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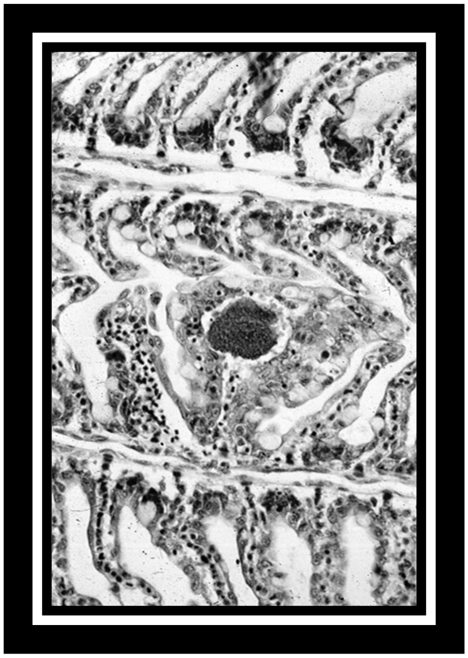
2015



40TH

MARCH 2 – 6, 2015
CHARLESTON, SC

Program of the:

40th Annual
Eastern Fish Health Workshop

2 March – 6 March 2015
Mt. Pleasant Holiday Inn
Mt. Pleasant, South Carolina

Foreword

Welcome to Mt. Pleasant and Charleston! We are so happy to be returning to this wonderful southern city full of history and charm. We are sure that those coming from the Northeast will be enjoying the lack of 100 inches of snow in the area. As we gather again this year, we hope to continue sharing aquatic animal health science and foster long-lasting relationships with our colleagues. Every year it is important to take a step back and appreciate the variety of topics that are presented, and to consider how important they are to aquatic animal health. 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, university personnel, and veterinarians working within marine and freshwater environments have always been encouraged. This meeting continues to be an entity unto itself and is not affiliated with any parent society. It is scientists hosting scientists. We hope you find the meeting enjoyable and the contributions relevant and important. Welcome to the 40th Annual Eastern Fish Health Workshop.

In Memoriam:

Dr. Stephen L. Kaattari
September 22, 1951- November 11, 2014

Dr. Stephen Kaattari, CSX Professor of Marine Science, Emeritus at the William and Mary-Virginia Institute of Marine Science (VIMS), passed away November 11, 2014.

Steve was raised in Menlo Park, California, and was married to Ilsa Kaattari, a molecular biology specialist in at VIMS. He earned a B.S. degree at University of California-Davis in 1973, a PhD in Microbiology at UC-Davis in 1979, and a post-doctorate fellow at Oregon Health Sciences University. Steve was a professor in the Department of Microbiology at Oregon State University where he served on the faculty for 10 years (1982-1992). He joined the William and Mary faculty in 1993 and had retired in January 2014. He served as the Chair of the Department of Environmental and Aquatic Animal Health from 2002 to 2004.

Steve was a highly respected and internationally recognized researcher, as well as an extraordinary teacher and mentor. His research program melded basic research in immunology with applied goals to provide innovative and practical new tools for the scientific community and the public. His research has provided new understanding of basic immune functions and has produced vaccines for use in aquaculture. His more recent work included development of real-time sensor platforms that are now being used to analyze pollutants in the Chesapeake Bay. Products from his research have resulted in new patents in the United States and Britain and have led to new areas of research that continue today. Steve advised 20 graduate students, 11 post-doctoral trainees, numerous undergraduates, and served on more than 75 graduate-student committees.

During Steve's tenure at VIMS, he secured more than $8 million in competitive extramural grants and contracts to support his research program. He authored or co-authored more than 90 technical articles and books, and reviewed more than 350 proposals and over 400 manuscripts for the scientific community. He also served on numerous advisory committees for professional societies. In 2004, Steve was awarded the Special Achievement Award by the American Fisheries Society.

http://www.vims.edu/newsandevents/topstories/kaattari_stephen.php

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Ross University, School of Veterinary Medicine, St. Kitts, West Indies

Cheryl Woodley
NOAA-National Ocean Service, Charleston, SC

2015 Distinguished Friend Of The Eastern Fish Health Workshop

Frederick Godfrey Kern, III

Frederick Godfrey Kern, III was born in Woodbury (NJ) on 28 December 1941 to Elizabeth and Frederick G. Kern, Jr. Soon thereafter, Fred's father accepted a position as a chemical engineer in Baltimore (MD), which was where young Fred (a.k.a. "Pug") spent much of his childhood. After his parents divorced, Fred moved to Oak Harbor (Washington) with his mother and step-father, Herbert Miles. There, he explored the Cascade Mountains and tidal pools of Puget Sound, which evoked a keen interest in science and the outdoors. In 1959, Fred entered the University of Maryland at College Park and also worked as a biological aide with the Department of Interior's, Bureau of Commercial Fisheries (BCF) facilities in Annapolis and Oxford (MD), which researched the health of shellfish species in Chesapeake Bay. Fred's part-time collegiate career was interrupted in 1964, when he was drafted into the U.S. Army and was stationed for 18-months in Munich, Germany. After serving his country, Fred returned to the University of Maryland and completed his B.S. in Biological Sciences in 1970. Following graduation, Fred became a food inspector with the U.S. Department of Agriculture Six months later, however, Fred accepted a position as a Biological Technician at the Oxford Laboratory, which was then a component of the National Oceanic and Atmospheric Administration's (NOAA), National

Marine Fisheries Service. During his tenure at Oxford, Fred was enrolled in NOAA's Senior Scientist Academic Program and attended Johns Hopkins School of Hygiene in Baltimore, where he did graduate study under the mentorship of Dr. Frederick Bang. For 42-years until his retirement in 2007, Fred rose through the ranks and retired from federal service as the Officer in Charge of the Oxford Laboratory. He served in that capacity from 1987-1995 and then again from 1999-2004. Most of his career was invested in research to understand, diagnose, and mitigate the effects of diseases associated with marine mollusks and prevent introductions of harmful, non-indigenous marine species. He became recognized as a national expert on invasive species introductions from ballast waters of foreign vessels and served as NOAA's representative on the Aquatic Nuisance Species Task Force and as the U.S. representative to the International Council for the Exploration of the Seas. Fred also chaired the Green Crab Control Working Group that led to the development of the federal management plan for the highly invasive European Green Crab. Fred was a member the Biological Review Team that was convened to review the status of Eastern oysters in response to a petition to list the oyster as a threatened or endangered species. For such efforts, Fred received the U.S. Environmental Protection Agency's 2004 Bronze Medal for Commendable Service. Fred also served on the National Delegation of the United States-Japan Natural Resources Aquaculture Panel through the U.S. Sea Grant Program. He was a Past-Treasurer and member of the Society of Invertebrate Pathology, the Atlantic Estuarine Research Society, and the Coastal and Estuarine Research Foundation. As a member of the Oxford Laboratory, which co-founded the Eastern Fish Health Workshop with the Eastern Fish Disease Laboratory, Fred not only attended the inaugural meeting but remained steadfastly dedicated to the workshop throughout his career. He has served the workshop as co-chairman on multiple occasions and was a perennial moderator, special session coordinator, and presenter. He tirelessly worked behind the scenes to facilitate site-selections, organize social events, and be the focal point to increase attendance of marine researchers. Fred and his wife, Sandra, have been married for 44 years and reside in Easton (MD). They have two married children, Melanie and Fred IV (a.k.a. "Fritz"), who have blessed their parents with five grandchildren.

Monday March 2

5:00 – 7:00 pm Registration and Reception
Hors d'oeuvres And Cash Bar

7:00 Yanong Welcome To The 40th Annual Eastern Fish Health Workshop

7:10 Gauthier Dedication Of The 40th Annual Eastern Fish Health Workshop

7:30 At Some Point, I Really Need To Get Around To Publishing This!

Dave Marancik chairs the annual opening evening session of baffling, bewildering, and bemusing case reports that showcase the ingenuity and smarts of some of our aquatic gurus as they deal with unusual maladies and pesky pathogens. It's opening night and we expect a magical mix of education and entertainment.

7:30	Marancik	Yet Another Rare Gram Positive Pathogen	1
7:40	Alsina	Red Flags In Red Cichlids (*Mesonatua festivus*)	2
7:50	Getchell	If You See Wigglers, Blame It On Their Diet	3
8:00	Dill	What's That Growing On Your *Halichoeres bivittatus*?	4
8:10	Camus	A Floundering Heart	5
8:20	Good	Salmon Strangeness: A Mysterious Case Of Systemic Granuloma In Atlantic Salmon *Salmo salar* Raised In Freshwater RAS	6

8:30 Break

9:00	McDermott	Casting A Broken Unicorn Horn	7
9:10	Khoo	When More Is Actually Less – The Tale of Chronic Ammonia Toxicity	8
9:20	Fogelson	This Betta Be Cool	9
9:30	Cornwell	Spring Chinook: A Needle In A Haystack	10
9:40	Bowser	Identification Of An *Edwardsiella* Infection In Largemouth Bass In A Closed Recirculation Aquaculture System	11
9:50	Viadanna	First Report Of Carp Edema Virus In South American Koi	12
10:00	Atkinson	It Came From The Willamette River!	13

10:20 Announcements
10:30 Adjourn

Tuesday, March 3

7:00 **Breakfast**

Special Session 1: The New Kid On The Block

Esteban Soto chairs a Step by Step look at some important aquatic emerging pathogens that are Hangin' Tough.

8:15	Reichley	Recent Advancements In Our Knowledge Of *Edwardsiella piscicida* And *E. tarda*	14
8:30	Griffin	Biotic And Abiotic Factors Associated With Outbreaks Of An Emergent Strains Of *Aeromonas hydrophila* In Catfish Aquaculture	15
8:45	Welch	Weissellosis: An Emerging Disease Of Farmed Rainbow Trout	16
9:00	Loch	Novel Fish-Associated Flavobacteria Continue To Emerge In The Great Lakes Basin	17
9:15	Dill	Francisellosis In The Aquarium – Histopathology And Diagnostics	18
9:30	Waltzek	Genomic Characterization Of Carp Edema Virus	19
9:45	Camus	Novel DNA Virus Genomes Associated With Papillomatous Skin Lesions In A Giant Guitarfish	20

10:00 **Morning Break**

Special Session 2: East And West Side Stories: Death Of A Sea Star

Marta Gomez-Chiarri and **Alisa Newton** chair this session on the sea star epizootics occurring on both sides of the country.

10:30	Hewson	Facing An Old Foe On New Turf: Densovirus Associated With Pacific Sea Star Wasting Disease	21
10:45	Miner	Documenting The Impacts Of Sea Star Wasting Syndrome Through Long-Term Surveys And Identifying Possible Factors Contributing To Disease Presence	22
11:00	DelSesto	Sea Star Wasting Disease In *Asterias forbesi* In The Northeast United States	23
11:15	Newton	Current Pathology Findings In The Pacific Coast Sea Star Wasting Disease Mortality Event	24
11:30	Eisenlord	Turning Up The Heat On Sea Star Wasting Disease	25

11:45 **Discussion**

12:00 **Lunch**

General Session 1: Rod Getchell – Moderator

1:00	Piesz	Secretory/Excretory Products Of *Lepeophtheirus salmonis* Regulate The Migration Of Atlantic Salmon Leukocytes	26
1:15	Di Natale	Type-4 Ice Structuring Protein In Rainbow Trout	27

1:30	Li	Adjuvant Effect Of *Quillaja saponaria* saponin (QSS) In Turbot (*Scophthalmus maximus*) Upon Bath Vaccination	28
1:45	Zayas	Laboratory Controlled Challenges Of *Streptococcus* in Tilapia (*Oreochromis* sp.)	29
2:00	Standish	A DNA Vaccine, Based On The Viral Hemorrhagic Septicemia Virus Genotype IVb Glycoprotein Gene Protects Muskellunge (*Esox masquinongy*)	30
2:15	Smith	Clinical Signs And Liver Pathology Associated with Microcystin-LR Exposure in Tilapia, *Oreochromis* sp.	31
2:30	Faisal	Disease Risks To Inland Fisheries: How Effective Are Current Policies In Preventing Fish Pathogen Spread	32

2:45 **Discussion**

3:00 **Afternoon Break**

Special Session 3: Catching Up On Crusty Critters

Karen Burnett chairs a session highlighting recent research on health and disease of marine crustaceans.

3:30	Burnett	Reading The Shrimp Transcriptome: Responses To Low Oxygen And High Carbon Dioxide	33
3:45	Lee	Black Gill In Penaeid Shrimp: Pathology And Transmission Studies	34
4:00	Frischer	Molecular-Enabled Identification, Detection, And Seasonal Pattern Of The Causative Agent Of Shrimp Black Gill In The Coastal Southeast USA	35
4:15	Fowler	Observations Of Black Gill In White Shrimp (*Litopenaeus setiferus*) From South Carolina: Molting, Mortality, & Mobility	36
4:30	O'Shaughnessy	Ecological Effects Of The Invading Rhizocephalan Parasite *Loxothylacus panopaei* In The Flatback Mud Crab *Eurypanopeus depressus*	37
4:45	Schott	Spatially And Temporally Variable Prevalence Of A Virus Fatal To The Blue Crab, *Callinectes sapidus*	38

5:00 **Adjourn**

7:00 **Dinner**
Induction of Fred Kern as Our "Distinguished Friend"

Wednesday, March 4

7:00 **Breakfast**

Special Session 4: Coral Health

Cheryl Woodley chairs this annual session that always provides up-to-date information on the health of these important animals.

8:15	Richardson	‘Omics Comes To The Coral World	39
8:30	Bhedi	We Have A Quorum, Now What Are We Saying?	40
8:45	Vroom	Regeneration In Corallimorpharia	41
9:00	Horricks	Regeneration In The Caribbean Star Coral *Montastrea cavernosa*	42
9:15	Peters	Molecular Madness: The Case For Phenotypic Anchoring In Understanding Corals	43
9:30	Leporacci	The Effects Of Ocean Acidification And Climate Change On The Virulence Of Black Band Disease	44
9:45	Waikel	Potential Role Of Dimethylsulfoniopropionate In Coral Disease	45

10:00 **Morning Break**

General Session 2: Julie Cavin- Moderator

10:30	Phillips	Comparison Of Hematology, Plasma Biochemistry, And Blood Gas Parameters Between Two Venipuncture Sites In Southern Stingrays (*Dasyatis americana*)	46
10:45	Hesami	*Carnobacterium maltaromaticum* In Stranded Pacific Salmon Sharks And Common Thresher Sharks	47
11:00	Dennis	Clinicopathological Features Of A Multi-Species Aquatic Animal Mortality Event Associated With A Large Scale Harbor Development Project	48
11:15	MacDonald	Molecular And Histopathological Disruption Of Wound Healing In An Ectoparasite Model	49
11:30	Contador	Streptococcosis In Lake Trout (*Salvelinus namaycush)*	50
11:45	Subramaniam	*Megalocytivirus* Infection In Fish	51

12:00 **Lunch**

General Session 3: Ron Miller– Moderator

1:15	Miller	Demystifying Antimicrobial Susceptibility Testing: Resistance Does Not Always Mean Resistant	52
1:30	Soto	The F Is For Fastidious… The Challenge In Antimicrobial Susceptibility Testing Of *Francisella noatunensis*	53
1:45	Gaunt	Use of Minimum Inhibitory Concentration And Disk Diffusion Zone Data To Determine Epidemiological Cutoff Values Of Antimicrobial Agents Against *Edwardsiella ictaluri*	54
2:00	Yen	*In Vitro* Synergistic Effects Of Florfenicol, Oxytetracycline, And Sulfadimethoxine: Ormetoprim Against *Edwardsiella ictaluri*	55

2:15	Purcell	Avermectin Treatment For *Lepeophtheirus salmonis* And Effects On Salmon Immunophysiology	56
2:30	Poley	Sea Lice (*Lepeophtheirus salmonis*) Responses To Concurrent Drug Treatments With Emamectin Benzoate And Cypermethrin	57
2:45	Mohammed	Black Seed *Nigella sativa* And Its Oil Extract Protects Against Columnaris Disease in Zebrafish And Catfish	58

3:00 **Afternoon Break**

General Session 4: **Jill Arnold- Moderator**

3:30	Warg	Aquatic Animal Disease Testing Added To The National Health Laboratory Network: Phase I Is Underway	59
3:45	Shavalier	Propagation Of Lake Trout (*Salvelinus namaycush*) Cells *In Vitro*	60
4:00	Seelye	*Danio rerio* T cell Receptor Alpha/Delta Locus Organization And Phylogenetic Analysis Of V Alpha/Delta Region	61
4:15	Baird	Potential Impacts Of The Fathead Minnow Nidovirus (Family: Coronaviridae, Order: *Nidovirales*) On The Laurentian Great Lakes Native Cyprinids And Rehabilitation Programs	62

4:30 **Adjourn**

6:30-9:30 **Optional Dinner and Tour at the South Carolina Aquarium**

Thursday, March 5

7:00 **Breakfast**

Special Session 5: The Empire Strikes Back

Grace Karreman returns to chair the second installment of a biofilms session.

8:00	Whipps	What Doesn't Kill You, Will Grow In Your Biofilm: Zebrafish Facilities And The Diversity Of *Mycobacterium* Species In Biofilms	63
8:15	Sanders	I Though They Smelled Bad On The Outside… Routine Evaluation, Monitoring, And Sanitation Of Automatic Reverse-Osmosis Watering Systems	64
8:30	Soto	Differential Resistance Of *Francisella noatunensis* subsp. *orientalis* To Virkon And Bleach By Biofilms And Their Planktonic States	65
8:45	Good	Attack Of The Snails: Adventures In Biofilm Remediation To Reduce Off-Flavor In RAS Salmonids	66
9:00	Arias	Effects Of Calcium On Biofilm Formation Of Fish Pathogens	67
9:15	Gomez-Chiarri	Fight Them Using Their Own Tools (And Some Others): The Role Of Biofilm Formation On The Probiotic Activity Of *Phaeobacter gallaeciensis* S4	68
9:30	Van Bonn	Characterization Of The Aquatic Microbiome Prior To, During, And Following A Ninety-Percent Water Change In An Artificial Salt Water System	69
9:45	Arnold	Monitoring *Enterococcus* In Public Aquarium Marine Fish Exhibits	70

10:00 **Morning Break**

General Session 5: Chris Good-Moderator

10:30	Trevey	The Spotfin Killifish *Fundulus luciae* Baird 1855: Interspecific Competition And Parasite Burden	71
10:45	Burge	Ecology Of A Forgotten Trematode Parasite, *Cercaria choanura*, In The Coquina Clam *Donax variabilis*	72
11:00	Alberson	A New Snail Host Identified For *Drepanocephalus spathans* (Digenea: Echinostomatidae) In Catfish Aquaculture	73
11:15	Frederick	Where Are All The Sea Lice? A First Glance At Sentinel Fish In Cobscook Bay	74

12:00 **Lunch**

Special Session 6: Alphabet Soup

Do the acronyms WGS, PFGE, AFLP, MLST, RAPD, rep-PCR, VNTR, SLST, or SNP mean something to you? **Ashley Haines** chairs a session on comparative genomics and molecular epidemiology of aquatic pathogens.

1:00	Griffin	Repetitive Sequence Mediated PCR And *gyrB* Sequence Demonstrates Intraspecific Genetic Variability Amongst *Edwardsiella ictaluri* Isolates From Different Hosts And Geographic Origins	75

1:15	Haines	Whole Genome Comparisons Of *Streptococcus parauberis* From Fish And Cattle	76
1:30	Fogelson	Speciation Of *Mycobacterium chelonae* Isolates By hsp65 And Whole Genome Sequencing	77
1:45	Gauthier	Comparative Genomics Of The Human And Animal Pathogen *Mycobacterium marinum*	78
2:00	Fast	NGS: Next Generation… Sturgeon, Developing Genomic Resources For Atlantic Sturgeon	79
2:15	Dove	Health Insights From Sequencing And Draft Assembly Of The Genome Of The Whale Shark, *Rhincodon typus*	80
2:30	Stilwell	Genomic Characterization Of A Novel Hepatitis E Virus In Three Salmonid Species (*Salmo salar, S. trutta*, and *Oncorhynchus mykiss*)	81
2:45	Steckler	Repeated Detections Of Ranaviruses In Aquaculture And The Development Of Improved Molecular Tools	82

3:00 **Afternoon Break**

General Session 6: **Isaure de Buron -Moderator**

3:30	Rosser	An Overview of Myxozoan Parasites Associated With Catfish Aquaculture	83
3:45	McElroy	In Life And Death: The Myxozoan *Kudoa inornata* Increases Spotted Seatrout (*Cynoscion nebulosus*) Swimming Performance But Also Causes Post-Mortem Myoliquefaction	84
4:00	de Buron	*Kudoa inornata*: A Seasonal Parasite?	85
4:15	Atkinson	Hunting For The Alternate Host Of *Kudoa inornata*, A Myxozoan Parasite Of Spotted Seatrout	86

4:30 **Adjourn**

6:30 **Cocktails**

7:00 **40th Annual Eastern Fish Health Dinner Banquet**
Best Student Presentation Award

8:30-11:00 **Dance!!**

Friday, March 6

8:00		Breakfast (for Continuing Education Class Participants)

"Crustacean Health and Disease" will discuss important biological and immunological principles with respect to crustaceans. These principles will then be explored through special topics such as histopathology of crustacean disease, important crustacean disease and their regulatory issues, and management of crustaceans in laboratory settings. This course is designed for people planning or currently conducting crustacean research and/or overseeing crustacean welfare at aquatic animal health facilities, aquaculture centers, and aquariums. Participants will earn 5.0 CE credit hours from the Fish Health Section (FHS) of the American Fisheries Society and receive the materials on a flash drive along with the Eastern Fish Health Workshop meeting booklet.

9:00	Fast	**Continuing Education Class** **Introduction, Welcome and Agenda**
9:10	Fast, Poley	Crustacean Physiology And Gender Impacts On Responses To Treatment
10:00	Burnett	Crustacean Immunology
10:50		**Break**
11:05	Frasca	Special Focus: Crustacean Histopathology Of Disease
12:00		**Lunch**
1:00	Frasca	Special Focus: Crustacean Histopathology Of Disease
1:50	Byrne	Special Focus: High Level Biocontainment Regulatory Research Involving Regulated Crustacean Diseases- Health, Management, And Disease Trials In A Laboratory Setting
2:40		Roundtable Questions With Expert Panel
3:00		**Adjourn**

Yet, Another Rare Gram Positive Pathogen

[1]David Marancik, [2]Scott LaPatra, and [3]Tim Welch

[1]Fish Vet Group, 350 Commercial St., Portland, Maine 04103; [2]Clear Springs Food, Inc., PO Box 712, Buhl, Idaho 83316; [3]NCCCWA-ARS-USDA, 11861 Leetown Rd., Kearneysville, West Virginia 25430

Chronic disease was observed in 4 year old post-spawned rainbow trout at a commercial hatchery resulting in significant morbidity and mortality. Gross necropsy performed on-site demonstrated fibrinous to fibrous adhesions and granulomatous exudate covering the heart, coelomic serosa and peritoneum of affected fish. Variable numbers of <1 mm to 1 cm nodules were present within organs. On microscopic examination, the serosal surfaces of the heart, spleen, kidney, liver and intestines were covered by a thin layer of fibrin progressing to profuse granulation tissue and severe granulomatous inflammation. The parenchyma of these organs were effaced by granulomas and areas of necrosis affecting up to 80% of the tissue. Bacterial cultures from internal organs incubated at 15ºC on TSA grew a monomorphic population of gram positive cocci. Bacterial isolates were identified by 16S rRNA sequencing as *Vagococcus salmoninarum*. Antibiotic treatment was largely unrewarding. Vaccination efforts are ongoing. Sporadic case reports from Europe describe similar case histories and suggest this chronic condition may be associated with stress and low water temperature.

Red Flags In Red Cichlids (*Mesonatua festivus*)

[1]Mayela M. Alsina, [1]Nicole D. Hatcher, [1]Tonya M. Clauss, and
[2]Alvin C. Camus

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

A flag cichlid (*Mesonauta festivus*) from the fresh water exhibit at the Georgia Aquarium presented moribund, with proliferative tissue at the base of the caudal fin and unilateral exophthalmia. Due to a poor prognosis, the fish was euthanized and a complete necropsy performed. Gross findings included friable kidneys, a pale heart, enlarged gallbladder and possibly obstructed lower intestine. Histopathological evaluation revealed a large, inflamed, fibrotic mass adhered to multiple coelomic organs. Contained within the fibrous tissue were variably sized nests of neoplastic epithelial cells, with indistinct borders and small to moderate amounts of finely granular to fibrillar basophilic cytoplasm. Some cells were distended by basophilic mucinous material. Nuclei were large, round to oval, and vesicular, with one to three prominent nuclei. Anisokaryosis was mild and mitoses were infrequent. Similar cell aggregates infiltrated the gastric muscularis, pancreatic islets, liver, and spleen. The intestinal submucosa contained multiple cross and longitudinal sections of a small nematode, with a series of esophageal gland cells (stichocytes) forming a stichosome. Inflammatory changes were limited to minimal numbers of lymphocytes in the sections examined. Additional findings included large numbers of granulomas with acid-fast bacilli in the liver, kidney and mesentery, and at the base of the caudal fin. Multiple myofibers in the gastric muscularis were expanded by microsporidial sporophorocysts filled by sporophorous vesicles containing refractile, approximately 3 x 4 um spores. The neoplasm in this cichlid was presumptively identified as an intestinal carcinoma. Morphologic features of the intestinal nematode were typical of a capillarid, at least 39 species of which are known to affect fish. Although the identity of the species involved in this case was unknown, in zebrafish colonies, *Pseudocapillaria tomentosa* is associated with lymphocytic enteritis and intestinal carcinoma. This fish was also affected by severe disseminated mycobacteriosis and gastric microsporidiosis. The presence of multiple spore containing vesicles within a large sporophocyst is suggestive of a *Heterosporis* sp.

If You See Wigglers, Blame It On Their Diet

[1]Rodman G. Getchell, [2]Geofrey E. Eckerlin, [2]Andrew D. Noyes, [3]Steven R. LaPan, [4]Dale C. Honeyfield, [1]Kelly L. Sams, [1]Hélène Marquis, and [1]Paul R. Bowser

[1]Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, 930 Campus Road, Cornell University, Ithaca, NY 14853; [2]NYSDEC Fish Disease Control Unit, 8314 Fish Hatchery Rd., Rome, NY 13440; [3]NYSDEC Bureau of Fisheries, Cape Vincent Fisheries Station, 541 East Broadway, P.O. Box 292, Cape Vincent, NY 13618; [4]Northern Appalachian Research Laboratory, U.S. Geological Survey, 176 Straight Run Road, Wellsboro, PA 16901

Adult steelhead trout returning from Lake Ontario to the Salmon River in Oswego County, New York are exhibiting signs of stress and elevated mortality rates. Steelhead *Oncorhynchus mykiss* provide economically important sport fisheries in the open lake and are especially sought after by anglers who fish in tributaries from fall through spring. Fishermen described capturing the lethargic “wigglers” with ease during the 2014 fall season. More recent reports indicate similar behavior in steelhead from other Lake Ontario tributaries. Moribund Salmon River steelhead were submitted to the Aquatic Animal Health Program to determine the cause of this disorder. Results indicated that fish pathogens were not responsible for the abnormal behavior and mortality. DEC scientists also injected three lethargic steelhead with thiamine, while a control group of three affected fish received a saline solution. The group given the thiamine boost was active and alert after 48 hours, while the controls remained listless and unresponsive. Steelhead liver and fillet samples were sent to the Northern Appalachian Research Laboratory for thiamine analysis. Results from the USGS Lab strongly indicate a severe thiamine (vitamin B_1) deficiency, which very likely is contributing to the steelhead mortality. Alewives, the main forage base in Lake Ontario, contain an enzyme, thiaminase, which breaks down thiamine. This year's acute deficiency is atypical in its severity. Histological evaluation of affected fish and five control steelhead from a Lake Erie tributary has been undertaken to find a more rapid diagnostic sign of this nutritional disorder. Preliminary findings suggest lower amounts of PAS-positive glycogen may be observed in hepatocytes of affected steelhead versus the five control fish, although only a very small sample size was examined. A summary of this case will be presented.

What's That Growing On Your *Halichoeres bivittatus?*

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Despite their indelicate name, *Halichoeres bivittatus*, commonly known as the slippery dick, are occasionally displayed in public aquariums. The small wrasse is native to shallow, tropical waters of the western Atlantic Ocean. This fish was one of three euthanized for evaluation of proliferative skin lesions grossly suggestive of lymphocystis disease or possible neoplasia in a newly arrived group undergoing quarantine observation. Grossly, there were friable, white, raised, irregular skin lesions on the caudal trunk and peduncle. Histologically, lesions were composed of locally extensive, well-demarcated, plaque-like to villonodular epidermal masses that variably expanded the epidermis up to 10 times its normal thickness. Arranged in broad trabeculae supported by a scant fibrovascular stroma, lesions often enveloped scales, but did not invade the underlying dermis. Contained within the proliferative lesions, were small to often massive numbers of round to polygonal cells, up to 15 x 20 um in diameter, with abundant, finely granular, pale eosinophilic cytoplasm. Distinct nuclei were large, round and centrally located, with dispersed, pale eosinophilic chromatin and a single, prominent nucleolus. Cytokeratin immunohistochemistry did not stain the cells, which were clearly delineated from positive staining host epithelium. Lesions were typical of so-called “X-cell pseudotumors.” First described in 1969, X-cell lesions have been reported from the gills, pseudobranchs, skin and rarely internal tissues of several, primarily bottom dwelling, teleost species. Often misidentified as neoplastic host cells, 18S rRNA sequence analysis indicates that X-cells are parasitic protozoans with no clear affinity to any known protistan groups. More recent molecular analysis, as well as apparent host and tissue specificities, support the existence of multiple closely related species. Direct transmission studies have been unsuccessful, suggesting life cycles may be complex.

A Floundering Heart

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Formalin fixed tissues were evaluated from a male Gulf flounder *Paralichthys albigutta* of unknown age. The fish became moribund and died after a 2 week period of anorexia and suspected gas supersaturation. The liver was pale and floated in formalin, while the bulbous arteriosus was gritty when trimmed. Microscopically, bulbar elastic bundles were extensively disrupted by acicular clefts, foamy macrophages, cell debris, and rare lymphocytes. Approximately 50% of glomerular mesangial areas were segmentally to globally enlarged by acellular, feathery, pale eosinophilic material and ill-defined vacuoles. Loss of capillary lumens was moderate to severe. Rare tubules were mildly ectatic and filled with proteinaceous fluid. Lipid vacuolation of hepatocytes was marked. Special stains (oil-red-O, Sudan IV) of frozen tissue sections revealed abundant lipid within bulbar and glomerular lesions. The diagnoses were bulbar atherosclerosis and glomerular lipidosis, unusual lesions with unclear pathogenesis in this flounder. In humans, atherosclerosis accounts for half of all deaths in Western countries. Age, gender and genetics are risk factors, as are modifiable causes, such as hypertension, diabetes mellitus and hyperlipidemia. There is also interest in the potential role of systemic inflammation. Lesions are characterized by fibrous capped lipid (primarily cholesterol) plaques within vessel walls. Glomerular lipidosis includes several poorly understood renal disorders associated with hyperlipidemia and disturbances of lipid metabolism. Common in human kidney biopsy specimens, lesions most commonly involve mesangial areas. Often interpreted as an incidental finding, significance varies with individual cases.

Salmon Strangeness: A Mysterious Case Of Systemic Granuloma In Atlantic Salmon *Salmo salar* Raised In Freshwater RAS

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Systemic granuloma was observed in sampled adult Atlantic salmon *Salmo salar* raised to harvest size in a freshwater recirculation aquaculture system. The prevalence of this condition was estimated at 10-20% of the population, with affected individuals grossly demonstrating pathology in varying degrees of extent and severity. Six downgraded fish were sampled at final harvest, and the following assessments were carried out: gross pathology and histopathology, bacterial culture and 16S PCR, and plasma chemistry. Granulomas were observed in all tissues examined, including brain and skeletal muscle; the most extensively affected tissues were liver and visceral adipose tissue. Special staining (Von Kossa) revealed a central core of mineral and/or necrotic debris in the granulomas. Culture and PCR did not reveal the presence of an agent or agents associated with this condition; likewise, plasma chemistry results were unremarkable. Overall, it is likely that the observed pathologies were related to mineral imbalance leading to widespread metastatic calcification; however, the true causation of the observed pathologies remains unknown, and further research needs to be conducted to complete our understanding. To our knowledge, this is the first description of systemic granuloma in Atlantic salmon.

Casting A Broken Unicorn Horn

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A 2.3 kg, Bluespine unicorn tang, *Naso unicornis,* presented for evaluation of an open, subacute, fracture of the distal portion of the horn. The fish was anesthetized for examination and treatment. Upon palpation, the fracture appeared stable; radiographs revealed a comminuted fracture. The distal 1 cm of bone was excised and a skin flap was sutured over the exposed bone. The fish was started on antibiotics and received a dose of corticosteroids for anti-inflammatory and analgesic purposes. Four days later it was noted that the surgical site had dehisced. The fish was anesthetized for wound debridement and treatment. Bone cement, impregnated with antibiotics, was placed over the exposed bone for stabilization and protection. Tissue glue was placed around the margin of the bone cement in order to secure it to the fish. One month post presentation the fish was euthanized due to abnormal behavior, anorexia, and abnormal buoyancy. At necropsy, the bone cement was still well adhered to the horn. There was a fungal infection present in the horn which may have been the inciting cause of the fracture or secondary due to exposed tissue. Although the fracture did not heal in this case, bone cement may be a viable option for treating fractures or open wounds in fish as it remained in place for 22 days.

When More Is Actually Less – The Tale Of Chronic Ammonia Toxicity

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A commercial catfish producer submitted 7 food-sized fish from a pond that was experiencing mortalities on Jan 24, 2014. The ~ 2.64 acre pond was stocked with hybrid catfish. No gross external lesions were evident except for linear erosions (scratches) on the skin that were thought iatrogenic in origin. Internally, 5 out 7 fish had intestinal intussusceptions and mildly congested spleens. Bacterial cultures of the brain and posterior kidney were performed and these were negative. Although Visceral Toxicosis of Catfish(VTC) was unlikely, bioassays were conducted because of the internal lesions and these were negative. Water quality analysis indicated an elevated Total Ammonia Nitrogen (TAN) - 5.0ppm. This pond was enrolled in production study in March of 2013 had been stocked at approximately twice the industry average with 12,915fish/acre with stockers weighing 98lb/1000fish. Water quality analysis conducted once every two weeks showed TAN levels above 10ppm starting Nov 6, 2013 to Jan 14, 2014 with a high of 11.1 ppm on Dec 3, 2013. Representative samples of brain, gill, spleen, GI tract, liver, eye, muscle and skin as well as the anterior and posterior kidney of 4 of the 7 fish were fixed in 10% neutral buffered formalin. Significant microscopic lesions were limited to liver and posterior kidney. Most of the pancreatic acinar cells were degranulated and there was severe to marked multifocal mononuclear pancreatitis. There was also mild to severe macro and microvesicular hepatocellular vacuolation. Renal tubular cells were vacuolated with rare accumulation of eosinophilic hyaline material in some of these tubules. Unfortunately, the losses occurred close to the time of harvest(survival 61.7%)adversely affecting the feed conversion ratio (3.62; above industry average) although the net production was still relatively good 13,233 lb/acre (twice industry average.

This Betta Be Cool

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An adult, male, *Betta splendens* was presented to the University of Georgia Aquatic Pathology Service for histologic evaluation of a rapidly growing, multilobulated, soft tissue mass located on the dorsum. Surgical removal was attempted via laser and a biopsy specimen was collected, however the fish died two days later. Gross examination of the 7.5 cm fish revealed a multilobulated, black and white, soft tissue mass cranial to the dorsal fin, approximately 0.75 cm at its longest margin. Exposed axial musculature circumscribed the lesion. Evidence of skeletal muscle and coelomic visceral involvement was apparent following decalcification and cross sectioning of the body. The head kidney was enlarged, misshapen, and effaced by friable white tissue. Up to 50% of the liver was also replaced by similar tissue. Histologic evaluation revealed a highly cellular, infiltrative neoplasm arranged primarily in sheets, with occasional streams, whorls, and interlacing bundles, supported by a fine fibrovascular stroma. The neoplasm effaced the local epidermis, dermis and hypodermis, invaded the subadjacent skeletal muscle, dissected between and isolated individual myofibers along the lateral aspects of vertebral bodies. Presumably by direct extension, the posterior kidney and an adjacent liver lobe were largely replaced by the neoplasm. Individual tumor cells were polygonal to spindle shaped, with indistinct cell borders and moderate amounts of eosinophilic granular cytoplasm containing abundant golden-brown, birefringent crystals. Nuclei were oval with clumped chromatin and a single distinct nucleolus. Mitotic figures were rare. Free melanin granules and scattered melanophores were present in superficial portions of the tumor. Degenerate myofibers, bacterial colonies, and mixed inflammatory infiltrates were associated with exposed muscle. Microscopic findings were consistent with an iridophoroma, a rare non-dendritic chromatophore tumor of neuroectodermal origin. Iridophores are found primarily in ventral white colored areas and are responsible for the iridescent quality of fish skin. Affecting both reptiles and fish, iridophoromas are characterized by the presence of birefringent anisotropic purine containing crystals.

Spring Chinook: A Needle In A Haystack

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A population of returning anadromous Spring Chinook salmon broodstock experienced a significant mortality event while being held prior to spawning at a large hatchery in Eastern Washington. As the fish arrived up the ladder, they were held in two large raceways supplied by deep reservoir river water. Early onset of clinical signs included lethargy, swimming at the surface, rolling, and sudden mortality. . On examination of the affected fish, they were almost ready to spawn, in good body condition but they had white to yellow tipped gills. Microscopic examination of the gills revealed numerous long flexing rod-shaped bacteria arranged in haystacks. The surviving fish were treated with Chloramine-T, a recently approved drug for use in aquaculture, the raceways were switched to well water to decrease the water temperature, and the oxygenation in the raceways was increased. Although mortality was initially high, some fish quickly recovered and successfully made it to spawning with treatment.

Identification Of An *Edwardsiella* Infection In Largemouth Bass In A Closed Recirculation Aquaculture System

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Largemouth bass being cultured in a closed recirculation aquaculture system were submitted to the Fish Disease Diagnostic Laboratory of the Aquatic Animal Health Program, Cornell University, due to a pattern of chronic mortality. Grossly visible signs included multifocal petechial hemorrhages on the skin and fins. Initial processing did not reveal any problematic pathogens on the skin, gills and fins. Large amounts of lipid deposits were present in the coelomic cavity. A bacterial culture was initiated from a sample collected from the posterior region of the kidneys and major organs were collected for histopathology. Small white bacterial colonies were observed on blood agar plates following 48-72 hours incubation. A wet mount revealed that the bacterial colonies were comprised of highly motile bacteria. Initial characterization of the isolate by MALDI-TOF mass spectrometry resulted in the identification of *Edwardsiella tarda*. The isolate was also evaluated by real-time PCR, and tested positive for *E. tarda* and negative for *E. ictaluri*. The identification of the isolate was complicated by the recent designation of two new species of *Edwardsiella.* Several strains of the new species were previously classified as *E. tarda*, and appear as *E. tarda in* GenBank and other databases. Within the most current classification scheme, three species (*E. piscicida*, *E. anguillarum*, and *E. ictaluri*) are phylogenetically closer to each other than any of them are to either *E. tarda* or *E. hoshinae*. Considering the sequenced portions of the 16s rRNA and rpoB genes, the bacterium isolated from the largemouth bass was genetically closest to *Edwardsiella piscicida*. This case highlights the need to be aware of the limitations of the reference databases available to support the most contemporary bacterial identification methodologies.

First report of Carp Edema Virus (CEV) in South American Koi

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In Brazil, koi (*Cyprinus carpio*) and goldfish (*Carassius auratus*) are the most important species reared for ornamental aquaculture. In 2014, one of the largest koi and goldfish producers in Brazil experienced substantial mortality in yearling koi (< 5 cm in total length) being reared in earthen ponds. The young koi were noted to be stratified in the upper layers of the ponds and in obvious respiratory distress. The mortality was estimated between 50-70% over a two week period in some affected ponds. During the outbreak, water temperature was around 22°C with other water quality parameters within normal limits. Wet mounts of affected fish revealed high levels of trichodinid parasites which were also observed upon histologic examination of external tissues. The most significant histologic lesions were observed in the gills where severe hyperplasia of the respiratory epithelium was noted leading to fusion of adjacent secondary lamellae and an associated inflammatory response. Gill tissue from moribund koi tested negative for koi herpesvirus (KHV) and positive for Carp Edema Virus (CEV) by conventional PCR. This study is the first detection of CEV in South America and further confirms the global spread of this lethal disease of koi.

It Came From The Willamette River!

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From 2011-2013, the OSU Department of Fisheries and Wildlife conducted electrofishing surveys of fish communities in the Willamette River, Oregon. All fish >50mm were stunned, netted and recorded; salmonids were returned live to the river but non-native fish species (bass, sunfish, carp, perch, etc.) were killed and passed on the Department of Microbiology for parasite examination. On 11 June 2013 we received a 588mm long male common carp, which had spectacular tumor-like nodules up to 15mm across, on dorsal surfaces of its head and on its tail and fins. Internal organs were normal, except for the presence of immature myxozoan plasmodial stages in the lumen of the gallbladder. In the oral cavity, nodules up to 25mm across covered the cartilaginous gill rakers; these nodules could be removed easily to leave normal-looking gills. The largest nodules on the body surface had small openings distally, which revealed white material within. Blunt dissection allowed us to readily extract a cohesive white mass from each lump. These masses resembled small brains or cauliflower florets. Samples were taken for histopathology, and given the purity of the pathogen material, fixed for electron microscopy and RNA/transcriptomics. Microscopic examination of fresh tissue squashes revealed the infectious agent.

Recent Advancements In Our Knowledge Of *Edwardsiella piscicida* And *E. tarda*

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Researchers have recently proposed the adoption of three distinct genetic taxa among bacteria previously classified as *Edwardsiella tarda*; namely *E. tarda*, *E. piscicida* and a taxon presently termed *E. piscicida*-like. Further research suggests that of the three, *E. piscicida* is more prevalent on Mississippi catfish farms than *E.* piscicida-like sp. or *E. tarda*. Current genomic investigations have demonstrated significant genetic differences between these phenotypically ambiguous taxa. To this end, real-time polymerase chain reactions were developed for the detection and quantification of *E. piscicida*, *E. tarda*, and *E. piscicida*-like species in catfish tissue and pond water. Under the conditions used in this study, the assays had a quantifiable limit ranging from 10^3 to 10^2 colony forming units and successfully detected their respective targets in both clinically and subclinically, experimentally infected channel catfish (*Ictalurus punctatus*). Subsequent *in vivo* studies using intracoelomic injections in catfish fingerlings (mean weight: 17.3g; range 7.3 - 40.9g) identified a median lethal dose (LD_{50}) of 3.9×10^5 CFU for *Edwardsiella piscicida* and 5.9×10^7 CFU for *Edwardsiella tarda*. Comparatively, only negligible mortality occurred in catfish injected with doses as high as 5.0×10^8 CFU of *E. piscicida*-like sp. Further research investigated the posterior kidney clearance rate of channel catfish exposed to sub-lethal doses of *E. piscicida*, *E. piscicida*-like sp., and *E. tarda*. This data, along with the histopathology of channel catfish infected with each bacteria will be discussed.

Biotic and Abiotic Factors Associated With Outbreaks Of An Emergent Strain Of *Aeromonas hydrophila* In Catfish Aquaculture

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In catfish aquaculture, *Aeromonas hydrophila* is predominantly considered an opportunistic pathogen, associated mostly with secondary bacterial infections. Recently a new strain of *A. hydrophila* has been implicated in significant losses in farm-raised catfish. Outbreaks attributable to this new strain began in Alabama in 2009 and have spread to Arkansas and Mississippi in recent years. These outbreaks mostly afflict market-sized fish, causing considerable losses in short periods of time. Comparative genomics revealed the epidemic *A. hydrophila* (EAh) isolates to be highly clonal and largely similar to isolates from diseased grass carp in China. These genomic studies identified genetic regions specific to EAh, and a qPCR assay was developed to detect the bacteria in fish tissues and environmental samples. Samples were collected from 28 ponds on 3 different operations with various histories of disease. Blood, gill and rectal swabs were collected from resident fish and analyzed for the presence of EAh. In addition, pond water, pond sediment, benthic oligochaetes and zooplankton were also collected and water chemistries and phytoplankton biomass were determined. A total of 74 ponds were sampled from August to November and fell into the following categories: ponds prior to outbreak, ponds with active outbreaks, ponds recovered from outbreaks, ponds with outbreaks the previous year, and ponds with no history of EAh. During active outbreaks EAh was detected by qPCR in pond water, sediments and resident fish. However, once an outbreak subsided EAh was detected only rarely and in low numbers. In ponds with prior histories of outbreaks, EAh was detected in resident fish but not the environment, suggesting a carrier state. Pond chemistries, zooplankton and phytoplankton communities were largely similar, although further sampling is required to rule out any associations between factors measured in this study and risk of *A. hydrophila* outbreak.

Weissellosis: An Emerging Disease Of Farmed Rainbow Trout

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Weissella ceti is a gram positive bacterium associated with Weissellosis, an emerging disease of farmed rainbow trout. This disease can result in high mortality in large fish (0.5-1.0 kg) and hence can cause significant economic loss. In the summer of 2011, severe Weissellosis outbreaks were identified at two commercial trout farms in North Carolina. The outbreaks subsided as water temperatures cooled in November; however, in the spring of 2012 the outbreaks recommenced thus demonstrating that this pathogen has the potential to persist in North Carolina through the winter months and suggesting that *W. ceti* could become an endemic disease problem in this area. Laboratory-based vaccination-challenge studies demonstrated that an aqueous vaccine formulation containing formalin-inactivated *W. ceti* whole-cell*s* conferred significant protection against experimental infection, suggesting that vaccination could be a viable means of controlling this pathogen. In 2012, all fish at the two affected farms were vaccinated using a custom bacterin vaccine produced and prescribed by a licensed veterinarian, with the exception of 10,000 fish that were left unvaccinated to serve as controls to aid in evaluation of vaccine performance. Challenge experiments performed approximately one year post-vaccination demonstrated that on farm vaccinated fish displayed significant protection when compared to unvaccinated controls (RPS = 65%). More importantly, after comprehensive vaccination was initiated in 2012 no cases of Weissellosis were detected on either of the farms impacted by the outbreak although the pathogen was detected at a nearby farm that had not used the vaccine. Additional surveillance performed in the summers of 2013 and 2014 on the 15 remaining farms in the county and in the counties contiguous to the original outbreak sites has also been negative.

Novel Fish-Associated Flavobacteria Continue to Emerge in the Great Lakes Basin

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Flavobacterial diseases in fish are caused by multiple species within the family Flavobacteriaceae and can generate devastating losses in wild and farmed stocks alike. The emergence of recent reports linking previously uncharacterized flavobacteria to systemic infections and mortality events in fish stocks of Europe, South America, Asia, and Africa is of major concern and has highlighted some of the difficulties surrounding the diagnosis and chemotherapeutic treatment of flavobacterial fish diseases. In the Laurentian Great Lakes, we recently described two novel flavobacterial species (*Flavobacterium spartansii* and *Chryseobacterium aahli*) that are capable of causing disease and mortality in multiple Great Lakes fishes. Herein, we describe four additional novel fish-associated flavobacteria, for which the names *Flavobacterium dolichovirga*, *Flavobacterium micropteri*, *Chryseobacterium magnalucas*, and *Chryseobacterium esocidense* are proposed.

Francisellosis In The Aquarium – Histopathology and Diagnostics

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Francisellosis is an emerging disease of marine and freshwater teleosts, most notably cod and tilapia, which increasingly affects ornamental species in public aquaria and must be differentiated from other more common causes of granuloma formation, such as mycobacteriosis. Currently, two subspecies of these highly pathogenic intracellular bacteria, *Francisella noatunensis* subsp. *noatunensis* and *F. noatunensis* subsp. *orientalis*, affect cold and warmwater fish species, respectively. To date, only *F. noatunensis* subsp. *orientalis* has been documented in aquarium settings. Clinical signs are non-specific and may include anorexia, exophthalmia and erratic swimming. Gross changes may be minimal, but can include renosplenomegaly accompanied by multifocal, small (~1 mm), white nodules. Consistent with gross changes, the kidney and spleen are most commonly and severely affected. Less often, lesions may be present in the liver, heart, intestine, skeletal muscle, ocular muscles and choroid, gill filaments and lamellae, and in the central nervous system. Typical lesions include multifocal necrosis, with pyogranulomatous inflammation or granulomas surrounded by epithelioid cells and/or degenerate macrophages containing low to moderate numbers of approximately 1 um, pleomorphic, coccobacilli within a single prominent vacuole. With chronicity, granulomas often coalesce, occupying extensive areas of tissue, and coagulative to caseous necrosis can be prominent. Additionally, bacterial numbers may decline confounding the diagnosis. Although discernable with hematoxylin and eosin (H&E), visualization of the organism is enhanced by Giemsa stains. The bacteria are Gram-negative and non-acid-fast. A presumptive diagnosis can be made on gross and microscopic findings, as well as failure of the bacteria to grow on routine (e.g. BHI or TSA) or mycobacterial media. Isolation of the fastidious organism requires special media, such as cysteine heart agar supplemented with blood or hemoglobin. The diagnosis can be confirmed by real-time PCR and immunohistochemistry specific for *Francisella noatunensis* subsp. *orientalis*.

Genomic Characterization of Carp Edema Virus

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Double-stranded DNA viruses are important pathogens of homeothermic and poikilothermic vertebrates. Seven families of such viruses have been characterized among homeothermic vertebrates, but only two (*Alloherpesviridae* and *Iridoviridae*) have been well studied among poikilothermic vertebrates (e.g. fish, amphibians, and reptiles). Here, we report the genomic characterization of a fish poxvirus, carp edema virus (CEV), which infects common carp varieties including koi. CEV is a globally emerging pathogen that is seriously impacting facilities that rear common carp for food, sport, or recreation. Our ultrastructural and phylogenetic studies provide unequivocal evidence that CEV is a novel member of the family *Poxviridae*. Transmission electron microscopy (TEM) showed that CEV displays the features of virion morphogenesis typical of poxviruses, with very large, spheroid particles observed within the cytoplasm of gill epithelial cells, and that virions possess a single lateral body, as reported previously for fish poxviruses. Phylogenetic analyses revealed that CEV is the sister taxon to the more extensively studied poxviruses of mammals and birds (subfamily *Chordopoxvirinae*). Preliminary comparisons of CEV with other recently discovered fish poxviruses suggest that these viruses represent a novel genus or subfamily in the family *Poxviridae*. Further research is needed to characterize the diversity of piscine poxviruses and to determine the full extent of the risk that they pose to farmed and feral fish stocks.

Novel DNA Virus Genomes Associated With Papillomatous Skin Lesions in a Giant Guitarfish

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A skin biopsy was collected from a 148 cm, 13.5 kg, juvenile, male giant guitarfish *Rhynchobatus djiddensis* with extensive, friable, raised, villonodular skin lesions affecting pigmented and non-pigmented skin of the caudal fin and ventrum, respectively. Microscopic examination revealed papillary proliferation of the epidermis, with marked karyomegaly. In approximately 90% of nuclei, chromatin was marginated by one to multiple, large, hyaline, amphophilic inclusions. Large numbers of unencapsulated, 75 nm, icosahedral viral particles were observed in affected nuclei using electron microscopy. Two novel DNA viruses were characterized using viral metagenomics and deep sequencing (Illumina Miseq). One genome shared sequence and morphologic similarities to the currently unclassified, 75 nm, "endothelial necrosis virus" of Japanese eel and contained a large polyomavirus-like T antigen. The second genome, present only in ventral skin samples, is phylogenetically divergent to all four genera of known mammalian and avian polyomaviruses, suggesting the existence of a new fish polyomavirus clade. Knowledge of viral disease is extremely limited in elasmobranchs. Viral papillomatoses have been suspected in multiple species, with no observation of viral particles or inclusions. Both adenoviral and herpesviral particles have been reported in cases of dermatitis in the smooth dogfish. Findings demonstrate the potential involvement of novel DNA viruses in elasmobranch skin disease. Further investigation into the pathogenicity of the two viruses and their relationship to the papillomatous skin lesions is ongoing.

Facing An Old Foe on New Turf: Densovirus Associated With Pacific Sea Star Wasting Disease

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Beginning in June 2013 and continuing to present, at least 20 asteroid species on the Northeast Pacific Coast have experienced an extensive outbreak of sea star (asteroid) wasting disease (SSWD). We performed an investigation into potential viral, bacterial, and eukaryotic microbial pathogens by direct comparison between symptomatic and asymptomatic sea stars. Bacterial communities analyzed by microbial fingerprinting techniques and metagenomics revealed the presence of abundant Gammaproteobacteria, Bacteroidetes, and Mycoplasmas in both symptomatic and asymptomatic individuals. Symptomatic sea stars had a higher abundance of several groups of bacteria which were also present in asymptomatic individuals, and those same groups were easily cultivatable on media containing sea star organic matter as sole C source. Viral communities were dominated by bacteriophage, but also revealed the presence of a densovirus (named sea star associated densovirus SSaDV) primarily in symptomatic animals. Quantitative molecular analyses across a large number of individuals demonstrated that SSaDV was in greater load and prevalence in symptomatic than asymptomatic individuals. Direct challenge of asymptomatic sea stars with viral-sized material (i.e. < 0.2 µm) from symptomatic tissues consistently resulted in SSWD signs, whereas animals receiving heat-killed (i.e. control) virus-sized inoculum remained asymptomatic. Quantification of SSaDV during transmission trials indicated that progression of SSWD paralleled increased SSaDV load. Transcriptomic analyses comparing asymptomatic to symptomatic body wall tissues demonstrated a pattern of enhanced apoptotic and tissues degradation processes and decreased energy metabolism, while signaling of death-related proteins was greater in asymptomatic (which represented preclinical infections since SSaDV was detected) and symptomatic tissues. Taken together our data demonstrate that SSWD is accompanied by infection by SSaDV, which in turn induces a rise in host apoptotic and degradative processes, and elevated abundance of bacteria normally inhabiting the sea stars.

Documenting the Impacts of Sea Star Wasting Syndrome through Long-Term Surveys and Identifying Possible Factors Contributing to Disease Presence

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An ongoing disease epidemic of unprecedented magnitude has devastated sea star populations along much of the west coast of North America. Sea star wasting syndrome (SSWS) was first documented on the Olympic Coast of Washington in June 2013, and has since resulted in massive sea star die-offs ranging from Sitka, AK to southern California. The Multi-Agency Rocky Intertidal Network (MARINe) collects population data for the ochre star, *Pisaster ochraceus*, at over 100 west coast sites as part of a long-term monitoring program, and communication among MARINe members was critical to the early detection of SSWS and a coordinated, coast-wide sampling effort. This effort was further expanded by enlisting the help of both citizen science groups and the general public to better document the extent and impact of SSWS. Population declines have been observed across all long-term sites, but recent recruitment in a few areas could aid in recovery if juveniles survive. The extensive MARINe/citizen science dataset was used to explore possible relationships between disease presence and several environmental factors. While temperature appeared to be important in some locations, it did not closely correlate with SSWS presence on a coast-wide scale. A better understanding of the possible connections between SSWS and environmental factors will be essential to developing accurate predictions about sea star population recovery and community-level changes resulting from sea star decline.

Sea Star Wasting Disease in *Asterias forbesi* in the Northeast United States

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As keystone predators, sea stars serve to maintain biodiversity and distribution through trophic level interactions in intertidal ecosystems. Sea Star Wasting Disease (SSWD) has caused widespread mass mortality of *A. forbesi* in locations along the Northeast Coast of the United States in recent years. A similar disease has been described in several sea star species from the West Coast of the US. Recently, a densovirus has been associated with wasting disease in West Coast sea stars and a few limited samples of *A. forbesi*. The goals of this research are to: a) describe the pathogenesis of SSWD in *A. forbesi* and other echinoderms in the Northeast Coast; and b) identify the causative agent through experimental challenges. Histological examination of *A. forbesi* tissues affected with SSWD has shown cuticle loss, edema, and vacuolation of cells in the epidermis. We see no evidence of bacterial agents in tissues examined. Challenge experiments by cohabitation and immersion in infected water suggest that the cause of SSWD is viral in nature, since filtration (0.22 µm) of water from tanks with SSWD does not diminish the transmission and progression of the disease. Death of challenged sea stars occurred 7-15 days after exposure to infected water or sea stars. On the West Coast, death occurred within 8-17 days of experimental exposure. The presence and levels of densovirus in samples of *A. forbesi* collected from wild populations and challenge experiments will be determined using polymerase chain reaction (PCR). Understanding the potential cause of this disease is a first step towards management and prevention of wide spread outbreaks.

Current Pathology Findings In The Pacific Coast Sea Star Wasting Disease Mortality Event

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Since June of 2013, at least 20 species of sea star (Asteroidea) have been affected by the devastating Pacific Coast sea star wasting disease (SSWD) mortality event. Both wild and captive populations in public aquaria, from Southeastern Alaska to Southern California, have been impacted including animals exported to aquaria in Europe. *Pisaster*, *Pycnopodia,* and *Evasterias* are the most affected genera with mortality approaching 100% in select species at certain locations. Clinical disease progression includes: 1) loss of body turgor (deflation) and weakness; 2) foci of body wall pallor and tissue loss; 3) sloughing of rays and/or rupture of the body wall; 4) death. To date (January 2015), 149 Pacific Coast samples, representing 14 asteroid species have been examined histologically by members of a SSWD pathology working group. Consistent changes include: 1) epidermal degeneration, necrosis and ulceration; 2) dermal edema, necrosis and inflammation. Comparative body wall composition analysis and mineral staining have also been pursued to investigate the pathogenesis of the clinical body wall dissolution. Links between the histologic lesions and candidate disease-associated organisms identified by viral and bacterial metagenomics remain under investigation by transmission electron microscopy and in-situ hybridization.

Turning Up the Heat on Sea Star Wasting Disease

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A sea star wasting disease (SSWD) epizootic, first reported in summer of 2013, has devastated populations of *Asteroidea* over thousands of miles of the Pacific Coast, SSWD affects at least 20 species of sea stars, leading to mass mortality events and localized extinction in some species. In the current event, a densovirus was identified as the causative agent of the disease, however, little is known about the environmental conditions that facilitate SSWD prevalence and intensity. One factor identified in previous SSWD events was temperature, although previous events were localized in scale. During summer 2014 ocean temperatures were anomalously warm in the San Juan Archipelago (SJA), Washington state coinciding with progression of disease into the SJA. We examined the impact of temperature on SSWD prevalence and severity in *Pisaster ochraceus*, a dominant intertidal species. To quantify this relationship, we conducted field surveys every two weeks during spring (pre-disease) to summer (disease) 2014 at multiple locations. Laboratory experiments indicate water temperature increased the severity and the rate of disease progression. Our results suggest temperature may play a significant role in the progression of SSWD in *P. ochraceous*.

Secretory/excretory Products Of *Lepeophtherius salmonis* Regulate The Migration Of Atlantic Salmon Leukocytes

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The salmon louse, *Lepeophtheirus salmonis,* evades elimination by its host, Atlantic salmon, by inhibiting inflammatory responses; however, the mechanisms of this activity are not well characterized. Chemokine-binding proteins identified in the saliva and salivary gland extracts of other arthropod ectoparasites inhibit neutrophil recruitment and activation *in vitro* and *in vivo* by interfering with chemotactic molecules, such as leukotriene B_4 (LTB4), blocking their interaction with receptors on the surface of neutrophils. To determine if *L. salmonis* is using similar mechanisms to evade the host's immune response, the effects of *L. salmonis* secretory/excretory products (SEPs) on LTB4 stimulated leukocyte migration *in vitro* were investigated. Atlantic salmon peripheral blood leukocytes were exposed to LTB4 (1E-9M) only, SEPs (5μg/ml) only, or LTB4 and SEPs simultaneously. Results showed an increase in leukocyte migration in response to LTB4 immune stimulation when compared to the control L-15 media. However, pre-incubation of LTB4 with SEPs for 1 hour reduced cell migration compared to LTB4 immune stimulation alone. This data suggests that *L. salmonis* may inhibit inflammatory responses in Atlantic salmon by secreting proteins with chemokine-binding or chemokine-degrading activity. An *in vivo* experiment was also designed to determine if *L. salmonis* SEPs affect the inflammatory response of Atlantic salmon to chitin exposure, a component of the exoskeleton of arthropods known to induce inflammation. Skin samples were collected 6 and 24 hours post chitin exposure and processed for histological analysis. The results will also be presented at the meeting.

Type-4 Ice Structuring Protein In Rainbow Trout

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The innate immune system, including the acute phase response, is essential to maintaining health in fish. An uncharacterized rainbow trout (*Oncorhynchus mykiss*) acute phase plasma protein, 9.5kDa, was identified using 2D-PAGE but mass spectrometry yielded limited amino acid sequence and no similar proteins were identified in Genbank. 3' and 5' RACE was used to isolate a cDNA sequence that contained a conserved apolipoprotein A-II domain and showed 73% sequence similarity to Type-4 ice-structuring protein (ISP) LS-12 from Atlantic salmon (*Salmo Salar*). The antifreeze activity of Type-4 ISP LS-12 is minimal and its function is thought to be related to binding a ligand other than ice. Apolipoproteins are known to contain lipid-binding domains and may act as pattern recognition receptors to bind lipid components of fish pathogens during the innate immune response.

Adjuvant Effect Of *Quillaja saponaria* Saponin (QSS) In Turbot (*Scophthalmus maximus*) Upon Bath Vaccination

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The adjuvant effect of QSS on protection of turbot fry was investigated with bath vaccination of formalin-killed *Vibrio anguillarum* O1 and various concentrations of saponin (5, 25, 45 and 65mg/L). Fish were challenged at day 7, 14 and 28 post-vaccination. Significantly high RPS [(59.1±13.6)%, (81.7±8.2)% and (77.8±9.6)%] were recorded in the fish received bacterin bath with QSS at 45mg/L which are comparable to the positive control group vaccinate by intraperitoneal injection(IP). Moreover, a remarkable higher serum antibody titer was also demonstrated after 28 days in the vaccinated fish with QSS (45mg/L) than those vaccinated fish without QSS ($P<0.05$), but lower than the IP immunized fish ($P<0.05$). Significant up-regulation of IgM gene expression has also been identified in the tissues of skin, gill, spleen and kidney from the immunized fish in comparison to the control fish. Taken together, the present study indicated that QSS could remarkably evoke systemic and mucosal immune responses in immunized fish. Therefore, QSS might be a promising adjuvant candidate for fish vaccination through bath administrating route.

Laboratory Controlled Challenges of *Streptococcus* In Tilapia (*Oreochromis sp.*)

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Streptococcus agalactiae, the etiological agent of streptococcosis in fish, is an important pathogen of cultured and wild fish worldwide. During the last decade outbreaks of streptococcosis have occurred in a wide range of cultured and wild fish. To gain a better understanding of the pathogenesis of streptococcosis in tilapia (*Oreochromis* sp.), fingerlings were challenged with *S. agalactiae* utilizing different delivery methods. Nile tilapia fingerlings were challenged via intraperitoneal injection, intramuscular injection, orally or by immersion with serial dilutions of *S. agalactiae*. The dose lethal to 50% of test fish 15 d post-challenge was of 120 CFU/fish after intraperitoneal, and of 10^5 CFU/fish after intramuscular challenges. Acute mortalities were present in both groups, but especially in the intraperitoneal challenged fish. Very low mortalities were observed in the fish challenged via oral or immersion routes. Histological analysis of survivors revealed classical lesions associated with fish streptococcosis including granulomatous epicarditis, granulomatous splenitis, meningitis, choroiditis, myocarditis, and exophthalmia. The information obtained from this study helps us get a better understanding of the pathogenesis of streptococcosis in fish, and provides useful information regarding controlled experimental infection in tilapia challenged with *S. agalactiae.*

A DNA Vaccine, Based On The Viral Hemorrhagic Septicemia Virus Genotype IVb Glycoprotein Gene Protects Muskellunge (*Esox masquinongy*)

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Since the initial discovery of Viral Hemorrhagic Septicemia virus genotype IVb in the Great Lakes basin, we have gained a better understanding of novel sublineage (IVb) ecology, phylogeny, pathogenicity and the host immune response. However, despite our work, we are still no closer to proactively preventing VHSV infections, specifically in dense naïve hatchery populations. This may be changing with the advent of improved aquatic vaccine technology within the last decade, particularly with the increasing efficacy of DNA vaccines. DNA vaccines have demonstrated considerable protection against numerous pathogens, including aquatic rhabdoviruses. Further, a DNA vaccine of another novirhabdovirus, the infectious hematopoietic necrosis virus (IHNV), has already been approved for use by the Canadian authorities. This technology could hold promise, not only for protection of hatchery fish, but also for the study of teleost immune responses when encountering the VHSV-IVb. In this study, we examined several vaccine innocula and vaccination regimens in their ability to confer protection for Great Lakes fishes against VHSV IVb. The presence of humoral anti-VHSV antibodies were evaluated throughout the study in muskellunge (*Esox masquinongy*) using a newly developed indirect-ELISA assay. The use of either a general cytomegalovirus (CMV) promoter or a carp (*Cyprinus carpio*) β-actin promoter was explored. Analysis shows that a DNA vector containing the VHSV IVb glycoprotein gene elicits a variable humoral immune response in muskellunge. The highest protection was observed in fish that received two doses of the DNA vector with the (CMV) promoter.

Clinical Signs And Liver Pathology Associated With Microcystin-LR Exposure In Tilapia, *Oreochromis sp.*

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Microcystins are cyclic heptapeptide, hepatotoxins released into water by lysis of actively growing or dying populations of cyanobacteria. Fish exposed to these toxins either by feeding or uptake through the gills of cyanobacteria and/or their toxins, can exhibit significant morbidity and mortality. In the present study, juvenile hybrid tilapia were intraperitoneally exposed to 700 µg/kg microcystin-LR (MC-LR). Blood and liver tissues were collected from a subset of the control and experimental populations on Days 1, 3, 5, 7, and 10 post-exposure. A number of fish appeared agitated and more aggressive toward tank mates, while others became listless, hyperpigmented and exhibited a loss of buoyancy on Day 1 post-exposure. Most of these clinical signs abated by Day 4 post-exposure with all fish appearing normal by Day 5 post-exposure. Microscopic examination of livers from Day 1 and 3 post-exposure fish revealed hepatocellular degeneration, individualization and necrosis that were regionally extensive to diffuse. By Day 5 post-exposure, there was evidence of regeneration with hepatocytes being more basophilic with large round nuclei and prominent nucleoli in close apposition to each other. A few remnants of hepatocellular necrosis and individualization were still present. It was difficult to differentiate the livers of Day 7 and Day 10 post-exposure fish from the controls as the repair and regeneration process was advanced. The degenerative changes due to microcystin were similar to those reported for other species of fish, but required a higher dose of toxin than reported for other species of fish to produce the same pathologic changes.

Disease Risks To Inland Fisheries: How Effective Are Current Policies In Preventing Fish Pathogen Spread?

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In their aquatic habitat, fish are exposed to a multitude of stressors including pathogenic microorganisms. When environmental factors fluctuate aggressively, fish host defense mechanisms become compromised and even the least virulent pathogen can cause substantial losses and long term impacts at both the population and community levels. The increasing transboundary movement of fish and high stocking density in aquaculture and stock enhancement programs seem to foster the eruption of disease outbreaks. Currently, fish diseases are considered one of the major impediments to aquaculture indicating that current policies need to be revisited to prevent the spread of fish pathogens. Therefore, fish health issues must be addressed by both proactive and reactive programs. One such issue is the increased movement of fish broodstock and fertilized eggs around the globe, which plays a major role in the emergence of new diseases in previously free zones. Efforts to prevent the spread of fish pathogens with sound management practices are essential at the farm, regional, national, and international levels. In this context, many national health plans have been proposed yet only a few have been implemented. Such plans have been confronted with major obstacles, including limited knowledge, shortage of funding, and absence of political will to execute these plans. International and national regulatory agencies share the responsibility along with the industry in managing aquatic animal diseases. Policies translated into well thought of guidelines will definitely lead to improved farm productivity, product quality, trade opportunities and ultimately profitability.

Reading the Shrimp Transcriptome: Responses to Low Oxygen and High Carbon Dioxide

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Acclimation to low O_2 in many organisms involves changes at the level of the transcriptome. Here we used high throughput RNA sequencing to explore the global transcriptomic response and specific involvement of new isoforms of hemocyanin (Hc) in the multistressor low O_2/high CO_2 response. Hepatopancreas mRNA of juvenile *L. vannamei* exposed to air-saturated water, low O_2, or low O_2/high CO_2 for 4 or 24 h, was pooled, sequenced (HiSeq 2500) and assembled (Trinity: 52,190 contigs) to create a deep strand-specific reference transcriptome. Annotation of the assembly revealed sequences encoding a small Hc subunit, three full-length isoforms of the large subunit, 8 partial sequences, and three intronic sequences. In addition to this, a previously unidentified full-length Hc subunit was discovered. Phylogenetic analysis demonstrated the subunit to be a ß-type Hc subunit, making this the first report of a ß-type hemocyanin subunit in the Penaeoidea. mRNA of individual shrimp was sequenced (6/treatment); resulting reads were quantified (eXpress) and regulated genes identified from pairwise comparisons at each time (DESeq2). GO term enrichment (Roff-Bentzen; $p<0.0001$) and PCA analysis demonstrated a distinct pattern of regulation between prolonged low O_2 alone and low O_2/high CO_2 treatments, showcasing the stabilization of energetically costly translational machinery, mobilization of energy stores, and downregulation of the ubiquitin/proteasomal degradation machinery. The addition of CO_2 to hypoxia resulted in an antagonistic effect on the transcriptomic response of all the Hc subunits to low O_2, except for the ß-type subunit. We are currently exploring the importance of this novel full-length subunit, as well as the other full-length and partial isoforms to the structural and functional response of Hc in low O_2 alone and with high CO_2 (NSF IOS-1147008).

Black Gill in Penaeid Shrimp: Pathology and Transmission Studies

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A severe outbreak of Black Gill has been noted in white shrimp (*Litopenaeus setiferus*) and brown shrimp (*Litopenaeus aztecus*) in late summer and fall along the south Atlantic coast of the United States. Large ciliates (70 µm in diameter), necrosis and melanized nodules were observed by light microscopy in shrimp showing the presence of Black Gill macroscopically. The large ciliate appears to be closely related to the apostome ciliate, *Hyalophysa chattoni*. The large ciliates were observed histologically in the months (April-June) before Black Gill was observed macroscopically (late summer and fall). In winter months (Jan-March) there was an absence of the large ciliate in the gill but small numbers of a smaller ciliate, tentatively identified as the peritrich ciliate, *Zoothamnium* sp. Transmission studies suggest that the Black Gill agent is infectious and promotes increased mortality and molting. An interesting aspect is that the formation of numerous nodules, host tissue necrosis and intense melanization in reaction to this ciliate by the shrimp are not reactions generally observed in crustaceans infested with *H. chattoni.* A similar agent was observed in penaeid shrimp with Black Gill from Georgia, Florida, North Carolina and South Carolina.

Molecular-Enabled Identification, Detection And Seasonal Pattern Of The Causative Agent Of Shrimp Black Gill In The Coastal Southeast USA

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Penaeid shrimp including *Litopenaeus setiferus* (white shrimp), *Farfantepenaeus aztecus* (brown shrimp), and *Farfantepenaeus duorarum* (pink shrimp) support one of the most valuable commercial fisheries in the US Southeast Atlantic. However, in the past two years the fishery has experienced a significant decline. The cause of this decline has been hypothesized to be due to a severe outbreak of a gill infection causing tissue melanization (Black Gill), but a significant relationship between shrimp landings and the prevalence of Black Gill has not been observed. DNA sequence-based analysis of the gill parasite 18S rRNA gene supports the hypothesis that the causative agent of the recent outbreak shrimp Black Gill in the coastal Western Atlantic is closely related to the apostomate ciliate *Hyalophysa chattoni*. In addition to the identification of the causative agent of Shrimp Black Gill, access to the parasite sequence has enabled the development of a new diagnostic assay and a survey of the seasonal pattern of occurrence of the Black Gill parasite and other micro-eukaryotic shrimp associates. Although symptomatic Black Gill is generally only observed from August through November, molecular analysis reveals that the infection begins earlier in the year and persists longer. When Black Gill is at its peak, microeukaryotic communities are dominated by *Hyalophysa*-related ciliates, but over the year highly diverse communities of gill associates including other potential ciliate, fungal, euglenozoid and amoeboid parasites are commonly observed. Improved molecular diagnostics and the ability to explore the diversity of potential parasites in shrimp provide important new insights into the relationship between the shrimp fishery and Black Gill.

Observations of Black Gill in White Shrimp (*Litopenaeus setiferus*) from South Carolina: Molting, Mortality, and Mobility

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Black gill disease (BGD), caused by an apostomate ciliate that induces inflammation, melanization, and necrosis of gill tissue, approaches nearly 100% prevalence in South Carolina's white shrimp, *Litopenaeus setiferus*, during summer and fall. While BGD does not impact shrimp taste, it is unsightly and fishermen claim that summer and fall catches are comprised mostly of dead BGD shrimp, which they cannot sell. How BGD directly impacts shrimp health and survival is poorly understood, but preliminary studies suggest it interferes with oxygen consumption. This has important economical and ecological implications, as BGD may impair normal physiological functions, which could contribute to higher predation rates and increased vulnerability to changing environmental conditions. Over four months in temperature controlled aquariums, we observed no mortality but an increase in molting frequency in BGD shrimp compared to control "clean" shrimp. In addition, BGD shrimp reached exhaustion more quickly (as early as 1 hour) and exhibited more exhaustive behaviors compared to control shrimp when exercised on a treadmill. Finally, whole body lactic acid concentrations were measured as an indicator of stress in both BGD and control exercised shrimp. These data will be used to discuss the ecological implications of BGD in white shrimp.

Ecological Effects Of The Invading Rhizocephalan Parasite *Loxothylacus panopaei* In The Flatback Mud Crab *Eurypanopeus depressus*

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The rhizocephalan barnacle *Loxothylacus panopaei* is a parasitic castrator of xanthid crabs and is invasive along the US Atlantic coast. It was introduced to the Chesapeake Bay by infected mud crabs associated with Gulf of Mexico oysters in the mid-1960s, and has since spread north to Long Island Sound, New York, and south to Cape Canaveral, Florida. Results of a 13-month study (January 2012–January 2013) at 3 sites in South Carolina (Waties Island, Murrells Inlet and North Inlet) found a total parasite prevalence of 24.2% among all 3 sites, with monthly prevalence as high as 51.6% at Waties Island (April 2012). The parasite was only found in *E. depressus* 5.8–16.3 mm carapace width, while unparasitized *E. depressus* ranged from 2.3–20.3 mm carapace width. Additionally, a foraging rate experiment compared prey consumption between parasitized (externa-bearing) and unparasitized (externa-lacking) *E. depressus* crabs 8–13 mm carapace width over a 72-h period. Parasitized crabs ($n = 43$) consumed significantly fewer (median = 2) mussels (5–9 mm shell height) than unparasitized crabs ($n = 29$, median = 4), suggesting the ecological role of *E. depressus* may be modified where *L. panopaei* is present.

Spatially And Temporally Variable Prevalence Of A Virus Fatal To The Blue Crab, *Callinectes sapidus*

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Unpredictable inter- and intra-annual variation in the abundance of the blue crab, *Callinectes sapidus*, poses a challenge to fisheries managers, who seek to allow maximum sustainable harvests. In addition to variable recruitment and predation, disease-related natural mortality of blue crabs may play a role in population fluctuations. A virus lethal to blue crabs, termed reo-like virus (RLV), is found at high prevalence in shedding system mortalities, and has been described in wild hard crabs. We are investigating the potential for RLV to impact wild crab populations throughout the northeastern US and Chesapeake Bay. In late summer and fall of 2011 and 2012, crabs were collected from five locations between Cape Cod and Delaware Bay and shipped to IMET for analysis. A total of 459 crabs were assessed for RLV using an RT-qPCR assay that has a detection limit of 10 virus genomes. Overall prevalence of RLV was 21%; however, prevalence was highly variable between locations and years, and varied from 0% to 78%. The RT-qPCR assay was then used to analyze frozen archived samples of crabs from five tributaries within the Chesapeake Bay. It was found that in 2010, prevalence of RLV varied from 0% to 42%, with an overall average of 21%. The observation that RLV is present at highly variable prevalences across time and space raises questions about the duration of infections, modes of transmission, and environmental factors that drive outbreaks. The overall RLV prevalence of 21% further suggests that the virus may indeed be a significant factor in natural mortality and therefore of intra- and inter-annual variation in blue crab abundance.

'Omics Comes to the Coral World

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We have begun using the tools of metabolomics and genomics to investigate the microbiology of a polymicrobial disease at the functional level. Our targeted disease is the cyanobacterial-dominated black band disease (BBD) of corals. In the past we have shown that BBD cyanobacteria produce the cyanotoxin microcystin, in particular the most toxic variant LR (MC-LR), and that exposure of the intact BBD community to microcystin results in stimulation of growth of BBD-associated heterotrophic bacteria as seen using SEM. The effect of MC-LR on individual (cultured) BBD bacterial isolates ranged from stimulation to inhibition, depending on the concentration of MC-LR used. Microcystin has also been implicated as a cell signaling molecule. To further investigate the role of microcystin in BBD at the mechanism level, we exposed the freshly collected BBD community to MC-LR (50 µg/l) and measured the metabolite pool at t = 0 (before exposure) and after 24 hours. A total of 135 metabolites were detected. Metabolomics revealed that exposure to MC-LR resulted in an increase in metabolites related to nucleic acid biosynthesis, peptidoglycan synthesis, and overall metabolic activity as indicted by an increase in oxidized NAD^+. We are also using metagenomics to compare the BBD community to a similar cyanobacteria-dominated community that is non-pathogenic. Within this data set over 13,000,000 sequences had predicted proteins with known function, including proteins associated with virulence and defense as well as cell-signaling. Genomes produced from laboratory cultures of the dominant cyanobacteria from both systems (BBD and the hot springs) were included in the analysis.

We Have A Quorum, Now What Are We Saying?

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Black band disease (BBD) is one of the most intricate and destructive coral diseases. It is comprised of four functional groups (photoautotrophs, sulfide oxidizers, sulfate reducers and heterotrophs) that act in collaboration in BBD progression. Of these, the heterotrophs have been shown to produce quorum sensing (QS) signal molecules, including AHLs (acyl homoserine lactones) which have been known to contribute to a number of virulence factors in infections worldwide. BBD is traditionally observed in summers when sea water temperature exceeds 28 °C. The overarching goal of this project was to assess the effect of temperature on QS signal production of heterotrophs isolated from BBD. 156 BBD isolates, 38 isolates from the surface mucopolysaccharide (SML) of healthy corals (HSML), and 36 isolates from the healthy SML of BBD infected corals (BSML) were tested for production of quorum sensing signal molecules by a *Chromobacterium violaceum* CV026 bioassay. Twenty-four QS positive heterotrophs were further exposed to varying temperatures (24°, 27° and 30° C), to assess the role of temperature on production of AHLs using LC-MS/MS at six timepoints throughout the day. A total of eight AHLs were detected, with 3OHC4 being the most abundant (17 of the 24 isolates), followed by the presence of C6 in 13 isolates. Statistical analysis of AHL production by the isolates at varying temperatures revealed that each AHL was affected to a varying degree, with 3OHC6 being the most significantly affected by temperature variation. While further analysis will be needed to determine the effect of temperature variation on AHLs and how this directly affects BBD etiology, these results are a strong indication that temperature variation does affect QS signal production by BBD heterotrophs. The effect of temperature on AHL production will further help in deciphering the role of quorum sensing in BBD pathogenesis and community structure, providing insights into the mechanisms of one of the most aggressive coral diseases.

Regeneration in Corallimorpharia

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Sessile animals are frequently at risk of injury due to predators, competition, environmental and anthropogenic impacts. The ability to regenerate lost tissue or new individuals is an important survival mechanism. Corallimorpharia species in the phylum Cnidaria are easily maintained and readily regenerate new individuals from small fragments of tissue. They are closely related to Scleractinia and possess the potential to be a model species for regeneration in corals. The process of regeneration and normal histology in this group is not described. A limited number of studies have used histology to describe the cellular process of regeneration in other corals. Fragmentation, followed by morphological description of the regenerative process will be performed over a period of several weeks. This work will create a basis for future research regarding the effects of environmental and intrinsic factors on regeneration of Corallimorpharia.

Regeneration In The Caribbean Star Coral *Montastraea cavernosa*

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Absolute coral cover in the Caribbean has decreased more than 70% since the 1970s. Given this extreme decline it is critical to determine the factors that most strongly affect coral regeneration in this region. *Montastraea cavernosa* is one of the most commonly encountered scleractinian species at intermediate (10-20m) depths despite the drastic reduction in coral cover that the Caribbean has sustained. 12mm diameter x 2mm deep circular lesions were made on 124 healthy *M. cavernosa* colonies at 10-12m depths in marine regions of interest in the coastal waters of Grenada and Carriacou. Images were captured every 0.5m along 0.5m x 30m long belt transects near targeted coral heads both inside and outside marine protected areas. Water samples were collected weekly for organic nutrient component analysis. Analysis of benthic diversity as a proxy for ecosystem heath, organic nutrient levels, and the presence of a marine protected area will be examined for their potential impact on *M. cavernosa* tissue regeneration rates. If any of these factors have a significant effect on coral regeneration a predictive model may be generated in order to determine which regions should be prioritized for their potential positive influence on scleractinian coral regeneration.

Molecular Madness: The Case for Phenotypic Anchoring In Understanding Corals

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Structure and function have been important partners in understanding the biology and pathology of living organisms. With the molecular age, structure has largely been ignored in favor of interrogating the molecular coding of DNA and RNA of genes, genomes and transcriptomes. Such studies have little or no structural context in terms of the phenotype of the organism being studied. New generations of scientists are unable to identify gross or microscopic organism structures and lack understanding of how the type, quantity, and arrangement of the organism’s molecules drive metabolism and can be damaged by disease. A growing number of molecular biologists are using the powerful science of genomics to determine DNA sequences of coral genes and genomes (genomics), putative proteins from their transcriptomes, and cell signaling molecules for communication, trafficking and other cellular and tissue functions. Many of the conclusions drawn from these studies are potentially flawed, however, because of assumptions made about their source material. The complexity and potential confounding elements of their samples have not been examined. Examples of concerns include using “healthy” samples that actually have microscopic lesions or parasites, comparing bleached samples that resulted from different modes of zooxanthellae or photosynthetic pigment loss, and what does up-regulation of genes shared with vertebrate genes (e.g., “cartilage and bone development,” “immunity”) mean in organisms that are morphologically dissimilar? Moving the pendulum back to consider structure and function equal partners will lead to improved interpretation of the true biology and pathology of corals.

The Effects Of Ocean Acidification And Climate Change On The Virulence Of Black Band Disease

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Black band disease is one of the most common diseases affecting scleractinian corals in the Florida Keys. In past studies the prevalence and incidence of the disease have been positively correlated with elevated sea water temperatures. However, the impact of decreasing ocean pH as a result of increasing atmospheric CO_2 levels, or ocean acidification, on disease dynamics has yet to be examined. Changes in organismal physiology because of environmental conditions may lead to increased disease susceptibility of the coral host or greater disease virulence for coral disease pathogens. This study tested effects of seawater temperature and pH on the progression rates of black band disease on three coral species (*Pseudodiploria clivosa*, *Orbicella faveolata,* and *Montastraea cavernosa*). We artificially inoculated 32 colonies of each species with black band disease and exposed them to two temperature conditions (low 26°C and high 30°C) and two pH conditions (high 8.1 and low 7.6). Individual coral colonies (n=8) were used under four treatment scenarios for 23 days. *O. faveolata* was most susceptible to black band disease with the highest rate of mortality. Low pH reduced progression of black band disease on all three species, suggesting that ocean acidification conditions may reduce black band disease virulence.

Potential Role Of Dimethylsulfoniopropionate In Coral Disease

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Dimethylsulfoniopropionate (DMSP), known to be abundant in the marine environment, was long thought to be produced solely by photosynthetic organisms, including the algal symbionts of corals. Recently, DMSP has been shown to be produced by the coral animal with increased production during thermal stress events. Higher disease prevalence in corals has also been correlated with thermal stress. Combined with recent metagenomic studies demonstrating that members of more than 65% of the microbial genera known to be involved in DMSP metabolism are associated with corals, this strongly suggests that DMSP may play a role in structuring coral-associated bacterial communities, specifically those of diseased corals. In addition to providing an energy source, DMSP was shown to serve as a chemical cue for motility in a known coral pathogen. By screening isolates for genes related to DMSP metabolism and assessing chemotactic response of those same isolates, we can further elucidate the role of DMSP in coral disease. To date, only four genes have been identified (*dmdA, dddD, dddL*, and *dddP*) that play a major role in DMSP degradation by coral-associated bacteria (CAB). Our current study aims to screen for three of these genes (*dmdA*, *dddD*, and *dddL*) in isolates of black band disease (BBD) and white plague type II (WPII). Additionally, chemotactic analysis of these organisms will be assessed by Adler capillary assays utilizing DMSP concentrations in the range of 15 µM to 100 µM. By correlating the presence or absence of DMSP metabolism-associated genes with chemotactic response data, we can determine the extent to which individual organisms are influenced by DMSP providing insight into the etiology of BBD and WPII as well as other coral diseases.

Comparison Of Hematology, Plasma Biochemistry, And Blood Gas Parameters Between Two Venipuncture Sites In Southern Stingrays (*Dasyatis americana*)

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The southern stingray (*Dasyatis americana*) is a batoid elasmobranch frequently exhibited in zoological institutions. Blood is most commonly collected from the caudal hemal arch at the tail base in stingrays from a ventral approach. Caution must be used while handling the tail due to the proximity of the sharp venomous barb to this venipuncture site. An alternative site that allows a dorsal or ventral approach without necessitating puncture of a cartilaginous structure has been identified between the cartilaginous pectoral fin rays (ceratotrichia). This site is currently being used with batoid elasmobranchs at various institutions. The objective of this study was to compare complete blood counts, plasma biochemistry values, and blood gases between caudal and pectoral fin venipuncture sites in the southern stingray. The study also aimed to determine the level of agreement between parameters to evaluate their utility for clinical use. Lithium heparinized blood samples were collected from the caudal and pectoral venipuncture sites from fifteen captive southern stingrays (ten females, five males) from four zoo and aquarium facilities. Data from iSTAT CG4+ cartridges, estimated leukocyte counts and differentials, and plasma chemistries were compared. Level of agreement for each parameter was assessed using Bland-Altman plots and determined by calculating the percent bias, or difference between the venipuncture sites, compared to the mean. The only statistically significant difference observed was higher creatine kinase (CK) values of pectoral fin samples. Levels of agreement between sampling sites were good or moderate for twenty-four of twenty-nine parameters assessed. Only alanine aminotransaminase, aspartate aminotransferase, CK, lactate, and partial pressure of oxygen (pO_2) had poor agreement between sites. The good agreement between sampling sites for the majority of parameters assessed and the lack differences that would alter clinical interpretation support the use of the pectoral site as a useful alternative to the traditional caudal venipuncture site in southern stingrays.

Carnobacterium maltaromaticum In Stranded Pacific Salmon Sharks And Common Thresher Sharks

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The Wildlife and Aquatic Veterinary Disease Laboratory received bacterial colonies on blood agar from two sharks in 2014. Colonies were isolated from brain, inner ear, and inflamed subcutaneous tissues surrounding the endolymphatic ducts of a juvenile common thresher shark (*Alopias vulpinus*), and from brain and peri-endolymphatic tissues of a juvenile salmon shark (*Lamna ditropis*). The two sharks were among 15 (12 salmon; 3 thresher) that stranded on CA beaches between 2011 and 2014, and were necropsied by the CADFW. Colonies were subcultured and subjected to biochemichal, phenotypic and molecular analyses. Based on morphological and biochemical characteristics, bacterial isolates were identified as *Carnobacterium maltaromaticum. C. maltaromaticum* is a gram positive rod that has been isolated from various sources. This bacterium inhibits growth of foodborne pathogens such as *Listeria monocytogenes* due to its ability to produce bacteriocins. Viable and heat-treated *C. maltaromaticum* have been used as protective cultures in various meat and seafood products. Sequence analysis of the 16S ribosomal RNA gene of all five shark isolates produced 1420 bp amplicons with identical sequences.The 16S rRNA gene sequences shared 99.7% (1416/1420 bps) identity with a *C. maltaromaticum* isolated from ripened cheese, and 99.7% (1013/1016 bps) sequence identity with *C. maltaromaticum* previously reported from 19 CA salmon sharks that stranded in 2002-2008. Identification of *C. maltaromaticum* in subcutaneous tissues surrounding the endolymphatic ducts of thresher and salmon sharks indicates that bacteria likely gain access to the inner ear and brain via the endolymphatic ducts. Genetic homology between *C. maltaromaticum* from sharks and the isolate from one human food source suggests that an anthropogenic etiology for CA shark strandings is at least a possibility. Determining whether or not the actual cause of otitis and meningoencephalitis in sharks stems from natural sources or aquatic contamination from human foods, remains to be seen.

Clinicopathological Features Of A Multi-species Aquatic Animal Mortality Event Associated With A Large Scale Harbor Development Project

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In order to accommodate industrial development, a harbor expansion project was initiated in the Port of Gladstone, Queensland, Australia in October 2010. Shortly after the project commenced, an unusual large-scale disease and mortality event occurred in the marine animal populations of the harbor and nearshore waters. Over a period of several months, fish kills involving many species of finfish occurred in several locations throughout the Harbor. In addition, fishermen reported catching large numbers of finfish affected with cutaneous and ocular lesions as well as increased sightings of sick and dying turtles, dugongs and dolphins, and frequent rejection of entire catches by retailers. At the peak of the fish illness, there were also reports of cutaneous lesions in over 40 humans, all of whom had contact with the harbor water or fish immediately prior to the onset of symptoms. This investigation, largely funded by private donations and the regional fishing industry, was undertaken to measure the extent and nature of disease of marine fishes in the affected harbor. Finfish were examined in sites representative of reported health problems and were caught as part of routine commercial fishing operations. Finfish were caught by gill net (n = 435) were compared to those caught at a reference site (n = 146), 250km north of the study site. Gross abnormalities were higher in study fish (25.5%) as compared to controls (5.5%; P <0.0001). Abnormalities included excessive mucous on skin and gills, erythema, heavy ectoparasitic infestations, cutaneous ulceration, corneal opacity or ulceration, and exophthalmos. Of finfish examined histologically, abnormalities were prevalent in those from the study area (36/28, 97.7%), but were uncommon in controls (3/22, 13.6%). Abnormalities included hyper-parasitism and noninfectious erosive to ulcerative dermatitis of variable chronicity. The prevalence of shell lesions was also higher in mud crabs from the study area (270/718, 37.6%) relative to those of the reference site (21/153, 13.7%; P <0.0001), and primary endocuticle degeneration, with pathogenesis thought to involve increased exposure to copper and zinc, was only histologically evident in crabs from the study area. The differential prevalences of disease between study and control groups suggest causal mechanisms are unique to the affected harbor, and the occurrence of ulcerative skin disease and hyperparasitism, together involving a wide range of species, raises serious concern for environmental stress. While a single causal mechanism cannot be established by this investigation, the influence of harbor development, particularly dredging, is an obvious important factor, given its spatial and temporal relationship with the disease outbreak, and its known detrimental effects on the aquatic environment. While not directly addressed by this investigation, water quality observations recorded by others support this hypothesis, including exceedances in turbidity, heavy metals, and documentation of algal blooms. To the authors' knowledge, a mortality and disease epidemic of this magnitude has not been previously reported in association with dredging. The unique geological and industrial history of the harbor, the scope of environmental modification, the disposal of dredge spoil within a reclamation site with a flawed bund wall, and proceeding flooding events are important contributing factors in this outbreak.

Molecular And Histopathological Disruption Of Wound Healing In An Ectoparasite Model

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Sea lice modulation of host immune responses disrupts wound healing capabilities of Atlantic salmon, preventing rejection of the parasite, prolonging time to wound healing, and increasing the risk of secondary infections and osmoregulatory stress. Resultant morbidity and mortality of parasitized fish is a major factor in the economic losses experienced by the aquaculture industry annually. In the present study, two immunostimulant feeds (confidential formulations), one with a single stimulatory compound (IS-1) and one with two stimulatory compounds (IS-2) were investigated for their effects on lice-induced skin lesions and wound healing in naïve Atlantic salmon. IS-1 and IS-2 fed fish were compared to fish fed a control diet. Head lesions of IS-2 fed fish were significantly more extensive at 27 dpi compared to IS-1 fed fish. Interleukin-1β was upregulated in infected skin sites and head kidney of IS-1 and IS-2 fed fish at 27 dpi and matrixmetalloproteinase-9 was also upregulated in the skin of both groups. Skin MMP9 upregulation coincided with a downregulation of collagen synthesis at infected skin sites. The levels of MMP9 tissue degradation may have contributed to disrupt wound healing. These data and additional immunological assessment of the host will be discussed with respect to the host-parasite interaction.

Streptococcosis In Lake Trout (*Salvelinus namaycush*)

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Streptococcosis is a complex of diseases caused by gram positive cocco-bacilli belonging to numerous genera and species. It is considered one of the more clinically important of the 'miscellaneous' bacterial infections of fish. Streptococcosis in cold-water is less widespread and clinically severe than in warm-water and affects primarily salmonids. The infections in colder water have been associated with chronic post-spawning stress and occurred at temperatures of 10 to 12 °C. An OMNR hatchery, which raises lake trout for stocking, experienced chronic mortalities in ~1 year old fish that were intended as broodstock. The aim of this report is to describe the gross and histopathological lesions and identification of the gram-positive cocci isolated from affected fish. Bacterial culture, histopathology and PCR assay with 16S universal and pSal-1 and PSal-2 primers were combined to establish the diagnosis.

Megalocytivirus Infection In Fish

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Aquaculture faces many challenges including those posed by emerging infectious diseases such as megalocytiviruses (MCVs). The MCVs are categorized as large double-stranded DNA viruses belonging to the family *Iridoviridae*. Members of this genus are divided into three major clades based on phylogenetic analyses: 1) infectious spleen and kidney necrosis virus (ISKNV) which is reported to cause disease in numerous marine and freshwater fishes, 2) red sea bream iridovirus (RSIV) that mainly infects marine fishes and 3) turbot reddish body iridovirus (TRBIV) that primarily infect flounder species. Currently, only RSIV is listed as an OIE reportable disease. MCVs are not limited to Asia but have also been described in North America and most recently in the Caribbean. They display low host specificity infecting greater than 100 species of freshwater and marine fishes. This has been confirmed by a number of cohabitation trials that have demonstrated MCVs can cross species boundaries. Although fish suffering from MCV infections display few external or behavioral disease signs, histopathological examination of various internal organs (e.g., spleen and anterior kidney) readily reveal basophilic inclusion body-bearing cells. The recent development of advanced molecular techniques such as conventional PCR, quantitative PCR and loop-mediated isothermal amplification has enabled specific and rapid detection of MCVs. In addition, several research laboratories are actively engaged in producing vaccines and biosecurity protocols to control these globally emerging pathogens.

Demystifying Antimicrobial Susceptibility Testing: Resistance Does Not Always Mean Resistant

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Internationally-harmonized, consensus-approved, standardized antimicrobial susceptibility testing (AST) methods are now available for testing most bacterial fish pathogens. These Clinical and Laboratory Standards Institute (CLSI) disk diffusion and broth microdilution methods with quality control parameters provide a means to monitor inter- and intra-laboratory variability - critical to maintaining consistency not just for the data generated, but how the data are interpreted. AST data can potentially be interpreted with two goals in mind: 1) to guide therapy (clinical breakpoints should only be used), and 2) provide antimicrobial resistance surveillance [epidemiological cutoff values (ECVs) or clinical breakpoints can be used]. Progress updates of CLSI Aquatic Animals Working Group's research will be provided on antimicrobial resistance surveillance of *Aeromonas salmonicida, Edwardsiella ictaluri, E. tarda, Yersinia ruckeri, Flavobacterium columnare,* and *F. psychrophilum.* Establishing these epidemiological cutoff values is the centerpiece of the Working Group's efforts, and will allow for detection of emerging antimicrobial resistance among bacterial species pathogenic to fish. Additional planned AST research by CLSI members and advisors will be discussed, as well as additional research areas that still need to be addressed.

The F Is For Fastidious… The Challenge In Antimicrobial Susceptibility Testing Of *Francisella noatunensis*

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Francisella noatunensis subsp. *orientalis* (*Fno*) is an emergent fish pathogen that can cause acute to chronic disease in a wide variety of fresh, brackish and marine fish. In this project we aim to provide a standardized protocol of testing antimicrobial susceptibility in *Fno* utilizing the broth micro-dilution method. Thirty four *Fno* isolates recovered from different fish species and locations and Escherichia coli ATTC 25922 reference strain were used. A cation adjusted Mueller Hinton broth supplemented with IsoVitalex and Glucose (MMH) was tested at pH of 6.5±1, 7.1±1, and 7.3±1. Growth curves generated for *Fno* indicated that MMH at a pH of 6.5 provided the best conditions for growth. However, the low pH in MMH appeared to interfere with the performance of potentiated sulfonamides (Sulfadimethoxine: Ormetoprim and Trimethoprim-sulfamethoxazole); and in some occasions with Gentamicin when using the *E. coli* ATTC reference strain. The minimal inhibitory concentration (MIC) of ten antimicrobials frequently used in aquaculture was similar in all *Fno* isolates. The *in vitro* susceptibility data provided here can provide a baseline for monitoring the development of antibiotic resistance among *Fno* isolates, as well as provide invaluable data for use in the development of potential therapeutics.

Use Of Minimal Inhibitory Concentration And Disk Diffusion Zone Data To Determine Epidemiological Cutoff Values Of Antimicrobial Agents Against *Edwardsiella ictaluri*

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At many aquatic animal disease diagnostic laboratories, bacterial diseases are the number one cause of accessions. To assist diagnosticians in treating fish bacterial diseases, it is important to determine the causative agent’s susceptibility to antimicrobials. Laboratory-specific clinical breakpoints are currently being used in each fish diagnostic laboratory to determine susceptibility of bacterial pathogens to antimicrobials and guide potential therapy. However, inter-laboratory variation in how susceptibility data are interpreted (susceptible, intermediate, resistant) can cause confusion especially in the literature. Standardized antimicrobial susceptibility testing methods approved by the Clinical and Laboratory Standards Institute are being used to establish laboratory-independent interpretive criteria to minimize this confusion, improve the detection of emerging resistance phenotypes, and potentially improve the antimicrobial therapy decision process. This report details the quest to establish much needed epidemiological cutoff values using frequency distributions of minimal inhibitory concentrations and zone diameters for over 100 *Edwardsiella ictaluri* isolates. Isolates were obtained from the US and Europe and were tested against 10 antimicrobials. These susceptibility data distributions will be used to develop epidemiological cutoff values (ECVs) critical to the detection of emerging antimicrobial resistant isolates. Eventually these ECVs may be combined with pharmacokinetic and pharmacodynamic data and clinical outcome reports to establish clinical breakpoints useful for guiding therapy.

In Vitro Synergistic Effects of Florfenicol, Oxytetracycline and Sulfadimethoxine: Ormetoprim against *Edwardsiella ictaluri*

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Aquaculture is the fastest growing food-production industry. However, as in many agricultural practices, infectious diseases pose a substantial constraint to the potential of fish farming. *Edwardsiella ictaluri*, a Gram-negative member of the family Enterobactericeae, is the known etiological agent of enteric septicemia of catfish, and it has been isolated from recent outbreaks in cultured Nile tilapia and zebrafish. Currently, only three antibiotics [florfenicol (FFN), oxytetracycline (OXY) and sulfadimethoxine: ormetoprim (PRI)] are approved by the United States Food and Drug Administration for use in U.S. aquaculture. In addition, there is very limited published data on antimicrobial susceptibility of fish pathogens and growing concerns on selection pressure for antimicrobial-resistant bacteria. Antimicrobial combinations are used to achieve synergistic activity against particular resistant strains, overcome bacterial tolerance, prevent the emergence of drug resistance, minimize toxicity, and prevent inactivation of an antimicrobial agent by enzymes produced by other bacteria. This study constructed concentration-response curves and assessed the *in vitro* interaction of drug combinations (FFN+OXY; OXY+PRI; and FFN+PRI) with the hope of identifying synergistic antimicrobial combinations against *E. ictaluri* isolates recovered from Nile tilapia, cultured zebrafish, and channel catfish. Using the broth micro-dilution method, the minimum inhibitory concentrations (MIC) of FFN (0.12µg/mL), OXY (0.25µg/mL) and PRI (1.19:0.06µg/mL) were similar among the different *E. ictaluri* isolates. When combined, the MIC for FFN+OXY (0.032+0.043 µg/mL) and for OXY+PRI (0.086+0.269:0.014µg/mL) were between 6- and 15-fold reduced compared to the MIC for each drug alone. At the highest concentration tested, FFN+PRI (0.127+0.538:0.028 µg/mL) did not inhibit bacteria growth. Further research into the *in vitro* and *in vivo* effect of antimicrobial combinations is warranted in order to provide more therapeutic options to the growing aquaculture industry.

Avermectin Treatment For *Lepeophtheirus Salmonis* And Effects On Salmon Immunophysiology

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Throughout the 1990s the in-feed treatment, SLICE™ (Emamectin benzoate-EMB) was so successful against *Lepeophtheirus salmonis*, it was used almost exclusively in the Canadian aquaculture industry, providing lasting protection against all life stages of the parasite. Recently this treatment has exhibited reduced efficacy due to the development of resistance by sea lice in Eastern Canada, Chile, UK and Norway. Reduced efficacy has resulted in increased dosages of EMB being used within the Bay of Fundy, New Brunswick, Canada as well as the returned usage of ivermectin, a macrocyclic lactone related to EMB, as an off-label, in-feed chemotherapeutant. Ivermectin has demonstrated significant efficacy, however there are serious issues relating to toxicity. The goal of this study was to determine the key parameters necessary to identify the role immune potentiation may be playing alongside the evolution of resistance to avermectins in sea lice populations in Eastern Canada. We investigated the effects of single (50 ug/kg), triple (150 ug/kg) EMB dosages and ivermectin treatments on immunological gene regulation and anti-viral responses in Atlantic salmon smolts, following stimulation with inactivated Infectious Salmon Anaemia Virus (ISAv). Different dosages of EMB and the repeated treatment with ivermectin did not affect feeding behaviour in salmon, however some neurotoxicity was observed in the ivermectin treated group. Impacts of avermectin treatment on expression of important anti-viral response genes in Atlantic salmon will be discussed.

Sea Lice (*Lepeophtheirus salmonis*) Responses To Concurrent Drug Treatments With Emamectin Benzoate And Cypermethrin

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The ectoparasitic copepod known as sea lice (*Lepeophtheirus salmonis*) is a major threat to global salmon aquaculture. Veterinarians are limited to few therapeutic options for sea lice control and consequently, overuse of these products has led to drug resistant populations. Treatment regimes have therefore shifted to incorporate concurrent and/or follow-up treatments using multiple drug options. Two of these compounds, emamectin benzoate (EMB) and cypermethrin, are still used globally despite the declining sensitivity of sea lice to their effects. To better understand the response dynamics of these treatments, a bioassay exposing sea lice to EMB with follow-up cypermethrin was designed. Adult sea lice of both sexes were exposed to a sub lethal dose (200ppb) of EMB or a seawater control for 24hrs. Surviving lice were then distributed to either a low (0.5ppb) or high (1.5ppb) dose of cypermethrin (or another sea water control) before assessing survival and collection for transcriptional analysis using a 38K oligonucleotide microarray. Principal component analysis indicated differential expression due to drug exposure was minor compared to differences between sexes. Furthermore, when compared to controls, EMB caused little perturbation in gene expression compared to cypermethrin. When sea lice were treated with both drugs, several genes showed a greater response when compared to either controls or lice treated with a single compound. This study demonstrates that concurrent treatment using multiple drugs could select for polygenic mechanisms of resistance and greater tolerance to chemical therapy.

Black Seed *Nigella sativa* And Its Oil Extract Protects Against Columnaris Disease In Zebrafish And Catfish

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Columnaris disease, caused by *Flavobacterium columnare*, is currently one of the most important bacterial disease affecting farm-raised channel catfish (*Ictalurus punctatus*) around the world. Conventional therapy for columnaris outbreaks is often through administration of medicated feeds with antibiotics, however, the excessive use of antibiotics in aquaculture in recent years has led to emergence of antibiotic resistance in many fish pathogens including *F. columnare*. Sustainable aquaculture requires safe and effective alternatives to increase fish survival while maintaining a healthy environment. *Nigella sativa* is a medicinal plant used in folk medicine by many cultures as a natural remedy for numerous disorders. Recently, we have discovered the antibacterial activity of the black seeds and seed oil from this plant against *F. columnare*. The goals of this study were to test the antibacterial effect of black seed oil against 25 *F. columnare* strains belonging to three genomovars (I, II and III) by disc diffusion method and to investigate the effect of black seeds and black seed oil dietary supplementation (5%) against experimental infection with *F. columnare* in zebrafish and channel catfish. Black seed oil showed a strong antibacterial activity against all *F. columnare* strains and yielded significantly larger zones of inhibition than those of oxytetracycline. For the *in-vivo* experiments, zebrafish juveniles and channel catfish were fed the experimental diets for 21 days before the immersion challenge with a highly virulent *F. columnare* strain following standard procedures. Supplemented diets were protective to fish in controlled laboratory challenges. Our results show a potent inhibitory effect of black seed oil on *F. columnare* and the protective potential against columnaris disease.

Aquatic Animal Disease Testing Added To The National Animal Health Laboratory Network: Phase I Is Underway

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The National Aquatic Animal Health Plan (NAAHP) is a roadmap on how to safeguard the health of our Nation's farmed and wild aquatic resources. It was developed by three federal agencies (APHIS, NOAA, and USFWS) under the auspices of the Joint Subcommittee on Aquaculture (currently Interagency Working Group on Aquaculture) with consultation and input from industry and other stakeholders. Key NAAHP recommendations concerning high consequence aquatic animal pathogens included preparation for potential disease outbreaks, prevention of pathogen introduction, pathogen monitoring, trade facilitation, and standardization of diagnostic assays across laboratories. In the United States, aquatic diagnostic testing has been provided by a variety of institutions including universities, research laboratories, private companies and Federal laboratories. Since the inception of the NAAHP many different proposals have been considered regarding the development of a national system that requires quality management including standardized testing and required reporting for aquatic animal diseases without adoption. One proposal discussed among aquatic health stakeholders set forth that testing for aquatic pathogens be phased into the existing National Animal Health Laboratory Network (NAHLN). The NAHLN is a USDA supported multifaceted network comprised diagnostic laboratories that focus on different diseases, using common testing methods and data standards to process diagnostic requests and share information. The APHIS Aquatic Animal Health Program along with NAHLN has moved forward with the inclusion of aquatic disease testing under the NAHLN in three phases. A brief update on inclusion of aquatic disease testing into the NAHLN will be provided.

Propagation Of Lake Trout (*Salvelinus namaycush)* Cells *In Vitro*

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The lake trout is the largest trout species native to the Laurentian Great lakes basin and is heavily sought after by both commercial and sport anglers. Great Lakes lake trout populations suffer from wide scale parasitism by the sea lamprey, which heavily impacts lake trout abundance and recruitment. In the 1980s, the declining lake trout populations further suffered from the emergence of a novel alloherpesvirus; the Epizootic Epitheliotropic Disease Virus (EEDV, salmonid herpesvirus 3). Studying the biological properties of this highly pathogenic virus is hampered by the inability to propagate EEDV on currently established cell lines, despite frequent attempts. This is surprising since other alloherpesviruses such as salmonid herpesvirus 1 and 2, koi herpesvirus, and channel catfish virus are capable of propagating in multiple cell lines. Currently, there are no available lake trout-derived cell lines. Hence, the aim of this study was to grow lake trout cells *in vitro* with the ultimate goal of testing their ability to support EEDV propagation. Primary cell cultures of lake trout were prepared from sac fry, skin, gonads, kidneys, and liver. A number of basal salt solutions as well as serum and serum-free supplements (including naïve lake trout serum) were compared for their ability to stimulate lake trout cell multiplication. Tissue culture vessels were coated with one or more cell attachment enhancers to encourage the formation of confluent monolayers. Results obtained will be presented and the potential to develop long-term lake trout cell cultures will be discussed.

Danio rerio T Cell Receptor Alpha/Delta Locus Organization And Phylogenetic Analysis Af V Alpha/Delta Region

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The goal of the current study was to identify the genomic location and organization of the T cell receptor alpha/delta locus of *Danio rerio* based on the most recent genome assembly, version 10. The locus was found on Chromosome 2 and spans roughly 600 Kb from nucleotides 36,664,000 to 36,110,000. The locus has a tentative organization of Vɑ/δ- Cɑ- Jɑ– Dδ -Jδ –Cδ, with the V segments being in reverse orientation to the other elements. This organization is similar to the locus in all teleost species in which it has been identified but is different from the traditional organization seen in higher species such as birds and mammals . So far we have identified a total of 144 theoretically functional V segments to date which have been grouped into approximately 51 families based on 70% nucleotide identity. This number represents the greatest amount of functional V regions previously known. Salmon have 128 functional Vɑ/δ, humans have 57, mouse has 98, and chickens have 70. Previous work by Schorpp et. al. and Haire et. al. identified two known Jδ segments, three Dδ segments and seven Jɑ segments. We are in the process of identifying additional V, D, and J segments. We have identified one Cδ segment and previous work by Haire et. al. had identified four Cɑ clones. We have further attempted to characterize the locus by obtaining expression data using a RACE library created from cells harvested from zebrafish spleen. This RACE library has only yielded one clone of Vɑ/δ- Dδ -Jδ –Cδ rearrangement. This confirms that zebrafish do express TCR delta, but apparently only at extremely low levels.

Potential Impacts Of The Fathead Minnow Nidovirus (Family: Coronaviridae, Order: *Nidovirales*) On The Laurentian Great Lakes Native Cyprinids And Rehabilitation Programs

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The Fathead Minnow Nidovirus (FHMNV), originally found in southern US states, has been found to be associated with muskellunge mortality events in two state fish hatcheries in Michigan and Wisconsin. The emergence of this serious pathogen in the Great Lakes region raised concerns regarding potential risks for native cyprinids as well as for piscivorous fish rehabilitation programs. This study was undertaken to ascertain the pathogenicity of this virus to representative Great Lakes fish species. Native cyprinids including spotfin shiner, creek chub and golden shiner were screened for their susceptibility to FHMNV by intraperitoneal (IP) injection, and the virus was found to be pathogenic to spotfin and golden shiners while creek chub appeared to be resistant to the virus. When spotfin and golden shiners were challenged by immersion, spotfin shiners were the only species which exhibited mortalities. Among piscivorous fish species tested (muskellunge, walleye, rainbow trout and largemouth bass), muskellunge were found susceptible both by IP injection and immersion. Follow up studies determined possible routes of infection, the virus tropism and shedding by infected muskellunge. The results of these studies are alarming, since FHMNV seems to impose a threat to a number of Great Lakes fish species of ecologic and economic importance.

What Doesn’t Kill You, Will Grow In Your Biofilm: Zebrafish Facilities And The Diversity Of *Mycobacterium* Species In Biofilms?

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Mycobacteria are considered ubiquitous in aquatic environments. Their waxy cell wall makes them one of the most hydrophobic groups of prokaryotes and allows them to resist changes in environmental conditions and readily form biofilms on surfaces. In zebrafish facilities, mycobacteriosis is a common finding in laboratory zebrafish, *Danio rerio*, and identical isolates have been collected from both fish and surface biofilms. The *Mycobacterium* species diversity is much greater in biofilms indicating that not all mycobacteria readily infect fish. An examination of biofilms, fish gut contents, and infections of internal organs shows a stepwise decrease in diversity, usually with only one species being able to cause infection. Thus, mycobacteria in biofilms does not directly equate to infections in fish. Dozens of species have been identified in biofilms that do not infect fish, and elimination of all mycobacteria from an aquatic system is likely not feasible because they can be free living and thrive even in extremely low nutrient conditions. It is desirable to understand if competition between species in biofilms could play a role in limiting the growth of pathogenic species. I.e., can a species not known to be a pathogen in zebrafish be used to establish a biofilm and limit the introduction of pathogenic species. Pros and cons of this approach will be discussed.

I Thought They Smelled Bad On The Outside…Routine Evaluation, Monitoring, And Sanitation Of Automatic Reverse-Osmosis Watering Systems

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In the terrestrial animal world, automated watering systems have been a great refinement in husbandry practices regarding the reliable delivery of water to animals housed in enclosures connected to these systems. However these systems must be adequately maintained per manufacturer's instructions to ensure that they continue to work correctly and deliver water of appropriate quality. Automatic watering systems supplied by reverse-osmosis (RO) water sources serve the individual ventilated caging (IVC) racks for several of our rodent vivaria. During routine environmental monitoring of our institution's watering systems, *Sphingomonas pauclimobilis,* formerly *Pseudomonas paucimobilis,* was cultured from the manifold lines of several IVC racks within one facility. These findings led to an increased evaluation of the entire RO water production and distribution system of that facility. Approximately two weeks after discovery in the water system, *S. pauclimobilis* was isolated from a peritoneal abscess of a B6.Cg-*Slc11a1*^{r} Rag1^{tm1Mom}/Cwi mouse housed in the same vivarium. Oropharyngeal cultures from related mice were obtained, and *S. pauclimobilis* was grown from 2 of 9 samples. Investigation into this situation revealed that routine rack and RO system sanitization was not being carried out, leading to biofilm accumulation within the automatic watering system. Rodents drinking from this system were likely exposed to fragments of biofilm on a random, but consistent basis. Such exposure put rodents at risk for oropharyngeal colonization and subsequent bacteremia, especially those strains that are immune compromised. This presentation will discuss the intricacies, including system sanitation and biocide application, of the husbandry and management-related events conducted to resolve this system's contamination issue.

Differential Resistance Of *Francisella noatunensis* subsp. *orientalis* To Virkon, Bleach And Hydrogen Peroxide By Biofilms And Their Planktonic States

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Francisella noatunensis subsp. *orientalis* (*Fno*) is an emergent pathogen in cultured and wild fish species in both marine and fresh water environments. Although it is recognized as a fastidious organism, the bacterium is suspected to persist in the environment even without the presence of a suitable fish host. In the present study, the resistance of *Fno* in biofilm and planktonic state to commonly used disinfectants used in the ornamental and aquaculture industry were compared. The minimum biofilm eradication concentration (MBEC™) assay was used to investigate the Minimum Biocidal Concentration (MBC), the Minimum inhibitory concentration (MIC), and the MBEC of Virkon, bleach and Hydrogen Peroxide in 48 h old *Fno* biofilms formed at 25°C. The MBEC for Virkon and bleach was significantly greater than the MBC or MIC of each disinfectant. The MBEC, MIC and MBC of hydrogen peroxide was $<0.045\%$ H_2O_2 when incubated for 10 min. This information needs to be taken into account if trying to eradicate the pathogen form aquaculture facilities or aquariums.

Attack Of The Snails: Adventures In Biofilm Remediation To Reduce Off-Flavor In RAS Salmonids

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Harvest-size fish in water recirculation aquaculture systems (RAS) often need to be depurated prior to slaughter in order to purge consumable tissues of undesirable “off-flavor” compounds. During a past trial culturing Atlantic salmon in a 150 m^3 semi-commercial scale RAS, native freshwater snails, *Physa gyrina*, inadvertently populated the water recycle loop; subsequently, harvested Atlantic salmon (4-5 kg) were found to have low to non-detectable off-flavor in the fillets. We investigated this phenomenon in a controlled 3-month study using 8 experimental RAS (0.5 m^3). Two treatments (4 systems/treatment) were evaluated while culturing rainbow trout: 1) systems stocked with snails and 2) systems without snails. Prior to stocking trout and snails, each culture system was brushed, cleaned, and disinfected using 250 mg/L hydrogen peroxide; thereafter, 30 juvenile rainbow trout were stocked in each culture tank, and snails were stocked several days later. Visually, a dramatic difference in biofilm was evident in RAS with and without snails; to quantify this difference, biofilm was scraped from the inside of a template held over several common areas of each sump, totaling 233 cm^2. Total solids per cm^2 resulting from the biofilm scraping was 0.03 ± 0.02 and 1.16 ± 0.16 mg/cm^2 for systems with and without snails, respectively. Replicated samples of water and rainbow trout fillets were collected from each experimental system monthly and analyzed for the off-flavor compounds, geosmin and MIB. Off-flavor results will be made available at the workshop. Data on bacterial densities and bacteria species present will also be presented.

Effect Of Ca^{2+} On Biofilm Formation Of Fish Pathogens

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The genera *Flavobacterium, Edwardsiella, and Aeromonas* contain important fish pathogens that cause great economic losses in aquaculture practice. Eradication of these pathogens is unrealistic as they are ubiquitous in freshwater environments. Interestingly, many aquatic bacteria are capable of forming biofilm and are more resistant to stressors such as disinfectants. Previous studies in our laboratory showed that bacteria in biofilm retained virulence and were able to infect channel catfish. Currently, we are investigating the role of Ca^{2+} in biofilm formation by *F. columnare*. We tested biofilm formation of *F. columnare* in glass flasks with different Ca^{2+} concentration (0 to 6.5 mM) and found that high concentrations of Ca^{2+} consistently enhanced biofilm formation. To test if the positive effect of Ca^{2+} in biofilm formation was a general mechanism, we quantified the production of biofilm in other fish pathogens at different Ca^{2+} concentrations including strains of *A. hydrophila, E. ictaluri, E. tarda* and *E. piscicida* using crystal violet and microtiter plates to quantify the biofilm. Preliminary data showed that bacterial response to Ca^{2+} was strain specific. Bacterial attachment to plastic surfaces under fluid conditions was also evaluated by using a microfluidic chamber. The microfluidic adhesion assay indicated that *F. columnare* can attach to plastic (polydimethylsiloxane) against higher effluent resistance at enhanced Ca^{2+} concentration. The biofilm colonized by beginning at the walls of the channels and progress to aggregate as a thick biofilm. It indicated the presence of Ca^{2+} can significantly increase the adhesion force of cells and thus promote the biofilm production. Our data indicate that the response of fish pathogens to Ca^{2+} was strain specific, and that increasing Ca^{2+} concentrations promote biofilm formation of *F. columnare* under both static and fluid conditions. The transcriptome of *F. columnare* exposed to different Ca^{2+} concentrations under fluid conditions is underway.

Fight Them Using Their Own Tools (And Some Others): The Role Of Biofilm Formation On The Probiotic Activity Of *Phaeobacter gallaeciensis* S4

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Probiotics are a promising option for the management of bacterial diseases in aquatic species. Several isolates from the genus *Phaeobacter* isolated from the marine environment have been shown to protect marine finfish and shellfish larvae against challenge with bacterial pathogens. *Phaeobacter* spp. have the ability to inhibit pathogen growth *in vitro* through production of the antibiotic tropodithietic acid (TDA). These species are also known for their ability to form biofilms and attach to phytoplankton. *Phaeobacter gallaeciensis* S4, isolated from the inner shell of an oyster from Rhode Island, is able to protect larval bivalve mollusks (oysters, clams, and scallops) against challenge with bacterial pathogens. In order to investigate the mechanisms of probiotic action of S4, we have constructed mutants targeting genes involved in antibiotic production and biofilm formation. Mutants unable to either produce TDA or deficient in biofilm formation showed a decrease in their ability to compete with the marine bacterial pathogens *Vibrio tubiashii, V. anguillarum,* and *Roseovarius crassostreae* on the formation of biofilms on glass or plastic surfaces. Moreover, these mutants showed a decrease in their ability to protect oyster larvae against challenge with vibrio pathogens. These results suggest that both TDA production and biofilm formation are involved in the probiotic activity of S4 against bacterial pathogens of larval bivalves.

Characterization of the Aquatic Microbiome Prior to, During and Following a Ninety-Percent Water Change in an Artificial Salt Water System

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As part of our developing Aquarium Microbiome Project, the bacterial community composition and structure of water from an established teleost fish system was examined before, during and after a major water change. The aim of this study was to explore the impact of such a water-change disturbance on the stability of the aquarium water microbiome. The diversity and evenness of the bacterial community significantly increased following a 90% water replacement, which was primarily influenced by a significant increase in the relative abundance of bacterial taxa related to four families. While the change in bacterial community structure was significant, it was slight, and was also weakly correlated with a change in the redox state of the water. Interestingly there was a significant shift in the correlative network relationships between operational taxonomic units from before to after the water replacement. We suggest this increase is due to an increase in the stochasticity and range of shifting relative abundances for bacterial taxa, which is supported by an increase in the variance in community structure in the water samples following replacement. This study illustrates the phenomenal power of contemporary molecular microbial ecology techniques and the ability to detect changes in aquatic microbial community structure with incredibly high resolution. These observations will inform future studies into manipulation of the microbiome by changing system environmental parameter values to optimize resident animal health.

Monitoring *Enterococcus* In Public Aquarium Marine Fish Exhibits

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Concern for diver safety, with respect to waterborne pathogen exposure, has prompted zoos and aquaria to use testing protocols established for public swimming beaches and/or marine mammal pools because none have been established for fish exhibits. Actual practices for water testing, interpretation, and corrective actions vary greatly. Weekly *Enterococcus* counts using the Idexx Enterolert test were performed at the National Aquarium for three large, mixed species fish exhibits. Biofilms from décor and tank substrate were periodically monitored. Positive test wells, indicated by fluorescence, were subcultured for preliminary identification by standard biochemical tests. Representative isolates from each exhibit were further identified using the Remel STR kit and a subset (n=19) were also analyzed by DNA sequencing. The STR kit and sequencing results matched for nine isolates (*E. faecalis*, *E. casseliflavus*, and *E. raffinosus*). Two isolates that did not key on the STR kit matched *E. casseliflavus* and two matched *E. pallens*. Six isolates matched a related genus, *Vagococcus fluvialis*. Numerous studies have reported the presence of *Enterococcus* from many sources, including mammals, birds, reptiles, insects, plants, soil, water, and food. Our results suggest that the usefulness of these tests to predict diver safety needs further consideration.

The Spotfin Killifish *Fundulus luciae* Baird 1855: Interspecific Competition And Parasite Burden

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The mummichog (*Fundulus heteroclitus*) has served as an excellent scientific model in numerous fields, but relatively little is known about the sympatric spotfin killifish (*Fundulus luciae*). *F. luciae* had been reported as rare or infrequently encountered throughout its range, however recent work suggests it may be locally abundant within intertidal marsh habitat, where it frequently co-occurs with *F. heteroclitus*. Pit traps installed at three intertidal salt marsh sites of northeastern South Carolina were dip-netted monthly from April – December 2014. *F. luciae* comprised 53.9 % [n = 654] of total fishes captured [n = 1214]. This is only the second time the spotfin killifish has been officially reported in South Carolina. Parasites play an important role in shaping host ecology in intertidal systems. Knowledge of *F. luciae* parasites is currently limited to a single survey of metazoans and documentation of a parasitic dinoflagellate, both in Virginia. Specimens of *F. luciae* will be examined to identify all ecto- and endoparasites, and quantified by measuring parasite intensity and prevalence. Seasonal variations in parasite burden will be examined. Early qualitative ectoparasite examinations have provisionally documented new parasite records for *F. luciae*, including species of the protist *Trichodina* and the monogenean trematode *Fundulotrema*. In Spring 2015 an enclosure experiment will be conducted to examine the effect of competition with *F. heteroclitus* on growth and parasite burden of *F. luciae*. Competition between species is an important ecological factor, and is especially pertinent for sympatric congeners.

Ecology Of A "Forgotten" Trematode Parasite, *Cercaria choanura*, In The Coquina Clam *Donax variabilis*

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The coquina clam *Donax variabilis* Say, 1822 is a familiar inhabitant of ocean-facing beaches throughout its range on the mid-Atlantic, southeastern and Gulf coasts. It is an important link in food webs from detritus and primary production to migratory and resident shorebirds and fishes. Its prevalence makes it an indicator of beach disturbances and anthropogenic impacts. A single study from the 1950s in Texas described a new species of trematode metacercariae from coquina, *Cercaria choanura* Hopkins, 1958, and documented relatively high prevalence and intensity of this larval parasite. As part of an undergraduate diseases and parasites class we have investigated the ecology of this "forgotten" host:parasite system in the Myrtle Beach area of South Carolina. Coquina clams were collected over seven months in 2013 from three area beaches to describe the density and intertidal zonation of hosts and parasites and to assess potential impacts of infection in the clam. Results to date indicate that demographics of coquina are highly variable across the sampled beaches, that all size classes of coquina are vulnerable to infection with intensity increasing with host shell length, and that there is seasonality of infection.

A New Snail Host Identified for *Drepanocephalus spathans* (Digenea: Echinostomatidae) in Catfish Aquaculture

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Digenetic trematodes are commonly associated with catfish aquaculture in the southeastern United States. Piscivorous birds are endemic to commercial catfish operations due to the regular availability of a confined food source. In addition, large numbers of potential snail intermediate hosts are often present, providing ideal conditions for the propagation of digenetic lifecycles. The double-crested cormorant (*Phalacrocorax auritus*), common on most catfish operations, serves as the definitive host for *Drepanocephalus spathans.* Meanwhile, the marsh rams-horn snail (*Planorbella trivolvis*), also endemic to catfish farms, serves as a snail intermediate host. In July 2014 marsh rams-horn snails and snails morphologically consistent with the ghost rams-horn snail (*Biomphalaria havanensis)* and later molecularly identified as *Biomphalaria obstructa* were collected from two separate farms in Noxubee County, Mississippi. Snails were placed in individual vials containing 10 ml of autoclaved spring water and observed for 48 hours for the presence of cercariae. Individuals from both snail types were actively shedding cercariae morphologically consistent with *D. spathans.* Genetic sequence analysis of cercariae from *B. obstructa* was a 99%-100% match to *D. spathans* from *P. trivolvis* across five different gene targets (18S, 28S, ITS, CO1, and ND1). Juvenile channel catfish (*Ictalurus punctatus*) were experimentally challenged with *D. spathans* cercariae shed from *B. obstructa* snails. Fish were individually exposed to inocula containing ~275 cercariae for a period of 4 hours, after which fish were transferred and held under flow-through conditions. Fish were necropsied 7 days post-exposure and the presence of metacercariae was confirmed by histopathology. This is the first report of *B. obstructa* serving as an intermediate host in the life cycle of *D. spathans.*

Where Are All The Sea Lice? A First Glance At Sentinel Fish In Cobscook Bay

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The nature and role of the interactions between sea lice (*Lepeophtheirus salmonis*) and wild and farmed salmon is an area of ongoing debate. Cobscook Bay represents one of the primary salmon farming regions in Maine for more than 20 years. The Denny's River feeds the inner Cobscook Bay and contains one of the endangered Atlantic salmon populations in Maine. Four sentinel cages have been placed throughout the bay to better understand the seasonal variability in sea lice infectious pressure. The cages have been stocked for 1 week a month since June 2013, except for January and March 2014 and October 2015. Environmental data including temperature, salinity, current speed, and direction have been collected. Preliminary data for the first year shows a seasonal increase in infection intensity and prevalence of lice infections and environmental data will help establish and model where and when sea lice infect migrating or farmed salmonids in a near shore ecosystem. The knowledge gained will provide vital information in understanding infectious pressures in the coastal zone, the role of wild fish as hosts for sea lice, and lice infection dynamics over an aquaculture production cycle. This will enable the evaluation and refinement of the success of industry driven collaborative IPM efforts and provide a scientific framework to inform lease-granting bodies and marine resource users of the actual sea lice risk factors associated with wild fish populations to salmon farms, or *vice versa*.

Repetitive Sequence Mediated PCR And *gyrB* Sequence Demonstrates Intraspecific Genetic Variability Amongst *Edwardsiella ictaluri* Isolates From Different Hosts And Geographic Regions

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The intraspecific genetic variability of *E. ictaluri* isolates from different origins was determined. Isolates were recovered from farm-raised catfish in Mississippi, tilapia cultured in the Western hemisphere, and zebrafish propagated in Florida, USA. These isolates were phenotypically homologous and antimicrobial profiles were largely similar. Genetically, isolates possessed subtle differences that could be exploited by repetitive-sequence mediated PCR (rep-PCR) and *gyrB* gene sequence. Of the four primer sets evaluated, the ERIC I & II, BOX and GTG_5 primers produced three distinct profiles unique to their respective groups. Analysis of the *gyrB* sequence was in agreement with these profiles, identifying three distinct *E. ictaluri gyrB* genotypes: one associated with catfish, one from tilapia, and a third from zebrafish. The biological significance of these findings is unclear and it is unknown whether these differences are driven by host species, culture practices, geography or other unidentified factors. This work identifies three genetically distinct strains of *E. ictaluri* from different origins that can be identified using rep-PCR and *gyrB* sequencing. In addition, this research demonstrates *gyrB* sequence provides greater resolution than 16S rRNA for identification and classification of the *Edwardsiella*.

Whole Genome Comparisons of *Streptococcus parauberis* from Fish and Cattle

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Streptococcus parauberis is a gram-positive, lactic acid bacterium that causes disease in dairy cattle and fish. It has been recognized as an aquaculture pathogen since 1995 and recently has been described infecting wild striped bass (*Morone saxatilis*) in the Chesapeake Bay. The genomes of eight *S. parauberis* isolates, including five from striped bass, were sequenced using the Illumina platform and assembled into advanced draft state. These genomes, plus five previously available, were analyzed and compared. Circular plots of the data facilitate the identification of similarities and differences between the genomes. These include metabolic genes, such as lactose utilization genes, which may influence survival in different host organisms, as well as numerous phages and transposons. These data contribute to our understanding of mechanisms of emergence in new hosts and can be used in the future development of vaccines against this emerging pathogen.

Speciation Of *Mycobacterium chelonae* Isolates By *hsp65* And Whole Genome Sequencing

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Mycobacterium chelonae is an important, rapidly growing acid-fast bacterial (RGB) pathogen that causes opportunistic disease in humans and primary fatal disease in a variety of aquatic animals. Members of the *M. chelonae* complex, *M. chelonae* and *M. abscessus*, are difficult to differentiate and numerous typing methods exist; however, many are time consuming, labor intensive, and difficult to assess or reproduce. Among captive aquatic species, fish of the Family Syngnathidae (seadragons, seahorses, and pipefish) are highly susceptible to mycobacteriosis, most commonly caused by *M. chelonae.* Histological lesions in syngnathids typically manifest as florid lepromatous disease, while other vertebrates, including teleosts, commonly form discrete granulomas. Isolates previously identified as *M. chelonae* by either 16S rRNA or *hsp65* gene sequencing were selected for *hsp65* and whole genome comparison. Whole genome sequencing of syngnathid (n=5), African cichlid (n=1), bluegill (n=1), snake (n=1), turtle (n=1), cow (n=1), and aquatic biofilm (n=3) isolates, and the *M. abscessus* ATCC® 19977™ type strain was performed. Multi-sequence alignment of the 441bp *hsp65* gene revealed key base arrangement differences leading to significant diversity among isolates from syngnathids, as well as between species. Results revealed two of the five syngnathid isolates had a 90.9% identity to *M. abscessus ATCC® 19977™ hsp65*, while the other three syngnathid isolates had a 99.7% identity to *M. chelonae* ATCC® 35752 *hsp65.* Two biofilm isolates had a 99.75% identity to *M. chelonae* ATCC® 35752™ and one had a 90.93% identity to *M. abscessus* ATCC® 19977™. The cow and snake isolates had a 97.9% and 100% identity to *M. abscessus* NC_010397 *hsp65 and M. chelonae* ATCC® 35752™ *hsp65*, respectively. When whole genomes are aligned to GenBank *M. chelonae* genome 1518 and *M. abscessus* genome NC_010397, isolates overall align closer to *M. abscessus* than *M. chelonae.* These results suggest that *hsp65* may not be a reliable method for delineation between *M. chelonae* complex isolates and that different strains of closely related *Mycobacteria* spp. cause disease in a variety species, with no host specificity. Additional whole genome analysis is warranted to properly elucidate significant differences between mycobacterial species.

Comparative Genomics of the Human and Animal Pathogen *Mycobacterium marinum*

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Major mycobacterial pathogens of humans, such as *M. tuberculosis*, *M. leprae,* and *M. ulcerans*, are thought to derive from generalist environmental ancestors. A great deal of research has focused on the genomics of these pathogens, however, much less is known about mycobacteria that largely retain capability to survive in the ancestral environmental niche. The *M. marinum*-like group of bacteria is representative of these environmental ancestors, in that its members retain relatively large genomes and diverse metabolic pathways for coping with diverse conditions, energy sources, and hosts. In this work, we present preliminary results of a whole-genome comparative study of over 30 strains of *M. marinum*, including genomic phylogeny and analysis of core and accessory genomes.

NGS: Next Generation...Sturgeon, Developing Genomic Resources For Atlantic Sturgeon

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The acipenserids are a primitive group of fish that contain some of the oldest extant ray-finned fish species. Therefore, a better understanding of these fish immune system will likely increase our understanding of its evolution, potential for vaccination and prophylactic management of disease. Unfortunately, there is a paucity of genomic information on sturgeon. Thus the generation of these resources may help to generate tools that will facilitate the study of immune function in sturgeon. In collaboration with the Atlantic Veterinary College at UPEI, the Center for Aquaculture Technologies Canada (CATC) used a whole transcriptome shotgun sequencing (RNA-seq) approach designed to identify Atlantic sturgeon genes that respond to lipopolysaccharide (LPS) stimulation. Pair-end stranded Illumina libraries produced from spleen mRNA of fish injected with LPS or PBS and sampled 24H post-injection were sequenced using the HiSeq 2000 technology. A custom assembly pipeline using digital normalization and multiple assemblers was developed to better deal with the highly duplicated nature of the Atlantic sturgeon genome. A reference transcriptome assembly for Atlantic sturgeon has been produced, to which nearly 70% of the pair-end reads can be properly mapped. Analysis of differentially expressed transcripts using edgeR and DESeq has enabled identification of sturgeon LPS-responsive genes such as interleukin-15, cathelicidin, interferon regulating factor 7 and inhibitory Kappa B Alpha. Interestingly, elements of both Major Histocompatability Class I and II antigen presenting system were identified, suggesting that it may have arisen early in vertebrate evolution, and likely responsive to immune enhancement through vaccination.

Health Insights From Draft Sequencing And Assembly Of The Genome Of The World’s Largest Fish, The Whale Shark: *Rhincodon typus* Smith 1828

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The whale shark (*Rhincodon typus*) has by far the largest body size of any elasmobranch species and is therefore also the largest extant species of the paraphyletic assemblage commonly referred to as “fishes”. As both a phenotypic extreme and a member of the group basal to the remaining gnathostomes, which includes all tetrapods and therefore also humans, its genome is of substantial comparative interest. Whale sharks are also listed as a “vulnerable” species on the IUCN Red List of threatened species and are of growing popularity as both a target of ecotourism and as a charismatic conservation ambassador for the pelagic ecosystem. A genome map for this species would therefore aid in defining effective conservation units by better understanding global population structure. We characterized the nuclear genome of the whale shark using next generation sequencing (454, Illumina) and *de novo* assembly and annotation methods, based on material collected from a male specimen from the collection at Georgia Aquarium in Atlanta. The data set consisted of 878,654,233 reads, which assembled into 11,347,816 contigs and 3,606,038 scaffolds. The estimated genome size was 3.44Gb. The proteome of the whale shark was most closely-related to the holocephalan elephant shark, as expected. The whale shark contained a novel Toll-like-receptor protein with sequence conservation to both the TLR4 and TLR13 proteins of mammals, but the ligands for this receptor remain unknown. The data are publicly available on a Galaxy bioinformatic server (http://whaleshark.georgiaaquarium.org). This represents the first shotgun elasmobranch genome and will aid studies of molecular systematics, biogeography, genetic differentiation, and conservation genetics in this and other shark species, as well as providing comparative data for studies of evolutionary biology and immunology across the jawed vertebrate lineages.

Genomic Characterization Of A Novel Hepatitis E Virus In Three Salmonid Species (*Salmo salar, Salmo trutta, Oncorhynchus mykiss)*

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Extensive research has been conducted on hepatitis E viruses (hepeviruses) in mammalian hosts including humans; however, hepeviruses remain relatively unstudied in aquatic animals. To date, the only recognized hepevirus in fish is cutthroat trout virus (CTV). We investigated novel hepeviruses isolated from asymptomatic Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*). Genomic sequencing for the three viral isolates was completed using a reverse transcription PCR and primer walking strategy. The genomes were approximately 7.3 kb in length and showed approximately 77% and 84-93% identity to CTV at the nucleotide and amino acid levels, respectively. The isolates exhibited between 94-95% nucleotide identity to each other forming a unique hepevirus clade as the sister group to CTV. Interestingly, all three isolates possessed only a fragmented homolog of a third open reading frame encoding the phosphoprotein found in CTV and other hepeviruses. Future research for this virus will involve determining viral pathogenesis and developing diagnostic assays.

Repeated Detections Of Ranaviruses In Aquaculture And The Development Of Improved Molecular Tools

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Ranaviruses have been detected with increasing frequency among aquacultured and wild fishes. To examine the impact and spread of ranaviruses, we conducted phylogenomic analyses to elucidate significant biologic and epidemiologic trends. Our comparative genomic analyses facilitated the design and validation of improved molecular diagnostic assays, including a quantitative real-time Taqman PCR capable of detecting all known ranaviruses while excluding other iridoviruses affecting fish, and an *in situ* hybridization assay to detect ranaviral DNA in tissue sections. These molecular tools will be used in future challenge studies examining whether stocking density and water temperature influence survival in aquacultured species. Improved diagnostics and a better understanding of ranaviral ecology and epidemiology in aquaculture will be imperative in the design of future mitigation strategies.

An Overview Of Myxozoan Parasites Associated With Catfish Aquaculture

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Myxozoans are among the most well-known parasites of fish worldwide, but with more than 2,000 described species little is still known concerning life histories and impacts that many of these species have on fish health. In the southeastern United States, the commercial catfish industry is persistently plagued by the myxozoan parasite that causes proliferative gill disease in channel and hybrid catfish, *Henneguya ictaluri* where outbreaks can result in excess of 50% mortality. To date there are no efficacious treatment or management strategies to prevent or cure fish with PGD. Although the production of channel × blue catfish hybrids is popular for their fast and uniform growth and inherent resistance to several importance diseases, hybrids still succumb to PGD. Recent research indicates that hybrids that survive a PGD outbreak may not serve as true intermediate hosts in the life cycle of *H. ictaluri*. In these shallow closed-earthen ponds, the catfish are brought into close proximity to actinospore shedding benthic oligochaete hosts, with the ubiquitous *Dero digitata* being of particular importance in the life cycles of myxozoans. In addition to being the definitive host for the pathogenic *H. ictaluri*, *D. digitata* is also a host to other myxozoan species that infect the channel catfish and potentially other fish species commonly found in commercial ponds. This talk will provide an overview on the past and present findings and future work on the myxozoans associated with catfish aquaculture in the southeastern United States.

In Life And Death: The Myxozoan *Kudoa inornata* Increases Spotted Seatrout (*Cynoscion nebulosus*) Swimming Performance But Also Causes Post-Mortem Myoliquefaction

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The spotted seatrout, *Cynoscion nebulosus,* is a tide dependent fish and an important trophic link in estuarine ecosystems at all stages of its life. It relies on swimming throughout its life and its success in the estuary depends on the ability to maximize swimming performance. However, a skeletal muscle dwelling myxozoan parasite, *Kudoa inornata*, whose myxospores compromise the integrity of the infected myofibers, commonly infects seatrout. During the life of the fish, infection by *K. inornata* is expected to compromise swimming performance. Myoliquefaction has been documented to soften the fillets of numerous teleost fish species and is associated with *Kudoa* infection*.* Fishermen reports of soft flesh in their catch in Charleston, SC. and the discovery of *K. inornata* in spotted seatrout suggests that post-mortem myoliquefaction may lead to decreased muscle firmness after death. Thus, *K. inornata* is expected to have negative effects on seatrout skeletal muscle in life and in death. Unexpectedly, increasing density of *K. inornata* myxospores in seatrout skeletal muscle was related to increased fish swimming performance. This experiment was repeated and confirmed these unexpected results. In a separate set of experiments we evaluated fillet firmness at 24 hours, 72 hours, and 144 hours post-mortem. Force testing revealed a significant decrease in flesh firmness in infected wild fish compared to the an aquaculture control group by 72 hours, and significantly higher firmness in unparasitized fish at all time points. Thus, even though the parasite appears to induce myoliquefaction of the muscle after the fish dies, in life *K. inornata's* effect on skeletal muscle results in enhancement of swimming performance.

Kudoa inornata: A Seasonal Parasite?

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Kudoa inornata is a muscle-dwelling myxozoan that infects the spotted seatrout, *Cynoscion nebulosus*. Over 80% of adult spotted seatrout in South Carolina wild populations are infected. The purpose of this study was to determine if infection of spotted seatrout by *K. inornata* follows a seasonal pattern. During a two year experiment (2013-2014), two replicates of 10 naïve sentinel seatrout were exposed monthly to raw water from Charleston Harbor. At the end of each four week exposure, the presence of myxospores was determined via microscopic observation of squashed muscle biopsies. When no myxospores were visualized, samples were screened for *K. inornata* rDNA using PCR. In both years, peak infection by myxospores was observed during the summer with no infection November through April. However, PCR results indicate the presence of *K. inornata* in fish muscle over a longer period of time (April through January). Because only naïve fish were used, these results indicate that actinospores of *K. inornata* are likely to be released by the annelid vector (yet to be identified) throughout the year except for the two coldest months. The results show that the seasonal presence of myxospores in seatrout muscle is a reflection of the different development rates of myxospores at different times of the year, most likely associated with water temperature. Future studies should focus on temporal quantification of waterborne actinospores and identification of the annelid vector.

Hunting For The Alternate Host Of *Kudoa inornata*, A Myxozoan Parasite Of Spotted Seatrout

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Kudoa spp. are widespread parasites of marine fishes. No *Kudoa* life cycles are known, but they probably require annelid worm alternate hosts. *Kudoa inornata* is a parasite of spotted seatrout, a commercially important species and an indicator of biological integrity of coastal resources. Both wild and captive seatrout become infected with *K. inornata* at high prevalence and intensity in Charleston Harbor, SC. To find the putative alternate host, we sampled annelids in summer 2014, by hand and by grab from the intertidal zone to 5m deep water. We examined 2287 polychaetes and 871 oligochaetes. We found a low diversity of oligochaete species (2 families, 5 species), but the level of overt myxozoan infections was relatively high: 45/871 (5%) with >20 genetically distinct actinospore types. For polychaetes, species diversity was very high (>20 families) but we found myxozoan infections only in 1 species of spionid 6/574 (1%); 0/1713 other polychaetes. We sequenced 18S rDNA from 34 infections. Four myxozoans were found twice each, but the rest were unique sequences, which indicated high local myxozoan diversity. The polychaete infections were genetically identical. BLAST searches showed no similarities >98% with GenBank myxozoan sequences. Phylogenetic analyses showed striking correlation between polychaetes as hosts of “marine” lineage myxozoans and oligochaetes as hosts of “freshwater” lineage myxozoans. This pattern leads us to hypothesize that the invertebrate host of *K. inornata* is a marine polychaete worm, probably a Spionid, Serpulid or Sabellid (which are known to host other myxozoan species). Collectively, these data are the first description of myxozoan infections in marine annelids from North America, and only the fifth species found in a marine polychaete worldwide.

Historical Record Of The Eastern Fish Health Workshop

1976	NOAA, National Marine Fisheries Service Laboratory	Oxford, MD
1977	NOAA, National Marine Fisheries Service Laboratory	Easton, MD
1978	Auburn University	Auburn, AL
1979	Department of Fisheries and Oceans	Halifax, N.S
1980	National Fish Health Research Laboratory	Leetown, WV
1981	Mississippi State University	Starkville, MS
1982	Sheraton Inner Harbor (with IAAAM)	Baltimore, MD
1983	National Fish Health Research Laboratory	Leetown, WV
1984	Auburn University	Auburn, AL
1985	Washington College	Chestertown, MD
1986	National Fish Health Research Laboratory (with FHS)	Martinsburg, WV
1987	Louisiana State University (with FHS &MWFHW)	Baton Rouge La
1988	University of Maine	Orono, ME
1989	University of Maryland (with FHS)	Annapolis, MD
1990	University of Prince Edward Island	Charlottetown, PEI
1991	National Fish Health Research Laboratory	Martinsburg, WV
1992	Auburn University	Auburn, AL
1993	NOAA, National Marine Fisheries Service (cancelled)	Woods Hole, MA
1994	Virginia Polytechnic Institute	Blacksburg, VA
1995	NY Dept of Environmental Conservation (with FHS)	Syracuse, NY
1996	Virginia Institute of Marine Science	Gloucester Pt, VA
1997	NFHRL, Royal Pavillion Resort and Conference Center	Atlantic Beach, NC
1998	NFHRL, John Carver Inn	Plymouth, MA
1999	NFHRL, Royal Pavillion Resort and Conference Center	Atlantic Beach, NC
2000	NFHRL, John Carver Inn	Plymouth, MA
2001	NFHRL, Clarion Inn and Conference Center	Shepherdstown, WV
2002	NFHRL, Holiday Inn – Patriot's Point	Mount Pleasant, SC
2003	NFHRL, Eisenhower Inn and Conference Center	Gettysburg, PA
2004	NFHRL, Royal Pavillion Resort and Conference Center	Atlantic Beach, NC
2005	NFHRL, Clarion Inn and Conference Center	Shepherdstown, WV
2006	NFHRL, Holiday Inn – Patriot's Point	Mount Pleasant, SC
2007	NFHRL, Eisenhower Inn and Conference Center	Gettysburg, PA
2008	NFHRL, Sheraton Oceanfront Hotel	Atlantic Beach, NC
2009	NFHRL, High Peaks Resort and Conference Center	Lake Placid, NY
2010	NFHRL, Clarion Inn and Conference Center	Shepherdstown, WV
2011	NFHRL, Holiday Inn – Patriot's Point	Mount Pleasant, SC
2012	NFHRL, High Peaks Resort and Conference Center	Lake Placid, NY
2013	NFHRL, Eisenhower Inn and Conference Center	Gettysburg, PA
2014	Clarion Inn and Conference Center	Shepherdstown, WV
2015	Holiday Inn – Charleston-Mt. Pleasant	Mount Pleasant, SC

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