Program of the:

33rd Annual
Eastern Fish Health Workshop

31 March – 4 April 2008
Sheraton Oceanfront Hotel
Atlantic Beach, North Carolina

Sponsored by:

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

Editor's Note

On behalf of the National Fish Health Research Laboratory, welcome to Pine Knoll Shores along the scenic Crystal Coast of Atlantic Beach, North Carolina. From the sands of the beach to the quaint streets of Beaufort, we hope that you enjoy the beauty and heritage of the Southern Outer Banks. The surrounding area was originally inhabited by the Tuscarora Indians and settled, during the early 1700's, by Huguenot, German, Scotch-Irish, French, and English immigrants mostly from northern American colonies. Area plantations produced tobacco, grains, salted meats, and fish that were shipped to England from the county seat at Beaufort. During the 18th and 19th centuries, the region around Beaufort was vulnerable to attack as "Blackbeard" and other infamous pirates plagued the seas and frequented the local areas. Wars with Spain, France and Great Britain provided further threat of coastal raids by enemy warships. When the War of 1812 prompted the federal government to organize a national network of coastal fortifications, Fort Macon (to our east) was constructed to guard Beaufort Inlet and Beaufort Harbor. During your stay at the Workshop, find time to explore the Beaufort State Historic Site, which portrays colonial life when Beaufort was the largest seaport in the Carolina colony. Visit Fort Macon State Park, which bears witness to America's attempts to protect her seacoasts and interprets this region's struggles during America's Civil War. Set sail to the North Carolina Maritime Museum, which pays tribute to the area's many bonds to the sea; -including the infamous Blackbeard, himself. Walk below the ancient oaks and over the sandy paths that wind between the weathered tombstones of the Old Burying Ground where early coastal residents were laid to rest facing eastwards; - "to be facing the sun when they arose on judgment morn." Join us when we visit and dine at the newly renovated North Carolina Aquarium where you can journey across the waters of North Carolina from its mountains to the sea.

In the shadow of this beautiful setting, we are proud to convene the 33rd Annual Eastern Fish Health Workshop. The very first workshop traces its roots to a small group of federal researchers, who gathered together on the Eastern Shore of Maryland to share their research, discuss issues, plan new studies, and enjoy the fellowship of each other. That first meeting was originally inspired by Dr. Stanislaus Snieszko of the Eastern Fish Disease Laboratory (precursor to the National Fish Health Research Laboratory) of the Department of the Interior and Drs. Carl Sinderman and Aaron Rosenfeld of the National Oceanic and Atmospheric Administration Laboratory of the Department of Commerce at Oxford, Maryland. Both laboratories were somewhat close geographically and both researched problems associated with fish diseases, but their emphasis differed between the freshwater and marine environments. Early workshops fostered communication between the two facilities and provided staff members with opportunities to plan and conduct collaborative studies. Within a few years, fish disease research became well established in the graduate programs of several major universities in the eastern United States and stimulated broader participation. Although the title of the workshop appears to be exclusive for fish, the workshop encourages broad participation in all aspects of aquatic animal health from invertebrates to mammals. Contributions from researchers, resource managers, biologists, aquarists, and university personnel working within both marine and freshwater environments are encouraged. Even though the Workshop has occasionally been held in conjunction with other organizations (e.g. - the Fish Health Section of the American Fisheries Society and the International Association of Aquatic Animal Medicine), it is not affiliated with any parent society. The meeting is an entity unto itself. Today, the National Fish Health Research Laboratory is extremely proud to continue the tradition of the Eastern Fish Health Workshop and serve as its annual host. The staff of the National Fish Health Research Laboratory welcomes you to the 33rd Annual Eastern Fish Health Workshop here at the Oceanfront Sheraton Inn. We hope that you will find the sessions interesting, informative, and productive. We also hope that you will have time to explore the sites, sounds, legends and lore that abound on the Crystal Coast!

Rocco C. Cipriano,
Editor

Monday , 31 March

Evening Session

5:00 – 8:30 pm **Registration And Reception**

Cash bar with house spirits, domestic beers, wine by the glass
Island Fruit, Cheese and Vegetable display

8:00 **Welcome to the 33rd Annual Eastern Fish Health Workshop**

Frank Panek, Scientific Director
National Fish Health Research Laboratory

8:15 The Aquatic Detective: Unusual Case Studies - John Lumsden, moderator

Tuesday, 1 April

7:00 **Breakfast**

Assorted fruit, chilled juice, country scrambled eggs, bacon strips and link sausage, hot buttered grits, breakfast potatoes and saw mill gravy, assorted breakfast breads, cold cereals, coffee, tea, milk

Special Session 1: **Crustacean Health – Gretchen Messick, moderator**

9:45	**Discussion**		
10:00	**Morning Break**		

General Session 1 **Al Dove, moderator**

10:30	Miller	Culture Transmission Studies Of *Hematodinium* sp. From *Callinectes sapidus*, A Key To Understanding This Enigmatic Life Cycle	17
10:45	Sokolowski	Preliminary Histological And Serum Chemistry Analysis Of Atlantic Sturgeon Infected With The Copepod *Dichelesthium oblongum*	18
11:00	Yost	Pathology Associated With Bolbophorus damnificus In Channel Catfish Fingerlings	19
11:15	Dove	Infestations Of Demersal Elasmobranchs In A Large Aquarium By The Leech, *Branchellion torpedinis*	20
11:30	Jørgensen	Immune Relevant Genes Expressed In Rainbow Trout After Immunization With A Live Vaccine Against *Ichthyophthirius multifiliis*	21
11:45	Bricknell	Monitoring The Infective Pressure Of *Lepeophtheirus salmonis* (Krøyer 1837) On Wild Salmonid Populations In Scotland	22

12:00 **Lunch** *Chicken Roma*

General Session 2: **Mohamed Faisal, moderator**

1:15	Lumsden	Chlamydia-Like Organism In Ontario Lake Trout	23
1:30	O'Brien	A Nodavirus Outbreak In Atlantic Cod: Effects Of Vaccine, Temperature And Dissolved Oxygen	24
1:45	Bebak	Continuous Exposure To Infectious Pancreatic Necrosis Virus (IPNV) During Early Life Stages Of Rainbow Trout	25
2:00	Getchell	Stability And Abundance Of VHSV In Great Lakes Diagnostic Submissions	26
2:15	Kurath	Development Of A System For Assessing Fish Virus Competition And Fitness in Rainbow Trout	27
2:30	Hanson	Going BAC And Forth To Make Channel Catfish Virus Recombinants	28
2:45	Warg	Orphan Viruses Detected During Fish Health Certification	29

3:00 **Afternoon Break**

General Session 3: **Jeffrey Wolf, moderator**

3:30	Rolland	The Current Status Of The U.S. National Aquatic Animal Health Plan	30
3:45	Jeon	Cloning And Characterization Of Phospholipase D From Olive Flounder	31
4:00	May	Introduction To The North Carolina Aquarium: Pine Knoll Shores	

4:30 **Adjourn**

5:00 **North Carolina Aquarium At Pine Knoll Shores**

6:30 ***Picken's Fest Dinner at the Aquarium:*** *includes chopped pork, fried chicken, fish, and shrimp, potato salad, cole slaw, baked beans, hushpuppies, tea and lemonade*

Wednesday, 2 April

7:00 **Breakfast**

Assorted fruit, chilled juice, country scrambled eggs, bacon strips and link sausage, hot buttered grits, breakfast potatoes and saw mill gravy, assorted breakfast breads, cold cereals, coffee, tea, milk

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Special Session 2: **Mollusks And Their Critters – Roxanna Smolowitz, moderator**

8:00	Walsh	How To Give a Clam a Check-Up: Designing Sensible Health Surveillance Programs In The Bivalve Realm	32
8:15	Sheppard	Exotic *Perkinsus* spp. Organisms In Ornamental Vietnamese Tridacnid Clams Imported Into The United States	33
8:30	Petty	Voluntary Quarantine Program To Establish *Perkinsus*-Free Stocks Of Imported *Tridacna* Clams	34
8:45	Smolowitz	Recent Knowledge Concerning QPX Infections In Hard Clams	35
9:00	Lyons	An Epizootiological Approach To The Study Of Marine Diseases	36
9:15	Peters	Comparative Histopathology Of *Mya arenaria* And *Tagelus plebeius* Clams In The Chesapeake Bay	37
9:30	Salger	Tissue Patterns Of A Matrix Metalloproteinase In Infected Eastern Oysters	38
9:45	Barord	Treatment Of Negative Buoyancy In A Captive Chambered Nautilus	39

10:00 **Morning Break**

Special Session 3 : **Emergent Organisms – Roy Yanong, moderator**

10:30	Yanong	Iridoviral Infections In Aquarium Fish	40
10:45	Waltzek	Molecular Phylogenetic Assessment Of A Systemic Iridovirus From The Banggai Cardinalfish	41
11:00	Mauel	Franciselliosis, One Of The New Kids On The Block	42
11:15	Soto	Highly Pathogenic *Francisella* sp. Causing Mortality In Farmed Tilapia Nilotica in Costa Rica	43
11:30	Hawke	Diagnosis And Management Of Streptococcosis In Intensive Aquaculture	44
11:45	Smith, S.	Pet, Ornamental, And Food Fish Zoonoses	45

12:00 **Lunch** *Pasta primavera*

General Session 4: **Paul Bowser, moderator**

1:15	Kapareiko	Effects Of A Shellfish-Larval Pathogen And A Prospective Probiotic Bacterium On Hemocyte Functions Of The Oyster: *In Vitro* Effects On Mortality, Phagocytosis, Adhesion And Oxidative Burst	46
1:30	Fast	Mutation Within The *Aeromonas salmonicida* Type III Secretion System Affects Host Leukocyte Activation And Downstream Immune Responses	47
1:45	Johnson	Benefits Of Hatchery Management Of Bacterial Kidney Disease Using ELISA-Based Culling Of Adult Chinook Salmon	48
2:00	LaFrentz	Development And Characterization Of Rifampicin Resistant *Flavobacterium psychrophilum* Strains And Their Potential As Live Attenuated Vaccine Candidates	49
2:15	Loch	Erratic Swimming Behavior Followed By Mortalities In Coho Salmon Fry Due To Infection By A Novel *Flavobacterium* sp.	50
2:30	Menanteau	Use Of Green Fluorescent Protein-Labeled *Edwardsiella ictaluri*	51

2:45	Williams	To Investigate The Pathogenesis Of Enteric Septicemia In Channel Catfish Characterization Of The *rrn* Operons In The Channel Catfish Pathogen *Edwardsiella ictaluri*	52

3:00 **Afternoon Break**

Special Session 4: Letting The Catfish Out Of The Bag: Updates From The South – Lester Khoo, moderator

3:30	Camus	Channel Catfish Anemia, A Review And New Insights	53
3:45	Pote	The Myxozoa: New Species And Old Species Behaving Badly	54
4:00	Wise	Impact And Treatment Of Trematodes In Catfish Culture	55
4:15	Terhune	Enteric Septicemia Of Catfish: The Commercial Catfish Industry's 'Thorn In The Flesh' For 30 Years	56
4:30	Hawke	Isolation, Culture, Susceptibility Testing, And Identification Of *Flavobacterium columnare*	57
4:45	**Discussion**		
5:00	**Adjourn**	Dinner on your own	

Thursday, 3 April

7:00 **Breakfast**

Assorted fruit, chilled juice, country scrambled eggs, bacon strips and link sausage, hot buttered grits, breakfast potatoes and saw mill gravy, assorted breakfast breads, cold cereals, coffee, tea, milk

Special Session 5: New Perspectives In Coral Disease – Garriet Smith, moderator

8:15	Cervino	The *Vibrio* Core Group Induces Yellow Band Disease In Caribbean And Indo-Pacific Reef Building Corals	58
8:30	Gochfeld	Antibacterial Chemical Defenses In Corals: Widespread But Selective Resistance To Bacterial Pathogens	59
8:45	Galloway	Antimicrobial Activity In The Common Seawhip	60
9:00	Kilgore	Preliminary Investigations Into Metabolic Profiling of Coral Surface Microbial Communities	61
9:15	Woodley	Coral Disease And Health Consortium(CDHC): Incorporating Virtual Slide Technology Into Coral Pathology	62
9:30	Smith, G.	Comparative Defense Mechanisms In *Pseudopterogorgia americana* And Other Gorgonians	63
9:45	**Discussion**		
10:00	**Morning Break**		

Special Session 6: What's Abnormal In Fish Histology And Histopathology? – Stephen Smith, moderator

10:30	Wolf	Histology And Histopathology Of The Fish Liver: Worst Case Scenario	64
10:45	Frasca	Histology And Histopathology Of The Nervous System Of Fish - This Is Your Fish's Brain On A Glass Slide	65
11:00	Terrell	Microscopic Examination Of The Cardiovascular System	66
11:15	Law	Pathology Of The Gills	67

11:30	Khoo	Piscine Kidneys - Tubular And Interstitial Response To Insults	68
11:45	Camus	When The Skin Wears Thin: An Introduction To The Histology Of Normal And Abnormal Fish Integument	69

12:00 **Lunch** *Sheraton trio salad*

Special Session 7: A Spoonful Of Finquel Helps The Medicine Go Down: Fish Medicine And Surgery – Caryn Poll, moderator

1:00	Poll	Two Shocking Cases: Anesthesia And Surgery Of The Electric Eel	70
1:15	Harms	Cryosurgery On A Premaxillary Fibrosarcoma From A Chain Pickerel Using An Over-The-Counter Wart Remover	71
1:30	Pouder	Dermal Fibroma On A Redtail Catfish	72
1:45	Berliner	Help! I've Got A Chicken Bone Stuck In My Ovisac: Considerations For Complex Ovariectomy In An Irish Lord	73
2:00	Clauss	Comprehensive Wound Care Management In A Cownose Ray, A Spadefish And A Blue Catfish	74
2:15	Hirokawa	Management Of Chytridiomycosis In *Dendrobates* At The New England Aquarium	75
2:30	Hadfield	Milbemycin Treatment Of Parasitic Copepods On *Acropora* Corals	76
2:45	Weber	The Veterinarian's Role With Aquatic Invasive Species	77

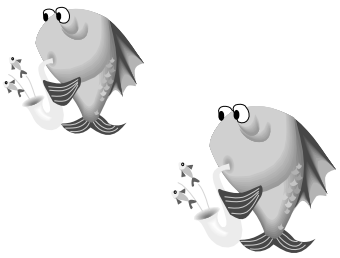
3:00 **Afternoon Break**

General Session 5: Frank Panek, moderator

3:30	Good	The Effects Of Rearing Density And Diet On Fin Condition, Intestinal Histopathology, And Fillet Quality Of Rainbow Trout	78
3:45	Allen	Fish Health Survey Of Ten Lake Winnipeg Species	79
4:00	Crosby	Disease Assessment And Mortality Of Channel Catfish Fingerlings Following Transport And Cage Stocking	80
4:15	Darwish	Laboratory Efficacy Of Oxytetracyline And Amoxicillin For The Control Of *Streptococcus iniae* Infection In Tilapia	81
4:30	Cipriano	Putative Bacterial Etiologies For Smallmouth Bass Lesions In Three Virginia Rivers During 2007	82

4:45 **Adjourn**

6:30 **33rd Annual EFHW Reception And Banquet**
6:30 *Cocktail reception (cash Bar)*
7:00 *Sheraton Beachside Luau*
8:00 **Presentation of the Best Student Paper Award**
Banquet Dance (cash bar)



11:30 **Adjourn**

Friday, 4 April

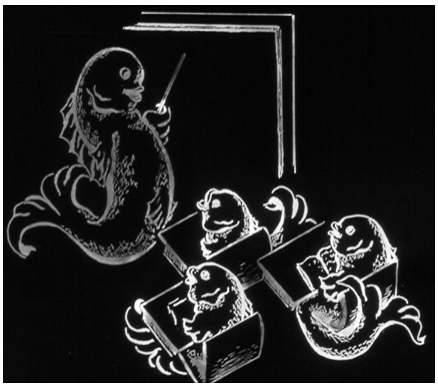
7:00 **Breakfast** (for Continuing Education Course participants)

Assorted fruit, chilled juice, country scrambled eggs, bacon strips and link sausage, hot buttered grits, breakfast potatoes and saw mill gravy, assorted breakfast breads, cold cereals, coffee, tea, milk

Continuing Education Session :

Viral Hemorrhagic Septicemia

8:00	Bowser	Introduction
8:05	Kurath	VHSV: The History And Basics Of The Virus And The Disease
8:45	Bowser	VHSV In The Great Lakes
9:15	Lumsden	The Canadian Experience
10:00	**Morning Break**	
10:30	Rolland	The USDA APHIS Perspective
11:15	Casey	Diagnostic Methodologies
12:00	**Lunch**	*Broiled flounder w/lemon and herbs*



A Mortality Event in Common Carp (*Cyprinus carpio*) in the Kawartha Lakes, Ontario

[1]Lowia Al-Hussinee, [1]Spencer K. Russell, [1]Veronique M. LePage, [1]Paul Huber, [2]Steve Lord, [2]Roselynn M.W. Stevenson and [1]John S. Lumsden

[1]Fish Pathology Laboratory, Department of Pathobiology, University of Guelph, Guelph, ON, Canada, N1G 2W1; [2]Fish Health Laboratory, Dept. of Molecular and Cellular Biology, University of Guelph

In June 2007 and continuing through July, there were die-offs of large numbers of common carp in Scugog, Sturgeon and Pigeon lakes in the Kawartha district in Ontario. This is prime cottage country and the large mortality event generated considerable public and media attention. Several groups of fish were submitted for examination. Gill necrosis was a common finding along with variable epidermal hemorrhage. Filamentous bacteria were noted in a few fish on gill wet mounts. No significant bacteria or viral agents were grown. There was marked diffuse branchial necrosis and variable hepatitis, nephritis and splenitis on light microscopy. A few fish with similar clinical signs were also submitted from Lake Ontario and the gross and light microscopic lesions were identical. Viral particles were noted on TEM of gill tissue.

An Unusual Lesion In The Gill Of A Walleye

[1]Lewis B. Bogdanovic, [1]Timothy L. Cushing, and [2]Paul R. Bowser

[1]Section of Anatomic Pathology, Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853; [2]Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

A walleye (*Sander vitreus*) captured by sport fishing was presented to the Fish Disease Diagnostic Laboratory, Cornell University, Ithaca, New York, USA for evaluation. The fish originated from Lake Erie near Dunkirk, New York (42.5^{o} N, 79.3 ^{o} W) and was captured on 14 August 2007. The sport angler noted a large mass in the region of the right gill arches. The fish was 58 cm total length and in good body condition (weight not provided by the angler). The head was presented for evaluation. Upon examination, a spherical to oval mass, approximately 5 cm X 5 cm X 4 cm was observed to be originating from the right gill arches. The mass was firm and multinodular. Upon microscopic examination, the lesion was identified as a compound odontoma. Odontomas are considered to be a relatively rare tumor in fish, with five confirmed cases in the archives of the Registry of Tumors in Lower Animals.

Circling And Cloudy Eyed Tilapia

Jennifer C. Haugland
North Carolina Veterinary Diagnostic Laboratory System, Rollins Animal Disease Diagnostic Laboratory, 1031 Mail Service Center, Raleigh, NC 27699

A Tilapia producer presented 200-300 gram fish to the diagnostic laboratory with a history of increased mortality and abnormal swimming behavior, which consisted of circling, darting, and were rested in the water column with the tails down and heads up. Approximately 10% of the fish were affected. The rate of mortality had peaked and the number of dead fish was decreasing each day. This tank of 200-300 gram fish was in an indoor recirculating system of 3 circular tanks. One of the tanks contained 49 gram fish that had been purchased and placed 3 weeks earlier. The 49 gram fish had similar clinical signs, which had started about 7 days after placement. The fish in this system had been treated orally for 6 days with oxytetracycline. At the time of submission, fish in separate system at this facility were also showing similar clinical signs. Gill snips and skin scrapings revealed only a few monogenean trematodes. Necropsy examinations revealed exophthalmia, cloudy corneas, enlarged and friable spleens, and enlarged and hemorrhagic testes. Gram stains of impression smears made from the brains, spleens, and testes revealed many gram positive cocci. Histopathology revealed encephalitis, ventriculitis, bronchitis, pericarditis, peritonitis, cholangiohepatitis, cholecystitis, pancreatitis, glomerulonephritis, submucosal edema of the stomach and intestine, and focal necrosis in the testes. Vascular thrombi were found in the gills and testes. Gram positive cocci were found in the brain, gill, liver, intestine, and adipose tissue of the peritoneum. *Streptococcus iniae* was cultured from the brain, spleen, kidney, liver, and testes.

The Case Of Quatto: The Conjoined Blue Catfish

[1]Casey Knight, [1]Jeff Terhune, [1]Karl Hayden, [1]Yolanda Brady, and [2]John Hathcock

[1]Southeastern Cooperative Fish Disease Lab, Department of Fisheries and Allied Aquacultures, 203 Swingle Hall, Auburn University, Alabama, 36849; [2]Auburn University Veterinary School, Department of Clinical Sciences, 612 Hoerlein Hall, Auburn University, Alabama, 36849

In June of 2007, a unique blue catfish (*Ictalurus furcatus)* was harvested from a commercial catfish pond at the Dean Wilson Farms, Browns, Alabama. The fish was removed from the processing line due to the severity of physical deformities that prevented further processing. The fish was submitted to the Southeastern Cooperative Fish Disease Lab, Auburn University (Case AL07-59). The fish weighed approximately 2.5 kg and physical deformities were due to a conjoined twin. X-rays, an MRI, and a necropsy were performed. Organs of the parasitic twin were rudimentary and not fully developed. Other than the grossly deformed physical attributes, reminiscent of Quatto from Total Recall, the fish was in apparent good health.

Stark Lake 'Slinky' Lake Trout (*Salvelinus namaycush*)

[1]Alex Reid, [2]George Low and [1]John Lumsden.

[1]Fish Pathology Laboratory, Department of Pathobiology, University of Guelph, Guelph, ON, Canada, N1G 2W1; [2]13 Riverview Dr. Hay River, NT X0E 0R7

Thin (slinky) Lake trout have been noted in Stark Lake and others near Great Slave Lake in the North West Territories, Canada. Several fish were collected for a variety of analyses including screening for known pathogens, parasitology, metal analysis and light microscopy. The fish pathology laboratory performed only the light microscopy portion of the study. The most marked alteration seen with light microscopy was massive red cell destruction evident as erythrophagocytosis and hemosiderin accumulation (hemosiderosis) of the spleen and kidney with the process less evident in other organs. Enlarged macrophages were evident in most tissues and were also visible within blood vessels. There was also atrophy/loss of splenic red and white pulp and renal interstitial atrophy. No organisms were associated with these lesions. A range of metazoans were also noted, some of which produced a notable tissue reaction.

”It’s Not A Tumor” In The Gill Arches Of A Bluntnose Stingray

[1]Vanessa L. Schumacher, [2]Stephen A. Bullard and [1]Salvatore Frasca Jr.

[1]Department of Pathobiology and Veterinary Science, Connecticut Veterinary Medical Diagnostic Laboratory University of Connecticut, 61 North Eagleville Rd, Storrs, CT 06269; [2]Gulf Coast Research Laboratory, University of Southern Mississippi, 703 East Beach Drive, Ocean Springs MS, 39564

A complete set of gill arches from a bluntnose stingray, *Dasyatis say*, was submitted to the Connecticut Veterinary Medical Diagnostic Laboratory for evaluation. The gill arches were severely distorted by an extensive, well demarcated, expansile, hard mass. Histopathologic evaluation revealed large, irregular foci of mineral deposition in the connective tissue of the gill arch. Smaller foci were scattered amongst the larger pieces. Often, there was fibrosis of the connective tissue surrounding the mineral deposition, and a chronic inflammatory infiltrate comprised of macrophages and lymphocytes was present. Cartilage of the gill arches was variably distorted, with foci of necrosis, mineralization and orderly proliferation, consisting of anastomosing projections along the perichondrium. Causes of abnormal tissue mineralization are typically categorized as dystrophic, metastatic or idiopathic. The underlying cause in this case is unknown; however, previous trauma was considered a strong possibility, as features of this lesion resemble those seen in calcinosis circumscripta of domestic animals. Mineralization that is locally extensive, as in this case, should be considered when evaluating lesions resembling tumors in skates and rays.

Periorbital Matrix Accumulations Of Captive Wild-Caught Scad (*Decapterus* spp.) From The Carolinas

[1]Shane M. Boylan, [2]Craig A. Harms, [3]Mike Garner, [2]J. McHugh Law, [1]Jason Cassell, and [4]Michael H. Fatzinger, [1]Sallie R. Miller

[1]South Carolina Aquarium, 100 Aquarium Wharf, Charleston SC 29414; [2]North Carolina State University, College of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, NC 27606; [3]Northwest Zoopath, 654 W. Main St. Monroe, WA 98272; [4]North Carolina Aquarium at Fort Fisher, 900 Loggerhead Road, Kure Beach, NC 28449

The North Carolina Aquarium at Fort Fisher and the South Carolina Aquarium have both experienced an unusual accumulation of a peri-orbital gel-matrix in wild collected scad (*Decapterus* spp). The condition first presents with bilateral accumulation of a clear to off-white, firm gel matrix accumulating cranial and occasionally caudal to the eye in a semi-lunar pattern. As the masses grow in size, the gel becomes abnormal in shape and covers the majority of the eye, adnexa, and operculum. Feeding and gilling are reduced which eventually leads to death. Histopathology, cytology, and transmission electron microscopy have not yet found an etiology, but the tissue appears to be a collagen secreting fibroblastic response reminiscent of a myxosarcoma. Current investigations are underway to ameliorate clinical symptoms which could allow scad species to be displayed.

Scleral Myxozoanosis In Lumpfish, *Cyclopterus lumpus*

[1,2]Akinyi Nyaoke, [1]Stephanie White-Hunt, [2,4]Julie Cavin, [2]Deana Edmunds, [2]Charles Innis, [3]E. Scott Weber, and [1]Salvatore Frasca Jr.

[1]Department of Pathobiology and Veterinary Science, Connecticut Veterinary Medical Diagnostic Laboratory, University of Connecticut, 61 North Eagleville Road, Storrs, CT 06269-3089; [2]New England Aquarium, Central Wharf, Boston, MA 02110-3399; [3]University of California Davis, School of Veterinary Medicine, Department of Medicine and Epidemiology, 2108 Tupper Hall, Davis CA; [4]Georgia Aquarium, 225 Baker Street, Atlanta GA, 30313

Lumpfish, *Cyclopterus lumpus*, are known to be susceptible to a wide variety of diseases including parasitic, fungal and bacterial infections. This case report describes lesions identified in two lumpfish from a commercial aquarium submitted to the Connecticut Veterinary Medical Diagnostic Laboratory. Both fish presented with histories of exophthalmos of one month duration affecting one or both eyes. There were multiple, white nodules along the surface of the sclera in one animal, and cytology of subconjunctival needle aspirates revealed myxozoan spores. Animals were euthanized, and necropsies performed. Tissues were fixed in 10% neutral-buffered formalin and processed routinely for embedment in paraffin and histologic sectioning. Histopathologic evaluation of the tissues revealed multiple expanded spaces that distorted the contour of the scleral cartilage. These spaces were filled with myxozoan developmental stages with or without inflammatory infiltrates and necrosis. In one specimen similar lesions were identified in cartilage at extra-ocular sites. The morphology of the spores in tissue section was that of a myxobolid-like myxozoan. Marine myxoboli have been identified in commercially relevant fish including lumpfish.

Electrosurgical Removal Of An Osteochondroma From The Operculum of A Lesser Amberjack *Seriola fasciata*

[1]Tonya M. Clauss, [2]Alvin C. Camus, [1]Aimee L. Berliner, [1]Julie M. Cavin

[1]Georgia Aquarium, 225 Baker Street, Atlanta, GA 30313; [2]Department of Pathology, University of Georgia, College of Veterinary Medicine, Athens, GA 30602

A young amberjack *Seriola fasciata* presented with a firm, 1.5 cm nodular mass on the left operculum. Needle aspiration was unproductive, blood parameters acceptable, and the fish continued to eat and behave normally. After enlarging for 5 months, the overlying epidermis eroded. Radiographs revealed a discrete mass of bone density surrounded by soft tissue. Punch biopsy revealed bone and collagen. Development of exophthalmia in the left eye prompted removal by electrosurgery. Healing was by second intention. Histopathology revealed a nodular mass of smooth multilocular bone in the hypodermis. Trabeculae were lined intermittently by osteoblasts and rarely osteoclasts. Hyaline cartilage and endochondral bone production was also present. Vascularized adipose filled spaces in the mass. Adipose, divided by broad collagenous septa, surrounded the mass and blended with adjacent muscle. Inflammation was minimal. Although osseous metaplasia cannot be entirely ruled out, the diagnosis of osteochondroma was preferred in the absence of inciting trauma or microscopic evidence of trauma or inflammation. Tumors of bone are uncommon in fish and their identification is a subject of debate. For 75 years, various bony masses have been described as exostoses, hamartomas, metaplasia, and osteomas. True osteomas of fish reportedly differ from those of mammals by developing from acellular bone and having a well developed fibrovascular or lipoid support structure. Due to this, some authors refer to these tumors as osteofibromas or osteolipomas.

Blue Crab Health Status: Results From An Ecosystem Health Assessment In Chesapeake Bay

[1]Gretchen Messick, [1]John Jacobs, [2]Joe Filipowicz, and [2]Sue Tyler

[1]Cooperative Oxford Laboratory, USDOC/NOAA/NOS/NCCOS/ CCEHBR, 904 S. Morris St., Oxford, Maryland 21654-1323; [2] JHT Inc. Contracting, Cooperative Oxford Laboratory, USDOC/NOAA/NOS/NCCOS/CCEHBR),904 S. Morris St., Oxford, Maryland 21654-1323

The Cooperative Oxford Lab is conducting an integrated biotic ecosystem assessment in Chesapeake Bay. The assessment measures multiple biotic indicators at varying levels of biological organization. Connecting changes at the sub-cellular level through pathological change in the organism to subsequent population level impacts allows for the realization of assessing the impact of specific anthropogenic stressors on living resources, and thus ecosystem health. Criteria used in indicator selection in crabs include population- community composition, shell disease, gill epibionts, parasitology and general pathology. Initial results found there was significant variation in prevalence of ciliates on crab gills among river systems and prevalence of shell disease was higher in the Corsica River than the Rhode or Magothy Rivers but the variation was not significant. The prevalence of shell disease was higher in crabs assayed during the autumn than spring or summer surveys and the prevalence of ciliates varied significantly among season. The prevalence and significance of pathologies such as inflammation and gill lesions along with prevalence of various pathogens in tissues including virus, microsporidian, and a gregarine will also be presented.

A Conceptual Model For Outbreaks Of *Hematodinium* In Populations Of The American Blue Crab

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Species of *Hematodinium* cause significant annual losses to several crab and lobster fisheries. During outbreaks, the prevalence of Hematodinium can approach 100% in affected populations. Outbreaks of *Hematodinium* share similarities between host-parasite systems. For example, outbreaks generally occur in areas with restricted water flow, or in areas with entrained water masses, and they often coincide with population level changes in host factors, such as mass molting events. We are developing a model for the blue crab - *Hematodinium* system that is intended to gage how physiographic features, fishing pressure and host factors contribute to outbreaks of disease. Fishing pressure and habitat degradation are severe and pervasive threats to marine ecosystems. Both are known to impact the dynamics of host movement, aggregation, nutrition, and mortality, and therefore the transmission of disease. However, the role of fishing pressure in facilitating the emergence and spread of diseases in marine systems is poorly understood. There is circumstantial evidence that overexploitation has contributed to the emergence of disease in several marine fisheries, but the effect of fishing pressure on disease has received little attention. We want to understand how fishing pressure and declining water quality combine with the hydrography of small coastal estuaries to promote outbreaks of a pathogenic parasite in blue crabs.

A Histopathological Examination Of *Hematodinium* sp. Infections In Blue Crabs (*Callinectes sapidus*)

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Blue crabs are an important commercial species in Chesapeake Bay. Epizootics of the parasitic dinoflagellate, *Hematodinium* sp., have been reported in the bay with prevalences as high as 70-80%. The parasite infects the hemolymph of blue crabs where it proliferates and ultimately kills its host. We examined the pathology of blue crabs collected from the Eastern Shore of Virginia and documented the histopathological alterations to the tissues. Pressure necrosis was evident in the soft connective tissues of the hepatopancreas, the blood vessels in most organs, and soft connectives in the eyestalks. In heavy infections little remained of the soft connective tissues around the hepatopancreas; however, the reserve inclusion cells appeared relatively intact. The typical structure of most organs was altered by the proliferation of the parasite and subsequent edema and loss of spongy connective tissues. Damage to the gills varied, but in some cases it was severe involving an apparent thinning of the cuticular layers and loss of host epithelial cells. Affected lamellae exhibited varying degrees of hypertrophy with the loss of trabecular cells, hemocyte infiltrations, and swelling along the distal margins. Large numbers of zoospores were located along the distal margins of affected lamellae suggesting that sporulation may result in the lysis or bursting of the lamellar cuticle to release spores. *Hematodinium* infections in the blue crabs are relatively short-term infections (40-80 d) that end in death during sporulation of the parasite.

Making Use Of The Rrna Genes Of The Blue Crab (*Callinectes sapidus*) Parasite, *Hematodinium* sp.

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Blue crab (*Callinectes sapidus*) populations in mid-Atlantic coastal bays and the lower Chesapeake Bay have experienced mortalities as a result of infections by the parasitic dinoflagellate *Hematodinium* sp. The episodic nature of outbreaks, and the existence of hotspots, suggests there are environmental or biotic reservoirs of the parasite. A powerful and flexible tool for detecting parasites in environmental samples is PCR-based amplification of ribosomal RNA genes of the species of interest. Previous work by COMB, University of MD Eastern Shore, and COL has used an 18S-targeted real time PCR technique to document *Hematodinium* sp. infections in blue crabs. However, 18S-targeted PCR may not be specific enough for water and sediment sampling analyses, which harbor hundreds of free-living dinoflagellate species that may have 18S rRNA genes similar to that of *Hematodinium*. Thus, to avoid the possibility of false-positives in PCR assays for this parasite, we are developing an assay targeted to the more variable ITS region of the rRNA gene cluster. To ensure that the region targeted is not variable between strains of blue crab *Hematodinium* sp., we have conducted a thorough characterization of the rRNA genes of multiple blue crab *Hematodinium* sp. infections from the MD coastal Bays and the Gulf of Mexico. Our analysis of ITS regions will be discussed in terms of geographic and intra-strain variability of the rRNA gene cluster.

Parasite Detection And Discovery By Denaturing HPLC (DHPLC)

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Recently (1997-2004) in coastal Georgia (USA), populations of blue crabs (*Callinectes sapidus*) were severely impacted by an epizootic caused by the parasitic dinoflagellate *Hematodinum* sp. To facilitate life history and disease ecology studies of *Hematodinium* we developed several parasite-specific PCR-based diagnostic assays that allow the rapid and unambiguous detection and quantification of *Hematodinium* in blue crabs, in water, and in other animal reservoirs. However, it remains a great challenge to discover, identify, and investigate unknown and emerging disease agents. Recently, we developed a new approach for detecting and identifying known and unknown parasites of the blue crab. Denaturing high-performance liquid chromatography (DHPLC) is used in combination with peptide nucleic acid (PNA) hybridization probes to detect PCR amplified 18S rDNA gene fragments from parasites against a background of host (crab) DNA. The validity of this assay was confirmed in the blue crab/*Hematodinium* model system by comparison to disease diagnoses using the *Hematodinium*-specific PCR assay. A comparison of 76 crabs diagnosed by PCR yielded good correspondence between the DHPLC and specific PCR assays. Several new blue crab infections were detected including infection by a Kinetoplastid-like parasite most closely related to *Procryptobia sorokin*, previously reported only as a free-living flagellate. To our knowledge this is the first report of this group of flagellates in any decapod crab. DHPLC technology appears to be a versatile and robust new molecular technology with broad potential for studies of parasitism and trophic interactions.

Effect Of Exposure To Bacteria On Metabolism During Exercise And Recovery In The Atlantic Blue Crab, *Callinectes sapidus*

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We have shown that exposure to bacteria reduces aerobic metabolism in resting blue crabs, while hemolymph lactate increases, suggesting an increase in anaerobic metabolism. In the present study we investigated how exposure to bacteria alters metabolism in crabs engaged in activity (walking on a treadmill). In saline-injected controls, O_2 uptake increased 2.5-fold during 30 min of walking at 8 m min^{-1} and returned to pre-activity levels within 40 min of recovery. Crabs injected with a sublethal dose of *Vibrio campbellii* (10^5 g^{-1}) 1 h prior to walking consumed significantly less O_2 during activity and 3 h recovery (31% and 32% less, respectively) than controls. Whole body lactate accumulated 1.8-fold during activity, however, *Vibrio*-injected crabs continued to accumulate more lactate even after the activity was completed, unlike controls that rapidly eliminated this anaerobic end product. *Vibrio*-injected crabs also accumulated higher amounts of hemolymph lactate than saline controls during 30 min of exercise; this elevation persisted for 2 h after activity. Exposure to bacteria did not alter the patterns of muscle ATP, ADP, AMP and arginine phosphate consumption/loss during exercise and recovery. Thus, saline- and *Vibrio*-injected crabs were engaged in activity requiring comparable energy, but exposure to bacteria altered the relative contributions of aerobic and anaerobic pathways to the overall metabolic pattern of energy consumption. This may have important consequences to the fitness of natural populations of overtly-healthy blue crab. (Supported by NSF IBN-0212921)

Visual Deficits In Clawed Lobsters (*Homarus americanus*) From Long Island Sound, NY

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In 2005, commercial landings of the American lobster on the east coast of the USA were valued at $414.2 million for 87.5 million pounds. Landings for New York and Connecticut were estimated $8.2 million for 1.9 million pounds, with much of its value taken from within Long Island Sound, NY (LIS). In 1999, a significant mortality occurred in lobsters from LIS, however the etiology of the mortality was unknown at the time. It was thought that parasitic infection, pesticides, or hypoxia were potential causes. Affected lobsters had physiological characteristics in varying degrees of severity including lethargy, integument discoloration, and "cloudy" gray eyes. In 2001 and 2004, eyes were histologically examined and shown to have idiopathic lesions affecting the nerve and cellular structure in the eyestalk and optic nerve. We present results from an electrophysiological study of the visual deficit linking eye function to the histological extent of damage. Single flash Electroretinograms (ERG) were employed to compare visual parameters between apparent healthy lobsters and those within the LIS. Deficits in vision were quantified using V log I curves and waveform analysis. Eyes were also histologically examined for lesions. Changes were apparent both physiologically and histologically via a reduction in the number of working ommatidia causing a reduced physiological response. The lesions, therefore, affect the visual capabilities in lobsters from Western Long Island Sound.

Culture Transmission Studies Of *Hematodinium* sp. From *Callinectes sapidus*, A Key To Understanding This Enigmatic Life Cycle

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Hematodinium species are parasitic dinoflagellates that propagate in the hemolymph and hemocoel of many decapod crustacean taxa. The parasites can cause significant mortalities, and epizootic outbreaks of these parasites have negatively impacted commercial crab fisheries by markedly reducing annual catches. There are only two species of *Hematodinium*, *H. perezi* and *H. australis*, but recent molecular work indicates the possibility of several more species. *Hematodinium* sp. is relatively prevalent in blue crabs (*Callinectes sapidus*) along the Atlantic seaboard of the United States, with seasonal prevalences of up to 80% reported. It resembles *H. perezi*, and not the species that has been cultured from the Norway lobsters. The lifecycle of the parasite from the blue crab has yet to be elucidated. Therefore, we have attempted to culture *Hematodinium* sp. from the hemolymph of *C. sapidus* using a modified *Nephrops* saline. This has revealed aspects of the life cycle that could not be inferred without *in vitro* culturing. Motile plasmodial filamentous trophonts removed from blue crab hemolymph give rise to amoeboid trophonts in culture, which proliferate by binary fission, forming chains and eventually large clump colonies. Alternatively, amoeboid trophonts obtained directly from host hemolymph may form arachnoid trophont or sporont colonies that produce presumptive sporoblasts. The life history stages of *Hematodinium* sp. observed *in vivo* and *in vitro* in *Callinectes sapidus* were compared with those reported from Norway lobsters, *Nephrops norvegicus* and boreal snow crabs, *Chionoecetes opilio* to explore possible life cycle, taxonomic, and pathological differences between these disparate hosts.

Preliminary Histological And Serum Chemistry Analysis Of Atlantic Sturgeon, *Acipenser oxyrinchus* , Infected With The Copepod *Dichelesthium oblongum*

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Atlantic Sturgeon (n=43) were sampled in October (n=26) and November (n=17) 2007 between Rockaway Beach and Jones Beach, Long Island, NY. The fish were examined for the presence of external parasites, blood was collected for subsequent serum analysis and select tissues were collected for histopathological evaluation. *Dichelesthium oblongum,* a parasitic copepod, was observed on 91% of the sturgeon sampled. During the course of these examinations, grossly visible lesions associated with the attachment and feeding of juvenile stages of *D. oblongum* were noted on the pectoral, dorsal and anal fins of infected fish. These lesions consisted of focal areas of ulceration with a raised border. Histologically the lesions were characterized by necrosis or complete loss of both epidermis and dermis as well as focal necrosis of the underlying musculature. In areas in proximity to the lesions, where the epidermis was intact, focal areas of spongiosis were sometimes observed. Serum chemistry of the most heavily infected animals indicated ion loading (i.e. sodium, calcium, magnesium) possibly as a result of water leakage through the epithelial breach.

Pathology Associated With *Bolbophorus damnificus* In Channel Catfish (*Ictalurus punctatus*) Fingerlings

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Natural infections of *Bolbophorus damnificus* in commercial channel catfish (*Ictalurus punctatus*) have been associated with mortalities and reductions in growth and feed intake. Preliminary research in our laboratory using artificial infections have demonstrated that mortalities can occur on day 5 post-infection when fish are challenged with 200 *B. damnificus* cercaria/fish. In order to better understand this infection and the subsequent pathology associated with this parasite over the course of 5 d, a titration study was done. Cercariae, confirmed by PCR to be *B. damnificus*, were collected from snails *(Planorbella trivolvis*), pooled and enumerated. Fingerling catfish (n=20/group) were individually exposed to 200, 100, 50, 25 or 0 cercariae for 2 h. After exposure, catfish were rinsed and held in flow-through tanks. Fish were necropsied at 3, 4, 5 and 6 d post-infection. Mortalities were first observed on day 4 in fish exposed to 200 cercariae (9% mortality) and continued on day 5 in this group (13% mortality). On day 6 post-exposure, mortalities were observed in the fish exposed to 200 (69% mortality), 100 (33% mortality) or 50 (7% mortality) cercariae with associated ascites in each group (53, 31 and 2%, respectively). At this point the trial was terminated, gross lesions were recorded, and all organs and muscle samples were collected for histopathology. Pathology results will be reported. Research supported by: USDA-Southern Regional Aquaculture Center: 2002-38500-11805 and USDA-NRI: 2002-35204-11678.

Infestations Of Demersal Elasmobranchs In A Large Aquarium By The Leech *Branchellion torpedinis*

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Clinically significant leech infestations are relatively uncommon. Except for their relatively long life span, however, leeches share many of the characteristics of problem pathogens in closed systems, including a simple life cycle and low host specificity. *Branchellion torpedinis* has become a significant challenge in the maintenance of a collection of demersal elasmobranchs in a large marine exhibit (24 million liters) at the Georgia Aquarium. The parasite sheds long-lived eggs free in the water column rather than adhering cocoons to hard surfaces like most leeches. It can attain body sizes over 4cm in length and shows a tropism for cryptic sites such as the orobranchial cavity, cloaca and nares. Transmission can occur by recruitment of new larvae but also by direct contact between hosts resulting in horizontal transfer of adult leeches. Profound anaemia has been observed in severe cases and the parasite has likely contributed to several deaths. An integrated disease management strategy has been instituted that relies upon the manual removal of adult leeches during periodic physical exams, combined with an intensive exhibit maintenance diving schedule aimed at reducing the number of eggs in the substrate. These efforts will be enlightened by continuing studies of the life history and biology of this unusual pathogen.

Immune Relevant Genes Expressed In Rainbow Trout After Immunization With A Live Vaccine Against *Ichthyophthirius multifiliis*

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Rainbow trout *Oncorhynchus mykiss* Walbaum, 1792 were immunised by i.p. injection using a live vaccine based on *Ichthyophthirius multifiliis* (Ich) theronts, which previously has been shown to confer protection against white spot disease. Samples were taken pre-vaccination and at day 1, 7, 21 and 28 d post-immunisation (p.i.). Expression of immune relevant genes in liver, spleen and head-kidney was monitored by qPCR. Specific antibody production was detected using ELISA. A series of genes encoding cytokines, complement factors, immunoglobulins, cellular receptors and acute phase reactants were studied and it was shown that a range of these genes became regulated following immunisation. Genes encoding acute phase reactants were up-regulated with SAA as the most pronounced. Complement factors C3, C5 and FB were found up-regulated on a number of occasions. Genes encoding immunoglobulins were not found up-regulated but a specific low titer IgM response (titer 25) against parasite antigens was detected by ELISA at 4 w post-vaccination.

Monitoring The Infective Pressure Of *Lepeophtheirus salmonis* (Krøyer 1837) On Wild Salmonid Populations In Scotland

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Controversy exists concerning distributions of salmon lice (*Lepeophtheirus salmonis*) within sea lochs. Some have suggested that low numbers of sea lice are distributed throughout the marine regions of a loch and *L. salmonis and only* infects in full salinity. Lice then cumulatively increase with time from low numbers to peak infection. Others believe that lice gather at river mouths in spring and early summer, then infect in a single event during low salinity conditions, which is less favorable for their long term survival. Neither model has been field tested. This paper examined where *L. salmonis* infection occurs within Loch Torridon, the seasonal variation in infectious pressure, and what stage infests the host. Sentinel cages were placed in the tidal zone of Loch Shieldaig in a tidal zone between Loch Torridon and the sea to correspond to salmonid migration patterns, currents, and access. At each station, 50 Atlantic salmon (*Salmo salar*) were introduced for 7 days, monthly. Parameters, including plankton, temperature, salinity, current speed and direction, were recorded. After exposure, fish were removed, euthanized, and the species and number of lice at each developmental stage were recorded. These data, which covers from March 2006 to December 2007, found that most of the lice were copepodids, with chalimus and pre-adult stages also recorded. The mean number of lice per fish increased even when numbers of lice on adjacent salmon farms was low. Results indicated that low numbers of mobile *L. salmonis* stages existed within the loch from spring to early summer, which increased through the winter and following spring without a winter decline in lice numbers (as previously reported). Copepodid and pre-adult stages initiated infection while adults played a minor role. In addition, a survey from 2005 and 2007 of *L. salmonis* burdens on >350 returning sea trout (*S. trutta*) compared Scottish coastal populations of sea trout. This was a direct comparison between lice burdens of wild salmonids in high farming (West coast) versus low farming (East coast) areas. In that study, the highest lice burden was found on East coast fish. This complex result suggested that the decline in sea trout on Scotland's West coast may not be due to increased sea lice burdens. Sea lice instead form part of a complex, multi-factorial problem and the reasons behind the decline in Scottish West coast sea trout remains poorly understood.

Chlamydia-Like Organism In Ontario Lake Trout (*Salvelinus namaycush*)

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Chlamydia-like (Neochlamydia) organism has been described in both Arctic charr and Atlantic salmon. It affects Arctic charr to a moderate degree in Ontario. In Lake trout however, it has become perhaps the single biggest impediment to enhancement culture of Lake trout at some OMNR facilities. The bacterial inclusions are small and difficult to discern with H&E stain. What does seem to be reasonably consistent however is the pattern of branchial lesions. There is prominent single-cell necrosis of leukocytes and epithelial cells and thickening and blunting of lamellae. Fish of any size from shortly after swim-up can be affected. Not surprisingly, surface treatments are not effective and a preliminary treatment trial with medicated feed (tribrissen and oxytetracycline) was performed.

A Nodavirus Outbreak In Atlantic Cod (*Gadus morhua*): Effects Of Vaccine, Temperature And Dissolved Oxygen

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Nodavirus, from the family Nodaviridae, affects juvenile Atlantic cod (*Gadus morhua*). There is no treatment or vaccine for the disease. Clinical signs include looping and spiraling swimming behavior, inappetence and high mortality. Diagnosis is based on virus isolation, reverse-transcriptase polymerase chain reaction (RT-PCR) and histopathology. Nodavirus positive populations require best management practices to prevent clinical manifestation of disease. Cod do not express humoral immunity with detectable specific antibodies in response to an antigen. Current research indicates that vaccines will stimulate nonspecific antibodies. This study describes a nodavirus outbreak in a hatchery population of approximately 10,500 Atlantic cod, five to fifteen grams in weight. Prior to the outbreak, six tanks were randomly allocated into treatment and control groups. The treatment groups were vaccinated with a commercially available dip vaccine against a group of bacteria that cause Vibriosis. The control groups were vaccinated with a sham vaccine (saline). By day 51, the cumulative mortality exceeded 60% and the population was culled. Survival analysis was used to describe the effects of temperature, oxygen, and vaccination effects on mortality.

Continuous Exposure To Infectious Pancreatic Necrosis Virus (IPNV) During Early Life Stages Of Rainbow Trout, *Oncorhynchus mykiss*, Walbaum

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Rainbow trout (*Oncorhynchus mykiss*, Walbaum) were exposed continuously to infectious pancreatic necrosis virus (IPNV) at 0, 10^1, 10^3, or 10^5 pfu/L of water to estimate the effects of chronic IPNV exposure on early life stages. Fish density averaged 35 fish/L (low) or 140 fish/L (high), and water flow rate through tanks was 250 mL/min. Virus exposure began at 6 d before hatch and continued until fish were 44 d old. Discrete-time event analysis was used to explore patterns of survival and mortality. Mortality that was significantly greater than in the 0 pfu/L (control) exposure did not occur until IPNV concentration was 10^5 pfu/L at low fish density and 10^3 pfu IPNV/L at high fish density. These results suggest that in the natural aquatic environment, where rainbow trout densities are likely to be considerably lower than in this study, mortality resulting from infection with IPNV will very likely not occur when ambient concentrations of virus are $\leq 10^3$ pfu IPNV/L. In aquaculture rearing units, trout density may be as high or higher than the densities used in this study, and under such density conditions continuous inputs of IPNV at concentrations $\geq 10^1$ pfu/L may result in IPN epidemics.

Stability And Abundance Of VHSV In Great Lakes Diagnostic Submissions

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Several groups of fish were collected and taken to the Aquatic Animal Health Program at Cornell University for the purpose of studying viral stability and abundance of viral hemorrhagic septicemia virus (VHSV). The sample groups included fifteen moribund, fresh dead (red gills), and decomposing (white gills) gizzard shad from Dunkirk Harbor on Lake Erie; six round gobies from Lake Ontario; one rock bass from Oneida Lake; and five round gobies from the St. Lawrence River. All of the fish came from separate mortality events, except for the final group of five round gobies, which was part of a surveillance collection of apparently health fish. Cell culture, RT-PCR, and quantitative RT-PCR (qRT-PCR) were used on pooled organ samples from the fifteen gizzard shad. The remaining groups of fish were tested using qRT-PCR and RT-PCR on spleen, kidney, heart, and liver to look at tissue tropism and freeze/thaw effects on VHSV. The three groups of five gizzard shad had low levels of VHSV as measured by qRT-PCR, but all were cell culture negative. There were no significant differences in tissue tropism and no effect of two freeze/thaw cycles with the round goby samples. Interestingly, even though all fish tested positive for VHSV with qRT-PCR, only samples with approximately 10^6 viral copy numbers tested positive with RT-PCR. This raises the issue of true detection of VHSV with the current OIE standard of cell culture and RT-PCR.

Development Of A System For Assessing Fish Virus Competition And Fitness in Rainbow Trout (*Oncorhynchus mykiss*)

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Competition and fitness are fundamental principles that shape the structure and balance of all biological systems. It is logical that this is just as true for viruses as for macro-organisms, and yet we know very little about viral competition. In fish, viruses compete in mixed infections with other pathogens, and with multiple strains of the same virus that may co-infect a host. It has been shown that ecological interactions within genetically diverse infections can influence disease severity, epidemiology, and pathogen evolution. Experimentally, virus fitness values have been measured as the relative ability of two competing viruses to produce infectious progeny in a given environment. Viral fitness has been explored in cell culture, but there has been little work *in vivo*. We have developed a system for competition assays to assess viral fitness in live juvenile rainbow trout (*O. mykiss*). Trout are co-infected with strains of infectious hematopoietic necrosis virus (IHNV) that can be distinguished genetically, and progeny virus populations are characterized after three days of in-host competition. To date these assays have shown that IHNV strains that co-circulate in an aquaculture setting are of equal fitness, and strains that differ in virulence also differ in fitness.

Going BAC And Forth To Make Channel Catfish Virus Recombinants

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Bacterial artificial chromosomes (BAC) containing herpesvirus genomes are extremely useful for molecular virology. These BAC clones can be extensively modified in *Escherichia coli* and then used to generate recombinant herpesviruses. Our laboratory has produced two infectious BAC systems for *Ictalurid herpesvirus* 1 (Channel catfish virus-CCV). One system utilizes overlapping BAC clones representing the CCV genome to reconstitutes virulent wild type CCV lacking BAC sequences. This system was used to insert a BAC cassette into the TK locus to generate the second system which is a single infectious BAC. This infectious BAC is TK deleted and displays moderate attenuation properties similar to CCVTK-. In another experiment gene 12 was deleted in a BAC clone using a PCR generated linear replacement cassette containing kanamycin resistance gene flanked by 33 bp virus sequences. The deleted BAC was used to generate gene 12 deleted CCV. This recombinant grew well in cell culture but was weakly attenuated in fish challenges. We incorporated the Gateway system (Invitrogen) into our overlapping CCV BAC system. With this we can utilize the LR-Clonase *in vitro*, to transfer the insert of a Gateway entry clone into our BAC system. Recombinants were produced by PCR amplifying the *lac Z* gene, directionally cloning it into TOPO entry vector, transferring it into a CCV Gateway destination cosmid using clonase and then generating the virus from by co-transfecting the cosmid with two overlapping BAC clones. Our ability to efficiently generate CCV recombinants will facilitate development of CCV as a vaccine vector and as a model for studying herpesviruses of fish and amphibians.

Orphan Viruses Detected During Fish Health Certification

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Orphan viruses are viruses that when originally isolated were not classified or specifically linked to a particular disease, but may exhibit pathogencity. The role that orphan viruses play in causing or contributing to disease is not specifically understood. Disease events (infectious salmon anemia, spring viremia of carp, and viral hemorrhagic septicemia) in U.S. finfish species and growing aquaculture industries have lead to increased efforts to certify the health status of fish prior to purchase or movement. Cell lines used to screen for specific viral pathogens can show sensitivity toward multiple viruses and have indirectly enabled the detection of a number of these orphan viruses during routine testing. This underscores the advantage that the use of cell culture in fish health certification testing can provide by adding the ability to potentially detect emerging pathogens. In addition to the increase in testing for health certification, public awareness of clinical signs in baitfish has also triggered additional investigations. Increased efforts are being made to characterize or identify viral isolates that in the past have been ignored or stored for later identification. Laboratory findings for a few select orphan viruses will be presented.

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

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Approximately six years after the Joint Subcommittee on Aquaculture assigned the National Aquatic Animal Health Task Force to develop a national aquatic animal health plan (NAAHP) for aquaculture in cooperation with industry, regional organizations, state, local, tribal governments and other stakeholders, the Plan is in the final stages of the clearance process. The NAAHP will foster and support efficient aquaculture; protect the health of our nation's wild and cultured aquatic resources; and meet both national and international trade obligations. Development of the NAAHP required input from technical working groups composed of individuals who represented certain sectors of the aquaculture industry. 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 implementation priorities will be presented and discussed.

Cloning And Characterization Of Phospholipase D From Olive Flounder (*Paralichthys olivaceus*)

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The phospholipase D1 (PLD1) cDNA, designated PoPLD, encoding a predicted protein of 1053 amino acids in olive flounder (*Paralichthys olivaceus*) has been cloned. The deduced amino acid sequence shares high identity with that of PLD1s and PLD2 in human, rat and mouse. The phylogenic analysis and sequence comparison of PoPLD with other PLD isozymes, suggest it to be closely related to the PLD1 isozyme in primary structure. Phospholipase D1 mRNA was predominantly expressed in the brain, gullet, muscle, stomach, head kidney, pyloric caeca, intestine and gill. The expression of the PoPLD gene was examined in various tissues of flounder by RT-PCR following stimulation with LPS and compared also with that of the inflammatory cytokines IL-1β and IL-8 in intestine and gill tissues. This provides indirect evidence that PLD1 might have a relevant role in immune responses against pathogens and in inflammation.

How To Give a Clam a Check-Up: Designing Sensible Health Surveillance Programs In The Bivalve Realm

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Few states/provinces/countries have easily accessible and user-friendly shellfish health regulations for transfer or introduction in place. So what should or do shellfish aquaculturists or hobbyists do when they are interested in meeting, exceeding or simply learning about shellfish health regulations? What if you are a "backyard" shellfish grower who just wants to know what diseases are out there and how to monitor for them? This talk is intended to give a brief overview of the current state of shellfish regulations in the Eastern U.S. (and beyond) and illustrate how aquaculturists and aquatic health professionals (including veterinarians) work together to protect the health of cultured and wild shellfish stocks and the environment.

Exotic *Perkinsus* spp. Organisms In Vietnamese Ornamental Tridacnid Clams Imported Into The United States

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Morbidity and mortality was observed in ornamental saltwater clams *Tridacna crocea* imported from the Vietnamese ornamental aquaculture industry into the U.S.A. and held in research facility aquaria, and long term in home aquaria. Clinical signs included an incompletely extended mantle, slow mantle responses to stimuli, and sloughing of byssal tissue beginning 2-5 days prior to death. Chronic infection without clinical signs until stressed was typical. At necropsy, the carcasses were moderately emaciated with visceral mass edema. Histopathology revealed marked inflammation and necrosis of visceral mass tissues containing large numbers of 10-15 µm extracellular round organisms identified as *Perkinsus* sp. trophozoites that were confirmed by tissue incubation in ARFTM with Lugol's iodine staining of hypnospores and genus specific Perkinsus PCR. Species specific PCR was negative for *P. marinus* and variably positive for the OIE reportable *Perkinsus olseni*, in a possible dual infection with a new Vietnamese *Perkinsus* sp. This confirms that importation of Vietnamese ornamental reef clams, which are not subject to testing or quarantine, has resulted in incursion of exotic *Perkinsus* sp. including *P. olseni* into the U.S.A. This finding has significant implications for national and international trade. The cross infection risk of the new Vietnamese Perkinsus to domestic shellfish is unknown. The sources within Vietnam, epidemiology of dissemination within the United States, characterization of the new *Perkinsus* sp. including its cross infection capability with domestic shellfish populations all require further investigation.

Voluntary Quarantine Program To Establish *Perkinsus*-Free Stocks Of Imported *Tridacna* Clams

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Tridacnid clams are an important marine ornamental mollusc imported into the U.S. from several Indo Pacific countries. In 2007, several *Tridacna crocea* were found to be infected with *Perkinsus olseni*, a protistan organism listed as a notifiable pathogen for molluscs by the World Organization for Animal Health (OIE). Prior to this finding *P. olseni* had not been reported in North America. After a meeting which included representatives from Federal and State of Florida agencies, University of Florida faculty, and representatives from relevant aquaculture industries, a plan was developed to establish a voluntary quarantine program which would allow for testing of quarantined *Tridacnid* clams to determine the presence or absence of *Perkinsus* spp. prior to distribution. Currently, there are no Federal health requirements for imported ornamental molluscs entering the U.S.; however, by appropriate quarantine procedures and testing the risk to commercial and wild susceptible livestocks is greatly minimized. This presentation will address concerns related to this pathogen as well as present an overview of the protocols recommended for quarantine and testing for *Perkinsus* in tridacnid clams.

Recent Knowledge Concerning QPX Infections In Hard Clams

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In 2005 the hard clam (Quahog, *Mercenaria mercenaria*) aquaculture industry produced $60 million worth of edible product in the U.S. Quahog Parasite Unknown (QPX) causes severe disease in both cultured and wild hard clams in the northeast U.S., especially in MA. Infection occurs most commonly in sub-market sized clams. Mantle edges and siphon base tissues are most commonly infected resulting in lack of function and dehabilitation. Mortality can occur throughout the warmer months but especially in the spring and fall. Up to 90% of an aquaculture plot of hard clams can be lost. QPX is a Labyrinthomorphid protist that proliferates in sea water, on surfaces of seaweed and in clam tissues, as well as in culture. The parasite produces a mucus coat that inhibits phagocytosis by hemocytes. Clam strains originating from latitudes south of the aquaculture farm were clams are cultured are more at highest disease risk. The agent is directly transmitted promoting disease spread within a plot. However, the pathogenesis of the initial infection has not been determined. Spring and fall temperatures as well as growth of water column associated QPX on dead clam tissues (which increased QPX proteases) may promote initial infection.

An Epizootiological Approach To The Study Of Marine Diseases

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The economically important marine bivalve mollusc, *Mercenaria mercenaria*, (commonly called a northern quahog or hard clam), has endured considerable mortalities due to a thraustochytrid pathogen called Quahog Parasite X (QPX). Data on the percent prevalence of QPX infections were compiled from published reports in order to describe the epizootiology of QPX disease. Advantages and disadvantages of an epizootiological approach to studying bivalve diseases will be presented in the context of our results for QPX. For example, the highest prevalences of QPX infections occurred in clams from samples with an intermediate size range (shell lengths 20-55 mm). This suggests clams of this size should be used in experiments regarding transmission of QPX. Likewise, our summary demonstrates that QPX infections occur in both male and female clams. Since infection prevalence does not appear to be correlated with sex or sex ratios, both male and female clams should be used in QPX-related experiments. Overall, our summary of information on QPX disease highlights the need for the collection of a specific type of information regarding factors believed to be associated with the presence and severity of diseases in bivalve molluscs.

Comparative Histopathology Of *Mya arenaria* And *Tagelus plebeius* Clams In The Chesapeake Bay

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Maryland initiated a histopathology survey of Chesapeake Bay softshell clams, *Mya arenaria*, and stout razor clams, *Tagelus plebeius*, in 2000, because softshells had declined and commercial clammers began to harvest razor clams for eel and crab pot bait. Results of the first four years of the survey revealed diverse lesions and parasites, raising questions about the animals' ability to survive in the Bay. Clams from Tangier Sound sites were in the best condition of all the clams sampled. *Perkinsus chesapeaki* was the most common parasite found in both species; however, hemocyte responses differed during parasite encapsulation and recovery (pink-layered cysts vs. acidophilic granuloma unknown [AGX]). New findings included intranuclear viral inclusion bodies in gill and digestive gland duct epithelia, suspect heart mesotheliomas, nuclear anomalies in hemocytes and renal epithelia, suspect new amoeba (rosy cell unknown, RCX) and ciliate parasites, and rickettsia in digestive gland or rarely in gill. Up to 70% of all razor clams greater than 30 mm shell length died between late 2003 and May 2004. Histopathological examinations of surviving spring-collected clams revealed gill, renopericardial canal, and kidney lesions; accumulations of calcium and lipofucsin-like materials; and one case of golden connective tissues; however, their roles in the mortalities are unclear.

Tissue Patterns Of A Matrix Metalloproteinase In Infected Eastern Oysters, *Crassostrea virginica*

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An understanding of the immune responses of eastern oysters to pathogens may reveal methods that may minimize disease mortalities in aquaculture. Matrix metalloproteinases (MMPs) are one potential mechanism of defense against disease. MMPs are a family of zinc-dependent proteases that break down the extracellular matrix and are involved in many functions of innate immunity in vertebrates, including activation of immune molecules, wound healing and cell migration. Immunofluorescent analysis of an oyster MMP, *Cv1*-MMP, was performed on histological sections from naturally and experimentally infected oysters. Interactions between *Cv1*-MMP and the pathogens causing dermo (*Perkinsus marinus*) or *Roseovarius* oyster disease (*R. crassostreae*) were analyzed using multiple labeling. *Cv1*-MMP labeling was observed in hemocytes infiltrating tissues and in epithelial cells of mucosal barriers. *Roseovarius crassostreae* labeling was restricted to the inner shell surfaces and mantle of oyster spat. In some cases, labeling of *P. marinus* in hemocytes of infected oysters co-localized with *Cv1*-MMP labeling in the cytoplasm of hemocytes. These results suggest that *Cv1*-MMP may provide a first line of defense for the oyster to invading pathogens. This research was funded by USDA-CSREES awards 2004-34438-15041 and 2007-35204-17810 and by use of the RI Genomics and Sequencing Center supported by NSF EPSCoR Grant 0554548 and the RI-INBRE Research Core Facility supported by NCRR NIH Grant # P20 RR16457.

Treatment Of Negative Buoyancy In A Captive Chambered Nautilus, *Nautilus pompilius*

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A single *Nautilus pompilius* was observed to have problems maintaining neutral buoyancy. This phenomenon is not well understood and not detailed in the literature. As a result of this condition, diagnostic techniques and treatments were employed that are not commonly performed on *Nautilus*. Procedures such as anesthesia and hemolymph removal were not recorded in the literature but necessary in order to properly care for the specimen. Anesthesia was obtained in an immersion bath via a 3% ethanol solution in seawater. While the animal was sedated, skin scrapings, tissue biopsies, and hemolymph were collected. Subsequent analysis of these samples revealed bacterial and parasitic infections. Radiography revealed no abnormalities of the shell structure, but an anomaly in the first chamber that appeared to be fluid filled as a reasonable cause for the negative buoyancy. The techniques used in this case, such as anesthesia and bleeding, were repeatedly performed with increased efficiency, and may be used in further diagnostic evaluation and treatment of *Nautilus*.

Iridoviral Infections In Aquarium Fish

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Iridoviruses are large, icosahedral, ds DNA viruses, infecting invertebrates and lower vertebrates. Taxonomy of Iridoviridae has been confusing, but is critical for understanding the epidemiology and pathology of members of each group. There are currently five ICTVdB-listed genera in the family Iridoviridae, but many iridoviral isolates have not yet been satisfactorily classified. *Iridovirus* and *Chloriridovirus* infect invertebrates. *Ranavirus* species (e.g., LMBV) infect fish, amphibians, and/or reptiles. *Lymphocystivirus* species infect fish, as do members of the relatively newly christened genus *Megalocytivirus*. For many years, the relatively benign disease lymphocystis was believed to be the extent of iridoviral pathology and significance. Identification of those viruses now classified in the *Megalocytivirus* has changed this. *Megalocytivirus* species are known to cause, and/or have been associated with severe systemic infections in fish, and histopathologically, necrosis associated with large, basophilic, inclusion body-bearing cells may be seen in multiple organs. *Megalocytivirus* infections have been identified in, or associated with a number of aquarium fish species, including African lampeye, dwarf gourami, blue gourami, and several species of cichlids, in some instances, marked by significant mortalities. Cases lacking virus isolation have been determined to be iridovirus-associated based on electron microscopic identification of iridoviral particles, typically in paracrystalline arrays. The *Megalocytivirus* species are an important group of iridoviruses requiring further study.

Molecular Phylogenetic Assessment Of A Systemic Iridovirus From The Banggai Cardinalfish (*Pterapogon kauderni*)

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In 2003-2005, a systemic iridovirus was identified in Banggai cardinalfish (*Pterapogon kauderni*) that were dying after transport and apparent acclimation to aquarium conditions. Throughout multiple tissues of affected fish, often recognizable beneath endothelium, were cytomegalic cells with basophilic granular cytoplasmic inclusions, which were determined by transmission electron microscopic examination to consist of arrays of viral particles of a size and shape consistent with those of previously described iridoviruses causing systemic infections of ornamental freshwater and marine fish. Partial DNA fragments of the DNA polymerase (DNApol), major capsid protein (MCP), as well as the full length adenosine triphosphatase (ATPase) genes were amplified by PCR from total genomic DNA extracted from fresh frozen tissues or formalin-fixed paraffin-embedded tissues. Molecular data permitted riboprobe generation toward in situ detection of virus in research and diagnostics, as well as molecular phylogenetic studies to gain insight into the epidemiology and evolution of this virus. Phylogentic analysis revealed the virus belongs to the genus Megalocytivirus within the family *Iridoviridae*.

Franciselliosis, One Of The New Kids On The Block

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During the 1990's, epizootics in tilapia caused by a fastidious, intracellular bacterium were reported in Taiwan, Hawaii and the United States. In addition, similar bacteria have been reported in three-lined grunt, Atlantic salmon and cod. The bacterium has been identified by molecular methods in hybrid striped bass and in a number of ornamental fish belonging to the Cichidae family. In populations with endemic chronic disease, the occurrence of mortalities is often associated with exposure to stressful conditions (e.g. poor water quality, rapid temperature changes or handling, etc.). Infected fish can display a variety of clinical signs and lesions (e.g. lethargy, inappetence, petechia, exophthalmia and abnormal swimming behavior). Multiple, variably-sized, white granulomas are generally detectable grossly in different organs (e.g. gills, spleen, kidney, testes, heart, ovaries and liver) with the spleen and kidney commonly enlarged. Mortalities can range from less than 1% to upwards of 90%. The bacterium is a Gram-negative, pleomorphic coccoid to short bacillus ranging from 0.5 -1.5 μm in diameter, is non-motile, and is a fastidious facultative intracellular organism. Similar to *Piscirickettsia salmonis,* these organisms are intracellular rickettsia-like pathogens of fish that replicate within membrane-bound intracytoplasmic vacuoles in infected cells. It is a member of the genus *Francisella*, and is grouped in the gamma subdivision of the proteobacteria.

Highly Pathogenic *Francisella* sp. Causing Mortality in Farmed Tilapia Nilotica (*Oreochromis niloticus*)in Costa Rica

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Francisella sp. is an emergent bacterial pathogen that causes acute to chronic diseases in warm and cold water cultured and wild fish species. During the past 3 years the bacterium has been detected in cultured tilapia nilotica (*Oreochromis niloticus*) cultured in Costa Rica. Infected fish presented non-specific clinical signs such as erratic swimming, anorexia, anemia, exophtalmia and high mortality. Upon macroscopic and microscopic examination, several internal organs (mainly spleen and kidney) were found enlarged with white nodules. Histological examination revealed the presence of multifocal granulomatous lesions, with the presence of numerous pleomorphic coco-bacilli. The bacteria was recovered from infected fish and grown in several media with and without antibiotics. Specific PCR primers to the *Francisella* genus were used to ensure the preliminary diagnoses. Interestingly, after a comparison with several bacterial 16S rRNA sequences, our isolate was found to share 99% similarities with other *Fransicella* sp. isolated from fish, and more than 97% similarities to the class A agent *Francisella tularensis*. Koch's postulates were also fulfilled after experimental intraperitoneal and gill exposure challenges, and a complete pathological, bacteriological and molecular examination confirmed the etiological agent, *Francisella* sp.

Diagnosis And Management Of Streptococcosis In Intensive Aquaculture

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Streptococcosis has been implicated as one of the limiting factors in intensive tilapia aquaculture worldwide. Methods of diagnosis and management of the important *Streptococcus* spp. agents affecting the tilapia *Oreochromis* spp. will be described. Streptococcosis may be caused by *Streptococcus iniae, S. agalactiae, S. dysgalactiae, Lactococcus garviae, or Enterococcus* sp. however, *S. iniae* has emerged as the first significant disease of cultured tilapia in the U.S. having been identified in 14 different states. Diagnosis of streptococcosis is made by isolation of the causative bacterium from diseased fish exhibiting clinical signs. Primary isolation is on tryptic soy agar (TSA) with 5% sheep blood and selective isolation is best achieved on Columbia colistin-nalidixic acid (CNA) agar with 5% sheep blood. Presumptive identification of *Streptococcus iniae* is done by biochemical testing and serology and confirmatory identification by PCR on pure cultures of the organism. Management of streptococcus infections centers on avoidance of the pathogen through careful selection of disease free fish and quarantine. Once the pathogen has invaded a facility, water quality parameters should be maintained in an optimum range and the use of vaccines should be considered. Although the bacterium is typically sensitive to various antibiotics, there are currently no USFDA approved antimicrobial agents for treatment of streptococcal disease in tilapia. Prevention of disease therefore is paramount.

Pet, Ornamental and Food Fish Zoonoses

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In additional to the multitude of pathogens that are transmissible to humans from aquatic species via consumption, there are also a number of specific pathogens that can be encountered during examination and handling of aquatic animals. The interaction of pathogens between humans and aquatic species is complicated because of the aqueous medium essential to the aquatic organism and the various routes of transmission. Many of the clinical signs of a potentially zoonotic disease in an aquatic species have little relevance to the clinical signs that may develop in humans affected with the pathogen, and some of the zoonotic pathogens are commensal organisms in the aquatic species and do not cause disease in the animal. As there are no reported parasitic, viral, or fungal zoonoses acquired from contact with aquatic species, bacteria are the only agents of concern for zoonotic infections from fish and includes both Gram-negative and Gram-positive bacteria. Aquatic animals live in a wide range of aqueous conditions, and the types of bacterial species that are associated with certain aquatic species varies depending on that environment. As with other diseases, prevention is a much more effective way of avoiding zoonotic diseases than responding to existing infections. Thus, implementation of an appropriate biosecurity plan for a home aquarium or aquaculture facility is important in reducing the introduction and minimizing the spread of a pathogen in an animal population. Avoidance of contact with the fish and water is the single most effective way to prevent human infection, therefore basic hygiene and thorough hand washing after contact with fish or water that contains fish is a good preventative protocol. This contact can be further decreased by the use of gloves when working with fish or aquatic systems, thereby reducing exposure to previous cuts or abrasions.

Effects Of A Shellfish-Larval Pathogen And A Prospective Probiotic Bacterium On Hemocyte Functions Of The Oyster *Crassostrea virginica*: *In Vitro* Effects On Mortality, Phagocytosis, Adhesion And Oxidative Burst

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Bacterial diseases are considered to be a major cause of mortality in shellfish larviculture. The use of probiotic bacteria for disease prevention and improved nutrition in shellfish hatcheries is becoming increasingly popular. Previous experiments at the Milford Lab have shown that a *Vibrio* sp. isolate (OY15)-- a naturally-occurring, potential probiotic candidate from the digestive glands of adult oysters -- had significant positive effects on the health and survival of oyster larvae. Mechanisms responsible for this effect were not elucidated in these past experiments. The objective of this *in vitro* study, therefore, was to determine the effects of probiotic candidate OY15, or the pathogenic strain B183, upon oyster hemocytes, the cells responsible for the internal-defense functions that respond to disease-causing organisms or environmental stressors. Hemocyte counts and four immune functions were assayed using flow-cytometric methods: hemocyte viability, phagocytosis, adhesion, and oxidative burst. Probiotic candidate OY15 caused no significant change in mortality of hemocytes, or upon percentages of granular and agranular cells. Probiotic OY15 enhanced hemocyte phagocytosis, and significantly decreased the number of non-adhering hemocytes. No significant changes in oxidative burst in granular hemocytes occurred when in the presence of probiotic candidate OY15. In contrast, oyster hemocytes exposed to bacterial pathogen B183 had higher mortality and unstimulated oxidative burst than pathogen control and probiotic treatments, as well as a significant increase in percentage of granulocytes, i.e., a decrease in numbers of hyalenocytes. Phagocytosis by granulocytes was unchanged upon exposure of hemolymph to pathogen B183. These findings suggest that the probiotic candidate enhances hemocyte functionality; whereas, the pathogen causes immunosuppression. Thus, these competing effects upon hemocytes may play a role in probiotic-pathogen interactions within larvae.

Mutation Within The *Aeromonas salmonicida* Type III Secretion System Affects Host Leukocyte Activation And Downstream Immune Responses

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Deletion mutants of *Aeromonas salmonicida* were constructed to determine whether the presence of the type three secretion system (TTSS) would affect host cell activation, microbicidal activity, and signalling. *Aeromonas salmonicida* parental strain A449 was used to generate mutant strains in the outer bacterial transmembrane pore, AscCΔ and in effector genes, Aop3Δ. Mutation had no effect on expression of the inflammatory mediators IL-8, IL-1 and TNFα at high bacterial concentrations (O.D. 0.1). Expression of IL-12 was significantly lower in AscCΔ such that there was no significant stimulation compared to PBS control, whereas A449 and Aop3Δ challenge resulted in an up-regulation of IL-12 in AHKLs 2- and 4- fold higher than PBS, respectively. The parental strain also elicited a significant increase in IL-10 expression at high bacterial concentrations (5.5x), whereas the two TTSS mutants had no effect on the expression of this cytokine. Inducible nitric oxide synthetase (iNOS) and arginase (I+II) genes were also significantly up-regulated in the parental and mutant strains. However, iNOS was significantly higher in Aop3Δ than the other strains, such that iNOS:arginase ratio was elevated in the effector mutant, whereas the ratio was depressed in the parental and AscCΔ strains, when compared to PBS control. Host cell invasion success and 24 h survival were also depressed in AscCΔ, as was 24 h survival in Aop3Δ, when compared to the parental strain. These results confirm that the TTSS is important in initial invasion and in survival within the host cell. It also suggests that *Aeromonas salmonicida* may enhance survival within the host cell through polarization of macrophages/leucocytes to an alternative activation state.

Benefits Of Hatchery Management Of Bacterial Kidney Disease Using ELISA-Based Culling Of Adult Chinook Salmon

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Idaho Department of Fish and Game (IDFG) has managed *Renibacterium salmoninarum,* the causative agent of bacterial kidney disease (BKD), in hatchery Chinook salmon *Oncorhynchus tshawytscha* dating back to 1969. We have successfully controlled clinical BKD by utilizing the following management strategy since 1993 consisting of: (1) intra-peritoneal injection of erythromycin to returning adult salmon; (2) iodophor disinfection of eggs during water hardening; (3) usually culling the eggs of females with ELISA optical densities greater than 0.25, to reduce the risk of vertical transmission; (4) application of two prophylactic feedings of erythromycin; (5) and when necessary, high BKD segregation groups are released separately from the low/negative BKD rearing groups. ELISA-based culling of broodstock is the cornerstone of this management strategy. The success of this program is documented by mortality records, routine inspection, and diagnostic sampling during the 18 month period of hatchery rearing and routine inspection sampling of returning adults. ELISA-based culling was implemented for three generations providing: (1) reduced pre-spawning adult mortality; (2) reduced mortality from ponding to release in juveniles from 7% to 1%; (3) increased smolt to adult survival; (4) and reduced the percent of eggs culled from 10% to 1%. Reduced risk of BKD epizootics at all five Chinook salmon hatcheries has prompted IDFG to examine ways to reduce erythromycin treatments of juveniles. The initial year of FAT and qPCR surveillance of mortalities during April through September has indicated that reduced treatments can be made without increased loss to BKD.

Development And Characterization Of Rifampicin Resistant *Flavobacterium psychrophilum* Strains And Their Potential As Live Attenuated Vaccine Candidates

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Previous studies have demonstrated that passage of pathogenic bacteria on increasing concentrations of the antibiotic rifampicin leads to the attenuation of virulence and these resistant strains may serve as live attenuated vaccines. Two rifampicin resistant strains of *Flavobacterium psychrophilum*, 259-93A.16 and 259-93B.17, were generated by routine passage on TYES plates containing increasing concentrations of rifampicin. Electrophoretic analysis of whole-cell lysates prepared from the parent and resistant strains identified numerous differences between the 259-93B.17 strain and parent strain, while there were no differences identified between the 259-93A.17 and parent strains. The LPS banding patterns were identical between all three strains. Bacterial challenges of rainbow trout (*Oncorhynchus mykiss* Walbaum) with the resistant strains demonstrated attenuation of the 259-93B.17 strain and reduced virulence of the 259-93A.16 strain at the challenge doses tested. Immunization of rainbow trout with the attenuated live 259-93B.17 strain by intraperitoneal injection and immersion resulted in significant protection against challenge with the virulent parent *F. psychrophilum* strain and fish exhibited elevated specific antibody titers. The results demonstrate that the attenuated 259-93B.17 strain may serve as an effective live vaccine for the prevention of *F. psychrophilum* infections.

Erratic Swimming Behavior Followed By Mortalities In Coho Salmon Fry Due To Infection By A Novel *Flavobacterium s*p.

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The Laurentian Great Lakes is a place of extreme complexity, in which its inhabitants are under constant threat from both endemic and invasive pathogenic organisms. Coho salmon (*Oncorhynchus kisutch*) fry being raised in a Michigan hatchery experienced elevated mortalities that eventually reached 30%. Moribund fish exhibited an erratic swimming behavior typical of Early Mortality Syndrome (EMS); however, treatment with thiamine did not decrease mortalities and thus excluded EMS as a likely cause. Bacterial cultures were taken from the kidneys and brains of moribund individuals using a needle, inoculated into Hsu-Shotts and Trypticase Soy Broths, and incubated at 22ºC for 48 hours. Bacterial growth was then sub-cultured onto Trypticase Soy and Hsu-Shotts agars for further purification. Brain cultures yielded pure, profuse growth that was effuse, filamentous, and yellow with spreading irregular margins on Hsu-Shotts agar. These Gram negative bacilli tested positive for the presence of flexirubin and catalase, but were cytochrome oxidase variable. Antibiotic sensitivity testing and subsequent treatment of the infection was a complete success. Amplification of portions of 16S rRNA and its subsequent sequencing and alignment with members of the family Flavobacteriaceae yielded 98-99% nucleotide homology with a novel *Flavobacterium* sp. (Y060) previously recovered from long fin eels (*Anguilla reinhardtii*) in South Africa. The results of experimental infections and the associated histopathological lesions will be presented. This report, as well as sequence data from other clinical cases involving *Flavobacterium* species, underscores the likelihood that numerous novel *Flavobacterium* species are capable of disease causation in fish and that the Great Lakes and its associated fauna will likely continue to be plagued by them.

Use Of Green Fluorescent Protein-Labeled *Edwardsiella ictaluri* To Investigate The Pathogenesis Of Enteric Septicemia In Channel Catfish

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Edwardsiella ictaluri carrying pAKgfp1 constitutively expresses green fluorescent protein (GFP). Here, GFP-labeled *Edwardsiella ictaluri* strain 93-146 was used to investigate the pathogenesis of enteric septicemia of catfish (ESC) following experimental immersion exposure. Channel catfish fingerlings were infected with fluorescent *E. ictaluri*, sampled at pre-determined time points, and flash frozen in liquid nitrogen. Through this technique, individual bacteria were visible within host tissues by fluorescence microscopy, and a complex infection cycle was detected. An initial bacteremia occurred just after bacterial penetration during which bacteria were phagocytized by blood leukocytes. Bacteria were transported to the melanomacrophage centers (MMC) in spleen and anterior kidneys. There, bacteria underwent replication. This was followed by a second episode of bacteremia and migration to the liver and posterior kidney, followed by colonization of other peripheral organs. *E. ictaluri* also migrated to the intestinal lumen, which appeared to constitute a route of bacterial dissemination into the environment. In addition to permitting us to describe the progression of ESC, this study confirmed the suitability of using GFP-labeled bacteria to study disease pathogenesis in channel catfish.

Characterization Of The *rrn* Operons In The Channel Catfish Pathogen *Edwardsiella ictaluri*

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Part of the *Edwardsiella ictaluri* genome sequencing project involved identification of ribosomal gene clusters (*rrn*) to ensure correct assembly of the genome. Using a genomic library constructed in pBK-CMV, portions of four *rrn* operons were identified and sequenced. Sequences were added into the working assembly to identify existing fosmid library clones containing entire *rrn* operons. Using this method, we determined that there are eight *rrn* operons with two of these being positioned in tandem to each other (169 bp separating them). The tandem *rrn* cluster was also seen in *E. tarda*. Discovery of this feature separates *E. ictaluri* and *E. tarda* from other sequenced members of the enteric family as they contain seven *rrn* operons. Complete characterization of the *rrn* operons allowed us to determine that there are 2 groups of *rrn* clusters based on the tRNA sequences in the intergenic spacer regions (ISR). We were able to clarify some findings cited in earlier studies regarding the homogeneity of isolates based on sequencing of ISR. We developed a diagnostic PCR method for *E. ictaluri* using the tandem *rrn* cluster and a previously identified intervening sequence that is a feature of *E. ictaluri* but not *E. tarda*. We tested this PCR method with 27 *E. ictaluri* isolates, 7 *E. tarda* isolates, and 12 other bacterial species that included other fish pathogens and other species in the *Enterobacteriaceae*.

Channel Catfish Anemia, A Review and New Insights

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Originally reported in 1983, channel catfish anemia (CCA), also "white lip" or "no blood," represents the most important noninfectious disease affecting production in MS. The disease is characterized by lethargy, anorexia, extreme pallor, and hematocrits often below 5%. Records from the National Warmwater Aquaculture Center reveal that on average, CCA was diagnosed in 4.0% of cases from 1997 to 2006. Despite its historical prevalence, a definitive cause for CCA remains elusive. Known infectious agents, parasites, and common water quality variables have been largely ruled out and no natural or anthropogenic contaminants have been found in feeds. Research has focused on feed related causes, primarily overt folic acid deficiency or microbially induced degradation of folic acid to the folate antagonist pteroic acid. However, anemias of the magnitude seen with CCA have not been reproduced by the feeding of experimental diets. Contrary to published reports, records indicate an insidious condition where fish in certain ponds have been diagnosed with CCA for up to four consecutive years and individual outbreaks have persisted for at least 5 months. The disease also occurs during winter months when feed is not being offered. These findings prompted an investigation into the iron status of CCA affected fish and revealed indices consistent with iron deficiency. Furthermore, the administration of parenteral iron produced complete recovery and returned iron indices to within the ranges of normal controls. Despite these new findings, factors predisposing a state of hypoferremia remain unknown and an area of active study.

The Myxozoa: New Species And Old Species Behaving Badly

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Although the first description of Myxozoa in fish occurred in the 19th century and there are currently over 2,000 myxozoan species described; much debate still remains about these parasites. While molecular data has aided in the completion of many myxozoan life cycles, it has also opened Pandora's box; bringing into question the taxonomy, host range, life cycles and validity of many of the described species. *Henneguya* spp. infections in channel catfish (*Ictalurus punctatus*) have proven to be excellent models to study the Myxozoa because of the number of species reported, their frequency of occurrence, and the significant pathology associated with one of these species, *H. ictaluri*. Research by our group has identified a new species, *H. sutherlandi*; the myxospore for *Aurantiactinomyxon mississippiensis*; reported a new host location for *H. exilis* (caudal fins and kidneys)and found differences in host susceptibility to *H. ictaluri* in channel catfish versus blue catfish, *I. furcatus*(less susceptible).

Impact And Treatment Of Trematodes In Catfish Culture

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The trematode identified as Bolbophorus sp can cause significant production losses in commercially raised channel catfish. A disease-monitoring and production efficiency study was conducted on a commercial catfish operation to assess the economic impact of this disease evaluate treatment strategies. Fish from each pond were sampled and each pond placed in categories of light, moderate, or severe infection. Ponds that did not contain samples of infected fish were categorized as negative. Of the 40 ponds sampled, 17 were categorized as negative, 7 as light, 5 as moderate, and 11 as severe. Fish from trematode positive ponds were shown to consume significantly less feed compared to fish from negative ponds. Relative to uninfected ponds, estimated fish production was reduced by 14, 35, and 40% in populations with light, moderate, and severe infections, respectively. Ponds in the negative category produced 6030 lbs/acre resulting in net returns of $617/acre. Net returns for ponds in the light category were reduced by 80.8% and production from ponds in the moderate and severe categories resulted in net losses of $506 and $631, respectively. A targeted approach to controlling snail populations in trematode positive ponds was developed using hydrated lime and copper sulfate. Hydrated lime was applied to the littoral zone at a rate of 1 lb/1 ft of pond bank during the summer months, followed by the application of copper sulfate at a rate of 3-5 ppm during the fall and early spring. All ponds were re-evaluated during the following production season and with exception to one pond, active infections were not detected in treated ponds. This study shows that trematode infections reduce fish production and economic returns, but the disease can be effectively controlled on a commercial scale with chemical treatments.

Enteric Septicemia Of Catfish: The Commercial Catfish Industry's 'Thorn In The Flesh' For 30 Years

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Enteric septicemia of catfish (ESC) was first documented in the late 1970's. The disease is caused by the Gram-negative enteric bacterium *Edwardsiella ictaluri*. Since its discovery, it has become endemic in the catfish industry causing disease outbreaks on over 60% of farms and resulting in tens of millions of dollars lost annually. Research in the past 10 years has focused on a number of areas due to the economic importance of this disease, but has primarily been in three general disciplines: 1) bacterium genomics, factors associated with virulence, and diagnostic techniques 2) host genomics, breeding, and immunological response 3) methods of prevention, management, and control including vaccination and antibiotic treatments. Research on this bacterium and disease is continuing with whole genome sequencing, fish breeding programs that include the use of hybrid blue catfish x channel catfish backcrosses, 2nd generation vaccines, and the potential exploration of biocontrol measures such as host-specific bacteriophages. This presentation will give an overview of the research progress made in the past several years and future directions.

Isolation, Culture, Susceptibility Testing, And Identification Of *Flavobacterium columnare*

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Media investigated for primary isolation of *Flavobacterium columnare* included both selective and non-selective Cytophaga agar (CA), Hsu-Shotts (HS), Shieh (S), tryptone yeast extract (TYE), dilute Mueller Hinton agar (DMH) and *Flavobacterium columnare* growth medium (FCGM). Selective cytophaga agar performed most consistently as a primary isolation medium although selective S and selective HS also performed well. The DMH medium formulation for disk diffusion susceptibility testing contained 4.0 g MH and 17 g of agar per liter and was fortified with 5% equine serum. This medium gave the best growth and the highest zone definition. Broth microdilution broth testing, utilizing the Sensititre® Aquaculture microtiter plates (Trek Diagnostic Systems, Inc.), was evaluated using clinical isolates of *F. columnare* and *Escherichia coli* ATCC 25922 as a quality control standard organism. Broth microdilution testing was performed using DMH medium at 28°C. Methods used successfully for the identification of *F. columnare* were the Griffin screen, and the polymerase chain reaction (PCR) Darwish et al. 2004. The API system was not found to be appropriate for the identification of *F. columnare.* Isolates of *F. columnare* from different geographic locations and different fish species were grouped into three major genotypes by random amplified polymorphic DNA(RAPD) analysis and 2 major groups by pulsed field gel electrophoresis (PFGE).

The *Vibrio* Core Group Induces Yellow Band Disease In Caribbean And Indo-Pacific Reef Building Corals

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Yellow band disease (YBD) is among the most significant coral diseases. The etiological agent of YBD is a consortium of *Vibrio* species, *Vibrio rotiferianus*, *V. harveyi*, *V. alginolyticus* and *V. proteolyticus*. This consortium affects the *Symbiodinium*, or zooxanthellae "*in hospite"* leading to degenerative and deformed organelle structures, vacuolated cell structures, and lack of photosynthetic pigments in coral specimens collected from the field and in laboratory. Infected corals show a decrease in the rate of zooxanthella cell division compared to healthy corals. These *Vibrios* have been isolated from diseased *Diploastrea heliopora., Fungia spp* and *Herpolitha spp.* reef building corals displaying pale yellow lesions, similar to those found on Caribbean *Montastraea spp.* with YBD. This consortium directly attacks the *Symbiodinium spp.* or zooxanthellae within the gastrodermal tissues, leading to degenerative and deformed organelle structures, and lack of photosynthetic pigments *in vitro* and *in situ*. Infected *Fungia spp*. also show a decrease in the rate of cell division compared to healthy zooxanthellae from 4.9% to 1.9%, (*P=>0.0024*) and a similar trend in infected *Diploastraea heliopora.* from 4.7% in healthy tissues to 0.7% (*P= >0.002*). The virulence of these pathogens is having a major impact on the survival of these important reef building corals.

Antibacterial Chemical Defenses In Corals: Widespread But Selective Resistance To Bacterial Pathogens

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One potential mechanism of resistance to disease in corals is the production of antibacterial chemical defenses. To determine whether corals produce antibacterial chemical defenses, we tested aqueous extracts from Caribbean and Pacific corals in bacterial growth assays using a series of test strains including known coral pathogens, potential marine pathogens found in human waste and bacteria identified from the surfaces of corals. Extracts from three species of Hawaiian corals exhibited high levels of antibacterial activity. Activity varied at the coral colony, population and species level, and was highly selective against different bacterial strains, rather than broad-spectrum in nature. In addition, some extracts stimulatory to certain bacteria. Caribbean corals also exhibited widespread antibacterial activity, and these were also highly selective in their activity against the different bacterial strains. Extracts from several Caribbean species stimulated the growth of Caribbean coral pathogens, providing evidence for a correlation between coral chemical defenses and disease incidence. Antibacterial chemical defenses clearly have the potential to provide corals with protection from bacterial pathogens. The high degree of selectivity observed is necessary so that the coral can maintain its naturally associated microbial community yet still ward off potentially harmful bacteria. Differences in levels or types of defenses may represent a mechanism by which variability in resistance or susceptibility to pathogens might be realized, and may provide insight into patterns of disease incidence and prevalence on coral reefs.

Antimicrobial Activity In The Common Seawhip, *Leptogorgia virgulata* (Cnidaria: Gorgonaceae)

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Antimicrobial activity was examined in the gorgonian *Leptogorgia virgulata* (common seawhip) from South Carolina waters. Detection of antimicrobial activity in crude extracts of *L. virgulata* was determined by liquid growth inhibition assays using several bacterial isolates. This represents the first report of antimicrobial activity in *L. virgulata*, a temperate/sub-tropical coral of the western Atlantic Ocean. Partial purification was accomplished by reverse-phase HPLC; identification of active compounds in the effluent was guided by growth inhibition assay of individual fractions. Further purification and isolation of active fractions was conducted by HPLC–mass spectrometry followed by structural characterization by 1H and 13C NMR spectroscopy. The presence of homarine and a homarine analog, well-known emetic metabolites previously isolated from *L. virgulata*, was confirmed in the antimicrobial-active fractions. This study provides evidence that homarine is an active constituent of the innate immune system in *L. virgulata*. We speculate it may act synergistically with cofactors and/or congeners in this octocoral to mount a response to microbial invasion and disease.

Preliminary Investigations Into Metabolic Profiling of Coral Surface Microbial Communities

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As part of a larger project involving coral reef restoration in the Florida Keys using colonies derived from aquacultured fragments, we attempted to characterize the metabolic diversity of the surface microbiota of seven species of Atlantic Scleractinia using Biolog® EcoPlates™ under different culture conditions (open ocean "control" site, land-based flow-through system, and greenhouse recirculating system) and during times of disease. Samples of the surface mucopolysaccharide layer (SML) from two fragments of each species from the two land-based facilities were obtained after a six-month period in culture (December 2006). These samples were obtained by direct suction with a syringe and used to inoculate the EcoPlates™. Turbidity and tetrazolium peak data were collected for each sample every 12-24 hours for a 192-hour period. At that same time, those fragments that passed a health certification process were then transplanted to the field site with the "control" fragments. Subsequent SML samples from all three groups were obtained in May, August, and December 2007. Microbial community analyses at the 72-hour time point using a Jaccard Index revealed similarities in the metabolic profiles of the coral SML at the two land-based culture sites as well as in comparing those to samples from the open ocean site. In contrast, comparison of healthy to diseased samples revealed differences in the metabolic profiles obtained.

Coral Disease and Health Consortium (CDHC): Incorporating Virtual Slide Technology Into Coral Pathology

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Virtual slide technology in the simplest terms is an advanced communication tool for microscopic anatomy and pathology that provides World Wide Web access to view, pan and zoom images via computer, thereby emulating the viewing of entire glass histology slides under a microscope. This technology may also be used to convene virtual conferences for case studies or for teaching purposes. The CDHC and the Marine Mammal Health and Stranding Response Program are working together to bring this capability to the marine conservation medicine community. Our goals are to facilitate the collection and dissemination of reference data; assist consultation and conferencing for cases, help standardize terminology and support outbreak investigative responses in unusual mortality events; train students, pathologists, and marine biologists through virtual rounds and standard cases; and provide distance learning for normal histology and pathology for threatened or endangered species. Although we are in the early stages of development for these systems, we hope to give researchers additional tools for improving conservation efforts to detect and assess trends in disease, determining the cause of diseases, and collecting and disseminating reference data on the health of all marine animals.

Comparative Defense Mechanisms In *Pseudopterogorgia americana* And Other Gorgonians

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A number of functions have been proposed for gorgonian surface sclerites. These include mechanical and protective functions. Sclerites can also be clear or pigmented. An increase in pigmented sclerites is indicative of stress or disease. Recently, the sclerite pigment produced by *Gorgonia ventalina* was shown to be a carotenoid with a polyene chain containing between 14 and 15 carbon double bonds. Carotenoids can act as antioxidants and have antifungal activity. Here, we performed a spectrophotometric comparison of pigments isolated from a number of gorgonian species. A variety of carotenoids were found among the different species. Further chemical characterization using Raman scanning electron microscopy is being performed.

Histology And Histopathology Of The Fish Liver: Worst Case Scenario

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The fish liver is a frequent target for infectious diseases, toxic insults, and carcinogenesis. This organ resembles the mammalian liver in many respects, although there are a few anatomical, biochemical, and physiological differences that may influence the comparative response of the fish liver to injury. Histopathologically, reactions of the hepatic and biliary systems to deleterious stimuli can include various types of degenerative and regenerative changes, patterns of necrosis, stages of inflammation, and/or categories of primary tumor formation. Developmental anomalies and metastatic neoplasms may also occur but are less commonly observed. Histopathologic examination of the fish liver can be an important tool for monitoring effects of environmental pollutants, and potentially useful parameters include the degree of hepatocellular vacuolization, the tinctorial quality of the liver cell cytoplasm, the relative number and size of pigmented macrophage aggregates, and the presence of altered hepatocellular foci and benign or malignant tumors. Accurate assessment of histopathologic changes, including the ability to distinguish these from histologic artifacts, is dependent on the use of appropriate methods for tissue collection and slide preparation, and adequate numbers of samples that incorporate control or reference site fish.

Histology And Histopathology Of The Nervous System Of Fish – This Is Your Fish's Brain On A Glass Slide

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Complete necropsy of fish, like that of other animals, requires gross and microscopic examination of the nervous system, e.g. brain, spinal cord and peripheral nerves. The brain of fish can be divided into five anatomic regions that provide structural and functional organization. Telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon are identifiable. Relative sizes and shapes of these five regions vary depending on the species; however, structures referable to each region can be identified grossly and microscopically. Similarly, the spinal cord is organized into a pattern of gray and white matter distinct from that of the various regions of the brain. Peripheral nerves are important components of tissues outside the central nervous system and are often identifiable in histologic sections. Cellular constituents vary morphologically and functionally as well, and are organized into particular patterns in specific regions of the brain and spinal cord. Changes in histologic presentation of cells may be the result of aging, reactivity to stimuli, or artifacts of processing. Lesions to nervous tissues may result from a variety of pathologic processes. Knowledge of normal gross and histologic anatomic features of the brain, spinal cord and nerves together with routinely encountered morphologic variation is essential for accurate assessment of nervous system lesions.

Microscopic Examination Of The Cardiovascular System

Scott P. Terrell

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The fish heart is unique in its physiology and function. Possessing only a single atrium and ventricle, the fish heart is the most primitive of the vertebrate hearts yet it functions as much more than just a "blood pump". The four components of the fish heart include the sinus venosus, atrium, ventricle, and bulbus arteriosus. All of the cardiac components are lined on the lumenal (endocardial) surface by endothelial cells and on the epicardial surface by mesothelial cells. The sinus venosus is composed of a thin connective tissue matrix separating the endocardium and epicardium. Rare myocardial cells may be seen as well. The atrium has a sac-like trabecular architecture. Atrial trabeculae are composed of branching thin myocardial fibers lined by atrial endothelial lining cells. It is here that the structure and function of the fish heart diverges from most other vertebrates. The atrial endothelial lining cells serve as a component of the reticuloendothelial system and play a role in phagocytosis and antigen trapping. As a result, these atrial endothelial cells are a component of the innate immune system in fish and thus are of vital diagnostic importance for the histopathologist. The ventricle has similar architecture to that of the atrium but the muscular trabeculae are much thicker. In addition, depending on the species, the ventricle may have an outer compact muscle layer surrounding the inner trabecular muscle. The compact muscle layer is more prominent in larger fish and fish that are active swimmers. The final component of the heart, the bulbus arteriosus, is comprised mostly of elastin fibers with interspersed myocardial fibers. This elastic organ plays a role in blood pressure regulation. Due to the complex nature of the elastin fiber organization, the bulbus may also play a role in the trapping of large antigens or infectious agents. The physiology and function the fish heart belies its relatively simple anatomic structure. It because of this physiology and function, that the fish heart is an important diagnostic specimen for the fish pathologist.

Pathology Of The Gills

Mac Law

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The gills of teleost fish are a rather unique respiratory system in the animal world. Yet, the fundamental reactions of the gills to tissue injury ("disease") are similar in many ways to other tissue types. Essentially a bag of delicate capillaries covered by a thin epithelial layer, they are continually exposed to a hostile aquatic environment. Thus, the gills are a particularly vulnerable target of disease. A significant factor in gill injury is loss of critical surface area for gas exchange. Like many tissues, the responses of gills to injury are often stereotypical, and include epithelial hyperplasia (thickening); increased mucus production; and atrophy, clubbing, and/or fusion of secondary lamellae. It is also important to note that, since morphological changes often lag behind functional impairment, lesion morphology does not correlate well in acute disease insults. This talk will highlight the basic mechanisms of gill injury, and then illustrate these lesions using several case examples.

Piscine Kidneys - Tubular and Interstitial Response To Insults

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The piscine kidney lies ventral to the vertebral column and is sometimes a fused, single body running just caudal to the head and to the trunk. However, with approximately 25,000 fish species, gross morphological and histological variations exist. Like mammalian kidneys, the typical piscine nephron consists of the renal corpuscle, the renal tubules, the collecting ducts and the ureters. The renal corpuscle consists of the glomerular tuft and Bowman's capsule while the renal tubule components include the neck segment, the proximal tubules, the intermediate tubules and the distal tubules. Inter and intra-species variations sometimes exist for the various segments and can complicate interpretation of lesions. The other significant differences from mammalian kidneys that make interpretation of lesions difficult include hematopoiesis that occurs mainly in anterior or head portion of kidney, the presence and numbers of melanomacrophage centers as well as endocrine elements(thyroid follicles, Chromaffin cells, corpusles of Stannius, and inter-renal tissue). Both hematopoiesis and melanomacrophage centers often make discernment of subtle inflammatory processes difficult while the latter group may be confused as neoplastic proliferations. The piscine kidney has a dual circulatory supply i.e. renal artery and renal portal system, which increases resistance to anoxic insults. However, like the mammalian kidney, it can be subjected to bacterial, viral, parasitic, toxic and neoplastic insults resulting in somewhat similar types glomerular and tubular responses. The interstitial responses are perhaps a little more unique to fish.

When The Skin Wears Thin: An Introduction To The Histology Of Normal And Abnormal Fish Integument

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The skin of fish consists of a living non-keratinized, stratified squamous epithelium that sits on a basement membrane and is supported by a dermis and hypodermis. The epidermis contains mucus secreting goblet cells that produce the surface cuticle and form a first line of defense against invading microorganisms, reduce drag, and aid in osmoregulation. The epithelium of some species (cyprinids and catfish) also contains pheromone producing alarm or club cells. When damaged these cells release a pheromone that frightens away fish of the same species. Additional structures found in the epithelium include taste buds, mechanoreceptors, and migrating leukocytes. Present beneath the epithelium is the connective tissue dermis that gives rise to the scales, contains chromatophores, and the mechanosensory lateral line system. A spongy hypodermis of connective tissue and adipose serves as a conduit for blood vessels and nerves and separates the dermis from underlying muscle. Skin lesions are common in fish due to its location and delicate nature. A host of parasites, bacteria, viruses, and fungi target the skin of many fish species either specifically or non-specifically. Regardless, once this primary barrier is breached, minor lesions can rapidly progress, leading to osmotic disturbances, systemic infections, and ultimately death.

Two Shocking Cases: Anesthesia And Surgery Of The Electric Eel, *Electrophorus electricus*

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Two, adult, electric eels, *Electrophorus electricus*, were presented on separate occasions to the Veterinary Service at the John G. Shedd Aquarium for evaluation of head masses. Diagnostics on each fish included anesthesia for radiography, ultrasonography, and surgical biopsy. The biopsy from the first case returned a diagnosis of primitive neuroectodermal tumor (PNET). The eel was anesthetized a second time for magnetic resonance imaging and surgical resection. The second eel had a subcutaneous pneumocyst of undetermined etiology which was treated surgically. Electric eels are strongly electric fish capable of producing discharges in excess of 600V and are obligate air breathers, making them a challenge to handle safely and efficiently for veterinary procedures. Specialized, non-conductive equipment for restraint and ventilation were constructed which allowed the eels to be safely handled by staff.

Cryosurgery On A Premaxillary Fibrosarcoma From A Chain Pickerel (*Esox niger*) Using An Over-The-Counter Wart Remover

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A chain pickerel (*Exox niger*) at a public aquarium developed a firm smooth raised 1 cm diameter mass on the right premaxilla that reduced the exhibit quality of the fish. An excisional biopsy was performed under general anesthesia with MS-222, with a single NSAID treatment for post-operative analgesia. Boney involvement prevented complete removal of the mass. Histologic diagnosis was a fibrosarcoma with incomplete margins. The mass rapidly recurred and enlarged. A radical excision was not considered feasible because of the likely disfigurement and impairment of feeding ability. Cryosurgery was elected as a means to devitalize the deep margins while retaining structural integrity of the premaxilla. Because of the facility location and logistics involved in transporting liquid nitrogen or a cryosurgery unit, an over-the-counter wart removal system based on a dimethyl ether/propane/isobutane canister was used. Two months following the initial biopsy, a deeper excision was performed. The surgery bed was subjected to three freeze-thaw cycles with a 2 mm blanch zone using the wart removal system. The surgical wound healed rapidly, with return to a cosmetically-acceptable appearance, and retention of normal function, although eventual recurrence is considered likely.

Dermal Fibroma On A Redtail Catfish, *Phractocephalus hemioliopterus*

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A redtail catfish, *Phractocephalus hemioliopterus*, in the reproductive research collection at the University of Florida Tropical Aquaculture Laboratory presented with large, coalescing masses on the dorsolateral caudal peduncle. The fish was anesthetized and the masses were surgically removed. Recovery was uneventful. Histopathological evaluation of the masses revealed a benign dermatofibroma. Although dermal fibromas have been reported in a variety of fish species, only one prior submission of a tumor from a redtail catfish, a benign lipoma, had been submitted to the Registry of Tumors in Lower Animals.

Help! I've Got A Chicken Bone Stuck In My Ovisac: Considerations For Complex Ovariectomy In An Irish Lord (*Hemilepidotus hemilepidotus*)

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A Red Irish Lord, *Hemilepidotus hemilepidotus*, presented with an acute history of anorexia, bloated abdomen, and tachypnea. Palpation revealed a firm partially moveable mass in the caudal abdomen. Diagnostics including gastroscopy, cloacoscopy, and contrast radiographs localized the mass to the reproductive tract. Surgical correction utilizing radiosurgery demonstrated a bi-lobed mass of necrotic material within the ovisac. Post-operative care included nutritional support, analgesia, antibiotics, and hormone therapy. Further communication has demonstrated multiple cases of dystocia in this species warranting further research into future monitoring and prevention options.

Comprehensive Wound Care Management In A Cownose Ray (*Rhinoptera bonasus)*, A Spadefish (*Chaetodipterus faber*) 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, a spadefish 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.

Management Of Chytridiomycosis In *Dendrobates* At The New England Aquarium

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Chytridiomycosis is a fungal disease, which is widely spread among wild and captive amphibians worldwide. It has been suggested as one of the causes for the rapid decline in amphibian populations worldwide. In 2006 ~ 2007, New England Aquarium experienced an outbreak of Chytridiomycosis in newly acquired *Dendrobates* species (*D. leucomela, D .auratus, D. tinctorius*). Clinical signs consisted of depression, anorexia, weight loss, and sudden death. Histological examinations of deceased animals' tissue revealed chytrid zoospores and thallis. Subsequently, 50% of the surviving animals and their tanks were swabbed for chytrid PCR testing. 80% of the tested animals and 3/4 of the tanks tested positive for chytrid fungus. All animals were treated daily with 5-minute, 0.1% itraconazole bath for 10 days, while maintaining the tank temperature at 27 ~ 28◦C. The tanks were disinfected daily with 1% Virkon. Only 5 out of 21 animals survived this outbreak. The current aquarium protocol includes swabbing of 50 % of incoming animals for PCR testing and prophylactic itraconazole bath on all animals. Since the institution of the current protocol, the recurrence of chytridiomycosis has been successfully prevented.

Milbemycin Treatment Of Parasitic Copepods On *Acropora* Corals

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'Red bug' (*Tegastes acroporanus*) is a harpacticoid copepod which lives and feeds only on corals in the genus *Acropora.* It can reduce polyp extension and coral growth. Milbemycin oxime has been used as a bath treatment by numerous coral hobbyists and professionals but has not been reported in the scientific literature. The parasite was first noted at the National Aquarium in Baltimore in a 6,400-gallon artificial seawater exhibit containing various hard corals (*Acropora*, *Pocillopora*, *Montipora*, *Caulastrea*), soft corals and anemones, echinoderms, molluscs, polychaetes, crustaceans and Perciforme fish**.** Following treatment trials on various fish and invertebrates, the tank was treated with milbemycin oxime (Interceptor, Novartis) at 16 µg/L as an 8-hr bath. This dose is approximately equivalent to one 23-mg-dose tablet for 51-100 lb dogs per 380 U.S. gallons. The drug was removed using flow-through water changes and the addition of activated carbon. This was repeated every seven days for three treatments. No adverse effects were noted in any corals, echinoderms, polychaetes, or fish. Mortalities were seen in hermit crabs (*Clibanarius tricolor* and *Paguristes cadenati*) and amphipods. Since treatment, no *Tegastes acroporanus* copepods have been seen. Amphipod populations have rebounded to pretreatment levels. The *Acropora* corals have shown an increase in polyp emergence and calcium consumption as well as improved coloration.

The Veterinarian's Role With Aquatic Invasive Species

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This lecture will explore the role of aquatic animal health specialists in public aquaria from water discharge for NPDES permits, to the migration and transport of invasive species as asymptomatic disease carriers, and for identifying new species outside of historical geographical ranges. In 2001, Dr. Judith Pederson, Director of MIT Seagrant, approached the New England Aquarium (NEA) to provide a lecture describing the role of public aquaria in invasive species for the MIT Seagrant Conference on Bio-Invaders, May 22, 2002. From that lecture, 'Bio-invaders in the Aquatic Realm-Cichlids to Lionfish", the NEA was invited to officially represent public aquariums on the Northeast Aquatic Nuisance Species (NEANS) Panel in 2003. This presentation highlights the aquatic animal health perspective from serving as both a panel member and co-chair for the Science and technology committee on NEANS from 2003 until May, 2007. Some highlights include a Citizens Monitoring of Marine Invasive Species workshop co-hosted with the MA Department of Coastal Zone Management on October 23, 2006, and development of a science program for the NEANS Panel meeting in November, 2006 in Portland, ME, which featured invasive pathogens of shellfish and showed the interconnectedness of hydrilla with a disease causing cyanobacteria in waterfowl and raptors.

The Effects Of Rearing Density And Diet On Fin Condition, Intestinal Histopathology, And Fillet Quality Of Rainbow Trout *Oncorhynchus mykiss*

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A 2X2 factorial study was conducted using four replicated groups of rainbow trout *Oncorhynchus mykiss* raised from sac-fry to 600g in twelve circular tanks (500 liters) with flow-through water at approximately 12.5°C. Each experimental group was reared at one of two densities (20-40 kg/m^3 or 40-80 kg/m^3), and each group received one of two different open-formula feeds (a fishmeal- and fish oil-based diet, or a grain-based diet). Both diets were formulated to have the same caloric content (40% digestible protein and 15% crude fat). Performance was evaluated through growth rate, feed conversion rate, and survival; fin condition was assessed by sampling 25 fish per tank and calculating fin indices (fin length standardized by fork length) for all rayed fins at approximately 15g, 150g, and 600g mean fish weight; distal intestines were sampled from 5 fish per tank for histopathological evaluation; and fillet quality was assessed through total PCB assays, fatty acid profiles, and pesticide screens at the study's end. Analyses of all these data are currently being performed, and results and conclusions will be presented.

Fish Health Survey of Ten Lake Winnipeg Species

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The Red River International Joint Commission has undertaken a three-year study of the impact of drainage from Devil's Lake to Lake Winnipeg, via the Cheyenne and Red rivers. The FPL undertook the light microscopy portion of the survey. Six hundred fish were examined in 2006-07 and 300 further fish (60 and 30, respectively of each species) in 2007-08. A particularly notable group of lesions included a proliferative endocarditis and multifocal myocarditis in walleye, and to a lesser degree in sauger, associated with few larval trematodes. Eggs were also seen rarely in the heart associated with the myocarditis and commonly in walleye lodged in lamellae. Sixty-two and forty-two percent of the walleye and sauger, respectively, from 2006-07 had lesions. Our goal is to use laser microdissection to attempt to classify this and other organisms noted during the survey.

Disease Assessment And Mortality Of Channel Catfish Fingerlings Following Transport And Cage Stocking

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Transport of catfish purchased to Virginia from out-of-state producers can require in excess of 20 hours causing stress on fingerlings. This study examined short-term (3-week) survival of catfish following transport and stocking into cages. Fish were delivered in June and September, 2007. Sixty fish were randomly sampled at initial stocking (Week 0) and at weekly intervals for three weeks post-stocking to assess the prevalence of parasites of the skin and gills. Catfish were free of clinical signs of diseases at the initial stockings, while gills of several of the June catfish (5%) indicated that Proliferative Gill Disease had been present. In June, fish started to die from external Columnaris within two days of the initial stocking and ESC was also recovered from these fish. Gill worms (*Ligictaluridus*) and *Henneguya* cysts were observed at Week 0 and *Ichthyophthirius multifiliis* (Ich) was present at Week 2 for both groups. Ich was observed among 18% of June fish and among 83% of September fish. Ich was no longer observed by Week 3 of the June study but was still present by the end of the September study. Total mortality was approximately 50% for the June group, whereas there was no significant mortality for the September group. It appears that the Spring fingerlings were subject to more environmental and handling stress compared to the Fall fingerlings.

Laboratory Efficacy Of Oxytetracyline And Amoxicillin For The Control Of *Streptococcus iniae* Infection In Tilapia

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Streptococcus iniae infection of tilapia is common in different parts of the world including the United States. *In vitro* and *in vivo* studies were conducted to assess the efficacy of oxytetracycline (OTC) and amoxicillin (AMX) for controlling *S. iniae* infection. *In vitro*, minimum-inhibitory-concentration testing of OTC and AMX against multiple *S. iniae* isolates revealed general sensitivity at concentration ranges of 0.25-0.5 μg/mL and 0.0156-0.5 μg/mL, respectively. *In vivo*, two separate experiments were conducted to assess the efficacy of OTC and AMX against an experimental *S. iniae* infection in tilapia; fish were challenged by waterborne exposure to *S. iniae* (after skin scraping). Each experiment had a nonchallenged nonmedicated group (negative control, a challenged nonmedicated group (positive control) and challenged groups fed different levels of the medicated feed. Oxytetracycline medicated feed was administered at concentrations of 25, 50, 75 and 100 mg active ingredient/kg of fish body weight (BW)/d for 14 d. Amoxicillin medicated feed was administered at concentrations of 5, 10, 30 and 50 mg active ingredient/kg BW/d for 8 d. The OTC at 50, 75 and 100 mg significantly increased the survival of challenged fish to 45, 85 and 98%, respectively, compared to 7% in the positive control. The AMX at 10, 30 and 80 mg significantly increased the survival of *S. iniae* infected tilapia to 45, 75 and 93.8%, respectively, compared to 3.8% in the positive control.

Putative Bacterial Etiologies For Smallmouth Bass (*Micropterus dolomieu*) Lesions In Three Virginia Rivers During 2007

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The Virginia Department of Environmental Quality and the Department of Game and Inland Fisheries, along with their partners on the Shenandoah River Fish Kill Task Force (including the USGS), have researched putative causes of the fish kills that have occurred in the Shenandoah River watershed among smallmouth bass (*Micropterus dolomieu*) and red breasted sunfish (*Lepomis auritus*) since 2004. The Shenandoah River has been one of the best smallmouth bass fisheries in the United States, but an estimated 80% of the adult smallmouth bass and redbreast sunfish were lost in 2004-2005. About 75 miles of the North Fork Shenandoah River were impacted in 2004 and 100+ miles of the South Fork and mainstem Shenandoah were affected in 2005. Adult smallmouth bass and redbreast sunfish exhibited bacterial lesions that resulted in mortality, following the first major precipitation/runoff event in the spring. At that time, other laboratories did not consider isolated bacteria to be anything more than secondary invaders in the disease process. In the spring and early summer of 2007, microbiologists at the National Fish Health Research Laboratory (NFHRL; - Kearneysville, WV) were asked to monitor bacterial populations on fish in the affected areas of the Shenandoah, Cowpasture, and James Rivers. In each case, *Aeromonas salmonicida* was unexpectedly cultured from lesion materials on smallmouth bass. In samples from the North Fork of the Shenandoah River, Aeromonas *salmonicida was* isolated from 8 of 10 fish with lesions and accounted for 65.2% of the total microorganisms (508) that were cultured. The remainder of the bacterial species consisted of facultative pathogens (*Aeromonas veronii* subsp. s*obria, Plesiomonas shigelloides, Aeromonas hydrophila, Aeromonas trota*, and *Aeromonas veronii* subsp. *Veronii*). *Aeromonas salmonicida* was also isolated from nine of ten gills examined from these same fish and constituted 50.3% of the total bacterial flora (n = 443) that were identified. Similar isolations were made in the South Fork, as well as from the James and Cowpasture Rivers. These isolations provided evidence that *A. salmonicida* was indeed the putative etiological agent of lesions and mortalities associated with smallmouth bass in the Shenandoah River watershed during the spring of 2007.