CHSE-214, CHH-1, KO-6 and CSE-119) at an optimal temperature of 18°C. No virus was produced at 24°C, or in the EPC, BB, AS or RTG-2 cell lines.

The virus was stable in minimum essential medium at pH 3-11, but not at pH 2. Replication was not affected by 50 ug/ml iododeoxyuridine suggesting the agent has an RNA genome. Infectious virus was recovered from fractions of a CsCl gradient with a density of 1.20 g/cc. The virus was inactivated by chloroform and caused hemagglutination of the erythrocytes from several species of fish, birds and mammals.

Thin sections of infected cells were examined by electron microscopy and revealed enveloped particles 125-250 nm in diameter containing a coiled nucleocapsid (Fig. 1). The nucleocapsids were extracted with freon and negatively stained. Electron micrographs of these preparations (Fig. 2) showed 18 nm helical structures over 1000 nm in length. Based on morphology and biochemical features, this new virus appears to be a member of the Paramyxoviridae.



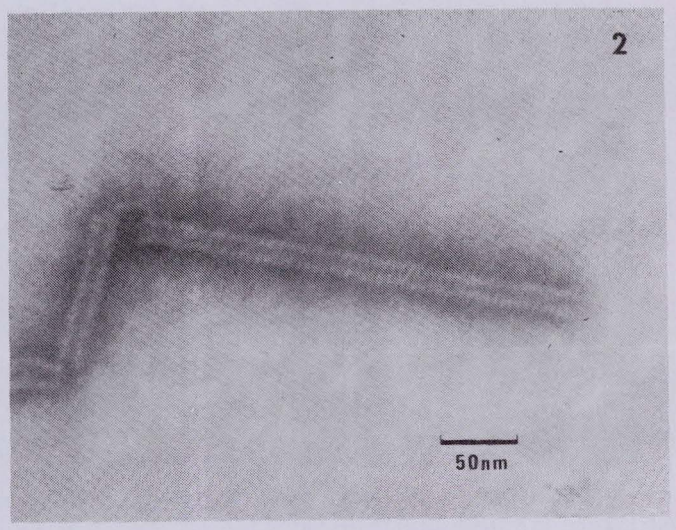


Figure 1. Thin section through an infected CHSE-214 cell showing the enveloped virions (arrows) containing coiled nucleocapsids.

Figure 2. Negative stain of a freon-extracted nucleocapsid. The helical nucleocapsid is 18 nm in diameter and over 1000 nm in some preparations.

ADENOVIRUS-LIKE PARTICLES ASSOCIATED WITH A DISEASE OF CULTURED WHITE STURGEON

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

An examination of juvenile white sturgeon, *Acipenser transmontanus* Richardson, suffering from a chronic mortality revealed the presence of enlarged nuclei particularly prominent in the epithelium of the straight intestine and spiral valve. These nuclei were often five times larger than those of uninfected cells and contained numerous virus particles with an average diameter of 74nm. Although nuclear changes induced by the virus are similar to those described for certain members of the family Herpesviridae, the virion morphology and absence of an envelope are more consistent characteristics shared with the Adenoviridae. Attempts to isolate the virus using established cell lines from selected freshwater fish and two lines recently developed from white sturgeon were unsuccessful. Nuclear enlargement indicating virus infection was, however, observed in juvenile sturgeon receiving intraperitonal injections of homogenates of viscera from infected fish.

OUTBREAK OF IHNV DISEASE IN A LABORATORY SETTING

Joseph R. Sullivan, Alaska Dept. of Fish and Game,
F.R.E.D. Division, Fish Pathology Section,
Anchorage, AL

Though IHNV disease has been a limiting factor in the culture of sock eye salmon (*Oncorhynchus nerka*) in hatcheries, unplanned outbreaks in small laboratories have been fairly rare. Such an outbreak did occur in two of ten tanks of 1983 Meadow Creek, Alaska sockeye salmon fry at the U.S. Fish and Wildlife Service Laboratory in Anchorage, Alaska in July. Tanks were fiberglass, 1.2 m in diameter, approximately 1.0 m deep, and were stocked with 1000 fry each. The same stock had suffered a 13% mortality to IHNV disease several months earlier at Alaska Department of Fish and Game's Big Lake Hatchery, but the disease appeared to have been contained and limited to fry from several incubators. Absence of detectable disease in the remaining fry allowed their release and use in the USFWS lab. Since the laboratory uses well water for fish culture, it is most probable that the virus was present in the fry when they were transported from Big Lake Hatchery two months earlier. Lack of detectability does not therefore guarantee absence of this pathogen. Laboratories using stocks with a history of IHN should make efforts to protect other stocks and projects from cross-infection and contamination.

BROOD STOCK SELECTION FAILS TO CONTROL IHN AMONG CHINOOK SALMON

Warren Groberg, Oregon Department of Fish and Wildlife, Department of Microbiology, OSU, Corvallis, OR 97331

The first isolation of infectious hematopoietic necrosis virus (IHNV) at an Oregon coastal location was made from adult fall chinook salmon at Elk River Salmon Hatchery (Oregon Department of Fish and Wildlife) in the fall of 1975. Fall chinook from the Elk and Chetco Rivers are propagated at this southern Oregon hatchery and progeny from both stocks underwent clinical IHN in the spring of 1976. Mortality occurred in most of the raceways but was not typically catastrophic for an IHN epizootic. For these and other reasons, the decision was made not to destroy fish in raceways known to contain IHNV infected fish and to attempt to "live with" IHN. This approach resulted in a quarantine being placed on these two rivers: progeny from either of these stocks could only be released into their indigenous waters. IHNV has been detected in either or both the Elk and Chetco River stocks in seven of nine years since it was first isolated. Juvenile mortality from IHN has occurred in only four of those nine years, and until 1984 the losses were not devastating and production goals were not impacted by the disease. The quarantine has remained in effect since 1976, however, severely constraining possible utilization of these valuable stocks outside their respective watersheds.

In the 1979-80 brood year, a brood stock selection program was begun and since that time only eggs derived from, and fertilized by, virus-negative parents have been hatched. No IHN mortality was identified in the progeny during the first four years of the brood stock selection program while the virus was isolated from adults in three of the five years since 1979-80. A feeling that the selection program by itself was successful began to prevail even though raw Elk River water continued to be used for egg incubation and all rearing at the hatchery. Elk River maintains a productive wild fall chinook population and IHNV has been detected in some feral adults. Some portion of the hatchery stock also spawn in the wild and the potential for water-borne transmission of IHNV from late spawning adults, with a high virus carrier rate in the river, to the earliest hatched juveniles, was probable.

This indeed seems to have been the case for the 1983-84 brood year, the first year in which IHN reached significant proportions producing a 25% loss in juveniles at Elk River Hatchery. The Elk River run-size for this year was the largest of the five years since brood stock selection began. Further, the virus was detected earlier and at a higher incidence for a longer period during the spawning season than in the previous four years. These factors undoubtedly produced higher levels of virus in the hatchery water supply than were present from 1979-80 to 1982-83. Fish in four of four raceways containing the earliest hatched juveniles developed IHN during the fourth week of January. Six of six groups ponded the following week broke with IHN and one of two, one of three and three of eleven groups developed the disease during the subsequent three weeks of ponding, respectively. The cumulative mortality was more typical of IHN epizootics and reached over 50% in most raceways when it was decided to destroy all fish in raceways with documented IHN. The proportion of raceways with fish developing IHN for a given week of ponding appeared to be directly correlated to the number and virus carrier rate of adults in the system. In weeks during which fish in all raceways developed IHN there were large numbers of spawning fish and the carrier rate was high. In later weeks when a small proportion of fish in raceways underwent IHN, the incidence of virus in adults remained high but the spawning population dwindled. Since all juveniles at the hatchery were progeny of parents determined to be virus-negative, it must be assumed the source of the virus was spawning adults above the water supply intake.

While it may be that brood stock selection mediated loss to IHN, two important conclusions can be drawn from this experience. First, brood stock selection for IHNV negative parents without concomitant elimination of virus from incubation and rearing water may be futile. A great deal of manpower, time and expense is incurred in individual mating pair crosses of virus-negative males and females to eliminate presumed, and as yet unproven, vertical transmission. This investment is questionable if horizontal transmission of virus via the hatchery water negates the anticipated benefits. Secondly, in spite of brood stock selection and a lack of known IHN mortality in the prior four years, the carrier rate in the 1983-84 brood was the highest known in these two southern Oregon fall chinook stocks. This suggests that brood stock selection contributed nothing towards reducing the carrier rate and further emphasizes the need to consider the role of horizontal transmission in the epidemiology of IHN. For specific data concerning IHN on the southern Oregon coast please contact the author.

IVERMECTIN EFFICACY TRIALS FOR NEMATODES PARASITIC TO FISH

Richard Heckmann, Department of Zoology, Brigham Young University, Provo, UT 84602

Ivermectin is one of the avermectin pharmaceuticals recently discovered in Japan. It is offered in the USA by Merck and Company, Rahway, New Jersey. Avermectin is one of the antiparasitic macrocyclic lactones originating from *Streptomyces avermitilis* (actinomycete). It has been efficacious for the treatment of gastro-intestinal and blood-dwelling nematodes and ecto-parasitic arthropods of sheep, cattle, dogs and chickens. There have been no published data for the use of Ivermectin against roundworms of fish. For the past eight months we have been conducting trials centered on the use of this pharmaceutical for fish parasites.

The avermectins paralyze nematodes and insects by blocking neuromuscular transmission, a GABA-related event involving increased chloride permeability. In addition to the inhibitory effects, a direct nematocidal effect against the larval stage has been shown which may occur during the third larval stage of development.

Mottled sculpin, *Cottus bairdi*, with a known infection of *Rhabdochona* sp. (Nematode) were collected from a nearby river and maintained in 20 gallon aquaria in the laboratory. Varying amounts of an injectable formulation (0.05% active compound) were given to the fish 1M or added into the water (bath).

Following is a summary of our conclusions:

1. Ivermectin is a moderately effective nematocide for mottled (*Cottus bairdi*). More work has to be done on delivery methods and dose levels to achieve maximum efficacy in mottled sculpin and other fishes.
2. Ivermectin can be used as either a "bath" or injected into host fish. Good results were achieved when Ivermectin was added directly to the water of the aquarium. Dosage has to be moderated whereby the best level can be attained.
3. Dosage levels for fish (sculpin) are critical. Fish can be killed with high levels of Ivermectin. Data to date show injection levels above 0.05 ml per sculpin (2-3 inches in total length) can kill the fish. For "baths" less than 1 ml per 20 gallons over a period of five days appears to be ideal. Our best results were achieved with 0.7 ml per 20 gallons (8 fish) over 5 days (0.1 ml, 0.2 ml, 0.2 ml, 0.2 ml).
4. The drug appears to be effective in controlling *Rhabdochona* sp. in mottled sculpin (*Cottus bairdi*).

STATISTICS AND THEIR SIGNIFICANCE

Stan Eisen, Christian Brothers College,
650 East Parkway South, Memphis, TN 38104

I would like to share with you an observation regarding the Fish Disease Workshop held last July in Little Rock, Arkansas and follow up that observation with a proposal.

The observation is that the use of statistical methods in verifying the significance of presented data was either inadequate or nonexistent.

To me, the lack of basic statistical information is a serious omission of an integral part of the research enterprise. Without certain information regarding the sample size and characteristics, the listener is left with the uncomfortable responsibility of deciding whether the results under discussion are meaningful or not.

Obviously, some studies do not lend themselves to statistical analyses, such as a description of the histopathology of a disease or condition of an individual organism. However, once the scientist is dealing with the prevalence or severity or disease symptoms, then there are some descriptive statistics which should be considered, including those of central tendency and those of dispersion.

I. STATISTICS OF CENTRAL TENDENCY. Here, I am referring to the mean, median and mode, as values which provide some type of average or 'typical' case, if such is possible.

1. The **mean** is defined as the quotient between the sum of observed values divided by the number of organisms counted. It is, by far, the most frequently used statistic of central tendency.
2. The **mode** is defined as the most frequently encountered value in the sample. In a symmetric distribution, the mean, mode and median will be identical. However, in a skewed distribution, each of these parameters will differ. A particular distribution may have more than one mode. This parameter is particularly useful in describing parasitic infections, where most hosts will either be uninfected or will have very few parasites.
3. The **median** is defined as that value which lies exactly halfway in the distribution, with half the other values being greater, and the other half being less than the median. This type of value is more frequently encountered in describing the salaries of teaching and research faculty than in the research findings.

II. STATISTICS OF DISPERSION. Statistics of dispersion are indicators of the degree of variation shown by a group of organisms with regards to the parameter under discussion. These indicators would include the range, variance, standard deviation and confidence intervals.

1. The **range** is defined as the minimum and maximum values encountered in the study. It is relatively unmeaningful because it does not give any information as to where the values are clustered.
2. The **variance** is defined as the mean of the squared deviations about the mean, and the **standard deviation** is the square root of the variance. These two values serve as the basis for parametric statistical tests and are generally the most useful in biological research.
3. The mean of a sample may not be truly characteristic of the entire population, so confidence intervals are calculated. This provides a range of possible values near the calculated mean which would include the true mean.

Certainly, if the researcher is interested in comparing the severity or prevalence of a disease among two different groups of host organisms, then statistical tests should be used. Many parametric tests, while being robust and useful, are subject to a number of underlying assumptions which may render their use invalid. In such cases, the same data may be analyzed using non-parametric tests.

I'll be the first to admit that the primary responsibility of any biologist reporting his/her findings is to discuss what the animals or plants are really doing, and not to confuse the listeners with a large mass of numbers. My only argument is that a small mass of numbers provides information regarding the meaning and significance of the researcher's findings. Therefore, the proposal is that a greater effort should be made to provide meaningful descriptive statistics and/or the results of statistical tests if the complexity of the experiment and the number of organisms in the study lend themselves to analysis.

FISH HEALTH AND WATER QUALITY IN SOUTH DAKOTA

Jerry Broughton, Hatchery Biologist, Cleghorn Springs State Hatchery, Rapid City, SD

At Cleghorn Springs State Hatchery stress factors associated with nitrogen supersaturation and the recirculation of water are suspected of leading to outbreaks of bacterial gill disease and furunculosis. Gill disease is primarly affecting fish smaller than 150 per kg. with recurring infections appearing at about 2 week intervals. Over the past 3 months an average of 3 raceways per day have required treatment. To date, furunculosis outbreaks have only been evident in brown trout, although other fish in the hatchery have been exposed to this disease through recirculation of the water. Therefore, carrier fish are a distinct possibility.

Presently our recirculation system has no filtration or ammonia removal devices. Suspended solid loads are high, especially after periods of raceway cleaning and flocculant material is abundant on gill tissues. Un-ionized ammonia levels vary but are usually near 0.01 mg/l. Nitrogen gas saturation levels range from 120% in the spring water to 104% in recirculated water. It is anticipated that removal of the stress factors by improving the water quality will alleviate the disease problems at this station.

INFORMATION REGARDING JOURNALS

Dr. Robert L. Kendall, managing editor for AFS publications, indicates that **Transactions of the American Fisheries Society** is encouraging high quality submissions in the area of fish health. Dr. Kendall feels that many microbiologists and fish health scientists overlook **Transactions** when submitting research articles from their work.

New--Journal of Applied Icthyology (Zeitschrift Fur Angewandte Ichthyologie)

Paul Parey, Scientific Publishers, 35 West 38th Street, #3 W, New York, NY 10018, announces that manuscripts for Volume 1 Number 1, March 1985 are welcomed now.

This international journal will publish original papers, short communications and book reviews in the field of applied ichthyology, aquaculture and marine fisheries as well as the management of fish resources. Application-oriented research is emphasized.

The journal is published quarterly, size of each issue 48 pages. Annual subscription price is $50 plus $4.50 postage. Sample copy of additional information may be obtained from Paul Parey (above).

The 26-member editorial board includes three United States members--J.E. Halver, G.L. Hoffman and K. Wolf.

New - Diseases of Aquatic Organisms

A new journal will be published by Inter-Research, POB 1120, D-2124 Amelinhausen, Federal Republic of Germany. The first issue should be available in the spring of 1985. The journal will publish original articles, review articles and notes in three primary areas: diseases of aquaculture organisms, diseases of aquatic animals affecting human health and diseases of aquatic animals as indicators of man's impact on the environment.

Additional information may be obtained from Professor , Dr. Otto Kinne, Managing Editor, Diseases of Aquatic Organisms, Ecology Institute Nordbunte 30, D-2124, Oldendorf-Luhe, Federal Republic of Germany.

BRIEF REPORTS

In the last issue of the Newsletter it was erroneously reported in the Brief Reports that it will be necessary to pass an examination for certification as a Fish Health Inspector. There will be no examination for Fish Health Inspector; the written test is required only for Certified Fish Pathologists. The editors regret this error and any confusion it may have caused.

PKD has been diagnosed in three locations in British Columbia, Canada. Two sites on Vancouver Island, Puntledge River and Robertson Creek and in the Chehalis River which is in the Fraser River system. It has been found in coho salmon and rainbow trout. An extensive survey of wild salmonids has not revealed the reservoir of infection. Gary Hoskins, Pacific Biological Station, Nanaimo, British Columbia, Canada V9R 5K6.

Preliminary tests of oxalinic acid indicate that this chemical is effective against furunculosis in coho salmon. To a population of 100,000 fry, 8.5 mg oxalinic acid / kg fish weight / day was fed in the diet. After one week mortality was decreased from 1000 fish / day to 29 fish / day. Losses in a control group which were administered terramycin were only slightly reduced. Oxalinic acid has potential in treating terramycin resistant *A. salmonicida*. Gary Hoskins, Pacific Biological Station, Nanaimo, British Columbia, Canada V9R 5K6.

Investigators at the Pacific Biological Station in Nanaimo are still finding high numbers of bacteria in fish feed (see FHS Newsletter 12:4). *Salmonella* sp. have been detected. Gordon Bell, Pacific Biological Station, Nanaimo, British Columbia, Canada V9R 5K6.

The La Crosse National Fishery Research Laboratory has a number of ongoing projects including: 1) Investigating the toxicity of chloramine-T and its efficacy under various environmental conditions as part of registration-related work on the use of chloramine-T as a control for bacterial gill disease; 2) An analytical method has been developed for the detection and quantification of Hyamine 3500 residues in water and fish tissues; 3) Testing of eggs and fry produced by adult Pacific salmon treated with malachite green yielded detectable residues; 4) Operation of a new packed column / vacuum degassing system at the lab was begun after the installation of the Koch ring columns in the water supply tower. Prior to treatment, well water was 131% saturated with nitrogen and contained only 2 ppm dissolved oxygen. After treatment in the tower, the nitrogen level dropped to 105% and the oxygen level climbed to 9.3 ppm. The vacuum degasser unit reduced the nitrogen level to 96.5% of saturation and the oxygen level was 9.2 ppm. Although these data are preliminary, the system appears to be functioning as expected. Fred Meyer, La Crosse National Fishery Research Laboratory, La Crosse, WI 54601.

Mortality attributable to PKD has been confirmed in fish at several commercial trout facilities in Idaho. Fry to market-sized fish are affected with mortalities from 30-500 fish per day. If PKD fish are medicated for a bacteremia, with either sulfamerazine or oxytetracycline, a synergistic relationship seems to develop causing mortalities to double or triple. Nancy Wood, International Aquaculture Research Center, Route 1, Box 264, Hagerman, ID 83331.

Fertilization and water hardening of spring chinook eggs in 100 ppm Argentyne for 15 minutes did not increase egg mortality or formation of aberrant fry. Eggs were 1) fertilized and water hardened; 2) water hardened in iodophor; 3) treated with iodophor after water hardening. All treatments were 15 minutes duration. Tony Amandi, Oregon Department of Fish & Wildlife, Department of Microbiology, Oregon State University, Corvallis, OR 97331.

Ceratomyxa shasta pansporoblasts containing up to sixteen spores were noted in ascites fluid from naturally infected rainbow trout. Jerri Hoffmaster, Department of Microbiology, Oregon State University, Corvallis, OR 97331.

Winter steelhead at an Oregon hatchery were immunized for protection against *Ichthyophthirius multifiliis* using a whole organism prepa ration of *Tetrahymena thermophilus*. Fish weighing approximately 3.5 grams were immersed in a solution containing 1×10^6 organisms per milliliter for 30 seconds. Winter steelhead at the hatchery generally require repeated treatment for "Ich" throughout the summer. No treatment was required for the immunized fish. Tony Amandi and Craig R. Banner, Oregon Department of Fish and Wildlife, Department of Microbiology, Oregon State University, Corvallis, Oregon 97331.

Do your preserved, parasitized fish fade? If so, try shielding them from your fluorescent lights or sunlight! Many of you are aware of my fish parasite collection. In 1976, some nice, glass door cabinets became available, so I joyfully prepared rather permanent displays of many fish and many fish parasites. Slowly fading items escape our attention and I didn't realize until too late that fluorescent light, even through glass, causes melanin to bleach. Valuable display material should be stored in opaque door cupboards. G.L. Hoffman, U.S. Fish and Wildlife Service, Fish Farming Experimental Station, Stuttgart, AK 72160.

Edwardsiella tarda was cultured from the kidney and lateral line of moribund striped bass being held at the National Fish Health Research Laboratory after transportation from Harrison Lake NFH, VA. Affected fish were lethargic, swimming near the surface, had pale gills, and the cranial area between the eyes became light colored. Histological sections revealed abscesses in the head kidney, hyperplasia and necrosis in the lateral line canals and epidermis of the head. Gram negative bacteria were associated with these lesions. Roger Lee Herman, National Fish Health Research Laboratory, Leetown, Box 700, Kearneysville, WV 25430.

RO-5, the potentiated sulfonamide consisting of sulfodimethoxine and ormetoprim is officially approved by the FDA for treatment of furunculosis in salmonids. It will be produced by Hoffmann-LaRoche with the brand name ROMET-30. The editors hope to publish an article by G. Bullock (USFWS) and G. Maestrone (Hoffman-LaRoche, Inc.) describing the fifteen years of work required for FDA approval of this drug.

Only 150 of the 500 FHS members have returned the directory information sheet to Rowan Gould. If you are too lazy to send it in, please return our stamps. FHS.

Cystine Heart Agar, normally used for tularemia, has been reported to be a rapid medium for growing BKD. In our laboratory, BKD did grow on CHA with and without supplement but at a rate similar to KDM2. Because CHA is more moist, contamination seemed to be worse than on KDM2. B. Larson, Edmonton, Alberta, Canada.

Paul Janeke, chairman of the Professional Standards Committee, has worked extremely hard for the Section in developing the program for recognizing Certified Fish Pathologists. He is to be commended for his efforts. EDITORS.

SPECIAL CONTRIBUTION

BACTERIAL KIDNEY DISEASE IN MAGIC VALLEY: A STUDY IN NON-COMMUNICATION

Nancy E. Wood, Fish Pathologist, International Aquaculture Research Center, Box 706, Buhl, ID 83316

Bacterial Kidney Disease (BKD), described as a chronic disease, can cause severe mortality in salmonid fishes, especially salmon. BKD manifests itself in kidney tissues with grayish-white lesions which are soft and contain a whitish pus. Rainbow trout are described as being the most resistant; however, a comment in Diseases of Fishes (Bullock, et al. 1971) indicates an isolation of BKD in trout that was more virulent than one from Pacific salmon. Transmission of the disease can be either horizontal (fish to fish) or vertical (fish to egg). Due to the chronic nature of the disease and the fact that the KD bacterium, *Renibacterium salmoninarum*, can occur intracellularly, it can be a difficult disease to treat. Erythromycin, the drug of choice, is not cleared for use in food fish. ". . . the best control measure is to prevent kidney disease from being introduced, . . . " (Bullock, 1971).

BKD, present in upper Columbia River drainage chinook salmon stocks, has been described as the single most important limiting factor in the success of federal Clearwater River hatcheries (BKD Project Proposal 1982). An old report on BKD (Warren 1963) indicates that BKD is more **manageable** in hard water hatcheries. Northern Idaho rearing facilities, at 25 ppm hardness, have soft water. Hagerman National Fish Hatchery (HNFH), with 160 ppm hardness, is defined as a hard water station.

0-10 ppm $CaCO_3$	= very soft
10-100 ppm $CaCO_3$	= soft
100-200 ppm $CaCO_3$	= hard
200 or more ppm $CaCO_3$	= very hard

In 1981, the University of Idaho and the U.S. Fish and Wildlife Service proposed rearing potential or known BKD infected chinook salmon at HNFH in order to evaluate Warren's hypothesis. The BKD Project Proposal (1982) stated that: "Studies are needed to determine if rearing in hard water or water with certain chemicals (whether natural or artificially added) results in more adult returns from hatchery fish." The 1981 proposal was successfully protested by the U.S. Fish and Wildlife Service pathologist and the manager at HNFH, on the basis that "you do not deliberately contaminate a facility with a disease new to that facility". In October, 1982, these objections were overruled. A three year study ensued during which known BKD positive chinook salmon eggs or fry were brought into HNFH. The 1982 stocking was with eggs from Kooskia National Fish Hatchery (KNFH) and Rapid River Hatchery. These chinook were at HNFH until May, 1983. Both state and private hatcheries are located on Riley Creek, downstream from HNFH. A copy of an "Updated BKD Work Plan" memo dated 5 January 1983, was sent to Idaho Fish and Game for their review by the project leader.

The eggs from the same lots sent to HNFH also were reared at KNFH and Red River Hatchery. They experienced heavy BKD mortalities. HNFH also had BKD mortality in their stocks, but it was less severe than at KNFH or Red River.

Because the incidence of BKD was lower at HNFH, known infected fish again were brought into HNFH in October, 1983 as swim-up fry. The fish were from Little White Salmon Hatchery in Washington. BKD mortality at HNFH in 1983-84 was less severe than 1982-83. These stocks were maintained at HNFH until May 1984.

In June-July, 1984, HNFH chinook salmon and steelhead with adipose and pelvic fin clips were electroshocked from Riley Creek water headraces at Hagerman State Hatchery (HSH). In July, rainbow trout from HSH were found with BKD infections. Notification was made to the Idaho State pathologist who notified the Boise office.

The third year of the BKD study at HNFH was terminated in August, 1984, partly at the urging of the Idaho Fish and Game Department. Also at that time, U.S. Fish and Wildlife Service personnel were notified and asked to notify the commercial trout farmers in the Hagerman Valley.

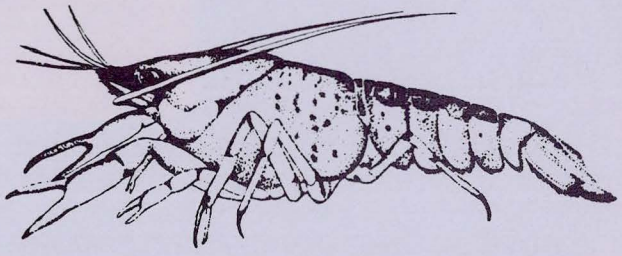
Just prior to a September 25-26, 1984 meeting of the Pacific Northwest Fish Health Protection Committee (PNFHPC), the Idaho Trout Growers Association (ITGA) representative was notified about the presence of BKD in the Hagerman/Magic Valley. He in turn asked several questions about BKD at the PNFHPC meeting. These questions were: (excerpted from PNFHPC report 9 October 1984)

1) Given the facts that BKD has demonstrated pathogenicity for rainbow trout and that it is not known to be endemic to the Hagerman Valley of Idaho, why was the decision ever made to begin this experiment in the first place and put such a large uncontrolled area of rainbow trout production at risk?
2) When the experiment was initially being proposed more than two years ago, why wasn't the state of Idaho and the Idaho Trout Growers Association consulted or at very least notified?
3) When BKD was actually confirmed in production rainbow trout at the Hagerman State Hatchery, why wasn't the Idaho Trout Growers Association immediately consulted or notified as to the potential threat to commercial operations in the drainage rather than finding out third hand after the fact?
4) What specific plans are being made to resolve this problem, prevent its repeat in the future, by whom, and when?

A statement in the original BKD Project Proposal designates that the study will "provide assistance to managers of Idaho and Lower Snake River Compensation Plan hatcheries in the formulation of updated production plans incorporating procedures to improve chinook rearing".

November, 1984: still with no notification to the Magic Valley commercial trout industry by either the U.S. Fish and Wildlife Service, the State of Idaho, or the ITGA, the topic was discussed at a federal coorination meeting in Seattle, Washington. A decision was made to terminate chinook salmon rearing at HNFH and to "tighten-up" to prevent future fish escapements. The U.S. Fish and Wildlife Service would be willing to help with clean-up measures; however, the state has jurisdiction over the water after it leaves HNFH so no specific program for clean-up or notification was established.

November, 1984: two commercial rainbow trout facilities have had confirmed BKD diagnoses in rainbow trout.



Is anybody out there? Pink Floyd, The Wall.

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

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