# A Brief History Of The Leetown West Virginia Laboratory

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In 1931, a United States Fisheries Center was developed on the site of a gristmill at Leetown, a small community in the eastern panhandle of West Virginia. A stone building was constructed that housed fish culture facilities, laboratories, office space, and a library. Circular pools, raceways, and warm water ponds were constructed to rear cold and warm water fishes. During these early days, Dr. H. S. Davis was the laboratory pathologist and he researched bacterial and parasitic diseases. Davis authored *Culture and Diseases of Game Fishes*, which became the first book on fish diseases that was published in the United States. In 1947, Dr. S. F. Snieszko became director of the laboratory and his leadership catalyzed the development of a comprehensive program, which brought the facility international recognition for its many contributions to fish health research and instruction. It was Dr. Snieszko's foresight that brought Dr. Ken Wolf and Dr. Glenn Hoffman to Leetown. It was Dr. Wolf 's who established the first continuously growing trout cell line (RTG-2 cells), isolated he first trout viral pathogen (IPNv), and published a definitive textbook on fish viruses and viral diseases. Together with Mrs. Maria Markiw, Dr. Wolf also completed the highly elusive life cycle of the whirling disease parasite *Myxobolus cerebralis*, a major parasite of salmonid fishes. Doctor Glenn Hoffman also became renowned for his work in fish parasitology. During his tenure at Leetown, Dr. Hoffman diagnosed the first outbreak of salmonid whirling disease in the United States and demonstrated its transmission. He would later publish an exhaustive compendium, which is used as a standard reference on parasites of North American fishes. Studies conducted within this facility were vitally important to the registration of the three antibiotics that were federally licensed for use on food fish in the United States. Throughout its history, the laboratory's staff has capably blended applied and basic science to resolve national issues associated with the identification, control, diagnosis and prevention of bacterial, parasitic, and viral diseases. Additionally, both short and long-term courses covering all aspects of fish health and pathology were developed. The excellence of this program is evident in more than 3,400 publications, to date.

# Pathologies In Demersal Fishes Of The Barents Sea And Adjacent Waters

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The Barents Sea and areas adjoining it to the north are zones of international fisheries. Although the Barents Sea fisheries are well researched, little information exists on the pathologies and diseases of marine organisms from this area. To work out a system that evaluated the health of commercial fish and the quality of their habitats, the Polar Research Institute of Marine Fisheries and Oceanography began to investigate the epizootic situation in the Northern seas and instituted a Russian database on pathologies of marine demersal fishes. Samples were performed on board research and fishing vessels. Techniques of clinical analysis including pathological and anatomical obduction plus histopathology were used. Some 89,516 were assayed that included plaice (*Pleuronectes platessa*), long rough dab (*Hippoglossoides platessoides*), Greenland halibut (*Reinchardtius hippoglossoides*), haddock (*Melanogrammus aeglefinus*), cod (*Gadus morhua morhua*), and wolffishes (*Anarchihas denticulatus*, *A. lupus*, A. *minor*). Ulcers, necroses, tumors and skeletal deformations were found and different forms of necrosis prevailed. Their proportion compared to other pathologies increased from 4% in 1999 to 80% in 2002. Tumors in fish occurred least often. Ppapillomas were most often evident in wolffishes with a level of morbidity of 3-8%. About 1% of plaices had pathologies. The next group consisted of cod and haddock, in which the prevalence of pathologies was less than 1%. An inter-annual increase of quantity of fish with pathologies from 0.5% in 1999 to 2.2% in 2002 was reported in the Barents and eastern Norwegian Seas. The morphological diversity and low occurrence of pathologies suggested that their appearance was caused by various endogenic and exogenic factors.

# The Volga Delta And Northern Caspian Sea: Fishing, Fish Health And Disease

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The Volga-Caspian basin contains one of the most important fisheries in Russia and yields 40.0% of the nation's freshwater (zander, common carp, bream, vobla, catfish, pike, etc.) and 70.0% of its sturgeon fisheries. There are also about 390,000 seals inhabiting the Caspian Sea. Russian and stellate sturgeons are the principal catches in the Volga River, and the stellate sturgeon is the predominant catch in the Ural River. Nine fish hatcheries operate around the Caspian Sea basin in the Russian Federation and their annual production averaged 55 million fry from 1997 through 2001. Hatchery production accounts for 98.0% of total beluga stocks, 56.0% of Russian sturgeon stocks, and 36.6% of stellate sturgeons. The most significant changes occurred in the second half of the 20$^{th}$ century when construction of hydropower stations caused sea level fluctuations. Today, the sea level is 192 cm higher than it was in 1997, which creates favorable habitat conditions for hydrobiont diseases caused by cestodes, nematodes and crustacea found in commercial catches from the Volga delta. Because the prevalence of these diseases was not high, records were not taken after 2000. Bacterial diseases were not noted, but viral fibrosarcomas in zander as well as cutaneous and fin papillomas in sheatfish were observed. The most important parasites included: *Anisakis schupakovi, Eustrongylides excisus, Apophallus muehlingi, Rossicotrema donicum, Paracoenogonimus ovatus, Pseudamphistomum truncatum, Opisthorchis felineus, Corynosoma strumosum*. Each of these with the exception of *C. strumosum* was detected in muscles. Unfavorable winter conditions in 1999-2000 initiated a mixed infection with Canine distemper virus, salmonellosis and pasterellosis that killed about 20,000 Caspian seals. Although viruses were not detected in stellate sturgeon brood fish used in aquaculture, bacteria were present in their gonads and they also had infestations of *Cocconema sulci, Polypodium hydriforme, Amphilina foliacea,* and *Eustrongylides excisus* that were clinically insignificant.

# Health Of Pacific Salmonids Held Under Natural And Artificial Conditions Of Reproduction In Kamchatka

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Results of bacteriological, virological, parasitological, histological and histochemical investigations of Pacific salmon (chum, pink, sockeye, Chinook and Coho) were compared among fish held under natural and artificial conditions of reproduction. Initiated in 1998, these investigations are ongoing in an effort to monitor salmon health and apply preventive treatments for infectious diseases. A total number of 100 eggs and 11,635 fingerlings were investigated at hatcheries. Another 2,110 fingerlings and 1,600 adult salmon were investigated in natural waters. Carriers of Infectious Hematopoietic Necrosis virus (IHNv) were detected among wild and hatchery adult sockeye salmon at the southwest rivers of the Kamchatka peninsula in 2001-2002 and an IHNv epizootic occurred among sockeye salmon fingerlings at one hatchery in 2002. Viral Erythrocytic Necrosis and Erythrocytic Inclusion Body Syndrome were also found. Eleven species of bacteria were isolated from wild and hatchery fish. *Aeromonas salmonicida* subsp. *salmonicida* and non-motile *A. salmonicida* subsp. *masoucida* were the most problematic but nine other secondary pathogens were noted. Four species of protozoan parasites *Ichthyobodo necator, Chilodonella piscicola, Apiosoma conicum* and *Trichodina truttae* were found among hatchery fingerlings of Pacific salmon. Thirty-one species of parasites from 15 classes were detected among young feral salmon. Nine of these species persisted within the fish during their time at sea. Thirty-six species of parasites representing eight classes were detected among adult fish from natural reservoirs. The $F_1$ generations that were produced by infected parents among nine of these species were also affected.

# Diseases Of Whitefish (*Coregonidae*) In The Russian North-West And North Caucasus

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The 1960's through the 1990's was a period of time in which whitefish (*Stenodus leucichthys leucichthys* Gueldenstaedt) were widely cultured within Russia. Infections produced by numerous diplostomatids, protozoa, cestodes and crustacea were often described in these fishes, but our data showed that there was actually a low prevalence of these parasites in coregonid fishes from the Russian North-West and North Caucasus. Today, diplostomosis is not considered to be a serious disease among whitefish in these latter regions. Although the prevalence of infection ranges between 1.3 to 89.2 %, parasite abundance is rather low and generally only 1 – 12 specimens may be found on an infected fish. The cause of the reduction in the severity of these parasitic infestations was a break in the parasite's development that led to reduced contact between cultured whitefish and other hosts, such as birds or mollusks. Toxicoses, alimentary disorders, and infectious diseases are currently the most problematic issues among whitefish. Round black tumor-like lesions have been found on the skin and in kidney of cultured juvenile whitefish at a fish farm located in North Caucasus. Light microscopy revealed that these growths were surrounded by a rim of hyperplastic epidermis that contained numerous branched, septate fungal hyphae and various host cells including erythrocytes, macrophages, and fibroblasts. The fungus was preliminarily identified as *Exophiala* sp. (*Hyphomycetes*) and this was the first record of the genus *Exophiala* that was reported as a pathogen in whitefish.

# Aquaculture And Fish Diseases In Russia And Former Soviet Union: Recent History And Current Situation

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The last decade of the 20th century was a critical time for aquaculture in what formerly was the USSR. All aspects of the industry (fish production, disease control and applied science) were affected and output dropped from 418,000 tons in 1990 to 108,000 tons in 1997. The decrease in Russia went from 260,000 tons in 1990 to 70,000 tons in 2000. Today, Russian aquaculture is poorly diversified when compared to that of the former Soviet Union. Although signs of revived activity are evidenced by the culture of sturgeons, salmonids and shellfish, cyprinid culture comprises about 84% of the total aquaculture production in Russia. Fisheries in Russia are oriented toward wild catches and there are no laws or national legislation that regulate the industry. Although many aquaculture enterprises have become joint stock companies, most are in urgent need of investment. The state veterinary service controls fish diseases in Russia and there is some specific veterinary legislation for certain fish disease issues. Unfortunately, few veterinary laboratories in the country can perform disease diagnostics and such tests are mostly done in research laboratories or universities. Even fewer of these facilities perform diagnostics certified to international standards. Although veterinary certificates normally accompany fish or egg movements, these are rarely based on laboratory data. Furthermore, federal programs for fish disease monitoring, reporting, risk analysis and contingency planning do not exist in Russia. Only six of ten research laboratories that dealt with infectious fish diseases in the former Soviet Union still exist today; - five of these facilities are in Russia. Twenty-one viral and virus-like agents or conditions and seven principal bacterial agents are present in wild and cultured fish. The most economically important viral diseases are SVC, VHS, IHN, The most serious bacterial diseases include vibriosis, furunculosis, streptococcosis, motile aeromonad septicemia, bacterial hemorrhagic septicemia and *Columnaris* disease. Contraction of aquaculture due to the national economy improved the epizootic situation for many infectious diseases, but it worsened some parasitic and bacterial epizootics caused by ubiquitous organisms (e.g.-aeromonads, pseudomonads, and flavobacteria). Current trends emphasize development and use of probiotics and vaccines, bur more efforts are needed to develop molecular diagnostic techniques.

# Atlantic Salmon Restoration In New England: Significance Of Certain Diseases

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Atlantic salmon (Salmo salar) once thrived in the northeastern part of the United States and ranged from Maine to the Housatonic River in Connecticut. Despite the large populations that once thrived in the Connecticut, Merrimack and Penobscot Rivers, Atlantic salmon essentially disappeared from New England by the mid 1880's. This decline was attributed to the construction of dams, pollution and overfishing; - problems associated with unfettered industrialization of the northeast. In the latter half of the 20th century, however, efforts began in earnest to restore the salmon based on legal justification provided by the U.S. Anadromous Fish Conservation Act of 1965. The act provided stability for a long-range restoration program involving the cooperation of numerous federal agencies, state department of fish and wildlife agencies, industry, and conservation groups. The current restoration program utilizes river-specific brood stock to maximize genetic diversity within the Connecticut, Merrimack, Penobscot and several other smaller rivers on the downeast coast of Maine. Following two to three years at sea off the coast of Greenland, mature Atlantic salmon return (around May) to spawn in their natal rivers. These fish are captured at dams or weirs, transported to holding facilities, and maintained as non-feeding captive brood until they become gravid (September/November). Thus, holding and production facilities are the cornerstones of restoration. Gametes, obtained from captive brood, are used to produce quality fry, parr and smolts that are stocked to enhance restoration. These fish spend their first two years of life in their natal rivers before they migrate to the ocean. Due to the unique genetic composition and depleted numbers of Atlantic salmon along the downeast coast of Maine, federal authorities have listed those stocks as an endangered species. In support of this listing several disease issues were cited that could deleteriously impact survival. Predominant among these concerns are: significant mortalities associated with Furunculosis, caused by *Aeromonas salmonicida*; vertical transmission of Bacterial Coldwater Disease, caused by *Flavobacterium psychrophilum*; the potential introduction of Infectious Salmon Anemia virus within restoration fishes; and the deleterious effects of Swimbladder Sarcoma Virus. Each of these issues will be discussed within this presentation.

# Mycobacteriosis And Chesapeake Bay Striped Bass: An Integrated Cooperative Research Program

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Mycobacteriosis is a bacterial disease of striped bass (*Morone saxatilis*), which can be disfigured by skin ulcers and internal lesions. Bass may also be emaciated by the chronic nature of this wasting disease. Recent evidence of polymicrobial infections in some striped bass suggested that the observed condition of infected fish might be caused by somewhat complex disease progression. While the extent of mortality associated with mycobacteriosis in wild striped bass is unknown, laboratory studies indicated that the bacteria are lethal when injected in biologically relevant doses. In the Chesapeake Bay and other regions of the United States, striped bass are a prized species for recreational anglers and commercial fishermen. The economic impact of mycobacterial infection could be significant. Data collected from 1998 to 2002 revealed that 70% of the bass taken from some locations within the Chesapeake Bay were infected and that the disease had persisted in these populations for at least five years. A cooperative research program to address research issues surrounding this mycobacteriosis epizootic began in 1999. This program, involving both state and federal entities, addressed issues associated with pathogenesis and immunity. Understanding the epidemiology of this disease is important to resource managers who must responsibly manage the striped bass and other fisheries in the Chesapeake Bay. It may also be important to those managers responsible for the overall health of the bay and interagency efforts to address concerns for environmental quality and the safety of bay resources for recreation.

# Diagnosis And Management Of Bacterial Diseases In Warmwater Aquaculture

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Warmwater aquaculture, particularly within the catfish industry, experienced tremendous growth in the last 25 years. Mariculture (marine aquaculture) is being examined as a means to compensate for over-fished natural stocks of marine fishes. Extensive culture practices, that were common before 1980, have yielded to more intensive pond culture and closed recirculating system culture. This increase in technology and production capability has also caused an increased incidence of disease in warmwater aquaculture. In this presentation, I shall describe methods to diagnose and manage important bacterial, viral, and parasitic agents that affect channel catfish *Ictalurus punctatus*, hybrid striped bass *Morone saxatilis*, and tilapia *Oreochromis niloticus*. Methods of diagnosis and control will be discussed based on procedures practiced at the Louisiana Aquatic Diagnostic Laboratory.

# Health And Disease Issues Associated With Fish Populations In The Great Lakes Basin

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In the Great Lakes basin, conservation and restoration efforts of fisheries depends primarily upon increasing the existing fish through stocking of hatchery-propagated fish. This practice requires collecting gametes from feral fish species and raising the offspring to a stage, which if released in the Great Lakes' environment, would yield a high survival rate. Maintaining the health of these fish is instrumental to the success of these efforts. This study describes a number of emerging fish diseases that threaten the success of the Great Lakes fisheries restoration efforts such as infection with the Largemouth Bass Virus (Ranavirus, Iridoviridae). This viral infection is spreading rapidly among the inland lakes of Michigan's lower peninsula. Infection with a *Piscirickettsia* sp has caused epizootic mortalities in the muskellunge population of Lake St. Claire. Visceral mycosis, primarily caused by a unique strain of *Phoma herbarum*, causes heavy losses in hatchery-reared Chinook salmon. Efforts to control the vertical transmission of bacterial kidney disease and coldwater disease have been successful.

# Disease Transmission From Cultured Salmonids To Wild Fish Stocks: Perspectives On The Alaskan Hatchery Program

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Geographic isolation, coldwater temperatures and protective fish health legislation have probably accounted for fewer indigenous salmonid pathogens in Alaska and have prevented the introduction of exotic disease agents through prohibition of fish imports. Nonetheless, prevalences of indicator pathogens such as infectious hematopoietic necrosis virus (IHNv) and *Renibacterium salmoninarum* (Rs) have approached 100% in wild fish stocks that have not had any exposure to hatchery fish or hatchery practices. In Alaska, all hatchery broodstocks have originated from wild salmonids. Disease interactions between hatchery and wild fish are minimized by stringent fish disease and genetics policies, hatchery and release site selection, and other management criteria. Examination has shown that there were no significant differences in IHNv or Rs prevalences between hatchery and wild stocks of salmonids. Furthermore, isolates of IHNv from hatchery and wild fish appear evolutionarily constrained with a low genetic diversity and restriction to sockeye salmon as a host species. Hence, these diseases in Alaskan salmonids have been relatively static with cyclical peaks and declines. Thus, there were no significant increases in pathogen levels and prevalences that could be attributed to hatchery practices and/or interactions with hatchery fish stocks.

# United States Department of Agriculture Services Related To Infectious Salmon Anemia And Spring Viremia Of Carp Aquatic Animal Health Programs

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The Office of the Secretary of Agriculture announced that an emergency threatens the livestock industry of this country, and United States Department of Agriculture (USDA) funds were used to establish Infectious Salmon Anemia (ISA) and Spring Viremia of Carp (SVC) programs to address the threat to the U.S. salmonid and ornamental fish industries. Approximately $8.3 million and 11.7 million was authorized for the Animal Plant Health Inspection Service (APHIS) Veterinary Services (VS) to implement an ISA and SVC control and indemnity program for farm-raised salmon and ornamental Koi carp in the United States. In addition to the payment of indemnity, these funds are to be used to assist the states of Maine, North Carolina and Virginia with program activities such as: depopulation and disposal, clean-up and disinfection, establishment of surveillance programs, epidemiology and diagnostic support, and training for producers and veterinarians. Federal assistance is deemed necessary to effectively control this disease, which poses a threat to animal health and the U.S. economy. Our goal is to control and contain ISA and SVC viruses through rapid detection and depopulation of salmon and koi that have been infected with or exposed to ISA or SVC. It is believed that these viruses can be controlled within high-risk zones through surveillance, and best management practices. This paper will outline those steps required for ISA and SVC indemnity, control, depopulation, cleaning and disinfection, surveillance and restocking of pens or ponds of commercial production operations.

# Theory And Practice Of Establishing Long-Term Cell Lines From Fish And Shellfish

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Potentially immortal continuous cell lines are of both scientific and pragmatic interest in relation to lower vertebrates and invertebrates. Since the 1960's, cell lines have been established from 300 finfish, 500 terrestrial invertebrates (insects) and soft-bodied or calcareous corals. However, the development of only one cell line from an aquatic invertebrate, (the freshwater mollusk, *Biomphalaria glabrata*), clearly indicates that success is still based largely on empirical findings rather than theoretical background. Experiences suggest that best results are achieved when cells are gently excised from donor organisms followed by placement in a comfortable environment that favors *in vitro* growth. There are at least five "bottlenecks" in which the most vulnerable of the explanted cells are lost. Such barriers include the donor animal itself, method of donor tissue decontamination, tissue dissociation techniques, composition of growth media, and quality of cell culture glass and plastic ware. In this context, the significance of the donor's immuno-physiological state, tissue selection, type of sample (individual or pooled), dissociation techniques, contributions of synthetic media (and sera), and glassware processing are reviewed. Additionally, this presentation assesses the importance of cell seeding densities, allogenic serum and conditioned cell culture media, role of "minicultures" and "chess-board" cell seeding, seeding of mixed cell or separated cell suspensions, and procedures for subculture. The work predominantly concentrates on spontaneously established cell lines, but new approaches for directed immortalization of primarily cultured cells will also be discussed.

# Retroviral Pathogens And Tumors In Coolwater Fish - Walleye Dermal Sarcoma

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Walleye dermal sarcoma is a dermal lesion found on walleyes *Stizostedion vitreum* during the cooler months of the year. The lesion develops in the fall, is carried by the fish through the winter and typically regresses in the early summer. Our laboratories have been investigating this tumor over the past 18 years in an effort to understand the mechanism(s) by which this retroviral tumor develops and regresses on a seasonal basis. The tumor can be transmitted in the laboratory by intramuscular injection, feeding or topical application of cell-free tumor filtrates from tumors collected in the spring. Attempts to transmit the tumor using filtrates from fall-collected tumors have met with little success. Tumors considered to be malignant have only been found on rare occasions. When laboratory challenges were performed with very young walleye (< 8 weeks post-hatch), invasive tumors were observed. During our studies at the New York State Department of Environmental Conservation Oneida Fish Hatchery, Oneida Lake, New York, typically 30-40,000 adult walleyes are brought to the facility. During that time, we have only observed three adult walleyes with tumors that were invasive. In the overwhelming majority of the cases, tumors will regress on fish. Field and laboratory studies suggest that, in most cases, a walleye that is tumor-positive in a given year will be refractory to development of a tumor in a subsequent year. The walleye dermal sarcoma virus is the first retrovirus of fish to have its complete sequence determined.

# Immune Sensor Based On Surface Plasmon Resonance For Express Control Of Fish Retroviral Infections

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Solving the problem of early diagnostics for retroviral infection is very important today because diseases induced by this infection are widespread among fishes, animals and humans. Bovine leucosis, AIDS, and some fish tumors are induced by retroviruses, which are identical in some conservative regions. We had earlier developed immune biosensors based on the surface plasmon resonance for express diagnostics of bovine leucosis. Some proteins of retrovirus were used as sensitive elements in this biosensor. In this report we intended to present the experimental results about our approaches for the development of the immune biosensor based on the above-mentioned transducer that detects retroviral infection among fishes in some reservoirs. In the Ukraine we have found tumors in pike [2], bream, walleye and mullet. At first, we investigated the possibility of obtaining retroviral antigens from some tumors of fish. In the course of these investigations, it was observed that retroviral antigens of pike were a complexed with antigen-specific antibodies. Second, it was revealed that these antigens cross-reacted with antibodies, which were induced by retroviruses that caused bovine leucosis. In all cases bovine leucosis virus antigen or purified fish retroviral antigen were spontaneously adsorbed on bare thin gold surface or preliminary lectin-treated one. After the selective binding of specific antibodies with the immobilised antigens, the specific signal of surface plasmon resonance was observed. At last, it was discovered that preliminary treatment of transducer surface by lectin allowed reception of a more stable and distinguished signal of the immune sensor. These investigations showed that the immune sensor could be used to diagnose retroviral infections among fishes. Moreover, such diagnostics may be done in field conditions as well as in real time. Our next experiments will investigate effective algorithm of fish retroviral antigen purification to study level of cross reactivity of fish and bovine retroviral antigens and to analyse the level of specific antibodies in the blood of fish and different stages of disease progression.

# Isolation Of Infectious Pancreatic Necrosis Virus From Young Atlantic Salmon (*Salmo salar* L.) In Russia

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Viral diseases cause significant economic losses and serious problems for Atlantic salmon (*Salmo salar L.*) raised in aquaculture. Infectious Pancreatic Necrosis virus (IPNv) is one of our more significant concerns. In this study, the viral cytopathogenic agent was isolated from young salmon at a fish farm of the Murmansk region in Russia. Chum salmon heart (CHH-1) and Chinook salmon embryo (CHSE-214) cell cultures were used to isolate the virus. Cytopathic alterations typically associated with IPNv were observed with the third passage after cell culture viral transformation. Distinctive inclusions in cell cytoplasm and larger pink inclusions typical of the RNA viruses were detected. The cells widened, displayed symplast formations, and had increased nuclear and cytoplasmic densities by the second day and cell monolayers were completely degraded by day three. The titer of virus in CHH-1 cell culture was up to $10^3$ $TCD_{50}$/mL and $10^{6.5}$ $TCD_{50}$/mL in CHSE-214 cells. The titer was as high as $10^8$ $TCD_{50}$/mL by the eleventh passage in CHH-1 cells. The optimal temperature for viral propagation was 15°C and the virus could be stored frozen at either –70°C or –20°C. Two types of viral particles were detected using electron microscopy; one resembled the family Birnaviridae. Serological identification of the virus was accomplished with an IPNv antigen-based enzyme linked immunosorbent assay that was conducted at the National Veterinary and Food Research Institute, Helsinki. Their data confirmed the presence of IPNv in the samples that were tested.

# Infectious Hematopoietic Necrosis Virus: Repetitive Isolation From Adult Sockeye Salmon (*Oncorhynchus nerka)* From River Bolshaya-Bistraya (Kamchatka)

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This study was conducted to detect the prevalence of Infectious Hematopoietic Necrosis virus (IHNv) among prespawning sockeye salmon *(Oncorhynchus nerka)* from the river Bolshaja-Bistraya. In 2002, 217 adult feral salmon were examined that were either caught on their natural spawning grounds or held as captive brood stock at a fishpond prespawning holding pool at the Malkinskiy Salmon Hatchery. Even though the IHNv carrier prevalence was 50% among the sockeye salmon from this population, pathological signs were not evident by visual examination. Samples of kidneys, spleens, brains and ovarian fluids were collected for virological examination. Samples were inoculated on CHSE-214 and EPC cell lines and cytopathic effects generally became apparent six days after inoculation. The virus was confirmed by neutralization assays using rabbit antiserum to IHNv obtained from the United States. The first sampling of the adult sockeye salmon revealed that 10% of the fish were infected, but the number of carriers increased to 75% before spawning ended. The titers of infectious virus in organs and ovarian fluid of the examined fishes was always at an epizootically significant level ($0.4?10^{5}$ to $0.4?10^{8.6}$ $TCD_{50}$/mL). Bullet-shaped viral particles, which measured approximately 150-220 x 60-80 nm were observed by electron microscopy. Morphological attributes indicated that the virus belonged to the family *Rhabdoviridae*.

# Genetic Typing of Infectious Hematopoietic Necrosis Virus

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Infectious hematopoietic necrosis virus (IHNv) is a rhabdovirus that causes severe disease and is prevalent in salmon and trout species in the northwestern region of North America. Since the 1950's IHNv has been known to cause epidemics in hatchery and farmed salmonids, and outbreaks in wild salmonids have also been reported. Genetic typing methods have been developed to characterize IHNv isolates on the basis of partial gene nucleotide sequences within the glycoprotein (G) and nucleocapsid (N) genes. Phylogenetic analyses of sequences from over 400 IHNv isolates from throughout the North American range of the virus have shown that there are three major genogroups of IHNv. These genogroups correlate strongly with geography, are they are therefore designated U, M, and L, to indicate that they are found in the upper, middle, and lower portions of the virus range. The IHNv genogroups vary in genetic diversity and evolutionary rate, suggesting varied evolutionary histories. Genetic typing also provides epidemiological insights, revealing complex virus traffic patterns and identifying potential sources of disease outbreaks. A subset of this data has been selected to generate phylogenetic trees of partial G (303 nucleotides) and partial N (412 nucleotides) sequences from 29 IHNv isolates. These trees were designed to include representatives of all IHNv genogroups and sub-groups that have been identified, so they can be used by other researchers to determine how new IHNv isolates fit into this family of IHNv.

# Diagnosis and Specific Prevention of Spring Viremia of Carp

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Spring Viremia of Carp virus (SVCv) is a rhabdovirus that causes severe disease, but remains largely untreatable. This research aimed to develop methods to detect and identify different SVCv isolates and evaluate recombinant vaccines that would prevent the disease. For diagnosis, an RT-PCR was developed that used primers to several SVCv genes. Most isolates of the virus were detected with three pairs of primers to the N gene. For detection of virus in clinical samples (tissues from fish, infected ZL4 strain SVCv), a semi-nested PCR was developed and the sensitivity of viral detection was $10^2 TCID_{50}/g$. The N1-N2 fragments were amplified, cloned, and sequenced. The aliments of N1-N2 fragments of the SVCv N gene and intergenic regions from a reference isolate (courtesy of Nikolai Fijan) and isolates from Czechia, Hungary and countries of Western Europe were very homologous. However, there were many differences in the N gene and intergenic region of Russian isolates. Three nucleotide substitutions lead to the amino acid placements that allowed differentiation between Russian and European isolates by PCR. Based on the sequence comparisons, restriction fragment length polymorphism (RFLP) analysis was used to type subgroups. A recombinant vaccine against SVCv, based on the viral glycoprotein gene, was evaluated. In initial experiments, a DNA vaccine (plasmid pcDNA3-G, constructed by means of insertion of the full-length viral G protein from an American isolate) was supplied by Joanne Leong. The vaccine was very protective and produced a relative percent survival of 49% among fish challenged at eight weeks post-vaccination and 80% among fish challenged after 10 weeks. Another DNA-vaccine based on G-gene of the Russian ZL4 strain under control of CMV or carp-beta-actin promoters (courtesy of N. Lorenzen) was constructed but there have been problems with efficacy due to impurities in the plasmids. Vaccine-candidates purified by different methods were prepared and their efficacy is under further study.

# First Report Of Spring Viremia Of Carp Virus At A Fish Farm In Moscow Province, Russia

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Although a cytopathogenic agent was first isolated from carp with infectious dropsy syndrome in the USSR during the early 1960's, viral etiology was confirmed eight years later and after the disease was first described in Yugoslavia. Since then, dozens of Spring Viremia of Carp virus (SVCv) isolations have been made in the Russian Federation, Ukraine, Belarus, Moldova, Georgia and Lithuania. These isolations geographically overlap natural areas where carp, *Cyprinus carpio*, are found in eastern Europe. Most of the Russian Federation isolates came south European Russia and other areas were considered to be free of SVCv. In May 2003, a heavy epizootic occurred among broodstock, 1+ and 2+ carp at the Lotoshinsky fish farm located in the northwestern part of Moscow Province. Mass mortality began soon after transfer of fish from wintering to summer brood and grow out ponds. Disease was triggered by water temperature fluctuations: from low to 20°C and back to 15°C in few days. Affected fish displayed pale gills, scale protrusion, exophthalmia, inflamed and protruded anus and skin ulcers. At necropsy, the liver and kidney were pale and swollen and ascitic fluid was present. Poloed liver, spleen and kidney samples were assayed from 11 diseased fish for virological examination and inoculated onto EPC cells. The isolated cytopathogenic agent was identified as SVCv using neutralization tests with rabbit antiserum (neutralization index $10^{4.25}$ at serum dilution 1:40). The disease was reproduced in yearling carp by intraperitoneal injection of the virus after its seventh *in vitro* passage. At water temperatures of 14.5–15.5°C, eight of nine infected fish had clinical signs of SVC and six of these fish died. More than an 80% mortality was observed on the farm at that same time in 8-day-old fry. Unfortunately, the diseased farm fish were not sampled. This was the first case of SVC detected in the Russian Federation after almost a decade-long collapse of national aquaculture, but carp culture is now an active industry in Moscow Province. The central position of the Province, the revival of aquaculture, weakened veterinary controls on fish movements, and peculiarities of the local watershed can contribute to the spread of SVCv into other regions.

# Intracytoplasmic Inclusions In Erythrocytes Of Pacific Salmon

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Investigations have been conducted for two years that revealed two types of intra-erythrocytic cytoplasmic inclusions from northern Okhotsk sea populations of chum, pink, and Coho salmon. These conditions were similar to those observed with Viral Erythrocytic Necrosis (VEN) and Erythrocytic Inclusion Body Syndrome (EIBS), which have been described in detail as among salmon abroad. Our investigations in northern Okhotsk-sea populations suggested that VEN was present in the chum, pink and Coho salmon, but EIBS was restricted to chum salmon fingerlings and brood fish. The number of affected breeders (of chum and coho salmon) increased rapidly during their maintenance within fish plants during the fall and just before spawning. To that extent, a seasonal character of infection in chum salmon was revealed. In the summer, the inclusions EIBS inclusions were predominant within fingerlings and breeders of chum salmon, but VEN inclusions became more prevalent in the fall. Such infections did not produce acute mortality, but compromised the immuno-physiological condition of chum fingerlings. This dehabilitation could potentially affect the survival of young fish after they are released into the river. Such infections should be considered to be epidemiologically dangerous to salmon aquaculture in the Far Eastern region of Russia.

# The United States Fish And Wildlife Service's National Wild Fish Health Survey: A New Tool For Fisheries Management

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The United States Fish and Wildlife Service's (USFWS) National Wild Fish Health Survey (NWFHS) has existed since 1996 and became available to the public in September 2001. The NWFHS comprises field collection, laboratory analysis and web-accessible database components, and is primarily a result of Federal, State and Tribal cooperative efforts. All field collections are conducted in accordance with published NWFHS standardized procedures. All laboratory procedures are, likewise, in accordance with the same published procedures, but are only conducted by USFWS fish health biologists stationed at our nine Fish Health Centers. The database can be queried relative to national fish pathogen distributions in free-ranging fish, the results of which can be displayed graphically and/or in tabular form, and overlaid on United States Geological Survey-based watershed maps. As of August 2003, the database contains entries from greater than 62,000 individual fish samples collected from more than 1,700 distinct geographic locations (each with an exact latitude and longitude based on global positioning satellite, or GPS, information) located in 44 States. The fish sampled are from 183 different species. Samples are routinely assayed for as many as 28 fish pathogens. The database has already been used by the USFWS and other aquatic resource managers to formulate, and implement, well-informed and more scientifically-based fishery management plans. As each year's samples are collected, analyzed and entered into the database, the NWFHS progresses closer to achieving its full potential. In addition to a review of the NWFHS, several examples of how the NWFHS has been successfully used by aquatic resource managers will be presented.

# *Aphanomyces invadans (= A. piscicida)*: A Widespread Problem In Wild And Cultured Fishes

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The disease now known by most investigators as epizootic ulcerative syndrome (EUS) has caused major losses in many countries for over three decades. It was first described in Japan as mycotic granulomatosis, a year later in Australia as red spot disease, throughout Southeast and South Asia as epizootic ulcerative syndrome and in the United States as ulcerative mycosis. These syndromes include the presence of ulcerative, dermal lesions in which invasive fungal (oomycete) hyphae have elicited a characteristic granulomatous response. In many cases, lesions extend deep into the musculature. Many species of freshwater and estuarine fishes are susceptible. Much controversy has occurred over the "cause" of these lesions. The role of other fungi or oomycetes, bacteria, viruses and toxic dinoflagellates in ulcer formation has been disputed. The presence of many opportunistic organisms in the open ulcers of affected fish, complicates isolation of a primary infectious agent; the lack, until recently, of molecular diagnostic techniques and reproducible models for experimental induction; and the lack of consistent methods for confirmatory diagnosis of EUS have contributed to this controversy. However, most investigators now agree that the cause is *Aphanomyces invadans (=A. piscicida)*, an aquatic oomycete. The state of our knowledge on the phylogenetic characterization of the organism, histopathological presentation of the disease, models for experimental induction of disease, environmental influences on the growth and sporulation of the organism, epidemiology and advances in molecular diagnostic techniques will be discussed. In addition, the outcomes of an expert consultation on EUS held during the Fifth Symposium on Diseases in Asian Aquaculture, Brisbane, Australia, November 2002, will be reviewed.

# Marine Mammal Stranding Response: Conservation Through Cooperation

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The southeastern region of Massachusetts, with more than 700 miles of coastline including Cape Cod, is a major area for the stranding of marine mammals. The Cape Cod Stranding Network, Inc. (CCSN) responds to strandings of marine mammals in this region. Our mission is to provide rapid response, assessment and humane care to stranded marine mammals; to effect appropriate release; and to conduct research and education in order to reduce suffering and prevent avoidable mortality. The CCSN is a non-profit organization formed by a group of like-minded animal welfare and scientific organizations functioning in a cooperative manner to provide effective responses to marine mammal stranding events with the added benefits of enhancing education about and conservation of marine mammals through innovative methodologies. As the holder of a Letter of Authorization from the National Marine Fisheries Service for the Cape Cod region, the CCSN is responsible for all strandings of marine mammals common to the many different habitats found along our shores. In 2002, the CCSN responded to approximately 300 stranded pinnipeds and cetaceans representing 16 species. In carrying out our mission, we work closely with federal, state, and local officials as well as other stranding networks in the Northeast Region. While our staff usually deals with single strandings, mass strandings require extensive cooperation between the CCSN, governmental agencies, and other networks in the region. The small permanent staff of the CCSN is enormously augmented with the contributions of more than 400 trained volunteers who assist at strandings of all magnitudes. Without the faithful cooperation of the staff of the various networks, the trained volunteers, and the several governmental agencies with jurisdiction over marine mammals in our waters, our mission could not be fulfilled.

# A Brief Review Of Some Important And Emerging Bacterial Diseases Of Pinnipeds And Cetaceans

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Bacterial diseases continue to play an important role in mortalities of both captive and wild marine mammals. Various authors have attributed up to 30% of wild marine mammal deaths and up to 50% of captive held marine mammal deaths to bacterial etiologies. A number of bacterial pathogens involved in mortalities in early zoological collections of pinnipeds and cetaceans continue to be recognized as the cause of death in contemporary captive collections. This presentation reviews the major bacterial diseases most frequently encountered in captive and stranded marine mammals. The clinical signs and courses of these diseases will be reviewed as well as the associated clinical pathologic changes and common treatment and prophylactic regimens. In addition, emphasis will be placed on two zoonotic bacterial diseases, either newly recognized, or emerging, in marine mammal populations (Brucellosis and Mycobacteriosis).

# Emerging Challenges In Public Aquaria

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In the past 25 years, public aquaria have gained popularity worldwide. Many major cities now host aquariums that educate and entertain visitors. Modern facilities house diverse aquatic and terrestrial collections requiring advanced life support systems, specific nutritional requirements, development of customized quarantine and husbandry programs, and the ability to recognize, diagnose and treat disease. Excellent water quality is required for fish health and clarity is also needed for public viewing. Providing water changes to large closed-water systems is expensive and, in some cases, not permitted due to elevated levels of phosphate, nitrates, or other pollutants. Alternate methods to manage potential toxins are in development. Additionally, fish utilize trace and macro elements from the water such as iodine, which is required to prevent goiter. The use of ozone, a common oxidant that disinfects and improves water clarity, alters the form of iodine that potentially decreases its absorption. Maintaining healthy elasmobranchs in captivity presents numerous challenges. There is a lack of standardized analytical methods for hematology and leukocytes morphologically differ among species. Because cell function is not understood, its relevance in health assessment is under investigation. Large sharks at some facilities have developed spinal deformities and there is a collaborative effort among clinicians and pathologists to determine its cause. Suitable anesthetic and surgical techniques are continually being developed for elasmobranchs. Oxygen carrying replacement fluids such as Oxyglobin®, a polyionic colloidal fluid made with ultra pure bovine hemoglobin, have been used in teleosts and elasmobranchs and show promise in emergency treatments. Seahorses and sea dragons (sygnathids) have become popular exhibits, but many of their diseases are not understood or described. For example, subcutaneous gas bubbles occur in many species, which often respond to treatment with antibiotics and a carbonic anhydrase inhibitor, but the cause is unclear. Mycobacteriosis is also a problem among captive sygnathids. Head and lateral line disease is commonly seen in captive teleosts. Although the cause is unknown, a new treatment offers hope for valuable specimens and may help determine its etiology. Everyday brings challenges to aquarium veterinary and husbandry staff, which present opportunities for collaborative research.

# DDT In Bottom Sediments And In The Liver Of Barfin Plaice *Pleuronectes pinnifasciatus*: A Factor Influencing Health Of Fishes From Amursky Bay (Peter The Great Bay, Sea Of Japan)

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In August andSeptember 2001, 15 samples of bottom sediments were collected in inner, middle and open areas of Amursky Bay near Vladivostok, Russia. Barfin plaice *Pleuronectes pinnifasciatus* were also sampled from the inner and middle parts of the bay. In the sediments and in the fish liver, the contents of chlorinated hydrocarbons, *p,p¢*- DDT and its metabolites (*p,p¢*- DDD and *p,p¢*- DDE) as well as α-, β- and γ-isomers of hexachlorocyclohexane (HCH) were determined using gas-liquid chromatography. The sediments contained negligible amounts of HCHs (0.2–0.8 ng/g dry weight), while DDT concentrations were quite high (1.7–16.3 ng/g dry weight). There were no differences in levels of pesticide contamination between the locations and our results resembled those reported for the Amursky Bay in the 1990s. Surprisingly, "fresh" DDT comprised 70–85% of total DDT content in the sediments from all locations studied in 2001. The DDT/DDE ratios were high (4.3–11.5). In fish livers, total DDT concentrations were 212.8 and 122.5 ng/g wet weight for the inner and the middle locations, respectively, and "fresh" DDT comprised 35 and 64% of DDTs, respectively. These results provide evidence for a recent input of DDT from an unknown source into Amursky Bay. Fish livers were examined for histopathological changes, which are biomarkers of toxic contaminants. Vacuolization of hepatocytes, coagulative necrosis of hepatocytes, necrosis of epithelial cells of bile ducts, and inflammatory reactions were evident. Diffuse vacuolization of hepatocytes was the most common type of pathology that was observed. On the whole, liver morphology as well as the occurrence of histopathological alterations were similar in the plaices from both locations. The only difference was that 23% of the plaices from the middle site had pathological changes of the bile ducts that were not detected in the fish from the inner site. This pathology is probably connected with an intensive metabolism of DDT by the fish.

# Global Climate And Large-Scale Environmental Influences On Aquatic Animal Health

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In the last 3 decades, numerous large-scale mortality events have occurred that affected a broad diversity of aquatic organisms in North America. Short-term forensic investigations of these events have sometimes characterized a causative agent or condition, but they have rarely provided insight sufficient to predict and manage animal health. Although traditional tools (microbiology, parasitology and pathology) are essential, technological advances in remote sensing, satellite imagery, and information management are required to understand these local biological phenomena in context to environmental conditions that change across broad regional and global scales. These additional tools will be invaluable for exploring the influences of climate, weather (e.g., precipitation), ultraviolet light exposure, Aeolian dust (transporting microorganisms and nutrients), and regional land use patterns on disease and host susceptibility. Ultimately, regional observing systems could forecast health risks to aquatic resources. One such system, under development in the Gulf of Mexico, will forecast red tide events that have extended along the Gulf coastal states of the United States and Mexico. But to further this approach, a meaningful understanding of large-scale environmental effects on disease is needed. Data collected from diseases of fish, corals, sea urchins, marine mammals, oysters, lobsters, and abalone can be examined in an integrated manner within broad temporal and spatial contexts for possible links with regional environmental factors. A pilot for this approach has been introduced but has not been utilized. Success will also require a comprehensive monitoring and data collection network, rather than the case-by-case, species-by-species reporting that exists. Disease and mortality data for multiple species must be gathered into an inclusive and comprehensive format. Again, a pilot for such a database exists, but has not been widely implemented. Together, application of these tools will allow development of epizootiological models to aid in understanding large-scale environmental influences on aquatic disease and will ultimately lead to management alternatives.

# Tumors Of Coregonid Fishes In The River Pechora

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The Pechora River is one of the largest watersheds in Northwest Russia, which is anthropogenically affected by oil pollution, heavy metal pollution, nuclear explosions, intensive navigation, and uncontrollable poaching. This investigation was based on fieldwork performed on the Pechora River and its tributary, the Usa River, which began in 1994 and continues to this day. Coregonid fish (*Coregonus lavaretus L., C albula L., C. peled Gmel., C. autumnalis Pall.,* and *C. nasus Pall.*) were used as a test subjects and pathologies were hitologically observed in functionally important organs. Fatty degeneration and vacuolar degeneration of the nuclei of hepatic cells were found in the livers of fish. Hemorrhage and necrosis were diagnosed in the kidneys. Neoplasias (hyperplasia and tumors) were the most dangerous form of pathology that was observed. Hyperplasia was found in epithelial cells of the gills. Skin, which had bactericidal properties, did not change. Tumors developed mainly in the kidneys. Pathological changes in blood vessels and circulation of the blood were found in all of the organs and tissues that were investigated. Infiltration properties and rapid growth testified to the malignant nature of tumors, which resulted in dystrophy and necrosis of the tissues of fish. All tumors were in the early stage of oncogenesis and their etiology was unknown. The pathological changes described were only diagnosed among Coregonid fish that were examined between 1994 and 1997. It was hypothesized that these pathologies were appearently caused by oil pollution.

# Disturbance Of Reproduction In Bottom Invertebrates And Fish Inhabiting Polluted Coastal Waters Near Vladivostok (Amursky Bay, Sea Of Japan)

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The coastal zone of Amursky Bay adjacent to Vladivostok city is one of the most polluted areas of Peter the Great Bay (Sea of Japan). Contaminants (heavy metals, oil hydrocarbons, pesticides etc.) have accumulated in surface bottom sediments. Bottom invertebrates and fish inhabiting bottom layers of the water column come into direct contact with these contaminated sediments and have been negatively impacted by such pollution. We studied the reproductive function in two common species of bottom invertebrates (the sea urchin *Strongylocentrotus intermedius* and the scallop *Mizuhopecten yessoensis*) and the barfin plaice *Pleuronectes pinnifasciatus* inhabiting Amursky Bay. In *S. intermedius*, reproductive function was examined using gonad index, gonad maturity index, frequency of pathological changes and heavy metal contents in the gonads, and quality of offspring (number of normal embryos and larvae and growth of larvae). In the sea urchins in the "near city" zone, severe anomalies were observed in the gonadal and offspring development. There was decreased gonadal maturity, a high frequency of histopathological changes in the gonads, increased concentrations of heavy metals, abnormal development, low growth rate and death of offspring at the early stages development. In the scallop *M. yessoensis* from the "near city" zone, similar disturbances were observed. In the ovaries of barfin plaice from the inner part of Amursky Bay, a mass destruction of the large vitellogenous oocytes was observed during the spawning period. This process was accompanied by degeneration of follicular epithelium; thus, follicular epithelial cells did not participate in oocyte resorption. This phenomenon is evidence of the loss of an important link in the process 'oogenesis – oocyte maturation'. We concluded that ecological conditions in the "near city" zone of Amursky Bay were greatly unfavorable for the reproduction of bottom invertebrates and plaices. Increased concentrations of heavy metals and DDT in bottom sediments and in the gonads apparently are the factors that negatively impacted animal reproduction.

# Tumors In Fish From Peter The Great Bay, Sea Of Japan

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A total of 2,000 fish belonging to 20 species commonly found in Peter the Great Bay (Sea of Japan) were examined for neoplastic lesions between 1995 and 2001. Tumors were detected in 60 individuals representing six species: black plaice *Pleuronectes obscurus*, striped flounder *P. pinnifasciatus*, longsnout flounder *P. punctatissimus*, Pacific needlefish *Strongylura anastomella*, sculpin *Myoxocephalus yaok*, and gizzard shad *Konosirus punctatus*. Only in three species, *P. obscurus, P. pinnifasciatus*, and *S. anastomella*, did morbidity by neoplasm develop a permanent character; tumor occurrence in the remaining species was sporadic. Tumors of skin, liver, and gills were detected. Among these neoplasia, eight different tumors were classified histologically: epidermal papillomas, rhabdomyosarcomas, hepatocellular adenomas, scirrhous carcinomas, chondromas, chondrosarcomas, mucoepidermoid carcinomas, and guanophoromas. The most frequently found tumors in fish from Peter the Great Bay were from the skin (4%) and gills (8%) of flatfish from Amursky Bay. Skin tumors were a complex of two heterogeneous tumors (epidermal papilloma and rhabdomyosarcoma) and ?-cells. Despite more than 30 years of studing ?-cells and ? -cellular lesions in fishes, the etiology of such pathology remains unknown. Gill tumors in flatfish resulted from the growth and neoplastic transformation of cartilage, which sometimes exhibited malignancies in adjoining tissues and metastases in other organs. Microscopic parasites of unknown etiology were found in gills that had neoplastic alterations. The parasites were localized in connective tissue or cartilage of filaments. A dense connective tissue capsule formed around the parasites and became large enough to deform gill filaments and lamellae. Cartilage grew around the encapsulated parasites and in the injured areas, which gave rise to tumors that were classified as chondromas and chondrosarcomas. When only one parasite was present in the gills, growth of cartilage occurred in several places and not just around the parasite, itself. In *P. punctatissimus*, which displayed such pathology, a tumor metastasis was detected as a nodule in the kidney that consisted of mature chondrocytes.

# Endocrine Disruption in Aquatic Environments: Effects on Fish Populations

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It has been recognized that the endocrine system of animals is affected by many environmental contaminants, often at very low concentrations. Wildlife, as well as human, health effects have been demonstrated as a result of exposure to these contaminants. The endocrine system is a multi-organ, multifaceted regulatory system that influences reproduction, embryonic development, growth, behavior and survival. Hormones are produced in an endocrine organ and move from their site of production into the bloodstream, usually bound to plasma proteins. There is a complex system of receptor-mediated feedback mechanisms that control release of these hormones. Once in the bloodstream the bioavailability of hormones is controlled by several factors, including plasma or tissue concentrations, sequestration by binding proteins, clearance and hepatic metabolism. Once hormones arrive at the target cells, they cross the cell membrane and elicit actions by binding to specific protein receptors. The receptor-hormone complex then binds to chromatin in the cell nucleus, stimulating the synthesis of specific RNAs and proteins. Endocrine-disrupting effects can occur along many sites of this complex, regulatory system. The rate of steroid excretion and biotransformation is also important to the regulation of hormone levels. A wide range of substances, both natural and synthetic, has been reported to be potential endocrine disruptors. These substances either interfere with the production, release, action or excretion of hormones or act as hormone mimics. Much of the research in this area has focused on the reproductive system. Biomarkers for reproductive effects include alterations in secondary sex characteristics (morphological changes), alterations in gonadal development and intersex (cellular changes), alterations in circulating sex steroid levels and induction of vitellogenin production by males (biochemical changes). It is believed these changes can and do translate to impaired reproductive capabilities and hence, population effects. Two other hormone systems that have begun to receive attention are the thyroid (thyroxine $T_4$ and triiodothyronine $T_3$) and the adrenal (glucocorticoids or stress hormones). These systems are involved in intermediary metabolism and hence growth, metamorphosis, osmoregulation and immunity. The differences between endocrine systems of mammals and fish, difficulties in assessing endocrine disruption and examples of studies in fish will be presented.

# Myxosporidia And Microsporidia From Salmon On Kamchatka

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Parasitological investigations were conducted within the five reservoir watersheds on the Kamchatka peninsula and in the Karaginsky Gulf of the Bering Sea. Five species of salmon belonging to the genus *Oncorhynchus* (Walbaum) were examined. These included chum salmon (*O. keta*), pink salmon (*O. gorbuscha*), sockeye salmon (*O. nerka*), Chinook salmon (*O. tschawytscha*) and Coho salmon (*O. kisutch*). Altogether 1,110 fry and 1,628 adult salmon were examined parasitologically from 1990 through 2001. Six species of myxo- and microsporideans were observed: *Henneguya zschokkei, Myxosoma dermatobia, Myxobolus arcticus, Chloromyxum wardi, Zschokkella orientalis, Leptotheca krogiusi* (*Myxosporea*) and *Pleistophora* spp. (*Microsporea*). *Henneguya zschokkei* formed ugly cysts in somatic muscle that spoiled the economic value of affected fish. It was found predominantly in Coho salmon, which had prevalences of infection from 1.7 to 20% in different years. *Myxosoma dermatobia* infected scale pockets of coho and chum salmon with prevalences from 6.7 to 13.3%. This parasite caused multiple, small dermal lesions in the skin that affected market values. *Myxobolus arcticus* was a nonpathogenic parasite of the central nervous system that ranged in prevalence from 33.3 to 80% in sockeye, 13.3 to 20% in Chinook and 13.3 to 40% in Coho salmon. It was also found in freshwater smolts at a prevalence of 33.3-36.7%, 6.7-10% and 6.7% in the same three species, respectively. *Chloromyxum wardi, Z. orientalis* and *L. krogiusi* were observed in chum and Coho salmon. The first two species were found in the gall, and the last species was observed in the urinary bladder at prevalences of 6.7 to 20%. They were not considered to be pathogenic. The microsporidians *Pleistophora* spp. Were found in chum and pink salmon in spawning reservoirs with prevalences ranging from 24.6 to 43.7%. It was found in pink salmoin in the Karaginsky Gulf of the Bering Sea. Individual meronts, sporonts, sporoblasts and spores of *Pleistophora* sp. were found in the kidneys of some fishes without visible pathology, and also in fish with superficial ulcers. A lot of plazmodias at different stages of development and mature spores were found in fishes with tumors or among those affected with Vibriosis.

# Whirling Disease in the United States: A Shifting Perspective in the 21st Century

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Whirling disease, caused by the myxosporean *Myxobolus cerebralis*, affects a number of salmonid fishes worldwide. In the United States, *M. cerebralis* has been recognized as a salmonid pathogen since the 1950's. As whirling disease spread within the United States during the latter 20th century, much research focused upon the means of dissemination, fundamental host-parasite interactions, potential methods of control and treatment, and elucidation of the parasite's life history. Because whirling disease was perceived to be problematic predominantly, if not exclusively, among populations of cultured salmonids, management techniques designed to reduce *M. cerebralis* transmission were developed and utilized among hatcheries. With the advent of these largely effective control measures, the associated level of scientific interest in whirling disease diminished. In the mid-1990's, however, population declines among wild rainbow trout in regions of the intermountain West. were associated with the occurrence of whirling disease. This discovery brought about a resurgence of scientific interest and correspondingly widened the scope of research efforts. Over the past decade, relatively novel avenues of whirling disease research include ecological and epidemiological approaches such as those that incorporate risk assessment and dynamic modeling tools to aid in our understanding of the occurrence of whirling disease in the natural environment. Molecular, genetic, and immunological techniques are also being increasingly incorporated to advance evaluations of disease pathogenesis and host immune responses as well as to recognize taxonomic differences in salmonid and oligochaete host susceptibility. At the National Fish Health Research Laboratory, a team of investigators has participated in whirling disease research for approximately six years. Specific investigations have examined species and strain-based differences in salmonid host susceptibility, physiological and immunological responses of rainbow trout to infection with *M. cerebralis*, environmental factors associated with infection of the intermediate host, and development of molecular techniques for evaluating infection status and host susceptibility.

# Myxosporian-Induced Alterations In Kidneys Of Siberian Roach (*Rutilus rutilus lacustris)* From Lake Baikal

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This study reports the occurrence of five myxosporean species from the kidneys of Siberian roach (*Rutilus rutilus lacustris)* in Lake Baikal (Chiviyrkuy Bay). *Myxidium rhodei* was localized in renal corpuscles, but rarely in the interstitium. In single infections, the parasite occurred in proximal segments of nephrons. The prevalence of infection was 71.4-100%; intensity was 3-15,070; and abundance was 40-2,209. Infection was characterized by hypertrophy, associated with atrophy of surrounding renal parenchyma. The glomeruli were gradually compressed and the parietal layer of Bowman's capsules was modified into a thin membrane. When very abundant (>3,000 plasmodia), the parasite was atypically present in the interstitium and a granulomatous inflammation was provoked in that tissue. The parasite destroyed renal corpuscles and reduced the volume of renal tubules. *Myxobolus ellipsoides* was located in the interstitium, but seldom under the parietal layer of Bowman's capsule. Prevalence of infection was 9-86,6%; intensity was 1-400; and abundance was 2–80. Plasmodia in the interstitium were surrounded with extra thin fibrillar capsules. Co-infection with *M. rhodei* under the parietal layer of Bowman's capsule provoked some degeneration of M. *ellipsoides* plasmodia. *Myxobolus muelleri* was found in surface connective tissue membranes of the kidney. Prevalence of infection was 6-87%; intensity was 1- 52; and abundance was 1–12. The ectoplasm of plasmodia was thin and difficult to observe and endoplasmic vacuoles contained considerable amounts of melanin. Capsules around parasites were absent. The parasites damaged the surface connective tissue membrane and other structures of the kidney. *Henneguya cutanea* was located in the interstitium of the kidney. Prevalence of infection was 7-10 % and intensity consisted of single plasmodia. The plasmodia were large (300µm) and not surrounded by a layer of connective tissue. Pathomorphological alterations were similar to M. *muelleri. Spirosuturia* sp. was present in renal tubules; prevalence of infection was 6-13% and intensity was that of single specimens. Early vegetative stages were situated intracellularly in the epithelium of renal tubules. Late stages were found in the lumina of tubules and caused dystrophy and destruction of epithelial cells.

# Parasites Of Fish In The Volga-Caspian Region: Organisms Pathogeneic For Humans And Animals

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Results of research conducted in 1996-2002 showed that eight epidemiologically important helminthes are parasitic on commercial fish in the Volga-Caspian region: *Anisakis schupakovi, Eustrongylides excisus, Apophallus muehlingi, Rossicotrema donicum, Paracoenogonimus ovatus, Pseudamphistomum truncatum, Opisthorchis felineus, and Corynosoma strumosum*. The acanthella *C. strumosum* were noted only in the body cavity of zander, catfish, asp, black-backed shad, big-eye shad, Caspian shad, and sturgeon. The most widespread parasites were *A. schupakovi* and *E. excisus* that invaded gonads and body cavities. However, *A. schupakovi* larvae also parasitized muscles of 3.3% of the asp, 1.6% of the bream, and 14.3% of the big-eye shad that were examined. *Eustrongylides excisus* was found in 2.0% of zander, 11.5% of pike, 78.6% of perch, 3.3% of asp, 5.0% of catfish and 2.1% of the sturgeon that were examined. The trematode metacercaria of *A. muehlingi* invaded the muscle tissue of rudd (11.5%), vobla (1.0%), bream (4.0%); *R. donicum* (28.0%) and zander (8.3%), but more often they parasitized cutaneous coverings and fins of those fish. Metacercaria of *P. ovatus* were found in muscles of tench (100%), rudd (92.2%), bream (55.2%), vobla (77.0%), asp (73.9%), common carp (54.2%), crucian carp (26.5%), zander (94.3%), pike (86.1%) and black-backed shad (0.3%). Metacercaria *O. felineus* (*Opisthorchidae* family) were revealed in muscles of tench (4.6%), bream (1.6%) and vobla (5.8%) while *P. truncatum* (*Opisthorchidae* family) was found in rudd (21.0%), bream (11.2%), tench (34.0%), and vobla (31.1%). Most of the aforementioned parasites, except for the trematodes of the family *Opisthorchidae*, rarely occur in humans. Diseases initiated by these parasites are often difficult to determine and diagnosed inaccurately. This necessitates a requirement for strict processing controls and decontamination of row fish and fish products.

# Similarities And Differences In Fauna Of Parasites Of Chars In Palaearctic (Eurasia) And Newarctic (North America)

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Chars of the genus *Salvelinus* are widely distributed through Eurasia and North America. In the Palaearctic there are *S. malma, S. albus, S. taranetzi, S. leucomaenis, S. levanidovi, S. alpinus, S. czerskii, S. boganidae, S. elgyticus, S. kronocius, S. schmidti, S. krogiusae, S. neiva, S. andriashevi, and Salvethymus svetovidovi.* Dolly Varden, arctic char, *S.fontinalis* and *S. namaycush* are commonly found in North America. The similarity index of Jaccard equaled 30% for comparisons of the parasitic composition of chars between Eurasia and North America. Fifty-two species of parasites were identified, which were also common to other fish that are closely related to chars. Data suggested that parasites have been actively exchanged between chars of Eurasia and North America and within each continent. Within the genus, *Salvelinus*, Dolly Varden and white spotted char (an anadromous Pacific species) are the most similar, but have diverse parasitofaunas. The next cluster included the lake endemic chars along the Asian coast, the Krogius char and the neiva (of Arctic origin). A third cluster was comprised of Arctic char and lake trout, mainly from lakes. The endemic brook trout and Schmidt char from Lake Kronotskoe in Kamchatka were more different from all of the other species. Dolly Varden had 132 species of parasites and its fauna was most diverse in Kamchatka, Chukotsky Peninsula and Primorye. The poorest parasitofauna was found in *S. malma* from Kuril Island (11 species), Alaska (8 species) and Oregon (3 species). Most parasites of Dolly Varden in Canada were found on both sides of the Pacific. An Asian origin of *S. malma* was postulated and most likely in Northern Asia on Kamchatka (probably in Chukotka). We could also distinguish between northern and southern subspecies of Dolly Varden. *Salvelinus leucomaenis* had 98 species of parasites throughout the area. The parasitofauna of white spotted char was most diverse in Kamchatka and Primorye. In Japan it had 33 species of parasites, which was twice as much as reported on Dolly Varden therein. The origin of *S.* leucomaenis was postulated to be somewhere within the Sea of Japan basin. Arctic char had 59 species of parasites and its parasitofauna was most diverse in Canada and the Kola Peninsula (Eastern Murman).

# Health Protection Of Fish In The European North Of Russia

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The Polar Research Institute of Marine Fisheries and Oceanography (PINRO) is the principal scientific institution of the fishing industry in the European North of Russia that studies diseases of marine and cultured fishes. When investigations are conducted microbiologic, histopathologic, hematological and physiologic techniques are used. In 1992-1999, mycosis of Atlantic-Scandian herring *Clupea harengus* L. in the Norwegian and Barents Sea caused by *Ichthyophonus hoferi* was investigated. The etiology, epizootilogy, mechanism and diagnosis of the Ichthyophoniasis problem were examined. This provided information on the impact of mycosis within the Atlantic-Scandian herring population. In 2001 – 2003, experience gained from studying Ichthyophoniasis was applied to diagnose disease in Atlantic salmon *Salmo salar* returning to spawn in rivers of the Kola Peninsula. In 1999, PINRO also monitored epizootics among populations of commercial ground fish in the Barents, Norwegian and Greenland Seas. Such investigations developed biological monitoring programs that evaluated fish health and habitat quality. Thus, the pathological status of eight species of marine fish has been achieved. Traditionally, research on diseases of cultured salmonids remains important but, until now, quarantine measures have not been enacted within the Northern region. Streptococcal and bacterial infections caused by relatively pathogenic microorganisms, some mycotic phenomena, alimentary diseases, and papillomatosis are commonly found in this area. Because of the severe climate in the Northern Region, epizootics mainly occur as chronic infections that persist for six months. The epidemiological sources of infectious agents in this area appear to come from natural reservoirs of pathogens in the water, itself.

# Disease On Coral Reefs – What Do We Know?

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Diseases on coral reefs were first reported in the early 1970's. Incidence of disease in coral reef organisms has greatly increased in the past 30 years, as has disease-related mortality, particularly in reef-building corals. Surveys have confirmed these observations throughout the world's oceans. Disease is now considered an important factor in the ecology of coral reefs. Of the coral diseases currently being monitored worldwide, most are in the Caribbean region, but increasing reports from the Pacific have shown this has become a global problem. Pathogens have been identified for some diseases: aspergillosis of gorgonians (*Aspergillus sydowii*); white plague of stony corals (*Aurantimonas coralicida*); white pox of acroporid corals (*Serratia marcescens*), all in the Caribbean; bacterial bleaching of *Oculina patagonica* (*Vibrio shiloi*) in the Mediterranean and *Pocillopora damicornis* (*Vibrio coralliilyticus*) from the Red Sea; black band of gorgonians and stony corals in the Pacific and Atlantic (cyanobacterial mat); and ciliate diseases of hard corals from the Great Barrier Reef. Fulfilling Koch's Postulates has successfully confirmed the etiology of a number of these conditions, but others remain elusive. Fungi and bacteria have been implicated in the majority of Caribbean diseases of coral reef organisms. What basic processes are driving the increases in incidence and number of diseases on coral reefs? Are biotic/abiotic interactions involved and if so, how? More questions remain than have been answered, even for those diseases with identified pathogens.

# Recent Observations On Aspergillosis Of Gorgonians

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Aspergillosis of Caribbean sea fans (*Gorgonia ventalina* and *G. flabellum*) has been known for almost a decade and the pathogenic agent has been identified as *Aspergillus sydowii*. Aspergillosis in the genus *Gorgonia* has now been reported from all areas of the Caribbean and is commonly considered in coral disease surveys. The impact of this disease has been significant in a number of locations, resulting in population shifts and overgrowth of competing organisms. *A. sydowii* that are gorgonian pathogens produce sydowinol and other secondary metabolites not produced by nonpathogenic strains. Sporogenesis does not occur in seawater, but does at the sea surface-to-air interface. Recently, a number of other gorgonians have been identified with aspergillosis. This has been confirmed in species of the genus *Pseudoterogorgia* and morphological evidence that the disease is much more widespread among gorgonians than previously thought. Gorgonian responses to infection by *A. sydowii* include nonspecific galling and the production of antifungal compounds. The relative severity of infection among various species and clones of gorgonians may be due to differences in their ability to react to infection.

# Integrated Research Into Species Of The *Ctenophora* Family: *Beroe Ovata* And *Mnemiopsis Leidyi*

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Introduction of live organisms in new geographic areas can damage the indigenous fauna. An example an incidental introduction was the invasion and expansion of the comb-jelly *Mnemiopsis leidyi* within the Caspian Sea. In order to curtail the expansion of *M. leidyi*, the state introduced *Beroe ovata* from the Black Sea to the Caspian Sea. Virological, parasitological and microbiological investigations were conducted on members of the *Ctenophora* family *(specifically, Beroe ovata and Mnemiopsis leidyi)* to guard against any detrimental effects. Viral cytopathogenic agents and parasites were not observed in specimens of *Beroe* that were examined. Results of microbiological investigations showed that total bacterial counts of the Black Sea comb-jellies and their habitats was about $10^4$ colony forming units (cfu)/g or mL. Microbial colonization of *Mnemiopsis* in the Caspian Sea was 1-2 orders lower than those in the Black Sea, which was attributed to differences in salinity and chemical composition. During the adaptation period (7 days) in mixed water, indices of quantity of mesophilic aerobes and facultative-anaerobes of *Beroe* decreased and averaged $3.8x10^3$ cfu/g. Decrease in microbe contamination of *B. ovata* was related to the displacement of certain bacterial species by others as a result of changes in salinity (from 18.0 to 12.0 $^0/_{00}$) and temperature (from 24.0 to 20.5$^o$? ). Qualitative composition of the *Ctenophora* microflora and water consisted mainly of vibrios and aeromonads in the Black Sea and of pseudomonads and acinetobacteria in the Caspian. Indigenous species of the Caspian microflora prevailed in microbiocenosis of *Beroe* after 7 and 12 days of acclimatization under laboratory conditions. Caspian ichthyofauna (*Acipenseridae, Gobiiedae, Mugilidae, Rutilus ritilus*) that were challenged with virulent and non-virulent strains of *Aeromonas hydrophila, Vibrio alginolyticus, Pseudomonas putida* and *Acinetobacter calcoaceticus* showed that microorganisms isolated from *Beroe* after 7 days of adaptation were not dangerous.

# Introduced Invasive Species: Shrimp, Shrimp Diseases, And Their Impacts On Native And Cultured Shrimp

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During the early 1980's, production of shrimp from aquaculture facilities increased worldwide. At the same time, reports of mortalities in shrimp aquaculture facilities were increasingly associated with newly described viral diseases. The developing multibillion-dollar shrimp aquaculture industry began to search for bigger and better brood stocks to produce a more palatable and larger product. This encouraged the vast international network of shrimp growers to exchange stocks during a variety of life cycle stages. Such unregulated exchanges brought about a concomitant exchange of viral agents that resulted in significant mortalities within the shrimp aquaculture industry. The resulting impacts of contagion within aquaculture facilities also raised concerns about the potential impact of these newly emerging diseases on native wild populations. The United States Joint Subcommittee on Aquaculture, therefore, convened a work group in 1996 charged with the responsibility to evaluate potential shrimp virus impacts on cultured and wild shrimp. This report will review the four viral species that were targeted in that study. These include Taura Syndrome Virus (TSV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), White Spot Syndrome Virus (WSSV), and Yellow Head Virus (YHV). The concerns identified by the Shrimp Virus Work Group, as well as the current status of natural shrimp populations and aquaculture production in the United States will be discussed.

# Impacts Of Hypoxia On Resistance Of Penaeid Shrimp To Bacterial Pathogens

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Levels of dissolved oxygen in aquatic production systems and natural habitats can vary on a daily, seasonal and annual basis. Hypoxia (low dissolved $O_2$) is often accompanied by an increase in dissolved carbon dioxide, called hypercapnia. The reaction of $CO_2$ with water produces carbonic acid that dissociates to bicarbonate ion and $H^+$. As a consequence, hypercapnia usually leads to a decrease in water pH. Dissolved $O_2$ levels are routinely monitored in well-managed aquaculture ponds, because hypoxia can be lethal. The effects of sub-lethal or moderate hypoxia (20–50% air saturation), although immediately less apparent, can have dramatic consequences on animal health. For example, moderate hypercapnic hypoxia or HH (low $O_2$, high $CO_2$, low pH) significantly increased pathogenicity of *Vibrio* sp. 90-69B3 administered intramuscularly into the penaeid shrimp *Litopenaeus vannamei*. Evidence suggests that the effects of HH on disease resistance may be mediated by suppression of cellular and humoral mechanisms of immune defense. In addition, the effects of HH on disease resistance may be aggravated by physiological responses that maintain blood gas and acid-base homeostasis in HH. We have shown that HH reduces the rate at which live 90-69B3 are cleared from the hemolymph of the blue crab, *Callinectes sapidus*. Preliminary data suggests that there is a similar effect of HH in *L. vannamei*. Hypercaapnic hypoxia decreases respiratory burst and bactericidal activity of fish macrophages as well as oyster and shrimp hemocytes. Crustaceans, unlike fish and mollusks, also rely on the prophenoloxidase (PPO) cascade as an immediate humoral defense against pathogens. On exposure to bacteria or fungi, the PPO cascade is rapidly activated to produce melanin. Melanin and other products of the PPO cascade help sequester and eliminate pathogens. We have now shown that levels of $O_2$ and pH that occur in the hemolymph of crustaceans held in moderate to severe HH reduce the activity of phenoloxidase, the terminal enzyme of the PPO cascade. These results suggest that HH may target both humoral and cellular components of the immune system in crustaceans, crippling both immediate and long term mechanisms of resistance to disease. (NSF-REU DBI-0244007, NSF-IBN-0212921, USDA CSREES 99-35204-8555).

# Possible Roles Of The Enzymes Produced By The Intestinal Obligate Anaerobic Microflora Isolated From Shrimp *Litopenaeus vannamei*

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The intestinal obligate anaerobic bacterial flora of shrimp remains relatively unknown. Our studies have isolated several obligate anaerobic genera from shrimp, which produce enzymes that possibly aid digestion. Previous studies in our lab have shown that diet and environmental factors influence the composition of the anaerobic intestinal flora of shrimp. The purpose of this on-going research is to continue to characterize this group of bacteria in order to determine their role in the digestive process of shrimp. Intestinal samples from shrimp were obtained from facilities in Hawaii. Samples were acquired from shrimp that were maintained on different feeding regimes and cultured in salinities ranging from 2-31 ppt. Shrimp were harvested at weekly intervals; mid- and hindguts were excised for recovery of anaerobic bacteria. Samples were grown in an anaerobe chamber supplied with a mixture of 5% $H_2$, 5% $CO_2$ and 90% $N_2$, plated on pre-reduced anaerobically sterilized media, and incubated at 28$^{o}$C. Cellular and colonial morphology, Gram stain, aerotolerance testing, and biochemical reactions were performed to identify isolates. Anaerobic bacterial isolates also were identified by fatty acid methyl ester (FAME). The majority of the isolates thus identified belonged to two species of the genus *Clostridium; C. bifermentans* GC subgroup A and *C. butyricum* GC Subgroup A. Enzyme analyses on the isolates were performed using the API ZYM panel. Anaerobic isolates produced *a*-galactosidase, *a*- glucosidase, *ß*-glucuronidase, *a*-fucosidase, trypsin, aminopeptidases, phosphohydrolase, esterases, and phosphatases. Several enzymes were similar to those produced by shrimp for the digestion and re-absorption of the exoskeleton. The possible roles of bacterial enzymes in the digestive process of shrimp will be discussed.

# Microbial Diseases And Population Status Of The American Lobster (*Homarus americanus*)

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The American lobster (*Homarus americanus)*, a crustacean of high commercial value, occupies coastal and marine areas of the northwest Atlantic along the northeastern coast of North America from southern Labrador (Canada) to Cape Hatteras (North Carolina). Canadian commercial landings reached an all-time high of 44 million kg in 1991 and declined to 36.7 million kg by the year 2000. United States landings peaked at 39.7 million kg in 1999 and declined to 33.4 million kg by 2001. Prior to 1973, the only recorded diseases in the American lobster were shell disease, that was expressed as an erosion of the exoskeleton putatively caused by several bacteria (first reported in 1937), and gaffkemia, a bacterial infection caused by *Aerococcus viridans*, which competes for the lobster's energy reserves and results in death (first reported in 1946). Shell disease affects the marketability of lobsters and since 1998 has become a serious problem in eastern Long Island Sound –as prevalent as 70% in April of 2001. *Aerococcus viridans* is always present in 5-7% of feral lobsters and sometimes causes deaths in up to 58% of pound-held lobsters. "Bumper-car disease", caused by the ciliated protozoan, *Anophryoides haemophila*, was recognized in 1973. It results in tissue destruction and death in up to 25% of lobsters during winter impoundment and is found in 0.4 to 20% of feral lobsters. Three fungal infections and one bacterial infection caused deaths in cultured larvae and post-larval juveniles (*Halipthoros milfordensis* and *Fusarium* sp. in 1975, *Lagenidium* sp. in 1976, and *Vibrio sp.* in *1979*). They were not proven to be serious infections in feral lobsters. Recently two additional diseases of adult American lobsters have emerged. From 1997 through 1999, lobsters valued at $2.5 million died from a bacterial disease in Maine coastal waters. It is unclear whether mortalities resulted from two different bacteria or if there was a conflict in classification; both *Vibrio fluvialis* and *Hyphomicrobium indicum* were implicated. In 1998 and 1999,1.0 to 1.4 million kg of lobsters died in western Long Island Sound from an amoeba infection multiple organs, but especially in nervous tissues. Confirmation of pathogenicity for this agent is complicated because it cannot be cultured; however, several studies are in progress.

# Pathogen And Disease Issues Associated With Imperiled Populations of Freshwater Mussels

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Freshwater mussels are important contributors to the health of aquatic ecosystems and are excellent indicators of system health. Because they are filter feeders, mussels are integral components of nutrient cycling and certain species can filter as much as 1.25 gallons of water/hour per individual. Their combined numbers can have a substantial impact on a given body of water, for example, filtering nearly 100% of the total volume daily. The life cycle of freshwater mussels involves a vertebrate host, i.e. fish, for transformation of glochidial larvae into juveniles; therefore, adverse impacts on mussels or their hosts affect mussel survival. Unfortunately, such impacts have made mussels one of the most imperiled fauna in the aquatic environment. There are nearly 1,000 species of freshwater mussels in the family Unionidae. Although the greatest diversity is in North America (300 species), more than 70% of the mussels from this region are categorized as endangered, threatened or of special concern. Federal and state agencies have expanded mussel conservation efforts to include the captive propagation of imperiled populations within disease-free refugia. Natural mussel mortalities in rivers have been reported with increased frequency and it is imperative to implement strategies that minimize the risk of pathogen introduction and transmission within such refugia. This research was initiated to prevent such disease progression. Methods were developed to isolate bacteria from mussels and it was found that their bacterial flora was quite stable in terms of total numbers, but species composition varied in response to environmental changes. The ability of mussels to harbor fish pathogens was also demonstrated by the isolation of *Flavobacterium columnare* (cause of Columnaris disease in fish) within two species of mussels that were obtained from the Ohio and Holston Rivers. Laboratory studies further indicated that the mussels *Amblema plicata* and *Fusconaia ebena* readily served as vectors for the fish pathogen *Aeromonas salmonicida*. If these mussels were depurated in specific pathogen-free water for less than 15 d, they did not act as vectors. Current studies are in progress to determine the etiology of natural mussel mortalities and develop non-lethal monitoring techniques.

# Monitoring *Flexibacter-Flavaobacterium* Infection In Sturgeon

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During the last decade, sturgeons became major objects of interest in Russian fisheries. Restoration, food marketing, and sport-fishing industries induced growth of many hatcheries increase and increased total production in different regions of Russia. High-density industrial-type facilities that often use power plant cooling waters were predominantly used for such culture. This study was conducted at one of the largest fish hatcheries in Russia, the Konakovo Sturgeon Center, which concentrated on freshwater sturgeon species such as Siberian sturgeon (*Acipenser baeri*), starlet (*A. ruthenus*) and their hybrids. Analysis of fish production over the decade showed the high level of mortality (up to 70 % loss of fry) in young sturgeons during the spring and summer months. We performed microbiological monitoring that showed the presence of bacteria in the water, mucus, gills, and kidney samples of affected fish. The number and prevalence of *Flexibacter-Flavobacterium* bacteria in the samples were correlated with water temperatures. In the absence of treatment, infections became septic. *Flavobacter johnsonae* was the predominant microorganism and it was isolated from 80 % of the kidney simples as a pure cultures. Virulence of the isolates was confirmed using challenge experiments on bacteria-free fish of the same species. About 50-60 % of the fish became infected via immersion challenges. In all cases symptoms of disease were similar to those that were observed among clinically ill fish at the hatchery and *F. johnsonae* was re-isolated as a "pure" culture. Oxytetracycline and Chloramine T treatments were recommended and such treatments effectively decreased (up to 100 times) the number of bacteria present in the water and on the gills of sturgeon. In addition, *Flexibacter-Flavobacterium* bacteria were totally eliminated from internal organs of the affected fish.

# Bacterial Diseases Of Herbivorous Fishes In Belarus

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Enhancement of aquaculture production in Belarus under the current economic situation is possible only by increasing the share of herbivorous fish culture. Investigations conducted in fish farms showed that during bacterial epizootics in carp, *Cyprinus carpio* L., other herbivorous fishes grown in polyculture with carp were also infected. When pseudomonad infections occurred under wintering ponds conditions in February and March, clinical disease symptoms in grass carp *Ctenopharyngodon idella* were identical to those in carp. These were somewhat different in bighead carp *Aristichthys nobilis*, and manifested itself as whitish cover on gills and petechial hemorrhages in fins. Pathogenic *Pseudomonas fluorescens* was isolated from all of the diseased fish (carp, grass carp and bighead carp). To treat bacterial infections during the winter period, we modified a method that directed the formation of bacterial plankton in ponds. A biological preparation containing a live bacterial culture of *Azotobacteraceae* was used as a bacterial fertilizer before fish were released into the ponds. After introduction in ponds, these bacteria grow well due to their high ecological plasticity and resistance to changes in environmental conditions. Introduced azotobacteria occupy a dominant position in pond bacterial plankton (about 30%) and suppress the growth of opportunistic bacteria like pseudomonads and aeromonads. The treatment reduces contagion, thereby decreasing the severity of bacterial disease.

# Characterization Of *Aeromonas Salmonicida* subsp. *salmonicida* Isolated From Salmon Spawned In Hatcheries On Kamchatka

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The bacterial pathogen of Pacific salmon *A. salmonicida* subsp. *salmonicida* was originally isolated on Kamchatka in 1989, from chum salmon (*Oncorhynchus keta*) of the Bering Sea. In 1990, the bacterium was also isolated from Coho salmon (*Oncorhynchus kisutch*) in the river Bistraya. Clinical signs of furunculosis were evident in both cases. Since 2000, bacterial control was developed for captive salmon spawners at hatcheries, because the pathogen can be transmitted via surface contamination of fertilized eggs. Five incidents of carriers of *A. salmonicida* subsp. *salmonicida* among spawning salmon populations were detected at two hatcheries in 2001-2002. One strain was isolated from chum salmon from river Paratunka and four strains were obtained from chum salmon and sockeye salmon (*Oncorhynchus nerka*) from the river Bistraya. Strains of *A. salmonicida* subsp. *salmonicida* were identified using the ??I 20? identification system. All of the strains were identical and produced acid from sucrose. This latter, somewhat "atypical" characteristic, was similar to reports of atypical strains isolated in Japan. Our investigation collaborates the assumption of T. Wiklund, who proposed a common origin for Kamchatka and Japanese isolates. In order to test the pathogenicity of the strain, an isolate from a chum salmon obtained in 2002 was inoculated into chum salmon fingerlings by two routes; oral and perbranchial deliveries. The pathogen was re-isolated from all dead fish and the highest mortality was observed among fingerlings that were infected through the gills.

# Molecular Mechanisms Of Host-Pathogen Interactions Between *Aeromonas salmonicida* And Atlantic Salmon, *Salmo salar*

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We are studying the molecular nature of host-pathogen interactions between Atlantic salmon (*Salmo salar*) and *Aeromonas salmonicida*. We are sequencing the entire genome of the bacterium, the analysis and assembly being carried out through the Canadian Bioinformatics Resource. Information gained from this large sequencing project will enable us to target potential virulence factors and other genes that may have application as vaccines or immunomodulatory candidates. Knockout mutants of *A. salmonicida* will help identify key virulence factors. We are also examining the functional genomics and proteomics of the *A. salmonicida* - *S. salar* relationship by examining expressed genes and their protein products of both the pathogen and the host. Salmon are challenged with *A. salmonicida* and samples of the bacterium and host are taken at selected times during the infection. From these samples, mRNA and proteins are isolated to determine which genes and proteins are differentially expressed and produced during the infection process. At the same time, immunological responses are measured. By linking genome sequencing, functional genomics, proteomics, carbohydrate analysis, and the immunological assays we are taking an integrated and innovative approach to pathogenesis research.

# Immune Parameters of Common Species of Aquatic Animals

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Comparative investigations were made of cellular and humoral immunity in aquatic animals from pollution-free regions of Rugozyorskaya inlet, Kandalakshskii Bay, White Sea within navaga *Eleginus navaga* Pallas, cod *Gadus morhua maris-albi* Derjugin, common mussel *Mytilus edulis* L., and starfish *Asterias rubens* L. Fish were assessed for 1) morpho-physiological parameters, 2) intensity and extensiveness of the acanthocephalan *Echinorhynchus gadi* Müller invasion, 3) bacterial symbionts on gills, 4) blood corpuscles concentration, erythrocyte sedimentation rate, hemoglobin concentration, blood colored index, serum total protein concentration and serum lysozyme concentration. Mussels were assayed for 1) morpho-physiological parameters, 2) bacterial symbionts, 3) hemocyte concentration in hemolymph and liver, 4) total protein concentration and lysozyme concentration. Starfishes were evaluated for 1) morpho-physiological parameters, 2) amoebocyte concentration in perivysceral fluid, 3) total protein concentration and lysozyme concentration in perivysceral fluid. The dependence of hematological, biochemical and immunological parameters on the intensity of acanthocephalan invasion was demonstrated. In infected fishes, the concentration of blood corpuscles was reduced two-fold. Blood hemoglobin concentration and blood colored index did not change. The erythrocyte sedimentation rate decreased to 2 mm/h in the most infected fishes. New protein fractions were found in electrophoregrams of serum from infected fishes, including a low-molecular weight fraction (approx. 14.5 kDa). Total serum protein concentration increased by 50% in navagas and by almost 100% in cod; serum lysozyme concentration increased 5-fold in navagas and 2-fold in cods. Phagocytic activity of hemocytes in mussel hemolymph and liver began to increase with 30 minutes of stimulation by the addition of Indian ink into the medium. Hemocytes in mussel hemolymph increased 1.5–3-fold after injection of BCA into mussels and 3-fold after the injection of HRBC. In liver, hemocyte concentration increased 3-fold after the injection of HRBC. Injection of various antigens into starfishes under normal conditions increased lysozyme concentration, whereas concentration of amoebocytes and total proteins did not change. Lower aeration reduced amoebocyte, total protein, and lysozyme concentrations in experimental and control groups.

# Advances in Fish Immunology: Identification and Characterization of Natural Killer-Like Cells in Channel Catfish (*Ictalurus punctatus*)

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Cytotoxic effector cells have been cloned from alloantigen-stimulated peripheral blood leukocytes of naïve channel catfish. These granular cells not only kill the stimulating allogeneic cells, but also unrelated allogeneic targets through a perforin/granzyme-mediated apoptosis pathway. They do not express T cell receptors $\alpha,\beta,\gamma$ or immunoglobulin $\mu$ transcripts, and are also negative for markers that define neutrophils, monocytes/macrophages, and nonspecific cytotoxic cells (NCC). For these reasons these clonal cytotoxic cells are operationally defined as NK-like cells. Further characterization of these NK-like cells revealed that some possess a putative Fc$\mu$ receptor, which can participate in antibody dependent cell-mediated cytotoxicity. These NK-like cells differentially express novel immune-type receptors (NITR), which have either one or two extracellular immunoglobulin-like domains. Some of the NITR have an immunotyrosine inhibitory motif (ITIM) in their cytoplasmic tail, whereas others contain a charged residue in their transmembrane domain, which suggests they may associate with an immunotyrosine activation motif (ITAM) containing accessory molecule. It is notable that each of the catfish NK-like cell lines expresses an ITAM-containing Fc$\varepsilon$R $\gamma$ chain homolog, which could potentially be used by activating NITRs and/or the putative Fc$\mu$R. The availability of these catfish NK-like cells lines will facilitate future functional studies in ways not currently possible with any other fish species.

# Vaccine Design And The Analysis Of Long-Lived Plasma Cell Production And Regulation

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The importance of long-lived plasma cells (LLPC) in the generation long-term immunity and memory in mammals has received considerable attention. Recent studies suggested that long-term immunity to infectious agents, such as viruses, may primarily be due to the expression of antibody from this unique class of plasma cells that reside within the bone marrow. These cells stand in distinct contrast to those found within the periphery, whose antibody response appears to be primarily generated by short-lived plasmablasts. Thus, the origin of these LLPC, their regulation within the bone marrow, and the possible qualitative differences in their antibody product may be of critical importance for effective prophylactic immunity (in both primary and memory responses). Of particular importance to fish vaccinologists is recent evidence that these same cell types and functions exist in rainbow trout (and likely in other teleosts as well). Our work has demonstrated that greater than 90% of the Ig-secreting cells in the periphery (blood and spleen) are plasmablasts, while the anterior kidney harbors a population of non-replicating LLPC. These LLPC persistently secrete specific antibody for greater than six months (and likely longer) whereas antibody secretion ceases in the periphery within two months after immunization. The implications of such B cell dichotomy in fish are profound for vaccine design. Given that long-term prophylaxis to certain pathogens may ultimately depend on the maintenance of a subset of B cells (LLPC) that strictly reside within the anterior kidney, the role that the supportive matrix may play within this tissue is likely critical to the continued antibody production by LLPC. Further, the anterior kidney is a complex organ that not only is responsible for hematopoiesis and lymphopoiesis, but it also possesses essential endocrine function. This dual role of endocrine function and as a supportive matrix for LLPC may determine the capacity of this organ to mount an effective response during vaccination.

# Stress In Fishes: Diagnosis And Corrective Treatment with "Piscin"

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Stress is especially important in intensive aquaculture in which fish are subject to numerous changes in physical, biological and environmental parameters. The goal of this study was to develop methods that diagnose and correct the negative influences of stress in different taxonomic groups of fishes, including *Cyprinus carpio, Hypophthalmichthys molitrix, Oncorhynchus mykiss,* hybrid Mozambique tilapia and Nile tilapia *(Oreochromis mossambicus x O. niloticus), Pterophillum scalare* and *Acipenser baeri.* The stress conditions that were studied included handling, hypoxia, and pH alterations. To reduce the negative influence of stress, a patented preparation made according to the author's technology was applied. This preparation "Piscin" is a water-soluble substance extracted from the skin of carp. Piscin was used in ultra low concentrations ($10^{-15}$) by means of its dilution in the water with experimental fish. Normalization of changes in physical and biochemical parameters caused by stressful conditions was monitored in each of the aforementioned fishes treated with "Piscin." The development of ecological methods for prophylactic treatment of stress presents new perspectives for future investigations in aquaculture.

# Aspects Of Application Of Vitamin-Coenzyme Fungal Complex At Early Stages Of Fish Ontogenesis

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A vitamin-coenzyme complex was developed based on selected strains of mytosporic fungi protein-vitamin producers. The complex contains vitamins $B_1$, $B_3$, $B_5$, E, ubiquinone $Q_{10}$, essential amino acids, and microelements, which was tested to see it use could increase the survivability of fish at early stages of ontogenesis. The complex was applied immediately after egg fertilization and was administered in combination with standard methods to increase egg viability using therapeutics of dye origin. The survivability of hatched larvae increased and fungal contamination was almost non-existent. Percent hatch was about 100%. The benefits of the complex were also studied in one-year carp, in which body mass of treated fish increased by 17.3%. Immunologically, the humoral titers of natural antibodies in serum of treated fish decreased from 4.4 to 2.9, as did the concentration of lysocine in serum. Immunological and parasitological studies showed that application of funga1 complex did not have any negative effects on the fish. A simultaneous decrease of complement level suggested that the complex intensified elements of the non-specific immune system.

# Metabolic Processes In The Livers Of Carp Cultured In Warm Water

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The effects of increased water temperature on the contents of basic vitamins and coenzymes and on the status of metabolic processes in carp are discussed. Experiments were conducted on two-year-old carp, which were divided into two groups. One group was kept in ponds at 37°C and the other group was held in similar ponds under natural conditions where the water temperature was 25°C. Growth inhibition, skeletal deformation, and sporadic decrease of immunity were noted in the first group of fish. Basic vitamin and coenzyme contents and activities of key enzymes on the metabolism in the liver of fish of both groups were studied to analyse causes of observed phenomena. We did not find any considerable changes in the contents of vitamins $B_1$, $B_2$, PP and their coenzymes. Simultaneous decrease of choline and reduced glutathione content was observed in the liver of fish kept at 37°C. Explicit changes in the activity of certain enzymes were revealed. Considerable increases were noted in the activity of pyruvatdehydrogenase and acetyl-CoA-carboxilase complexes, whereas decreases in the activities of oxidative enzymes of electron transport chain, particularly of α-ketoglutaratdehydrogenase, were observed. An increase of general lipid content free fatty acids was noted in the livers of carp kept at 37°C. These changes provide unambiguous evidence for an intensification of lipogenesis and for the decrease in velocity of oxidative processes in carp that were maintained at higher temperatures.

# Influence Of Fodder Additions That Lower Lipogenesis On The Development And Survival Of Fish Maintained In Warm Water

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Comparative studies of metabolic processes in tissues of carp maintained under artificial and natural conditions show that the intensity of oxidizing processes is reduced and lipogenesis is activated by warm water cultivation. Changes in metabolism cause diminished growth, reduced immunity, and accumulation of fat in tissues. In this study, certain biologically active compounds were added to the diet and attempted to correct the aforementioned metabolic. Based on experimental data, a biologically active fodder additive was developed that contained a vitamin complex and other biologically active compounds (aminoacid, microelements) that promote activation of vitamin exchange and synergistically act upon cellular metabolism. This fodder premix (and also some of its modified variants) had been tested, particularly within in Uzbekistan, on various age groups of fishes under differing climatic regions. Research has confirmed that it was efficient to use the premix to enhance the development of fish held under various conditions. When this premix was tested on carp that were developing under artificial conditions, the lipid content in tissues of all age groups decreased up to 40 % and processes of protein synthesis were activated. Periodic introduction of the premix in forage initiated when fish are young can significantly prolong the active period of their development. Thus the fish's survival and the efficiency of aquaculture were increased significantly. Administration of the premix enhanced the quality of inexpensive forages and, under certain conditions; it can replace standard vitamin premixes.

# Thiamine Deficiency Complex In Fish And Aquatic Animals

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Thiamine deficiency in fish and aquatic animals has been identified in several geographical locations. Early life stage mortality in salmonids in the Laurentian Great Lakes and New York Finger Lakes is such a significant problem. The mortality of swim-up fry dying from early mortality syndrome (EMS), a non-infectious disease, ranges from 0 to 100% depending upon the lake, the salmonid species, stock within a species, and year. The syndrome is characterized by lack of vitamin B1 (thiamine). Adult salmonids feeding on prey species (e.g. – alewife and rainbow smelt) containing thiaminase produce eggs low in thiamine and fry die between hatch and swim-up. Thiamine treatment of eggs or fry is effective, but it is only practical in species that migrate to weirs where eggs are collected and reared in the hatchery. Since lake trout eggs are not collected, thiamine treatment is not an option. Characteristic thiamine deficiency brain lesions have been observed in lake trout that survived overt mortality and in Atlantic salmon from the Baltic Sea, where the malady is called M74. Recent data suggested that thiamine deficiency affects neural function (visual acuity). Both predator avoidance and prey capture were impaired in lake trout fry with low thiamine. Data also showed that adult Coho salmon, steelhead trout, and lake trout are dying as a result of thiamine deficiency, which has a negative impact on sustaining adequate numbers of spawning animals. Thiaminase in alewives consumed by salmonids is highly variable and the cause of that variability is under investigation. Naturally occurring thiamine deficiency appears to occur in Florida alligators foraging on gizzard shad. The data suggest that thiamine deficiency in wild aquatic top predators has a significant impact on sustainable reproduction.