Laboratory Efficacy Of Florfenicol Against *Streptococcus iniae* Infection In Sunshine Bass

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Abstract. – An experimental feeding trial was performed to evaluate the efficacy of florfenicol (FF) in controlling *Streptococcus iniae* infection in sunshine bass (SB). Doses of FF tested were 0, 5, 10, 15 and 30 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 virulent *S. iniae*. The FF medication was continued for 10 consecutive days, followed by a 25 d post treatment observation. Oral administration of FF medicated feed for 10 d at 5, 10, 15 and 30 mg FF/ BW/d significantly increased ($P<0.05$) the survival of *S. iniae* infected SB from 4.2 % in the challenged non-medicated positive control group to 69.2, 86.7, 94.2 and 94.2 %, respectively. The survival rate was significantly higher in the 15 and 30 mg treatments (94.2 and 94.2 %, respectively) than the 5 mg treatment (69.2) but there was no significant difference among the treatments 10 mg and higher. At the conclusion of the experiment no carriers were detected in any challenged group receiving FF medicated diet while the bacterium was recovered from the non-medicated challenged survivors of the infection.

Comparison of Lipopolysaccharide And Protein Profiles Between *Flavobacterium columnare* Strains From Different Genomovars

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Lipopolysaccharide (LPS) and total protein profiles from four different strains of *Flavobacterium columnare* were compared in this study. These strains belonged to genetically different groups. *F. columnare* ALG-530 and ARS-1 are highly virulent strains that belonged to genogroup I and II respectively. *F. colulmnare* FC-RR is genogroup III avirulent mutant. ALG-03-063 is a clinical strain included in the same genogroup as FC-RR. Electrophoresis of LPS showed differences among the four strains. Only low molecular weight LPS bands were displayed. ALG-530 shared a similar banding pattern with ARS-1, while FC-RR and ALG-03-063 LPS profiles were more complex. In addition, anti-ALG-530 and anti-FC-RR sera were generated from channel catfish to perform immunoblotting analysis. Analysis of LPS by immunoblotting revealed that the avirulent mutant lacked the higher molecular bands in the LPS. Total protein analysis displayed by immunoblotting showed differences between the strains analyzed. However, some common bands were shared by all the isolates. ARS-1 displayed the highest number of antigenic proteins. FC-RR lacked two distinct common bands shared by the other three strains. For both LPS and total protein analysis, each strain displayed almost identical immunoblotting banding pattern regardless of antisera used. Based on the differences of LPS and total protein profiles, it is possible to discriminate between the avirulent mutant FC-RR from other clinical *F. columnare* strains. According to our results, LPS may play a major role in *F. columnare* pathogenicity.

Evaluation Of Medium Components For Growth Of *Flavobacterium psychrophilum*

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Coldwater disease has, and continues to warrant consideration when rearing and managing important sport and restoration fishes in the Great Lakes Region. Fish health specialists continue to be plagued with difficulty in primary isolation of the causal bacterium *Flavobacterium psychrophilum*, particularly among low-level, asymptomatic fish encountered in health inspections. The thought is that weak *F. psychrophilum* colony growth and overgrowth by garden-variety bacteria and fungi on primary isolation plates often lead to false negative conclusions. Because of this problem, we are trying to develop a medium to improve primary isolation. Our approach is two-fold: enhance the growth of what we desire to isolate, *F. psychrophilum*, and inhibit/retard what we do not desire to isolate. The recipe for the medium was extracted from the "best of the literature". We have done 72 h growth curves evaluating (in broth and on agar): cell diluents; Anacker and Ordal Cytophaga vs. EAOA plus serum vs. experimental medium "#2"; individual medium components; and four different types of serum. Compared to Cytophaga medium, #2 and to a lesser extent EAOA plus serum, yielded superior growth for many *F. psychrophilum* isolates. More luxuriant colony growth, more quickly was evident on #2 plate medium.

Antimicrobial Resistance Patterns Of Ontario *Flavobacterium psychrophilum* Strains

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Flavobacterium psychrophilum is the causative agent of bacterial cold water disease and rainbow trout fry syndrome. Coldwater disease has a considerable economic impact in Ontario aquaculture operations especially during the wintertime. Antibiotics in many operations are used to reduce the economic impact of disease. Limited information is available regarding antimicrobial resistance of North American (NA) strains of *F. psychrophilum*. Ninety-two isolates of *F. psychrophilum* were collected during an 8 year period from rainbow trout, Arctic charr and Atlantic salmon. These isolates were characterized phenotypically, biochemically and serologically and were found to be relatively homogeneous. A multiplex PCR using primers for 16S ribosomal RNA and subunit B of DNA gyrase is used to aid in primary isolation and to confirm strain identity. To determine antimicrobial resistance patterns of these *F. psychrophilum* strains, the minimum inhibitory concentrations (MICs) were assessed using TREK SENSITITRE susceptibility plates for aquaculture. The MIC values of three antibiotics frequently used on farms in NA were high. For trimethoprime/sulfa-methoxazole (STX), 90.9% of isolates had MIC>1/19 (range 0.015/0.3 to 1/19 µg/ mL), for sulphadimethoxine/ormetoprim (PRI), the MICs of 78% were >76/4 (range 0.15/0.008 to 76/4 µg/ mL) and for oxytetracycline (OXY), the MICs of 44% were >8 (range 0.015 to 8µg/mL). In contrast, the MIC of flofenicol (FFN) for 63% of isolates was low, between 0.25 and 4.0 (range 0.03 to 16µg/ mL). These results are similar to those in a number of European studies except that the values for florfenicol for NA strains are moderately higher. Moderate MICs to florfenicol occurred on farms that had never used the antibiotic. In addition, 56 to 70% of the strains tested had high MIC values for gentamicin, oxolinic acid, and ampicillin, and low MICs for erythromycin, enrofloxacin and flumequine—although none of these drugs are currently used in NA fish farms. The *in vivo* significance of these results needs to be studied with infection trials.

Application Of Polyphasic Taxonomy To The Study Of Bacterial Fish Pathogens

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Bacteria identification constitutes one of the main challenging areas in microbiology. Identification of bacterial pathogens at the species level should be rapid and accurate. Moreover, pathogen identification at strain level is crucial in most epidemiological studies. The best approach for strain identification is the combination of fingerprints generated by both phenotypic and genotypic techniques. In our lab we are using four techniques based on phenotypic characters and up to five genomic methods in order to fully characterize each bacterial isolate. Phenotypic methods include Fatty Acid Methyl Ester (FAME) analysis and miniaturized commercial systems (API, VITEK). Genomic methods comprise fingerprinting methods such as Amplified Fragment Length Polymorphisms (AFLP) and Enterobacterial Repetitive Intergenic Consensus(ERIC) sequences analysis, as well as sequence-based methods such as 16S rRNA gene and internal spacer region (ISR) analysis. All information generated is compiled into a centralized database which allows data integration from different typing methods. Practical examples of polyphasic taxonomy applied to fish pathogens (*Edwardsiella ictaluri*, *Flavobacterium columnare* and *Yersinia ruckeri*) are presented. *E. ictaluri* behaves as a clonal species although high resolution genotyping methods revealed differences between catfish and madtom isolates. *F. columnare* is a phenotypically uniform but genetically diverse species. Atypical *Y. ruckeri* isolates were identified as biogroup 2 by polyphasic taxonomy methods.

Using DNA Microarrays To Compare Clinical Isolates Of The Fish Pathogen *Aeromonas salmonicida*

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Aeromonas salmonicida has been isolated from numerous fish species, and shows wide variation in virulence and pathogenicity. In order to compare isolates from select geographic and host locations, an amplicon-based DNA microarray was constructed using a subset of 2068 genes from the draft genome sequence of an *A. salmonicida* wild-type strain. The microarray included genes encoding known virulence-associated factors in *A. salmonicida*, and homologues to virulence genes of other pathogens. The microarray was used in comparative genomic hybridizations (CGH) whereby genomic DNA from selected *A. salmonicida* sbsp. *salmonicida* isolates, as well as other *Aeromonas* species and subspecies was compared with that of the sequenced strain. Results showed that variation among the virulence associated genes increased across sub-species and species boundaries. Preliminary data showed no correlation between geographic region and degree of genetic variation. Our data indicate that typing methods that rely on phenotypes do not always correlate with the CGH data. Genome variability studies, including microarray CGH, provide essential information for the selection of targets for candidate vaccines and for other health management tools.

From Sequence To Sickness: Pathogenesis In *Aeromonas salmonicida* Subsp. *Salmonicida*

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Aeromonas salmonicida subsp. *salmonicida*, the etiological agent of furunculosis, an infectious bacteremia of salmonids has been known since the late 1800s. It was the subject of vaccination trials in the 1940s and was the first bacterial species to have its outer surface protein coat described (the eponymous A-layer). After over a century of study the virulence factors of *A. salmonicida* subsp. *salmonicida* remain unclear and more than one report in the literature describes the lack of a relationship between genotype and a virulent phenotype. We used three different methods to identify putative virulence factors; scanning the genome manually, comparative genomic hybridization (CGH) and proteomics. Of the many systems and genes identified in *A. salmonicida* subsp. *salmonicida* and implicated in virulence in other bacterial pathogens, three systems were chosen for further study. These systems were Type IV pili, iron scavenging and sequestration and Type III Secretion. Isogenic knockout mutants were created and tested for virulence *in vivo* and for virulence-like behavior *in vitro*. Two adhesins, the Type IV pili Tap and Flp, were found to be important in, but not absolutely required for virulence in Atlantic salmon. *In vitro* studies showed that the A-layer is still an important adhesin. Iron scavenging and sequestration is of vital importance to pathogenic bacteria as *in vivo* iron levels are significantly lower than those required to support microbial growth. Three siderophore receptors; FstA, FstB and HupA were identified. In contrast to the observations of others at least one of these receptors, FstB, was required for virulence. In common with other workers the Type III secretion system (TTSS) was found to be absolutely important for the virulence of *A. salmonicida* subsp. *salmonicida*. Systematic deletion of three identified TTSS effectors revealed only subtle effects on virulence. These data, drawn from a number of systems, have allowed a more understand more coherently the pathogenesis of furunculosis. This research was partially supported by the National Research Council of Canada's Genomics and Health Initiative.

Bacterial Infections In Representative Lake Whitefish *(Coregonus clupeaformis)* Stocks In Michigan

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Lake whitefish are an indigenous species that are highly sought after in both the commercial and recreational fisheries within the Great Lakes region. Within the last four decades, a notable deterioration in the overall condition of this economically important species has occurred, with both nutrition and disease being implicated as possible contributing factors. In order to elucidate the role that pathogens may play in this decline, wild, adult, Lake whitefish were collected from four sites in lakes Michigan and Huron and were subsequently subjected to extensive pathological examinations. Bacteriological cultures were taken from the kidneys and any internal or external lesions and then inoculated onto each of the following media; Brain Heart Infusion and/or Trypticase Soy Agar, Coomassie Brilliant Blue Agar, Modified Kidney Disease Medium, and Hsu-Shotts Agar. Any growth of interest was then struck for isolation and subjected to exhaustive biochemical testing. Concurrently, kidney tissues were sampled and tested via Q-ELISA in order to assess the level of *Renibacterium salmoninarum* antigens, the etiological agent of Bacterial Kidney Disease. Q-ELISA results have illustrated definitive patterns associated with *R. salmoninarum* infections that fluctuate by sample site, season, and year, yielding a vivid picture of spatial and temporal prevalence/intensity that at times, have reached upwards of 80% infection. In addition to *R. salmoninarum*, other retrieved isolates include multiple species of gram-negative bacteria belonging to three complexes within the genus *Aeromonas*, the ubiquitous facultative pathogen known as *Pseudomonas fluorescens*, and the gram-positive lactic acid bacterium, *Carnobacterium piscicola*. Thus far, 31 representative *Aeromonas* isolates, 17 representative *C. piscicola* isolates, and five representative *Pseudomonas* isolates have been retrieved, some of which were associated with both internal and external clinical lesions. Of significant interest are the isolates of *C. piscicola*, which are being reported from this host and region for the first time. In all, this study is making great strides in elucidating the role pathogens play in the current declines occurring within the indigenous Lake whitefish populations of the Great Lakes region.

Quantification Of A 16kda Defense Lectin (Ladderlectin) In Rainbow Trout (*Oncorhynchus mykiss*) Plasma

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Extracellular lectins play a critical role in the innate immune response through recognition of carbohydrate patterns on microbial surfaces. Soluble, multimeric lectins, such as the mannan-binding lectins (MBL) and ficolins, enhance pathogen opsonization, directly activate complement and augment phagocytosis. Although much of the evidence for defense functions of soluble lectins is based on mammals, several functionally homologous molecules have been identified in fish. Rabbits were immunized with a synthetic peptide generated from amino terminal sequence of the reduced 16kDa subunit of rainbow trout ladderlectin (RT-LL). Antiserum specificity was confirmed by Western blots of whole rainbow trout plasma. Similarly, bacterial binding activity of RT-LL to a variety of fish pathogens including *Aeromonas salmonicida* sbsp. *salmonicida*, *A. hydrophila*, *Yersinia ruckeri* and *Pseudomonas* spp. was confirmed by Western blotting. Finally, we have developed an enzyme immunoassay to compare individual and population-level variation of RT-LL in the plasma of healthy rainbow trout. Similar to investigations of porcine plasma MBL and ficolin, RT-LL revealed considerable variation ($p<0.0001$) amongst individual fish, as well as between populations screened to date ($p<0.05$). Ongoing investigations will focus on individual and temporal variations of RT-LL in high and low abundance fish during experimental infection with *A. salmonicida*. Our goal is to monitor high and low abundance individuals to determine if plasma lectin concentration correlates with increased (or altered) disease resistance.

Characterization Of Ladder Lectin And An Intelectin In The Plasma Of Rainbow Trout (*Oncorhynchus mykiss*)

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To investigate lectin mechanisms in susceptibility and resistance to disease, we isolated and characterized ladder lectin (RTLL) and intelectin (RTIntl) from rainbow trout (*Oncorhynchus mykiss*) plasma based upon their carbohydrate-dependent affinity. Both RTLL and RTIntl bound to whole *Aeromonas salmonicida subsp. salmonicida*, chitin and artemia and this activity was calcium-dependant. RTLL was characterized by reducing 1 and 2D SDS-PAGE as a 16 kDa monomer with three isoforms between pI 5.0 -5.3. Nucleotide sequence of RT-LL was deduced from the N-terminal amino acid sequence of the protein and by 3'rapid amplification of cDNA ends. The deduced amino acid sequence displayed high homology to the Atlantic salmon mannan-binding lectin. Mannose binding specificity was confirmed by the presence of and EPN amino-acid motif in the C-type carbohydrate recognition domain. RTLL was identified by immuno-histochemistry in the gill epithelium, renal interstitium, heptic sinusoidal cells, and leukocytes. RTLL was also intimately associated with *A. salmonicida* microcolonies. Reducing 1 and 2D SDS-PAGE revealed RTIntl to be a 37kDa monomer with five isoforms between 5.0 – 5.6. Nucleotide sequence of cDNA encoding this lectin and deduced amino acid showed moderate sequence similarity with a human galactofuranose binding intelectin. RTIntl amino acid sequence does not display a C-type lectin motif but rather a fibrinogen-like motif that may involve carbohydrate binding. Immuno-histochemical RtIntl positive cell were present in the skin, gill, intestine and swim bladder. The functional significance of these lectins is now being investigated to determine their roles as innate immune molecules.

B Lymphocytes And Antibody Secreting Cells In Channel Catfish Skin

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The skin of fish is both the point of entry and site of infection for many pathogens, and mucosal antibodies play a major role in host defense against infection by parasites such as *Ichthyophthirius multifiliis*. To demonstrate that antibody-secreting cells (ASC) and B lymphocytes are present in fish skin, methods were developed to isolate and culture leukocytes from the skin of channel catfish (*Ictalurus punctatus*). ASC were identified by ELISPOT. Immediately after isolation of leukocytes from fish unexposed to *I. multifiliis*, ASC were found to comprise approximately 0.06% of these cells. As previously shown, in fish vaccinated against *I. multifiliis*, 1.35**%** of skin ASC recognized i-antigen, the major surface protein of *I. multifiliis*. This suggests that ASC residing in skin are the source of mucosal antibodies that confer protection against infection by *I. multifiliis*. To determine if skin contains functional/mature B cells, leukocytes were isolated from skin, placed into cell culture media ± LPS for 5 days, and ASC numbers determined by ELISPOT. Initial results showed that LPS treatment stimulated polyclonal activation, i.e. replication of B cells (measured by BrdU incorporation) and differentiation into ASC, as the number of ASC per 10^6 cells increased significantly after 5 days in culture. These data demonstrate that both ASC and B cells are resident in skin of fish.

The Impacts Of Redox Structure On Trout Antibody Affinity

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Studies to date indicate that although trout serum antibody is comprised predominantly of large molecular weight (800kDa), tetrameric Igs, these antibodies possess considerable structural diversity (redox structure) due to non-uniform disulfide cross-linking of the monomeric subunits. There is limited data available on the possible functionality of these different antibody structural forms. We have begun explore the effects of this redox diversity on antibody affinity by the selective adsorption of trout Igs onto a TNP-BSA carrier with varying ratios of hapten. This selective adsorption revealed significant skewing of affinity profiles of antibody subpopulations from the same anti-serum. The average affinity of the low-density hapten-carrier conjugates (TNP1-BSA) adsorbed antibodies possessing a higher apparent affinity constant (aK) than that of those adsorbed by the high density conjugates (TNP13-BSA). Further, subsets of antibodies demonstrated different redox profiles. TNP1-BSA preferentially bound the higher order redox forms (i.e. those wherein the tetramers were completely disulfide polymerized). However, TNP13-BSA captured significantly fewer higher order forms and possessed increased concentrations of lower order forms (i.e. less disulfide cross-linked). Redox / affinity associations were also observed with antibodies produced from *in vitro* culture of trout lymphocytes with the antigen, TNP-LPS. Antibodies secreted by the lymphocytes assumed the typical redox structure observed with serum antibodies; however, the intracellular antibody tetramers are strictly composed of halfmer subunits (100kDa). This intracellular form is surprisingly restricted in affinity, compared to the secreted antibody which expresses a broader range of affinities. This is surprising in that the repertoire of affinities should be identical between both forms as 1) they arise from the same cells and 2) affinity is supposedly dependent only upon the antibody binding site. These distinctive affinity repertoires are highly suggestive that oligomerization of the antibody molecule leads to complex allosteric effects upon the antibody binding sites. In other words oligomerization confers much of the affinity heterogeneity observed in the antibody repertoire. The linking of the binding affinity to redox structure is discussed.

Characterization And Utilization Of Affinity-Purified Atlantic Sturgeon (*Acipenser oxyrinchus*) Immunoglobulin

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Immunoglobulin (Ig) was isolated from the serum of healthy Atlantic sturgeon (*Acipenser oxyrinchus)* by affinity purification and concentrated by centrifugation. The molecular weight of the reduced and alkylated heavy chain was determined to be 71.1 kD by SDS-page chromatography, while the two light chains were determined to be 22.5 and 27.7 kD. Therefore, the calculated molecular weight of the unreduced native tetrameric IgM-like immunoglobulin using the predominant 22.5 kD light chain was estimated to be approximately 750 kD. A rabbit antiserum against the affinity-purified Atlantic sturgeon Ig was produced and shown to react by gel diffusion with the serum Ig of numerous species of North American sturgeon (gulf sturgeon, *A. oxyrinchus desotoi*; white sturgeon, *A. transmontanus*; shortnose sturgeon, *A. brevirostrum*; lake sturgeon, *A. fulvescens*; green sturgeon, *A. medirostris*; shovelnose sturgeon, *Scaphirhynchus platorynchus*; pallid sturgeon, *S. albus*) and paddlefish (*Polyodon spthula*), while serum from taxonomically unrelated species of fish showed no reaction. The specificity of the rabbit anti-Atlantic sturgeon antiserum recognized both the heavy and light chains of the Ig by Western blot analysis. The affinity-purified Ig was further used in an indirect enzyme-linked immunosorbent assay (ELISA) to assess serum Ig concentrations in three different species of cultured sturgeon (Atlantic sturgeon, shortnosed sturgeon and white sturgeon) It is anticipated that this ELISA will be useful in assessing the health status of wild populations of sturgeon.

The Health Condition Of Impinged And Open Water Fish From The Mobile River

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Studies investigating the general health status of impinged and open-water fish were conducted at Barry Steam Plant located on the lower Mobile River, Al. Species studied, due to high rates of impingement and mitigation value, were threadfin shad, freshwater drum, blue catfish, and channel catfish. Fish were sampled for the health studies during the spring and fall of 2005 over a five to six week period in each season. Fish suitable for necropsy were measured for total length, weighed, and wet mounts prepared of gill samples and skin scrapes. Additionally, trunk kidney and liver samples were removed and streaked on to bacterial growth media to determine prevalence of bacterial pathogens. Impinged fish were compared to open-water fish that were obtained from within 5 km of the vicinity of the intake area. A total of 938 fish representing the impinged (N=457) and open water (N=481) populations were sampled. Results from this study support the general hypothesis that impingement may select compromised fish. Lower body weight was demonstrated to be significant (at equivalent lengths) for all species except for channel catfish. Both protozoan parasites (*Icthyobodo necator* and *Chilodonella* sp.) and bacterial pathogens (*Flavobacterium columnare* and *Aeromonas* sp.) were found at a significantly higher prevalence in impinged fish versus fish from the open water population.

The South Carolina Aquarium Sea Turtle Rescue Program

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Just after opening its doors in May 2000, the South Carolina Aquarium responded to a call from the South Carolina Department of Natural Resources to care for a sick loggerhead sea turtle. That turtle was the start of a new endeavor for the Aquarium. With strandings on the rise in the Southeast, there exists a strong regional need for a stranding center. Although sea turtle rescue and rehabilitation was not in the initial design of the Aquarium, a stranding program seemed to fit naturally with its conservation mission. For several years, lack of space, staff and resources limited the numbers of sick sea turtles the Aquarium could house. Each year, holding tanks would reach maximum capacity and additional stranded turtles would be transported a minimum of 6 hours to the nearest rehabilitation center. The animals washing up on the beaches are in critical condition and immediate treatment increases their chances of survival. In response, the SCA Sea Turtle Rescue Program has grown in its capacity to house and care for these threatened and endangered animals and in 2005 reached its goal in taking in every turtle that needed medical attention. Standardized treatments administered to stranded turtles include taking blood for complete blood counts, chemistries, cultures and sensitivities, performing radiographs, analyzing fecal samples and removing ectoparasites. Additional procedures may include blood transfusions, fluid therapy, tube feeding, surgeries and physical therapy. Recovered turtles are released off the coast of South Carolina.

Characterization Of A Systemic Iridovirus From The Banggai Cardinalfish (*Pterapogon kauderni*)

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During the period from 2003-2005, specimens of Banggai cardinalfish (*Pterapogon kauderni*) were submitted to the Connecticut Veterinary Medical Diagnostic Laboratory (Storrs, CT) to investigate mortalities of multiple cohorts of fish after transport and apparent acclimation. Histopathologic examination revealed large cells with basophilic granular cytoplasmic inclusions located beneath endothelium throughout the tissues of affected specimens, and transmission electron microscopy revealed inclusions to consist of paracrystalline arrays of viral particles with features characteristic of previously described iridoviruses causing systemic infections of ornamental freshwater and marine fish. Subgenetic fragments of the DNA polymerase (DNApol) and adenosine triphosphatase (ATPase) genes were amplified, cloned and sequenced from total genomic DNA extracted from fresh frozen tissues by the Fish Health Laboratory (University of California, Davis, CA), and, later, a segment of the major capsid protein (MCP) gene was amplified, cloned and sequenced using DNA extracted from formalin-fixed paraffin-embedded tissues. Molecular data will allow probe generation toward in situ detection of virus in research and diagnostics, as well as molecular phylogenetic studies to gain insight into the epidemiology and evolution of these viruses.

Coryphaena hippurus: The Challenges Of Collecting And Exhibiting This Pelagic Species

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During the winter of 2002 the South Carolina Aquarium started planning to diversify the collection of pelagic species in our Great Ocean Tank, a 385,000 gallon exhibit. The species we decided to try first was *Coryphaena hippurus* (aka. dolphin, mahi, or dorado) due to its availability offshore and proven success in a handful of other aquariums and aquaculture facilities. The acquisition process was broken down into 5 main areas of focus: collecting, transport, acclamation, quarantine and exhibiting. Each phase of this project had unique challenges that would be vital to the success of our efforts. Collecting, quarantine, and exhibiting the animals ended up being the easiest aspects while transport and acclimation were the hardest. To date we still are fine tuning the methods that work for mahi and we have begun to experiment with other pelagic species.

Amyloodinium In Elasmobranchs

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Protozoal infections in aquarium fishes have always been a significant problem. In particular, *Amyloodinium ocellatum* is persistent and difficult to remove from marine systems. The life cycle of *Amyloodinium* consists of an active form that attaches to the fish's tissue called a trophont, a free swimming form known as a dinospore, and a tomont or cystic form that retires to the bottom. The tomont is resistant to chemical medical prophylaxis and treatment. Copper is traditionally the therapy that helps control *Amyloodinium* in systems and on teleost fish. Previously, there had been little written on the subject of this protozoon infecting elasmobranchs. Copper, of course, is a threat to elasmobranches. Recently, *Amyloodinium* was diagnosed and treated in two public aquaria. The vector for the infection appears to be three shipments of stingrays, *Dasyatis americana*, from the same source. The fish were successfully treated with 10ppm chloroquinone disphosphate.

Laparoscopic Liver Biopsy In Fish: Equipment, Technique, And Preliminary Assessment Of Biopsy Quality

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As an initial investigation of the utility of laparoscopy in fish, laparoscopy and laparoscopic liver biopsy were performed post-mortem or peri-mortem in six fish that died or were euthanized due to advanced age or illness. In addition, these procedures were performed in two living fish for diagnostic reasons. A 2.7mm diameter, rigid endoscope with a surgical sheath and cup biopsy instruments were used. Insufflation of the coelom was achieved using 0.9% sodium chloride solution delivered via the surgical sheath. Anesthesia was induced and maintained with MS-222 at 90ppm. In all cases, good visualization of the coelomic viscera was achieved and biopsies were easily performed. In two terminal cases, the spleen was biopsied in addition to the liver. A size 5 French biopsy instrument provided better purchase and biopsy size than the smaller 3.5 French instrument. Histopathologic evaluation of biopsies revealed good correlation between biopsies and tissues obtained at necropsy. In two cases, the target organ was incorrectly identified, and biopsy of testicle and granulomatous ovary were obtained. Surgical incisions in the two living patients were closed with a single cruciate nylon suture. Recovery of these patients was uneventful, although they were later euthanized due to persistence of their illness.

Challenges Of Managing Horseshoe Crabs (*Limulus polyphemus*) In An Interactive Exhibit

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The Children's Discovery Corner at the National Aquarium in Baltimore is a 'touch tank' housing horseshoe crabs, channeled whelk, knobbed whelk, bay sea stars, green crabs, and pencil urchins. It is an 800-gallon recirculating artificial saltwater system, which is divided into a primary exhibit tray and four back up trays. Since June 2005, the following lesions were identified in horseshoe crabs: soft shells, gill erosion, abraded compound eyes, and torn telson musculature. Differentials for these problems include conspecific trauma, improper handling, infectious diseases, poor water quality, and malnutrition. A review of lesions, histopathology, and medical treatments will be presented. Challenges identified in adequately managing this species included: inadequate record keeping and communication regarding animal rotation, diseases, and mortalities; inability to perform routine water changes; inappropriate handling of animals by volunteers and members of the public; and lack of sufficient numbers of animals for program needs. Medical and husbandry staffs have addressed these concerns by increasing water change frequency, improving all aspects of record keeping and communication, and educating volunteer staff on proper animal handling. Details regarding these issues will be presented. Invertebrates should not be considered "disposable" resources. Every effort should be made to ensure that all animals, vertebrate or invertebrate, are provided with the same standards of care.

Medical Management Of Cold-Stunned Sea Turtles Stranded On Cape Cod

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Although most species of sea turtles inhabit warm waters, over the last decade increasing numbers of stranded sea turtles have occurred annually in the late autumn and early winter on the northeastern shores of Cape Cod. Most of these animals are juvenile Kemp's Ridleys, but representatives of several other sea turtle species are also recovered. Because the pelagic stage of Ridleys is unknown, the reason animals strand on Cape Cod during this time of year can only be conjectured as related to changes in ocean currents, tides, wind trajectory, and weather/storm patterns coupled with the geographical anomaly of the Cape's formation. In autumn, the Massachusetts Audubon Society monitors the beaches for stranded animals based on weather conditions, prevailing wind, and high tides. Live animals are then transported to the New England Aquarium's (NEA) Rescue and Rehabilitation Department. At NEA, animals receive an initial health assessment and veterinary care to treat the most immediate and life-threatening conditions of hypothermia, dehydration, pneumonia, and trauma. From 1999 – 2005, NEA has responded to 563 stranded sea turtles and has had over an 88% survival rate in individuals that are warmed to ambient temperature in an Intensive Care Unit (ICU) over the first three days post arrival. The following offers a brief overview of initial triage, diagnostic findings, treatments, and release of rehabilitated cold-Stunned sea turtles at NEA.

Vaccine Design

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Reduction in catches in commercial fisheries operations is putting ever-increasing demands on aquaculture to satisfy market demand. This emphasis on increased farm production has led to a dramatic expansion in aquaculture operations globally. One of the key factors that can define the success or failure of an aquaculture operation is control or prevention of disease. For many years aquarists have turned to antibiotics as a means to control disease, but there is considerable resistance developing against their continued use. Vaccines, however, are seen as one of the most cost effective and efficacious ways to prevent disease outbreaks in commercial operations; however successful commercial vaccines are rare. Recent research in our laboratory has found that the induction of a certain cell type, the long-lived plasma cell, is important for the long-term maintenance of humoral immunity. In the absence of these cells, antibody titer diminishes, and humoral immunity is lost. Furthermore, within the logistics of an aquacultural setting, often a single immunization is all that can be afforded. In such cases it is critical that this single immunization produce titers of the greatest longevity. Therefore it is important when designing a vaccine that the researcher not only determines the protective epitope to generate the vaccine, but also administers the vaccine via a route and at the correct dose to optimize the production of long-lived plasma cells. This seminar will discuss the new research being conducted on long-lived plasma cell generation, which will allow more efficient design, dose and delivery of vaccines.

Beneficial Use Of Attenuated Vaccines In Aquaculture

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The success of safe and effective vaccines in controlling disease and enhancing profitability in livestock production has been profound. Because fish farmers operate on narrow profit margins, cost effectiveness is of paramount importance in any vaccination program. In aquaculture, the best vaccines should offer ease of administration with the minimum of stress, long-term protection, and lowered production cost. Within these parameters, the vaccines should be efficacious in protecting fry that are most vulnerable to disease-causing agents until the pond-stock is ready for the market. With these objectives in view, two new vaccines for immunization of catfish against *Edwardsiella ictaluri* [the cause of enteric septicemia in catfish (ESC)] and *Flavobacterium columnare* (cause of columnaris disease) were developed, field tested, and licensed for immunization of 7-10 day post-hatch fry by immersion exposure. The USDA, Agricultural Research Service developed vaccines licensed to Intervet and marketed under the trade names AQUAVAC-ESC® and AQUAVAC-COL® are used for protection of fish against ESC and columnaris disease respectively. The vaccines have augmented survival rates and provided protection up to periods of 1 to 2 years. The cost benefits of vaccination with AQUAVAC-ESC alone, is estimated by Intervet to be $1,706 per pond acre compared with similarly farmed non-vaccinated catfish. Notably, attenuated vaccines have a proven record of safety and efficacy for vaccination of poultry, swine and cattle. Since many bacterial pathogens are ubiquitous in the aquatic environment, attenuated vaccines should afford adequate long-term protection until fish are ready for market distribution.

DNA Vaccines And Environmental Safety

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In 2005, Novartis Animal Health (NAH) was issued a license by the Canadian Food Inspection Agency (CFIA) for the commercial release of APEX IHN©, a plasmid DNA vaccine targeting the infectious hematopoietic necrosis (IHN) virus of salmonids. Safety evaluation of APEX IHN© included a study examining the potential for plasmid DNA transfer to the intestinal aerobic microbial flora of vaccinated Atlantic salmon. Microorganisms isolated from the feces of APEX IHN© vaccinated (N=40) and non-vaccinated (N=10) Atlantic salmon were selected on the basis of their resistance to the aminoglycoside kanamycin (kan) owing to the presence of a phosphotransferases (3')I selective marker within the APEX IHN© vector. A total of 109 and 58 Kan+ microorganisms were isolated from the feces of vaccinated and non-vaccinated fish respectively. Further characterisation based on morphology, Gram stain, and total protein profiling, indicated that the isolates belong to nine microbial species. Presence of the APEX IHN© plasmid in the isolates was assessed by polymerase chain reaction (PCR) using plasmid specific primers. Only DNA extracts with positive amplification for 16S rRNA confirming the quality of the DNA template were considered for PCR analysis. The expected 406 bp amplicon could not be detected in 64 DNA extracts from vaccinated or 48 extracts from control fish. The results therefore indicated that resistance to kanamycin observed in the isolated microorganisms was derived from innate or acquired resistance mechanism(s) and not due to the transferred of APEX IHN©.

IPNV Cohabitation Challenge Of Atlantic Salmon Post-Smolts

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Infectious pancreatic necrosis virus (IPNV) causes high mortality in Atlantic salmon post-smolts. An experimental saltwater IPNV cohabitation challenge model using a Canadian strain of Atlantic salmon post-smolts was developed. Virus isolate and fish stock both affected mortality. Injection of a Scottish IPNV isolate caused 36-45% mortality in Scottish salmon. In a new trial using Canadian St. John River salmon, the Scottish IPNV isolate was avirulent, but a Norwegian IPNV isolate caused 50-80% mortality when given by injection. Naive fish cohabited with Norwegian IPNV-injected fish had a delayed onset of mortality but a cumulative mortality of 44-60%. The number of injected fish (15-25) placed with 25 naive fish did not appear to alter mortality of the naive fish to a great extent. To evaluate new vaccine constructs, cohabitation of five Norwegian IPNV-injected fish with fifteen vaccinated fish resulted in more variable mortality in the injected fish (40-100%), but the model was adequate for assessing the efficacy of some of vaccines. In the third trial, 20 injected fish cohabited with 25 vaccinates resulted in more consistent mortality in the injected fish and allowed for a realistic evaluation of vaccine dose. Critical parameters for cohabitation IPNV challenge include fish stock, virus isolate and the ratio of injected:vaccinated fish. Precocious males should be removed. The effect of injected virus dose and fish density will be evaluated in future studies to develop a fully reproducible IPNV challenge for post-smolts.

Ichthyophthirius multifiliis: Challenges In Vaccine Development

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The ciliate *Ichthyophthirius multifiliis* is a highly virulent obligate parasite that infects freshwater fish. Channel catfish (*Ictalurus punctatus*) are very susceptible to infection, but can be routinely immunized in the laboratory by exposure to sub-lethal infection. Immunity develops within 2 to 4 weeks and is long-lived, lasting for at least one year. Following infection, fish mount an antibody-mediated immune response directed primarily against a class of abundant 40- to 60- kDa *I. multifiliis* surface membrane proteins, referred to as immobilization antigens, or i-antigens. Antibodies recognizing i-antigens are present both in blood and skin, where they are produced by resident antibody secreting cells. Vaccination with purified i-antigen also confers protective immunity against parasite challenge. Thus, two important criteria for the development of a vaccine against this parasite exist: the development of a naturally acquired adaptive immunity following infection and the identification of specific protective antigens. A number of practical challenges remain to be resolved before a commercial vaccine becomes available. These include: the presence of different serotypic strains of *I. multifiliis*, the production of antigen in quantities sufficient for vaccination, and a method of vaccine delivery that optimizes the induction of cutaneous mucosal antibodies and stimulates long-term immune memory.

Structural Analysis And Epitope Mapping Of *Ichthyophthirius* I-Antigens

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The parasitic ciliate *Ichthyophthirius multifiliis,* expresses abundant GPI-anchored membrane proteins known as immobilization antigens (i-antigens) that are important in the host immune response. These antigens vary in natural populations and at least five i-antigen serotypes (A–E) have now been identified. In connection with efforts at vaccine development, we are interested in the structure of these proteins, and have begun to map the determinants responsible for eliciting immobilizing and protective antibodies in the host. A characteristic of these proteins is a series of tandem repeats of ~80 aa each that span their length. However, tandem repeat copy number varies, and the i-antigens thus far analyzed share only ~50% homology at the level of primary sequence. We have used computational modeling to deduce a structure in which the tandem repeats are stabilized by disulfide bonds, and are separated by flexible hinge regions. This in silico approach further predicts that the N-terminal repeats adopt a fold similar to the laminin-type epidermal growth factor-like module (L-EGF) with a high degree of certainty. To experimentally map the conformational epitopes that interact with protective antibodies, we have over-expressed the genes for these proteins in the free-living ciliate *Tetrahymena thermophila*. Cells expressing chimeric gene products are immobilized by antibodies that are specific for different i-antigen variants allowing regional mapping of the epitopes responsible for eliciting protective immunity in the host. In the long term, identification of such epitopes should contribute to the development of effective multivalent vaccines.

Ecological Determinants In Outbreaks Of Bitter Crab Disease (*Hematodinium* sp.) In Snow Crabs, *Chionoecetes opilio,* From Conception Bay, Newfoundland, Canada

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The parasitic dinoflagellate *Hematodinium* sp. causes bitter crab disease (BCD) in snow crabs, *Chionoecetes opilio*, and Tanner crabs, *C. bairdi*. As implied, crabs infected with BCD are unmarketable due to their bitter flavor. We surveyed the distribution of BCD in Conception Bay, Newfoundland from 1997 to 2004. The disease has become firmly established, starting with a prevalence well below 1% in 1997 to an epizootic in 1999 that persisted through 2000 reaching prevalences of over 2% to 9% in trapped and trawled male crabs and from 19% to 26% in trawled and trapped female crabs, respectively. Infections were highest in females and small males. In 2004, there was a shift in the dynamics of the disease. An epizootic occurred primarily in adult males. This coincided with increased temperatures and mass molting events that had not occurred in previous years. Temperature, benthic substrate, depth, host size and sex were all correlated with prevalence of the BCD during outbreaks. Patterns in the molting cycle and prevalence of infection indicate that transmission occurs during the post-molt condition, and that overt infections probably develop two to four months after infection, lasting three to four months thereafter.

Molecular Diagnostics For *Hematodinium* And Other Protozoa In Water And Crustacean Hosts

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Diseases that effect commercially important crustaceans represent a threat to commercial fishing, recreational fishing and regional tourism. However, little is known about many of the diseases, parasites and pathogens that occur in the estuaries of the South Atlantic Bight. During a recent drought (1999-2003) we observed an outbreak of hematodinium disease in coastal Georgia blue crabs caused by the parasitic dinoflagellate, *Hematodinium* sp. Monitoring of Hematodinium infection was facilitated by the development of a PCR diagnostic assay specific for *Hematodinium*. Using this technique we were able to detect *Hematodinium* sp. in several crab species, including *Callinectes sapidus, Neopanope sayi, Libinia emarginata, Menippe mercenaria*. The assay involves extraction and purification of genomic DNA, amplification of a 187bp 18S rDNA fragment using Hematodinium-specific primers, and detection by gel electrophoresis. This PCR diagnostic assay is rapid and more sensitive, by several orders of magnitude, than the histological assay. In our studies we observed a seasonal pattern of *Hematodinium* sp. infection with low levels of *Hematodinium* sp. in the winter and intense infection during the spring and fall. We also used this assay to detect *Hematodinium* sp.in the water column, providing the first evidence for a free-living life cycle stage of *Hematodinium* sp. More recently we developed and validated a quantitative Real Time PCR assay utilizing the *Hematodinium* sp. primer set. Comparison of quantitative PCR results to direct hemolymph microscopic counts from heavily infected crabs indicated a direct relationship between the two techniques validating the assay. To expand our research into other parasitic protozoan species infecting crustaceans we plan to use denaturing high performance liquid chromatography (dHPLC) to detect and identify unknown protozoan parasites. The protocol involves purification of genomic DNA from crustaceans, amplification of separated parasite 18S rDNA fragments using universally targeted primers and identification of parasite 18S rRNA gene fragments after separation on a DNA binding chromatography column.

Lysozyme Gene Expression As A Molecular Marker Of Hemocyte Location In Pacific White Shrimp, *Litopenaeus vannamei,* After Injection With *Vibrio*

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The purpose of this study was to develop a genetic molecular marker of hemocyte distribution among tissues of the Pacific white shrimp, *Litopenaeus vannamei*. We used this marker to investigate the immune response of shrimp to a bacterial pathogen. We quantified by real-time PCR the gene expression of lysozyme, an anti-bacterial protein expressed by shrimp hemocytes. Tissue-level transcript expression also was detected by *in situ* hybridization of mRNA in circulating hemocytes and within tissues with high hemocyte concentrations. Lysozyme gene expression was quantified in 5 tissues and in circulating hemocytes for 48 h following intramuscular injection of shrimp with *Vibrio campbellii*. Lysozyme mRNA significantly decreased within 4 h in circulating hemocytes and peripheral tissues after injection of bacteria due to hemocyte trafficking to the site of injection during the early course of the immune response. Lysozyme signal increased significantly in the muscle at the site of injection during this early period and remained high for the duration of the time-course. These data monitoring hemocyte localization by lysozyme mRNA expression indicate that after injection of bacteria, hemocytes accumulate at the injection site, which supports the contention that the composition of the circulating hemocyte pool is modified by bacterial injection. Supported by NSF Grant No. IBN-021292

Changes In Metabolism And Performance In Crustaceans Fighting Bacterial Infections

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We were interested in determining if mounting an immune defense against a sublethal dose of pathogenic bacteria affected other functions in crustaceans. Injecting shrimp (*Litopenaeus vannamei*) with *Vibrio campbellii* caused a 19% reduction in O_2 uptake that was rapid and persisted for 24 h. The blue crab *Callinectes sapidus* experienced a 43% decline in O_2 uptake following injection of *V. campbellii*. This decline in blue crabs is associated with lower hemolymph oxygenation and elevated vascular resistance, consistent with the idea that hemolymph flow through the gill is occluded by the formation and trapping of hemocyte aggregates. The low aerobic metabolism resulting from bacterial injection does not appear to be compensated with anaerobic energy production. We have begun to assess the impacts that the overall lowering of metabolism might have on the ability of both shrimp and crabs to perform sustained periods of activity such as swimming or walking, activities critical to survival in the field. Shrimp placed on treadmills sustained swimming activity for hours. Saline-injected shrimp built up lactate in their tissues (10.6 $\mu mol\ g^{-1}$ tissue) and secreted lactate into the surrounding water (1.4 $\mu mol\ g^{-1}\ h^{-1}$) during 30 min of swimming. Surprisingly, *Vibrio*-injected shrimp accumulated similar amounts of lactate in the tissues, but excreted much less (0.5 $\mu mol\ g^{-1}\ h^{-1}$) than saline controls. After walking on a treadmill for 30 min, saline-injected blue crabs produced higher hemolymph concentrations of lactate (8 mmol/L) (3.7 mmol/L) than *Vibrio*-injected crabs. Lactate excretion by saline-injected crabs was much higher (13.9 $\mu mol\ g^{-1}\ h^{-1}$) than by shrimp, and significantly higher than *Vibrio*-injected crabs. These data suggest anaerobic pathways are important during exercise, but that bacterial challenge interferes with either the overall metabolism and/or the excretion of lactic acid. This research was supported by NSF IBN-0212921 to LEB & KGB.

White Spot Syndrome Virus (WSSV) In Wild *Litopenaeus setiferus* and *Callinectes sapidus* From The U.S. South Atlantic Coast

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White spot syndrome virus (WSSV) was discovered in shrimp aquaculture facilities in South Carolina in 1997. The disease is known to cause devastating mortality in cultured shrimp in Southeast Asia and its discovery in SC prompted concern for the health of wild populations. An initial study conducted in 2000 found detectable virus in wild stocks of the Atlantic white shrimp, *Litopenaeus setiferus* and the brown shrimp, *Farfantepenaeus aztecus* with incidence and severity levels lower in the latter species. One *L. setiferus* individual collected offshore displayed a significant level of acute disease. The current study was designed to further explore interactions between ovarian development, spawning and incidence of WSSV in wild populations of *L.setiferus* and the blue crab, *Callinectes sapidus*, using existing molecular diagnostics, bioassay, and immunoassays techniques. A total of 1,808 *L.setiferus* and 300 *C. sapidus* specimens at various stages of reproduction were examined for WSSV infection using a commercially available immunoassay diagnostic test kit (Shrimple). Shrimple detects viral infection by recognizing vp28, a viral structural protein. A subset of the collected specimens was tested for WSSV using real-time PCR and bioassays. Although 87 of shrimp and 11 of crabs tested positive with the Shrimple kits, none of the shrimp and only one crab was found to carry viable virus at levels significant enough to cause infection in injection bioassays. Thus, although continuing to be present, current incidence of WSSV in wild shrimp and crab populations along the Southeastern Atlantic coast is minimal even during the physiologically rigorous reproductive cycle.

Diagnosis Of *Panulirus argus* Virus 1 (PaV1) In The Caribbean Spiny Lobster Using Fluorescence *In Situ* Hybridization

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Panulirus argus virus 1 (PaV1), the first virus identified in the Caribbean spiny lobster, *Panulirus argus* from Florida Keys, is highly pathogenic to juvenile spiny lobsters. The monitoring of the virus in wild populations and study of its behavior in the laboratory depends upon reliable diagnostic tools. A sensitive and specific fluorescence *in situ* hybridization (FISH) assay for diagnosis of PaV1 was developed in this study. The lower limit of the 110 bp DNA probe in a dot blot hybridization for PaV1 DNA was 10 pg of cloned template of PaV1 DNA and 10 ng of genomic DNA extracted from hemolymph of diseased spiny lobster. The fluorescein (FITC)-labeled probe hybridized to PaV1-infected cells in all tissues tested, producing the unique green fluorescent signal inside infected cells. Most FITC-stained objects were observed accumulated around the inner periphery of the nuclear membrane, with a few distributed in a more dispersed pattern inside the nuclear. The probe did not hybridize with host tissues of spiny lobsters; nor did it cross-react with possibly related viruses. This assay will facilitate understanding of pathogenesis of the viral disease and help in monitoring efforts directed at determining the prevalence of PaV1 in juvenile nurseries for the lobster.

An Overview Of Disease Surveys In Crustaceans From The Chesapeake Bay And United States Mid-Atlantic Coast

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NOAA's Cooperative Oxford Laboratory conducts research on health of crustaceans from the Chesapeake Bay and US Mid-Atlantic coasts to provide information to researchers and resource managers. Current projects include investigations on the prevalence of the parasitic dinoflagellate *Hematodinium* sp. in blue crabs and other crustaceans, an experiment to assay mortality in crabs infected with *Hematodinium* sp., the presence of *Vibrio* spp., *Vibrio parahemolyticus*, and virulent strains in blue crab hemolymph, and a comparison of the general health of crabs collected from a pristine NERRS site and a site near Baltimore Harbor in the Chesapeake Bay. Assay methods include histology, microbiology, electron microscopy and molecular techniques. This presentation will provide an overview of results to date on these various research efforts.

Manganese Induced Immune Suppression In *Nephrops norvegicus*

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Manganese (Mn) is one of the most abundant elements in soft bottom sediments, where the metal is predominantly bound in a four-valent colloid state (MnO_2). During hypoxic conditions MnO_2 can be reduced and released in its bioavailable state (Mn^{2+}). In such situations the Mn^{2+} concentration can increase by a factor of 1000 and reach 20-22 mg l^{-1} in the bottom waters. The bioavailable fraction can remain even after hypoxia, since Mn^{2+} needs to attach to particles to reoxidize. In bottom living organisms, such as the Norway lobster, *Nephrops norvegicus*, Mn can reach neurotoxic levels. The main transport of Mn out of mitochondria occurs via a slow, energy-requiring Na^+-dependent efflux mechanism. Thus, the metal accumulates, primarily in the nervous tissue but also in the hemolymph of *N. norvegicus*. In the hemolymph Mn accumulated three times the exposure concentration and as much as 80-90% of the metal was associated to the protein fraction, mainly consisting of hemocyanin but also including the immune active hemocytes. Recently, we discovered that a surplus of Mn affected several immunological processes of *N. norvegicus*. These are the first reports pointing out Mn as immune toxic. When exposed to relevant concentration of 20 mg l^{-1} Mn we found that the number of haemocytes of lobsters significantly decreased by 44-50 %. Despite the great loss of haemocytes renewal through increased proliferation of the haematopoietic stem cells did not appear. Additionally, maturation of stem cells to immune active haemocytes was inhibited in Mn exposed lobsters. The degranulation of haemocytes, crucial for release of prophenoloxidase system (ProPO), was also significantly suppressed after Mn treatment. Furthermore, the activation of the ProPO by the non-self molecule, lipopolysaccharide, was blocked in Mn treated lobsters. Most recent study have revealed that apoptosis in both haematopoietic precursor cells and circulating haemocytes is an important contributing factor to the Mn induced hemocytepenia observed in *N. norvegicus*. Animals were exposed to different concentrations and time intervals (0, 5, 10, 20 mg l^{-1} Mn^{2+}; 5 and 10 days). Legible results showed a gradual increase in apoptosis ratio from 0 mg l^{-1} Mn^{2+} to 20 mg l^{-1} Mn^{2+} after both the time intervals. These results together may increase susceptibility to bacteria and infectious diseases in naturally living animals.

Clinical Aspects Of Water Biology And The Effects On Water Quality In Koi Ponds

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As the new specialty practice of pet fish medicine evolves, important factors influencing the health care of individual species will begin to help the practitioner develop a diagnostic protocol and database of norms. Sufficient information about the chemical aspects of water quality of koi ponds has been written, yet very little has been published about the biological aspects of water quality and the relationship with disease. As a clinician, bacterial disease, from a point-of-view of both morbidity and mortality, remains one of the most frequent causes of health problems and reasons to contact the fish veterinarian. When parasites and water chemistry have been ruled out as sources of the bacterial disease, problems of water biology should be considered. As a routine protocol, both the water column and the benthic zone should be evaluated. The water column is evaluated quantitatively for turbidity and qualitatively by microscopy using a protocol developed by the author. The benthic zone is evaluated by direct microscopy of scrapings. The findings and their effects on water quality will be discussed.

Selected Cases In Fish Medicine In Private Practice

Sandra F. Yosha

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Fish presented to a private practice veterinary clinic are often pets or are individually important. Owners have often developed a human-pet fish bond that supersedes estimated dollar value of the animal. As pets, they have names and are provided a work-up similar to what is provided for other companion animals. The 20 year old giant gourami named "baby" was presented with a history of several months of "bloat" and recent anorexia. This fish was examined, anesthetized, and submitted for x-ray and ultrasound. Although a pre-mortem diagnosis was not determined, a poor prognosis prompted the owners to elect euthanasia. Post mortem analysis was compared to pre-mortem diagnostic tests to arrive at a probable diagnosis of ileus. The results are discussed and compared to a previous case of anorexia in a green moray eel.

Frozen Fish: Private Fish Medicine In Buffalo, NY

Helen E. Roberts

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According to the American Pet Products Manufacturers Association 2005-2006 National Pet Owners Survey (APPMA), Americans own 139 million freshwater fish and 9.6 million saltwater fish. Treating pet fish is a unique and challenging combination of herd health and individual pet medicine in a field where a necropsy is currently the gold standard for diagnosis. Veterinary care is sought for several reasons including the deep emotional bond the owners have with their pets and the financial cost of their fish. Veterinarians can provide diagnostic services that can ensure a more accurate diagnosis and increased survivability of these pets. Two detailed case reports of malignant melanoma in *Carassius auratus* will be presented. The cases will demonstrate the bond that clients have with their fish and the diagnostic and treatment options open to private practitioners.

Dermal Mesenchymal Neoplasms In Goldfish, Koi And Carp

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Dermal mesenchymal tumors are not uncommon in fish, however, the prevalence of such neoplasms appears to be especially high in goldfish Carassius *auratus auratus*, koi *Cyprinus carpio carpio* (ornamental), and common carp *Cyprinus carpio carpio*. Diagnostic criteria tend to place these tumors into two major categories: pigment cell neoplasms and peripheral nerve sheath tumors. However, these piscine neoplasms contain phenotypic characteristics which do not appear to be mutually exclusive within each of the tumor categories. Despite previous descriptions in other studies, differential morphologic criteria for these lesions remain unclear. Consequently, ancillary tests such as cytochemical and immunohistochemical and/or transmission electron microscopy may be required for more definitive diagnoses. The National Cancer Institute's Registry of Tumors in Lower Animals (RTLA) contains more than 60 examples of pigment cell and nerve sheath neoplasms in goldfish, koi, and carp. These challenging cases will be reviewed with the salient features elucidated and illustrated.

Laser Surgery To Remove Skin Tumors in Goldfish (*Carassius auratus*)

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Various types of mesenchymal neoplastic dermal tumors have been described in goldfish and other teleost species. These tumors rarely metastasize but can grow quickly and are usually aesthetically unpleasing. These growths can also put the affected fish at risk of secondary infection and/or blood loss if the tumor becomes traumatized. In our experience traditional surgical excision, and even intralesional chemotherapy in one case, have been unrewarding (the tumors usually recur). Laser surgery, combined with surgical debulking of the mass, has promise as a curative treatment regimen for dermal tumors in goldfish. Laser is an acronym for: Light Amplification by Stimulated Emission of Radiation. A carbon dioxide (CO_2) laser was used in the goldfish cases to be discussed during the presentation. One of the patients has now been grossly without tumor recurrence for 29 months (since September, 2003). In addition to general anesthesia with tricaine methanesulfonate (MS-222), a local anesthetic such as 2% lidocaine is recommended. Post-operative care may include topical silver sulfadiazine cream on the surgical site and an injectable analgesic agent such as butorphanol or ketoprofen.

Safe And Effective Control Of Ozone In Aquarium Life Support Systems

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Ozone's chemical properties make it a valuable resource for use in managing water quality in aquarium life support systems. As an effective disinfectant and micro-flocculent, it provides excellent control of microorganisms, dissolved organic molecules, and other contaminants that threaten fish health such as heavy metals. When applied carefully ozone can remove organic pollutants and disinfect aquarium water through two processes: flocculation in protein skimmers, in the range 0.01-0.05mg.l^{-1}, and oxidation in contact chambers, in the range 0.10-1.00mg.l^{-1}. When improperly applied and controlled, ozone can be severely harmful to species held within the system, and can even pose a threat to human health. By understanding and using the concepts of applied ozone dose, redox potential and redox controllers, and by using best practices for husbandry staff; ozone can be effectively applied and controlled to achieve desired benefits. Ozone and some of its by-products, collectively referred to as total residual oxidants (TRO), are toxic to aquatic life so great care must be employed in its use. A safe ozone dosing regime can only be achieved by monitoring all of the following parameters: (1) applied ozone dose (2) oxidative redox potential; (3) TRO; (4) turbidity; (5) husbandry (i.e. feeding and cleaning activities); and (6) animal behavior.

The Microbial Diversity Associated With The Gorgonian Coral, *Pseudopterogorgia americana*

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Corals harbor an active microbial community in their surface mucopolysaccharide layer (SML) that may be specific to the host coral over spatial and temporal scales. We used both culture-dependent and culture-independent approaches to characterize bacteria associated with the SML of a healthy gorgonian coral, *Pseudopterogorgia americana*, from Rocky Point Reef, San Salvador, Bahamas. Of 53 microbial isolates obtained, 30 were distinct ribotypes based on 16S rDNA sequence analysis, and all of the isolates clustered within the *Bacillus*, *Gamma* and *Alpha Proteobacteria*. Additionally, 10 of the SML isolates tested positive for quorum sensing signaling molecules and 2 tested positive for antifungal activity against *Aspergillus sydowii*. Following extraction of SML DNA, PCR amplification of the microbial community 16S rRNA genes was followed by denaturing gradient gel electrophoresis (DGGE) and 16S rDNA cloning. Of the DGGE sequences obtained, the majority clustered within the *Beta Proteobacteria*. The 16S rDNA clone library was dominated by the genus *Sphingomonas*, with almost half of the sequences identified belonging to this genus. Thus, we observed little congruency between the microbial communities observed in culture-dependent and culture-independent approaches.

Assessment Of Microbial Communities Associated With An Acroporid Coral Disease Outbreak

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Acroporid coral populations in the Western Atlantic have been impacted by numerous disease events over the past two decades, contributing to region-wide declines in these formerly dominant reef-building species. One such mortality occurred in the Florida Keys National Marine Sanctuary, Biscayne National Park, and Dry Tortugas National Park during the late spring of 2003. *Acropora cervicornis*, *A. palmata*, and *A. prolifera* displaying similar disease signs were observed and sampled from these locations during the mortality event. Aseptically obtained tissue, mucus, and water samples were used to cultivate bacteria on non-specific marine media. Samples were also analyzed for total microbial composition by construction and sequencing of PCR-based 16S rDNA libraries. The presence/absence of known coral pathogens was determined by PCR assay using primer pairs designed to specifically target such organisms. Sequencing of 16S rDNA libraries indicates co-occurrence of two distinct *Photobacterium* spp. groups preferentially within diseased corals. Isolation of culturable bacteria additionally produced several *Photobacterium* spp. from diseased samples. Ongoing research will further assess potential roles of these microbes to the disease.

Investigations Of *Serratia marcescens* As A White Pox Pathogen In The Florida Keys

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Serratia marcescens causes white pox disease in the Caribbean elkhorn coral, *Acropora palmata.* Although *S. marcescens* is common in freshwater and terrestrial environments and in the intestines of humans and other animals, the prevalence of the bacterium in the marine environment and the source of the coral pathogen are unknown. Nearshore marine and coral reef environments in the Florida Keys and raw sewage and treated effluent from an advanced wastewater treatment plant were screened for the presence of the bacterium. *S. marcescens* was identified using selective agar media and species-specific PCR. RFLP-PFGE was used to determine the diversity of *S. marcescens* and to elucidate a potential source of the coral pathogen. A total of 257 *S. marcescens* isolates produced 118 different pulse-field patterns, indicating that *S. marcescens* is genetically diverse in environments of the Florida Keys. While the bacterium was rare in marine environments (0.3% prevalence), it was common in untreated human sewage (100% prevalence) and canals (76.5% prevalence). The majority (84%) of *S. marcescens* was isolated from raw sewage and contaminated nearshore sources. The source of the known acroporid serratiosis pathogen (strain PDL100) was not identified, but a second strain of *S. marcescens* (strain PDR60) was isolated from disease lesions and found to be identical to a *S. marcescens* strain isolated from human sewage.

Coral Disease And Genetics Survey In St. John, USVI Using Non-Destructive Methods

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In August, 2004, USGS Scientists with the help and cooperation of NPS personnel, sampled healthy and diseased *Montastrea annularis* along transects at Tektite Reef, St. John Island, US Virgin Islands. Importantly, noninvasive, nondestructive sampling methods were employed to accomplish sampling needs and get statistically significant numbers with minimal impact on the reef organisms. Sterile, foam swabs were used to sample healthy and diseased coral, and material was transferred to Whatman FTA cards for storage and transport to the laboratory. This card-based sampling method allows room temperature storage of DNA samples for years and eliminates problems with often-used low temperature storage and transport from remote locations. Bacterial 16S ribosomal genes and zooxanthellae ITS-1 genes were readily amplified by polymerase chain reaction (PCR) from card samples. In particular, positive PCR signals for *Aurantimonas sp*. (the presumptive causative agent of White Plague) were widely detected from apparently healthy corals. Additionally, *Serratia marcescens* (presumptive causative agent of White Pox) was also detected from healthy as well as diseased *Acropora* at Hawksnest Bay. The samples analyzed to date preceded the peak of the large bleaching event that occurred in 2005. The genotypes of zooxanthellae present before this bleaching event are being determined and will be compared with post-bleaching recolonizations. Additional samples have been taken over time and across reef tracts and are being tested using molecular methodology for the presence of pathogenic bacteria and examined for possible correlations of pathogen presence/absence with host (coral) and symbiont (zooxanthellae) genotypes. The sampling methods developed and tested are easily employed in the field under a variety of conditions.

Zooxanthellae Shifts And Yellow Band Disease

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Many outbreaks of Yellow Band Disease have been reported Caribbean-wide, affecting mainly the *Montastrea annularis* complex, one of the main components of the reef framework of the Caribbean. Several authors have discussed the possibility that the pathogen(s) infect the algal symbiont rather than the coral tissue. Experiments looking for possible pathogens yielded a number of *Vibrio* strains. Observations of possible shifts in the clade composition of corals with the disease support this hypothesis. In order to study zooxanthellae clade composition and changes due to the presence of the disease, affected and unaffected portions of *Montastrea faveolata* corals were extracted from La Parguera, Puerto Rico and evaluated using Denaturing Gradient Gel Electrophoresis of the ITS region of the zooxanthellae. Our results showed changes in zooxanthellae composition in corals affected by Yellow Band Disease, shifting from C7/C12 and B1 clades to A1.1 in most cases. Although these patterns do not seem to hold in other parts of the Caribbean, based on similar studies in other parts of the Caribbean, it is interesting that changes occur in the zooxanthellae composition. This supports the suggestion of a zooxanthellae disease. This was the first time that clade A1.1 was isolated from a coral sample, showing the possibility that this zooxanthellae is opportunistic.

White Band Disease In The Caribbean

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The white band disease (WBD) epizootic event of the early 1980's resulted in significant changes in the structure and composition of coral communities throughout the wider Caribbean. During the fall months of 2003 and 2004, samples of *Acropora cervicornis* with signs of white band disease type II (WBD-II) were collected in Mario reef, La Parguera, southwest coast of Puerto Rico. Bacteria extracted from these samples were isolated in TCBS agar, grown in Glycerol Seawater agar, and then used to infect healthy-looking colonies at the same locality. Signs of the disease were produced in the inoculated corals, which were subsequently sampled and the bacterial agent re-isolated to be identified, thus fulfilling Koch's postulates. Preliminary results confirmed that the cause of WBD-II is a *Vibrio* species very close to *Vibrio harveyi*.

Molecular Diversity Of Microbes Associated With Black Band Diseased Coral *Siderastrea siderea*

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Biodiversity and abundance of reef-building corals are substantially declining worldwide and coral disease is believed to be a major contributing factor. Black band disease (BBD) is one of the diseases, which plays an important role in the decline of coral reefs especially in Caribbean. The causative agent for BBD has not been identified, and the earlier studies showed that it is a 'polymicrobial' disease. In the present study, microbes associated with BBD infected *Siderastrea siderea* corals collected from two reefs in the Bahamas (Horseshoe and Rainbow Reefs) and one in the Florida Keys (Watson Reef) were assessed by 16S rRNA gene cloning, sequencing, and amplicon-length heterogeneity (LH)-PCR community profiling. Partial and full-length 16S rRNA gene sequences were retrieved from positive clones and used for comparative sequence analyses. Results showed that the clone libraries were dominated by α-proteobacteria (53-87%) followed by δ-proteobacteria (6-20%), verrucomicrobia (2-11%), cytophaga-flavobacterium-bacteroidetes (CFB, 6-9%), cyanobacteria (0-6%) and other bacterial groups (0-6%). These results were compared with LH-PCR whole community profiles to confirm their presence. Sequences related to bacteria associated with toxin-producing dinoflagellates, and sulfate-reducing bacteria like *Desulfovibrio* were consistently present in all the clone libraries. Sequence types of bacteria associated with juvenile oyster disease (JOD) and known toxin-producing cyanobacteria were found in at least two sampling sites. This study provides a better understanding of BBD associated microbes as compared to previous molecular studies.

Defining The Reservoir Of The Pathogen *Aurantimonas coralicida* By Fluorescence *In Situ* Hybridization

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The three dimensional structure of coral reefs creates many different microhabitats that can be used by coral disease pathogens as a reservoir. In the last decade, the coral disease white plague (WP) has dramatically reduced the population sizes of its coral hosts. Although advances in the study of this disease include the isolation and identification of the causative agent (*Aurantimonas coralicida*), there are still many unknown aspects to its etiology. These include the mode of transmission, the reservoir of this bacterium, and the infection mechanism that initiates the disease. Fluorescence *in situ* hybridization (FISH) with an oligonucleotide probe specific to *A. coralicida* has been developed based on the 16S rRNA gene sequence of the isolate. This probe has been used to diagnose WP in several reefs in the Caribbean and has proven to be efficient for the identification of *A. coralicida*. Using FISH and the species-specific oligonucleotide probe, reef sediment samples, including in association with the surfaces of the macroalgae *Halimeda* and *Dictyota spp*., and water samples, from three different Caribbean reefs have been analyzed. *Halimeda* was recently proposed to play a role in WP infection on the reef. Results showed that *A. coralicida* is present in both the water column and the sediments around these reefs, and is not exclusively associated with any algal species. This suggests that the reservoir of *A. coralicida* may include both the sediment and the water column as well as the surface mucopolysaccharide layer (SML) of healthy but WP susceptible corals.

Differential Diagnosis: A Need For The Use Of Multidisciplinary Tools To Investigate The Coral Disease White Plague Type II

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Microbial community composition in apparently healthy and white plague type II (WPII)-diseased colonies of *Montastraea annularis* (complex) from St. Croix, U.S. Virgin Islands and The Bahamas was characterized using a combination of molecular fingerprinting, microbial culturing, 16S rDNA sequencing, and histopathology. Principal coordinate analysis (PCO) of amplicon length heterogeneity (ALH) fingerprints of the bacterial community on unaffected corals and apparently healthy tissue from diseased corals were similar within each geographical location but differed between locations. Diseased samples from both sites were more similar to each other than to the other samples. The major bacterial Operational Taxonomic Units from coral genomic DNA extracts proved to be culturable (32% match) bacteria. 16S rDNA sequencing suggests that this culturable community is dominated by the *Pseudoalteromonas* and *Vibrio* groups. *Aurantimonas coralicida*, the causative agent of WPII (amplicon length 313.1 bp), was found in relatively low abundance on both diseased and healthy tissue on affected corals from The Bahamas. Tissue changes varied with the sample. These findings conflict with previous reports on WPII pathogenesis and suggest that the same, or similar, disease signs could be caused by different organisms, thus highlighting a need for the application of differential diagnostic techniques in the identification of coral diseases.

From Leidy To Snieszko: Pioneers Of Fish Health In The US (1850-1950)

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The achievements of seven fish health pioneers from Joseph P. Leidy to Stanislas F. Snieszko are discussed. Leidy (1823-1891), one of the most prominent 19th century scientists, published more than 600 papers; working at the fringe of his interests he published 23 papers on fish parasites making him the most prolific fish health writer of the 19th century. He was one of the first to recognize that stress predisposed fish to disease. Livingston Stone (1836-1912), clergyman turned premier fish culturist, described the first fish treatment (salt) and 23 diseases of domesticated trout and drew sketches of microscopic fish parasites. Stephen A. Forbes (1844-1930), one of the founding fathers of ecological studies was the first to use histological and bacteriological techniques in fish diagnostics. Edwin Linton (1855-1939), the father of fish parasitology in the United States, produced more than 60 publications about fish parasites. Millard C. Marsh (1872-1936), the first fish pathologist for the U. S. Fish Commission published 19 papers that were directly beneficial for the control of fish diseases; these included information on bacterial characterization and diseases description, disinfection and treatments, blood parameters, immunity, tumors, and several non-infectious diseases. Herbert S. Davis (1875-1958), often called the "Father of Fish Health" in the US, published on viral, bacterial, parasitic, fungal, nutritional, and environmental diseases and established a disease diagnostic service and the world renowned fish disease laboratory at Leetown, WV. Snieszko (1902-1984), the gentleman-ambassador for US fish health, published more than 200 fish health papers and is best known for his work with bacterial pathogens, the interaction of the host, pathogen, and environment, fish health training, and disease diagnostic and certification programs.

The Current Status Of The United States National Aquatic Animal Health Plan

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The Joint Subcommittee on Aquaculture's National Aquatic Animal Health Task Force must develop and implement a national aquatic animal health plan (NAAHP) for aquaculture in cooperation with industry, regional organizations, state, local, tribal governments and other stakeholders. The NAAHP will foster and support efficient aquaculture; protect the health of our nation's wild and cultured aquatic resources; and meet both national and international trade obligations. The NAAHP requires input from working groups composed of individuals who represent a certain sectors of the aquaculture industry. A pivotal chapter of the plan has been drafted on guidelines for the surveillance of fish diseases. The NAAHP is anticipated to be completed in the spring of 2007. The plan will not be codified into regulation; however, implementation of certain elements, such as import requirements, may require revisions to existing laws, regulations or policies. In this presentation, a summary of key milestones will be presented and discussed with regard to future goals.

Fish Health Medicine Certificate Program

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The "Fish Health Medicine Certificate Program," was developed by the Wisconsin Department of Agriculture, Trade & Consumer Protection (WDATCP), the University of Wisconsin-Madison (UW-Mad), and national fish health experts. The course is constructed as a series of modules for veterinarians leading to certification. The full certificate program involves five online modules and a final hands-on training module. The online lectures use narrated PowerPoint presentations and supplemental reading materials delivered using new educational technology software. The course is available through the continuing education (CE) portal of the University of Wisconsin, School of Veterinary Medicine, http://vetmedce.vetmed.wisc.edu/FishCertificate/. Each module has a post-test automatically generated and computer scored. Successful completion of each module will result in the award of CE credit that can be used to satisfy state veterinary licensure requirements. The final module of the certificate program is a hands-on one-day course called "Aquaculture Veterinary Medicine for Practitioners." It is an intensive program designed to provide practical training in field techniques for sample collection and field diagnostics reinforcing the online training. Selected online training modules will also be made available individually as well as part of the full certificate program. This project was made possible by funding from the National Risk Management Feasibility Program for Aquaculture, a partnership program between the USDA Risk Management Agency and Mississippi State University. This certificate program is expected to reduce risk of disease on fish farms by increasing the number of trained veterinarians available to provide services to fish producers.

Natural Content And Processing Of Alternative Protein Sources: Histologic Effects In Fingerling Channel Catfish (*Ictalurus punctatus*)

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Significant effort has been made to utilize alternative protein sources like cottonseed meal (CSM) and soybean meal (SBM) to replace fishmeal in channel catfish (*Ictalurus punctatus*) feed. These sources are readily available and have high nutritional value, but contain anti-nutritional factors (ANF) and toxins (ex. trypsin inhibitors in SBM and gossypol in CSM) that may be detrimental to fish. We studied the effects of these factors by evaluating histopathologic changes in fingerling catfish fed diets containing various levels of CSM, gossypol-acetic acid, or SBM for 10-12 weeks. Changes associated with ANF/toxins were variable often in a dose-dependant manner, but included hepatic glycogen deposition, pancreatic necrosis, pancreatic vacuolization, loss of enterocyte supranuclear vacuolization, and pigmentation of the spleen. Processing methods commonly used to inactivate or detoxify CSM or SBM ANF/toxins in mammalian diets were also studied; iron failed to reduce gossypol toxicity and heat-treatment failed to significantly reduce trypsin inhibitor toxicity. These studies indicate that ANF/toxins naturally present in SBM and CSM may cause histologic changes in fingerling channel catfish. Further refinement of processing techniques to inactivate or eliminate these ANF/toxins and minimize their adverse effects on fish is warranted.

Growth Response And Acquired Resistance Of *Streptococcus iniae*-Recovered Nile Tilapia, *Oreochromis niloticus*

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The growth performance and acquired resistance of *Streptococcus iniae*-recovered Nile tilapia was determined. Tilapia were challenged with three doses of *S. iniae* (8.8×10^3, 8.8×10^4 and 8.8×10^5 CFU/fish for low, medium and high challenge, respectively). Groups of non-injected and tryptic soy broth-injected fish were maintained as controls. Significantly ($P < 0.05$) higher mortality (45.0 %) occurred in the high challenge treatment than in the low challenge treatment (29.6 %). The medium challenge group had mortality of 36.3%. The *S. iniae*-recovered tilapia, which were used to assess growth performance, were selected from survivors without clinical signs of disease. Fish were randomly stocked at 30 fish per 57-L aquarium in triplicate and fed to apparent satiation for 8-weeks. No differences were detected in weight gain, feed intake, feed efficiency ratio or survival between *S. iniae*-recovered tilapia and the control treatments following the 8-week performance trial. Following the 8-week feeding study, the tilapia were challenged with 1×10^6 CFU/fish of *S. iniae* to assess acquired immunity. Mean cumulative mortality was significantly higher ($P < 0.05$) in the control treatments (41.7 % and 43.3 %) than in the low, medium and high challenge treatments (7.4, 3.3 and 8.3 %, respectively). The results suggest that *S. iniae*-recovered tilapia not showing overt disease signs gained acquired immunity and performed as well as non-infected tilapia.

Hurricane Katrina And The Mississippi Catfish Industry, A Little Good News For A Change

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In the past five years, commercial catfish aquaculture in Mississippi has faced a number of challenges, including historically low farm gate prices, competition from foreign imports, and the ongoing cost of disease losses. As hurricane Katrina approached the Gulf Coast, dire predictions were made concerning potential losses to the catfish industry. In August, oxygen budgets in ponds are critically low and supplemental aeration, supplied in the form of electric and tractor driven aerators, is typically required for fish survival. On August 29, 2005, Katrina moved through central Mississippi causing catastrophic damage in the southern third of the state. Despite widespread power outages over much of the state's catfish producing areas, through cessation of feeding ahead of the storm's arrival, a major disaster was averted. Mississippi's poultry industry was not so fortunate and suffered major losses. Although there were fuel shortages, damage to the distribution system for processed fish, as well as temporary losses of power to processing facilities and feed mills, these problems were quickly resolved. The major impact of Katrina on the catfish industry appears to have been the loss of markets in devastated coastal communities. A number of these areas were supplied with fish donated by processors to aide in relief efforts. At present, the price of fish remains strong.

Bacterial Endocarditis In The Giant Pacific Octopus, *Enteroctopus dofleini*

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Giant Pacific Octopus, *Enteroctopus dofleini*, are extremely popular, interesting members of many public aquaria's collections. They are intelligent and easy to fall in love with. Octopus manifest characteristics of personality, mood, and behavior that attract the keeper's attachment. Unfortunately, octopuses are relatively short lived and are sensitive to water quality issues and diseases that we are, just now, beginning to understand. This presentation refers to the first octopus that Tennessee Aquarium housed and exhibited. Temperature changes and water quality associated with starting up a new cold water marine exhibit may have contributed to its demise. Ultimately, the octopus exhibited signs of senescence that resembled the typical geriatric problems that other institutions had experienced. Post-mortem examination followed by bacteriology and histopathology (Connecticut Veterinary Medical Diagnostic Laboratory) helped determine that the octopus succumbed to a bacterial endocarditis associated with coliform bacteria.

Enrofloxacin Resistant Meningoencephalitis In A Tiger Shark (*Galeacerdo cuvier*)

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A 7 foot 2 inch 163 lb female tiger shark (*Galeacerdo cuvier*) had a history of abrasions on its dorsal fin due to scraping exhibit rock work. To prevent further abrasions, the animal was transferred to a new exhibit and was started on a course on enrofloxacin. A few months later, the shark started intermittently exhibiting abnormal swimming behavior consisting of holding its head above and tail below the horizontal plane. The shark was given several more rounds of enrofloxacin. The shark became anorexic for 1 week, the veterinarian was notified and a physical examination was performed. On physical exam, the animal was extremely cachectic, had exposed cartilage on the cranial edge of the dorsal fin, and the edges of the pelvic, caudal, and anal fins, and had severe ventral abrasions. Hematology and chemistries appeared to be within normal limits. The blood cultured enrofloxacin resistant *Staphylococcus cohnii*. A fecal wash collected a small amount of stool in which no parasites were present. The animal was administered vitamin E, vitamin B complex, ceftiofur sodium, and prednisolone sodium succinate. The animal continued to deteriorate the following day and methylprednisolone acetate and oxygen therapy was given. The next morning, the animal was hyporesponsive, and euthanasia with MS-222 and quinaldine sulfate was elected. At necropsy, a sterile cerebral spinal fluid tap was performed. The fluid was clear, transparent, of moderate tenacity, and contained a few pieces of white flocculent material, crystals, and other nondescript debris. The fluid cultured enrofloxacin resistant Streptococcus acidominimus. The GI tract was devoid of food, and the colon contained numerous tapeworms. Histopathology revealed meningoencephalitis and nephritis, and branchitis, gastritis, and coelomitis associated with parasitism.

Koi Herpesvirus In New York State: The 2005 Common Carp Mortality Event

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In June, 2005 a mortality event was reported to our Fish Disease Diagnostic Laboratory by the New York State Department of Environmental Conservation. The mortality event involved common carp (*Cyprinus carpio*) on Chautauqua Lake, New York. The mortality event lasted approximately 2 weeks, during which time an estimated 25,000 adult carp weighing an estimated 20 – 30 pounds each died. In consideration of the number of dead fish floating on the lake as the July 4th holiday weekend approached, a massive clean-up effort was undertaken by the New York State Department of Environmental Conservation and the Chautauqua Lake Association. According to a local newspaper, "The rafts of dead carp are being buried in trenches next to the local landfill." Our diagnostic processing of the specimens submitted revealed the Koi Herpesvirus (KHV) by both cell culture in Fathead Minnow (FHM) cells and by quantitative PCR. Because the virus grew in FHM cells, we forwarded a sample of virus to the USDA APHIS National Veterinary Services Laboratories (NVSL) in Ames, Iowa to rule out the potential of Spring Viremia of Carp Virus. Results of testing at NVSL were consistent with our diagnosis of KHV. This was the second consecutive year that KHV has been found in this watershed. In 2004 approximately 8,000 adult common carp died in the Chadakion River, the river that drains Chautauqua Lake.

Pancreatic Amyloidosis In Captive Tricolour Sharks (*Balantiocheilus melanopterus*)

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A captive adult tricolour shark from a freshwater display was found dead. Gross post mortem revealed an enlarged abdomen that was expanded by approximately 2ml of bloody fluid. Skin scrapes and gill biopsies were unremarkable. Significant histological lesions were limited to the peritoneal cavity. The exocrine pancreas comprised an unusually large proportion of the peritoneal parenchyma. This expanded exocrine pancreatic tissue was composed of areas of well organized pancreatic acini interspersed with a population of a poorly differentiated, basophilic, angular, spindle-shaped cells arranged in cords that compressed and effaced normal parenchyma. These cells possessed large pleiomorphic nuclei, prominent nucleoli and basophilic cytoplasm. Some of these cells appear to have small accumulations of cytoplasmic zymogen granules. Within mesenteric tissues surrounding the mass, there is marked, multifocal expansion of collagen fibers by macrophages, hemorrhage and small numbers of neoplastic cells similar to those described above. The tunica media of large blood vessels was greatly expanded by accumulations of a lightly eosinophilic, hyaline, homogeneous, acellular material. Similar material was present expanding the fibrovascular connective tissue of the pancreas, liver and mesenteries. No organisms were identified with a Brown and Brenn modified gram stain. The homogeneous, acellular material was light-orange and exhibited birefringence upon polarization when stained with Congo red and was light-pink with toluidine blue. This material appeared bright-green upon microscopic examination using 255nm ultraviolet light after staining with thioflavine-T. Exocrine pancreatic neoplasia (pancreatic adenocarcinoma in this case) is uncommon in fish and most reports describe lesions following experimental exposure to carcinogens. Amyloidosis is a very uncommon finding in fish and to our knowledge has not been described associated with a neoplastic lesion. In secondary amyloidosis (secondary to chronic inflammation or some types of neoplasia) the fibrils are composed of altered serum amyloid A. The composition of the present deposit is not known but will be investigated using laser micro-dissection, polyacrylamide electrophoresis and MALDI-tof mass spectrometry.

Progressive Erosive Stomatitis In Green Moray Eels: A Case Study

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Two green moray eels (*Gymnothorax funebris)* from a public aquarium collection were presented to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL). In this presentation we describe the clinical skin ulcerations that developed in these two eels. The second eel was necropsied and samples of skin, gill, tests, kidney, liver and serum were collected. Skin samples were stained with H&E and Periodic Acid Schiff and observed with light microscopy. Additional skin samples were submitted in 2.5% glutaraldehyde for electron microscopy evaluation. Under light microscopy the skin appeared thickened with scattered foci of ulceration and erosions with an increased number of goblet cells. No infectious agents were identified ultrastructurally and no significant tissue alterations were found in the other tissues examined. Similar lesions in moray eels have been caused by hypoxic conditions, exposure to infectious agents or exposure to toxic compounds, however these were ruled out through water quality tests and histopathology. The absence of identifiable infectious agents in any of the tissues examined, as well as, no history of contact with toxins or irritants leave the underlying etiology undiagnosed. It is believed that stress induced hypercortisolemia may have been an inciting factor.

The Big Belly Blues

Andrew J. Mitchell

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Abdominal enlargements (big belly) occur for several reasons: when host tissues proliferate, when air bladders over-inflate, when fluids fill the body cavity, when egg production becomes much greater than normal, when the stomach becomes over-filled with food, and when invading organisms grow or multiply within the body cavity. Some causes for abdominal enlargement in fish have not been determined while others have defined etiologies or associated pathogens including several caused by bacteria, viruses, and parasites. Several parasites that are associated with abdominal enlargements in warmwater cultured fish include: a zygomycete fungi (microsporidian) -- *Gluglea pimephales*; a myxosporean called the kidney bloater -- *Hoferellus carassii*; a nematode -- *Eustrongylides* sp.; visceral tapeworms -- *Ligula intestinalis*, *Diphyllobothrium sebago* and *Schistocephalus solidus*; the Asian tapeworm -- *Bothriocephalus acheilognathi*; and the white grub -- *Posthodiplostomum minimum*. These parasites will be briefly discussed with emphasis placed on the Asian tapeworm and the white grub. Comments on control measures for these latter two parasites will be given.

Pathology Associated With *Coleps* Spp. In Tilapia

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Tilapia (*Oreochromis niloticus*) were submitted to the Pennsylvania Animal Diagnostic Laboratory System from a commercial producer who was raising the fish in a recirculating system. The major complaint was that production in the various tanks of the system was not up to expected standards. No major mortalities were noted at this time. Fish were submitted live and were subjected to a full diagnostic work up which included gill biopsies, skin scrapes, necropsy, and microbiological culture of the kidney and histopathological examination of samples of all the various tissues collected in 10% neutral buffered formalin. Microscopic examination of the skin scrapes and gill clips revealed a large number (approximately 15 to 20 per high power field) of a small (approximately 75um X 30um), barrel shaped holociliate that was later identified as *Coleps* sp. The gills also appeared swollen, congested and slightly blunted. Although *Vibrio angullarum* was isolated from one fish (out of five), there were no corresponding histopathological findings that would indicate a bacterial infection. Significant histopathological findings were renal tubular mineralization as well as lamellar edema and epithelial erosion of the gills. Occasionally, sections of organisms consistent with *Coleps* sp. were also present in between the gill lamella. It was thought that branchial lesions might be a result of a combination of factors including water quality (the total ammonia nitrogen was reported to be high), formalin treatments and as well as possible irritation by the protozoa. Similar appearing organisms were also rarely seen in the affected renal tissue. *Coleps* sp. are protozoans that have distinctive protective calcerous plates. They are mainly regarded as free living scavengers that feed on dead and dying tissue and microorganisms. However, they have been reported to kill zebra fish (4-9 day old) larvae in production systems as well tropical fish and rainbow trout.

Three Disease Cases: Acid-Sulfate Soil, Largemouth Bass Wintertime Sudden Death, And Channel Catfish "Pimples"

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Acid-sulfate soils in the watershed of largemouth bass ponds in Ohio were exposed to air and rain after deforestation. Acid run-off into the bass ponds decreased pH and caused mortalities ranging from 100% in ponds closest to the exposed acid-sulfate soil to 0 - 1% in ponds near the end of the hollow. Red iron oxide and hydroxide precipitate marked the ponds experiencing high mortalities. In another case, largemouth bass in Kentucky are experiencing high mortalities this winter (2005-2006). They display lethargic and disoriented swimming, and when startled, they thrash vigorously at the water surface and die in less than a minute with the pectoral fins rigid at a 90º angle from the body. Daily mortalities range from 20 to 40 out of 4,000 fish. In a third case, digital photographs of channel catfish with red lesions resembling pimples or blisters were sent to my lab in January 2006. In previous similar cases, no cause of this condition was found.

Unusual Spinal Deformity In Newly Hatched Yellow Perch

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In June, 2005 a diagnostic case involving yellow perch (*Perca flavescens*) was submitted to our Fish Disease Diagnostic Laboratory. The fish were newly hatched yellow perch that had originated from eggs collected from Oneida Lake, New York. These fish were to be used in a research project. The complaint associated with the case was that the fry from certain egg groups were experiencing moderate to high mortalities, while fry from other egg groups experienced no such mortality. Microscopic examination of the fish revealed severe curvature of the spine. A standard practice of placing used Christmas trees into the lake to serve as spawning substrate for yellow perch was employed in this case. A search of the literature revealed studies with the agricultural pesticide metam, in which similar spinal abnormalities were observed in young zebrafish exposed to the compound. Metam is considered to be the third most widely used agricultural pesticide in the United States. One of the major uses of metam is in the production of ornamental conifers. While we do not have a direct cause and effect of metam in this case and we currently consider the diagnosis to be open, we consider exposure to metam as a cause deserving further consideration.

Atypical Clinical Signs Of *Streptococcus agalactiae* In Nile Tilapia (*Oreochromis niloticus*)

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After injection challenge with *Streptococcus agalactiae*, a male Nile tilapia, *Oreochromis niloticus*, exhibited an erratic corkscrew swimming pattern and assumed a "C"-shaped body posture. The fish did not die after challenge but developed a grossly-observable "hunchback" at the level of the cervical spine. Radiographs revealed multiple spinal curvatures along the length of the spinal column with vertebral lordosis and kyphosis. The male fish also developed a cranial cavitation on the dorsal aspect of the head, forming a depression between the eyes. The fish subsequently mated and helped produce fry that initially appeared normal. The fry population soon experienced high mortalities, and moribund fish exhibited reddened gills, presumptively over-inflated bladders, and spun on their longitudinal axis, often with their head pointed down (tail-up swimming). Surviving fry had stunted or absent fins, deformed gill opercula, and/or cranial cavitation, but did not show vertebral deformities. Gram-positive bacteria identified as *S. agalactiae* were isolated from the fry. *Streptococcus agalactiae* appears to be the causative agent of these skeletal anomalies among the male broodfish and the fry, and this is the first report of hunchback skeletal abnormalities in *S. agalactiae*-infected fish. Furthermore, the results demonstrate the vertical transmission of *S. agalactiae* to the fry.

A Tale of Two Tumors: The Shrimp's And The Clam's

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Diagnosing neoplasms in the invertebrates presents many challenges. Solid and disseminated tumors have been recognized in the Arthropoda and Mollusca; however, the cells involved and changes seen are not always consistent with vertebrate neoplasm characteristics. A recent contribution to the RTLA provides further perspectives on rare crustacean cases in the collection. An epidermal papilloma on *Litopenaeus vannemei* featured thin, elongated abnormal epidermal cells with hypertrophied nuclei and prominent nucleoli, mitotically active areas, abnormal formation of chitin, and cystic, hemolymph-filled stroma in the verrucose mass. In contrast, disseminated neoplasia (DN), a proliferation of anaplastic, hypertrophied circulating cells (hypothesized to be hemocytes, and in many respects similar to leukemias) can be found in up to 90% of some populations of bivalves, occurring in 15 species. Histopathological examinations of razor clams, *Tagelus plebeius*, from Chesapeake Bay, revealed normal-appearing hemocytes and circulating cells with hypertrophied nuclei and scant cytoplasm. Mitoses were few but abnormal nuclear morphologies (binucleate, C-shaped) were common. Razor clams from Delaware Bay also contain hypertrophic circulating cells with normal-appearing hemocytes. Is this a normal cell type for this species? Can the binucleated cells be interpreted based on vertebrate leucocyte variations?

Molecular Detection And Identification Of *Hematodinium* Species

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The parasitic dinoflagellates *Hematodinium* spp. and *Hematodinium*-like species are economically significant pathogens of decapod Crustacea. Previous methods used to detect infections included external assessment for signs of infection, a microscopic index (pleopod diagnosis) and several immunoassays. The current study describes the development and application of two sets of *Hematodinium*-specific polymerase chain reaction (PCR) primers and DNA probes based on *Hematodinium* ribosomal RNA (rRNA) sequences. The first set of PCR primers detects *Hematodinium* infections in *N. norvegicus, C. puber, P. bernhardus* and *C. opilio*. The second set of PCR primers detects infections in *C. sapidus, L. depurator and P. trituberculatus*. Using the latter set of primers, a post-PCR restriction enzyme digestion of the PCR amplification products can be used to differentiate between the *Hematodinium* species infecting *C. sapidus, L. depurator and Portunus trituberculatus*. Sequencing and phylogenetic analysis of the first internal transcribed spacer region (ITS1) of the rRNA region from several *Hematodinium* isolates indicate two genetically distinct groups of *Hematodinium/Hematodinium*-like organisms.

Aspects Of The Pathology Of *Hematodinium* Infections In Snow Crabs (*Chionoecetes opilio*) From Newfoundland, Canada

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Bitter crab disease (BCD) of snow crabs, *Chionoecetes opilio*, is caused by a parasitic dinoflagellate, *Hematodinium* sp. The disease has increased in prevalence in Newfoundland's commercial fishery since it was first recorded in the early 1990s. We examined the pathology of infected snow crabs and documented histopathological alterations to the tissues in heavy infections and alterations to the gills during sporulation of the parasite. Infections of *Hematodinium* can lead to high densities of parasitic cells in the hemolymph (10^8 cells ml^{-1}) that lead to host death. Pressure necrosis was evident in the soft connective tissues of the hepatopancreas, the blood vessels in most organs, and soft connectives in the eyestalks. In heavy infections, little remained of the soft connective tissues around the hepatopancreas. Damage to the gills varied, but in some cases it was severe, involving a loss of host epithelial cells and fusion of the membranous layer of adjacent gill lamellae. Lamellae exhibited hypertrophy with the loss of trabecular cells, hemocyte infiltrations, and clubbing along the distal margins. Large numbers of zoospores were located along the distal margins of affected lamellae suggesting that sporulation may result in the lysis or bursting of the lamellar cuticle to release spores. It is unclear how dinospores transmit the disease to the next host. Hematodinium infections in the snow crab appear to be chronic, long-term infections that end in death during sporulation of the parasite.

Effects Of Hypercapnic Hypoxia On The Clearance Of *Vibrio campbellii* In The Eastern Oyster, *Crassostrea virginica*

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In order to understand the role of marine organisms as vectors of human disease pathogens, we investigated the effects of hypercapnic hypoxia (HH) on the clearance of live pathogenic bacteria in *Crassostrea virginica*. Oysters were held in normoxic (Po_2 = 20.0-20.7 kPa or 98% air saturated, pH 7.8-8.0) or HH (Pco_2 = 1.8 kPa or 2% CO_2, Po_2 = 3.9-4.2 kPa or 19-20% air saturation, pH 6.2-6.5) conditions at 25 °C for 4 h, then injected in the adductor muscle with 10^5 live *Vibrio campbellii*. Culturable *V. campbellii* (CFU) were quantified in the whole oyster, including all tissues and fluids, at 10, 30 and 60 min post-injection. CFU decreased rapidly in all animals, however, oysters held in HH retained significantly higher CFU than animals held in air-saturated water over the test period (2-way ANOVA, $p<0.001$). At 60 min post-injection, animals held in normoxic conditions retained less than 15% of the injected bacteria as compared to animals held in HH, which retained 30% of the injected bacteria. These results indicate that HH dramatically reduces the rate at which oysters can render bacteria non-culturable. We suggest that poor water quality can increase the risk that oysters will harbor and transmit bacterial pathogens hazardous to human and ecosystem health.

Putative Identification Of Expressed Genes Associated With Attachment Of The Zebra Mussel (*Dreissena polymorpha*) Using Suppression Subtractive Hybridization cDNA Library

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Zebra mussels (*Dreissena polymorpha*) continue to cause economical and ecological devastation in the Great Lakes basin. A potentially effective method to control the spread of this invasive species is to prevent its attachment to substrates under water. In this study, a Suppression Subtractive Hybridization (SSH) cDNA library of the zebra mussel foot has been developed. This array contains genes involved in attachment, exocrine excretion, host defense, protease-antiprotease cascades, signal transduction, cell division and development, metabolism, cell structure maintaining, and DNA/RNA synthesis. The relation between those putative genes and zebra mussel attachment is being analyzed. This study provides the first cDNA microarray of zebra mussel and is an important resource toward the bio-control of this invasive species.

Recent Advances In Our Understanding Of The Host-Parasite Relationship Of *Lepeophtheirus salmonis* And Atlantic Salmon (*Salmo salar*)

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The inability of Atlantic salmon to mount a significant inflammatory response against *Lepeophtheirus salmonis* infection has led to the belief that immunomodulatory compounds exist in this parasite's secretions. With the use of dopamine stimulation, compounds within *L. salmonis* secretions have been identified and biologically characterized. Among those compounds identified, trypsin and prostaglandin E_2 have been linked to immunosuppression and anti-inflammatory actions. More recently, pooled secretions separated by size-exclusion chromatography have shown differing degrees of anti-inflammatory activity, measured by down regulation of lipopolysaccharide-induced IL-1β expression. Multiple *L. salmonis* genes (SL-0903 and SL-0858), encoding proteins identified in these secretions have also shown up-regulation following host attachment and infection. Further isolation of fractions within these pools revealed that fraction 1-2 could fully account for the inhibition of IL-1β expression in SHK-1 cells observed in pooled fraction 1. This provides evidence for the presence of immunomodulatory compounds, not related to trypsin or PGE_2, in the secretions of *L. salmonis*, which suppress expression of Atlantic salmon immune-related genes *in vitro*. The implications of this data with respect to the host-parasite relationship and possible development of future vaccine formulations will be discussed.

Unusual Trematode "Egg Nests" In The Stomach Tissues Of Goggle-Eyed Scad, *Selar crumenophthalmus* (Bloch, 1793)

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A shipment was received by the Georgia Aquarium of wild goggle-eye scads from waters off of the Florida Keys and during quarantine 3 mortalities were presented for necropsy. On gross dissection, brown patches of discoloration were evident in the stomach wall of all 3 animals examined. Histological examination showed these to be "nests" of trematode-like eggs in the lamina propria and muscularis mucosa. This presentation is extremely unusual for enteric digeneans, which normally discharge their eggs to the exterior with the host's faeces. Egg nests were frequently associated with granulomatous inflammation and a proliferative connective tissue response. Focal and diffuse lymphocytic infiltrates and blood vessel dilation were also observed, consistent with more acute inflammatory reaction. Connective tissue necrosis led to sloughing of the overlying gastric mucosa in at least one case. Parasitological examination showed infections in the stomach with a small unidentified hemiurid digenean, and in the intestine with a fellodsitomid digenean matching the genus *Tergestia* and an unidentified opecoelid digenean. Histozoic digeneans more often associated with parenteral egg deposition (basically sanguinicolids) were not observed and egg nests were not observed in organs other than the stomach. Egg measurements were made using digital image analysis of histological sections and of whole mounts of each worm type, in order to identify the source of the eggs. Eggs from the nests did not match the dimensions of those from any of the three identified trematodes, suggesting the existence of a fourth trematode species parasitic in this host, the identity of which remains unknown. In two cases, digeneans were seen in histological section in the muscularis mucosa, suggesting that the responsible agent is truly histozoic. . This presentation is a result of independent research and does not represent the findings of the U.S. Food and Drug Administration

Disease and Adaptive Fishery Management: The Role of the Fish Health Professional

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Knowledge of fish disease processes, fish disease defense mechanisms and ecology are important to the development of comprehensive fishery management strategies. This is especially true where either epizootic disease or chronic disease influence fish at the population level. In such instances, successful resource management depends upon the ability of resource managers to properly diagnosis disease, to predict population level risks, and to take appropriate management actions. Adaptive management is a process from which scientific knowledge is generated and subsequently utilized to formulate management strategies. It is an iterative strategy that relies upon cooperation, collaboration and communication. However, fishery managers and fish health professionals do not always communicate effectively making it often difficult to integrate aquatic animal health concerns in fishery management decisions. A review of the peer reviewed literature from selected journals over the past 10-years suggests that communication and collaboration between aquatic animal health professionals and fish culturists is more effective than that between the former and fishery management biologists. Of the 109 papers reviewed in the Journal of Aquatic Animal Health approximately 20% specifically provided recommendations and or applications for fish culture while 14% provided comparable management recommendations to fishery managers. A similar trend was noted in 103 papers published in Diseases of Aquatic Organisms; 16% provided fish culture recommendations and 14% recommended fishery management actions. Perhaps more interesting is the publication practices of the fish culturists and fishery managers. Thirty percent of 65 papers in the North American Journal of Aquaculture specifically addressed a disease issue and provided fish culture disease management recommendations. However, of the 112 papers reviewed in the North America Journal of Fish Management only 8% mentioned or discussed fish disease as a factor in fishery management. The seven (7) components required to address adaptive fishery management are discussed along with the recommendations for enhancing the role(s) of aquatic animal health specialists and veterinarians.

Drugs For Domestic Aquaculture: Background And Session Strategy

Thomas A. Bell

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Activities in the U.S. aimed at obtaining new approved drugs for aquaculture species have been ongoing, in some shape or form, for several decades. However, true concerted efforts have only occurred in the last 10-15 years. Historically, and still to this day, developing new drugs for the U.S. aquaculture industry (private and public) has been very low on the priority list of nearly all drug companies; the bottom-line being that anticipated domestic profits fall far short of expected R&D costs. Consequently, a significant portion of the investment in data generation to support new drugs for aquaculture species has had to come from the public sector. This session will attempt to not only inform you of progress to date, but also to provide some insight into why we have progressed as far as we have, but not as far as most would like. Just as importantly, we hope to also shed some light on how very well we have done, considering the odds against it. Our strategy for the session is to very briefly contrast progress pre-1992 with the present, followed by the FDA process and their view of our progress. We will then shift gears to provide a glimpse behind the scenes in two labs conducting pivotal studies. We will close the session with a realistic view of many of the reasons why we are not as close to gaining those approvals as all of us would like, but nonetheless accomplishments for which we can take great pride.

Drug Approval Status: Then And Now

Rosalie (Roz) Schnick

National Coordinator for Aquaculture New Animal Drug Applications, Michigan State University, 3039 Edgewater Lane, La Crosse, WI 54603

Efforts from 1964 to 1994 produced limited New Animal Drug Application (NADA) approvals for five aquaculture drugs: formalin, MS-222, oral oxytetracycline, Romet-30®, and sulfamerazine. These NADA approvals were gained with very limited funds. In 1990, the U.S. Food and Drug Administration (FDA) surveyed the aquaculture community and determined that we lacked proper and broad approvals. That set the stage for concerted efforts to gain the extensive funding needed to meet the data requirements of FDA's Center for Veterinary Medicine to prove that a drug is safe and effective for the intended use. We are currently pursuing a full spectrum of drugs for NADA approval and have made tremendous progress. Information comparing the studies completed toward approval in 1994 as compared to 2006 will be presented for each aquaculture drug actively being pursued and funded for NADA approval.

The INAD/NADA Process: That's Not A Train, But A Light At The End Of The Tunnel

Donald A. Prater

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Currently there are only a small number of drugs approved for use in aquaculture. The lack of availability of drugs approved for use in cultured fish species has significant impact on the health and welfare of these animals and severely hampers the productivity of this burgeoning industry. The reasons for a lack of approved drugs for aquaculture are several and include: the economic value of marketed products is perceived to be small by major pharmaceutical and chemical companies, the course of regulatory approval can be long and costly, and there are additional challenges in designing and conducting the required safety and effectiveness studies in aquaculture systems. Drugs used in aquaculture fall into one of several categories, approved, unapproved, low regulatory priority (LRP), regulatory discretion, or extralabel. Approved drugs require a new animal drug application (NADA). The NADA is composed of major and minor technical sections including effectiveness, target animal safety, environmental safety, human food safety, chemistry, manufacturing and controls, labeling, and all other information. Most work drug development work is conducted under an investigational new animal drug (INAD) exemption file. Once a drug is approved there is a system for monitoring for adverse reactions, including lack of effectiveness. Some drugs are available over-the-counter (OTC) while others are available by prescription or veterinary feed directive (VFD). Some approved drugs may be used extralabel, although medicated feeds carry additional restrictions for extralabel use. The Minor Use and Minor Species (MUMS) Animal Health Act of 2004 provides new incentives for sponsors of drugs for minor species, such as aquatic species. The major provisions of the legislation are 1) designation which qualifies a drug for additional periods of exclusivity and potential grants, 2) conditional approval which allows a drug to come to market before completing the required effectiveness section, 3) indexing which creates a new process for establishing legally marketed, unapproved new animal drugs. These new incentives should help increase the availability of new drugs for use in aquaculture.

Efficacy And Target Animal Safety Studies: Who Said This Was A Cake Walk?

Jim Bowker

Aquatic Animal Drug Approval Partnership Program, U. S. Fish and Wildlife Service, 4050 Bridger Canyon Road, Bozeman, Montana 59715

The approval of drugs for use in aquaculture is regulated by the U.S. Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM). Although culture and management strategies used typically minimize the need for drug use, at times, fish culturists and fisheries managers need access to approved therapeutants, anesthetics, marking agents, or spawning aids. The process to gain FDA approval is lengthy and costly, and includes FDA acceptance of data to demonstrate that the drug is effective, safe to humans, fish, and the environment, and can be manufactured consistently. Ideally, completion and acceptance of these "Technical Sections" will result in FDA approval to allow use of a new drug on all fishes for all purposes (i.e., claims). Although completing each Technical Section is theoretically straightforward, researchers designing and conducting studies to demonstrate that a drug is safe to fish and effective for the specified claim are often faced with unique biological and logistical challenges. The FDA requires that the effectiveness of a drug be demonstrated under field and "quasi-production" conditions and that test fish are "naturally" infected with the disease pathogen. Variables associated with the onset of a disease, combined with the necessity of having no secondary disease pathogen, make successful completion of these pivotal studies problematic. In addition, logistical challenges, such as trying to get staff and equipment to the study site in a timely manner, result in a success rate that is akin to a good major league baseball batting average. We could boost our "batting average" by utilizing disease models to initiate disease outbreaks. However, at present, use of such models can not be used in place of these pivotal studies. Requirements for successful completion and CVM acceptance of target animal safety (TAS) studies include different but equally challenging issues. This presentation will provide an overview of challenges associated with conducting efficacy and TAS studies in support of a new animal drug approval, and will demonstrate how CVM acceptance of study results isn't a cake walk.

Trials And Tribulations Developing Environmental Safety And Human Food Safety Data For Aquaculture Drug Approvals

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During the aquaculture drug approval process, data are submitted to the U.S. Food and Drug Administration (FDA) to fulfill data requirements of six technical sections. The six technical sections include (1) Product Chemistry, (2) Labeling, (3) Animal Safety, (4) Effectiveness, (5) Environmental Safety, and (6) Human Food Safety. Studies conducted to support the Environmental Safety and Human Food Safety technical sections (aka, data packages) are often the most time and money consuming projects associated with the drug approval process. Initially, Environmental Safety data packages were challenging to fulfill primarily because there was little guidance to draw upon to generate the required information. Initial submissions required implementing new and innovative techniques to conduct hatchery surveys, chronic toxicity studies, and develop drug discharge models. Original submissions eventually led to the generation of FDA supported templates that will serve as the basis for future aquaculture drug Environmental Safety submissions. Human food safety data packages are also challenging to fulfill primarily because of the scope of data that this data package requires. This data package draws upon data generated in five scientific fields including toxicology, microbial food safety, drug residue/metabolism, analytical method validation, and drug residue depletion. Studies conducted in these scientific fields are generally technically complicated and time consuming.

Drug Approval Status: Why We Are Where We Are And Not Where You Thought We Should Be

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The business of New Animal Drug Application (NADA) approvals is expensive, time-consuming, and difficult to achieve because of specific factors that are either under our control and others which are not. Examples of such factors will be given to include (1) new Center for Veterinary Medicine (CVM) requirements, (2) changes in CVM staffing, and (3) acquisition of extensive funds to generate the expensive studies needed for approval. In spite of the many factors that have been an impediment to the approval process, we have made great strides and have many label claims on the verge of NADA approval. Information will be given on the current status of each aquaculture drug label claim being actively pursued and funded toward NADA approval.

Monitoring Hawaiian Invertebrates (Crustacea) For White Spot Syndrome Virus (WSSV)

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April 14, 2004 marked the first identification of white spot syndrome virus (WSSV) in Hawaii. This outbreak occurred at a commercial shrimp facility on the island of Kauai. A second confirmation of WSSV on the island of Oahu occurred in late 2005. This case involved shrimp that were maintained in an inland primary quarantine facility that received unfiltered seawater from Kaneohe Bay. WSSV has a wide host range and has been shown, both experimentally and naturally, to infect many species found in Hawaiian waters. A preliminary survey for the presence of WSSV in Hawaiian decapod crustaceans was performed to establish whether a larger scale survey is warranted. Samples were collected from Oahu and Kauai and tested for WSSV using a two-step PCR protocol. A low percentage of samples from Oahu were WSSV positive, to include *Palaemon debilis* (grass shrimp), *Pachygrapsus* sp. (shore crab), and *Thalamita crenata* (mangrove swimming crab). These results highlight the requirement for a larger scale survey to assess WSSV prevalence, identify virus-susceptible species, as well as the necessity of experiments to better understand modes of viral transmission.

Comparative Susceptibility Of Early Life Stages Of The Bullfrog (*Rana catesbeiana*) To The Tadpole Edema Virus

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Investigations concerning the worldwide decline of amphibian populations have revealed a number of potentially related causal factors, including non-infectious and infectious diseases. Among these, viral pathogens of the family Iridoviridae (genus Ranavirus) have been isolated from an increasing number of species of dead and clinically diseased amphibians over an expanding geographic range, sometimes in association with significant levels of mortality. These viruses, including Frog Virus 3 and nearly homologous isolates such as the Tadpole Edema Virus (TEV), represent potentially important pathogens of many amphibians. Still, there are considerable unknown factors related to their transmission, virulence, and pathogenesis in and among the host species. Here we investigate the comparative susceptibility of pre-hatch and post-hatch life stages of the bullfrog, *Rana catesbeiana*, to TEV. Replicate groups of fertilized bullfrog eggs or tadpoles were exposed to TEV at 0, 10^5 or 10^6 $TCID_{50}$/ml in 50 ml spring water (21^oC) for 20 hours, at either 3 days pre-hatch or 3 days post-hatch. Mortality was recorded daily, dead tadpoles and eggs collected for 7 days following exposure, and surviving tadpoles were humanely euthanized. Tadpoles and eggs were evaluated for presence of virus using a quantal viral infectivity assay to determine 50% endpoint in cell culture. No virus was detected among any control groups, dead eggs, or tadpoles exposed pre-hatch. Virus was detected among the post-hatch exposure groups, ranging from 10^7-10^8 $TCID_{50}$/g for mortalities and 10^5-10^7 $TCID_{50}$/g for select survivors.

Largemouth Bass Virus In New York State

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During 2004 and 2005, we undertook an effort to identify natural bodies of water in New York State that contained fish infected with Largemouth Bass Virus (LMBV). Fish samples collected by the New York State Department of Environmental Conservation and the Cornell Biological Field Station during the course of their fisheries studies were made available to us for testing. A quantitative PCR (Q-PCR) test was used to individually screen individual fish. Samples that were Q-PCR positive were subsequently processed for virus isolation using Fathead Minnow (FHM) cell cultures. In 2004, 91 largemouth bass (*Micropterus salmoides*) from 13 bodies of water were tested using the Q-PCR. LMBV was detected in 7 fish from 4 lakes. Cell culture was performed on some samples, however no cytopathic effect was observed in any inoculation. In 2005, an additional 54 Largemouth Bass from 6 bodies of water were tested. LMBV was detected in 15 fish from 3 areas. Inoculations of these samples in fathead minnow cell produced cytopathic effect in 2 samples. Additionally, a small sample of 7 smallmouth bass (*Micropterus dolomieui*) from a single river was tested with the Q-PCR, and 6 positive results were obtained. Subsequently, cytopathic effect was observed in 2 samples after inoculation in cell culture. This survey confirms the presence of LMBV within wild fish populations in geographically diverse areas of New York State, although no mass mortality events to date have been associated with this virus in this state.

Isolation Of Viral Hemorrhagic Septicemia Virus (VHSV) From Muskellunge (*Esox masquinongy*) From Lake St. Clair, Michigan

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Viral hemorrhagic septicemia virus (VHSV) was isolated from muskellunge (*Esox masquinongy*) caught from the Clinton River area and the Grosse Point Yacht site in Lake St. Clair, Michigan, USA. Affected fish exhibited widespread edema of the skin, fin hemorrhages, sunken eyes, and congestion of internal organs. The inner wall of the swim bladder was thicker than usual, with numerous fluid-filled vesicles. A virus was isolated using FHM and CHSE cell lines inoculated with a homogenate of kidney and spleen tissues from affected fish. Focal areas of cell rounding and granulation appeared as early as 24 hr post-inoculation and expanded rapidly to destroy the entire cell sheet by 96 hr. Electron microscopy revealed virions having the typical bullet shape morphology of rhabdoviruses. Viral particles ranged in size from 165-185 nm in length and 55-75 nm in width. The virus was confirmed as VHSV by RT-PCR and sequence analysis of the nucleoprotein gene and a portion of the glycoprotein gene revealed the virus was a member of the North American genotype of VHSV and nearly identical to an isolate from freshwater drum in Lake Ontario. This report documents the first isolation of VHSV from *Esox masquinongy* in Michigan waters. The impact of this virus on the muskellunge population of Lake St. Clair remains to be determined.

Isolation Of Viral Hemorrhagic Septicemia Virus Type IV From A Mortality Event In Lake Ontario Drum (*Aplodinotus grunniens*)

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Large numbers of dead and dying freshwater drum were observed within the Bay of Quinte (Lake Ontario) and later towards the St. Lawrence River entrance. Moribund fish appeared lethargic, were unable to maintain proper orientation and were easily caught by hand. Externally, all fish exhibited multifocal to regionally extensive hyperemia and hemorrhages at fin bases and ventral surfaces. All fish exhibited mild to moderate bilateral exopthalmia, and several had hyphema. Internally, there was copious serosanguanous ascitic fluid and there was extensive serosal and sub-mucosal gastrointestinal hyperemia. The spleen of several of the fish was moderately enlarged. Skin scrapes and gill biopsies were unrevealing. No growth was observed from spleen and kidney on cytophaga, tryptic soy and blood agar following incubation at 20 and 37°C for 48-72hrs. All drum examined had a similar pattern of histologic lesions that were consistently oriented on the vasculature. Fibrinoid vasculitis (arteritis most prominently but also a phlebitis) was most notable in the kidney and meninges. Consistent lesions noted were as follows; severe necrotizing myocarditis, epicarditis, and meningoencencephalitis, mild to moderate multifocal necrotizing splenitis and interstitial nephritis and diffuse enteritis. No organisms were identified in H&E sections. All mouse inoculations using pooled tissues were negative for botulism toxin. There were numerous clusters of viral particles ~50x150nm present in myocardial tissue fixed in 2% glutaraldehyde and examined by transmission electron microcopy (TEM). TEM and negative staining of filtered fathead minnow culture supernatants revealed similar virions with typical rhabdovirus morphology. RNA polymerase gene (L) sequence results indicated the virus to be 91% homologous to a viral hemorrhagic septicemia virus isolate from Japan (type IV strain).

Kinetics Of The Natural Microbial Populations Of Atlantic Salmon In Relation To Infection And The Recuperative Process

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Certain bacterial fish pathogens exist in/on affected animals in two sequential phases; an external phase on the skin, gills or other external surfaces and an internal or systemic (circulating) phase. As in the cases of *Aeromonas salmonicida* subsp. s*almonicida* and *Yersinia ruckeri*, it is typically the systemic phase of the infective process that initiates extensive pathological changes within vital organs and produces mortality. Although the external phase is not nearly as traumatic for the host, it also produces subtle, albeit measurable changes that can be nonlethally detected by monitoring a pathogen's interactions with the microbial flora resident within a host's dermal mucus. As a horizontally transmitted or water-borne pathogen initiates disease, it becomes more prevalent and displaces the normal microflora; - most of which are gram-negative, glucose - -inert or -oxidative bacteria. Replication of the pathogen continues on the dermis until that bacterium dominates the external microflora, overcomes host defenses, and thereby initiates systemic infection. During this increase in pathogen load, the pathogen is shed into the water where contagion, both in the wild and under conditions of intensive culture, is density dependent. Depending upon the virulence of the pathogen and host resistance, a fulminating septicemia develops with resultant mortality that necessitates antibiotic treatment. In the recovery process, this microbial situation is reversed and the normal bacterial flora re-establishes itself to pre-challenge levels. Hence, understanding the importance of key microbial species and the kinetics of their bacterial distributions may provide a novel approach toward assessing population health. It is my hypothesis that the assessment of surface bacterial flora (the distribution of species and the relative proportion of a pathogen to other microorganisms) can be used to predict an imminent disease epizootic prior to development of clinical signs. Similar measurements are also vital towards understanding the relative importance of other microbes, often thought of as facultative pathogens (e.g. – motile aeromonads and *Pseudomonas fluorescens*), in the recuperative process. The hypothesis set forth in this abstract will be explained using the interplay between *A. salmoncida* and the normal flora of Atlantic salmon (*Salmo salar*) as a model.