FHS
NEWSLETTER
FISH HEALTH SECTION
AMERICAN FISHERIES SOCIETY

Volume 23, Number 3 Page 1 July, 1995

FHS 23(3) 1995

Isolation of a New Rickettsia-like Organism from Atlantic Salmon in Chile

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In 1989, a rickettsia-like organism (RLO), commonly referred to as " UA " (for " unidentified agent "), was causing serious losses among coho salmon in Chile and only in seawater pens. The disease it caused, " Coho Salmon Syndrome " , was first reported in 1989 by Bravo and Campos. In 1989, the responsible organism was isolated and described for the first time and the disease renamed " Salmonid Rickettsial Septicemia " (SRS) because it was detected also in chinook and Atlantic salmon and rainbow trout (Cvitanich, Garate, and Smith, 1990). In 1992, the organism was formally named *Piscirickettsia salmonis* (Fryer, Lannan, Giovannoni, and Wood, 1992). Generally considered a seawater disease, SRS has been recently confirmed in freshwater by IFAT in rainbow trout from Lake Llanquihue (Bravo, 1994) and by IFAT and isolation in cell culture from coho salmon and rainbow trout from Chiloe Island (Gaggero, Castro, and Sandino, 1995).

During December 1994 and January 1995 (summer in Chile), a new RLO was observed and isolated from Atlantic salmon by pathologists at the TROUW-Chile fish pathology laboratory in Puerto Montt. Fish, ~ 20-100 g, were experiencing a disease outbreak in net pens in Lake Llanquihue (water ~16^0 C) and seawater pens (water ~14^0 C) into which they were transferred.

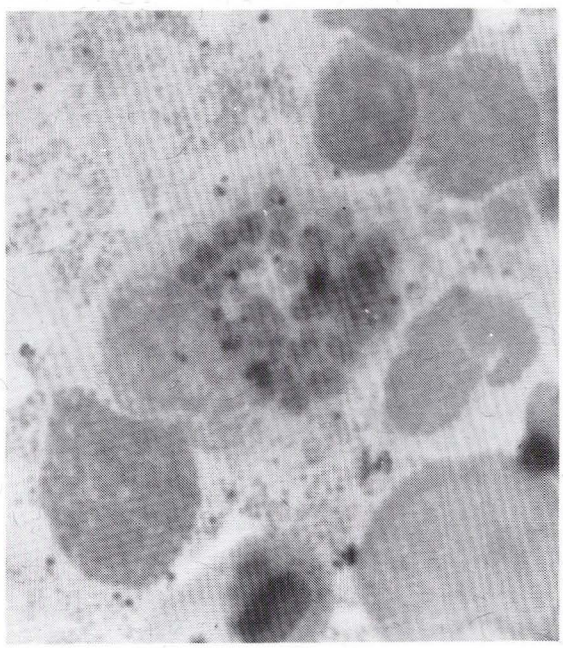

Figure 1. Spleen imprint from an infected Atlantic salmon showing rickettsia-like organisms extracellularly and also intracellularly in variable-sized clusters within the cytoplasm of one cell.

Pathologists at the laboratory had seen the organism only once before at the same freshwater site (water ~ 10^0 C) in July, 1994, in Gram stains prepared from a few moribund Atlantic salmon (~10 g). The population was not experiencing any mortality but was obviously infected. This RLO is also an unidentified agent and, until it is fully characterized and identified, we have chosen to call it UA-2 or " U2 " for short.

Rickettsias are bacteria characterized as growing only in the living cells of the host tissues they infect and not on common laboratory media. So far, U2 has grown in 7 fish cell lines (EPC, CHH-1, CHSE-214, RTG-2, FHM, BB, and BF-2) at temperatures of 15-27^0 C, but has not grown on 7 common bacteriological media incubated at 15^0 C and 21^0 C. U2 produced a detectable cytopathic effect in EPC's in only 2 days, and extensive CPE in 4-5 days. U2 has not produced CPE in the EPC, McCoy (mouse fibroblasts), or P388D1 (mouse macrophage) cell lines at 33^0 C. Like UA, U2 was susceptible to streptomycin and gentamicin but not penicillin or amphotericin B (Fungizone), and was retained by both 0.2 and 0.45 μm filters. For these reasons, neither U2 or UA can be isolated from field samples prepared for virus screening and isolation if they have been centrifuged, filtered, or incubated in a typical antibiotic-containing cell culture system. These facts have been critical to the isolation of both organisms.

Microscopically, U2 was observed to be a non-motile, Gram-negative bacterium, spherical to coccoid in shape, often pleomorphic, typically variable in size, and ranged from ~ 0.2 - 0.8 μm in diameter. It was PAS, acid fast, and Gimenez negative. It has been observed both extracellularly and intracellularly. Variable-sized clusters of U2 were present within the cytoplasm of infected kidney and spleen cells in May-Grunwald Giemsa-stained imprint preparations (Figure 1) and electron micrographs. Preliminary EM findings indicate that these clusters do not appear to be in membrane-bound vacuoles as is seen with UA infected cells. In a DFAT, U2 did not cross-react with FITC-labeled antisera prepared against UA cells or *Renibacterium salmoninarum*. Chlamydia, bacteria also requiring a living host cell to replicate in, have a characteristic developmental cycle not seen with U2. Furthermore, in 3 tests used to identify chlamydia (culture in McCoy cells, ELISA, and Gimenez stain), U2 was negative in each. Taxonomically, U2 appears to belong to the order Rickettsiales and family Rickettsiaceae, however, further work will be required to identify or classify this fish pathogen.

Mortalities from U2 occurred at temperatures ranging from 9 - 17^0 C and ranged from an estimated 4-12%/week in freshwater and continued in seawater to about week 6. In contrast, mortality from UA generally begins in seawater about 6 weeks after fish have been transferred from freshwater. Fish heavily infected with U2 had normal or darker coloration and were sometimes smaller and somewhat emaciated. Kidneys showed little or no swelling and often had a darker gray coloration. The spleen was often swollen and sometimes had white spots.

Infections appeared to originate in the kidney and spleen, eventually producing a septicemia and a systemic infection with lesions developing in several tissues and organs. Anemia was present in all fish, producing pale gills and hematocrits of ~ 20%. The organism was not visibly

present in stained blood smears, unlike UA. Histologically, in U2 the most significant pathological changes were noted in the kidney, liver , and spleen. Kidney and spleen were typically the most heavily infected tissues and could contain > 10^9 U2/g. Fish heavily infected with UA typically have 10^7-10^8 UA/g. In fish with severe infections, the kidney tubule epithelium showed mild necrosis and renal casts were occasionally seen. Numerous melanomacrophages were diffusely scattered throughout hematopoietic tissue. Some contained large amounts of melanin while many had few granules and were engorged with extremely small microorganisms that stained lightly with both H&E and Giemsa. Livers showed a mild diffuse necrosis and exhibited a generalized hepatitis with numerous focal granulomatous areas. Infected melano-macrophages were distended with organisms and scattered throughout the liver, but were more common in the granulomatous tissue. Hepatocytes were sometimes infected and often swollen. Severe vasculitis was noted in most vessels within liver sections. Spleens had thickened, edematous capsules, were devoid of lymphocytes, and were diffusely granulomatous. Small focal areas of infected melanomacrophages were also common. Heart, intestine, pyloric caeca, muscle, gills, pseudobranch, nasal capsule, choroid of the eye, and brain were also infected in some fish but with lower concentrations of U2.

U2 has been isolated or observed from diseased Atlantic salmon at 6 seawater, one freshwater, and one estuary site belonging to 5 different companies. Two of these companies purchased smolts from freshwater from another company who sells smolts, so it is likely that other companies also have U2. It appears that this is a freshwater disease that is transferred to seawater, but perhaps only heavily infected fish continue to die, however, it may be too early to make this conclusion. The largest infected Atlantic salmon recorded in seawater is ~ 300 g. Recently, U2 was detected in Gram stains from an ~500 g rainbow trout and an ~300 g coho salmon in seawater off the east coast of Chiloe Island. In a few cases, U2 was observed in mixed infections with UA or the microsporidian, *Enterocytozoon salmonis*. U2 appears to be very different from UA.

Current research efforts are focused on (1) antibiotic sensitivities and field trials, (2) a fluorescent antibody reagent for diagnostic and research purposes, and (3) the identification of U2. This work has been supported by TROUW-Chile.

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Ribosomal DNA Comparisons of *Henneguya salmonicola* and *H. zschokkei* from Salmon and Whitefish.

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Henneguya zschokkei (Gurley, 1894) Doflein, 1901 and *H. salminicola* Ward, 1919 are morphologically similar myxosporeans that infect flesh of fishes in the order Salmoniformes. *Henneguya zschokkei* was first described from several species of whitefish *Coregonus* spp. (family Coregonidae) from Switzerland. This myxosporean has been identified in various whitefish species in other parts of Europe, including Russia (Donets and Shulman, 1984; Lom and Dykova 1992), and in North America (Margolis and Arthur 1979; Smothers, 1993; McDonald and Margolis 1995). *Henneguya salminicola* was first described in coho salmon (*Oncorhynchus kisutch*) from Alaska, and infects various *Oncorhynchus* species (family Salmonidae) in the Pacific Northwest of North American (Margolis and Arthur 1979; McDonald and Margolis 1995). The separation of these two organisms into separate species has not been accepted universally, and in two major reviews on myxosporea, *H. salminicola* has been considered a junior synonym of *H. zschokkei* (Donets and Shulman 1984; Lom and Dykova 1992).

Comparisons of nucleotide sequences of ribosomal DNA (rDNA), particularly in the 18S region, have been extensively used for taxonomic comparisons in many phylogenetic groups, including the Myxosporea (Smothers 1993), and we have initiated examination of rDNA sequences of *H. salminicola* and *H. zschokkei* to resolve the relationship of these myxosporeans. *Henneguya salminicola* was collected from sockeye salmon (*O. nerka*) from Vancouver Island, British Columbia and *H. zschokkei* was collected from whitefish (*Coregonus lavaretus*) near Oslo, Norway. The 18S rDNA was amplified by PCR using universal primers 18e and 18g, and cloned. At present, we have compared about 350 base pairs sequenced at the 5' end of the 18S with our isolates and with the rDNA sequences of Smothers (1993) obtained from " *H. zschokkei*" from mountain whitefish (*Prosopium williamsoni*) in Idaho. Genetic distances were estimated using the Jukes-Cantor method with pairwise elimination of detection/insertion sites.

Based on these 350 base pairs of the 3 isolates, *H. salminicola* shows 8.21 % genetic divergence from *H. zschokkei* from whitefish of Norway. The isolate from whitefish of North America showed a similar divergence (9.98 %) from the Norwegian isolate. Interestingly, *H. salminicola* was more closely related to "*H. zschokkei*" from whitefish from North America, showing only 2.91 % divergence.

These preliminary data suggest that the *Henneguya* isolates cluster by geographic region, rather than by host families, which implies that "*H. zschokkei*" and *H. salminicola* from North American should be included in the same species assemblage. Furthermore, these results suggest that *H. zschokkei* from Europe (i.e. Norway) may be distinct from the *Henneguya* species from North American salmoniforms.

Further analyses of the rDNA of these isolates and additional specimens from whitefish and salmon from different geographic areas will help clarify the relationship of the *Henneguya* complex from fishes of the order Salmoniformes. This work is in progress in our laboratories, and we would appreciate receiving frozen or ethanol- fixed specimens of *Henneguya* species from either salmon or whitefish to include in our analyses.

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In Memoriam

Professor Dr. Phil. Wilhelm Schäperclaus (1900-1995). On January 3, 1995, Prof. Dr. Phil. Wilhelm Schäperclaus died at the age of 95. He was considered one of the finest fish pathologists of this century. He received his PhD at the University of Münster in 1925 and began his research at the Institute for Fisheries in Berlin-Friedrichhagen. His studies included carp dropsy, antibiotic therapy, whirling disease, *Chilodnella* infections, *Eimeria* infections and ichthyophthiriasis. He was also the first scientist to describe VHS in trout and its supposed viral etiology. His *Handbook on Fish Diseases* and *Textbook on Pond Culture* are among his best known publications. He became Director of the Institute of Fisheries and Head of the Division of Fisheries Science at Humboldt University in Berlin, in 1956. He continued in these positions until his retirement.

DEADLINE FOR SUBMITTING ARTICLES TO THE NEXT ISSUE OF THE FHS NEWSLETTER IS SEPTEMBER 8, 1995 !!!!!

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POSITION ANNOUNCEMENTS

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Fish Disease Research and Diagnostic Service:
The College of Veterinary Medicine at Mississippi State University is seeking nominations and applications for a tenure track faculty position in fish disease diagnostics and research. The successful candidate will provide research and diagnostic services to the catfish industry. Primary responsibility includes contributing the fish health monitoring component of the total pond management program conducted by Mississippi State University for the catfish farmers of Mississippi. In addition, activities consultation with farmers, diagnosis of fish diseases and fish kill problems and providing treatment recommendations. Research in aquatic pathobiology to address the problems of catfish farmers may include toxicology, microbiology, immunology, parasitology, physiology, epidemiology, nutrition or management or any combination of these. Candidate must be capable and willing to work with farmers, research scientists of other departments and divisions of the university, graduate students and residents. Minimum qualifications are M.S. degree with advanced training in aquatic pathobiology required or D.V.M. (or equivalent) or Ph.D. degrees with experience in warm water (catfish) disease problems and fish pathologist certification or eligibility. Salary is commensurate with qualifications and experience. Applications will be accepted through July 1, 1995, or until a suitable candidate is identified. Qualified persons should submit a letter of application briefly outlining qualifications and pertinent experience along with a resume, transcripts and three (3) letters of reference to : Dr. H. Graham Purchase, Director of Research, College of Veterinary Medicine, Box 9825, Mississippi State University, Mississippi State, MS 39762-9825.**Mississippi State University is an AA/EOE Employer**

Aquaculture/Fish Health Management:
Academic-year appointment (Assistant Professor), Fish and Wildlife Resources, College of Forestry, Wildlife and Range Sciences, University of Idaho, Moscow, Idaho.
Qualifications: The appointee must possess a PhD or DVM degree in aquaculture/fish disease or closely related field. A commitment to teaching excellence is required. Desired qualifications include post-doctoral or agency experience and demonstrated research productivity. The successful applicant will teach the undergraduate course in aquaculture, fish diseases and a graduate course in his/her area of interest and will establish a nationally recognized research program. Salary commensurate with experience and the starting date is in the fall 1995. Closing date for application: 3 July or until a suitable candidate is selected. Send letter of application, curriculum vitae and three letters of recommendation to: Aquaculture Search, Department of Fish and Wildlife, College of Forestry, Wildlife and Range Sciences, University of Idaho, Moscow, ID 83844-1136, Phone (208) 885-6434, FAX (208) 885-9080. The Department of Fish and Wildlife has one of the largest enrollments of undergraduate and graduate students at the University of Idaho. The Department has an international reputation for teaching and research in the natural resources-based College of Forestry, Wildlife and Range Sciences. AA/EO employer and education institution.

ABSTRACTS AVAILABLE:

Papers on Fish Parasites: At the annual meeting of the Rocky Mountain Conference of Parasitologists (May 18-20) held at Idaho State University, there were 12 scientific papers presented on fish parasites and diseases. Copies of the abstracts are available. Write: Dr. Richard A. Heckmann, 109 WIDB, Zoology Department, Brigham Young University, Provo, UTAH 84602

MEETINGS

AUGUST, 1995

Aquaculture Europe'95. "Quality in Aquaculture" is the main theme for the international conference Aquaculture Europe '95, which will be held together with the Aqua Nor '95 exhibition in Trandheim from **August 9-12, 1995.** The conference is organized by the **European Aquaculture Society (EAS)** and is the first time these two major biennial events have joined forces. The conference is arranged with plenary and parallel sessions. Over 275 contributions from 45 countries have been accepted for presentation in the sessions or in the adjoining poster sessions. Among the invited plenary speakers will be Dr. John Joyce, chief executive of the Irish Salmon Growers' Association, who will focus on ethical quality in aquaculture; and Dr. Beatrice Chatain of IFREMER on developing technologies for improving the quality of juveniles. Other plenary talks will cover nutrition and feeding, the influence of harvest procedures, and development of post-harvest technologies. On the final day, there will be a special session to consider Improvement of Salmonid Production. **Additional meetings during Aquaculture Europe will include one (in English) on smoltification, and one (in Norwegian) on fish health.** For further information contact: EAS secretariat, Coupure Rechts 168, B-9000 Gent BELGIUM, Fax 32-9-223-7604.

SEPTEMBER, 1995

European Association of Fish Pathologists Seventh International Conference on Diseases of Fish and Shellfish. September 10-15, 1995. Palma de Mallorca, Spain. Contact: Eva-Maria Bernoth, CSIRO Australian Animal Health Laboratory, P.O. Bag 24, Geelong, VIC 3220, AUSTRALIA; eva@aahl.dah.csiro.au.

OCTOBER, 1995

Fourth International Symposium of Ichthyoparasitology. October 3-7, 1995. Munich, Germany.Contact: Prof. Dr. R. Hoffman and Dr. M. El-Matbouli, Institute of Zoology, Fish Biology and Fish Diseases, University of Munich, Kaulbachstr 37, 80539 Munich, GERMANY. phone: 39-89-2180-2687 Fax: 39-89-2180-5175.

Immunological Response of White Sturgeon to IHN Virus

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The polyculture of white sturgeon, *Acipenser transmontanus,* and rainbow trout, *Oncorhynchus mykiss*, was examined from 1989-1994. Empirical observations suggest that sturgeon and trout can successfully cohabitate. This type of polyculture may enhance hatchery environmental conditions, but there are concerns regarding disease transmission. One of the most lethal diseases of rainbow trout is caused by a rhabovirus, infectious hematopoietic necrosis virus (IHNV). Sturgeon can be exposed to IHNV by cohabitating and consuming infected rainbow trout. Although no sturgeon mortality has been reported due to IHNV infection, a cell line derived from white sturgeon will support IHNV replication. An objective of the study was to evaluate serum from sturgeon exposed to different culture environments for IHNV neutralization activity.

White sturgeon (4-6 years old) cohabitated in raceways with rainbow trout or held in single-pass spring water and fed a dry pelleted diet were bled and serum tested for the presence of IHNV neutralizing activity using a complement dependent 50% plaque neutralization titer assay. Additionally, six yearling sturgeon approximately 30 cm were prebled and injected with 0.5 ml of IHNV (concentration, 10^6 plaque forming units [pfu] IHNV/mL). An additional six fish held in a separate aquaria were handled similarly but injected with cell culture media only. This regime was repeated a 4 week intervals over a 20 week period. Blood was collected at each interval and serum samples were tested for IHNV neutralization activity.

Serum from sturgeon was also tested for its capacity to protect rainbow trout against IHNV after passive serum transfer. Briefly, rainbow trout (mean weight, 1 g) were anesthetized and injected intraperitoneally with 50 μL of sturgeon serum with no detectable IHNV neutralization activity or saline (titer <20), or with sturgeon serum with moderate (titer, 160) or high (titer, 640) neutralization activity. An additional group of fish was injected with hyperimmune rabbit serum made against IHNV (positive control). Replicate 25-fish groups were challenged with 10^4 pfu of IHNV/mL by standard procedures and monitored for 21 d for mortality. Cumulative percent mortality of the replicates was examined by analysis of variance (ANOVA) on transformed (arcsin $\sqrt{}$ percentage) data and pairwise comparisons were done with the Student-Newman-Keuls Method using the Sigma Stat statistical software (Jandel Scientific Co.)

Virus neutralization activity was detected in 30% (7/23)of the sera obtained from adult sturgeon cultured with rainbow trout exposed to IHNV but not in sturgeon kept in a pathogen-free environment and fed a manufactured diet. Neutralization titers ranged from 20-640. Titers (PNT, <20) were not detected in fish injected with cell culture media. Sturgeon injected with virus had no detectable titers at 4 weeks post-

injection but appeared to respond to IHNV at the later times (Table 1).

Table 1.

Bleed	Week	1	2	3	4	5	6
1[a]	0	<20	<20	<20	<20	<20	<20
2	4	<20	<20	<20	<20	<20	<20
3	8	20	<20	80	<20	40	20
4	12	40	40	40	40	40	80
5	16	<20	80	20	<20	<20	80
6	20	80	40	20	40	<20	80

[a]Numbers were randomly assigned to serum samples not individual sturgeon.

Passive immunization of rainbow trout with sturgeon serum having IHNV neutralization activity provided significant ($p < 0.05$), dose-dependent protection against IHNV challenge. Passive immunization with IHNV hyperimmune rabbit serum provided complete protection (Table 2). Rainbow trout injected with saline exhibited mortality (28% 13/47) that was not significantly different than fish injected with sturgeon serum with IHNV neutralization titer <20. Virus was isolated from 86% (30/35) of all dead trout examined from this test. Virus concentrations in tissue homogenates ranged from $10^{2.0}$ to $10^{6.6}$ pfu/g (mean, $10^{5.5}$ pfu/g)

Juvenile sturgeon consume an array of benthic invertebrates, but larger fish are primarily piscivorous. In the laboratory we have observed that sturgeon as small as 15 cm, fed an artificial diet since larval stage, rapidly adapt to a fish diet. Food available to sturgeon in rainbow trout raceways consists primarily of wasted feed. However, sturgeon also utilize dead and dying fish as a food source which could enhance their exposure to pathogenic microorganisms. If a serological response is elicited that is detectable, sturgeon could be useful in determining the distribution of finfish pathogens in aquatic ecosystems.

In this study virus neutralization activity was detected in serum from adult sturgeon cultured with rainbow trout exposed to IHNV and in sturgeon injected with virus, but not in sturgeon kept in a pathogen-free environment of injected with cell culture media. Sturgeon serum with IHNV neutralizing activity that was used to passively immunize rainbow trout provided significant protection against IHNV challenge.

Future studies should evaluate the susceptibility of sturgeon to other finfish pathogens and serological responses that may occur. Sturgeon may be useful in epizootiological studies or as models for understanding the immune systems of more advanced teleosts.

Table 2.

Serum source	PNT[a] titer	Rainbow Cumulative mortality	Relative protection
Sturgeon[b]	<20	32% (16/50)	0
Sturgeon	160	16% (8/50)	50%
Sturgeon	640	8% (4/50)	75%
Rabbit	640	0 (0/50)	100%

[a] IHNV neutralization titer determined by plaque neutralization test (PNT).

[b] Cumulative mortality was significantly different ($p < 0.05$) than other treatment groups.

PLEASE HELP CONFINE AND ELIMINATE TILAPIA WASTING DISEASE

We have isolated and are describing a new primary pathogen and disease of tilapia (Tilapia Wasting Disease). This disease has killed wild fishes in fresh and brackish waters, and cultured fishes in a variety of freshwater systems. We have reports from Puerto Rico and the continental USA. Infected fishes appear emaciated with sunken bellies and large heads. In advanced stages the skin may appear rough and fins frayed. The internal organs are riddled with golden to brown, various sized cysts (microscopic to 1-2 mm). This disease was first mentioned in our book, "Parasites of Puerto Rican Freshwater Sport Fishes", p. 112-113. Copies are available upon request from the senior author, Dr. Lucy Bunkley-Williams, POB 9088, Lajas, PR 00667 USA, please indicate choice of English or Spanish Editions. Please report any suspected cases of Tilapia Wasting Disease to us. We are trying to trace the origin and distribution, and determine if this devastating disease can be contained. We would not want to see world tilapia culture become as self-limiting. **Please send information and/or formalin preserved samples of the spleen or other organs of infected tilapia with cysts to:**

Dr. Lucy Bunkley-Williams or Dr. Ernest H. Williams, Jr
Caribbean Aquatic Animal Health Project
Department of Marine Sciences, University of Puerto Rico
P.O. Box 908, Lajas PR 00667 USA
Phone (809) 899-2048, FAX 809-899-5500
E-mail (INTERNET) "E_Williams@RUMAC.UPR.CLU.EDU"

Praziquantel for Treatment of Grass Carp, Ctenopharyngodon idella, Infected with Bothriocephalus acheilognathi

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

During 1993 grass carp, Ctenopharyngodon idella, were allowed in the state of Utah for the first time. This shipment was distributed to three ponds for aquatic vegetation control. Two ponds in the valley near Provo, Utah and one pond near Big Water, Utah (Lake Powell region). The grass carp were not treated for the Asian fish tapeworm (Bothriocephalus acheilognathi) previous to shipment. This in not required by law but should be common practice to purge the fish of tapeworm before introduction to new areas, especially if it is a two host tapeworm like B. acheilognathi. The Asian fish tapeworm, was introduced into this country through shipments of grass carp from China to help control aquatic vegetation in ponds (Hoffman and Schubert, 1984). The Asian fish tapeworm has spread from its initial introduction in the southern part of the United States to the western part through introductions of infected fishes. (Heckmann et al., 1986). The Asian fish tapeworm in fishes from the Virgin River in Utah and Nevada was first reported in 1986 (Heckmann et at.), and later confirmed in other species of fish (Heckmann et al., 1987). The Asian fish tapeworm is also found in states bordering Utah due to the introduction of grass carp, and the use of live minnows for fish bait.

Bothriocephalus acheilognathi is considered one of the most dangerous pseudophyllidean cestodes for cultured carp in Europe (Hoffman and Schubert, 1984). This species, first described from fish in Japan, is common in the intestine of young grass carp cultured in South China. From China, cestode infections have followed grass carp imports into Europe, Russia, and the United States (Hoffman and Schubert, 1984). The major management problem for fish infected with B. acheilognathi is that this cestode is non-host specific and uses two host (copepod, fish) in its life history instead of the usual three hosts for cestodes. The adult stage occurs in fish and the larval stage in copepods. There are other cestodes in fish that look like the Asian fish tapeworm, thus suspected infections of the Asian fish tapeworm should be confirmed by a qualified fish parasitologist. The best known carp parasite transported to the fish ponds of many countries with the Chinese carp is Bothriocephalus acheilognathi (=B. gowkongensis =B. opsalichthydis). All European countries that culture carp in large quantities now have this pathogen.

The Asian fish tapeworm is characterized by viper-like or arrow shaped scolex and numerous microtriches. This parasite, spreading into new localities, results in heavy infections of young fishes during the first year after it appears. B. acheilognathi can infect many fish species, but particularly results in heavy infections in cyprinids.

In Europe, it has been found in European guppies (Poecilia reticulata), mosquito fish (Gambusia affinis), and other species. In the United States it has been found in

golden shiners (Notegmigous crysoleucas) and fat-head minnows (Pimephales promelas), as well as in grass carp (Ctenopharyngodon idella), Colorado squawfish (Ptychocheilus lucius), and mosquito fish (G. affinis). In the western United States, B. acheilognathi has been found in golden shiners (N. crysoleucas), fat head minnows (P. promelas), grass carp (C. idella, mosquito fish (G. affinis), roundtail chub (Gila robusta),speckled dace (Rhinichthys osculus), Virgin spindace(Lipidomeda mollispinis), woundfin minnow, (Plagopterus argentissumus), and Colorado squawfish, (Ptychocheilus lucius) and recently in red shiners (Cyprinella lutrensis).

Praziquantel (Droncit, Bayvet Div., Cutter Lab) has been successfully used in veterinary and medical experiments to treat cestodes and trematodes in mammals and larval Digenea in fishes, and can be used for treating fishes infected with the Asian fish tapeworm. Fish can be purged with a treated bath (12 to 24 hours) of Praziquantel (2 ml., injectable per 200 L water) (Heckmann 1984). Various other drugs have been tried but to date the drug of choice is Praziquantel (Hoffman 1983, Heckmann 1990). European fish farmers control this parasite by drying the ponds annually or treating drained wet ponds with calcium chloride or calcium hydroxide to kill the copepod intermediate hosts, and treating the fish with anthelmintics (Hoffman 1983). Valuable fish can be fed anthelmintic drugs such as Praziquantel.

Suggested Treatments

Ponds containing fish infected with B. acheilognathi should be dried completely after fish removal. Drying or freezing kills the tapeworm eggs. In ponds that can not be dried completely, lime (calcium hydroxide) should be sprinkled heavily (enough to visibly cover, probably about 2 tons per acre on wet pond bottom; this kills the copepods that transmit the tapeworm. Europeans use calcium chloride (chloride of lime) at about 400 lbs/acre. Calcium hypochlorite (HTH) could be used if the chlorine is allowed to dissipate before filling the pond; allow several days to dissipate. HTH will kill fish, copepods, tapeworm eggs etc. (Hoffman, 1983). Remember the danger of HTH for fish.

Because the tapeworm larvae must be carried by free-living copepods ("water fleas") another control method is possible by reducing the population of the copepods thus breaking the life cycle. However, this is not possible on a long term basis in a fish pond. It would be possible to reduce the copepod numbers during spawning and transfer of egg collecting mats. Mastoten (Dylox) at 0.25mg/L (13.5 oz per acre foot) will reduce the copepod population in ponds (Hoffman, 1983). There are other chemicals applicable for copepod and vegetation control.

The Utah Report

The grass carp brought into Utah were not quarantined but the 16 fish sent to Big Water, Utah were treated with Praziquantel added to a water bath (2 ml. per 200 L water). An injectable formulation was used (56.8 mg active ingredient/ml.solution). After treating the fish at Big Water with a water bath of Praziquantel for 24 hours, tapeworms were

seen and identified in the bottom of the container. The grass carp introduced into ponds in the Provo, Utah region were not treated and the examination of one dead fish indicated a tapeworm infected specimen.

During 1995 it was determined that the grass carp at Big Water were no longer necessary for the ponds. We examined eight of the grass carp for parasites and found no tapeworms. The purge of Praziquantel had effectively eliminated all the Asian fish tapeworms with one treatment during 1993. The tapeworm cycle had not been established in the pond thus no re-infection. A water bath of Praziquantel is effective in treating grass carp infected with the Asian fish tapeworm. It is important for readers to remember Praziquantel has not been approved by FDA for use with food fish or in culinary water systems.

Acknowledgment: We received excellent help on this project from the Utah Division of Wildlife Resources especially from Wayne Gustaffeson and his staff at Lake Powell.

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1995 Snieszko Student Travel Award Recipients

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Kenneth D. Cain, Department of Animal Science, Washington State University, Pullman, WA.

Christine Densmore, NFHRL, NBS, Leetown WV and Department of Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Craig Shoemaker, Department of Fisheries and Allied Aquaculture, Auburn University, Auburn, AL.

Deborah Siegel, Department of Fish and Wildlife, University of Idaho, Moscow, ID.

Congratulations !!!!!!

ANNOUNCEMENT

There has been some concern expressed by the membership regarding an Elisa workshop planned during the Syracuse joint meeting of the AFS/FHS and the Eastern Fish Disease Workshop. The purpose of the workshop is to formulate standard procedures for ELISA which ultimately will be included in the Blue Book. Because the workshop has not been widely advertised some folks have been concerned that they were not invited. However, out of necessity the number of attenders has been kept to a minimum in order to facilitate coverage of an ambitious agenda in a very short period of time. This will be a working session only.

Most importantly, please note that any draft document resulting from this work session will be sent out for review and comment to all interested FHS members as announced in a later issue of the newsletter. Again, everyone will have a chance to review and comment.

Ted Meyers
FHS President

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

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DEADLINE FOR NEXT EDITION: September 8, 1995

FHS/AFS Newsletter
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