Program of the:

32nd Annual
Eastern Fish Health Workshop

18 – 22 June, 2007
Eisenhower Inn and Conference Center
Gettysburg, Pennsylvania

National Fish Health Research Laboratory
USGS - Leetown Science Center
11649 Leetown Road
Kearneysville, West Virginia 25430

Monday , June 18

5:00 – 8:30 pm **Registration And Reception**
Lee/McClellan/Grant Salons

Hors d'oeuvres and Cash Bar

8:00 Welcome to the 32nd Annual Eastern Fish Health Workshop

8:30 Unusual Diagnostic Experiences, chaired by Andy Noyes and Mark Matsche

Longstreet Salon

8:30	Jones	Esocid Lymphosarcoma In Lake Champlain	1
8:40	Bowser	The Case Of A Worm In A Bottle	2
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9:10	Groman	Unusual Myocardial Pathology Of Farm Reared Atlantic Halibut (*Hippoglossus hippoglossus*)	5
9:20	Wolf	A Walk on the Wild Side: SelectiveHistopathologic Findings From Endocrine Disruption Studies	6
9:30	Warg	An Epizootic Of Cyprinid Herpesvirus 2 (Goldfish Herpesvirus) In A Goldfish-Koi Production Farm	7
9:40	Walker	Right Treatment; Wrong Results	8
9:50	Knight	Pigmented Tumors In Gizzard Shad From The Black Warrior River, Alabama	9
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10:20	Bakal	Current Status Of The U.S. National Aquatic Animal Health Plan	11

10:40 **Adjourn**

Tuesday, June 19

7:00 **Breakfast** **Lee/McClellan/Grant Salons**
Assorted fruit, chilled juice, country scrambled eggs, bacon, fruit crepes, pancakes with syrup, assorted biscuits and muffins, cold cereals, coffee, tea, and milk.

Proceedings **Sheridan/Longstreet Salons**

Emerging Disease Problems in Crustaceans, chaired by Jeff Shields

8:00	Shields	Comparison Of The Biology Of *Hematodinium* Infections In Blue Crabs (*Callinectes sapidus*) And Snow Crabs (*Chionoecetes opilio*)	12
8:15	Ratzlaff	Characterization Of *Panulirus argus* Virus 1 From The Caribbean Spiny Lobster	13
8:30	Li	Primary Culture Of Hemocytes From The Caribbean Spiny Lobster, *Panulirus argus* And Their Susceptibility To *Panulirus argus* Virus 1 (PaV1)	14
8:45	Messick	Ailments In Captive Crabs	15
9:00	Kiryu	Parasites And Diseases Of The Blue Crab *Callinectes sapidus* From Tampa Bay, Florida	16
9:15	Schott	Aspects Of The Molecular Biology Of *Hematodinium* sp.	17
9:30	Burge	Shrimp Hemocyte Immunity: Peroxinectin Expression In Response To *Vibrio* Infection	18

5:00 **Adjourn**

6:30 **Country Cookout Reception and Dinner with the 2nd South Carolina String Band**

Lee/McClellan/Grant Rooms

6:30 Reception with Cash Bar
7:30 Dinner

Steamship round of beef, fried chicken, barbecued country pork ribs, chili and crackers, baked potatoes, cole slaw, ambrosia, corn on the cob, corn bread and biscuits, butter and honey, dessert table, coffee, tea, and iced tea.

Music of the 2nd South Carolina

Wednesday, June 20

7:00 **Breakfast** **Lee/McClellan/Grant Salons**
Assorted fruit, chilled juice, Western scrambled eggs, bacon, sausage links, French toast with syrup,, assorted biscuits and muffins, cold cereals, coffee, tea, and milk

Proceedings **Sheridan/Longstreet Salons**

Diving Deeper: The pursuit of truth in aquarium medicine, chaired by Charlie Innis

8:00	Nyaoke	Advances In Our Understanding Of Phaeohyphomycosis	39
8:15	Frasca	Histopathologic Interpretation Of Lesions In Cephalopods	40
8:30	Weber	Microbiology Testing For Aquatic Systems: Findings And Potential Implications	41
8:45	Gargan	Phagocytosis In Nurse Shark (*Ginglymostoma cirratum*) Peripheral Blood Leukocytes	42
9:00	Corwin	Head And Lateral Line Erosion Syndrome (HLLES) In Ocean Surgeonfish, (*Acanthurus bahianus*), Current Efforts To Determine Etiologies	43
9:15	Hadfield	Gas Supersaturation In A Large Artificial Reef Exhibit: An Unusual Presentation And Resolution	44
9:30	LePage	Retrospective Summary Of Diseases Affecting Captive Seahorses At The Toronto Zoo	45
9:45	Poll	Surgical Treatment Of Lymphoma In A California Moray Eel, *Gymnothorax mordax*	46

10:00 **Morning Break** *Coffee, tea*

The cutting edge of cutting fish: surgery and anesthesia, chaired by Greg Lewbart

10:30	Lewbart	Fish Sutures: Tying Things Together	47
10:45	Roberts	To Cut Is To Cure: Surgical Cases In Fish Medicine	48
11:00	Bakal	Endoscopic Sex Determination And Sterilization Of Gulf Sturgeon (*Acipenser oxyrinchus* Desotoi)	49
11:15	Innis	Ophthalmic Surgical Procedures In Public Aquarium Fishes	50

12:00 **Lunch** **Lee/McClellan/Grant Rooms**
Sautéed beef tips with blended rice, honey glazed carrots, sherbet, coffee, tea, and iced tea.

General Session 3 - Renate Reimschuessel, moderator

3:00 **Afternoon Break** *Coffee, tea, sodas*

General Session 4 - Al Camus, moderator

5:00 **Adjourn**

Dinner on your own

5:30 **Gettysburg National Historic Battlefield Tour**

Thursday, June 21

7:00 **Breakfast** **Lee/McClellan/Grant Salons**
Assorted fruit, chilled juice, country scrambled eggs, bacon, sausage links, pancakes with syrup,, assorted biscuits and muffins, cold cereals, coffee, tea, and milk.

Proceedings **Sheridan/Longstreet Salons**

Unraveling Complexities of Coral Pathogens and the Etiology of Coral Diseases, chaired by Laurie Richardson

General Session 5 – Paul Bowser, moderator

General Session 6 - John Lumsden, moderator

2:45	Densmore	Tadpole Edema Virus: Comparative Susceptibility Among Early Life Stages of the Fowler's Toad (*Bufo fowleri*)	86

3:00 **Afternoon Break** *Coffee, tea, sodas*

Putting It All Together: A Manager's Perspective On Aquatic Animal Health, chaired by Frank Panek

3:30	Panek	Pathogens Associated With Native And Exotic Trout Populations In Shenandoah National Park And The Relationships To Fish Stocking Practices	87
3:45	Wiggins	Perspectives for Trout Management and Fish Health in the Batten Kill	88
4:00	Reeser	CSI Shenandoah: The Importance Of Understanding Fish Health In A Riverine Fish Kill Investigation	89
4:15	Jacobs	Bio-Indicators Of Estuarine Health: A Multi-Variate Approach Linking Source To Resource	90
4:30	Cipriano	Evaluating Population Health Of Endangered Fishes By Non-lethal Analysis Of Microbial Diversity	91

4:45 Discussion

5:00 **Adjourn**

6:30 **32nd Annual EFHW Reception and Banquet**
Sheridan/Lee/McClellan/Grant Rooms

6:30 Cocktail reception (cash Bar)
7:00 *Sliced roast beef with mushroom sauce, oven baked chicken, sliced baked sugar cured ham, sliced roast turkey breast, pasta primavera, tossed salad, fruit salad, pasta salad, vegetable medley, parsley buttered potatoes, assorted pies, cakes and puddings, coffee, tea, and iced tea*

8:00 **Presentation of the Best Student Paper Award**
Banquet Dance (Cash Bar)

11:30 **Adjourn**

Esocid Lymphosarcoma In Lake Champlain

Thomas E.Jones

Vermont Fish & Wildlife Department, 103 South Main Street, 10 South, Waterbury, Vermont 05671-0501

Esocid lymphosarcoma has been observed on northern pike in Lake Champlain for a number of years by governmental fisheries management agencies as well as the sport fishing public. Through physical observation of adult pike caught during electrofishing activities during the spring months, estimates of prevalence of the disease are as high as 20%. Observations of lesions on pike appear to be seasonally dependant with the majority of lesions reported during late fall, winter and spring months. Beyond this account, little is known about the impact of this disease on northern pike or other Esocids in Lake Champlain.

The Case Of A Worm In A Bottle

Paul R. Bowser

Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853-6401

A case submission of 28 June 2005 to the Fish Disease Diagnostic Laboratory at Cornell consisted of what appeared to be a nematode. The sample was presented by a Cornell University faculty member who received it from a colleague in Minnesota. The sample was submitted with the request that the organism be identified and with the question of potential risk to humans. The nematode had been found in a fresh Chinook salmon fillet purchased in a grocery store. The nematode was identified as *Anisakis simplex,* also known as the "sushi worm." The parasite does pose a zoonotic risk. Signs in humans may include gastrointestinal pain, nausea and vomiting. The infection is often misdiagnosed.

'Bloat' – The Most Abused Term In Fish Health?

John S. Lumsden, Shohreh Hesami, Spencer Russell and Véronique M. LePage

Fish Pathology Laboratory, Department of Pathobiology, University of Guelph, Guelph, ON, Canada, N1G 2W1

How many different diseases or pathophysiologic pathways can you think of that might lead to the clinical presentation of a fish with a distended abdomen? 'Bloat', a colloquial term used widely for the clinical symptom of a distended abdomen, is used not just by hobbyists and on the internet but by aquaculturists and by diagnosticians in the literature. 'Bloat' is used to describe superficially similar syndromes in a variety of species of farmed salmonids that are pathophysiologically distinct. Using gastric dilation and air sacculitis (GDAS) in salmonids as one pertinent example of 'bloat' and outlining a novel occurrence of this syndrome in cultured sturgeon in Ontario; the pitfalls of superficial pathologic descriptions will be illustrated.

Not Your Average Patient: Whale Shark Medicine 101

[1]Tonya Clauss, [1]Alistair D.M. Dove, [1]Christian Schreiber, [1]Timothy Binder, [1]Christopher Coco, [1]Ray Davis, [1]Ashlie Vinson, [1]Nicole LaBove, [1]Michele Moses, [1]Michael Maslanka, [1]Michael Walsh, and [2]Jill Arnold

[1]Georgia Aquarium, 225 Baker Street, Atlanta, Georgia 30313; [2]National Aquarium in Baltimore, Pier 3, 501 East Pratt Street, Baltimore, Maryland 21202

The whale shark, *Rhincodon typus*, is the largest fish in the world; at seven meters, a whale shark may weigh as much as 1500 kilograms. This sheer size creates numerous logistical challenges when handling these animals for medical procedures. Principal among these is capture and restraint in a manner that is safe for the people and the animal involved. Additional challenges include the collection of morphometric information and diagnostic samples, diagnostic imaging techniques and the delivery of medications, supplements or fluids. Perhaps the most significant of the obstacles, however, is analysis of the biological specimens and subsequent interpretation and action. There is currently no database of clinical information on whale sharks and there remains much to learn about the basic anatomy, physiology and ethology of this species. Nonetheless, the experiences gained from husbandry and veterinary practices with the four whale sharks acquired by the Georgia Aquarium will be invaluable in future efforts to better understand and appreciate these enigmatic elasmobranchs in both aquariums and their natural environment. We have overcome many of the challenges involved in handling and performing medical procedures with these animals in a captive setting. Exciting collaborations doubtless lie ahead with the other institutions maintaining whale sharks, in a collaborative effort to increase the knowledge base information and conservation efforts for this species.

Unusual Myocardial Pathology Of Farm Reared Atlantic Halibut (*Hippoglossus hippoglossus*)

[1]Peter Sykes, [2]David Groman, and [1]Larry Hammell

[1] Center for Aquatic Health Sciences and [2] Aquatic Diagnostic Services, 550 University Ave., Atlantic Veterinary College, UPEI, Charlottetown, P.E.I. C1A4P3 Canada

Production of Atlantic halibut (*Hippoglossus hippoglossus*) is an ongoing alternative species aquaculture industry in Norway, Iceland, Scotland and more recently Atlantic Canada. Inflammation within the epicardium and on the pericardial surfaces of aquaculture reared fingerling halibut has been documented in Norwegian, Icelandic and Canadian stocks, with suggestion of a nutritional or bio-mechanical etiology. To date there has been no clear linkage to pathogens. Recent observations of "grower" halibut, reared in production systems in Atlantic Canada, have revealed an unusually high prevalence of gross pericardial nodules and adhesions. Bacteriology and virology analysis of tissue from affected fish have proved negative for infectious agents. Histological examination of heart tissue from a range of fish with gross lesions has revealed a mix of proliferative, inflammatory and vascular changes. Gross and histopathology data will be presented with hypotheses as to etiology.

A Walk On The Wild Side: Selected Histopathologic Findings From Endocrine Disruptor Studies

Jeffrey C. Wolf and Marilyn J. Wolfe

Experimental Pathology Laboratories, Inc., 456000 Terminal Drive, Sterling, Virginia, 20166

Although the concept of endocrine disruption (ED) is decades old, research efforts in this area have intensified during the past fifteen years primarily due to government mandates and heightened public awareness. Increasingly, chemical production companies and regulatory agencies find themselves under pressure to rapidly provide data for use in ED risk assessment analysis. A downside of this accelerated pace of investigation is that the biological characteristics of a test species may not be fully understood prior to the creation and performance of ED experiments. Potential consequences of this are suboptimal study design and misinterpretation of study results, which may in turn lead to misconceptions that persist in the scientific literature. A specific problem in aquatic animal bioassays is that information concerning the occurrence of spontaneous histopathologic anomalies in control or reference site animals is often lacking. The primary focus of this presentation will be the illustration and brief discussion of various reproductive tissue abnormalities, some of which could be considered "background" lesions that have been identified in toxicological studies.

An Epizootic Of Cyprinid Herpesvirus 2 (Goldfish Herpesvirus) In A Goldfish - Koi Production Farm

[1]Janet V.Warg, [2]Jennifer Haugland, and [3]James W. Provo

[1] USDA-APHIS-VS, National Veterinary Services Laboratories, Diagnostic Virology Laboratory, P.O. Box 844, Ames, Iowa 50010; [2] North Carolina Veterinary Diagnostic Laboratory System, Raleigh, NC; [3] USDA-APHIS-VS,930 Main Campus Drive Raleigh, North Carolina 27606.

The cause of goldfish mortalities at a goldfish-koi production farm was investigated. The clinical signs and lesions were consistent with those of herpesviral hematopoietic necrosis. The goldfish were lethargic, swimming at the top, and gathering around the diffuser. Three dead and 3 live goldfish were received for diagnostic work-up. Petechial hemorrhages of the skin were observed. The gills were pale pink to tan and some had pinpoint white foci within the filaments. No fluid in the peritoneum was found. Some fish had pinpoint white foci in the spleen and kidneys. Other kidneys were pale and small. A few fish had mildly enlarged spleens. One fish had exophthalmia and the corneas were cloudy. Histopathological examination revealed subacute inflammatory lesions and intranuclear inclusion bodies in the brain, gills, liver, intestine, pancreas, and adipose tissue. The caudal kidney and spleen exhibited lymphohematopoietic necrosis and intranuclear inclusion bodies. A 10% tissue homogenate made from a pool of kidney and spleen from four goldfish was used for virus isolation. A sample of cell culture fluids from koi fin cell cultures demonstrating CPE was processed for electron microscopy and nucleic acid extraction for use in PCR. Testing performed in the diagnostic workup including histopathology, bacteriology, virus isolation, electron microscopy, and molecular characterization of the viral isolate supported the diagnosis of cyprinid herpesvirus 2. This is the first report of successful cyprinid herpesvirus 2 isolation on KF-1 cell cultures in the United State.

Right Treatment; Wrong Results

[1]Carolyn Gunn, [1]Peter Walker, [1]Mandy Colburn, and [2]Charlie Smith

[1]Colorado Aquatic Animal Health Laboratory, P.O. Box 128, Brush, Colorado 80723; [2] 212 Story Hill Road, Bozeman, Montana 59715

Flavobacterium psychrophilum was isolated from the spleens of 10-centimeter rainbow trout in a lot of 160,000 at Pueblo State Fish Hatchery in south central Colorado in late December, 2006. The fish quickly responded to a regime of oxytetracycline administered in the feed, but mortalities returned to normal for less than two weeks before a second round of bacteremias of much greater severity broke out. This time virtually all fish were affected. Clinical signs included fluid-filled blisters progressing to open sores, exophthalmia, pale gills and livers, enlarged spleens, and grossly swollen and pale renal kidneys. Histopathology of the hind kidney demonstrated necrosis of some kidney tubules characterized by pyknotic nuclei. Many cells within hematopoietic tissue were enlarged and had large, often pleomorphic nuclei that often contained 2 and occasionally 3 nucleoli. Liver cells were also swollen and often displayed double, swollen nucleoli, while others were degenerate demonstrating karyorectic nuclei. Macrophages were swollen and commonly seen within blood vessels often attached to vessel walls primarily of of livers and kidneys. Steiner staining showed that macrophages were engorged with short rods of two distinct morphologies. Cultures from kidney and spleen on TYES Agar failed to grow *F. psychrophilum*; however, two species of gram negative, gelatinase positive, beta-hemolytic short rods were isolated on Blood Agar. These were identified as strains of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. Both isolates were totally resistant to oxytetracycline, Romet-30, and florfenicol. The entire lot of trout was buried and the raceways disinfected.

Pigmented Tumors In Gizzard Shad From The Black Warrior River, Alabama

[1]Amelia C. Knight, [1]Jeffery S. Terhune, [1]John M. Grizzle, and [2]William E. Garret

[1]Department of Fisheries and Allied Aquacultures, Auburn University, Alabama, 36849; [2]Alabama Power Co. Environemntal Affairs, P.O. Box 2641, Birmingham, Alabama

Some types of neoplasia in wild fish populations are thought to be linked to environmental contaminants. However, other types of neoplasia may be caused by viruses or inherited traits. In our study, gizzard shad (*Dorosoma cepediaum)* were examined (n=200) from the Black Warrior River at Plant Gorgas, a fossil fuel electric steam plant operated by Alabama Power Company, in the summer of 2006. Four gizzard shad had raised, pigmented tumors, varying in size from 0.2 to 0.4 to 0.7 cm to 1.0 x 1.6 x 1.7 cm, located in the skin of the frontal bone, and less frequently in the skin of the fins. These tumors were cutaneous melanomas, and this diagnosis was verified by the Registry of Tumors in Lower Animals. Since the 2006 summer sample, an additional 400 gizzard shad have been collected and subsequent pigmented tumors were observed in six additional fish. Histological examination of these latter samples is pending. Similar tumors in gizzard shad have been described from several lakes in Oklahoma. Genetics, viruses, and chemical carcinogens have not to been linked to the tumors in Oklahoma. Likewise, the cause of the gizzard shad tumors in Alabama is presently unknown.

Online Fish Disease Course At Kentucky State University

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Phone (502)597-6581

The Kentucky State University Division of Aquaculture plans to offer Fish Diseases as a 3-credit graduate (AQU 511) and undergraduate (AQU 411) course in the spring 2008 semester. Course lectures were video recorded when the course was taught in the fall of 2006. These lectures will be made available to students taking the class in 2008 in Flash video format and will be downloadable onto hand-held iPods. Students who have taken other aquaculture courses in this video format preferred it over online courses that were presented in text and pictures only. They were able to re-watch lectures to reinforce and/or review certain concepts that they may have missed after the initial viewing of the lectures. For fish diseases, video format has the advantage of being able to present moribund behavior of sick fish and videos of fish parasites and bacteria. In addition, course documents including video clips of fish pathogens and infected fish will be posted for student access online on Blackboard®. Messages to students and grades will be accessible on Blackboard®, and timed exams will be administered online. Offering Fish Diseases online will make the course available to a greater number and wider range of students compared to teaching the course only to on-campus students. Online courses in aquaculture at KSU have typically had more than 20 students enrolled compared to the typical class size of about 9 for on-campus only courses. If enrollment increases by 11 students when teaching a course online and tuition is $900.00, university income will increase by almost $10.000.00 per course per semester. And students who may not be able to quit their job and go back to school full time will be able to take this online course during their free time during evenings and weekends.

The Current Status Of The U.S. National Aquatic Animal Health Plan

Robert Bakal

U.S. Department of Interior, Fish and Wildlife Service, National Aquatic Animal Health Coordinator, 4700 Hillsborough St., Raleigh, NC 27606

The Joint Subcommittee on Aquaculture's (JSA) National Aquatic Animal Health Task Force was tasked with developing a national aquatic animal health plan (NAAHP) for aquaculture in cooperation with industry, regional organizations, state, local, tribal governments and other stakeholders. The NAAHP is intended to 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. Development of the NAAHP required input from working groups composed of individuals who represent certain sectors of the aquaculture industry. The final draft of the NAAHP was completed and submitted to the JSA in June 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.

Comparison Of The Biology Of *Hematodinium* Infections In Blue Crabs (*Callinectes sapidus*) And Snow Crabs (*Chionoecetes opilio*)

[1]Jeffrey D. Shields, [2]David M. Taylor, and [1,3]Hamish J. Small

[1]Virginia Institute of Marine Science, Gloucester Point, VA 23062; [2]Department of Fisheries and Oceans, CP5567, White Hills, St. John's, Newfoundland, CANADA A1C 5X1; [3]Centre for Environment, Fisheries & Aquaculture Science, Weymouth Laboratory, Weymouth, Dorset DT4 8UB, UK

Differences in the patterns of infection, life history and histopathology between disparate hosts may lead to a better understanding of the biology of *Hematodinium*. We compare *Hematodinium* infections in the temperate blue crab, *Callinectes sapidus*, with that in the boreal snow crab, *Chionoecetes opilio*. In heavy infections, crabs become weak and lethargic and die from handling stress, but blue crabs do not show a discoloration of the carapace that is a hallmark of infected snow crabs. Snow crabs infected with *Hematodinium* sp. develop an unusual bitter flavor that does not occur in infected blue crabs. We speculate that both the discoloration of the carapace and the bitter flavor result from the duration of the infection. In the blue crab, infections result in a relatively short (<40 d), acute disease, whereas in the snow crab, infections are a lengthy (12 mo+), chronic process. Snow crabs live in temperatures as low as –1.3° C, which decreases metabolic rates, perhaps resulting in longer infections. Histological findings indicate potential differences in the life histories of the parasites in their disparate hosts. The plasmodial filamentous trophont occurs in early infections in the hemolymph of blue crabs but does not occur in the hemolymph of snow crabs. However, a different sheet-like plasmodial stage occurs in the hepatopancreas of snow crabs but not in that of blue crabs. Such life history differences can be used to further taxonomic and pathological comparisons between these infections.

Characterization Of *Panulirus argus* Virus 1 From The Caribbean Spiny Lobster

[1]Robert E. Ratzlaff, [1]Megan M. Montgomery-Fullerton, [1]Michelle Todd, Bill [2]Newcomb, [2]Jay Brown, [3]Jeffrey D. Shields, and [1]Roland A. Cooper

[1]Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529; [2]Department of Microbiology, University of Virginia School of Medicine, Box 800734, Charlottesville, VA 22908; [3]Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062

Panulirus argus virus 1 (PaV1) is a pathogenic virus that infects the Caribbean spiny lobster (*Panulirus argus*) in the Florida Keys. PaV1 was isolated from Florida Bay spiny lobsters with signs of disease. Purified virions were morphologically similar to PaV1 previously reported in tissue biopsies and were infectious to healthy lobsters. PaV1 shares similarity with Herpesviridae, Iridoviridae, and Ascoviridae families based on BLAST characterization of DNA fragments cloned from the virus.

Primary Culture Of Hemocytes From The Caribbean Spiny Lobster, *Panulirus argus* And Their Susceptibility To *Panulirus argus* Virus 1 (PaV1)

Caiwen Li and Jeffrey D. Shields

Virginia Institute of Marine Science, The College of William and Mary, Gloucester Pt, VA 23062

Primary cultures of hemocytes from the Caribbean spiny lobster *Panulirus argus* were developed for studies on the *in vitro* propagation of *Panulirus argus* Virus 1 (PaV1). A modified Leibovitz L-15 medium supported the best survival of hemocytes in *in vitro* primary cultures. However, degradation of the cultures occurred rapidly in the presence of granulocytes. A Percoll step gradient was used to separate hemocytes into three subpopulations enriched in hyalinocytes, semigranulocytes, and granulocytes, respectively. When cultured separately, hyalinocytes and semigranulocytes maintained high viability (~ 80% survival) after 18 days incubation compared with granulocytes, which degraded over 2-3 days. Susceptibility of the cell types was investigated in challenge studies with PaV1. Hyalinocytes and semigranulocytes were susceptible to PaV1. Cytopathic effects (CPE) were observed as early as 12 h post-inoculation, and as the infection progressed, CPE became more apparent, with cell debris and cellular exudates present in inoculated cultures. Cell lysis was noticeable within 24 hrs of infection. The presence of virus within cells was further confirmed by *in situ* hybridization using a specific DNA probe. The probe gave a unique staining pattern to cells infected with PaV1 24-h post inoculation. Cells in the control treatment were intact and negative to hybridization. This assay was further applied to the quantification of infectious virus in hemolymph using a 50% tissue culture infectious dose assay ($TCID_{50}$) based on CPE. These tools will now allow the quantification of PaV1 using established culture-based methods.

Ailments In Captive Crabs

Gretchen Messick

Cooperative Oxford Laboratory, Center for Coastal Environmental Health & Biomolecular Research at Charleston, USDOC/NOAA/NOS/NCCOS, 904 S. Morris Street, Oxford, Maryland 21654-1323

The blue crab *Callinectes sapidus* is an essential component of Chesapeake Bay's ecology and supports an important commercial fishery. Research indicates that extended captivity can impart stress on blue crabs, leading to enhanced prevalence and intensity of disease. Captive crabs can have a high incidence of shell disease. Although initial mortalities are often attributed to generalized bacterial infections associated with inflammation and nodule formation in tissue, mortalities occurring in crabs held in captivity for prolonged periods may also be due to viral infections. Viruses that have been encountered in captive crabs during investigations include reolike (RLV) virus and rhabdolike (RhVA) virus, a virus sequenced as aquareovirus reoviridae, Chesapeake Bay virus (CBV) and Bifaces virus (BFV). In addition to bacterial and viral infections, other pathogens in captive crabs have included microsporidians, fungus, rickettsia-like infections and ciliates. Histological abnormalities have also been observed, including scab-like gill lesions and an unusual strand-like structure in the lumen of the hepatopancreas. Some or all of these parasites or manifestations of disease may be latent in the crab until stress causes infections to become patent. The development of molecular detection methods for viruses or other parasites would provide diagnostic tools needed to assess environmental impacts on crab populations in the Chesapeake Bay, and monitor the health status of captive crabs.

Parasites And Diseases Of Blue Crab, *Callinectes sapidus* From Tampa Bay, Florida

Yasunari Kiryu, Jan H. Landsberg, Anne L. McMillen-Jackson, Charles R. Crawford, Susan D. Gerhart, Angie M. Machniak, Jay Abbott, Catalina E. Brown, and Noretta E. Perry

Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 100 Eighth Avenue Southeast, Saint Petersburg, Florida 33701-5095

In spite of recent significant declines in blue crab, *Callinectes sapidus*, commercial fishery landings, we have little knowledge about the general health status of this species in Florida. A preliminary study was conducted from June 2004 – July 2006 to monitor the general health of blue crabs in Tampa Bay (Florida central west coast). Blue crabs were collected weekly from traps routinely monitored at five locations: BH, Bayboro Harbor; AB, Apollo Beach; AR, Alafia River; AP, St. Petersburg/Clearwater International Airport; and SH, Safety Harbor. At each site, randomly selected live blue crabs were brought back to the lab for necropsy. Hemolymph was drawn and observed by light microscopy (LM) (using Giemsa stain) and bacterial isolations were attempted. Internal tissues were prepared for wet mount LM, routine H&E histology. From histology and stained hemolymph slides, the prevalence of the dinoflagellate parasite *Hematodinium* was 9.5% (BH; N=21), 13.0% (AB; N=23), 20.0% (AR; N=20), and 7.3% (AP; N=41). Prevalence of the microsporidian parasite, *Thelohania* sp. was 8.7% (AB), 10.0% (AR), and 9.8% (AP). Neither *Hematodinium* nor microsporidia were detected from SH (N=32). *Microphallus basodactylophallus* metacercarial cysts were detected at all sites (10.0% – 30.4%). Tetraphyllidean cestodes were ubiquitous, with a high prevalence at AR (45.0%). *Vibrio* was commonly isolated from the hemolymph. Results will be reviewed for potential management applications.

Aspects Of The Molecular Biology Of *Hematodinium* sp.

[1]Eric Schott, [2]Gretchen Messick, and [1]Allen Place

[1]Center of Marine Biotechnology, 701 East Pratt Street, Baltimore, MD 21202; [2]Cooperative Oxford Laboratory, (CCEHBR) USDOC/NOAA/NOS/NCCOS, 904 S. Morris St., Oxford, Maryland 21654

Hematodinium sp. is a parasitic dinoflagellate that infects the blue crab (*Callinectes sapidus*) and other crustaceans. Blue crab populations in the MD and VA coastal bays, and the lower Chesapeake Bay, have experienced occasional losses as a result of *Hematodinium* sp. infections. While *Hematodinium* sp. is most prevalent in high salinity, it may be carried by crabs as they migrate into and out of water of lower salinity. We describe the use of a highly sensitive, quantitative PCR assay (Q-PCR) that can detect parasite DNA corresponding to less than a single cell. We are using the Q-PCR assay in parallel with histology to assess *Hematodinium* sp. prevalence and intensity in blue crabs from Chesapeake Bay and MD coastal bays. In a retrospective study, we have also applied the Q-PCR assay to archived DNA samples of crabs from mesohaline areas of the Chesapeake Bay, and have detected a 10% prevalence of light infections. The Q-PCR assay is directed towards the multicopy rRNA locus. By comparing Q-PCR standard curves of parasite cell dilutions versus Q-PCR standard curves on dilutions of the cloned rRNA gene target (gift of R. Lee, SKIO) we find evidence for nearly 40,000 copies of the rRNA gene cluster per cell. Investigations are underway as to whether there is variation in the DNA sequence among these copies, and whether structural genes of *Hematodinium* sp. also exist in multicopy.

Shrimp Hemocyte Immunity: Peroxinectin Expression In Response To *Vibrio* Infection

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The purpose of this study was to examine changes in the tissue-level hemocyte distributions of the Pacific white shrimp, *Litopenaeus vannamei*, in response to challenge with a bacterial pathogen. We quantified by real-time PCR the gene expression of peroxinectin, a multi-functional immune protein expressed by shrimp hemocytes. Biological functions of peroxinectin include roles in cell adhesion, degranulation, opsonization, encapsulation and peroxidase activity. Peroxinectin gene expression (as mRNA) was quantified in 5 tissues and in circulating hemocytes for 48 h following intramuscular injection of shrimp with *Vibrio campbellii*. Peroxinectin signal increased significantly in the muscle at the site of injection between 1 and 4 h and returned to control levels within 24 h of injection. Surprisingly, changes in peroxinectin expression in the circulating pool of hemocytes were not correlated with *Vibrio* injection, suggesting that recruitment of hemocytes to the site of injection is from existing tissue localized hemocyte subpopulations or that recruited hemocytes substantially upregulate peroxinectin within 4 h of infection. *Vibrio*-injected shrimp experience an increase in peroxinectin expression within the gill by 48 h post-injection, supporting the hypothesis that localization and encapsulation of bacteria in the gill is an important aspect of crustacean immunity to bacteria. Supported by NSF Grant No. IBN-021292 to KGB and LEB.

The Role Of Microorganisms In Development Of Epizootic Shell Disease In The American Lobster

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Epizootic shell disease found at high levels in wild populations of the American lobster appears to have bacterial etiology. The microbial community associated with epizootic shell disease lesions consists of two to five dominant strains of bacteria. DGGE of PCR amplified 16S rRNA genes (PCR-DGGE) from DNA isolated from lobster lesions, indicated that lesions of lobsters always harbor at least two microorganisms: a strain of *Aquimarina* sp. and either an unidentified a α-proteobacterium or a *Pseudoalteromonas* sp. strain. Although our attempt to verify the Koch postulate for *Aquimarina* sp. was not successful, the importance of *Aquimarina* in pathogenesis should not be underestimated. It is conceivable that other precursory pathogens weaken the immune system of lobsters, preventing effective defence against epizootic shell disease. If such pathogens existed, they must cause a latent, otherwise unnoticeable infection. Necropsies of over 60 lobsters carried out over the last two years, demonstrated that lobsters with shell disease have cubicula lesions but are otherwise healthy. To investigate the possibility that unknown eukaryotic microbe may be involved, we carried out PCR-DGGE analysis of 18S rDNA amplified from DNA isolated from lesions and hemolymph of diseased lobsters. No other eukaryotic DNA is amplifiable from hemolymph except lobster's one. 18S rDNA of a bacteriovorus nematode (*Geomonhysteria disjuncta*) was detected in lesion of all tested lobsters, and that of barnacles, bryozoans and protozoans in lesions of some.

Effects Of Lipopolysaccharide On Total Hemocyte Counts Of The Blue Crab, *Callinectes sapidus*

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In contrast to vertebrates, the crustacean immune response does not include an adaptive component based on immunological memory, yet their immune systems are extremely complex, efficient and highly developed. In crustaceans, circulating hemocytes perform essential roles in immunity; phagocytosis, encapsulation, and lysis of foreign cells. Lipopolysaccharides (LPS), or endotoxins, are an integral component of the outer membrane of gram-negative bacteria. LPS exposure induces rapid degranulation of hemocytes resulting in haemocytopenia (hemocyte depletion). We investigated the effects of injected LPS on circulating hemocytes of the blue crab, *Callinectes sapidus*, to determine: 1) the concentration which induced the most severe haemocytopenia, 2) the time to reach maximum haemocytopenia, and 3) the time course of recovery from sub-lethal doses. Severe haemocytopenia was induced at 0.5µg/ul per gram of wet weight; LPS concentrations of 1, 2 and 5µg/ul resulted in mortality. At 0.1µg/ul LPS maximum haemocytopenia was reached within 2 hours post-injection, with recovery to pre-injection levels by 12 hours. Our data showed that dramatic decreases in the number of circulating hemocytes can be induced after exposure to non-self molecules such as LPS. Haemocytopenia induced during infection constitutes a serious threat to the health of an animal by compromising its ability to fight off invading microorganisms therefore, the rapid recovery of hemocytes is essential to combat infections. The ability of the blue crab to rapidly replenish hemocytes may be a necessity given the environment in which it lives.

An Overview Of Clam Health In The Chesapeake Bay

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Mollusks are infaunal benthic organisms important to the ecology of estuaries. Clams and other members of the benthic community are reliable and sensitive indicators of habitat quality because they reside in bottom sediments where exposure to contaminants and oxygen stress frequently occur. Benthic organisms are known to respond to multiple types of environmental stress and to reflect environmental conditions that vary over time. In 2006-2007, native and exotic clams were collected monthly from relatively clean and polluted sites in the Chesapeake Bay and their tissues examined for histopathology. An overview of the health status of several clam species will be presented. For example, infection prevalence and intensity of *Perkinsus* sp. parasites increased in the late summer in Baltic clams at two sites in the Tred Avon River. High mortalities of Baltic clams were associated with increasing parasite burdens coupled with heat stress. Disseminated sarcomas were also observed in clam tissues. Insights into historical and current impacts of potential pathogens on *Macoma balthica*, *Rangia* spp., and *Corbicula fluminea* will enhance the development of diagnostic and predictive tools needed to more adequately assess ecosystem health.

Antimicrobial Peptides From The Suppression Subtractive Hybridization cDNA Library Of Zebra Mussel (*Dreissena polymorpha*) Byssus Glands

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As a special apparatus located at the root of the zebra mussel (*Dreissena polymorpha*) foot, byssus maintains the adhesion through its entire life span. However, the current studies on the attachment mechanisms employed by zebra mussels are extremely limited. In this study, 2418 Expressed Sequence Tags (ESTs) were obtained from the Suppression Subtractive Hybridization (SSH) cDNA library constructed with zebra mussel foot. 750 new aligned ESTs remained after the analysis of sequences alignment program, Contig Assembly Program 3 (CAP3). The homologues search with BLASTx reveals that 470 ESTs had putative functions, while 280 of them were unpredictable. All the ESTs were divided into 5 groups according to the putative functions of their homologues, which included attachment related genes, exocrine gland secreted genes, defense related genes, normal cell functioning genes, and unknown byssal genes. The full length antimicrobial peptides genes were cloned and sequenced by Rapid Amplified CDNA Ends (RACE). The expression analysis of the molecules induced by different stimulation was analyzed by Real-time PCR and the recombinant expression of the antimicrobial peptides was applied in the biological activity assay to detect the inhibition of the growth of Gram-positive and Gram-negative bacteria. As a result of this study, a number of previously undiscovered zebra mussel byssal gland ESTs were added to the ESTs database providing a powerful tool for the further study of zebra mussel attachment mechanism.

Identification Of Microbial Communities Associated With The Zebra Mussel (*Dreissena polymorpha)*

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Although much research has been conducted concerning the ecology and physiology of zebra mussels since they were first introduced into the Great Lakes region, little is known concerning microbial communities associated with zebra mussels in Great Lakes ecosystems. In these studies, microbial community diversity was investigated in zebra mussel gill, gut, and hemolymph samples collected from inland lakes and tributaries in Michigan's Lower Peninsula. To determine microbial diversity we applied two molecular techniques – sequencing of 16S rRNA genes through the construction of clone libraries and community analysis using T-RFLP (Terminal-Restriction Fragment Length Polymorphism) genotyping. Clone library analysis identified ecologically diverse bacteria from 11 phyla and revealed distinct microbial assemblages associated with each sample type. Cyanobacteria accounted for 24.1% of bacteria isolated from gut samples. Members of the Microbacteriaceae family were only found to be prevalent in gill samples. T-RFLP may represent a powerful tool to be used to identify and track disease in zebra mussel populations. These findings have implications for the fields of biological control, limnology, and water quality management and warrant further investigation.

Health Evaluations Of Impinged Fishes At Steam Generating Power Plants

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Section 316(b) of The Clean Water Act of 1972 Section 316(b) requires that the best available technology (BTA) be implemented in location, design, construction, and capacity of cooling water system intake (CWSI) structures in efforts to reduce adverse environmental impacts (AEI). Since its enactment the interpretation of how to define and measure BTA's and AEI's has been highly debated. A major component of AEI is impingement. Impingement occurs when larger organisms, unable to pass through the screens or trash racks of the CWSI, are trapped against the intake screens by the water velocity of the intake. Impinged organisms trapped against the screen and may incur injury and possibly death. Previous studies have examined factors that may affect impingement rates of fishes including: CWSI velocity, temperature, turbidity, pH, and dissolved oxygen. The purpose of this study is to (1) to evaluate the prevalence of disease in impinged fish versus designated treatment and open water river reference fish from the steam generating plants of interest, (2) to evaluate disease prevalence in impinged fish versus open water river reference fish populations over multiple seasons and geographic regions. The overall goal of this study is to obtain a better understand how and why fish are impinged, and to ultimately lower the impingement rates of fish at steam generating power plants. Study sites include four plants in Alabama and four plants on the Ohio River. Impinged fishes were collected from all plants, and reference fish collected at each plant by electroshocking and gill netting. The complexity of the CWSI structure at Plant Gorgas (Alabama Power Co.) called for two additional control treatments, the intake canal, and the intake pit. Preliminary results suggest there are significant differences between impinged and reference fish, as well as additional Plant Gorgas treatments in pathogen prevalence ($p<0.001$). Further interactions between treatments and power plants, species, and disease factors will also be examined and discussed.

Strategies For Field Sampling When Large Sample Sizes Are Required

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Estimates of prevalence or incidence of infection with a pathogen endemic in a fish population can be valuable information for development and evaluation of aquatic animal health management strategies. However, hundreds of unbiased samples may be required in order to accurately estimate these parameters on a catfish farm, which is an especially inhospitable environment. The high ambient summer air temperatures, lack of shelter and electrical power, and the large number and size of the ponds contribute to the challenging conditions. One strategy that can be employed to reduce the number of samples needed is the use of the "herd", or epidemiological unit, concept. Even with the use of the epidemiological unit, however, hundreds of samples will most likely be needed, so it will be impractical to process each fish at the pond side. Quick freezing fish on dry ice and storage at -70°C for assay at a later time may be a viable option, but should be validated before use in the field. The "herd" concept and the validation of the use of frozen fish as samples for pathogen assay will be discussed during this presentation.

Genomics And Proteomics: What Do These Add To Fish Health Research?

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Genomics and proteomics technologies have resulted in an explosion of data in the biological sciences. Examples of tools and methods are Expressed Sequence Tags (ESTs), whole genome sequencing, Serial Analysis of Gene Expression (SAGE), microarray-based transcript profiling, mass-spectrometry analysis of protein expression. These can all provide important data to understand the biochemical and molecular mechanisms of infection. However, caution must be exercised when contemplating using these methods. Capital equipment can be costly, operating expenses are high and advanced training is usually required. Moreover, some of the data that are generated must be analysed by specialized methods. This session will examine some of those technologies and will illustrate the advantages and disadvantages of each, using presenters' research programs as examples. The objective of this session is to show when, why, and how genomics and proteomics techniques can be used to benefit fish health research programs.

Genomic Approaches For Studies Of Fish Responses To Pathogens And Pollutants

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Pathogens and pollutants are serious threats to fish populations. Genomic techniques, such as high-complexity cDNA library construction and microarray hybridizations, have been used to identify fish genes responsive to pathogens (bacterial, viral, fungal, protozoal) or environmental toxicants (e.g. heavy metals, pesticides, dioxins). There are excellent genomic resources available for several fish species. For example, there are more than one million zebrafish (*Danio rerio*) expressed sequence tags (ESTs) and over 436,000 Atlantic salmon (*Salmo salar*) ESTs in the National Center for Biotechnology Information (NCBI) EST database. Available fish DNA microarrays include a ~16,000 gene (16K) salmonid cDNA microarray from the consortium for Genomic Research on All Salmon Project (cGRASP) and commercially available resources such as a 21K zebrafish oligonucleotide microarray (Agilent). While currently available fish DNA microarrays can provide valuable information on the molecular pathways altered by exposure to pathogens or toxicants, these resources are missing some important defense-relevant genes. A complete understanding of the molecular mechanisms by which infections and pollutants alter fish physiology will require the development of new genomic resources such as suppression subtractive hybridization (SSH) cDNA libraries generated from immune- and toxicant-challenged fish tissues. SSH libraries have been shown to be effective means of identifying novel fish genes responsive to immunogenic stimuli. Functional genomic research may lead to the development of new tools for detecting and combating emerging diseases of fish.

Application Of Genomics To Study Host-Pathogen Interactions In Oysters

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Oysters are one of the oldest and most productive segments of US aquaculture. Unfortunately, habitat degradation, over-fishing, disease, and lack of successful sets have severely impacted oyster populations in United States coastal waters. Parasites like *Perkinsus marinus* and *Haplosporidium nelsoni* place a large economic burden on the oyster industry. Basic knowledge of innate immunity and the mechanisms used by pathogens to evade host responses is crucial to develop strategies for promoting disease resistance and managing epizootics. The recent development of genomic resources for oysters has provided the means for an explosion in oyster disease research. These resources include several collections of expressed sequence tags (ESTs) from a variety of laboratories, subtractive libraries of genes differentially expressed in response to infection, oyster microarrays, and BAC libraries. More importantly, the Joint Genome Initiative has agreed to sequence 150,000 cDNA clones from the Pacific oyster and 133 BAC clones. We are using these resources to isolate and characterize genes involved in host-pathogen interactions in oysters. Although genomics resources, combined with other more traditional approaches, have already led to the identification and characterization of several molecules with immune function in oysters, the large majority of the genes in oyster databases do not match sequences in other databases and have unknown functions. Unraveling the biological function of the large amount of genes derived from the oyster genome project will be a major challenge.

Real-Time Immunology: Analysis Of Gene Expression To Determine Fish Health

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The bane of the fish health field has long been the paucity of biomarkers and reagents to accurately assess immunological status for diverse fish species. As we slowly address the issue of antibody and ELISA approaches to quantify immune effector molecule regulation, expansion in fish genomics have opened a door to study host-pathogen interactions in another way. The use of suppression subtractive hybridization and microarray analysis are powerful tools for isolating and identifying, 10s to 100s; to possibly 1000s of differentially expressed transcripts, over a short period of time. Real-time PCR is a technique, which can build upon SSH and microarray analysis to accurately quantify these expression differences. It has an accurate dynamic range of 7 to 8 log orders of magnitude without post-amplification manipulation, can discriminate between mRNAs with nearly identical sequences and is extremely sensitive (less RNA required). Here we will discuss the considerations and pitfalls of this technology, highlighting the different chemistries, many types of normalization, selection of housekeeping and effector genes and most importantly how these have and will be applied to fish health.

DNA Microarrays To Study Bacterial Pathogenesis In Fish Health Research

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Using a draft sequence of the genome of *Aeromonas salmonicida* subsp. *salmonicida*, we designed and constructed a DNA microarray of 2068 genes selected on the basis of "involvement in some aspect of virulence". This microarray was used to study the comparative genomics of several wild and clinical isolates of *A. salmonicida* as well as to study gene expression of the pathogen grown under *in vitro* low iron conditions and within *in vivo* implants. The latter experiments were performed to test the hypothesis that that genes associated with iron regulation would be differentially expressed in *A. salmonicida* grown under low-iron *in vitro* culture, and inside *in vivo* implants, as compared to under iron-replete *in vitro* conditions. Many of the genes observed to be up-regulated by *in vitro* growth in low iron are involved in iron acquisition or transport, heme synthesis, contain iron-sulphur clusters, or are iron-regulated. Complementary proteomics studies of the same *A. salmonicida* samples indicated that the levels of >60 proteins increased or decreased by at least 50% in low iron medium. Many of the up-regulated proteins are involved in iron homeostasis, and several match genes up-regulated in our low iron microarray experiments. We have also identified additional genes and proteins, not associated with iron regulation, that are differentially expressed under *in vivo* implant growth conditions. We are correlating these results with data from other studies from our laboratories to determine molecular mechanisms of *A. salmonicida* pathogenicity.

Metabolomics; Continuing Post-Genomics Refinement

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Despite the relative novelty of genomics it is often said that we are already in the post-genomics era, defined by gene function rather than gene presence. Transcriptomics, proteomics and metabolomics respectively attempt "global" surveys of the successive molecular events occurring in a biological system under given conditions. Metabolomics aims to measure the amounts and fluxes of low molecular weight metabolites, which are products of complex interactions among cellular components that originate from the protein products of RNA transcription and subsequent post-translational modifications. Metabolites may be measured principally by Mass Spectrometry (MS) or Nuclear Magnetic resonance (NMR) spectroscopy. This talk will concentrate on proton (^{1}H) NMR-based metabolomics, which has advantages that complement other "–omics" technologies. Usually applied to biofluids or tissue extracts, it measures signals from all hydrogen-containing small molecules in proportion to their concentrations, provided that the molecules are in free solution and are not adsorbed to macromolecular or solid surfaces or otherwise restricted in their motional freedom. In some circumstances, ^{1}H NMR can be applied to whole organisms, organ preparations or cell cultures. It permits metabolites to be surveyed with little or no bias, relative to other spectroscopic or spectrometric methods. Although the proportionality of signal area to concentration is close to constant for each hydrogen atom in a molecular species, the detailed shapes of peaks in ^{1}H NMR spectra are complicated by coupling and exchange phenomena, which are in turn indicative of molecular structure. Unless combined with separation techniques or specialized pulse sequences that selectively detect particular molecular classes, the method produces spectra, which are sums of the spectra of individual metabolites, and concentration changes in minor components may be obscured by overlapping signals from major components. Comparisons can be made between individual samples, or between two or more groups each consisting of multiple individuals, by means of principal components analysis or other statistical techniques. Metabolites contributing significantly to inter-group differences can be chemically identified. Metabolomics is a valuable complement to existing nucleic acid and protein-based technologies, with numerous uses in human medicine. Increasing applications to aquatic systems include studies of aquatic animal health from which illustrations will be drawn.

Expression of Antimicrobial Peptide Genes (Hepcidin 1 And 2) In Largemouth And Smallmouth Bass

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The coincidence of intersex and fish lesions in largemouth (*Micropterus salmoides*) and smallmouth (*M. dolomieu*) bass in the Potomac and Shenandoah Rivers suggests a putative relationship between the presence of estrogenic endocrine-disrupting compounds (EEDCs), endocrine disruption, and altered immune function. Estrogen or EEDCs, which may cause intersex in smallmouth bass, may also interfere with the expression of genes involved in innate immunity, *e.g.* hepcidin, thus increasing susceptibility of fish to bacterial infection. We have identified two distinct hepcidin sequences in both largemouth and smallmouth bass (four hepcidin genes). Hepcidin is a small, cationic, disulfide-bonded antimicrobial peptide that exhibits antibacterial and antifungal activity *in vitro*. In mammals, hepcidin is an important iron regulatory hormone that blocks uptake of iron from the intestine and release of stored iron from macrophages, thus decreasing available iron. Expression of mammalian hepcidin is stimulated by interleukin-6 and expression of interleukin-6 is inhibited by estrogen. Hepcidins have been identified in several species of fish and are predominantly expressed in the liver. Expression of these antimicrobial peptides is induced by bacterial infection (or structural components of bacteria, including lipopolysaccharides). We will discuss the constitutive and inducible tissue-specific expression of two hepcidin genes in largemouth and smallmouth bass and the effect of estrogen on these expression patterns.

Immunohistochemical Localization Of Two Putative Defense Lectins, Rainbow Trout Ladderlectin And Intelectin In Diseased Rainbow Trout (*Oncorhynchus mykiss*) Tissue

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To investigate the role of lectins 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 *Aeromonas salmonicida* subsp. *salmonicida*, *Aeromonas hydrophila*, *Yersinia ruckeri*, *Pseudomonas* spp, chitin and artemia cyts (chitinous walls). As both lectins appear to be associated with peripheral blood leukocytes, their role in recognition and/or processing of foreign material is suggested. We used immunohistochemistry for the localization of rainbow trout ladderlectin and intelectin in tissue from rainbow trout submitted for examination from clinical cases of infectious disease. Based on these results, we discuss the possible biological and defensive function of these lectins.

Analysis Of A Novel And Phylogenetically Conserved Pattern Recognition Receptor (PRR)

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Pattern recognition receptors (PRR) are important components of the vertebrate immune system for recognition of microbial structures and the subsequent inflammatory responses to danger signals. We have previously described a member of the PRR family (scavenger receptor) which is present on nonspecific cytotoxic cells (NCC) of catfish. NCC are teleost NK-like cells important in innate immune mechanisms against parasites, viruses and bacteria. In the present study we describe the expression of another PRR in NCC which is a novel and phylogenetically conserved molecule. This new PRR is expressed on the membrane, in the cytosol and in granule extracts from NCC and it is referred to as nonspecific cytotoxic cell cationic antimicrobial protein-1/ncamp-1. Its name derives from its bifunctional activities: on the membrane ncamp-1 binds ligands (dinucleotide, polyguanosine, etc.) and in recombinant form ncamp-1 lyses bacteria. The relevance of ncamp-1 in innate immune responses was recently supported by finding a mammalian orthologue on mouse leukocytes. Using an anti-ncamp-1 monoclonal antibody we identified its expression in subsets of mouse leukocytes. These studies demonstrated that teleosts, similar to mammals express heterogeneous families of PRR that may be relevant in the design of future vaccine formulations against infectious agents. Research supported by BARD and USDA.

A Novel Pattern Recognition Receptor On Nonspecific Cytotoxic Cells Has Bimodal Functions Of Membrane Expression And Bactericidal Activity

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A novel cationic antimicrobial protein (nonspecific cytotoxic cell cationic antimicrobial protein-1/NCAMP-1) was identified from catfish (cf) NCC. Ncamp-1 is believed to have a bimodal action: as a membrane protein it may be a pattern recognition receptor (PRR) and in soluble recombinant form is a potent antimicrobial. When expressed in recombinant form, ncamp-1 has bactericidal activity. Expression of ncamp-1 as full length or truncated peptides and testing in killing assays determined that the bactericidal activity against $Gram^-$ and $Gram^+$ pathogens was associated with expression of lysine based motifs of the synthetic protein. To assess the biological significance of ncamp-1 in cf innate immunity, clinically relevant bacteriological isolates from 8 clinical cases and a vaccine strain of *Edwardsiella ictaluri* were tested in *in vitro* antimicrobial assays. The bactericidal activity of recombinant ncamp-1 was also determined against *E. coli* from chickens and from bovine mastitis. In order to evaluate the relationship between ncamp-1 membrane expression and immune functions, an anti-ncamp-1 monoclonal antibody (mab 9C9) was derived. This mab was utilized to investigate the potential signaling activity of ncamp-1. Flow cytometric analysis, using mab 9C9 binding, indicated that PMA activation up-regulated the membrane expression of ncamp-1 on NCC. The *in vivo* role of ncamp-1 in *Edwardsiella* infection models is currently being investigated. Research supported by USDA and Veterinary Medical Experiment Station.

Immune Response Of Hybrid Striped Bass (*Morone saxatilis x M. chrysops)* To A Commercial *Vibrio* Vaccine

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Striped bass (*Morone saxatilis*) and hybrid striped bass (*Morone saxatilis* X *M. chrysops*) are both a sport fish and food fish, with current commercial aquaculture significance. Striped bass and their hybrids are susceptible to a diverse variety of pathogens, including viral, bacterial, fungal and parasitic agents. Of these pathogens, bacteria of the genus *Vibrio* are a major pathogen of cultured marine species. This study was undertaken to evaluate the immune response of hybrid striped bass to vaccination with a commercial *Vibrio* vaccine (Vibrogen 2, Aqua Health Ltd.). Two test groups (IP injected and bath immersion) and a PBS control group of fish (n=80 fish each) were evaluated for antibody levels to the *Vibrio* vaccine for 56 days post-vaccination. An ELISA was developed to assess specific immunoglobulin levels using a previously developed affinity-purified rabbit anti-striped bass antibody as one of the ELISA reagents. Fish (n=8) were non-lethally bled on days 0 (pre-), 4, 7, 14, 21, 28, 35, 42, 49, and 56. Blood was allowed to clot, the serum separated by centrifugation, and frozen until ELISA analysis. ELISA results of the three groups were compared to each other over the course of the experimental period.

Girlie Boyz: Low-Level Endocrine Disruption In Fatheads

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The ability of male fathead minnows, *Pimephales promelas*, to compete for, maintain, and defend a spawning substrate is paramount to reproductive success. This study quantified alterations in male fathead minnow reproductive behaviors after exposure to environmentally-relevant concentrations (0, 10, 20 or 40 ng/L) of 17α-ethinylestradiol (EE_2) for 21 days. A video-based behavioral quantification system was used to examine changes in male-male competitive behaviors (i.e., chasing and head butting), and the ability of males to maintain spawning substrates (i.e., nibbling and scrubbing). Male behaviors analyzed included time under the spawning substrate, frequency of substrate cleaning, and conspecific aggression. Plasma hormone levels (11-KT, T, and E_2), vitellogenin (VTG), secondary male characteristics (tubercle count and dorsal nape pad rank), gonadal somatic index (GSI), and gonad histology were also evaluated. Exposure to 40 ng/L EE_2 decreased the ability of exposed males to compete with control males for spawning substrates ($p = 0.09$). Further, exposed males displayed reduced frequency of substrate cleaning activities as well as chasing male competitors ($p \leq 0.05$). 11-KT, T, and E_2 were lower, and VTG was notably higher, in EE_2-exposed males compared with control males ($p \leq 0.03$). EE_2 exposure in males was also associated with reduced number and size of tubercles, lower GSI and gonadal maturity ranks, and presence of an ovipositor ($p < 0.001$ for each endpoint). These data reveal clear alterations in male reproductive behavior that coincide with decreased hormone levels and secondary sex characteristics. Behavioral endpoints to discern potential ecological consequences in fish exposed to low concentrations of endocrine disrupting compounds may provide sensitive and functional indices of exposure.

Assessment Of Environmental Nitrate Stress Effects In Fishes

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Aquatic environments are in decline worldwide due to both natural and anthropogenic stressors including coastal development, agricultural runoff and sewage. Chronically elevated cortisol in response to stress can lead to impairment of immune response, reproduction and growth. Cortisol is frequently determined in plasma samples as a measure of stress. Since some free cortisol is excreted in feces, measurement of fecal cortisol can serve as a stress response indicator. Fecal samples offer ease of collection, minimal associated stress and elimination of potentially misleading acute hormone spikes seen in blood. The aim of this study was to determine the effects of nitrate, a common aquatic pollutant, on fecal cortisol levels in fishes. Butterfly koi (*Cyprinus carpio*) were chosen as a conservative model species, since they are hardy and tolerate poor water quality. Fishes were maintained group-wise (n=7) in 96 L aquaria. Nitrate (as sodium nitrate) was added to the water in 0.4 g/L increments approximately weekly beginning at 0.4 g/L and ending at 1.6 g/L. Fecal samples were collected daily and alternative-day samples were extracted and assayed for cortisol by EIA. Nitrate effects were compared in both baseline (12 days) vs. treatment (12 days) and control group (n=7) vs. treatment group (n=7) conditions. Nitrate treatment significantly (t-test, $p<0.0001$) increased cortisol excretion (9-fold, from a pre-treatment mean of 49.5 pg cortisol/g dry feces to a treatment mean of 329.1 pg cortisol/g dry feces). Additional nitrate increments produced no further increases in cortisol. This study demonstrates that fecal cortisol measurement in fishes can be useful as an indicator of nitrate stress in the aquatic environment.

Advances In Our Understanding Of Phaeohyphomycosis

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Melanized, also known as phaeoid, fungi have been identified as agents of cutaneous lesions or systemic infections with greater frequency among submissions from aquaria to the Connecticut Veterinary Medical Diagnostic Laboratory (UConn, Storrs, CT) during the last 3 years. In conjunction with several commercial aquaria, a concerted effort to culture tissue samples from specimens identified as having fungal lesions has led to identification of several *Exophiala* species, including a novel species isolated from seadragons (*Phyllopteryx taeniolatus* and *Phycodurus eques*). Recent efforts have resulted in isolation of *Exophiala angulospora* from lumpfish (*Cyclopterus lumpus)* with cutaneous and systemic lesions. Association of *E. angulospora* with lesions in a vertebrate animal represents a novel finding regarding this species, which is a well-known environmental isolate. Phylogenetic analyses of a broad assortment of melanized fungi, including *Exophiala* species from aquarium sources, have provided insights into relationships between these fungi, their hosts and growth conditions.

Histopathologic Interpretation Of Lesions In Cephalopods

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Cephalopod mollusks, which include octopus, squid, cuttlefish and nautilus, have unique features regarding their neurobiology, immunology, anatomy and behavior that impact management of these invertebrates and the occurrence of disease in aquaria. Reports describing lesions and mortalities associated with captive cephalopod populations have highlighted integumentary and ophthalmic lesions as among those frequently encountered by biologists, clinicians, researchers and pathologists. Consistent with previous reports, mantle ulcers, erosive and ulcerative dermatitis involving bacteria and protozoa alone or in combination, and ophthalmic lesions involving both globe and lens, were among the most common findings from aquarium submissions to the Connecticut Veterinary Medical Diagnostic Laboratory (UConn, Storrs, CT) during the period from 2002 to 2007. At the MBL (Woods Hole, MA), the most common dermal problems resulted from trauma-induced erosions and ulcerations. Recognition of salient histologic features of the skin and eyes of several cephalopod species contributes to an improved histopathologic interpretation of lesions and is essential to meaningful comparative assessments of histopathologic changes. This may in turn provide useful insight into the management of disease conditions in tank and other aquarium environments.

Microbiology Testing For Aquatic Systems: Findings And Potential Implications

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Analyzing environmental quality for aquatic systems is an essential component for preventive health. Most aquarists measure water for a standard set of physical and chemical parameters that typically includes: temperature, pH, salinity, DO (Dissolved Oxygen), ammonia (Total Ammonia Nitrogen), nitrite, nitrate, phosphate, and alkalinity. Depending on resources and laboratory capabilities additional chemical and physical testing can also be done to check more comprehensive water quality for more complex systems (i.e. coral reef exhibits), monitor chemical/treatment additions, and identify toxic metal/chemical contamination. In public aquaria, microbiology is also performed to assess total coliform levels for systems designed to maintain marine mammals for display purposes. At the New England aquarium, microbiology testing has been expanded to test for Enterococcus and total coliforms in aquatic systems, which house only pokilotherms. The following presentation will discuss some preliminary findings for these systems, problems associated with high levels of bacterial detection, and potential implications for animals and staff.

Phagocytosis In Nurse Shark (*Ginglymostoma cirratum*) Peripheral Blood Leukocytes

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Chondrichthyans (elasmobranchs and holocephalans) are the oldest extant class of vertebrates to have a fully functional adaptive immune system. Molecular studies examining the immune system elements are ongoing. However, studies on phagocytosis and evaluation of the function of peripheral blood leukocytes (PBL) have been confusing due to nomenclature discrepancies as well as variations in species of elasmobranch used, targets, and treatment of cells. This study attempts to begin standardization for the identification of cell types based on function, using a cell atlas derived from sandbar shark (*Carcharhinus plumbeus)* PBL. Whole PBL were exposed to fluorescein isothiocyanate (FITC) conjugated bacterial targets and phagocytosis was evaluated by flow cytometry, microscopic examination, and differentiation of cell types. Bacterial species examined included *Escherichia coli*, *Staphylococcus aureus*, and *Vibrio alginolyticus*. Opsonization of the bacteria with shark plasma and IgM blocking studies were also performed. Results showed the most actively phagocytic cell to be the coarse eosinophilic granulocyte (CEG). Interestingly, some lymphocytes were also shown to be phagocytic. Opsonized *E. coli* and *Vibrio* sp. were taken up by cells, but nonspecifically. Opsonized *S. aureus* were taken up in significant amounts by washed PBL when compared to unwashed PBL, and blocking studies to determine the involvement of shark antibodies in the uptake suggested that other serum factors were involved.

Head and Lateral Line Erosion Syndrome In Ocean Surgeonfish (*Acanthurus bahianus),* Current Efforts To Determine Etiologies

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Head and lateral line erosion syndrome (HLLES) or chronic erosive dermatopathy (CED) in fish has long been a frustration of the aquarium and pet industry. The anatomy of the head and body regions along the lateral line has been clearly described as well as the pathology of a disease. The etiology of this syndrome, however, is still unknown and has been a focus of much debate. Implicated causative agents include (not all-encompassing) activated carbon, parasites, carbon dust, heavy metals such as copper, electrical currents, endotoxins, ozone exposure products, ultraviolet radiation exposure products, as well as nutrient deficiencies including vitamins A and C. Of these, the authors could only determine that heavy metals have been formally studied in a controlled situation. Over the last several years, staff from The Seas at Epcot and the University of Florida has been systematically examining possible etiologic agents for HLLES in ocean surgeonfish (*Acanthurus bahianus)*. To date, in-line ultraviolet radiation, activated carbon, foam fractionation, ozone, and vitamin A have been preliminarily examined with controls. Even though these applications need to be examined in more detail, only carbon and ozone have produced dermatitis in distinct and consistent areas of the skin, including the lateral line region. Future studies plan to investigate causative agents in further detail by varying applications rates and concentrations of these agents.

Gas Supersaturation In A Large Artificial Reef Exhibit: An Unusual Presentation And Resolution

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A 335,000-gallon artificial seawater exhibit containing 380 teleost fish (Perciformes, Tetraodontiformes, and Beryciformes) and three stingrays has been in operation at the National Aquarium in Baltimore for 26 years. In early September 2006, acute mortalities were seen in two blue runners (*Caranx crysos*). The animals were in good body condition, and showed recent food intake and no evidence of significant pathogens. The gills showed gas emboli within the blood vessels and telangectasia. Other clinical signs in the exhibit included decreased food intake and increased gilling rate and effort in the medium-to-large sized fish. Total gas pressure was recorded at 102-103% with a peak on September 27 of 105-106%. Two blue runner mortalities occurred on September 27 and October 1. Mortalities were not seen in any other species. Acute gas supersaturation in an aquarium is usually due to air injection into a pump that allows gas to become trapped under high pressure. The five pumps on this system were working normally. The source of the gas supersaturation was an elevated water level in the exhibit that created flow within a 40-ft vertical overflow pipe into the biotower; there was insufficient off-gassing before the pumps brought the water to the exhibit. The water level was corrected on October 7. Total gas pressure has subsequently been 99-100% and no further clinical signs have been seen.

Retrospective Summary Of Diseases Affecting Captive Seahorses At The Toronto Zoo

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Wild seahorse populations are in sharp decline from over-fishing for aquaria, curios and traditional medicine. Captive rearing of seahorses has been practiced for decades and husbandry has improved yet seahorses still provide a challenge for aquarists. Infectious disease and management deficiencies are common causes of morbidity and mortality but are often poorly documented. We are conducting a five-year retrospective review of seahorse cases submitted by the Toronto Zoo. To date, approximately half of cases (of 100 total) have been reviewed. The major reoccurring problems are; a bilaterally symmetrical multifocal chronic active myopathy, enteritis with bacterial overgrowth, bacterial dermatitis ('redtail'), a suspect viral enteritis, protozoan dermatitis and enteritis, and a variety of systemic bacterial lesions, among other less common diagnoses. The myopathy was found to be the most prominent problem, occurring to varying degrees in approximately 80% of cases reviewed thus far. Because the myopathy was found to be bilaterally symmetrical, it is presumed that it is nutritional in origin. Supplementation of the diet (brine shrimp primarily) did seem to resolve, but not eliminate the myopathy. Lesions caused by bacteria and protozoa were observed in approximately 20 and five percent of cases, respectively.

Surgical Treatment Of Lymphoma In A California Moray Eel, *Gymnothorax mordax*

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An adult California moray eel, *Gymnothorax mordax*, was presented to the Veterinary Service at the John G. Shedd Aquarium for evaluation of an ovoid, intracoelomic mass. Diagnostics including plain and contrast radiography, ultrasound, and fine needle aspiration with cytology and bacterial culture suggested an undifferentiated, malignant neoplasm of mesenchymal origin. The eel was anesthetized and surgery was performed to resect the mass, which was associated with the gastrointestinal tract and spleen. A section of stomach and intestine was resected and anastomosed in a 3.5 hour surgery. Medical and nutritional post-operative care along with the eel's natural wound healing ability resulted in an uneventful recovery. Histopathology confirmed the mass as a high-grade lymphoma.

Fish Sutures: Tying Things Together

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Many suture types have been used successfully in fish. Monofilament absorbable skin sutures are generally recommended to decrease the risk of wicking bacteria into the wound (synthetic absorbable sutures may not be readily absorbed in fish). Other factors in suture selection are tissue reactivity and healing time. Needles with a cutting tip facilitate skin penetration. Simple continuous, simple interrupted and continuous Ford interlocking patterns have all been used for skin closure with satisfactory results. Continuous patterns provide less surface area for bacterial and epibiont colonization, and subjectively have been associated with less suture reaction than interrupted patterns, but if not placed with adequate tension or without excellent knot security at either end, dehiscence is more likely. Single or two-layer closure can be employed depending on the thickness of the body wall. Studies have shown that surgical adhesives (e.g. cyanoacrylate), either alone or in combination with sutures, can contribute to dehiscence, delay healing, and cause local irritation. Results from a study in koi (*Cyprinus carpio*) indicate that monofilament materials like nylon and polyglyconate (Maxon®) are slightly superior to braided suture. Once sutures have been placed, a topical antimicrobial, like povidone iodine (Betadine®) ointment or triple antibiotic ointment, can be applied with care to the wound. In many cases suture materials are removed in 10-14 days; fish wounds heal quickly when optimal environmental parameters are present.

To Cut Is To Cure: Surgical Cases In Fish Medicine

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Surgical treatments have been historically used commonly in many companion animal species, including dogs and pets. With the increasing awareness of the availability of companion animal/exotic animal veterinarians that are willing to treat pet fish, a few procedures have become relatively common in pet fish surgery. Epidermal mass removals, enucleations, repair of traumatic injuries and exploratory celiotomies are examples of the typical surgeries that may be seen in companion animal practice. Fortunately, fish make excellent surgical candidates. A few select case reports of surgical cases seen in private aquatic veterinary practice will be presented.

Endoscopic Sex Determination And Sterilization Of Gulf Sturgeon (*Acipenser oxyrinchus* Desotoi)

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Seventeen Gulf sturgeon underwent endoscopic sex determination and various procedures as part of a study to determine whether sterilized animals could serve as sentinels. The fish were anesthetized with tricaine methanesulphonate (MS-222) and maintained on a re-circulating anesthesia machine. A 6 mm cannula was placed just off ventral midline, midway between pectoral and pelvic fins, which allowed insertion of a 5 mm telescope. Emptying the swim bladder coupled with CO_2 insufflation provided excellent visualization of the celoemic cavity. Two additional cannulae were placed laterally, cranial and caudal to the first cannula for introduction of surgical instruments. Sex determination was successfully performed in all fish; with 5 of 17 fish requiring gonadal biopsy. Bilateral ovariectomy or orchidectomy was successfully performed in 3 males and 4 females. Unilateral ovariectomy and bilateral ligation of the müllerian ducts was accomplished in an additional three females. Endoscopy has provided tremendous advantages over classical surgical techniques allowing us to minimize trauma to the animals and improve our success rates.

Ophthalmic Surgical Procedures In Public Aquarium Fishes

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Ophthalmic disorders, including corneal abrasions, ulcers, exophthalmia, buphthalmia, uveitis, hypopyon, hyphema, cataracts, lens luxation, gas bubble disease, proptosis, and various retrobulbar disorders are very common in aquarium fish. Deep corneal ulcers may require surgical debridement and grid keratectomy. Protection of the ulcer with cyanoacrylate surgical glue has been effective in some cases, but appears to have caused tissue reaction in other cases. Lens removal may improve vision, decrease pain, and eliminate uveitis in cases of lens luxation or advanced cataracts. While advanced techniques such as phacoemulsification could be used, good results are often seen via simple keratectomy and lens extraction. The cornea is closed with 6-0 to 8-0 suture. Unilateral or bilateral pseudobranch ablation via bipolar cautery has shown approximately 50% success in cases of recurrent gas accumulation in the anterior chamber or retrobulbar space. Enucleation is a reasonable alternative for incurable ocular disease, but closure is challenging in a fish with a very large orbit. Several reports of successful ocular prostheses in fish have been described.

Anesthesia Of Fish: I Don't Want To Make The Wrong Mistake (Yogi Berra)

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Many substances have been used to anesthetize fish. Tricaine is probably the most commonly used agent and is the only one approved by the Food and Drug Administration for use in food fish in the United States, however, it has a withdrawal time of 21 days. Depending on the specific drug and the circumstances they may be administered by intramuscular injection intravenous injection, orally, the drug may be put in water and fish placed in water or the drug may be put in the water and the water pumped into the fish's mouth and over the gills. It needs to be pumped fast enough to deliver enough anesthetic and to ventilate the fish i.e. maintain normal arterial oxygen and carbon dioxide concentrations. The system should have an aerator stone in it which bubbles air into the water. The basic scheme of stages of anesthesia in the fish can be described as: awake, excited, turn to lateral or dorsal recumbency, sink, anesthetized. Heart rate may be monitored by use of an ECG, a Doppler flow probe, or a pulse oximeter. These different monitors worked inconsistently between fish and sites in each fish, but when they work they are very useful. For recovery the fish should be ventilated with water free of anesthetic until they make strong opercular movements and then be put in the recovery tank. The water should have an aerator stone in it, which bubbles air into the water. There is much discussion and disagreement about the question of pain in fish. Until the issue is resolved with assurance, analgesics should be administered.

Comprehensive Wound Care Management In A Cownose Ray (*Rhinoptera bonasus)* And A Blue Catfish (*Ictalurus furcatus*)

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The primary function of the integumentary system is to provide a protective barrier between the internal organs and the environment. Fish have integumentary adaptations that permit them to live in an aqueous habitat. Anatomical or physiological insult to the skin may compromise homeostasis, immune competency and sensory mechanisms leading to increased morbidity and mortality. Wounds of varying severity and origin are one of the most common problems in captive aquatic species. The unique and sophisticated characteristics of fish integument and exposure to a constantly moist substrate enable fish to heal rapidly. However, factors such as wound severity, medical condition of the animal, nutrition and water quality can influence healing time. Comprehensive wound management in fish may entail mechanical procedures such as debridement or closure, local and systemic chemotherapeutics, stress reduction, proper nutrition and provision of optimal water quality. At the Georgia Aquarium, the use of Tricide-Neo, a potentiated antimicrobial preparation, and Regranex™, a recombinant platelet-derived growth factor, were used to treat a cownose ray and a blue catfish with injuries sustained from tank mate aggression. The complicated nature of their wounds required a multifaceted approach to control infection and facilitate healing.

Comparison Of Lethal And Non-Lethal Sampling Techniques For The Detection Of *Aeromonas salmonicida* And Other Fish Pathogens In Populations Of Ontario Salmonids

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To monitor bacterial fish pathogens for the Ontario Ministry of Natural Resources (MNR), the Fish Health Laboratory routinely uses conventional bacteriological culture of fish organs collected by lethal sampling. As a non-lethal approach, we used culture, PCR and PCR-Denaturing Gradient Gel Electrophoresis (DGGE) to detect *Aeromonas salmonicida* and other fish pathogens in fish mucus. Wild chinook and coho salmon (n=60 and 52) from the Credit River and lake trout (n=27) from a MNR culture station were sampled lethally and non-lethally. *A. salmonicida* was isolated on Coomassie Brilliant Blue agar from mucus of 43 chinook and 31 coho, while it was cultured from kidneys of 28 chinook and 15 coho. PCR for the *vap*A gene was positive in the mucus of 30 chinook and 21 coho. *A. salmonicida* was not detected in mucus or kidney tissue of the lake trout by either culture or PCR. When mucus samples were used for a nested PCR amplification of a 193bp segment of eubacterial 16S rDNA, 5 lake trout had bands coincident to the migratory distance of the bands amplified from control cultures of *A. salmonicida.* Bands were also found suggesting *Y. ruckeri* (4 fish) and *A. hydrophila* (9 fish). As DGGE can separate species-specific sequences from a mixed microbial community, PCR-DGGE may be useful in indicating the presence of multiple pathogens in fish mucus simultaneously. However, analysis of results may be complex, in terms of matching band migration patterns and discerning the presence of bacteria with multiple band patterns, including *A. salmonicida*.

Efficacy Of Common Disinfectants Against *Aeromonas* spp. And *Edwardsiella* spp.

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Bacterial pathogens are one of the main disease-causing agents of the aquaculture industry. As many potentially pathogenic bacterial species are water-borne, disinfection is essential for both disease control and for enhanced biosecurity. This study examined a variety of commercially available compounds for their efficacy to reduce or eliminate specific bacteria from the water, and included Clorox (50,000 ppm, 200 ppm, 100 ppm, 50 ppm), ethanol (70%, 50%, 30%), Roccal (1:256), Lysol (1%), iodine (100 ppm, 50 ppm), formalin (250ppm), Chloramine-T (15mg/L), glutaraldehyde (2%) and Virkon (1%). Each compound was tested for various time intervals against *Edwardsiella ictaluri, E. tarda*, *Aeromonas hydrophila*, *Aeromonas salmonicida*, and *Aeromonas salmonicida subsp achromogenes*. In addition, the three *Aeromonas* species were tested at various temperatures (22C, 15C, 10C, 5C). Both species of *Edwardsiella* were killed within one minute of contact time by all compounds except Chloramine-T and formalin, which were both ineffective, even after 60 minutes. Similarly, all compounds, except Chloramine-T and formalin, killed the three *Aeromonas* species within one minute of exposure time regardless of temperature or organism. The effects of Chloramine-T were temperature-dependent, and also varied with the organism. *A. hydrophila* was killed within five minutes exposure regardless of temperature. *A. salmonicida* was also killed within five minutes, but only at 22C, 15C and 10C, while an exposure of 10 minutes was required at 5c. In contrast, *A. salmonicida subsp achromogenes*, survived up to 30 minutes exposure at all temperatures, while no growth was detected at 60 minutes. Formalin was the least effective disinfectant tested, producing less than a one log reduction in bacterial growth even after one hour of exposure regardless of temperature or organism.

Florfenicol Uptake And Depletion In Tilapia (*Oreochromis niloticus*) Of Various Sizes

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Nile tilapia *Oreochromis niloticus*, of three different size groups were medicated with florfenicol (Aquaflor, Schering-Plough) via a medicated ration at 15 mg florfenicol/Kg fish body weight/day for 10 days to compare elimination kinetics of the test article. This study was a part of a larger effort to develop a species-grouping concept for the labeling of therapeutic compounds for cultured fish. Tests were conducted on tilapia weighing 100 gm, 250 gm and 500 gm at a temperature of 25º C to determine if depletion of florfenicol was impacted by fish size. Animals were acclimated for a minimum of 7 days before administration of the medicated feed and held for up to 28 days after the 10-day medication period. Fifteen fish were sampled at the following time points: 5 days after the initiation of the medicated feeding period (mid-point of medication period) and at 1 hr, 1 d, 2 d, 4 d, 8 d, 14 d and 28 d post-medication period. During acclimation and after the medicated feed was administered a control diet was given. Results indicated that for tilapia at 25º C there is a trend for more rapid elimination in smaller fish. For both 100 and 250 g fish, concentrations of florfenicol declined below the action level within 8 days and for 500 g fish, within 14 days.

Minimum Inhibitory Concentration Testing of *Flavobacterium columnare*

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A simple, accurate and reliable microdilution method has been developed to test the susceptibility of *Flavobacterium columnare* to antibiotics. The method has been used to determine the minimum inhibitory concentration (MIC) of 23 *F. columnare* isolates. The developed method conducted at 28 °C for 48 h used standardized inoculum, Mueller Hinton broth at 1/5 the full strength (4g/l), reference isolate, a positive and a negative control wells and a standardized custom made microtitre plates, Sensititre® Susceptibility Plates for Aquaculture (Trek Diagnostic Systems, Inc.). *Escherichia coli*, ATCC25922 was used as the reference isolate and produced MIC values within the range published by the Clinical and Laboratory Standards Institute (CLSI), formally the National Committee for Clinical Laboratory Standards (NCCLS). Mueller Hinton broth at 1/5 the full strength (4 g/l) was found to support significantly better growth for *Escherichia coli*, ATCC25922 and *F. columnare* type strain, ATCC23463, than Mueller Hinton broth at 1/7 the full strength.

Sequence Analysis Of The *Yersinia ruckeri* Multidrug Resistance Plasmid pYR1

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Antimicrobial resistance in *Yersinia pestis* constitutes a significant threat considering that antimicrobials are used for both plague treatment and for the prevention of human-to-human transmission. For this reason, the discovery of a multiple antimicrobial resistant (MDR) isolate of *Y. pestis* (strain IP275) in 1997 caused considerable alarm. This isolate contains a large self-transmissible plasmid (pIP1202) that confers resistance to at least eight antimicrobials including several of the drugs recommended for plague treatment and prophylaxis. Comparative analysis of the DNA sequence of pIP1202 revealed an IncA/C plasmid backbone that is shared by MDR plasmids isolated from *Salmonella enterica* serotype Newport SL254 and the fish pathogen *Yersinia ruckeri* YR71. The high degree of sequence identity and gene synteny of this shared plasmid backbone suggests recent acquisition of these plasmids from a common ancestor. In addition, PCR-based plasmid typing and rapid detection methods were used to screen a collection of MDR isolates of *Salmonella*, *Escherichia coli* and *Klebsiella* for the pIP1202 backbone. Strains tested were isolated from retail meat samples collected between 2002 and 2005. The *Y. pestis* pIP1202-like plasmid backbone was detected in 65 isolates, including several serotypes of *Salmonella* as well as several *Escherichia coli* and *Klebsiella* strains. The plasmid-positive strains were found in bacteria associated with beef, chicken, turkey and pork, and were found in samples from the following states: CA, CO, CT, GA, MD, MN, NM, NY and OR. These studies reveal a common plasmid backbone that is broadly disseminated among zoonotic pathogens associated with agriculture. These plasmids represent a reservoir of mobile resistance determinants and have the potential to disseminate to *Y. pestis* and other human and zoonotic bacterial pathogens of public health importance.

Pharmacokinetics Of Oxytetracycline In The Horseshoe Crab

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The American horseshoe crab, *Limulus polyphemus*, is commonly maintained in research laboratories and public aquaria. Concerns over the health of these captive animals make the clinical diagnosis and treatment of pathological conditions in *L. polyphemus* essential. This study investigated the pharmacokinetics of oxytetracycline in male and female adult horseshoe crabs following either intravenous or oral dosing. A non-compartmental model was developed to describe the pharmacokinetics of oxytetracycline in the horseshoe crab. The following parameters were determined for a single intravenous bolus of 25 mg/kg oxytetracycline: AUC = 9524.60 µg*h/mL, MRT = 443.65 h, Cl_b = 0.044 mL/min/kg, $V_{d(ss)}$ = 1.164 L/kg, $t_{1/2}$ = 128.3 h, C_{max} = 55.90 µg/mL, C_{ave} = 27.39 µg/mL. After a single oral bolus of 20 mg/kg, the following parameters were determined: AUC = 5861.81 µg*h/mL, MRT = 395.89 h, Cl_b = 0.071 mL/min/kg, $V_{d(ss)}$ = 1.688 L/kg, $t_{1/2}$ = 210.0 h, C_{max} = 7.83 µg/mL, C_{ave} = 2.89 µg/mL, F = 61.56%. As compared with the intravenous route (i.e. 100% bioavailability), oral absorption of oxytetracycline was observed at a rate of 61.56% in the horseshoe crab. Because absorption is significantly reduced in the oral route as evidenced by the relatively low bioavailability, it is recommended that intravenous injection is a more appropriate route for therapeutic dosing of oxytetracycline in the horseshoe crab. This preference in route of administration is further supported by the ease with which an intravenous bolus can be delivered into the cardiac sinus of horseshoe crabs.

Use Of 17-Alpha-Methyltestosterone For Masculinization Of Ornamental Fish

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The compound 17-alpha-methyltestosterone (MT) has been used in aquaculture for many years. In the U.S., MT is available through an FDA INAD for use as a masculinizing feed additive for more efficient production of tilapia. MT has additional potential applications in ornamental species. Many ornamentals, including swordtails, African cichlids, dwarf gouramis, rosy barbs, and rainbowfish are sexually dimorphic, and phenotypic males often having greater economic value than phenotypic females. MT may also provide a mechanism to reduce population growth in aquarium exhibits, by allowing stocking of all males/male phenotypes and/or by sterilization of fish, which may also be desirable in other situations. Under a current FDA INAD for ornamental swordtails, ongoing studies in Florida through the Tropical Aquaculture Laboratory have demonstrated 100% masculinization in fish fed 3% or 5% body weight of a 60 mg MT/kg feed, as defined by development of a caudal fin extension ("sword") of seller size phenotypic female fish during in-tank trials. Commercial field trials for swordtails are currently planned. Other laboratory trials using limited combinations of different life stages (~2-week-old, ~6-week-old, and broodstock) and treatment regimens (10% or 5% BW feeding of 60 mg MT/kg feed; for 28, 56, or 84 days)in zebra danios have also been completed. Histological analysis of representative fish as well as breeding experiments demonstrated differences in effectiveness depending upon the size/age of fish exposed, with 2-week-old fish appearing to be masculinized most successfully. Additional studies are planned for other species.

Preliminary Investigations Into The Use Of Metomidate Hydrochloride (Aquacalm®) In Ornamental Cichlids

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Metomidate hydrochloride (Aquacalm™) is used for anesthesia or sedation of fish for procedures such as tagging, weight sampling, spawning, and transportation. However, its usefulness as a sedative during transport of ornamental fish has yet to be fully determined. Based on the results of several pilot studies, convict cichlids (~4.5 cm TL) *Archocentrus nigrofasciatus* (Cichlidae) were chosen as a test species to evaluate sedation with metomidate during transport. Three metomidate concentrations (0.2 ppm, 0.5 ppm, and 1.0 ppm) and a control were evaluated. Fish were stocked at a density of twenty-five per bag, and eight replicate bags were run for each metomidate concentration. After approximately 24 hours of exposure to metomidate (including 4.5 hours of air transport), the fish in each shipping bag were transferred to a 5-gallon "recovery" tank for data collection over a 7-day observation period. Water quality data (including total ammonia nitrogen, dissolved oxygen, temperature, and pH), mortality data, and appearance and behavior scores were collected at various times throughout the observation period. Statistical analyses of cumulative mortality data as well as mean ranks of appearance and behavior scores revealed that a high dose (1.0 ppm) of metomidate resulted in fewer mortalities and improved appearance during shipping.

Gene Discovery In *Ichthyophthirius multifiliis*: A Complementary DNA Resource

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Ichthyophthirius multifiliis (Ich) is a major pathogen of freshwater fish with substantial impact on commercial aquaculture. No vaccines are currently available for this agent, and treatment options are restricted to the use of chemicals that are both costly and toxic to the environment. We have assembled and analyzed a comprehensive collection of 24,741 ESTs representing genes expressed at 2 different stages of the Ich life cycle, the mature trophozoite (trophont) and the free swimming infectious theront. Analysis of these sequences has resulted in the identification of 5311 unique transcripts (UniScripts), 3666 from the trophont stage and 2083 from the theront stage. We have documented significant differences in gene expression between the stages examined, which strongly reflect the predominant physiological activities occurring at each developmental stage. This annotated EST collection should aid in the discovery of potential drug and vaccine candidates for the treatment and prevention of "white spot" disease, and in the elucidation of metabolic pathways that control virulence and parasitism in *I. multifiliis* and more distantly related parasitic protozoa within the alveolate clade. Expression profiling of the genes that are active at different stages of the life cycle, along with comparative genomic analyses with free-living ciliates and apicomplexans, should also lend fundamental insight into the genes involved in ciliate pathogenesis.

Cormorants As Mechanical Vectors Of *Heterosporis* sp.

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Heterosporis sp., an emerging microsporidial fish pathogen in North America, has a wide host range and infects skeletal muscle rendering it unpalatable for human consumption. The potential for cormorants (*Phalacrocorax auritus) to* spread this parasite was investigated. Eight cormorants were used: three control birds; and five experimental birds each fed 20g of *Heterosporis* sp.-infected yellow perch (*Perca flavescens*) muscle daily for three days. The affected yellow perch muscle was confirmed to contain *Heterosporis* sp. by nPCR and sequencing of the product obtained. Feces were collected daily from each group of birds for one week after the first bolus of infected muscle was given. Fathead minnows (*Pimephales promelas*) were used to detect the presence of viable parasites. Two tanks housed control fish that were fed daily for one week; brine shrimp mixed with 2 ml of fresh feces from the control birds. Two tanks of fish were exposed to feces from cormorants fed infected muscle following a similar procedure. One tank of fish was given on two successive days, 0.1 ml of feces *per os*, from birds fed infected muscle. Another tank of fish was fed macerated infected yellow perch muscle for three days, and served as positive controls. At approximately monthly intervals over four-and-a-half months, groups of fish were euthanized and examined for evidence of *Heterosporis* sp. by histology and nPCR. *Heterosporis* sp. was detected only in the fish fed infected muscle tissue. At least under the experimental conditions employed here, cormorants do not appear to be mechanical vectors of *Heterosporis* sp.

Renal Trematodiasis Of Largemouth Bass And Development Of PCR Methods

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Acolpenteron ureteroecetes (suborder Monopisthocotylea, family Caleostomatidae), is a monogenean trematode that lives in the renal tubules of large mouth bass, *Micropterus salmoides*. In July, 2005, a new fresh water MA aquaculture venture purchased fingerlings from a commercial hatchery. The fish were placed in recirculating raceways upon arrival in MA. In order to get the best growth of the large mouth bass in this system, and to make the venture economically profitable, the temperature of the water was held at 27ºC. By November, 2005, when the fish averaged 9" in length, mortality counts of 75 animals/day were routine. Moribund fish from the facility showed lesions consistent with renal trematodiasis. The water temperature was lowered to 24ºC and mortality decreased significantly. But, the growth rate also decreased resulting in a poor, long term economic outlook. The grower requested a method for screening fingerlings. A PCR based diagnostic test provides a quick and sensitive method. DNA was initially amplified using family specific primers. Species specific primers were then developed and used on samples of infected kidney tissue, trematodes only and eggs only. Results showed that the primers selected for our PCR test method were successful at identifying parasites and eggs. This test could provide uninfected fish that can be maintained at higher, growth promoting temperatures which will decrease time to market and will increase economic viability of bass aquaculture.

Biology And Management Of A Monogenean Trematode (*Acolpenteron ureterocetes*) Infecting Largemouth Bass (*Micropterus salmoides*)

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Recently a fish farm in Massachusetts experienced mortalities in largemouth bass (*Micropterus salmoides*) starting three months after delivery as fingerlings from a commercial hatchery. Upon examination, Dr. Roxanna Smolowitz, DVM found numerous trematodes in the kidney and ureters. The diagnosis indicated a unique and understudied monogenetic trematode, *Acolpenteron ureterocetes*, as the primary cause of fish mortality. Bass fingerlings from the same cohort, housed at Roger Williams University, were also examined and also found to host the trematode but with less severity of infection. To better understand the etiology of this parasite, the major focus of this project was to develop a rapid and accurate diagnostic protocol to evaluate the degree of parasite load in fish. A fresh tissue smear technique was developed and compared with a histopathological inspection/evaluation protocol. The quicker, easier and less expensive fresh smear protocol proved to be reliable and a quantifiable indicator of parasite prevalence. The effects of the commercial anti-trematode treatment, Praziquantel, were also studied. This research focused on the tissue uptake of the drug, administered under controlled conditions (1.5mg/L with 24 hour exposure) to largemouth bass fingerlings (8-10cm) and compared to an ethanol control treatment (112 ppm with 24 hour exposure). The body burden of praziquantel in the bass kidney, liver, gills, and muscle was measured by HPLC, UV/Vis spectroscopy (λ =220nm) and fluorescence spectroscopy (λex=260nm and λem=285nm, 100ng detection limit). The degree of uptake by the four bass tissue compartments was quantified along with the elapsed time post-treatment where detection of residual praziquantel and metabolites in the fish tissue were measurable.

Prevalence And Impact Of Leeches Parasitizing Fish Of Lake St. Clair, Michigan

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Lake St. Clair (LSC) plays a critical role for recreational and conservation fisheries. The purpose of this study was to identify the most prevalent leech species parasitizing LSC fish and to assess damage caused by the leeches to their host. Fish and leeches were collected via trapnets by the MDNR during May 2006. Leeches were identified morphologically according to several keys. Three species of leech were identified: *Actinobdella pediculata*, *Myzobdella lugubris*, and *Placobdella montifera*. Of the three, *M. lugubris* (83.29%) was the most widespread. Freshwater drum, *Aplodinotus grunniens,* (18.21%) and channel catfish, *Ictalurus punctatus*, (18.13%) were the most prevalent leech hosts. The pectoral fins (29.21%) were the most common attachment site. Leech attachment sites were associated with epidermal erosion, hemorrhages, edema, massive expansion of the connective tissue, and congestion. These findings show that leeches do cause damage to their hosts and could potentially be assisting other pathogens to cause harm.

Toxin Production And Virulence Factors In A Polymicrobial Disease Of Corals

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Black band disease (BBD) of corals is a complex, polymicrobial infection that migrates as a distinct mat across the surface of coral hosts, lysing coral tissue. We have been investigating the etiology of this disease using microbiological, molecular, and analytical techniques. It appears that multiple toxins and virulence factors are involved in disease pathogenesis. First, aerobic respiration by BBD community members results in permanently anoxic zones in the middle and base of the 1 mm thick band, extending up into the diffusive boundary layer above the mat at night. The anoxic conditions select for sulfate-reducing bacteria, which generate toxic sulfide that accumulates to concentrations in the band that are lethal to coral. Experiments using the inhibitor Na molybdate revealed that while sulfide production is not required for coral tissue lysis and disease progression in an actively migrating band, it is required for disease initiation. We have found a second BBD toxin, the cyanotoxin microcystin. Using HPLC/MS we detected microcystin in 22 field samples of BBD from regions of the northern Caribbean, but not from the eastern Caribbean or the Philippines. Microcystin was also produced in cyanobacterial cultures isolated from BBD. Toxic activity was confirmed by ELISA and the protein phosphatase inhibition assay. In addition to the above, we have consistently detected numerous 16S rRNA gene sequences in clone libraries constructed from BBD samples that are most closely matched to bacteria associated with Paralytic Shellfish Toxin (PST) producing dinoflagellates suggesting additional toxins.

Antibacterial Activity Of Cyanobacteria Associated With Black Band Disease

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One of the most common coral diseases is black band disease (BBD). This complex microbial community contains a great number of taxonomically and metabolically diverse microorganisms among which cyanobacteria are dominant. It is currently believed that the disease is caused by synergistic action of the members of this consortium. Cyanobacteria are known to produce a vast number of different biologically active compounds. In this work, we are testing the "Coral Probiotic Hypothesis" according to which the "right" microbial population associated with coral mucus and tissue can provide resistance to pathogens. To assess the potential of coral reef cyanobacteria to affect bacterial growth, we tested 19 cyanobacterial strains that were isolated from BBD and non-BBD associated cyanobacterial mats on the reef. Their effect was tested on 10 strains of heterotrophic bacteria isolated from BBD and 10 strains of heterotrophic bacteria from the mucus of apparently healthy corals. Taxonomic identification of all microorganisms was performed by 16S rRNA gene sequencing followed by a BLAST search. Of 19 cyanobacterial strains tested, 10 showed antibacterial activity against at least one target organism. The most active cyanobacterial strain was a *Leptolyngbya* isolated from a non-BBD source, and the second most active was a BBD *Geitlerinema*. The most susceptible bacterial strain was *Vibrio harveyi* that was isolated from BBD and that is known to be a common pathogen for many marine organisms. These preliminary data indicate that cyanobacteria indeed produce antibacterial compounds effective against potential BBD pathogens.

Comparison of Collection Methodology, Location, Coral Species, and Health Status on Acroporid Coral Microbial Community Composition

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Acropora spp., once dominant corals on Caribbean reefs, have suffered region-wide declines over the past two decades. *A. palmata* and *A. cervicornis* are the first corals to be listed as threatened species under the U.S. Endangered Species Act. These corals harbor diverse microbiota, likely participants in coral health and disease processes. Several investigators have previously attempted to ascertain the structure of microbial communities associated with these corals by various methods, often with conflicting results. This study used a larger-scale genomic approach to analyze the microbiota. Replicate biopsies from apparently healthy and diseased acroporid colonies were collected at six sites in the Florida Keys and Dry Tortugas. Three different processing methods were used in parallel to process the samples. Individual 16S rRNA gene libraries were generated from each of 56 samples with over 17,000 clones sequenced to date. Results indicate that the methodology employed to process a given sample introduces significant variability into the microbial community composition detected. Of the three methods used, coral mucus samples reveal the most consistent microbial composition between similar samples, with minor variation introduced by differing geographic location and coral species. Several groups of bacteria that were differentially detected in healthy versus diseased samples are currently under investigation.

Defense Mechanisms In Gorgonians Against Infections By *Aspergillus sydowii*

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Gorgonian defense mechanisms against infections include accumulation of amebocytes, increased production of antifungal compounds and elevated production of gorgonin, melanin and chitinase. The most apparent response, however, is the production of pigmented sclerites. Until now the chemical nature of the pigment remained unknown. We report here, using confocal Raman microscopy, that the pigment is a carotenoid with a polyene chain containing between 14 to 15 carbon double bonds. Antifungal activity of carotenoids has been reported and we are currently testing this against the gorgonian pathogen *Aspergillus sydowii.*

Histopathology Of White Plague-Diseased Corals

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Knowledge of the microscopic structure of cells and tissues provides clues to their function and is necessary in the study of diseases in all organisms. One concern about the health of the scleractinian or stony corals since the early 1970s has been the rapid loss of polyps and coenenchymal tissue resulting in completely denuded exoskeleton within a matter of hours. Using standard histological techniques and examining the stained tissue sections by light microscopy revealed bacterial aggregates in some affected coral species and not in others, leading to the separation of the white band and white plague diseases. Coral tissues have been sampled during disease outbreaks on reefs in the Caribbean and Indo-Pacific during the last 10 years. Histopathological examinations have shown that similar gross signs of tissue loss can have apparently diverse etiologies, including bacteria, non-bacterial-associated apoptosis, and macropredators. Refined protocols that trap the receding tissue margin of diseased corals and stain the tissue for bacteria and mucus have resulted in a clearer visual image of white plague, at least as it affects Caribbean corals of the genus *Montastraea*, but have obfuscated the etiology. Corals having signs matching the current field diagnosis of "white syndrome" require the application of histopathological and microbiological methods to distinguish the etiologic agents responsible for rapid tissue loss and to help explain mechanisms of action.

Large-Scale Geographical Comparisons Of Bacterial Communities Associated With Corals Exhibiting Signs Consistent With White Plague Type II

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The objectives of this project focus on fundamental questions pertaining to a possible white-disease syndrome affecting hard corals on reefs throughout the greater Caribbean region. Ultimately, this investigation aims to establish the cause of this disease by characterizing the microflora associated with diseased and apparently healthy coral tissue spanning the reported geographic range of white plague type II: the North Atlantic Ocean (Bermuda), the Tropical Western Atlantic (Lee Stocking Island, The Bahamas, the Dry Tortugas, and the Florida Keys), the Caribbean Sea (Little Cayman and St. Croix, USVI), and the Gulf of Mexico (the Flower Garden Banks National Marine Sanctuary). Samples of coral tissue, skeleton, and mucus were collected from colonies of the *Montastraea annularis* species complex exhibiting signs consistent with white plague type II. Using culture dependent and culture independent techniques, the bacterial communities associated with diseased and apparently healthy coral tissue and skeleton were characterized and quantified. Preliminary results suggest that microflora associated with diseased and apparently healthy tissues are not dominated by a single organism and differ on both small and large spatial scales.

Molecular Genetic Screening For Putative Antimicrobial Genes In Anthozoans: A Possible First-Line Defense Against Coral Disease Pathogen

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Coral reefs worldwide are undergoing a significant decline in species abundance and changes in community structure. Coral disease is now thought to be a major cause of reef decline and epizootics have been reported for several coral species. To date, 18 coral diseases affecting at least 150 anthozoan and hydrozoan species have been described from the Caribbean and the Indo-Pacific. Antimicrobial peptides and pore-forming toxins are widespread in nature and are synthesized by microorganisms as well as by multi-cellular organisms. These naturally occurring agents form a first-line of host defense against pathogens and are involved in innate immunity. Preliminary results of genetic screening by PCR and sequencing of putative genes in antimicrobial agents will be presented for corals and sea anemones. Conserved sequence motifs in these genes in anthozoans will be compared to those same genes that have been characterized in other organisms. Target genes to be screened in this study include antimicrobial peptides (metalloproteinases, cystine and serine proteases) and pore-forming toxins (equinatoxin, hydralysin, neuroysin). This study will better our understanding of disease mechanisms and to assess the status coral health.

Correlation Between API-ZYM And 16S rRNA-RFLP Profiles Of Ontario *Flavobacterium psychrophilum* Isolates

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Two genetic lineages (I and II) of *F. psychrophilum* have been described. The goal of the present study was to identify these lineages in 75 isolates *of F. psychrophilum* that were collected from farmed salmonids with coldwater disease in Ontario, Canada. The Ontario isolates were morphologically and serologically homogeneous but two distinct biovars were identified by API-ZYM. A 194 bp PCR product was generated from all Ontario isolates and was digested individually with MaeIII or with MnlI. PCR-RFLP analysis demonstrated four restriction patterns with a correlation between biovar I and digestion with MaeIII (lineage II), and between biovar II and digestion with MnlI (lineage I). MnlI digestion produced two fragments of 105 and 62 bp in 16 of 33 isolates belonging to biovar II, but digested only two of the PCR products from biovar I isolates. Digestion with MaeIII gave rise to fragments of 128 and 64 bp in 40 of 45 isolates belonging to biovar I whereas only one biovar II isolate had a PCR product that was digested with MaeIII. One isolate of biovar I was cut by both, and two isolates of biovar I and 16 isolates of biovar II was cut by neither, of the restriction enzymes. Sequence analysis of the 194 bp ribosomal RNA fragment led to the conclusion that *F. psychrophilum* isolates from Ontario are composed of at least six different PCR-RFLP clusters indicating the existence of polymorphism among the isolates.

Cold-Inducible Proteins Of *Flavobacterium psychrophilum,* The Cause Of Coldwater Disease

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Coldwater disease is a worldwide problem in temperate freshwater aquaculture and is caused by the Gram-negative bacterium *Flavobacterium psychrophilum.* There are numerous manifestations of this organism including tail rot/peduncle disease, necrotic myositis, osteochondritis/scleritis and rainbow trout fry syndrome. As the name 'coldwater disease' suggests, low water temperatures are characteristic of all of the presentations. Our hypothesis is that there will be differential expression of proteins from a given strain of *F. psychrophilum* grown at 8°C vs. 18°C and that these proteins may be associated with virulence. 2D-PAGE is used to examine spot-pattern differences of two strains (ATCC and Ontario) of *F. psychrophilum*. The spots of greatest interest are those that are only present at 8°C or those that are more prominent at 8°C vs. 18°C. So far the conditions for 2D-PAGE of *F. psychrophilum* have been established and the identification of spot differences is being evaluated using SYPRO- and Coomassie-stained gels and densitometric quantification. Spots will be manually excised from gels for analysis by mass spectroscopy (MALDI-tof) and MS/MS (tandem mass spec.) for internal amino acid sequence.

A Prospective Case-Control Study Of Bacterial Gill Disease Outbreaks In Ontario, Canada Government Salmonid Hatcheries

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Bacterial gill disease (BGD) is an important concern in freshwater aquaculture. It has been a persistent problem at Ontario Ministry of Natural Resources (OMNR) salmonid hatcheries and the Alma Aquaculture Research Station (AARS), where outbreaks have been associated with rapid and high mortality levels. The causative agent of BGD, *Flavobacterium branchiophilum*, is considered ubiquitous in fresh water; therefore, outbreaks are thought to be precipitated by environmental conditions favoring opportunistic infections. Despite the importance of BGD, little epidemiological research has been conducted to examine risk factors for this disease. This paper presents a 14-month (July, 2002 – September, 2003) prospective case-control investigation at five OMNR hatcheries and the AARS, to identify and quantify important risk factors for BGD outbreaks. Daily data for all early-rearing (<9 months of age) units were collected, and all outbreaks of BGD were confirmed at the Fish Health Laboratory (University of Guelph). Control tanks were matched to individual case tanks based on time, hatchery, and species, and the case-control data were analyzed using multivariable logistic regression modeling. Results of the final model indicated that tanks with confirmed BGD outbreaks were significantly more likely to have lower fish numbers, lower individual fish weights, higher mortality levels and higher feeding rates during the week preceding observed BGD outbreaks than were asymptomatic control tanks. Refinements in the observation and manipulation of these factors can therefore aid in the prevention of fish losses associated with BGD outbreak mortality spikes.

Unique Mycobacterial Resistance And Clearance In Channel Catfish (*Ictalurus punctatus)*

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A novel model for studying *Mycobacteruim marinum* was compared with an existing fish model to help expand the knowledge base of the fish immune function to this bacterial pathogen. Juvenile channel catfish (*Ictalurus punctatus)* and hybrid striped bass *(Morone saxatilis x M. chrysops)* were injected I.M. with *M. marinum* at 3.84 X 10^3 colony forming units per gram of fish weight. Splenic bacterial counts, macrophage assays (i.e. respiratory burst and phagocytosis), histopathology, clinical signs and mortality were observed over an 84 day period. Significant differences were seen between the two species with hybrid striped bass exhibiting progressive classical signs of pathology and disease. In contrast, channel catfish developed minimal pathology at the injection site and no internal organ lesions, based gross observations and on histopathology. Splenic bacterial counts in the channel catfish remained low through Day 21 and were completely absent of *M. marinum* growth after Day 35. There are no reports of mycobacteriosis in channel catfish, and no other fish species has been reported to spontaneously clear a mycobacterium infection. Due to the unique nature of the complete clearance of bacteria from splenic counts in channel catfish, in conjunction with the occurrence of minimal histopathology, the channel catfish offers a new model to study *M. marinum*.

DNA Fingerprinting of *Edwardsiella ictaluri* Using Pulsed Field Gel Electrophoresis And Repetitive Sequence-PCR

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As a result of the current *Edwardsiella ictaluri* genome sequencing project, we have discovered the presence of numerous mobile elements. The sheer number of these mobile elements has led us to investigate how these strains have changed molecularly, if at all. An epidemiological analysis of archived *E. ictaluri* isolates was performed. A total of 88 isolates of *E. ictaluri* were analyzed by repetitive sequence PCR using BOX and GTG-5 primers, and 20 of these strains were subsequently analyzed by restriction enzyme digest with *Pme*I and *Spe*I followed by separation by pulsed field gel electrophoresis. These strains were isolated between 1978 through 2006 from Mississippi, Louisiana, Alabama, Arkansas, Georgia, and South Carolina.

Pseudokidney Disease In Captive Salmonine Broodstocks

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Carnobacterium maltaromaticum, the etiological agent of pseudokidney disease, has recently been isolated from captive lake trout (*Salvelinus namaycush*), rainbow trout (*Oncorhynchus mykiss*), and brown trout (*Salmo trutta*) brood-stock experiencing elevated mortality rates in Michigan. All isolates were Gram-positive, nonmotile, facultatively anaerobic, asporogenous rods arranged in palisades. They did not produce catalase, cytochrome oxidase, or H_2S, and did not reduce nitrate to nitrite. Isolates hydrolyzed esculin, produced arginine dihydrolase, produced acid from inulin and mannitol, and grew on both Tryptic Soy Agar and Cresol Red Thallium Acetate Sucrose Inulin Agar, a selective and differential medium for *Carnobacterium* spp. External clinical signs observed in infected individuals include ulceration, melanosis, varying degrees of exophthalmia, and ocular hemorrhage. Internal signs included pseudo-membrane formation, hepatic and renal hyperemia, splenomegaly, nodule formation within the kidney, and the presence of a transparent mucoid exudate within the lumen of the swim-bladder. Sites from which this bacterium was recovered include the liver, spleen, kidney, swim-bladder exudate, and external ulcerations. Molecular characterization and phylogenetic analyses of the isolates using 16S and 23S rRNA regions specific to *C. maltaromaticum* will be presented. The impact of this infection on broodstock will be discussed.

Molecular Cloning, Expression And Genome Organization Of Channel Catfish (*Ictalurus punctatus*) Matrix Metalloproteinase-9

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In the course of studying pathogenesis of enteric septicemia of catfish, we noted that channel catfish matrix metalloproteinase-9 (MMP-9) gene was up-regulated after *Edwardsiella ictaluri* infection. In this study, we cloned, sequenced and characterized MMP-9 gene. The sequence of the CC MMP-9 cDNA gene consisted of 2562 nucleotides that potentially encode a 686 amino acid peptide with a calculated molecular mass (without glycosylation) of approximate 77.4 kDa. In addition, analyzed by the SiganlIP 3.0 program, the CC MMP-9 included a signal peptide with a cleavage site at positions 20 and 21 (AWS-HP). CC MMP-9 analyzed by the NetOGlyc 3.1 program had potentially heavy *O*-glycosylation sites between positions 447 and 490. CC MMP-9 consisted of many structural domains, including a signal sequence, matrix metalloprotease domain, matrixin, fibronectin type II domain 1, fibronectin type II domain 2, fibronectin type II domain 3 and hemopexin-like repeats. In addition, the pair-wise comparison of amino acid sequences showed that the MMP-9 was highly conserved among fish species (70% - 74%), but not in mammalian MMP-9 (e.g. 55% with human MMP-9). The complete sequence of the MMP-9 genomic DNA consisted of 5663 nucleotides. Analysis of the sequence revealed that channel catfish MMP-9 contains 13 exons (#1 - #13). The numbers of base pairs of each exon are as follows (exon 1 – exon 13): 160, 236, 151, 130, 177, 174, 177, 159, 236, 142, 155, 107 and 108, respectively. These results will provide information for further exploration of the role of MMP-9 in early stage of infection.

Viral Hemorrhagic Septicemia Virus (VHSV) Type IV′b′ Experimental Infection In Ontario Rainbow Trout

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The purpose of this study was to determine the effect of VHSV IV 'b' (freshwater drum isolate) on an outbred strain of Ontario rainbow trout used by commercial aquaculture operations to stock freshwater netpens in Georgian Bay. Fish were held for five months before the experiment and were determined to be healthy (VHSV-free) by virus isolation, tissue RT-PCR, bacteriology and histopathology. Treatment groups used (triplicate tanks of 40, ~10-12g fish) were intraperitoneal (i.p.) injection and three graded waterborne dosages that received $TCID_{50}$ titres of $10^{7.5}$/0.1ml, $10^{8.5}$/ml, $10^{6.5}$/ml, and $10^{4.5}$/ml, respectively; with three tanks of control fish (media). Fish exposed in the water were held in 2L of aerated water for two hours before being returned to the tanks. Fish were housed in 60L tanks with flow through water at 12°C and three fish per tank were sampled at weekly intervals. We evaluated morbidity (behavior indices), mortality, histopathology and immunohistochemistry for VHSV, RT-PCR on tissues, and virus isolation on EPC cells. As early as seven days post infection, the virus was isolated from trout in the i.p. and the highest waterborne dosage treatment groups. The infection trial is presently still in progress.

Viral Hemorrhagic Septicemia In New York State

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In May 2006 mortality events of Round Gobies (*Neogobius melanostomus*) occurred in the St. Lawrence River and Lake Ontario. Investigation revealed the isolation of Viral Hemorrhagic Septicemia virus of the IVb genotype which has previously been described in Muskellunge in Lake St. Clair. A survey of multiple bodies of water across New York State was performed using inoculations on cell culture and a newly developed quantitative PCR technique. The results indicate the presence of Viral Hemorrhagic Septicemia in a wide range of species in all the Great Lakes waters of New York State and one inland location, Lake Conesus.

Emergence And Spread Of VHSV In Michigan

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Viral hemorrhagic septicemia virus (VHSV) was isolated from muskellunge (*Esox masquinongy)* caught from the northwest portion of Lake St. Clair, Michigan from 2002-2005. Affected fish exhibited congestion of internal organs associated with hemorrhages in the inner wall of the swim bladder. From mortality episodes affecting Lake St. Clair gizzard shad and muskellunge in the spring of 2006, VHSV was retrieved in high titers from these two fish species. Histopathology and electron microscopy provided evidence that VHSV has caused such mortalities. VHSV was also isolated from other fish species (northern pike, freshwater drum, rock bass, shortnose redhorse and silver redhorse suckers) not experiencing mortalities, yet exhibiting clinical signs. The identity of all isolates was confirmed to be the North American of VHSV by RT-PCR and gene sequencing. VHSV seem to have spread to Lake Huron and caused mortalities in walleyes and lake whitefish. Molecular studies performed on isolates determined they are members of the North American genotype of VHSV; however, they are sufficiently distinct to be considered a separate sub-lineage. The impact of VHSV on Lake St. Clair and the Great Lakes fish stocks remains to be urgently determined.

Federal Regulatory Issues Regarding Viral Hemorrhagic Septicemia (VHS)

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A Federal Order (originally effective October 24, 2006 and amended Nov 14, 2006) issued by USDA APHIS stipulated the conditions under which specified VHS-susceptible live fish may be imported into the United States from two Canadian provinces (Ontario and Quebec) or be moved interstate from the eight states bordering the Great Lakes. The Federal Order was not intended to remain in effect on a long-term basis, especially as the epizootiology of VHS in Canada and the United States is still only partially understood. New regulations in an interim rule would establish requirements for importation and interstate movement of live VHS-susceptible fish from affected areas. This presentation is intended to provide an update of the current status of Federal regulatory issues regarding VHS.

Molecular Correlates Of Infectious Salmon Anemia Virus Virulence

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Infectious salmon anemia virus (ISAV) is classified in the family *Orthomyxoviridae*, genus *Isavirus*. Although virulence variation of ISAV can be demonstrated experimentally in fish, virus strain identification is ambiguous because the correlates of pathogenicity and/or antigenicity of ISAV are not well defined. Thirteen ISAV strains isolated from different locations and characterized for ability to kill fish were used to search for markers of virulence. Genetic analyses revealed two genotypes of ISAV, North American and European. The European genotype was further separated into two genogroups, real-European and European-in-North America. Deletion/insertion of ≤ 13 amino acids and the presence of two specific motifs in the HE protein, in combination with a specific motif very close to the trypsin-cleavage site of the F protein were correlated with reduced cytopathogenicity and reduced virulence for Atlantic salmon. A novel phylogenetic software program, BACKTRACK, we wrote estimated that the North American and European genotypes diverged between 1879 and 1891, whereas the European-in-North America genogroup diverged from the real-European genogroup between 1976 and 1988.

Diagnostic Methodologies For Detection of Beta-Nodavirus Infections Of Atlantic Cod (*Gadus morhua*)

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Current methodologies for detection of Betanodavirus in marine fin-fish included virus culture in SSN-1 or E-11 cells and confirmation by RT-PCR using a single primer set designed to amplify the T4 variable region (427 bases) of *Striped Jack Nervous Necrosis Virus* (SJNNV) coat protein gene. *Atlantic Cod Nervous Necrosis Virus* (ACNNV) has routinely been isolated and identified using similar techniques (SSN-1 cells and primers specific to the same region in ACNNV). We undertook a study to compare the efficiency of virus isolation between SSN-1 and E-11 cell lines. In addition, we compared the use of Real-time PCR with RT-PCR technologies using a new primer set constructed from a conserved region of the coat protein gene of all 4 existing Betanodavirus clads. Our results indicated that the use of E-11 cells provided a minimum of 24 hr. time advantage over the SSN-1 cells for virus isolation. Similarly, we confirmed that the Real-time PCR increased sensitivity of detection by 1 log in titration assays with these new primers. We propose an improved protocol for isolation and detection of ACNNV which utilizes E-11 cells and Real-time PCR which targets a conserved coat protein primer set. Supporting data is presented.

Tadpole Edema Virus: Comparative Susceptibility Among Early Life Stages of the Fowler's Toad (*Bufo fowleri*)

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Tadpole edema virus (TEV) is one of a group of ranaviruses pathogenic to amphibians. Ranaviruses have been isolated from both non-diseased, and diseased or deceased amphibians. Factors that influence virulence, host susceptibility and pathogenesis are not well described. Here, we investigate the comparative virulence of TEV among aquatic life stages of the Fowler's toad, a common anuran species, from hatch to metamorphosis. A single mass of fertilized Fowler's toad eggs was field collected, and the hatched tadpoles reared under laboratory conditions. Replicate groups of untreated control eggs/tadpoles or groups challenged with TEV (aqueous exposures of 10^5 $TCID_{50}$/ml for 24h) were maintained for 14 day trials for the following approximated life stages: Gosner stage 20 (hatchlings), Gosner 25 (mouth parts developed), Gosner 36 (rear limbs developing), and Gosner 42 (metamorphosis). Mortality was recorded daily, and dead tadpoles and representative survivors were evaluated for presence and titer of virus using a quantal viral infectivity assay to determine 50% endpoint in cell culture. Mortality levels were negligible for all control groups, and no virus was detected among any control groups or the pre-screened eggs and embryos. Among the TEV-challenged groups, there was variability in mortality and associated detection of virus among the 4 life stages. High mortality associated with TEV was observed among the earliest and latest life stages (Gosner 20 and Gosner 42) while lower mortality levels were observed from the intermediate aquatic life stages examined. Viral titers among exposed survivors and tadpoles dying with TEV ranged from 10^3 to 10^6 $TCID_{50}$/g. Susceptibility appeared greatest during the periods of heightened physiological stress near hatch and metamorphosis.

Pathogens Associated With Native And Exotic Trout Populations In Shenandoah National Park And The Relationships To Fish Stocking Practices

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Threats to native fish populations from practices such as fish stocking and from invasive and non-native species and their associated disease concerns are important natural resource management issues in National Parks. Prohibitive or restrictive fish stocking policies in National Parks were developed as early as 1936 in order to preserve native fish assemblages and genetic diversity. Despite recent efforts to understand the effects of non-native or exotic fish introductions, park managers have limited information regarding the effects of these introductions on native fish communities. Shenandoah National Park (SHEN) was established in 1936 and brook trout restoration within selected streams in the park began in 1937 in collaboration with the Virginia Department of Game and Inland Fisheries (VDGIF). The only known records associated with park streams stocked during the 1930s were in the survey reports completed by the US Fish and Wildlife Service in Leetown, West Virginia. These reports suggest that most accessible streams were annually stocked by the Commonwealth of Virginia with hatchery reared fingerlings and catchable size trout through 1949. This practice was continued by the US Fish and Wildlife Service through 1955. We know that all fish stocked within Shenandoah National Park originated from a limited number of hatcheries including the former Fish Culture Station at what is now the Leetown Science Center, the Erwin National Fish Hatchery in Erwin, Tennessee and the Montebello Fish Cultural Station in Nelson, Virginia operated by the VDGIF. A preliminary sampling of brook, brown and rainbow trout from 28 streams within SHEN from 1998-2002 revealed the presence of several pathogens. These pathogens included *Renibacterium salmoninarum*, *Yersinia ruckeri*, and infectious pancreatic necrosis virus (IPNv). In order to investigate the relationships of the occurrence of fish pathogens with stocking histories we classified the streams in three categories: 1) streams within the park with no known stocking records, 2) streams within the park that were historically stocked, and 3) streams within the park that were historically stocked and presently stocked downstream of the park boundary by the VDGIF. The occurrences of the three pathogens above were summarized relative to this stocking history. In general, we found an increase in the occurrence of pathogens in brook, brown and rainbow trout associated with stocked streams.

Perspectives For Trout Management And Fish Health In The Batten Kill

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The Batten Kill stands out as one of Vermont's most famous trout streams. Between 1994 and 1996, wild brown trout abundance (≥ 6 inches) declined precipitously despite continued evidence of abundant reproduction. During this same period, wild brook trout populations remained relatively stable. In 2002, both brook and brown trout were found positive for the causative agent of Whirling Disease (*Myxobolus cerebralis*). This was the first documented occurrence of the pathogen in Vermont waters. In 2000, the Vermont Fish & Wildlife Department (VTFWD) established an inter-agency Batten Kill Study Team charged with investigating a list of likely causes for the brown trout decline. Of all the possible causes for the brown trout population decline that were investigated over the next six years, the most compelling finding was that fish cover in the river is inadequate. Nonetheless, the existence of WD in the Batten Kill has potential implications for its resident trout populations but more broadly is a risk to other trout waters in the region. Therefore, the VTFWD is committed to monitoring for fish pathogens, including WD in the Batten Kill as well as other state waters. Additionally, the VTFWD continues efforts to increase public awareness of the issue, and inform user groups of their role in curbing the potential spread of pathogens to other waters.

CSI Shenandoah: The Importance Of Understanding Fish Health In A Riverine Fish Kill Investigation

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Between 2004 and 2006 unexplained fish kills occurred throughout the Shenandoah River in Virginia. These mortality events involved only the adults of mainly three species *Micropterus dolomieu*, *Lepomis auritus* and *Hypentelium nigricans*. Small numbers of other species were also affected. In some cases an estimated 80% of the adult *Micropterus dolomieu* and *Lepomis auritus* perished in long (>50 km) reaches of the river. The kills involving *Micropterus dolomieu* and *Lepomis auritus* occurred in the early spring and *Hypentelium nigricans* mortality took place in late May and again in early December. Some acute mortality was associated with the beginnings of these kill events, and these fish showed no outward signs of stress. However, the bulk of the mortality was chronic with fish exhibiting bacterial lesions, eroded fin rays, excessive surface mucus production, and fungal infections. Fish kill episodes lasted from several days to three months. Diagnosis by fish pathologists indicated that the observed symptoms were "secondary effects" and not the origin of stress. To date the cause of these fish kills is unknown. Suspect causes being investigated include: environmental stressors, pathogens, contaminants, and immune system suppression. Much of the data collected thus far points to multiple stressors leading to mortality. Researchers with the Shenandoah River Fish Kill Task Force and the Environmental Protection Agency's Casual Analysis/Diagnosis Decision Information (CADDIS) team have placed a high priority on fish health information in determining the cause(s) of fish mortality. Histopathology, virology, bacteriology, and parasitology of fish have been conducted and analysis is pending. Investigators hope to use fish health diagnostics to solve the mystery of fish stress and mortality in the Shenandoah River.

Bio-Indicators Of Estuarine Health: A Multivariate Approach Linking Source To Resource

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There is a recognized need among management entities for descriptive indices that simply and concisely convey ecosystem health. However, individual indicators are often inadequate for estuarine systems because of the inherent variability in these environments and the inability to infer causality from observed responses. These issues may become more apparent as assessments are performed over broader spatial scales. The NOAA/NOS Oxford Lab, in cooperation with federal, state, and academic partners, is implementing an integrated biotic ecosystem assessment on a sub-watershed (14-digit Hydrologic Unit Code) scale in Chesapeake Bay. Sub-watersheds were chosen based on statistical analysis of land use patterns to represent a gradient from developed to agricultural. A random stratified design was developed and sampling approaches coordinated within this structure to allow for robust system comparisons. The approach is hierarchal, with metrics chosen to represent a range from community to cellular level responses across multiple organisms. Particular attention is focused on the use of pathobiology as a tool for assessing environmental condition. By integrating the biotic component with water quality, sediment indices, and land-use information, a holistic evaluation of ecosystem health will provide management entities with information needed to inform local decision-making processes and establish benchmarks for future restoration efforts.

Evaluating Population Health Of Endangered Fishes By Non-lethal Analysis Of Microbial Diversity

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When a bacterium initiates infection and disease, and is eventually cleared from an affected population, it influences the normal bacterial flora on external surfaces of individual fish. Understanding how microbial diversity is affected by the presence, replication and clearance of a pathogen can provide an index of population health. Such analysis is non-lethal, which is necessary when working with an endangered species. To further understand pathogen and normal flora interactions, Lost River (*Deltistes luxatus*) and Shortnose (*Chasmistes brevirostris*) suckers were studied in the Upper Klamath Lake (OR). Widespread habitat destruction and water quality degradation have caused drastic declines in both species, which were listed as endangered in 1988 (53 FR 27130 27134). Occurrence of diseases within the lake, especially Columnaris Disease, threatens population recoveries. Analyses of the microbial flora affecting these fishes become important metrics to monitor population health and environmental quality, which are vital to species' recovery plans. In this study, gills and skin of Lost River and Shortnose suckers were evaluated from spawning adults (4/06), juveniles (9/06), and in adults prior to winter (10/06). The microbial flora from blue chubs (*Gila* coerulea) was also compared to adult suckers (8/06) to determine if the former species could serve as a surrogate model. Results were based upon 49,421 total bacterial identifications. Neither sucker species was significantly impacted by bacterial infections in 2006; - no bacterial epizootics were reported to this laboratory nor were any obligate pathogens isolated. *Flavobacterium columnare,* cause of Columnaris Disease, was not isolated from any sucker and was isolated only from the gills of one chub. Although there were instances where the incidences of facultative pathogens (mostly motile aeromonads) increased among adult suckers, they rarely supplanted the normal hierarchical order of the normal flora and were not deemed to severely impact population health. There were strong hierarchical shifts among juvenile fish (9/06) and adults obtained during August in which motile aeromonads actually displaced the normal flora of the suckers. When combined with certain environmental stressors, such shifts could potentially impact survival. Finally, the microbial flora associated with blue chubs differed from those of the suckers; therefore, these fish were not appropriate surrogates for the suckers.