Mycobacterium sp. Infection in Cultured Cobia (*Rachycentron canadum)*

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Juvenile cobia, *Rachycentron canadum*, were presented to the Aquatic Medicine Laboratory of the Virginia-Maryland Regional College of Veterinary Medicine with the complaint of chronic losses over a two-week period. Fish, approximately 15 to 20 cm in length, were representative of the affected population and presented with emaciation, lethargy, ulcerative dermal lesions, exophthalmia, hyperpigmentation and hypopigmentation. Necropsies were performed on the fish and bacterial cultures were taken from the posterior kidney and skin for culture on TSA and BHI media. Representative tissues were obtained for histopathology, preserved in 10% neutral buffered formalin and processed for standard H&E staining. Bacterial cultures from the skin grew *Aeromonas hydrophila* and *Citrobacter* sp., but bacterial cultures from the posterior kidney grew only *Aeromonas hydrophila*. Histopathology of the gill tissue demonstrated mild to moderate lamellar hyperplasia, while skin lesions showed epithelial ulceration and deeper dermal necrosis with a few well-formed granulomas. Numerous granulomas were also observed throughout the spleen, liver, and anterior and posterior kidney. A modified acid-fast stain (Fites) revealed numerous acid-fast bacteria within the granulomas and granulomatous inflammation of the tissues, suggesting a *Mycobacterium* sp. infection. In addition, bacterial cultures grown on Middlebrook agar developed small, white colonies in seven days that stained acid-fast positive. Though the fish were externally and systemically infected with several other species of bacteria, it was thought that these were secondary to the underlying *Mycobacterium* sp. infection. The producers were immediately advised to depopulate and disinfect the systems holding the fish.

Mycobacterium Infections In New York Striped Bass And Bluefish

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Due to the epizootic of mycobacteriosis in Chesapeake Bay striped bass over the past several years and the mixing of the Chesapeake Bay and Hudson River striped bass stocks, a survey for mycobacteriosis in the New York bight area (n=26) and Hudson River (n=53) was conducted. Spleen, liver, and kidney samples were collected for histopathology and the remaining spleen tissue was homogenized for bacteriological culture. The prevalence of acid-fast positive bacteria in the spleens (n=2, 2.5%) was low, with one positive from each site. Granulomas that were suggestive of mycobacterial infections but were acid-fast negative were found in the New York bight striped bass spleens (n=3, 11.5%) and liver (n=11, 42.3%) and in Hudson River striped bass spleens (n=6, 11.3%), liver (n=22, 41.5%) and kidney (n=1, 1.9%). Thus the prevalence of definitive and suggestive lesions was very similar in fish from both locations. *Mycobacterium lentiflavum*, a pathogen known previously only from people, was the only isolate to be successfully cultured, although mold contamination hampered culture efforts. The biology of striped bass and bluefish is similar in several respects including distribution, prey, and parasite assemblages. For this reason spleen, liver, and kidney samples were also collected for histopathology from bluefish (n=51) in the mid-Atlantic bight area. The prevalence of acid-fast positive bacteria in splenic granulomas was low (n=2 or 3.8%) but higher than for striped bass; no other granulomas were observed that were acid-fast negative. Bluefish and striped bass in NY waters are both infected by mycobacteria, although the species identities of isolates in each fish remain to be determined.

Mycobacterial Infections In Striped Bass (*Morone saxatilis*) From Delaware Bay

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A study was initiated in 2003 to determine if mycobacteriosis is occurring in Delaware Bay striped bass (*Morone saxatilis*). Eighty striped bass were obtained from gill-nets off Woodland (n = 70) and Bowers (n = 10) Beach in December of 2003. Data obtained from these samples were compared to those obtained in November 2003 from striped bass captured in pound nets located in the Chesapeake Bay at the mouths of the Potomac (n = 50) and Nanticoke Rivers (n = 50). Tissues were examined by selective culture for the presence of mycobacteria and associated pathology by histology. Pooled data from Delaware Bay indicated an infection rate of approximately 17% and infection intensity (Log mean ± S.E.) of 3.398 ± 0.576 Log CFU g^{-1} of spleen in the infected striped bass. Infection rates in Delaware Bay were significantly lower ($p \leq 0.025$) than those observed at the Chesapeake Bay sites where the rates were 80.4% and 60.4% for the Potomac and Nanticoke Rivers respectively. Infection intensity in impacted striped bass was significantly lower than observed in the Potomac but not the Nanticoke River where the infection intensities (Log mean ± S.E.) were 5.054 ± 0.318 and 4.772 ± 0.384 Log CFU g^{-1} of spleen respectively. The fish were in good condition overall with only one sampled fish exhibiting a relatively heavy infection (over 10,000 CFU g^{-1} of spleen). The isolate from this heavily infected fish, identified as *Mycobacterium chelonae*, was the only isolate to clearly fit current species descriptions. Other isolates appear to be similar to recently described species obtained from Chesapeake Bay striped bass.

Coldwater Disease Caused By *Flavobacterium psychrophilum* In Ontario; Characterization Of Strains And Antimicrobial Resistance

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Flavobacterium psychrophilum is the causative agent of bacterial coldwater disease in North America and rainbow trout fry syndrome in Europe. BCWD has a considerable economic impact in Ontario aquaculture operations; however our limited understanding of bacterial population structure and epidemiology is an impediment to improved management strategies. Eighty-five isolates of *F. psychrophilum* were collected during an 8 year period from farmed salmonids with tail rot, necrotic myositis and cephalic/scleral osteochondrosis. These isolates were characterized phenotypically, biochemically and serologically. Two distinct biovars were identified by API-ZYM, however the isolates were relatively serologically homogeneous, with 80% recognized by antiserum raised against *F. psychrophilum* ATCC 49510. To assist identification of *F. psychrophilum* from clinical samples, a multiplex PCR based on intergenic 16S rRNA and DNA gyrase was adapted. Antimicrobial susceptibility profiles of Ontario isolates were determined using a commercial multi-well plate system. We plan to monitor temporal trends in sensitivity as antimicrobial use evolves in the Ontario aquaculture industry. Management strategies used to reduce the impact of bacterial coldwater disease will also be discussed.

Detection Of Flavobacteria On Gills And Skin Of Salmonids By PCR And DGGE

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Skin and gill diseases involving species of the genus *Flavobacterium* present major and constant problems in freshwater aquaculture. Routine diagnosis of Bacterial Gill Disease (BGD) is almost entirely by microscopic examination of gill tissues for long rods, as culture of *F. branchiophilum* is difficult. PCR amplification with primers for 16S rRNA sequences was used to identify species of flavobacteria present on gills and skin. We tested DNA isolated from 82 gill samples from fish with bacterial gill disease, representing 10 separate cases. Of these, 27 showed an amplification product with the PCR primers for *F. branchiophilum,* BRA1 and 1500R. There was no amplification with PSY primers for *F. psychrophilum.* DNA from bacterial colonies cultured from gill samples on cytophaga agar did not amplify with either primer set. DNA from 32 of 70 gill samples was amplified with eubacterial rRNA GM5 primers (with GC clamp). Denaturing Gradient Gel Electrophoresis (DGGE) analysis showed 26 samples had bands coincident with the product from DNA of *F. branchiophilum*. Bands resembling *F. psychrophilum* were in two samples. Amplifying DNA from cytophaga agar cultures from gills with GM5 primers gave a product in 69 of 70 samples. On DGGE gels, the bands indicated mixed cultures of bacteria, including 9 samples with bands coincident with *F. branchiophilum*, and 32 with patterns similar to *F. psychrophilum*. With PSY-primers and DGGE, *F. psychrophilum* was detected in skin scrapings of diseased fish.

Application Of DGGE Techniques As A Potential Approach To Non-Lethal Testing For Fish Pathogens

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Non-lethal sampling procedures are desirable for detecting pathogen carriers among broodstock fish. Using the method of Cipriano *et al.*(1992), *Aeromonas salmonicida* could be cultured from the skin mucus of Ontario freshwater Chinook and coho salmon and rainbow trout. To broaden the range of bacteria that could be detected in mucus samples, we used denaturing gradient gel electrophoresis (DGGE) to analyze 16S ribosomal RNA obtained from PCR-amplification of bacterial cultures and from mucus directly. First, 25 cultures were isolated from the mucus of salmon and trout from the Credit River, Dorion Hatchery, Chatsworth Hatchery and Hagen Aqualab to assess diversity of bacterial communities associated with fish mucus. Physiological tests carried out with Biolog GN2 and GP2 plates identified 18 species of bacteria, predominantly gram negative. These isolates were used to establish the biodiversity of mucus isolates. No bacterial species were common to all sources. Eubacterial GM5 primers with a GC clamp were used to amplify a 200 bp segment of 16S rDNA directly from fish mucus and also from cultures of *Aeromonas salmonicida*, *Flavobacterium branchiophilum*, and *Yersinia ruckeri*. Mucus of fish from two sources did have bands corresponding to those of *A. salmonicida* and *Y. ruckeri*, but sequences of the bands are required for confirmation, as related non-pathogens may share conserved sequences.

Signature-Tagged Mutants For Studies Of Pathogenic Mechanisms Of *Yersinia ruckeri* In Rainbow Trout

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The virulence factors and the pathogenic mechanisms that enable *Yersinia ruckeri* to cause Enteric Red Mouth disease in salmonid fish are still unclear. To identify possible virulence-related genes, a library of 1056 transposon mutants of a serotype 1 strain of *Y. ruckeri*, RS1154, was prepared and screened for mutants unable to survive in rainbow trout for 7 days after a bath-immersion infection. For efficient screening, the mutants were prepared using 11 signature tags present on pUT miniTn5 km2 transposon vectors. Groups of 11 mutants with different tags were screened by infecting two adult rainbow trout by bath immersion for 10 min in 10^8-10^9CFU/ML of each mutant in challenge dose. At 7 days post-infection, fish kidney tissue was cultured, and recovered mutants were screened by PCR for missing tags. In initial screening, 132 mutants were missing in both fish. New pools of these mutants were re-screened in fish, and 24 mutants (2.2% of the library) were selected for further characterization. Southern blots with the kanamycin marker indicated each had only a single mutation. Cloning and sequencing the mutations allow homology searches for genes that may help explain the persistence of *Y. ruckeri* in carrier fish, and identify the key pathogenic processes that are triggered in the stressed host.

Estimation Of The Genome Size Of *Renibacterium salmoninarum* ATCC 33209 By Pulsed-Field Gel Electrophoresis.

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Renibacterium salmoninarum is a salmon and trout pathogen that belongs to the high G + C subgroup of gram-positive bacteria. Little is known about the molecular genetics of this microorganism. Here, we used pulsed-field gel electrophoresis to estimate the genome size of the ATCC 33209 type strain. Digestion of genomic DNA with *Pme*I generated four bands containing five restriction fragments with a cumulative size of 3.4 ± .1 Mb. Seven previously identified *Renibacterium* genes were mapped to the *Pme*I fragments by Southern blotting. Both *hly* (metalloprotease) and one copy of *msa* (major soluble antigen or p57) were mapped to *Pme*I fragment I, while the other copy of *msa*, *lysB* and three IS994 insertion-element flanking-genes were mapped to *Pme*I fragments II/III. Unlike the duplicated *msa* gene, only single copies of *hly*, *lysB* and the three IS994 insertion-element flanking-genes were identified by Southern blotting. In summary, these data suggest that the genome size of *R. salmoninarum* ATCC 33209 is close to the average for prokaryotes (3.2 Mb), and that most *R. salmoninarum* genes are not duplicated.

An Introduction To Canadian Aquaculture

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This presentation will highlight the current status of commercial aquaculture in Canada, providing information on production data and commercial focus for future development. A comparison to the current status of the United States aquaculture industry will be provided. Information regarding key emerging and re-emerging infectious disease problems facing commercial fin-fish and shellfish (molluscan) aquaculture in Canada will be detailed.

The Current Status Of *Loma salmonae*, Disease And Control In Farm Raised Chinook Salmon, *Oncorhynchus tshawytscha*

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Microsporidial Gill Disease (MGD), caused by the pathogen *Loma salmonae* is an important disease affecting the culture of Pacific salmon in British Columbia. The industry, which currently produces 16,000 metric tonnes annually and has a value of $40 million dollars, undergoes seasonal losses of up to 13% of the stock attributed to MGD. In addition to research into vaccination and treatment, an important consideration is understanding the nature of the host inflammatory response to the disease. This is particularly relevant for MGD because morbidity and mortality begin late in the course of infection, prompted by the severe host reaction elicited by the rupture of the spore-filled xenomas. A study of the ultrastructural pathology of MGD has revealed several novel findings including (1) the discovery of dendritic-like cells within acute lesions, (2) arterial thrombosis, and (3) chronic inflammatory lesions with neovascularization. This suggests that rupturing xenomas cause an inflammatory cascade that start as tissue destructive acute lesions which persist and develop into chronic lesions characterized by macrophage infiltration and neovascularization. Based on this, future work into treatment will also focus on determining the therapeutic benefit of anti-inflammatory agents during xenoma rupture and resolution.

Emerging Infectious Diseases Of Atlantic Cod (*Gadus morhua*), Haddock (*Melanogrammus aeglefinus*) And Halibut (*Hippoglossus hippoglossus*)

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Alternative marine species culture of cod, haddock and halibut has resulted in the detection and investigation into disease issues not commonly encountered in salmonid production. Nodavirus and Loma have been detected in stocks cultured in eastern Canada. Fish health practitioners have adapted terrestrial mammal and salmonid biosecurity practices to mitigate or negate these threats to the continued commercialization of these species. Extensive applied research endeavors are underway to remove or control these pathogenic agents. This presentation will highlight current and emerging infectious viral, bacterial and parasitic threats to these coldwater marine finfish species in Atlantic Canada.

Multiple Drug Resistance In *Aeromonas salmonicida* Infected Salmon Fry And An Update On Infectious Salmon Anemia In The Bay Of Fundy Region, Atlantic Canada

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In May 2005, the New Brunswick Department of Agriculture Fisheries and Aquaculture (DAFA) was informed of an *Aeromonas salmonicida* (furunculosis) outbreak at a hatchery in the northeast section of the province. Investigation indicated that *A. salmonicida* had massive resistance to most known antibiotics. including all potentiated sulfonamides, erythromycin, naladixic acid, amoxicillin, enrofloxacin, florfenicol, oxytetracycline, oxylinic acid, streptomycin, ceftiofur, and chloramphenicol. The DAFA immediately generated a plan to prevent persistence or spread of this bacterium into the surrounding environment. The plan consisted of five major pillars; risk to workers health, risk to human health (consumers), environmental risk, risks associated with depopulation, and needs for immediate and future studies. The facility was depopulated within 7 days of notification and the case was submitted to DFO, CFIA and Health Canada. Since the outbreak, DAFA determined, with the help of personnel at RPC laboratories, that the *A. salmonicida* was infiltrated with a first class fully integrated integron. Integrons are defined as potentially mobile DNA elements that comprise a site-specific recombination system capable of capturing and expressing genes contained within cassette structures. The integron is over 100 kbp's in size and further DNA analysis has uncovered the genetic sequence of the resistance packages within each of the cassettes. The presence of such a diverse array of resistance genes has not been reported previously for *A. salmonicida.* This is the first isolation of this class of beta-lactamase in *A. salmonicida* and is identical to the plasmid pSCR1 identified in *Salmonella* isolated in China and the United States. The most significant finding was that the MDR strains possessed a multiple gene cassette, which demonstrated a very high degree of sequence homology and structural organization with the antibiotic resistance cassette of SXT element of *Vibrio cholerae* found in India. The resistance pattern was not associated with irresponsible or indiscriminant use of antibiotics. Resistance packages in many bacteria could be the result of untreated effluent from hospitals being discharged into local river systems, in addition to the ease at which these integrons are accepted by nonpathogenic aquatic bacteria. An update on the status of Infectious Salmon Anemia (ISA) will also be presented. In addition new information will be provided on the typing of segments 5 and 6 of the ISA structure.

Identification Of A New HPR Group Of Infectious Salmon Anemia Virus In New Brunswick: What Does It Mean?

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Infectious salmon anemia (ISA) is a highly fatal disease of marine-farmed Atlantic salmon caused by ISA virus (ISAV). The virus also infects several marine salmonid and non-salmonid fish species resulting in asymptomatic carriers of the virus. Such benign infections in the wild fishery are considered to be the source of virulent strains that cause clinical disease in marine-farmed Atlantic salmon. It has been hypothesized that the ISAV variant without any deletion in the haemagglutinin-esterase (HE) protein, designated HPR0 to indicate a full-length highly polymorphic region (HPR) in HE, represents the avirulent archetype of the virus from which the virulent strains arise by deletion of several nucleotides in the HPR. Because HPR0 virus has not been isolated in cell culture, it remains a source of diagnostic confusion, particularly in surveillance programs with ISAV RT-PCR-positive fish that are negative on virus isolation. This is compounded by the variations in susceptibility of the different salmonid cell lines used to detect ISAV. Our laboratory has been typing ISAV isolates from disease outbreaks on Atlantic salmon farms in New Brunswick, Canada, in order to gain detailed knowledge about the epidemic strains of ISAV. We report here the identification of a new European HPR group of ISAV which replicates poorly in the ASK-2 cell line. Of all HPR groups reported to date, this new HPR group is the closest to HPR0 in terms of the pattern of deletion associated with this unique phenotype, and indicates a direct molecular relationship between cytopathogenicity of ISAV in cell culture and ability to cause clinical disease in farmed Atlantic salmon.

Current Status Of Canada's National Aquatic Animal Health Program (NAAHP)

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The Office of Aquatic Animal Health (AAH) of the Canadian Food Inspection Agency (CFIA) in conjunction with Science Branch of Fisheries and Oceans Canada (DFO) are implementing the federal component of the National Aquatic Animal Health Program (NAAHP). On February 23, 2004, $59 Million in funding over 5 years was announced in the federal budget for the NAAHP, which, when implemented, will secure seafood production and trade, improve governance on aquaculture that meets domestic and international standards, protect Canadian aquatic resources (wild and farmed) from serious infectious diseases and help ensure access to international markets on an on-going basis. The federal NAAHP will apply to all reportable diseases of aquatic animals (currently of finfish, molluscs, crustaceans), and will cover all sectors that use fisheries resources in Canada (e.g. aquaculture, the capture fishery, seafood processing, and live fish and seafood trade. The program will add legislative and infrastructure support for aquatic animal health and seafood certification that is consistent with other food sectors. Roles and responsibilities of the CFIA include program direction, trade certification, international negotiation, risk analysis, aquaculture surveillance, contingency planning, zonation, and the incorporation of new aquatic animal health requirements into the existing Regulations under the federal Health of Animals Act (HAA). Roles and responsibilities of DFO include management of a national diseases database, diagnostics, technology development, disease research and wild surveillance. An Aquatic Animal Health Committee, co-chaired by DFO and CFIA, has been established to coordinate broad NAAHP initiatives.

The National Aquatic Animal Health Plan: Briefing And Update

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The Joint Subcommittee on Aquaculture's National Aquatic Animal Health Task Force has been charged with the mission to develop and implement a national aquatic animal health plan (NAAHP) for aquaculture in partnership and cooperation with industry, regional organizations, State, local, tribal governments and other stakeholders. The purpose of the NAAHP is to foster and support effective and efficient aquaculture; to protect the health of our nation's wild and cultured aquatic resources; and to meet our national and international trade obligations. The NAAHP is being developed via input from a series of Task Force-associated working groups. Each working group consists of 10-20 experts, each representing a sector of the aquaculture community. Each work group focuses on a specific element of the NAAHP such as: roles and responsibilities of health professionals, laboratory methodologies and species-specific working groups that address issues for that sector. Several work groups have met and recommendations are considered when drafting the NAAHP. The first complete draft of the NAAHP is expected to be completed in the spring of 2006, with refining and implementation to follow. The NAAHP in itself will not be codified into regulation; however, implementation of certain elements, such as import requirements, may require revisions to existing laws, regulations or policies.

The Proposed Use Of The Non-Native Oyster *Crassostrea ariakensis* In Chesapeake Bay

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The abundance of the native oyster in Chesapeake Bay, *Crassostrea virginica*, has declined drastically over the last four decades because of mortality caused by two protistan diseases, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo). Annual harvest levels in Chesapeake Bay between 1930 and 1959 averaged about 5.5 million bushels, but have declined to less than 50,000 bushels in the last decade. Interest in the use of a non-native oyster to rehabilitate the fishery and to provide ecological services began in the 1990s. Field trials with sterile, triploid Pacific oysters, *Crassostrea gigas*, revealed that they did not grow well in the low to moderate salinities of Chesapeake Bay. Interest shifted to the Suminoe oyster, *Crassostrea ariakensis*, and field trials using sterile triploid oysters demonstrated faster growth and greater survival compared with the native oyster. *Crassostrea ariakensis* seems to be totally refractory to *H. nelsoni* as no infections of this pathogen have ever been observed. Infections of *P. marinus* are acquired, but intensity levels remain low and no mortality has been observed caused by this parasite. Taste tests have demonstrated wide acceptance of the product in the marketplace. Virginia had originally proposed the use of sterile, triploid *C. ariakensis* as an aquaculture product to prevent inadvertent introduction of reproducing non-native oysters. Maryland was originally opposed to any use of *C. ariakensis*, but a new governor elected in 2004 has reversed this position and now Maryland is proposing the introduction of diploid, reproducing *C. ariakensis* to rehabilitate the public oyster fishery in Maryland. A decision has recently been delayed from March 2005 until July 2005 to allow more research. Maryland Department of Natural Resources and NOAA have provided over $3 million for research.

Detection And Identification Of Oyster Herpes Viruses (OsHV) And *Perkinsus* Species From Oysters In Asia: Relevance To Chesapeake Bay

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Decline of native *Crassostrea virginica* populations in the Chesapeake Bay region due to overfishing, disease, and habitat destruction, along with continued heavy disease pressure has led to interest in using the relatively more disease tolerant Asian oyster, *Crassostrea ariakensis*, for aquaculture development, fishery resource enhancement and habitat restoration. *Crassostrea ariakensis* oyster samples from China, Japan, Korea, and from US hatcheries were surveyed for OsHV and *Perkinsus* species using molecular diagnostics. Although several oysters were also examined for parasites and potential pathogens by histology, very few were found. Two cases of *Perkinsus* spp. were found at Beihai, in southern China, by histopathology. PCR screening revealed *Perkinsus spp.* and/or OsHV DNA in many additional samples. Of 18 Asian sites surveyed, 9 showed evidence of OsHV and 13 evidence of *Perkinsus* spp. None of the hatchery samples contained OsHV DNA, but local *Perkinsus* spp. were detected in VIMS hatchery groups. Sequencing of PCR products from Asian oysters positive for *Perkinsus* indicated that two species of *Perkinsus* not currently found in Chesapeake Bay were present; *P. olseni*, and a new, undescribed species. We are conducting studies to determine the potential impact these non-native pathogens could have on local bivalve species in case of accidental introduction. Live oyster samples from southern China were recently brought into quarantine at our laboratory in order to further characterize the pathogens through challenge and transmission studies and to develop in vitro cultures of *Perkinsus* species.

Epizootiology Of *Bonamia* sp. In Non-Native Oysters, *Crassostrea ariakensis*, In North Carolina, And Implications For Aquaculture

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At Bogue Sound, North Carolina, in July and August 2003, very high mortality (> 85%) was observed in experimental lots of hatchery-produced *Crassostrea ariakensis*. A microcell parasite was observed at high prevalence and intensity in moribund oysters fixed during the height of the epizootic, and in subsequent samples from an October deployment to the same area. PCR amplification confirmed this parasite to be a *Bonamia* sp., and DNA sequencing established it as a possible new species. This *Bonamia* sp. has been monitored in experimental *C. ariakensis* and wild *Ostrea equestris* in Bogue Sound (the only area in which it has yet been detected) since November 2003. It has persisted in *C. ariakensis*, and has been found also in *O. equestris*, which may be a reservoir of the parasite; it has been transmitted, with new infections established, from at least May (2004 data) through October (2003, 2004); and it has shown a greater infectivity towards smaller (< 40 mm) oysters. The latter finding, combined with information on parasite salinity tolerance, may allow aquaculturists to avoid the parasite or mitigate its impact by using separate nursery and grow-out locations: maintaining oysters through their window of susceptibility in more growth-marginal but parasite-free environments, then transferring to more growth-optimal locations for culture to market size.

Salinity Effects On The Persistence Of *Bonamia* sp. In C*rassostrea ariakensis*

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The Asian oyster *Crassostrea ariakensis* is a candidate for introduction to Chesapeake Bay in part because it resists diseases caused by the parasites *Haplosporidium nelsoni* and *Perkinsus marinus*. Its susceptibility to *Bonamia* parasites, however, emerged as a key issue in 2003 after the finding of a new *Bonamia* sp. in *C. ariakensis* dying in Bogue Sound, North Carolina. This parasite has been observed in high salinity environments and it is not clear whether it would be infective in lower salinity waters of Chesapeake Bay. To address this issue, experimental trials were designed to investigate the persistence and potential transmission of *Bonamia* sp. under three salinities: 30, 20 and 10 psu. Salinity tolerance was also investigated *in situ* by deploying *C. ariakensis* along a salinity gradient from Bogue Sound to Neuse River through Core Creek Canal. Field and experimental trials results have suggested that salinity plays a significant role in *Bonamia* sp. dynamics. Field sites that experienced salinities lower than 30 ppt were characterized by an absence of infection acquisition, while in the laboratory trials oyster mortality, parasite prevalence, and salinity were positively correlated.

Polydora-Associated Shell Disease: Recent Distribution In Native And Non-Native Oysters, And Implications For A *Crassostrea ariakensis* Introduction.

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The Asian oyster species *Crassostrea ariakensis* is favored by some for introduction to Chesapeake Bay because it is "disease resistant". This oyster's "disease resistance" does not necessarily extend beyond native oyster parasites *Haplosporidium nelsoni* and *Perkinsus marinus*, however, and other results suggest *C. ariakensis* may not even be more resistant than *Crassostrea virginica* to all disease agents active in Chesapeake Bay. The shell-burrowing polychaete *Polydora websteri* is very active in mesohaline environments like Chesapeake Bay and a common pest of native *C. virginica*. Its burrows impair oyster shell structure, leaving the oyster potentially more vulnerable to crab predation; when the inner shell surface is penetrated, the host responds with production of conchiolin blisters, an energetic cost that could leave the oyster more vulnerable to parasitic infection. Side-by-side trials since 2003 at eight sites in and around Chesapeake Bay have compared *P. websteri* infestation and conchiolin shell blistering in *C. ariakensis* and *C. virginica*. While infestation levels, heaviest inside Chesapeake Bay, are similar between hosts, conchiolin blistering is much more intense in *C. ariakensis*, a probable function of this oyster's production of a thin and easily penetrated shell. The repercussions of this for aquaculture are profound. Besides potential losses to crab predation, a serious question is whether enough *C. ariakensis* may be produced and sold to high-end markets to make aquaculture of this species in Chesapeake Bay a viable enterprise.

Recent Trends In The Distribution And Abundance Of *Haplosporidium nelsoni* (MSX) And *Perkinsus marinus* (Dermo) In Native Oysters In Chesapeake Bay

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Haplosporidium nelsoni is an introduced pathogen that began causing mortality in lower Chesapeake Bay in 1959; it rapidly spread up the Bay throughout areas where salinity was greater than 15 psu. *Perkinsus marinus* has probably always been an associate of oysters in the Bay, but intensified and spread throughout the Bay during drought periods of the late 1980s. These two pathogens combined have caused a drastic reduction in the abundance of the native oyster *Crassostrea virginica*. Within Chesapeake Bay, *H. nelsoni* distribution and abundance are controlled by salinity with few infections below 10 psu. *Perkinsus marinus* is distributed throughout all oyster beds in Chesapeake Bay, but its abundance and impact fluctuate with salinity. Generally, for both pathogens, their abundance and impact decrease in wet years and increase in dry years. Beginning in summer 1998 the Chesapeake Bay watershed experienced over four years of consecutive drought conditions. During only three months over that entire period was streamflow above average. These drought conditions and resultant increased salinity caused a dramatic increase in abundance of both *H. nelsoni* and *P. marinus*, and widespread oyster mortality. Intensity of *P. marinus* infections was the highest ever observed in 40 years of monitoring. Unfortunately, funds for restoration of native oysters also increased during this period, so we were trying to restore native oysters under the worst possible disease pressure. 2003 was an extremely wet year, as was the summer of 2004. With the resultant decline in salinity, abundance of both *H. nelsoni* and *P. marinus* declined significantly and mortality from these pathogens was low during 2003 and 2004.

Coral Diseases: An Overview And Update On Black Band Disease In The Bahamas And The Florida Keys

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Over the past twenty years, a dramatic increase in coral diseases has caused massive mortalities in many species of corals worldwide. The species and the number of coral infected can vary from year to year, but an overall increase is evident. Black band disease (BBD) is one of several coral diseases that contribute to coral reef degradation. Since 1972, BBD has spread throughout the western Atlantic, Indo-Pacific, the Red Sea, and the Great Barrier Reef. We have been studying the prevalence, disease incidence, and severity of BBD on reefs near Lee Stocking Island, Bahamas and the Florida Keys. It known that the biofilm responsible for BBD is composed of a diverse microbial community. As the disease consortium moves across the coral surface it creates an "extreme" environment of anoxia and sulfide that destroys the host tissue. Earlier characterization of BBD was based on bacterial morphology, selective media and light microscopy that identified the cyanobacteria, sulfate-reducers and other members of the microbial mat. More recently, we have cultured an assortment of heterotrophs and cyanobacteria from different BBD sampled in both Lee Stocking Island, Bahamas and the Florida Keys. In addition to molecular community profiling, we have started to clone and sequence many of the isolates from BBD samples. The preliminary results of the members of the community, including a comparison of cultivative and non-cultivative approaches, will be presented. Insight as to which populations of microbes are members of this pathogenic consortium will provide more definitive assessment of the disease and its dominant members.

Comparison Of Bacterial Communities Between Geographically Separated Corals Infected With White Plague Type II

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White plague type II (WPII) is a disease of scleractinian corals which characteristically destroys tissue beginning at the edge of the colony. This investigation probed fundamental questions about the bacterial community associated with WPII and the tissue/cell responses by comparing WPII-diseased corals with apparently healthy controls. We collected tissue cores from healthy and diseased *Montastraea annularis* (complex) from the U.S. Virgin Islands (USVI) and the Bahamas. The tissues were analyzed using a combination of molecular (amplicon length heterogeneity – LH-PCR) fingerprinting, microbial culturing, 16S rRNA gene sequencing, and histological examination. We hypothesized (1) that the causative agent(s) is/are opportunistic pathogen(s) normally present in the host or its environs rather than a novel, obligate pathogen; (2) that corals exhibiting WPII disease signs from different geographical regions harbor differing microbial communities in healthy and diseased tissue; and (3) the WPII disease process is the result of a broad shift in the microbial community (dysbiosis). The LH-PCR fingerprints indicate that the bacterial diversity in healthy *M. annularis* tissue from the Bahamas was significantly greater than in samples from the USVI. In the Bahamas bacterial diversity was lower in the diseased tissue compared to healthy tissue, but that was not true for the USVI. Principal component analysis of LH-PCR data from pairs of corals suggest that the microbial community on control corals and apparently healthy areas of diseased corals were similar at each geographical location, but the microflora on the Bahamian corals differed markedly from that on the USVI corals. Similarly, the fingerprints of the active diseased samples from each geographic location clustered together, but there was little clustering between the different locations. An amplicon corresponding to that of the bacterium *Aurantimonas coralicida* was found in tissue from diseased and healthy coral from the Bahamas but not from the USVI.

Prevalence And Potential Origin Of The Acroporid Serratiosis Pathogen In The Florida Keys

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In the Florida Keys, populations of elkhorn coral, *Acropora palmata,* have been decimated by white pox. The fecal enteric bacterium, *Serratia marcescens,* has been identified as a cause of the disease. To reflect its etiology, when white pox lesions are associated with *S. marcescens,* the disease is referred to as acroporid serratiosis. While *S. marcescens* is common in clinical settings and in the guts of vertebrates and invertebrates in terrestrial and freshwater environments, little is known of the prevalence and ecology of this bacterium in the marine environment. Terrestrial and reef environments in the Florida Keys were surveyed for *S. marcescens.* Samples were collected from *A. palmata*, *Coralliophila abbreviata*, *Hermodice carunculata*, parrotfish feces, reef water, beach water, canal water, wastewater influent and final effluent (Key West Wastewater Treatment Plant), and seabird guano. Samples were plated onto MacConkey Sorbital agar (MCSA). Colonies appearing pink to red on MCSA were tested for DNase activity. Isolates were confirmed to species level using PCR directed at 16S rDNA. More than 250 *S. marcescens* isolates were confirmed, and the majority (90%) originated from human sewage and polluted nearshore sources. Molecular fingerprints for each confirmed isolate were generated by restriction enzyme digestion followed by pulsed field gel electrophoresis. Band patterns between isolates were compared using presence and absence of specific DNA fragments to determine the potential origin of the acroporid serratiosis pathogen.

The Role Of *Vibrio* spp. In Coral Disease: From Normal Microbiota To Pathogens

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Various species within the genus *Vibrio* have been reported as associates of corals for some time. Members of this genus were indicated as normal inhabitants of the surface mucopolysaccharide layers from a number of coral species. In addition, the relative population levels of bacteria with carbon source utilization patterns consistent with *Vibrio* changed with the health of certain corals. More recently, a number of *Vibrio* isolated have been identified as pathogens for specific coral diseases. In some cases more than one species was required to cause significant disease signs. This paper discusses the range of *Vibrio* species shown to play a role in disease and proposes testable mechanisms of how this may take place, primarily, through environmental induction of toxin genes.

Use Of Fluorescence *In Situ* Hybridization For The Identification Of *Aurantimonas coralicida* In Environmental Samples

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One of the most virulent coral diseases, white plague type II, was first described in 1995 on coral reefs of the Florida Keys. The pathogen for this disease has been identified as *Aurantimonas coralicida*. A fluorochrome-labeled probe was designed based on the full sequence of its 16S rRNA gene, and used with a fluorescence *in situ* hybridization protocol. The probe was optimized using pure cultures of *A. coralicida* and tested for specificity against closely related bacteria. A sensitivity test was conducted to determine the concentration of *A. coralicida* needed in a sample to result in a positive identification. Results revealed that 4 out of 10 cultures tested hybridized with the probe in question. One of the four may be present in the reef environment. The concentration of *A. coralicida* cells necessary for a positive identification varied with the method used. One method, which concentrated cells by settling and centrifugation, required at least 2250 cells/ml for detection by the probe, while a modified filter method could detect *A. coralicida* down to 10 cells/ml. Currently the probe is being tested on environmental samples from the disease line of infected colonies, healthy tissue on diseased colonies, healthy colonies, and sediment from the reefs. Results are being used to determine not only the presence/absence of the pathogen in coral populations, but also in the determination of the pathogen's reservoir.

Coral Mucous And Indigenous Bacteria: A Possible First-Line Defense Against Coral Disease Pathogens

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Coral reefs worldwide are undergoing a significant decline in species abundance and changes in community structure. A contributing factor to this decline is bacterial infections of scleractinian corals. Recently, a bacterial pathogen, *Serratia marcescens*, has been identified as the etiological agent for acroporid serratiosis (white pox disease). The current hypothesis for the development of this disease is based on a dose-response relationship between *S. marcescens* and the affected host, *Acropora palmata*. However, mechanisms of natural resistance associated with coral systems have not been determined. To address this issue, bacterial isolates (n=150) recovered from mucus samples collected from corals in the Florida Keys were screened for their ability to inhibit or prevent growth of *S. marcescens* and *E. coli*, using a soft agar overlay method. Isolates that demonstrated inhibition or prevention of growth of either pathogen were further characterized using Biolog GN2 plates and sequencing of the 16S rRNA gene. Approximately 5% (n=7) of the isolates demonstrated pathogen inhibition. All isolates were biochemically identified as *Vibrio alginolyticus*. However, their relative 16S rRNA sequence data positioned two isolates as closely related to *V. alginolyticus*, while the remaining isolates were related to *V. harveyi* (n=1), *V. proteolyticus* (n=2), and *V. fisheri* (n=2). All of the characterized *Vibrio* species were capable of preventing or inhibiting the growth of *S. marcescens* and *E. coli*. These results indicate bacterial disease mechanisms in coral reef systems are much more complex than the current model indicates and that the coral mucus layer acts as a primitive immune system, possibly providing a first-line defense against the establishment of pathogens.

Customs-Confiscated Corals: Gifthorses Requiring Oral Examination.

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Corals are currently exhibited in the Aquarium at the London Zoo and are propagated in anticipation of the 2008 opening of "BIOTA!" a new aquatic-themed facility in east London. Live corals will feature strongly in "BIOTA!" exhibits to highlight coral reef conservation. The Zoological Society of London (ZSL) has policy statements for the acquisition of all animal species. At irregular intervals, ZSL is offered animals that have been seized by HM Customs. Their accession to the collection falls within ZSL acquisition policies. A large proportion of ZSL's coral specimens have been thus acquired. Many of the coral species traded legitimately are the hardier, more easily propagated species. The care of confiscated corals can be challenging. Many of them are the more delicate hermatypic species and have been almost invariably taken, often illegally, from the wild. During subsequent quarantines and holding, individual colonies of *Euphyllia* spp. and *Goniopora* spp. have been found with burdens of the protozoan *Helicostoma* sp. Areas of tissue death have also been noted on a number of colonies of *Montipora* spp. and *Porites* spp. caused by nudibranch predation, all believed to be members of the genus *Phestilla*. Treatment of these coral colonies was to be further complicated by the desirable presence on the *Porites* of the commensal Christmas tree worms *Spirobranchus* spp. (tube-dwelling serpulid polychaetes). Treatment regimes for these conditions will be described. In the light of ZSL's experience with these confiscated corals, protocols for examination and management are being reviewed. Quarantines and routine quarantine treatments will in the future be more proactive, in particular as coral holding and propagation capacity will increase in the run-up to the opening of "BIOTA!" The London Zoo Aquarium is land-locked. Above and beyond risks to corals within the quarantine holding and, more broadly, within the zoological collection, the potential future risk of the release of potential pathogens, via waste water, to the wild has to be addressed. This risk is going to be greatest at coastal institutes where identical or similar species of coral occur in the wild.

Preparation Of Coral Tissues For Histological Examination: A Primer On Fixation And Decalcification

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Microscopic examination of tissues and cellular structure has long been a component in the diagnostic process of human and veterinary pathology, and there is a critical need to investigate the cellular alterations associated with coral diseases. Achieving optimal fixation of coral tissue is the essential first step in this diagnostic process. Numerous chemical formulations are available which preserve tissue morphology, yet each has a varying impact on the integrity of tissues with implications for interpretation of normal histology and the description of lesions in corals. Selection of a fixative is determined primarily by the objectives of the study; however, the logistics of specimen collection often require a balance between achieving optimal preservation of tissues with ease of use in the field. Selection of a decalcifying solution for traditional paraffin processing will also affect the final product and success of subsequent procedures. In this study, the quality of tissue fixation in *Porites astreoides*, *Siderastrea radians*, and *Plexaurella fusifera* was compared using six fixative formulations and three decalcification solutions. Evaluation included effect on tissue architecture, staining quality, nuclear morphological characteristics, and impact on zooxanthellae. In addition, some field situations necessitate the temporary storage of collected coral specimens in ambient seawater prior to fixation. The length of time between collection and subsequent immersion in fixative has a negative impact on tissue quality, as observed in samples of *Porites compressa*, *Montipora capitata*, and *Pocillopora damicornis* fixed in seawater-diluted Z-Fix concentrate at intervals of 10, 100, and 200 minutes after collection.

The Control Of Fish Parasites And The Eradication Of Asian Tapeworm Infections In Grass Carp Using Praziquantel Bath Treatments

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There are few studies on the control of fish parasites and even fewer effective or accepted methods for the direct or indirect control of these parasites. When parasite eradication from fish stocks is the goal, the probability for success is very low. Condition of host, life cycle of parasite, water quality, temperature, and chemical formulation, dose, and contact time are some of the factors that affect the outcome of control methods. In this session, presentations will be made on the use of chemicals to control alternate hosts, pre-parasitic phases, and parasites on the hosts. Presentations will also be given on management techniques to limit the number of pre-parasitic phases in a water system, to prevent parasite infection through disinfection, and to potentially enhance the production of natural host-produced substances that kill invading parasites. A number of states require that imported fish be free of the Asian tapeworm *Bothriocephalus acheilognathi*. Eradication of this tapeworm from the intestines of infected fish can be achieved by Praziquantel bath treatments. To obtain successful treatment, consideration must be given not only to dose, but also to treatment duration, fish holding concentration, and the intensity of the tapeworm infection. At a low fish density (6 g fish/L of water) a 1.5 mg/L Praziquantel bath treatment for 24 h is completely effective in eliminating tapeworm infections from heavily infected grass carp *Ctenopharyngodon idella* (30-150 tapeworms/fish).

Sea Lice Control In The Maine Aquaculture Industry

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The first epidemic outbreak of sea lice, *Lepeophtheirus salmonis* and *Caligus elongatus*, in Maine occurred in 1994. Since that time the Maine aquaculture industry initiated a program to control the sea lice population on farmed Atlantic salmon. From 1996-2000 the control program relied on the use of Excis which has cypermethrin as the active ingredient under an Investigative New Animal Drug (INAD) application. From 2001 to the present, the program switched to SLICE, again under an INAD, which has emamectin benzoate as the active ingredient. In addition to chemotherapeutant use, a strong emphasis has been placed on continuous refinement of industry best management practices (BMP). These include regular use of practices such as site and/or bay fallowing and single year class sites and zones. Both the Excis and SLICE INADs have relied on the establishment of a monitoring program on all farms which desire to treat their animals under the INAD. This monitoring has provided almost a decade of information on sea lice populations around Maine's salmon farms. Continuous improvement in industry best management practices and the use of Slice have helped to bring the management of sea lice populations under control.

The Role Of Antimicrobial Polypeptides In Resistance To Parasitic Diseases Of Fish

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Antimicrobial polypeptides (AMPP) are host-produced innate defenses that typically have potent, broad-spectrum activity against many serious pathogens. We have identified several types of AMPP in fish. One group of these AMPP comprise the histone-like proteins (HLPs), small (~12-25 kDa), highly basic proteins that have been isolated from channel catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*) and hybrid striped bass (*Morone saxatilis* x *M. chrysops*). HLPs are highly lethal to the important marine ectoparasite *Amyloodinium ocellatum* and interestingly target the trophont (feeding) stage, which is highly resistant to conventional drug treatment. Piscidins are small ~2.5 kDa AMPP initially isolated from hybrid striped bass. We now have evidence that piscidin-like AMPP are probably present in a taxonomically wide range of fish species, especially members of the Perciformes. Both HLPs and piscidins are expressed in high concentrations in healthy fish at portals of entry for potential pathogens such as skin, gill and gastrointestinal tract. Piscidins display highly potent activity against a number of fish pathogens, including viruses, bacteria (gram-positive and gram–negative) and parasites. Unlike most AMPP isolated from other animals, piscidins are also effective in the presence of significant salt concentrations, suggesting that they function in both marine and freshwater environments. The sensitivity of certain AMPP to chronic stress indicates their potential usefulness as health biomarkers in many fish species.

Further Elucidation Of The Life Cycle And Pathology Of The Digenetic Trematode, *Bolbophorus damnificus*

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The digenetic trematode *Bolbophorus damnificus* has been associated with high mortalities in channel catfish in the Mississippi Delta. The American white pelican and the snail *Planorbella trivolvis* have been identified as hosts for this parasite based on life stages found in each host. In this research, a series of studies were done to: confirm stages of this life cycle in a single study; determine if other fish- eating birds and snail species could serve as hosts; and confirm the pathogenicity of this parasite. An artificial infection in which American white pelican and *P. trivolvis* were exposed to *B. damnificus* metacercariae and ova, respectively, demonstrated that the American white pelican shed *B. damnificus* ova for 4 months post-infection; snails shed cercaria 23 days post-infection, and fish mortality occurred 13 days post-exposure to cercariae. In a series of studies where the snails *Physella gyrina* and *P. trivolvis* (Mississippi Delta) and *P. trivolvis*, subspecies (North Dakota) were exposed to *B. damnificus* ova, only *P. trivolvis* became infected. A pen study in which American white pelicans, cormorants, great blue herons, and great egrets were artificially infected with *B. damnificus* metacercaria, demonstrated that the American white pelican was the only bird to develop a patent infection. A series of studies where fish were exposed to 200 *B. damnificus* cercariae resulted in mortalities at 6 d post-infection. These studies confirmed: stages of the *B. damnificus* life cycle; cormorants, great egrets and blue herons and *P. gyrina* are not hosts for this parasite; and 200 *B. damnificus* cercaria can cause high mortalities in catfish 6 days post-infection. Research supported by grants: USDA/NRI: 2002-35204-11678; USDA/IPM: 2002-34103-11858; USDA/SCRAC: 2002-38500-11805.

Evaluation Of Copper Sulfate To Control Snail Numbers In Catfish Ponds Affected By *Bolbophorus* Trematodes

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Bolbophorus damnificus emerged as a threat to channel catfish aquaculture in 1995. Fingerlings rapidly succumb to heavy infections while sublethal infections decrease feed consumption and increase susceptibility to *Edwardsiella ictaluri* in fish of all sizes. The digenetic trematode's life cycle involves ram's horn snails *Planobella trivolvis* and catfish as first and second intermediates, respectively, and American white pelicans *Pelicanus erythrorhynchos* as final hosts. Control has focused on eliminating snails from ponds. Chemical methods rely on hydrated lime or copper sulfate applied in high concentrations along pond edges. These treatments are effective, provided snails are concentrated on pond margins, but fail if they are dispersed away from the bank. A 24-hr LC50 of 2.4 mg/L $CuSO_4 \cdot 5H_2O$ was determined for snails, independent of water alkalinity. Studies were conducted to evaluate the efficacy and safety of 2.5-5.0 mg/L treatments applied to entire pond volumes with alkalinities averaging 239 mg/L as $CaCO_3$. In 0.25-10-acre ponds, greater than 95% of caged snails were killed with only minor fish losses. In a heavily infested commercial pond treated with 5.0 mg/L $CuSO_4 \cdot 5H_2O$, 96.5% of caged snails and 99% of snails along the bank died. Prior to treatment, 20,000 lbs of fish had been lost to enteric septicemia and columnaris disease. An additional 2,000 lbs died within 24 hrs of treatment. Although some risk of fish losses exists, in high alkalinity waters typical of the Mississippi Delta, results suggest copper sulfate applied above traditional guidelines (alkalinity ÷ 100) effectively salvages production in ponds heavily impacted by *Bolbophorus* trematodes. Research supported by grants: USDA/IPM: 2002-34103-11858; USDA/SCRAC: 2002-38500-11805.

Integrated Control Program For The Sea Lamprey, *Petromyzon marinus*, Macroparasite Of The Great Lakes

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The sea lamprey (*Petromyzon marinus)*, an ancient parasitic eel-like fish native to the Atlantic Ocean, was first discovered in Lake Ontario in 1835. Construction of the Welland Canal in 1919 provided sea lampreys access to the remaining Great Lakes and by 1947 they were prevalent throughout the entire Great Lakes basin. Sea lampreys prey on commercially important species such as lake trout (*Salvelinus namaycush*) by living off blood and body fluids of adult fish. During its life as a parasite one sea lamprey can kill 40 pounds or more of fish. Coupled with commercial over-harvest, sea lampreys were a major cause of the collapse of the Great Lakes fishery in the 1940s and 1950s. In response to this collapse, an extensive bi-national effort by the United States and Canada and headed by the Great Lakes Fishery Commission was launched in the late 1950s to control the sea lamprey. Today, sea lamprey populations are maintained at about 10% of their peak level in the 1960s by an integrated pest management control program that includes the use of selective chemicals, mechanical and electrical barriers, traps, and sterilization and release of spawning males. In this talk, we will provide background on sea lamprey, evolution of the control program, and new control methods now under development.

Control Measures For *Heterosporis* Species

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Heterosporis sp. is a newly identified microsporidan parasite that infects muscle cells of fish. Although first discovered in wild yellow perch, *Perca flavescens*, in Wisconsin, Minnesota, Michigan and the province of Ontario, other natural infections have since been confirmed in walleye, northern pike, rock bass, pumpkinseed, burbot, sculpin and trout-perch. Laboratory studies have shown that rainbow trout, Coho salmon, brook trout, brown trout, lake trout, white suckers, mosquito fish, channel catfish, fathead minnow, and largemouth bass are also susceptible to infection. Bluegill, lake sturgeon, smallmouth bass and golden shiners exposed to spores remained uninfected. Fish are infected by consuming live, infected fish or pieces of infected muscle, and by contact with spores suspended in water. Until its appearance in North America, *Heterosporis* sp. infections were described in cichlids and angelfish from Europe, bettas from Thailand and Japanese eels from Taiwan. There is potential for routine fisheries activities to accidentally spread the parasite to new lakes. Several control measures have been tested and the following were found to inactivate *Heterosporis* spores: 1) Complete desiccation for 24 hours; 2) Freezing at -20^{o}C for 24-hours; and 3) immersing spores in a chlorine solution for 5-minutes (3 cups household bleach [6% chlorine] in 5-gallons of water). Nets, raingear, live wells and boats can be treated using these protocols to reduce the risk of moving spores to new locations. This parasite has the potential to infect multiple species wherever it is introduced. Although there is no evidence that *Heterosporis* sp. can infect people, it is likely that anglers will discard infected fillets due to changes in texture and quality.

Some Effects Of *Philometra saltatrix* On Bluefish Ovaries

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The prevalence and intensity of an ovarian nematode parasite, *Philometra saltatrix*, and its effect on bluefish (*Pomatomus saltatrix*) reproductive potential was investigated. Prevalence of live *Ph. saltatrix* in ovaries of adult bluefish (n = 168) captured in 2004 was highest in July at the height of the spawning season, when 39 % of sampled fish were infected with live worms. Intensity of infection was also highest in July at an average of 2.4 worms per female fish. Standard histological examinations of ovaries revealed pathologies including hemorrhage, inflammation, and follicular atresia associated with the infection. Damage to ovarian tissues may affect bluefish fecundity. The highest prevalence of live worms in young-of-the-year (YOY) fish obtained in 2004(n = 243) was 22.2%, which occurred in August. All infections of YOY were in the pericardial cavity except for two fish in which worms were found in the body cavity. The first intermediate hosts in all known philometrid life-cycles are cyclopoid copepods, and it is possible that YOY bluefish, while still zooplanktivorous, first become infected by feeding on these copepods. Alternatively, bluefish may acquire the parasite once they have become piscivorous through either a paratenic or a second intermediate fish host. This is supported by the fact that the smallest infected specimen was 59 mm in length, and certainly piscivorous.

Gross Observations From A Chesapeake Bay Fish Survey

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A variety of fishes, including striped bass (*Morone saxatilis*) and Atlantic menhaden (*Brevoortia tyrannus*), were sampled during 2004 from the Chesapeake Bay. External lesions were observed including areas of reddening, fin erosion, as well as ulcers. Skin scrapes and gill biopsies for parasitology were performed, and a variety of protozoan and metazoan parasites were found. Striped bass were more heavily parasitized than menhaden, and consequently, parasites of striped bass represented more taxa than those of menhaden. The most common parasites observed from striped bass were ciliates (*Trichodina*, *Ambiphyra)* and copepods (*Ergasilus*) on the skin and gills, acanthacephalans (*Pomphorhynchus bulbocolli*) in the distal gut, and nematodes in the viscera and peritoneum. Other parasites observed from striped bass included *Ichthyobodo* and *Trichophyra* in the gills and on the skin, and red nematodes in the gut. Leeches and the branchiuran *Argulus* were also observed from the skin of several species. Interestingly, the majority of menhaden sampled were free of parasites, except for observations of isopods (*Olencira praegustator*) commonly found in the oral cavity of fish from multiple river systems. While parasitology concentrated on observations from striped bass and menhaden, findings from other fish species included an abdominal mass in spot, cataracts in striped bass and white perch, and lordosis in white perch. These findings illustrate the breath of observations that can be found in wild fish populations.

Spinal Deformity In Triploid Grass Carp *Ctenopharyngodon idella*

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In mid-2004, grass carp, *Ctenophayngodon idella (*Valenciennes*)*, showing evidence of spinal deformity were presented to the Aquatic Animal Health Program, Cornell University. The carp were from two separate locations in New York State. The first case, involving several fish, was submitted by the New York State Department of Conservation (NYSDEC) staff. The second was a single affected individual from a backyard pond in the Ithaca region. In early 2005, another fish showing similar signs was submitted from a hatchery in a third location. In all cases, the fish were at least seven years of age and had been stocked for weed control. In the first case, there was concern that electroshocking for biological surveys may have caused the spinal lesions however fish from the backyard pond and hatchery had never been shocked. Radiographs and Computer Tomography (CT) scans revealed the deformities to be of bony origin. This paper discusses general causes of spinal deformity in cultured and wild fish, and the methods by which spinal deformity may have occurred in the submitted fish.

Deaths associated with vascular pathology in hybrid striped bass

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Low level mortalities (about 10 of 3600 fish) began appearing in approximately one year old hybrid striped bass that had been raised in recirculating systems consisting sixteen 1136-L tanks configured in clusters of four. These fish were purchased as approximately 1-cm fingerlings and were part of a feed trial consisting of four commercial diets that varied in the level of protein and fat. Diets did not appear to be of consequence since there were affected fish from different tanks on each of the diets. Larger fish were most often affected. There was an acute onset of clinical signs with affected fish appearing listless and separating from the rest of the fish in the morning. They were sometimes unable to maintain equilibrium and hold an upright position in the water column. By the afternoon, these fish had developed exophthalmia (usually bilateral), pendulous abdomens and were sometimes dead. Upon necropsy, significant gross findings included a coelomic cavity that was filled with clotted (sometimes unclotted blood). Source of the blood could not be grossly determined. These fish also had pale, friable livers and pale gills. Bacterial cultures of the kidneys did not reveal any known pathogens. Histologic examination of the formalin fixed tissues revealed irregular proliferative growths on the adventitial surfaces of the heart (bulbus arteriosus, ventricle and pericardium), serosal surface of the GI tract, and the capsular surface of the liver. These lesions consisted of incompletely blood filled channels lined by variably plump spindle shaped cells arranged haphazardly. These cells had large condensed or open faced nuclei (the latter with prominent nucleoli) and scant cytoplasm. Mitotic figures were rare. Tumor cells were supported by a stroma consisting of fine reticulin fibers. These changes were consistent with hemangiosarcoma. The cause of the neoplasms in these fish was unknown but there are reports of similar chemically induced neoplasms in medaka and zebrafish using N-methyl-N'-nitro-N-nirtosoguanidine. Other significant lesions in these fish included erythrocyte depletion in the spleen and hyperplasia of hematopoietic tissue in the kidney. The changes in the liver were consistent with hepatocellular degeneration and pancreatic metaplasia with general loss of hepatocellular fat and glycogen.

Widespread Deformities In An Inland Yellow Perch Population In Michigan

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Widespread skeletal deformities in a yellow perch (*Perca flavescens*) population have been noticed in a major inland lake in Michigan. The deformities involved all adult fish examined (300 fish). Fishermen report that 100% of adult yellow perch, but not other fish species, in this lake are affected. Radiographic analysis revealed the presence of an unusual complex spinal column deformity consisting of consecutive repetition of lordosis and, scoliosis from head to tail. Most spinal curvatures occurred in two specific sites along the vertebral column. Lordiosis was characterized by V-shaped dorso-ventral curvature; however, all lordotic fish possessed inflated functional swimbladders. Additional malformations such as vertebral fusion, absence of one or both operculum, and bent-jaw were also observed albeit at much lower prevalence. The cause of these deformities and their impact on the population are currently unknown.

An Extreme Example Of Common Seahorse Diseases

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Sygnathid fishes, e.g. seahorses, seadragons and pipefish, have become popular and commercially important exhibit animals. As a testament to the value of these fish to the aquarium industry, the pathology service of the Connecticut Veterinary Medical Diagnostic Laboratory (UConn, Storrs, CT) has seen 296 sygnathid fish from 2000-2005, representing at least 11 different seahorse species, 2 species of seadragon, and 5 separate pipefish species. From this submission experience, several pathological conditions have been encountered with frequency in aquarium-maintained sygnathid fish and include mycobacteriosis, erosive to ulcerative bacterial dermatitis, uronemosis, and more recently cutaneous or systemic phaeohyphomycosis. This case presentation involves an aquarium-maintained male potbellied seahorse (*Hippocampus abdominalis*) that exhibited several of these frequently seen pathologic conditions concurrently. Histopathologic findings included focal phaeohyphomycotic dermatitis, locally extensive bacterial ulcerative dermatitis with uronema, and mycobacteriosis. Fungal cultures and DNA sequence analysis have determined the fungus to be *Exophiala pisciphila*. Albeit these several conditions do not constitute the entirety of diseases seen in aquarium-maintained sygnathid fish and presentations may vary significantly between specimens, pathologists and fish health clinicians should be aware of them and be cognizant that their occurrence may be concurrent.

Pathology Associated With Large Cavernous Lesions In Chinook Salmon From Lake Ontario

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Chinook salmon (*Onchrhynchus tshawytscha*) from Lake Ontario have been observed since 1999 to have large focal cavernous, fluid-filled lesions in their muscle. The disease appears to be limited to adult Chinook salmon. Fish were presented for evaluation by the sport fishing public or by the staff of the Salmon River Fish Hatchery (Altmar, New York, USA) of the New York State Department of Environmental Conservation (NYS DEC) during the spawning season (October to December). All of the examined fish originated from Lake Ontario, and we are not aware of fish presenting with this lesion from any other body of water in New York State. The affected fish ranged in size from 4.5 – 9.1 kg. Fish presented for evaluation typically had large raised, poorly demarcated, oval areas in their dorsal musculature near and posterior to the dorsal fin. These lesions ranged in size from 70 to 100 mm long x 40 to 60 mm wide x 20 to 30 mm deep. Some contained as much as 300 mL of variably clear to slightly turbid, yellow to yellow-brown odorless fluid. The ability to culture bacteria from the fish was variable, with *Aeromonas salmonicida, Klebsiella* sp., or no bacteria being isolated. In some cases a parasite similar to *Spironucleus* was found in the fluid from the cavernous lesions and in the blood.

A Case Of Mistaken Sexual Identity And Function: Pitfalls Of Methyl-Testosterone Sex-Reversal In Aquaculture

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The compound, 17-Methyl-testosterone (MT) is sometimes used in Tilapia aquaculture to produce mono-sex all male populations of fish, which results in better production efficiency. However care should be exercised in proper handling and labeling of MT-medicated feed when used in aquaculture. Accidental exposure of young Tilapia destined for use as brood stock to MT-treated feed during the early development of the fish resulted in a case of mistaken sexual identity during later brood-stock selection. The subsequent failure of a recirculation aquaculture facilities brood-stock to meet production expectations resulted in the case presented here.

Mass Mortalities In The Brook Stickleback (*Culaea inconstans*) Exhibiting Abnormal Abdominal Tumor

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Widespread tumor-like lesions along with 100% mortalities were reported from a Brook stickleback population residing in a 4-acre stocking pond in northern Michigan. No other species were affected at the time of outbreak. Affected fish exhibited relatively large, symmetrical abdominal swellings that disabled fish swimming. Microscopic examination of the tumor contents revealed the presence of large numbers of oval spores that measured 5 um length and 2 um width with a prominent posterior vacuole. Electron microscopy indicated that the tumor-like lesion is a typical xenoma microsporidians and that the spores and developmental stages are those of *Glugea anomala*.

Life Is Never Dull At College Park

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Three case reports and one on-going project will be presented. The first case involved tilapia from an aquaculture facility presented with bulging eyes that literally fell from their respective sockets. A second case, also involving tilapia, presented with gills filled with *Leptospira*. The source of infection is believed to be fish food contaminated with rat urine. The third case involved sturgeon with a severe case of mites. In our on-going project, we have also isolated more than 450 Mycobacteria over the last few years from both cultured and wild caught fish. Mycobacteria were cultured and submitted to fatty acid methyl ester (FAME) analyses using gas chromatography. Bacteria were grown on Middlebrook agar and incubated at 28 C until there was sufficient growth for analysis. A dendrogram of these isolates was produced to allow cluster analysis. This dendrogram, which measures the relatedness of isolates based on several characteristics, allows identification of strains or species, some of which appear novel. The dendrogram groupings will be supported by polymerase chain reaction results and sequencing. Life is never dull.

Effects Of Elevated Suspended Sediment On Stress, Growth And Gill Condition Of The Spotfin Chub And The Whitetail Shiner

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North America's freshwater fish fauna continues to decline, in part due to excessive sedimentation in streams and rivers. The objective of this research was to investigate the effects of elevated suspended sediment concentration (SSC) on the spotfin chub (*Erimonax monachus*), an imperiled southern Appalachian minnow, and on a surrogate species, the whitetail shiner (*Cyprinella galactura*). I investigated the effects of SSC (0, 25, 50, 100, and 500 mg/L) on the stress response (cortisol concentration), specific growth rate (percent change in mass per day), and gill condition (i.e., lamellar thickness and interlamellar area) of young-of-year (YOY) spotfin chubs and whitetail shiners. Exposure of YOY to elevated SSC caused a significant increase in cortisol levels, in both species and a significant decrease in growth rate at three life stages. Increased SSC elicited a stress response in spotfin chubs 3-fold higher than controls; this response was similar to previous accounts of rainbow trout exposed to acute handling stress. For spotfin chubs, a 15-fold decrease in specific growth rate occurred at the highest SSC (500 mg/L). Gill damage observed by quantitative confocal microscopy was minimal at 0, 25, and 50 mg/L, moderate at 100 mg/L, and severe at 500 mg/L. Specific growth rate was significantly and inversely related to increasing gill lamellar thickness. The sublethal effects documented here support the hypothesis that elevated suspended sediment contributes to the imperilment of southeastern native fishes.

Infectious Hematopoietic Necrosis Virus In Atlantic Salmon Seapens In British Columbia

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Atlantic salmon were introduced to British Columbia (BC) for aquaculture production in the mid 1980's. In 1992, infectious hematopoietic necrosis (IHN) was first diagnosed in pen-reared Atlantic salmon, and over the next 5 years spread to 14 net-pen sites in a very localized area on Vancouver Island. IHN was not reported again until August 2001 at a location near the 1992 index case. Since then the virus has been detected in over 25 net-pen sites over a wide geographic area around Vancouver Island. IHNV isolates were collected from Atlantic salmon in seapens, and from Pacific salmon in Vancouver Island and mainland BC watersheds. Sequence analysis of the midG region, a 303 base sequence in the middle of the glycoprotein (G) gene, separated the recent seapen IHNV isolates into two sequence types within the U genogroup of IHNV. Comparisons of historical IHNV isolate midG sequences from throughout BC from 1974 to 2001 with the recent Vancouver Island Atlantic salmon sea pen outbreaks will be presented.

Virulence, Monoclonal Epitope, And Genome Analyses: A Conjoined Assessment Of Infectious Pancreatic Necrosis Virus Isolates Endemic And Exotic To The Great Lakes Basin

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Attendant to the "Strategic Vision of a Healthy Great Lakes Ecosystem," the Great Lakes Fish Health Committee is developing a risk assessment decision-making process to estimate the impact on the ecosystem of various biota, including fish pathogens. An appraisal of the virulence of isolates of infectious pancreatic necrosis virus (IPNV), endemic and exotic to the Great Lakes Basin, is a component of this program. We began with a collection of 129 IPNV isolates, representing historic and contemporary recoveries from epizootics and routine fish health inspections involving diverse species of fishes and invertebrates. Conjoining analyses of virulence (mortality ranging from <10% to near 100%), monoclonal epitope (19 Birnadent groups) and genome sequencing (phylogenetic analyses of nucleotide and deduced amino acid sequences of the VP2 variable region), we winnowed our collection to evaluating 40 IPNV isolates. Virulence estimates are being developed in 8 species of salmonid fishes using standard immersion challenges, IPNV-carrier excretion exposures, and defined chronic waterborne exposures.

Largemouth Bass Virus In New York State: An Update

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We have undertaken an effort to determine the distribution of Largemouth Bass Virus (LMBV) in New York State. The virus had been found in Lake Champlain, on the New York–Vermont border, in 2002 through the efforts of the Vermont Fish and Wildlife Department. Our efforts began in the summer of 2004. Largemouth bass were provided by the New York State Department of Environmental Conservation and by the Cornell Biological Field Station, Oneida Lake. The fish were collected in connection with previously scheduled fish population monitoring efforts. Our goal was to test a minimum of 5 fish per source. The samples were screened using the fathead minnow cell line. In addition, a quantitative PCR test for the LMBV was developed and used to conduct parallel screening of all samples. During 2004, 107 largemouth bass were collected from 12 lakes in New York State. Based on cell culture efforts in FHM cells, all fish were negative. All cell cultures were maintained for 28 days and had gone through 2-3 blind passages before being scored negative. Quantitative PCR testing indicated a positive largemouth bass from Hyde Lake. In a related effort, we are developing a cell line from the largemouth bass. We hypothesize that this cell line may be a more sensitive cell culture system for the culture of the largemouth bass virus.

First Report Of Koi Herpesvirus In Wild Common Carp In The Western Hemisphere

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Koi herpesvirus (KHV) is a recently discovered virus infecting koi and common carp *Cyprinus carpio*. Most cases of KHV disease have been reported in the ornamental fish industry and farmed common carp, but KHV has been reported in wild populations of common carp in the United Kingdom and Japan. During April and May 2004, thousands of wild common carp died in the Santee-Cooper Reservoir system in South Carolina. Three moribund fish were captured having ventrally located reddened areas of skin and severe necrosis of the gills. *Flavobacterium columnare* was observed in both wet mounts and histological preparations of gills. Additionally, *F. columnare* was isolated on agar plates from both from the gills and internal organs. Histological lesions characteristic of KHV disease were not observed in any organs in any of the fish. Cell culture of samples from each fish on fathead minnow cells, epithelioma papulosum cyprini cells, and koi fin cells also failed to produce any cytopathic effect. Organ samples were tested for KHV with a polymerase chain reaction assay. All samples from the 3 fish collected during the outbreak were positive for KHV. To our knowledge, this is the first report of KHV infection in wild common carp other than in the Western hemisphere.

Diagnosis Of Goldfish Hematopoietic Necrosis (*Cyprinid herpesvirus 2*) By Quantitative PCR

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Koi and goldfish producers and hobbyists are very concerned about koi herpes virus (KHV) and spring viremia of carp virus (SVCV), but have overlooked other viruses that may be equally important. The CyHV-2 virus (associated with hematopoietic necrosis disease in goldfish) produces high mortality and has been reported in Japan, Taiwan, Australia and the United States, but this pathogen is rarely reported. This is because diagnosis of the disease has been based only on electron microscopy and histological findings. Recently, degenerate PCR of viral DNA polymerase genes and sequencing showed that tissues from moribund goldfish, sampled during three epizootics in North America, carried the CyHV-2 genome. This work established that the disease is widespread in the United States and demonstrated the need for a sensitive and specific assay for CyHV-2. In our laboratory, we have developed a TaqMan PCR method for CyHV-2 that targets the viral DNA polymerase gene. Using a cloned version of the PCR product and North American cases as controls, we have validated the assay and determined that it is specific for CyHV-2, detects as few as 200 copies of the viral genome per reaction, is quantitative over 8 logs of template number, and detects the virus in asymptomatic fish and brood stock.

Spring Viremia Of Carp Virus In Rainbow Trout: Possible But Uncommon

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Generally, the cyprinid vesiculovirus SVC (genogroup I) does not readily infect salmonids; however, there are no epizootiological constraints that prevent such transmission and the susceptibilities of salmonid cell lines (RTG-2 and ASE) to SVCV suggests that this is feasible. We repeatedly isolated SVCV from a cohort of rainbow trout held in our laboratory that were bath infected with VHS virus (>2 years earlier) to monitor viral cycling. The isolation was originally believed to be contamination from SVCV-M2 infected common carp. Isolations were done from skin, fins, gills, brains, and pooled kidneys, spleens, and livers, which yielded an isolate (FB1/03) from internal organs. It was not neutralized with IHNV or VHSV antisera. The CPE was delayed but not neutralized by M2-SVCVantiserum and by M2-SVCV mixed with VHSV antisera, suggesting that the virus mutated in its novel host. Using two primer pairs to viral N-gene, RT-PCR identified the agent as SVCV. Data from N-gene based RT-PCR and RFLP suggested that the isolate was a mix of two genetically distinct strains. Independent G-gene sequencing at CEFAS provided similar evidence indicating that one strain belonged to the "parent" genomic subgroup Id and another belonged to subgroup Ic. Interestingly, FB1/03 caused low morbidity absent mortality when injected in yearling common carp, but produced acute disease (70% mortality) in rainbow trout (~1g) compared with 55% mortality produced by M2-SVCV. Both strains were recovered from moribund fish indicating that SVCV multiplied in the rainbow trout ($10^{7.85}$–$10^{8.1}$ $TCID_{50}$/g tissue). After a two week bath challenge in M2, only one of 50 rainbow trout (~0.3g) died absent clinical signs but with high virus load ($10^{8.85}$ $TCID_{50}$/g tissue). Neither disease nor mortality was evident among common carp cohabited with these trout. Another SVCV strain from common carp, KU/0204, killed 2 of 96 bath infected rainbow trout producing hydrocephalus and skin hemorrhages with moderate viral titers in tissues ($10^{4.35}$–$10^{7.6}$ $TCID_{50}$/g). Carp injected with virus re-isolated from the dead rainbow trout fry had reduced morbidity and mortality compared with the original KU/0204 strain. By injection, this strain produced 50% mortality in 3-mo trout (~1.3g) with signs of acute exsudative hemorrhagic syndrome and high virus titers ($10^{8.35}$ $TCID_{50}$/g tissue). The virus did not cause disease in 4-mo rainbow trout (~4g) and was not recovered from occasional mortalities or surviving fish, however, semi-nested RT-PCR detected it in 20–50% pooled tissue samples, 34 days post injection.

In Vitro Sensitivity And Specificity Analysis Of Spring Viremia Of Carp Virus Diagnostic Tests

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Spring viremia of carp (SVC), which was considered a foreign animal disease in the US, was first detected in April 2002. The viral disease continues to pose a major threat to the carp industry, as well as free ranging cyprinid species. There are several diagnostic tests that are available for the detection of SVCV. However, the tests have not been validated. *In vitro* sensitivity and specificity for several SVCV diagnostic tests were evaluated. Serial dilutions of SVCV virus as low as 10^1 $TCID_{50}$/mL were used to evaluate the *in vitro* sensitivity of SVCV diagnostic tests. *In vitro* specificity was evaluated by serially diluting five different strains of fish rhabdoviruses and testing using SVCV diagnostic tests. The study showed that a minimum viral titer of 10^5 $TCID_{50/mL}$ is required to detect the virus in cell culture within 48 hours. Molecular and serological based techniques (RT-PCR, ELISA and IFAT) proved to be more sensitive and were able to detect the virus in less time than virus culture. Among these tests, RT-PCR was the most sensitive and detected the virus at 10^4 $TCID_{50}$/mL. RT-PCR and ELISA assays did not detect any fish rhabdovirus other than SVCV. *Although In vivo* studies are pending, the preliminary findings of this study indicate that RT-PCR could be recommended as a test of choice for rapid and accurate diagnosis of SVCV.

Cutaneous Infection Of Channel Catfish By Microscopic Nematodes

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In January 2005, two samples of channel catfish (*Ictalurus punctatus*) were submitted by an Arkansas fish farmer. Focal, raised, firm 3-5 mm lesions were scattered over the entire tegument of the fish. Fish that were 3-years-old (1.5 kg) had many lesions (20 up to 400) while younger fish in the same holding facilities were not affected. The farm manager reported that the lesions ruptured and became hemorrhagic over the winter months and then healed over during summer. In two of the larger fish submitted, many of the lesions were ulcerated and hemorrhagic. The farmer reported no changes in feeding behavior and no mortality. Necropsy revealed that the lesions were located within the dermis layer of the skin and that live microscopic nematodes were present. Histopathology showed cross sections of nematodes associated with necrotic muscle debris, lymphocytes and macrophages. No other nematode life stages or nematode species were found in any other fish tissue. To our knowledge this nematode species has not been previously identified. Historically, there have been twelve cases of this skin condition reported in Arkansas since 1965 and two cases in Mississippi. We will be working in collaboration with Dr. Al Dove, of Stony Brook University, to identify the nematode species using molecular techniques.

Widespread Skin Ulcerations In The Yellow Perch Population Of The Lake St. Claire

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Widespread skin ulcerations were reported exclusively in Yellow perch collected from Lake St. Clair, Michigan, during summer 2004. The case has directed public attention and concerns about zoonotic potentials have been raised. In almost all fish submitted for analysis, shallow ulceration in posterior side of their body surrounded by hemorrhagic rim were observed. The affected fish were emaciated exhibiting sunken eyes and rough, dry skin. Bacteriological examination revealed consistent isolation of *Pseudomonas fluorescens* from skin lesions and kidneys of affected fish.

A Moribund Lake Sturgeon From The Niagara River, New York

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A moribund lake sturgeon *Acipenser flavescens* was found on 1 January 2005 in shallow water along the edge of the Niagara River near Wheatfield, NY, upstream from Niagara Falls. The fish was still alive on 2 January, but died on the morning of 3 January and was transported by the New York State Department of Environmental Conservation to Cornell University for examination. Upon presentation, the fish was 229 cm and 95.3 kg (7' 6" long, weighed 210 lbs). Evaluation of a cross-section of the anterior fin ray of the left pectoral fin revealed that the fish was 75 years old. Gross examination of the fish revealed some petechiation of the ventral body surface, which was likely due to it resting on the bottom of the river for several days. No other gross pathology was noted. Bacterial cultures from the posterior kidneys were negative for growth. An attempt at virus isolation on the white sturgeon cell line (courtesy of R. Hedrick, UC Davis) was negative. Histopathology of major organs was unremarkable. A primary rule-out was botulism type E as this condition has become problematic in the Lower Great Lakes. Gut contents, liver, kidney, spleen and blood were evaluated for presence of botulism type E with a quantitative PCR test. The results of the botulism testing were negative. The fish was found in a location known to have been heavily contaminated with hexachlorobenzene.

A Fish Kill Of Common Carp In New York State And An Unusual Koi Herpesvirus

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Koi herpesvirus (KHV), a highly contagious and lethal virus affecting both koi (*Cyprinus carpio koi*) and common carp (*Cyprinus carpio*) was isolated in 1998 from two outbreaks of koi suffering mass mortality in the New York State, USA and in Israel. However, the disease was described as early as 1996 in Europe. In July 2004, this virus was found to be associated with a mass mortality event in wild common carp in the Chadakoin River in western New York State. Affected fish typically showed marked hyperplasia of gill tissues and severe multifocal to diffuse external haemorrhages. The viral isolate found in this outbreak was somewhat unusual in that it grew well on fathead minnow FHM cells giving initial rise to the fear that the virus was Spring Viremia of Carp Virus (SVCV). Testing at the National Veterinary Services Laboratories, Ames, Iowa, USA, confirmed the virus's identity to be that of KHV. KHV is not currently on the OIE (Office International des Epizooties) list of notifiable diseases however is capable of causing mass mortality in susceptible fish at permissive temperatures.

Encounters With Fungi In Sygnathid Fish

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From 2000-2005, sygnathid fish represent the largest number of submissions from aquaria to the pathology service of the Connecticut Veterinary Medical Diagnostic Laboratory (UConn, Storrs, CT). With greater frequency in recent years, phaeoid fungi have been identified as agents of cutaneous lesions or systemic infections. A desire to characterize these phaeoid fungi has been the incentive to culture an increased number of specimens suspicious of having fungal lesions. The result has been morphologic and molecular identification of several *Exophiala* species, a novel one isolated from systemic lesions of seadragons (*Phyllopteryx taeniolatus* and *Phycodurus eques*), and another (*E. pisciphila*) infecting potbellied seahorses (*Hippocampus abdominalis*). More frequent attempts at fungal culture have led to isolation of several notable hyaline hyphomycetes, e.g. *Paecilomyces lilacinus*. Active fungal testing is necessary to understand both the range of fungi, as well as the range of clinical and pathological manifestations, encountered in certain aquarium-maintained fish.

Furunculosis In The Sea Lamprey (*Petromyzon marinus*) In Lake Ontario

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For the last six decades, the Laurentian Great Lakes have been plagued with parasitic sea lamprey that invaded the basin causing devastating losses in salmonid fisheries. To reduce the number of sea lampreys, the Great Lakes Fishery Commission began a large-scale program based on trapping male sea lamprey, sterilizing them, and subsequently releasing the sterile males back into streams where they compete with fertile males for spawning females. These transfers of lamprey to different locations may additionally transfer various pathogens concurrently, which have raised major concerns regarding the possibility of resident fish populations becoming infected. During health inspection of a group of sea lampreys, collected from Lake Ontario, for their suitability to translocation within the basin, lampreys with obvious furuncles (1-2 cm in diameter) were noticed. Most of the furuncles occupied the dorso-lateral musculature of affected lampreys. From the furuncles, spleens, and kidneys of affected fish, typical *Aeromonas salmonicida* subsp. *salmonicida* isolates were retrieved. Additional isolates were retrieved from clinically asymptomatic fish. Biochemical profiles and variations between the isolates were compared among each others and among those isolated from other fish species in Michigan. It is highly probable that sea lamprey play a role in the temporal and spatial spread of *A. salmonicida* in the Great Lakes basin.

Lesions Caused By *Pomphorhynchus* spp. (Acanthocephala) In Bluefish And Striped Bass

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In New York waters, both striped bass (*Morone saxatilis*) and bluefish (*Pomatomus saltatrix*) are infected by acanthocephalans of the genus *Pomphorhynchus*, but whether or not the parasites are the same species has not yet been determined. *Pomphorhynchus* spp. prefer the rectum, where they can occur in very large numbers. Striped bass and bluefish were examined for pathologies associated with pomphorhynchid infection and to investigate a potential role for this parasite in the epidemiology of mycobacteriosis. In both hosts, worms penetrate all layers of the rectum with a narrow extension of the trunk, which is inflated in the coelom and thereby acts as a permanent anchor (the spiny proboscis probably plays little part in attachment). Presence of worms was associated with complex mixtures of pathologies and host responses. The former included interstitial hemorrhage, necrosis and sloughing of the rectal epithelium, whereas the latter include thickening of the *lamina propria*, multiple layers of epithelioid cells, and fibrous connective tissue encapsulation of parasites. Sites of attachment were also associated with multifocal granulomatous inflammation. In one bluefish these granulomas were acid-fast positive and acid fast bacilli were observed in the spaces between the parasite and the layers of host response. The penetration of the host rectum by *Pomphorhynchus* spp. may play an important role in providing mycobacteria in the digestive stream with a patent conduit to the coelom.

An Outbreak Of Phaeohyphomycosis In Hatchery-Reared Chinook Salmon Fingerlings

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A hatchery experienced a major die-off of fingerling Chinook Salmon (*Oncorhynchus tschawytscha*) during Feb. and March, 2003. Clinical signs included abnormal swimming behavior, loss of appetite, exophthalmia, distended abdomens, and redness surrounding the vent. Internal examination revealed pale livers, distended stomachs filled with yellow fluid, swim bladders which were collapsed and contained white necrotic material. Mortality was estimated at 8-12%, with nearly 100,000 presmolts dying. Wet mount smears of the swim bladder contents revealed large numbers of branching, septate fungal hyphae. Mycotic growth appeared in 3 days when swim bladder contents were inoculated onto Sabouraud's dextrose agar media. The small subunit ribosomal DNA (18S ssu-rDNA) was amplified by PCR, sequenced, and compared with sequences in GenBank, and found 100% homology with of *Phoma herbarum*. Histologically, the fungus appeared to enter the host swim bladder forming a dense mat of septate, irregular branching, non-pigmented hyphae. These hyphae obliterated the swim bladder wall, and extended into the stomach, kidney, gonads, coelom, blood vessels and mesentery. Normally, *P. herbarum* is a plant saprophyte; it has been reported from the water, soil, diseased and dead plants worldwide. It has rarely been associated with disease in fish; a single previous report from a die-off of hatchery chinook and coho salmon occurred in Washington state in 1975. Interestingly, the hatchery in this report was free of the disease in 2004, but experienced a recurrence in March, 2005. Phaeohyphomycosis due to *P. herbarum* may be an emerging disease of hatchery-reared salmon, and fish health personnel should be on the alert for this pathogen.

Livid Lesions In Lumpfish (*Cyclopterus lumpus*) Associated With A Fungus *Exophiala pisciphila*

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During the preceding 40 years, The Registry of Tumors in Lower Animals (RTLA) has been the fortunate recipient of many excellent diagnostic cases. Quite often, submitted specimens contain tumor-like lesions that are ultimately discovered to be non-neoplastic. The present case involves two captive lumpfish (*Cyclopterus lumpus* Linnaeus 1758) from the Woods Hole Science Aquarium, Woods Hole, MA, that were originally collected in the Gulf of Maine during routine biological sampling by NOAA Fisheries Service vessels. While in captivity, these fish were maintained in a cold water (average T = 49-50^{o}F) aquarium system. Signs of disease in the submitted fish included skin ulcers and sudden death. Skin scrapings yielded fungal elements and paramecium-type protozoa. Necropsy revealed abundant peritoneal fluid, and black, red, or green nodules (0.5-2.0cm) in multiple organs, including the kidney, cardiac ventricle, spleen, liver, gill, stomach, and skin. Microscopically, the nodules were determined to be infarcts (areas of necrosis caused by vascular obstruction) in various stages of progression. Thrombi associated with the infarcts contained abundant narrow, branching, septate, brown-pigmented, angiocentric, fungal hyphae. Cultures from two lumpfish on CM+ yielded colonies of the same fungus. The fungus was identified as *Exophiala pisciphila* based on colonial and microscopic morphology, and comparison with reference isolates of this species.

Coldwater Disease Outbreak At A West Virginia Rainbow Trout (*Oncorhynchus mykiss*) Farm: An Unusual Presentation

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In 2004, a WV trout farmer reported mortality in three month old rainbow trout fingerlings reared in flow-through single-pass tanks. The groundwater supply was 12°C and good quality for trout culture. Mortality was >100 fish per tank per day in a population of 35,000. The problem was noticed two weeks before when fish stopped feeding in one tank and then fish in 15 other tanks went off feed. Fish were lethargic with exophthalmia, thin, had pale red gills and kidneys, red-tinged coelomic fluid and pale brown livers. Some were laying on the bottom of the tank with bilateral pigmentation. The clinical diagnosis was bacterial or viral septicemia. Samples were collected for virology and bacteriology. The spread from tank to tank was consistent with the cleaning routine used by personnel. The option of antibiotic-medicated feed was declined by the farmer. He was instructed to remove all dead and dying fish twice a day, work on sick fish last, use separate equipment for each tank, chlorine for equipment, gloves or hand-washing for fish handlers, block off the area with tape and cones and transfer fish to clean, disinfected tanks. The mortality rate declined quickly after changes were implemented. Histologic changes were consistent with bacterial septicemia, and involved spleen, heart, gills, bone, and cartilage. The distribution of bone lesions was typical of cold water disease. The organism stained with Warthin-Starry silver stain, but not with Brown and Brenn tissue Gram stain. Three *Flexibacter*-like isolates from three fish were cultured. One was confirmed as *Flavobacterium psychrophilum* by biochemical characterization and all three were *F. psychrophilum* as assessed by 16S sequencing.

Probable Branchiomycosis Among A Captive Population Of Horseshoe Crabs, *Limulus polyphemus,* From Delaware Bay

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A laboratory maintained population of horseshoe crabs, *Limulus polyphemus*, experienced an acute high-level mortality event in the summer of 2004 following the introduction of additional animals taken from the Delaware Bay. Necropsy of affected animals revealed multifocal, pale, often circumscribed lesions over the ventrum, particularly concentrated on the gills. Microscopic examination of affected branchial tissues revealed the consistent presence of dense aggregates of branching, aseptate hyphae with associated tissue reaction including amoebocyte cell infiltration. Treatment was attempted for the remainder of the population through manipulation of water quality parameters and freshwater baths, but these measures were not successful, and mortality continued at a slower rate throughout the fall and winter. An oomycete-like organism repeatedly isolated from affected branchial tissues is the presumed pathogen. Work to identify and further characterize this organism is currently ongoing.

The Role Of The Anterior Kidney In Generating Long-Term Immunity

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Recent work in our laboratories has been dedicated to the resolution of various stages of antibody secreting cell development, including the production and dissemination of plasmablasts, long- and short-lived plasma cells, as well as putative memory precursors. These studies have demonstrated that long-term antibody production is not uniformly distributed throughout all immune tissues, but exclusively resides within a pool of long-lived plasma cells that are maintained in the anterior kidney. It is striking that two developmentally distinct functions which reside in the bone marrow of mammals (hemopoiesis and plasma cell maintenance) both solely reside within the anterior kidney of teleost fish. Such a dual occurrence prompts questions as to the underlying reasons for the preservation of dissimilar functions with the same tissue. Using markers for transcription factors such as Pax-5, Blimp-1, and surface and cytoplasmic Ig we plan to resolve the various developmental and trafficking processes as well as the role that critical niches play within the teleost anterior kidney for the production of these differentiated lymphocytes.

Development Of *In Vitro* Methods To Determine Antibody Gene Specificity In Salmonids

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Salmonids utilize at least eleven different families of VH immunoglobulin genes in the production of antigen binding sites. Individual VH gene families, however, have yet to be correlated with their antigenic specificities. To determine whether family specificity occurs, we are developing *in vitro* immunization techniques that result in production of antibody with high specificity and affinity. Concurrently, we are examining the mRNA from cultured cells secreting antibody, utilizing such molecular techniques as 5'RACE with primers for secreted antibody, cloning, and sequencing. Currently employed antigens include *Vibrio anguillarum* lipopolysaccharide (LPS), phosphorylcholine (associated with streptococcal species), G protein of infectious hematopoietic necrosis virus (IHNV), p57 of *Renibacterium salmoninarum*, as well as the common anthropogenic antigen, TNP. Strategies for B cell stimulation incorporate recent data delineating the regional development of plasmablasts or plasma cells from specific tissues. In summary, this work may lead to improved genetic markers for disease resistance as well as estimates of robustness of resistance within salmonid populations.

Unique Mechanisms Employed By Trout To Generate Antibody Functionality

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The predominant trout serum Ig is a tetrameric molecule which varies in the degree of inter-monomeric disulfide crosslinking. This non-uniform crosslinking leads to considerable structural diversity (redox forms) of the tetrameric product, with either fully crosslinked tetramers or tetramers composed of non-covalently associated monomers, dimers, and trimers. There is limited data available on the role of such structural diversity amongst teleost antibodies in regard to antigen binding or effector function. To address this, we have studied the relationship of affinity distribution and redox structure using selective adsorption of Ig using a TNP-BSA carrier with varying haptenation ratios. We have observed that selective adsorption can cause significant skewing of affinity profiles of antibody subpopulations from the same serum sample. The average affinity of the low-density hapten-carrier conjugates (TNP_1-BSA) adsorbed antibodies possessed the higher apparent affinity constant (aK) than that of those adsorbed by the high density conjugates (TNP_{13}-BSA). Further, there selective antibodies demonstrated different redox profiles. TNP_1-BSA preferentially bound the higher order redox forms (i.e. those wherein the tetramers were completely disulfide polymerized, or containing a substantial proportion of cross-linked trimers). However, TNP_{13}-BSA captured significantly less higher order forms and possessed increased concentrations of lower order forms (i.e. unlinked monomers and dimers). The linking of the binding affinity/avidity to redox structure is discussed, and an alternate structural model of antibody affinity maturation is explored.

Unique Difficulties In The Development Of Serological Reagents For Striped Bass Antibody Responses

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The development of serological reagents against striped bass Ig is pivotal in the monitoring of humoral immune responses in this species. Despite extensive effort on the part of a number of investigators, these reagents have not been forthcoming due to what we believe to be the unique characteristics of the striped bass Ig. The difficulty in producing these reagents appears to stem from the degree to which striped bass Ig is glycosylated. We have found that, originally all Mabs generated were against carbohydrate epitopes, which exhibit a large degree of cross-reactivity with other serum components as well as some Mycobacterial antigens. Deglycosylation of striped bass Ig allows the production of heavy chain and light chain specific Mabs, which all appear to be specific for the Fab region of the antibody. Thus such epitopes become blocked as the antibody binds to antigen, rendering the mAbs useless for determining antibody titers. A three step process was therefore developed to generate Mabs specific for striped bass Ig that would work in various formats. Mice were immunized and boosted with highly purified, denatured and reduced striped bass light chains. Four days prior to fusion the mice were IV boosted with affinity-purified striped bass antibody and fused with standard techniques. All screening was performed using anti-TNP striped bass antibody bound to TNP-BSA coated plates. This technique has allowed the development of a suite of antibodies specific for native striped bass Ig, that has been successfully used in ELISA, western blotting and immunohistological applications.

Mechanisms of Antibacterial Resistance by Nonspecific Cytotoxic Cells

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Nonspecific cytotoxic cells (NCC) are the teleost equivalent of mammalian NK cells. NCC kill tumor and viral transformed cells via receptor mediated target cell recognition. The role of NCC in innate anti-bacterial immunity has recently been suggested by finding a scavenger receptor homologue as well as a new pattern recognition protein on these cells. This new protein (referred to as NCC cationic antimicrobial protein-1/ncamp-1) binds bacterial DNA, oligodeoxynucleotides and polyguanosine. Sequence comparisons of this protein indicated similarity to zebrafish histone family member 1-X and (to a lesser extent) to trout H1. Inspection for signature sequences or repeats in ncamp-1 indicated the presence of multiple lysine based motifs composed of AKKA or PKK repeats. Membrane expression of ncamp-1 on teleost cells was confirmed using a rabbit polyclonal antiserum raised against the recombinant protein. Binding competition experiments demonstrated that both the polyclonal and oligodeoxynucleotide bound to the same protein (i.e. ncamp-1) on NCC. In recombinant form, ncamp-1 not only binds to the oligodeoxynucleotides, but also kills certain Gram positive and Gram negative bacteria. We suggest that ncamp-1 may belong to a new class of pattern recognition receptors. As a membrane protein it binds bacteria to exacerbate inflammatory responses, whereas in soluble form it elicits bactericidal activity. Research supported by BARD US-3159-99C.

Effector Molecules Of Cell Mediated Cytotoxicity In Catfish And Tilapia NCC

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The existence of cell-mediated cytotoxicity in lower vertebrates has been well documented but the molecules involved in this process had not been identified until recently. We have previously reported the functional significance of non specific cytotoxic cell (NCC) activity in catfish and tilapia. As part of the innate immune system, these cells are important in the defense against viral, bacterial and parasitic infections. We hypothesized that the multiple roles of NCC activity would require diverse pathways to kill target cells. We now show molecular evidence that NCC indeed employ several mechanisms to kill target cells with granule-exocytosis as well as TNF superfamily mediated effectors. These cells express multiple granzymes along with accessory molecules like perforin, granulysin and serglycin. The recombinant granzymes were expressed and substrate specificities were assessed using various thiobenzyl esters revealing granzymes with tryptase and chymase activities. We had previously demonstrated functional evidence for the secreted form of Fas ligand in NCC, which is capable of inducing cell death in susceptible target cells. Now we also show that NCC express the membrane-bound form of TNF-alpha, which induces cytotoxicity in susceptible targets. Recombinant TNF (rTNF) has been expressed and has been tested for its effects on NCC. rTNF appears to function in the regulation of NCC activity by protecting from activation-induced cell death and by increasing the expression of granzymes. These data show that NCC have pleitropic activities with multiple pathways of target cell death providing diverse roles in innate immune mechanisms. Research supported by BARD # US-3159-99C and by grants from the Veterinary Medical Experiment Station of the State of Georgia.

Mannose-Binding Lectin Of Channel And Blue Catfish

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The channel catfish (*Ictalurus punctatus*) is extensively used in aquaculture in the Southeast U. S. and is susceptible to bacterial infections acquired from its pond environment. Research is needed to understand innate immunity and the catfish defensive response against bacterial pathogens. We purified by affinity chromatography a mannose-binding C-type lectin (MBL) from channel catfish serum and also isolated MBL from blue catfish (*Ictalurus furcatus*) serum. MBL in mammals acts as an opsonin for phagocytosis by macrophages and also activates the lectin complement pathway of the innate immune response. This leads to killing of Gram-negative bacteria and enveloped viruses. Another objective of the research was a quantitative comparison by the ELISA technique (using guinea pig anti-MBL IgG) of channel catfish, blue catfish, and other catfish strains for serum MBL as a resistance marker for innate bacterial resistance in catfish. Functional studies of catfish MBL binding to *Edwardsiella ictaluri* using the immunoperoxidase ELISA method were done. This research in catfish describes for the first time the existence of serum MBL and lectin complement pathway and adds a new component of innate immunity to catfish defense. MBL could be used as a molecular resistance marker for selection of innate bacterial resistance in the various strains of catfish used in fish farming.

Plasma Proteomic Analysis Of The Acute Phase Response Of Rainbow Trout To Intraperitoneal Inflammation And LPS Injection.

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An acute phase response was induced in rainbow trout (*Oncorhynchus mykiss*, Walbaum) by inflammation triggered by intraperitoneal administration of purified *A. salmonicida* LPS emulsified in Freund's incomplete adjuvant (LPS/FIA) or a commercial oil-based *A. salmonicida* vaccine. Acute phase proteins were characterized by comparative densitometry of plasma proteins separated by 2D-PAGE and identified by MALDI-TOF and ESI MS/MS mass spectrometry. In one experiment, plasma samples were compared between treatment and control groups in which fish were terminally bled. In another experiment, individual fish were sampled repeatedly. Proteins with increased expression were those whose normalized value increased three-fold or greater between pre- and post-stimulus. Proteins with reduced expression were those whose normalized values decreased two-fold or greater. Constitutive proteins were those that were not altered or did not meet either of these criteria. Proteins that were absent in pre-stimulus gels but present in post-stimulus profiles were considered to be induced. Only those proteins that were altered in all fish for a given treatment were considered. In both experiments, protein p36 was increased up to 13-fold and several proteins were detected that had not been previously. In all fish treated with LPS/FIA, p9.5 was consistently increased an average of 75-fold in plasma. The only proteins similar to previously identified proteins were pre-cerebellin-like (p24a), transferrin (p37) and apolipoprotein (p10.5, p24c and p28).

Cross-matching Of Elasmobranch Blood And A Transfusion Trial Within Atlantic Stingrays (*Dasyatis sabina*)

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Bite wounds have lead to mortalities in several species of shark due to hemorrhage and sepsis. Whole blood transfusions are a potential supplement to elasmobranch-adapted isotonic crystalloids and hemoglobin products. Cross-matching of red blood cells and sera was carried out between nineteen individuals from seven species: sandbar shark (*Carcharhinus plumbeus*), sandtiger shark (*Carcharhinus taurus*), common nurse shark (*Ginglymostoma cirratum*), white-spotted and brown-banded bamboo shark (*Chiloscyllium plagiosum* and *C. punctatum*), zebra shark (*Stegostoma fasciatum*) and spotted wobbegong (*Orectolobus maculatus*). Prior to the trial, the authors identified a suitable washing solution, anticoagulant and positive controls. No positive cross-matches were seen between individuals of the same species within sandbars, sandtigers and common nurse sharks. Positive cross-matches were seen as hemolysis or agglutination between several of the species, suggesting these would not be suitable as donor-recipient combinations. A subsequent trial involved transfusion of heparinized whole blood between five Atlantic stingrays (*Dasyatis sabina*). Two control animals received an auto-transfusion or crystalloids. No morbidity or mortalities were associated with the trial.

Reproductive Problems, Diagnoses And Treatments In Teleosts

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Many species of fish do not readily breed in captivity. Because of environmental threats and decreasing habitat, breeding fish in captivity may become necessary for the survival of several species in the wild. Fish reproductive problems and anomalies are a common occurrence in public and home aquaria. Many of these cases had been previously diagnosed on necropsy. With advances in fish medicine, reproductive problems are being diagnosed pre-mortem to allow for treatments. Diagnosis of reproductive related diseases begins with a thorough history and water quality evaluation, as well as knowledge of the individual species reproductive physiology. Some diagnostic modalities that can be used to evaluate the fish patient are CBCs, blood chemistries, ultrasound, radiology, computer assisted tomography, general palpation, laparoscopy, cystocentesis, and cloacal endoscopy. The most common problems encountered at the New England Aquarium in order of prevalence include: dystocia or egg binding, infectious disease, organ prolapse, organ trauma, and neoplasia. Specific treatments for reproductive problems in fish can include hormonal treatment, surgery, antibiotic or antifungal therapy, environmental changes, and nutritional adjustments. Some individual cases will be highlighted to examine diagnostic approach and treatment.

Ecology Of The Fish Pathogen *Mycobacterium marinum*

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Mycobacterium marinum poses serious health and economic problems to the fish industries. Biofilters and walls in the aquaculture recirculating systems can be potential sites of colonization by this pathogen once it is introduced into the aquaculture tanks. Colonization of surfaces by bacteria requires initial attachment to the surface and increasing evidence suggests that this process can be modulated at the molecular level by the bacteria's microenvironment. The purpose of this research was to examine attachment of *M. marinum* to hydrophobic surfaces in minimal-defined culture medium and to determine whether the nutrient content and age of the medium plays any role in influencing this interaction. As part of this study we determined the strength of hydrophobic interaction between *M. marinum* cell and the surface of polypropylene by using solvents of different polarities to disrupt this interaction. Only highly non-polar organic solvents were able to efficiently remove the attached bacteria from the surface of polypropylene. It was also found that the ratio of mycobacteria in the population that attached to the polypropylene decreased with the age of the culture and this was correlated with accumulation of capsular material on the cell surface of the bacteria. An examination of the protein profiles of cells from exponential and stationary phases by Two-D gel electrophoresis indicated significant upregulation of a ~ 40 kDa protein in the stationary phase. We identified this protein as the alanine dehydrogenase of *M. marinum* by LC MS/MS analysis. This enzyme is thought to be secreted or associated with the cell surface and may be involved in response to stresses such as hypoxic or microaerophilic conditions. Elucidation of the molecular events that regulate attachment and capsular biosynthesis in *M. marinum* will lead to development of efficient and logical strategies for preventing colonization and spread of this pathogen in fish tanks.

Mycobacteriosis In Wild-Caught, Laboratory-Maintained Menhaden: A Case Study

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Mycobacteriosis is a chronic wasting disease found in many species of fish examined from wild-caught, cultured and aquarium-reared populations. A menhaden (*Brevoortia tyrannus*) with an open ulcer from a wild-caught, laboratory maintained population was sampled for microbiology. *Mycobacterium fortuitum* complex and *Mycobacterium* spp. were recovered from the spleen and the ulcer of this fish, respectively. Subsequently, twenty menhaden were sub-sampled from this population to determine the extent of the infection. Bacteriology samples were taken from the brain, liver and kidney. Spleens were homogenized and plated on media enriched for mycobacterial growth, and full necropsies were performed and samples were taken for histology. Bacteriology results revealed a 100% rate of mycobacterial infection in this population of menhaden. Three species of mycobacteria were isolated from the spleen tissue: *M. marinum*, *M. fortuitum* complex, and *M. gordonae*. Histology results revealed that granulomas, characteristic of mycobacteriosis, were most prevalent in livers, but were also found in spleen, posterior kidney and heart. In addition, *Vibrio hollisae* and *Photobacterium damsela* were isolated from internal organs. This report adds menhaden to the list of susceptible wild species to mycobacteria. Ecologically, menhaden are an important prey species, and their susceptibility indicates the potential for disease transmission.

Chloramine Removal From Municipal Water Supply For Use In Recirculating Aquatic Life Support Systems

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Municipal water authorities are beginning to use chloramine, which has greater residual than chlorine. For Fishes and Invertebrates, chloramine creates equivalent deleterious health conditions as chlorine. Removal of chloramine at point-of-use is more difficult than removal of chlorine; it cannot be effectively removed by activated carbon. Catalytic activated carbon types break the amine bond but leave ammonia in solution. At the National Aquarium in Washington, D.C., a chloramine removal method employs catalytic activated carbon, zeolite, a nitrate specific resin and aeration. Catalytic activated carbon is shown to effectively break the amine bond and subsequently remove chlorine. Zeolite is effective at removing ammonia; however some ammonia is oxidized to nitrite before reaching the zeolite bed. A nitrate specific ion resin effectively removes both nitrite and nitrate. Although the combination of activated carbon, zeolite, and the nitrate resin effectively remove chloramine and its residual nitrogen species, frequent regeneration of the various media beds result in occasional spikes of nitrite and/or ammonia into the product water stream. Aeration is added as a final step to mitigate these spikes in nitrogen species.

Disaster Planning For Aquatic Animal Facilities

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Disasters can cause catastrophic damage and loss of life in aquatic animal facilities. Preparing for major building damage, loss of support infrastructure, inaccessibility during a crisis, and utility failure and subsequent life support challenges can minimize the impact of a disaster. Developing a detailed contingency plan that provides support for both staff and animals is key to ensuring a successful outcome—or at least minimizing the damage. This plan must include a designated disaster response team with established leadership and a chain of command, clear roles and responsibilities for each team member, methods of communication prior to and during a disaster, and a mechanism to provide essential utilities (power and water). Establishing affiliations and a working relationship with local emergency management service (EMS) will increase the likelihood that needed resources will be made available during the event. Next, resources must be identified, and equipment and supplies obtained. All equipment, such as generators and radios, must be tested on a routine basis to ensure it is in working order. Finally, training and drills must be conducted to ensure that all staff are prepared for a disaster. In most facilities housing fishes the loss of dissolved oxygen and temperature control are the most immediate concerns when power is interrupted. Emergency generators can be used to power air pumps and chillers. If no power is available, supplemental air can be added using SCUBA tanks or compressed oxygen. To maintain water temperatures in chilled systems, fresh or saltwater ice blocks can be placed in the tank. Propane or oil-fired water heaters can be used as a back-up to electrical units for warm-water systems. The accumulation of nitrogenous waste can also be deadly to fish. The risk can be minimized by reducing or stopping feeding for 1 to 2 days in advance of forecasted events such as hurricanes. If life support is interrupted, most fishes can be held for one or more weeks without food, as necessary, to maintain water quality. Disaster planning should be undertaken by all aquatic animal facilities. Common sense, coordination, communication, and training will help minimize the impact of any disaster. When disasters do occur, and life returns to normal, take the time to review and improve your plan, and make the most of the lessons learned.

Cooperative Investigations Of Suspect Disease Cases In Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*)

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The U.S. Fish and Wildlife Service, Northeast Fishery Center (NEFC-Lamar, PA) has taken a lead role in researching culture technology for Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*), an anadromous species which has declined in population size in eastern rivers of the United States. Beginning in 1991, NEFC developed methods of capturing, spawning wild adult Atlantic sturgeon and rearing juvenile sturgeon from the egg stage to age 12. The water supplies used for these efforts have included a recirculated saltwater system, surfacing spring water, as well as raw creek water. Circular tanks, ponds and serial use raceway rearing units have been employed. During the course of the sturgeon capture and propagation activities, several pathological situations became evident, some of which required assistance from researchers at the USGS National Fish Health Research Laboratory (NFHRL – Leetown, WV). Infections with *Saprolegnia* and other parasitic micro-organisms diagnosed and treated by NEFC staff are discussed. Winter kill, pathology associated with ingestion of *Bufo americanus* (American toad), and swim bladder inflation syndrome contributed to experimental culture mortalities. In addition, pathology associated with experimental yearling sturgeon on raw creek water was investigated. Findings of on site, small scale pathogenicity studies as well as findings of research assistance from the NFHRL will be presented.

Asesing Susceptibility Of Atlantic Sturgeon (*Acipenser oxyrinchus*) To *Aeromonas salmonicida,* Cause Of Furunculosis

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Restoration of Atlantic sturgeon (*Acipenser oxyrinchus*) is a goal of the Atlantic States Marine Fisheries Commission and the United States Fish and Wildlife Service has researched methods at its Northeast Fishery Center (NEFC-Lamar, PA) to enhance populations stocked into mid-Atlantic rivers. In two instances, *Aeromonas salmonicida* was isolated from brood fish from the Hudson River, but the pathogenic significance was unclear. This study assessed virulence of *A. salmonicida* for Atlantic sturgeon and attempted to transmit the pathogen to sturgeon by co-habitation with infected trout. Juvenile Atlantic sturgeon were found to be specific pathogen free after sampling 60 fish failed to isolate any bacteria systemically and only *Acinetobacter* sp., *Pseudomonas* sp., *Moraxella* sp., *Enterobacter* sp. and motile aeromonads were isolated externally. Nine of 12 *A. salmonicida* isolates injected at 10^5 cfu/sturgeon (3 fish/isolate) induced mortality within 14-days. Isolate 3.155, originally obtained from coho salmon (*Oncorhynchus kisutch*), produced complete mortality within 5-days and had $LD_{(50)}$ values of 2.3 cfu in brook trout (*Salvelinus fontinalis*) and $2.3x10^4$ cfu in Atlantic sturgeon. Therefore, sturgeon were susceptible to infection, but more resistant to *A. salmonicida* than a typical salmonid host, such as the brook trout. Brook trout were then injected with the bacterium and cohabited with Atlantic sturgeon. All trout died within 8-days and Atlantic sturgeon began to die at day 14. On day 21, surviving Atlantic sturgeon (n=21) were assayed and the pathogen was cultured from mucous (n=20), gills (n=17), kidneys (19), hearts (14), livers (n=13), spleens (n=7), and guts (n=3) of these fish. Thus, *A. salmonicida* was horizontally transmitted, established infection, and induced mortality in Atlantic sturgeon cohabited with infected trout. A natural furunculosis epizootic among juvenile Atlantic sturgeon at the NEFC is also discussed. These studies indicated that *A. salmonicida* is pathogenic for Atlantic sturgeon and suggested that these fish can contract furunculosis, especially when cultured where *A. salmonicida* is enzootic.

Pathogen Binding Proteins In Plasma Of Salmonid Fish

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Bacterial surface polysaccharides can be recognized by various humoral and cell-surface lectins exhibiting direct antibacterial activity or opsonizing capabilities. Identification, quantification and determination of the potential importance of lectins as immune-active proteins in fish, however, are recent activities. To assess specific binding affinities, plasma proteins from rainbow trout (*O. mykiss*), Atlantic salmon (*S. salar*) and Arctic char (*S. alpinus*) were isolated based on calcium and carbohydrate-dependent binding to synthetic matrices and pathogenic strains of *Aeromonas salmonicida* and *Flavobacterium pyschrophilum*. Several saccharide- or calcium-elutable proteins were isolated from all three species including those consistent with serum amyloid P and C-reactive protein. Three other proteins (16kDa, 37kDa and 40kDa) were also isolated from each species. The rainbow trout 37kDa protein exhibits sequence homology to human intelectin, which recognizes galactofuranose within carbohydrate chains of bacterial cell walls**.** The 16kDa rainbow trout protein was isolated by direct bacterial binding assays to both *A. salmonicida* and *F. psychrophilum*. Western blots have confirmed the specificity of a rabbit antiserum to the 16kDa protein that will be used for enzyme immunoassay quantification of individual and temporal variation during experimental infections. Our program goal is to determine the functional significance of these plasma lectins in salmonid resistance and/or susceptibility to infectious disease.

Laboratory Efficacy Of Amoxicillin For The Control Of *Streptococcus Iniae* Infection In Blue Tilapia

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Experimental feeding trials were performed to evaluate the efficacy of amoxicillin (AMX) in controlling *Streptococcus iniae* infection in blue tilapia, *Oreochromis aureus*. Doses of AMX tested were 0, 5, 10, 30 and 80 mg active ingredient per kilogram of fish body weight (BW) per day. Administration of medicated feed started within 22-24 h post challenge by waterborne exposure to *S. iniae* (after skin scraping) and continued for 12 consecutive days, followed by a 17 d post treatment observation. Oral administration of AMX medicated feed for 12 d at 10, 30 and 80 mg AMX/ BW/d significantly increased ($P<0.05$) the survival of *S. iniae* infected tilapia from 3.8 % in challenged non-medicated positive control to 45, 75 and 93.8 %, respectively. The survival rate was significantly higher in the 80 mg treatment (93.75 %) than the 10 mg treatment (45%) but there was no significant difference between the 30 and 10 mg treatments (75 and 45 %, respectively). At the conclusion of the experiment no carriers were detected in any challenged group receiving AMX medicated diet while the bacterium was recovered from the non-medicated challenged survivors of the infection.

Development Of A Tuberculosis Model Using The Small Fish Medaka (*Orysias latipes*) And *Mycobacterium marinum*

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Human infection by *Mycobacterium tuberculosis* (TB) is endemic, with approximately 2 billion people infected and is the leading cause of fatality with about 3 million deaths annually. Because of its slow growth rate (>24hrs) and infection risk to researchers, other species of *Mycobacterium* have been employed as alternative model systems for TB. Nucleotide sequence analysis has shown that *Mycobacterium marinum* is a closely related species to TB and has proven to be a good model for tuberculosis infection, conferring TB-like infections in some fish. Medaka (*Oryzias latipes*) have long been used for toxicological research, are easily propagated, and offer many strains, good genetics, and transgenic constructs. These include a variety that carry a shuttle vector for studying *in vivo* mutagenesis. We are currently working to test if medaka can provide an alternate model for tuberculosis-like chronic infections by *M. marinum*. We are able to induce both acute and chronic infections depending on bacterial dose. Bacterial colonization of specific organs has been observed by colony counts and can be visualized within organs using a *M. marinum* strain expressing green fluorescent protein (GFP). The immunological manifestations of infection are being surveyed by classical histology. With this new model for *M. marinum* infection, we will be able to study the possibility of a connection between chronic infection and mutagenesis, the role played by the DNA repair network in bacterial survival within its host, and explore other aspects of mycobacterial infections.

Localization By In Situ Hybridization Of A Chlamydia-Like 16S Ribosomal RNA Gene Sequence Within Branchial Epithelial Cells Of Cultured Arctic Char (*Salvalinus alpinus*) With Epitheliocystis

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Epitheliocystis refers to a gill disease associated with yet uncultured, gram-negative, intracellular, chlamydia-like or rickettsia-like bacteria that causes morbidity and mortality in a wide range of fish. Histologically, the agent forms intracytoplasmic, basophilic, granular inclusions in branchial epithelial cells. Attempts to develop Arctic char aquaculture have been plagued by epitheliocystis. Molecular techniques have been used successfully to characterize an agent of epitheliocystis in Atlantic salmon (Draghi et al. *Journal of Clinical Microbiology*, Nov. 2004, 42 (11):5286-5297). Those same techniques have been used to elucidate one possible etiological agent of epitheliocystis in Arctic char. Samples from the Fresh Water Institute were taken for histopathologic, ultrastructural and PCR studies. Histopathology was typical of epitheliocystis. Special stains commonly used to detect chlamydia reacted positively with branchial epithelial inclusions of char. Transmission electron microscopic studies revealed the existence of chlamydia-like life stages (reticulate bodies). The 16S rRNA gene signature sequence amplified from histologically positive gill samples using chlamydiales order-specific primers branched with members of the order Chlamydiales as opposed to those of *Rickettsia* and *Ehrlichia* in molecular phylogenetic studies using parsimony and distance methods. Amplicons were cloned into a dual-promoter vector and transcribed to generate digoxigenin-labeled riboprobes of 295-bp. These digoxigenin-labeled sequence-specific riboprobes when applied to infected arctic char gills labeled chlamydia-like inclusions within epithelial cells. No labeling was seen in the same tissue with a *Mycoplasma gallisepticum* 16S rRNA gene riboprobe, or in those sections not receiving riboprobe. To the best of our knowledge, this is the first example of a chlamydia-like sequence identified by in situ hybridization in char, and confirms earlier data suggesting that a chlamydial-like bacterium is associated with epitheliocystis in Arctic char.

Quantitation Of Immobilization Antigen-Specific Antibody Secreting Cells In The Skin And Head Kidney Of *Ichthyophthirius multifiliis* Immune Channel Catfish Using ELISPOT Assay

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This study provides the first quantitation of antibody secreting cells (ASC) isolated from the skin of channel catfish (*Ictalurus punctatus*). ELISPOT assays for the detection and quantification of ASCs were developed and the isolation of viable leukocytes from skin is described in detail. The density of leukocytes in skin was found to be between 2.5×10^4 and 3.5×10^5 cells/cm^2. Preliminary ELISPOT assays indicate that immobilization antigen-specific ASCs occur in the skin and head kidney of catfish immune to infection with the ciliated protozoan pathogen *Ichthyophthirius multifiliis*. The percentages of specific to non-specific ASCs in skin and head kidney are 1.35% (n=1) and 1.97% (n=2), respectively. Ongoing studies are focused on the ontogeny and kinetics of the cutaneous antibody response of fish following infection with the parasite.

Intersex In Smallmouth Bass From The South Branch Of The Potomac River: Implications Of Endocrine Disruption

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For the past few years, our laboratory, in collaboration with multiple agencies has been involved in a multidisciplinary study in the South Branch (SB) of the Potomac River. This work was initiated in response to observations of skin lesions, occasional fish kills and a perceived population decrease of smallmouth bass (SMB) by resource managers, fishermen and the public. During 2003 SMB were collected from multiple sites in the South Branch drainage and processed for histological evaluation. Internally, perhaps the most interesting finding was the high incidence of intersex in male fish at a number of sites. Intersex in male gonochoristic fishes is typically an indication of exposure to estrogens or other endocrine disrupting chemicals (EDCs). Thus this observation led us to suspect that such chemicals may be present in the SB. During the 2004 sampling seasons we collected SMB and other species in order to confirm the observation of intersex male SMB and investigate whether this condition was unique to SMB. Intersex was identified in SMB and largemouth bass (LMB), but not suckers during 2004. The frequency of intersex in SMB was high and ranged from 69-100% in pre-spawn fish. Preliminary screening of water and sediment samples has identified the presence known endocrine disrupting chemicals, but specific sources and seasonal concentrations are currently unknown. The high incidence of intersex in SMB may be associated with sediment or waterborne EDCs, but this suspicion has not been confirmed. Ongoing research has been designed to investigate the possible impacts of environmentally relevant concentrations of EDCs identified in this watershed on SMB and LMB reproductive development.

Bacteriological Studies On Freshwater Mussels: The Flora Present In Free-Ranging Animals And Pathogen Depuration By Two Species, *Amblena plicata* And *Fusconaia ebena*

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We report on studies to further understand the nature of the bacterial flora of freshwater mussels. A number of Agencies are involved with conservation, propagation and population augmentation of listed mussel species. One dimension is collection and captive-rearing of feral animals at fish hatcheries. Our effort is to study the potential for pathogen vectoring (i.e. contamination) to hatcheries and to natural habitats upon restoration. Seasonal mussel die offs have been observed in the Holston and Clinch Rivers in Virginia; an infectious agent(s) is suspected, however, none have been identified. We will report on the bacterial flora from healthy mussels from these rivers, which was studied to allow for subsequent comparisons with the flora from diseased mussels. We have found the bacterial flora to rapidly change in response to a change in the animals' water supply. However, in a study that employed a model to transmit *Aeromonas salmonicida* between mussels and fish, it took between 9 and 15 days for mussels to depurate this pathogen, emphasizing the need for some quarantine of mussels prior to their introduction to hatcheries. Another study has begun to develop techniques for non-destructive examination of mussels for pathogens so that health inspections and management strategies may be implemented to reduce the risk for introduction and transmission.

Roles Of Astaxanthin In The Pathobiology Of Lobsters, *Homarus americanus*

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During the summer in western Long Island Sound (LIS), a confluence of natural and anthropogenic factors often reduce dissolved oxygen concentrations in the epibenthic water layer, which in turn leads to increased levels of stress for the American lobster, *Homarus americanus*. Anecdotal evidence suggested that at these times, the carotenoid astaxanthin was present in higher concentrations in the circulating hemolymph of lobsters. We hypothesized that astaxanthin is mobilized to the plasma of lobsters during periods of stress, because of its anti-oxidant properties. During the summer/fall of 2004, therefore, 207 lobsters were sampled from three different trap lines in western LIS to investigate the dynamics of astaxanthin in lobster hemolymph and tissues. Hepatopancreas, antennal gland, muscle, shell epithelium, plasma, and hemocytes were all analyzed for astaxanthin concentrations by spectrophotometry. A strong seasonal signal for plasma, shell epithelium, and hepatopancreas astaxanthin was evident, with highest levels observed in early September. Astaxanthin concentration in hemocytes showed an increase until mid August and then decreased as plasma, epithelium and hepatopancreas concentrations peaked. Lobsters taken from the more western trap lines, where environmental stress is typically greater, showed significantly higher astaxanthin concentrations in hemocytes but not other tissues. Observations on dynamics and spatial patterns of astaxanthin in lobsters were consistent with the hypothesized role of this hemolymph carotenoid as a stress-coping mechanism. The possibility that astaxanthin release into the hemolymph is a disease symptom rather than a specific immune response could not be excluded, but if this is so, then it would represent a novel disease sign.

Toxicopathology Of Resmethrin In Larval And Juvenile American Lobsters (*Homarus americanus*)

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The American lobster (*Homarus americanus*) population in western Long Island Sound suffered mass mortality in the fall of 1999. Pesticide spraying, temperature/ hypoxia anomalies and *Neoparamoeba* infection (or some combination) were proposed as possible explanations. The focus of this study was to examine the response of larval (stage I and II) and juvenile lobsters to insecticides, and determine if temperature stress enhanced toxicity. Lobster larvae were exposed for 24 to 96 h to nominal resmethrin concentrations up to 900 ng L^{-1} at both non-stressful (16^{o}C) and stressful (24^{o}C) temperatures in a flow-through system. Resmethrin toxicity was time dependent: LC50s decreased from >300 ng L^{-1} in 24-h exposures to 95 ng L^{-1} in 96-h exposures. Significant pathologies were evident in the hepatopancreas of moribund larvae in the highest treatments after 48 h, including nuclear and cellular hypertrophy and epithelial sloughing. Elevated mortality was observed in all groups at 24^{o}C. Resmethrin was significantly more toxic than malathion or methoprene to the larvae. The 48-h 16^{o}C LC50 for malathion was 3700 ng L^{-1}, whereas methoprene exposure of up to 10,000 ng L^{-1} resulted in no mortality. Juvenile lobsters were exposed continuously for 7 d to resmethrin and malathion at sublethal concentrations of 30 ng L^{-1} and 1000 ng L^{-1}, respectively, at both stressful (22°C) and non-stressful (16°C) temperatures. Hemocyte phenoloxidase (PO) activity was measured to assess the immune response. Activity of PO was significantly reduced at 30 ng L^{-1} of resmethrin in only one out of three experiments. Exposure to malathion failed to elicit a significant response. Elevated temperature, however, caused a 65% increase in PO activity ($p \leq 0.006$) separate from the effect of pesticide exposure. Quantitative histological analyses of tissues collected from juveniles are in progress.

Laboratory Culture And Health Care Of The Horseshoe Crab (*Limulus polyphemus*)

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The "American" horseshoe crab, *Limulus polyphemus*, is a unique marine organism that has served as a laboratory research model to study the structure, function, embryology and physiology of invertebrates. Horseshoe crabs can be maintained in a wide variety of systems ranging from glass aquaria to large fiberglass tanks with various types of mechanical and biological filtration systems. Some systems may also employ protein skimmers, UV filtration units, and ozonation to help reduce potentially harmful bacteria from the water. Adult horseshoe crabs are tolerant of a wide range of environmental conditions, ranging in temperatures from –5°C to 35°C and salinities from 5-ppt to 35-ppt, with optimal temperatures between 15°C and 21°C and salinity around 27-ppt. In captivity, horseshoe crabs are commonly fed dead fish, squid, small crabs, clams and frozen brine shrimp. Horseshoe crabs also readily consume commercially-prepared artificial shrimp/fish diets, but the nutritional value of these synthetic formulations is not known. Though the horseshoe crab has been used for many years as a laboratory animal, few reports exist describing their diseases or syndromes. Non-infectious problems range from water quality problems, developmental problems and traumatic injuries. Infectious etiologies include algae, fungus, colonial and filamentous cyanobacteria, Gram (-) negative bacteria and a variety of protozoan and metazoan parasites. Clinical evaluation of a horseshoe crab can be problematic as the hard, non-transparent carapace makes examination and sample collection difficult. In addition, very little is known concerning the treatment and medical management of horseshoe crabs, though a few external treatments have been suggested to remove ecto-commensals and external parasites. To date, antibiotic treatment of bacterial infections in horseshoe crabs has not been reported.

Histological Techniques For Marine Bivalve Mollusks And Crustaceans

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In 1983 a NOAA Technical Memorandum "Histological Techniques for Marine Bivalve Mollusks" was published by Howard and Smith. The publication received worldwide circulation and another 500 copies were reprinted in 1997. Due to the popularity and continued requests for the publication, a second edition was planned and published in December 2004. Like the original, the updated publication is designed and written as an instructional manual for scientists and technicians investigating the health and diseases of coastal ocean and estuarine invertebrates; however, many of the techniques may be applied to studies of fish and other aquatic animals. The 230-page manual provides detailed instructions for the handling and processing of many bivalves and crustaceans for histological examination in a step by step manner and includes color photographs and illustrations of the processes, information on invertebrate anatomy, signs of disease, recommended tissue fixatives, color micrographs of results expected from routine and specialized stains, and cryosection techniques. Chapters have been added on histocytology, diagnostic techniques for blue crabs, miscellaneous techniques and methods for mollusks, diagnostic techniques for the softshell clam, and special techniques. An overview of the manual will be presented.

Plasma Cortisol In White Perch: Associations With Sampling Method, Dissolved Oxygen, And Water Temperature

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Cortisol is the main corticosteroid in fish released upon exposure to stress. Plasma concentrations were quantified in white perch *Morone americana* from the Hackensack River (NJ) and Pocomoke River (MD) by way of competitive ELISA. Cortisol concentrations in white perch from the Pocomoke River were significantly greater than in Hackensack River fish. Fish were sampled using otter trawl, trap nets, gill nets, and seining. Though differences in cortisol levels were seen based on sampling method used, there were no significant differences. Although increases in water temperature are associated with reduced cortisol concentrations, these results were not significant. There were weak significant negative correlations of cortisol with dissolved oxygen. Concentrations of cadmium and lead in muscle were significantly positively correlated with cortisol, whereas there was a significant negative correlation of mercury with cortisol. Though pooled results showed no significant associations of PCBs, organochlorine compounds, or DDT and related compounds with cortisol, when females were analyzed separately, a significant negative correlation between DDT related compounds and cortisol was seen.