FISH HEALTH SECTION AFS NEWS LETTER

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ADULT CATFISH RESPOND TO WATER-BORNE EXPOSURES TO CHANNEL CATFISH VIRUS (CCV)

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Channel catfish virus (CCV) is known to cause substantial losses in juvenile channel catfish (*Ictalurus punctatus*) under certain conditions. Fish exposed to the virus that survive infection develop specific neutralizing antibody. Whether these fish are latent carriers of the virus has yet to be established, but in California, growers are encouraged to use broodstock that are free of anti-CCV neutralizing antibodies.

In this study we examined the response of adult catfish (2.5 years old) to exposures to CCV in the water under laboratory conditions to determine if exposed fish would mount a stronger humoral response than previously unexposed fish. Two groups of fish were used: one that survived CCV challenge as juveniles (3 months old) and a second of the same lineage (reared in the laboratory) but never exposed to the virus. Approximately one-half of the fish in each of these groups was exposed to 3.3×10^1 PFU/ml of CCV in the water for 1h. The remaining fish in each group received a sham challenge with tissue culture medium. The fish were then held in 4 ft circular tanks receiving well water heated to 80°F.

The fish were bled at 1,3,6 and 9 weeks following exposure to estimate the levels of anti-CCV antibody in the serum and to determine if virus could be recovered by co-cultivation by leucocytes with CCO cells (Table 1). An unexpected result of these experimental challenges was the death on day 7 (post exposure) of one fish from the previously unexposed group that was challenged with CCV. The fish (wt 0.8 kg) showed clinical signs typical of CCVD. Upon necropsy, no other causes of death were detected (exams for parasites and bacterial cultures and smears were negative).

Tissue concentrations of virus indicated a heavy infection with CCV (Table 1). These concentrations are similar to those observed in moribund juveniles. A histological exam of the organs from the adult catfish were indicative of CCV infection. In addition to the fish that succumbed to CCVD, a second fish in the same group became infected as determined by co-cultivation of its leucocytes with CCO cells at 1 wk.

An examination of the antibody response of catfish to CCV exposures (the original purpose of the study) showed that fish, not previously exposed to the virus, began to produce detectable levels of activity within 3 weeks (Table 1). These fish continued to show measurable levels of antibody at 9 weeks when the experiment was terminated. Fish previously exposed (survivors of challenges as juveniles) continued to show anti-CCV antibody but there was no apparent increase detected following re-exposure at any of the sample times. Fish from the same group but not re-exposed continued to show baseline activity (Table 1). Catfish never exposed to the virus showed no detectable antibody or virus (by co-cultivaltion) throughout the entire study (Table 1).

In conclusion, catfish adults exposed for the first time to CCV can be susceptible, even to low concentrations of virus, showing typical signs and concentrations of the virus associated with CCVD in juveniles. Furthermore, these fish respond by producing anti-CCV antibody first detected at 3 wk and still present 9 wk following exposure to the virus in the water. Fish previously exposed to the virus do not seem to mount a stronger response to the virus upon second or subsequent exposure. Of continued concern is whether antibody-positive fish represent potential carriers of the virus.

However, because adults can become infected, horizontal transmission may have a more substantial role than previously thought in the spread of virus among older animals and subsequently to their progeny. In speculating on the reasons for the susceptibility of adults reared in the laboratory, it seems that although age may confer some resistance, that immune mechanism (elicited by previous sublethal exposures to CCV) are responsible for the apparently stronger resistance seen in pond-reared fish. We are continuing our studies on the effects of size, age and previous exposures on the resistance of channel catfish to CCV with the goal of better understanding the carrier state or lack thereof, following CCV infection.

Table. 1. Concentrations of anti-CCV antibody in the serum of channel catfish upon primary and secondary exposures to channel catfish virus (CCV) in the water.

Treatment	Average concentration of antibody (virus recovery)				
	Pre-exposure	1 wk	3 wk	6 wk	9 wk
Previously unexposed no re-exposure	0(-)	0(-)	0(-)	0(-)	0(-)
Previously unexposed primary exposure	0(-)	0(+)	44.4(-)	25.8(-)	42.7(-)
Previously exposed no re-exposure	28.6(-)	9.1(-)	34.3(-)	18.0(-)	26.0(-)
Previously exposed re-exposed	44.8(-)	5.6(-)	68.0(-)	17.6(-)	124.0(-)

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AWARDS & RECOGNITION

Thomas L. Wellborn, Jr. was honored by the Catfish Farmers of America with their Distinguished Service Award. Tom received the award during Aquaculture '86 in Reno at the joint meeting of the Catfish Farmers of America, U.S. Trout Growers Association, the Fish Culture Section of the AFS and the World Mariculture Society. The award is given to individuals who have made major contributions to the catfish industry. Tom has worked for more than 15 years with catfish farmers. One of his many accomplishments is the establishment of a diagnostic laboratory at Stoneville, MS.

LETTER TO THE EDITORS

Dear Editors:

The Fish Health Section has an important role in aquaculture and in fisheries. We have the responsibility for ensuring competent fish disease specialists are trained and that these persons maintain a suitable level of competency throughout their careers. We also have the responsibility to foster high caliber research not only in the specific area of fish diseases, but also in other areas of fish biology that may impact our knowledge or ability to insure adequate fish health. These responsibilities require a commitment by all members to maintain active participation in Section activities, and to recruit new members who benefit from us and we from them.

The recent change in our section bylaws was unanimously approved by the voters. Unfortunately, less than 10% of our membership took time to cast their ballots. This may mean either that most felt the bylaws change was of little consequence, or that most members did not care.

Regardless of the reasons for the poor voter response, the Membership and Balloting Committee urges all members to vote. We will shortly be electing new officers. These persons will be charged with overseeing the activities of this section and representing the membership. The responsibility of selecting the officers who might best represent our section rests with the membership. The implications of poor voter turnout are obvious.

The Membership and Balloting Committee also urges each member to recruit at least one person into our section. That person need not be directly involved in fish health. Researchers and educators involved in fish biology, fish culture, or water quality are all important for the success of our organization. We feel that our membership can greatly assist and benefit from such close association.

Membership and Balloting Committee

PASSAGES

Guy L. Tebbit, who was the laboratory director at Wildlife Vaccines, has become a regulatory specialist at CIBA-GEIGY. His new address is P.O. Box 18300, Greensboro, NC 27419, phone 919-292-7100.

* * * * * *

James R. Wood has retired from the Washington Department of Fisheries. He left no forwarding address. We wish Jim all the best.

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James E. Sanders has also retired. Jim worked many years for the Oregon Department of Fish and Wildlife and the Department of Microbiology at Oregon State University. He seems too young for retirement, but he looks old.

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Burt Lidgerding, cell culturist at the National Fish Health Research Center at Leetown, has taken a position at

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Bert Lidgerding, cell culturist at the National Fish Health Research Center at Leetown, has taken a position as chief of the hybridoma laboratory, U.S. Army Medical Research Institute of Infectious Diseases, Virology Division. His address is Building 1425, Ft. Detrick, Frederick, MD 211701-5011. His FTS number is 935-7241.

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Dan Mulcahy of the National Fisheries Center, Seattle, will soon be transferring to USFWS, The National Wildlife Health Laboratory, 6006 Schroeder Rd., Madison WI 53711, ph. 608-271-4640.

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The fish health profession will miss these individuals.

POSITION ANNOUNCEMENTS

AQUATIC BIOMEDICINE GRADUATE POSITION

The School of Veterinary Medicine at North Carolina State University is seeking applications for a graduate student position leading to the M.S. or Ph.D. degree with a major in Veterinary Medical Microbiology. Applicants should have the B.S. or D.V.M. or an equivalent degree.

Strong biomedical support facilities provide ample opportunities to apply the latest techniques to the understanding and solution of disease problems in both aquaculture and the commercial fishery. Major research emphases include the characterization of spontaneous dermatological disease problems affecting the freshwater and estuarine fisheries and the development and use of *in vitro* culture systems for economically important fish ectoparasites. A minor in biotechnology is available.

Positions are renewed on an annual basis subject to satisfactory progress. Those interested should contact Dr. Edward J. Noga, Department of Companion Animal and Special Species Medicine, telephone (919) 829-4236 for further information. Application material (Ref: Position #026) should be obtained through the Office of Research and Graduate Studies, School of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606. Closing date for completed application is June 1, 1986, or until a suitable applicant is found.

North Carolina State University is an equal opportunity employer.

RESEARCH ASSISTANT

The Institute for Marine and Aquarium Studies, division of Sea Research Foundation, Inc., is seeking applicants for the position of Research Assistant. This 12-month grant-supported position will be available 01 July 1986, with possible continuation depending on funding. The applicant should have a B.S. in Biology, with one to two years of experience in an aquaculture-related field. Working knowledge of toxicology, pharmacology, and microbiology highly desirable. Duties and responsibilities include maintenance of closed seawater aquariums, seawater analysis, cultivation of parasitic protozoans, and conducting bioassays using marine fishes. Salary $13,000+, depending on qualifications. Please submit letter of application, resume, educational transcripts, and three letters of recommendation to:

Carol E. Bower, Director
Institute for Marine and Aquarium Studies
Biology Department
Eastern Connecticut State University
Willimantic, CT 06226
Phone: 203/423-7007

REPORTS — PAST MEETINGS

January 19-23, 1986 - Aquaculture Reno '86 was attended by approximately 1500 participants from at least 40 countries. The meeting was sponsored by the World Mariculture Society, the Catfish Farmers of America, the Fish Culture Section of the AFS and the U.S Trout Growers Association. The meeting featured reports on commercial developments, research reports, panel discussions, poster sessions, and a trade show. There were several technical papers on aspects of fish health. A special session entitled "Certification, Transplantation and Catastrophic Diseases: Their Role in Success of Aquaculture" was divided into two parts. One afternoon was reserved for papers on this subject which gave the perspective of university researchers, governmental officials, and commercial growers which are involved in the aquaculture of shellfish, shrimp and finfish. These talks stimulated much discussion which was held on the second day of the session.

MEETING ANNOUNCEMENTS

The 17th Annual Conference for the International Association of Aquatic Animal Medicine will be held May 4-7, 1986 at the Biloxi Hilton, Biloxi, MS. Further information may be obtained by contacting Dr. Mobashir Solangi, Institute for Marine Mammal Studies, P.O. Box 4078, Gulfport, MS 39502; phone (601) 864-2511. (An optional day in New Orleans including the Audubon Zoo, French Quarter and a Riverboat Ride will follow on May 8, 1986.

The scientific program includes medical, surgical, and research presentations pertinent to species of whales, seals, otters, shellfish, marine and fresh water fishes, alligators, sea turtles, and seabirds.

Included in the $75.00 U.S. registration fee ($50.00 for students) is an Icebreaker Reception, the IAAAM Annual Banquet, and a dinner boat trip to the Barrier Islands in the Gulf of Mexico. Special Conference room rates as well as airline fares are available.

1986 ANNUAL MEETING OF THE FISH HEALTH SECTION OF THE AMERICAN FISHERIES SOCIETY AND ELEVENTH ANNUAL EASTERN FISH HEALTH WORKSHOP

National Fish Health Research Laboratory
U.S. Fish and Wildlife Service
Kearneysville, WV 25430

The combined meeting of the Fish Health Section of the American Fisheries Society and the Eastern Fish Health Workshop will be sponsored by the National Fish Health Research Laboratory of the U.S. Fish and Wildlife Service. The proceedings will be held at the newly constructed Sheraton Inn in Martinsburg, WV.

The meeting is planned for 4 days:
Monday, July 21 , 5 pm - registration and social reception
Tuesday-Thursday, July 22-24 - formal proceedings, poster sessions and panel discussions

Participants are encouraged to contribute research topics in the following categories:

1. Infectious and noninfectious diseases of fishes, shellfishes, and crustacea.
2. Virulence mechanisms of pathogens affecting aquacultural organisms.
3. Drug registration studies.
4. Vaccination and immunomodulation in cold-blooded vertebrates.

Every effort will be made to accommodate other topics on either a formal or an informal basis. The final program will be organized when proposed abstracts have been received.

A registration fee of $25.00 will be charged to cover the formal proceedings and social events. Included in the cost of registration are dinners on both Tuesday and Wednesday evenings. Dinner, Tuesday evening, will be held on the grounds of the National Fisheries Center where a night of outdoor fun and entertainment has been planned. On Wednesday evening, dinner will be held at the Sheraton Inn.

For those traveling by air, free ground transportation will be provided from Dulles International Airport to the Sheraton Inn. More details about airline travel will be given in our next announcement.

For further information contact Dr. R.C. Cipriano, National Fish Health Research Laboratory, Route 1, Kearneysville, WV 25430

MYXOSPOREANS DETECTED IN NON-SALMONID FISHES FROM WATERS ENZOOTIC FOR PROLIFERATIVE KIDNEY DISEASE (PKD)

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Recent evidence indicates that PKX, the causative agent of proliferative kidney disease (PKD), is a myxozoan parasite of salmonid fishes. Myxosporeans are ubiquitous parasites of fishes but also have been found in other cold-blooded vertebrates. Because the PKX parasite seemingly fails to complete sporulation and invokes such an intense immune response, salmonids may be aberrant hosts for the pathogen. In searching for the reservoir of the parasite, our efforts have concentrated on resident fish populations in water enzootic for PKD.

The Hot Creek Hatchery in the Sierra-Nevadas of California (U.S.A.) has provided a unique opportunity to examine the source of the infective stage of PKD. This hatchery is supplied by water from springs 100-200 m from the production ponds where PKD was first detected in March of 1985 among cultured rainbow trout (*Salmo gairdneri*). Live box studies have shown the presence of the infective stage in this short run of stream between the springs and the ponds. An examination of the fish population in this area showed that an abundant native cyprinid, the tui chub (*Gila bicolor*), was parasitized by a myxosporean with similar sporogonic stages to those we have detected in fish recovering from PKD (Figure 1). The early sporogonic stages first detected in August were later seen to develop in the chub into a mature *Sphaerospora* sp. in September (Figure 2). Sphaerospores were most prominent among the chub but a few were detected in one rainbow trout (Figure 3). As opposed to previously observed spores in salmonids, the chub parasite had distinct valves observable in fresh material and by electron microscopy (Figure 4a and b).

PKX and *Sphaerospora* have similar developmental stages that occur in the blood and renal tubules (Kent, 1985). At this time we can only speculate whether the parasites in the chub and trout are identical but cross-infection studies in progress should provide some insight into their relatedness.

An additional parasite observed in the tui chub was a *Myxobolus* sp. which formed aggregates in the kidney (usually associated with melanomacrophages), spleen, and liver (Figure 5). The spores are similar in size and shape to those described for *Myxobolus cyprini* by Molnar and Kovacs-Gayer (1985). This parasite develops in the muscle and spores are then dispersed via the circulatory system to various organs.

We have also detected myxosporean parasites in the kidneys of sticklebacks (*Gasterosteus aculeatus*) from waters enzootic for PKD in Washington State U.S.A. In these fish, we have observed the early blood stages of a *Sphaerospora spp.* that resemble PKX (Figure 6a). As with the chub, completely developed sphaerospores were seen in the renal tubules (Figure 6b). Spores of *Myxobilatus* sp. were also observed in the lumens of the kidney tubules of several sticklebacks. Although the spores of both myxosporeans were described by Lester (1979), we believe our observations of the early blood stages of *Sphaerospora* are the first to be reported in North America. This lends support to the hypothesis of Lom et al. (1985) that these early blood stages may be found in all *Sphaerospora* spp.

The connection between the sphaerospores in non-salmonid species and PKX and PKD in salmonids is yet to be determined. Studies in our laboratory, however, will hopefully provide clues into the sources and reservoirs of the infective stage of PKX.

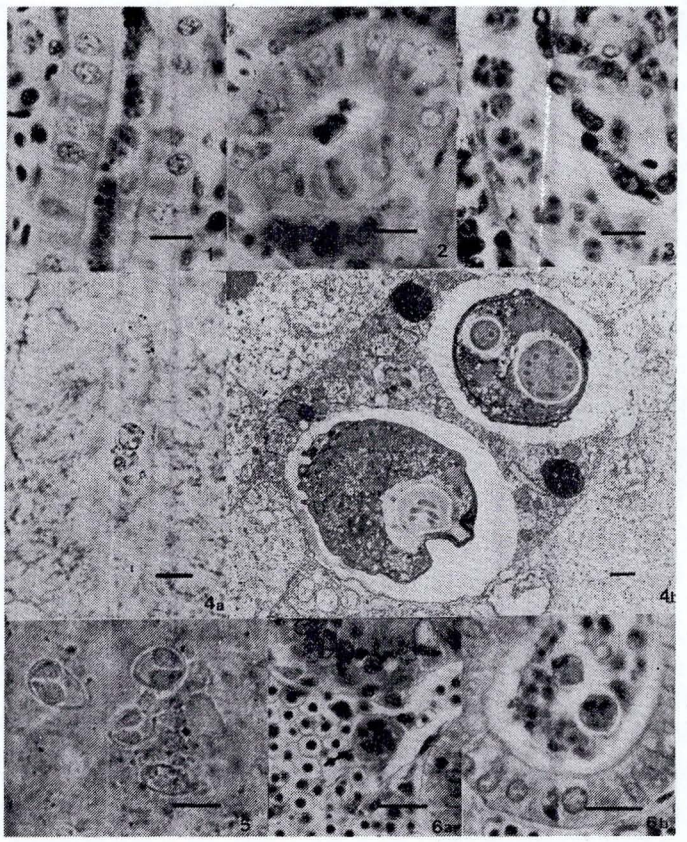

Figure Legends

Figure 1. Myxosporeans found in the kidney of rainbow trout convalescing from proliferative kidney disease (PKD) at Hot Creek Hatchery. Giemsa, bar = 10u.

Figure 2. Myxosporean in the kidney of tui chub from the water supply at Hot Creek Hatchery. Giemsa, bar = 10u.

Figure 3. Sphaerospores in the kidney of rainbow trout from the water supply at Hot Creek Hatchery. H&E, bar = 10u.

Figure 4. *Sphaerospora* sp. from the kidney of tui chubs at Hot Creek Hatchery. (a) fresh mount, bar = 10u and (b) electron micrograph, bar = 1u.

Figure 5. *Myxobolus* sp. found in the kidney of tui chub from Hot Creek Hatchery, fresh mount bar = 10u.

Figure 6. *Sphaerospora sp.* from sticklebacks from Lake Quinalt, Washington State (U.S.A.) showing (a) early blood stages similar to PKX and (b) mature spores. H&E, bar = 10u.

PLEROCERCOIDS OF CUTTHROAT TROUT, *SALMO CLARKI*, FROM YELLOWSTONE LAKE, YELLOWSTONE NATIONAL PARK, WY

Richard A. Heckmann, Department of Zoology
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Hilda L. Ching, Hydra Enterprises, LTD.
Vancouver, B.C., Canada V6B 4M9

The last comprehensive survey of parasites from a species of fish in Yellowstone Lake, Yellowstone National Park, WY, was published in 1971 (R. Heckmann 1971, Prog. Fish. Cult. 33:103-106). During 1985 a brief survey was completed for the same fish and locality. One of the main goals for the current project was to determine the species of plerocercoid inhabiting the cutthroat trout, *Salmo clarki,* from Yellowstone Lake. Previously all plerocercoids from this host have been lumped into one species, *Diphyllobothrium cordiceps,* based on Scott's description (Scott 1935, J. Parasitology 21: 443 and Scott 1955, Rev. Iber. Parasit., Tomo Extraord. 33: pp 47-70). Recent research by H. Ching in Canada and K. Andersen in Norway warranted the re-evaluation of the cestode larvae.

During 1985 Heckmann examined 23 cutthroat trout from two locations in Yellowstone Lake for parasites emphasizing the taxonomic question of the plerocercoids. The fish ranged in size from 310 to 400 mm TL and weighed 200 to 450 grams. All fish were infected with plerocercoids with two definite size groups noted. The plerocercoids were fixed in both buffered 3.0% gluteraldehyde and AFA after relaxation in cold water. Specimens were sent to both H. Ching and K. Andersen as well as extensive examination both with light and electron microscopy by the senior author. Attempts were made to infect gerbils with cestode larvae for development to the adult stage. There appeared to be two, possibly three, species of plerocercoids inhabiting cutthroat trout. *Diphyllobothrium ditremum, D. dentriticum* were confirmed by Heckmann, Ching and Andersen (Figures 1 and 2). Additional specimens will be necessary to determine if *D. dendriticum* is valid for this piscine host. These plerocercoids replace *D. cordiceps* for the parasite list.

Besides the cestode larvae, the following parasites were found for the examined *S. clarki.* Protozoa: *Trichophrya clarki, Myxosoma* sp.; Trematodes: *Crepidostomum farionis, Diplostomum baeri bucculentum* (metacercariae); Nematodes: *Bulbodacnitis scotti;* Copepods: *Salmincola* sp.; Hirudinea: *Piscicola salmositica.* In comparing previous surveys, three protozoa species were not observed this time (*Costia pyriformis, Trichodina* sp., *Haemogregarina* sp.) as well as one species of acanthocephala (*Neoechinorhynchus rutili*). These four parasites were uncommon (0.4% to 2.0%) from the 1971 survey. For the 1985 survey, five parasite species were added to the previous list. Fungal infections were noted on two trout.

Another goal of this project was to determine drug efficacy of Invermectin and Praziquantel towards the helminths of the cutthroat trout. Invermectin is effective for the treatment of roundworms in *S. clarki* and Praziquantel is lethal towards the cestodes and trematodes of the same host.

REVISED BULLETIN ON CATFISH DISEASES

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The bulletin "Principle Diseases of Farm-Raised Catfish, Southern Cooperative Series Bulletin No. 225, 76 pp." edited by J.A. Plumb has been revised and includes up-to-date information on ESC and "hamburger gill disease." It covers the viral, bacterial, fungal, and parasitic diseases and contains a chapter on the role of stress in disease and one on control and therapy. It is an excellent publication and is a must for anyone working with diseases of catfish. The cost if $3.00 and may be ordered from Research Information, Alabama Agricultural Experiment Station, Auburn, AL 36849.

LOW IMPACT OF THE MYXOSPOREAN PARASITE *CERATOMYXA SHASTA* ON SURVIVAL OF FRASER RIVER, BRITISH COLUMBIA, CHINOOK SALMON SMOLTS

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To determine the prevalence of *Ceratomyxa shasta* and assess the impact of this pathogen on survival of downstream migrant chinook salmon in the Fraser River, British Columbia, weekly field sampling was conducted from late March to early August 1985 at several sites in the main and north arms of the lower Fraser River. Samples were collected by beach seine and, based on the capture of some coded-wire tagged fish, consisted of both hatchery-produced and wild stocks.

More than 1200 chinook were sampled, slightly more than 70% of which were caught prior to June 11. No juvenile chinook were caught after July 23. Approximately 350 fish were examined within 1-2 days of capture; the remainder were maintained live in seawater tanks at the Pacific Biological Station for up to 4.5 months. Only 20 (1.65%) of the sampled fish were found to be infected; all were caught after June 10. Judging from the beach seine catches, the post-June 10 samples represented a significantly smaller proportion (less than approximately 30%) of the total downstream migration than did the earlier samples. Even assuming that all infected fish would ultimately die of ceratomyxosis (some died of this disease during captivity), these results suggest that *C. shasta* was not likely a major cause of mortality among juvenile chinook salmon migrating out of the Fraser River in 1985.

These findings are in striking contrast to those reported by Ching and Munday (1984, Canadian Journal of Zoology 62:1075-1080; 1081-1083) and Ching (1984, Canadian Journal of Zoology 62; 1423-1424), who obtained mortality rates of 72-100% among juvenile chinook exposed between mid-May and mid-November (1981-1983) to lower Fraser River water either directly in cages or in tanks away from the river. Results from such experimental exposures, therefore, should not be extrapolated to naturally migrating stocks of juvenile chinook.

It is planned to repeat the sampling of downstream migrating chinooks in the Fraser River during the 1986 migration.

IMMERSION TREATMENTS AGAINST THE ASIAN TAPEWORM (*BOTHRIOCEPHALUS OPSARICHTHYDIS*) IN GRASS CARP

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Fish Farming Experimental Station, P.O. Box 860
Stuttgart, AK 72160

Several tests were conducted to determine the effectiveness of Masoten and praziquantel for controlling the Asian tapeworm in grass carp. Masoten was ineffective: live worms were found in fish after 24 hours at treatment levels up to 50 ppm. Results from preliminary 24 hour bath treatments with praziquantel were much more promising. Praziquantel was put into solution by first dissolving 0.1 mg of the 100% active ingredient in 20 ml of isopropyl alcohol. After a number of initial tests to determine the range for effective treatments, several tests were conducted with grass carp stocked at 69 grams/liter (a maximum commercial holding tank stocking density) at 5, 10, 15, 20, 25, and 30 mg of prazinquantel/kg of fish in 40 liter tanks. The results indicated that worms were controlled at a dose rate of 10 mg/kg as effectively as at higher levels. In most cases, drug levels from 10 to 30 mg/kg reduced tapeworms from a pretreatment incidence of 60 to 85% to 0% by 48 hours after treatment. On one occasion small deformed tapeworms were found in 2 of 27 fish 48 hours after treatment at the 15 mg/kg level. Whether or not these would have died is unknown. After 96 hours no tapeworms were found in treatments of 10 mg/kg or above. Drug levels of 40 mg/kg were necessary for effective treatment if the treatment period was reduced to 12 hours. Unfortunately praziquantel is not presently available for further testing on fish.

SUMMARY OF THE 1985 FISH KILLS INVESTIGATED BY THE MCES FISH DISEASE DIAGNOSTIC LABORATORIES

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Robert M. Durborow and M. David Crosby
P.O. Box 142, Stoneville, MS 38776

During 1985 the Mississippi Cooperative Extension Service Fish Disease Diagnostic Labs investigated 1,771 fish kills involving 11 species: channel catfish (1,748), largemouth bass (5), bluegill (4), golden shiners (5), blue catfish (3), grass carp (1), smallmouth buffalo (1), goldfish (1), black bullhead (1), yellow bullhead (1), and brown bullhead (1). The biggest problems encountered were *Edwardsiella ictaluri* and *Flexibacter columnaris*. The major diseases found are given. In addition to the ones listed, a few problems involving miscellaneous protozoan and metazoan parasites, water quality, toxic materials, etc. were diagnosed. Many of the cases involved more than one etiologic agent.

Major disease problems diagnosed by the MCES Fish Disease Diagnostic Laboratories during 1985 are summarized as follows:

Disease	Number of Occurrences	Percent Occurrence
Edwardsiella ictaluri	876	49.4
Flexibacter columnaris	597	33.7
Trichophrya	231	13.0
Fungi	226	12.8
Aeromonas sobria	169	9.5
Trichodina	120	6.8
Hamburger Gill Disease	97	5.5
Ichthyobodo	92	5.2
Ambiphrya	82	4.6
Aeromonas hydrophila	40	2.3
Channel Catfish Virus	35	2.0
Winter kill	29	1.6
Anemia	24	1.4
Edwardsiella tarda	12	0.7
Ichthyophthirius	11	0.6

USE OF PERCOLL GRADIENT ULTRA-CENTRIFUGATION AND SEPHACRYL S-1000 COLUMN CHROMATOGRAPHY TO PURIFY INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS

Don Richter, Hiro Sata, Farhad Hashemi and Vern Winston
Campus Box 8094, Idaho State University, Pocatello, ID 83209

Infectious Hematopoietic Necrosis Virus (IHNV) is normally purified using sucrose gradient ultracentrifugation. We have developed a new way of purifying the virus which uses ultracentrifugation of the virus on gradients of Percoll. Percoll consists of colloidal particles of silica coated with polyvinylpyrrolidone. Gradients of Percoll are self-forming, and they are iso-osmotic. We purified the virus from crude lysates of infected cells by first removing the debris by slow speed centrifugation. The supernatant was concentrated by ultrafiltration and the concentrated material was placed over a layer of Percoll in an ultracentrifuge tube. Virus was centrifuged into the Percoll (22,500 rpm, 1hr). The peak of virus activity in the Percoll was determined by an enzyme-linked immunosorbent assay and virus-containing fractions were subjected to column chromatography on Sephacryl S-1000 to remove the Percoll. Assays in cell culture demonstrated that the virus remained viable after purification, SDS-PAGE analysis and electron microscopy were also used to demonstrate that the virus remained intact.

FISH DISEASE SURVEY OF THE SOUTHEASTERN UNITED STATES

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In July of last year (1985) the Southern Division of the American Fisheries Society released its 1984 fish disease survey. A total of 2,690 cases were included in the survey and involved 3,211 disease agents or other problems (the number of disease agents is greater than the number of cases reported because often more than one disease agent contributed to the mortality). Bacterial problems were identified in 48.4% of the cases, parasites in 28.1%, fungi in 6.3%, viruses in 2.4%, noninfectious problems in 14.7%, and there were 19.2% unsolved cases. The following infectious and noninfectious agents occurred as problems in one percent or more of the cases.

Flexibacter columnaris	18.0%
Edwardsiella ictaluri	15.0%
Aeromonas hydrophila	9.9%
Trichodina	7.3%
External fungus	6.2%
Ichthyobodo	3.3%
Oxygen depletion	3.2%
Ambiphrya	2.9%
Trichophrya	2.7%
Gyrodactylus	2.1%
Channel catfish virus	1.8%
Anemic condition	1.6%
Malnutrition	1.6%
Ammonia toxicity	1.3%
Nitrite toxicity	1.2%
Cleidodiscus	1.0%
Henneguya spp.	1.0%

MORTALITIES AMONG STRIPED BASS (*MORONE SAXITILIS*) CAUSED BY *MYCOBACTERIUM MARINUM*

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An examination of a chronic mortality among cultured striped bass (*Morone saxitilis*) underyearlings showed signs typical of mycobacteriosis. Losses over a 6-month period approached 50% of the original population. Diseased fish showed few to no outward signs of infection. Internal signs included granulomas in the liver, kidney and spleen. Histological analyses confirmed the nature of the tubercules. Numerous acid fast bacteria were associated with these lesions and were easily observed in stained smears from affected organs.

The acid-fast bacteria was cultured on Petragnani's medium where it produced rough yellow colonies in 5 to 8 days at 20C. The organism grew at 37C. The isolate is presumed to be *Mycobacterium marinum* and further characterization is underway. As with mycobacteriosis in aquarium fish, the isolate from striped bass can produce substantial losses. This is a particular problem for groups intended for extended rearing for broodstock. The source of infection is unknown but was presumed to be prior to movement to the culture facility that utilizes well water. A comparison of our isolate to that reported by Sakanari et al (Transactions of the American Fisheries Society 112:565-566, 1983) from subclinically infected feral striped bass is planned.

SQUASH PLATE TECHNIQUE FOR IDENTIFYING OR DETECTING INTESTINAL WORMS

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A method for the detection of tapeworms, nematodes, and other intestinal worms has been developed by modifying a procedure used for the diagnosis of *Trichinella* (Schmidt and Roberts 1977; Healy, et al., 1984). Although the method is useful on fish up to 20 cm, best results are obtained on fish less than 13 cm in total length.

The head is severed from a fish just posterior to the opercular flap. A blunt scissors tip is inserted into the body cavity ventrally and the ventral wall is cut to the anus, with care to not sever the intestine. The intestinal tract is cut loose at the anus and anteriorly and removed with forceps or fingers. It is carefully uncoiled and the attached viscera is removed with the fingers. The intestinal tract should not be broken because parasites can be lost through the break. If the intestinal tract is small and difficult to work with, it can be placed directly on a microscope slide or glass plate without further processing. The glass plates used are squares of 1/8 inch thick glass 9 cm square. The processed intestinal tracts from larger fish can be straightened and placed on a slide or plate and folded if they are longer than the slide or plate. Another slide or plate of the same size is placed over the specimen(s). The use of two or four 1/4-inch binder clips placed on opposite sides aids the flattening of the intestines between the glass. Small specimens (less than 3 cm) may not require binder clips for flattening. The use of a dissection scope with reflected light at 25±5X is usually adequate to reveal parasites. The scan objective on a binocular scope works well if the specimen is small enough to be placed between two slides. Once small worms are recognized by this method they will be consistently spotted. The anterior third of the intestinal tract should be examined thoroughly because helminths, particularly tapeworms, are most likely to occupy this portion. Also it is advisable to starve fish for 24 hours to allow time for the passage of food particles in the gut that may obscure worms.

For fish larger than 20 cm the anterior third of the gut can be removed, slit open lengthwise and the inside portion scraped with a scalpel. The contents can then be flattened between two slides (no binder clips necessary) and examined under the scan objective of a binocular scope.

NEW BOOK ON CATFISH FARMING

T.L. Wellborn
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A new book, "Channel Catfish Culture", edited by Dr. Craig S. Tucker is the definitive text on the culture of channel catfish. It contains thirteen chapters (658 pages) covering all aspects of catfish farming. It has an excellent chapter on infectious diseases of channel catfish, one on non-infectious diseases and one on water quality. It is not a "how-to" manual and is written in a technical rather than a practical style. The chapters on diseases and water quality should be of particular interest to anyone working in the field of fish health. One drawback is the cost: $98.25. Copies may be ordered from: Elsevier Science Publishing Company, Inc., P.O. Box 1663, Grand Central Station, New York, NY 10163.

BRIEF REPORTS

Approximately 1.5 million rainbow trout (1/3 of British Columbia's hatchery stock) were destroyed at the Summerland Hatchery, Okanagan Lake, after IHN was detected. Sockeye salmon in the watershed may have been the reservoir for this disease. G.R. Bell, Pacific Biological Station, Nanaimo, B.C., Canada V9R SK6.

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A cooperative study on isolation of specific DNA from tomites of *Ichthyopthirius multifiliis* is being done at the University of Georgia by the Department of Medical Microbiology and Molecular Genetics. The objective is to clone the gene responsible for coding of immunizing proteins with the long term objective of expressing the gene in a bacterial vector. Dr. Craig Findley, a molecular geneticist, and Dr. John Gratzek are principal co-investigators. Dr. Harry Dickerson, a post-doctoral associate working with Dr. Findley, is assigned full-time to the project. The researchers' long term goal is to construct engineered vaccines for fish which are specific for both bacterial and specific parasites such as "Ich'.

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Emmett Shotts is in need of recent isolates of *Yersinia ruckeri.* He would appreciate receiving any available at the Dept. of Medical Microbiology, College of Veterinary Medicine, U. of Georgia, Athens, GA 30601.

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The BLUE BOOK is still available to Fish Health Section members at a special rate. Use the enclosed form to order your copy. The price goes up in July.

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Applications for membership in the AFS and the Fish Health Section can be found in Fisheries Magazine. Use these as you recruit new members.

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Herpesvirus salmonis has been isolated from adult steelhead trout returning to Warm Springs Hatchery on the Russian River in Northern California. R.P. Hedrick, Aquaculture and Fisheries Program, U.C. Davis, Davis, CA 95616.

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Systemic saprolegniosis was detected in juvenile white sturgeon, *Acipenser transmontanus.* Findings suggested that under certain conditions, Saprolegnia infections can originate in the gut and then invade the coelomic cavity causing substantial mortality in sturgeon. C.S. Friedman and R.P. Hedrick, Aquaculture and Fisheries Program, U.C. Davis, Davis, CA 95616.

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Monoclonal antibodies capable of neutralizing isolates of IHNV have been developed at Oregon State University. These reagents recognize differences among strains of the virus and should be usefu l diagnostic tools. J.R. Winton, Marine Science Center, Newport, OR 97365.