

Book of Abstracts



Fifth Florida Marine Mammal Health Conference

June 2-4, 2015
Gainesville, Florida



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Welcome From the Organizers

Dear Aquatic Ecosystem Health Conference Attendees,

The Organizing Committees for the Third International Ranavirus Symposium and the Fifth Florida Marine Mammal Health Conference would like to extend a warm welcome to each of you. We are excited to share how wonderful Gainesville, home to the University of Florida, is for gatherings such as this! Gainesville is the perfect venue with exceptional weather for these sister meetings as it offers a blend of small town manners, a big college atmosphere, with unlimited entertainment and dining options. Check your swag bags for food and activity guides. Please feel free to ask those in the blue volunteer shirts manning the conference registration desk or the hotel staff for help finding after hours food, fun, and relaxation.

The Third International Symposium on Ranaviruses and the Fifth Florida Marine Mammal Health Conference offer scientists, managers, wildlife biologists, veterinarians, policy makers, and students the opportunity to network and learn from fellow contributors, researchers, and clinicians. Although the content of each of the two meetings is unique, both meetings underscore the importance of multidisciplinary research efforts in tackling global aquatic ecosystem health issues. The meetings offer lectures, topical discussions, poster sessions, wet labs, and a social evening at the Florida Museum of Natural History. In an effort to make these meetings as “green” as possible we have limited the use of paper. Please note, the proceedings are available on the conference website and onsite at the registration desk via USB drives.

We are extremely grateful to the Ranavirus Symposium and Marine Mammal Health Conference Organizing Committees for their time and energy in bringing this event to life. A special thanks to all the speakers and session chairs who have given their time to ensure the success of this event. We are also very thankful to the Gainesville Hilton Hotel and our many donors whose generosity has made this event possible. Finally, we thank you for your attendance and for contributing your passion and expertise during the conference. We wish you all safe travels and we hope this venue will help you forge lasting friendships and meaningful partnerships in aquatic ecosystem health and conservation.

Sincerely,

The Third International Ranavirus Symposium Organizing Committee

The Fifth Marine Mammal Health Conference Organizing Committee

The Aquatic Animal Health Program

The University of Florida, College of Veterinary Medicine

About Aquatic Ecosystem Health 2015

The University of Florida's Aquatic Animal Health Program is proud to host **Aquatic Ecosystem Health 2015**, consisting of two back-to-back conferences: the **Third International Symposium on Ranaviruses** and the **Fifth Florida Marine Mammal Health Conference**.

Florida is the perfect location to host a joint discussion addressing global aquatic ecosystem health issues because its ecosystems, economy, and heritage are inextricably linked to its diverse water resources. The Florida ecosystem faces many of the global challenges affecting aquatic ecosystems worldwide, such as population growth, rising temperatures, ocean acidification, chemical contamination of surface waters, an unprecedented number of unexplained marine mammal mortality events. Furthermore, significant disease epizootics in freshwater habitats, including ranavirus infections in amphibians, have been increasingly documented. These conferences provide a forum for Marine Mammal and Ranavirus experts to address the complexity of global aquatic ecosystem health in a broad perspective.

About the Fifth Florida Marine Mammal Health Conference

The purpose of this conference is to address aquatic ecosystem health issues impacting marine mammals and other aquatic species in Florida. This conference provides a forum for issues affecting Florida's aquatic species and provides an opportunity to review the impact environmental change has on their current health status, discuss future directions of investigation, and determine actions likely to produce benefits to their health and survival.

The conference is brings together scientists, veterinarians, managers, policy makers, planners and partners who are actively involved in and/or affected by all aspects of aquatic ecosystem health. Participants will interact in an interdisciplinary setting to summarize and review state-of-the-art science, healthcare, and management activities, with a focus on formulating goals and strategies for improvements. Attendees will have ample opportunities to share information regarding issues that affect marine mammals during topical discussion periods, poster sessions, and networking functions.

Conference Organizers

Thomas Waltzek

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Conference Coordinator
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University of Florida
College of Veterinary Medicine

Detailed Program Agenda

Monday June 1, 2015			
4:00pm - 8:00pm	Registration		Registration Desk
4:00pm - 8:00pm	Poster Set-Up		Century Ballroom B/C
Tuesday June 2, 2015			
7:00am - 5:00pm	Registration		Registration Desk
7:00am - 5:00pm	Poster Set-Up		Century Ballroom B/C
7:00am - 8:00am	Morning Refreshments		Century Ballroom B/C
7:50am-8:00am	Welcome from Dr. James Lloyd, Dean of the College of Veterinary Medicine		Century Ballroom A
8:00am-8:30am	Keynote Address - Dr. Jan Landsberg Healthy Aquatic Ecosystems and Tipping Points		Century Ballroom A
8:30am - 9:00am	Keynote Address - John Reynolds Marine Mammal Conservation: The Role of Health Professionals		Century Ballroom A
Aquatic Ecosystem Health Assessments – Cetaceans			
Session Chair: Dr. Craig Pelton			
9:00am - 9:15am	Evaluating Ecosystem Impacts On The Health Of Indian River Lagoon Bottlenose Dolphins During The 2013 Unusual Mortality Event	Wendy Noke	Century Ballroom A
9:15am - 9:30am	Health Assessments in North Atlantic Right Whales (<i>Eubalaena glacialis</i>): From Individuals To Population Level Projections	Scott Kraus / Rosalind Rolland	Century Ballroom A
9:30am - 9:45am	Augmenting Capture Based Dolphin Health Assessments	Craig Pelton / Michael Walsh	Century Ballroom A
9:45am - 10:00am	Cost, Benefits And Solutions: An Overview Of Planning And Executing Marine Mammal Health And Environmental Risk Assessments And Interventions	Stephen D. McCulloch	Century Ballroom A
10:00am - 10:30am	Applying Dolphin Population Health To Conservation	Randy Wells	Century Ballroom A
10:30am-10:45am	Break		Century Ballroom B/C
Aquatic Ecosystem Health Assessments - Manatees			
Session Chair: Dr. Martine de Wit			
10:45am - 11:00am	One Health At The Terrestrial-Aquatic Interface	Jenifer Chatfield	Century Ballroom A
11:00am - 11:30am	The Use Of Hematology And Chemistry Analyses For Monitoring Health In The Florida Manatee (<i>Trichechus manatus latirostris</i>) And Its Contribution To Understanding Biological Population Data	Nicole Stacy	Century Ballroom A
11:30am - 11:45am	Disseminated Toxoplasmosis And Serologic Survey For <i>Toxoplasma gondii</i> In The Wild Florida Manatee (<i>Trichechus manatus latirostris</i>)	Lauren Smith	Century Ballroom A
11:45am - 12:00pm	Establishing Thromboelastography Reference Intervals In Wild Florida Manatees (<i>Trichechus manatus latirostris</i>) To Enable Further Understanding Of The Pathophysiology Of Cold Stress Syndrome	Ashley Barratclough	Century Ballroom A
12:00pm - 1:30pm	Lunch		Albert's Restaurant

Tuesday June 2, 2015 (cont'd)			
Aquatic Ecosystem Health Monitoring - Sea Grass, HABS, GIS Satellite Use			
Session Chair: Dr. Jan Landsberg			
1:30pm - 1:50pm	Harmful algal bloom monitoring to protect public and animal health in Florida	Alina Corcoran	Century Ballroom A
1:50pm - 2:10pm	Brevetoxin-associated mass mortality event of bottlenose dolphins and manatees along the east coast of Florida	Spencer Fire	Century Ballroom A
2:10pm - 2:30pm	The rise and fall... and rise again of the benthic macrophyte communities in the Indian River Lagoon	Lori Morris	Century Ballroom A
2:30pm - 3:00pm	Florida Dept. of Health water quality monitoring	Bob Vincent / Danielle Stanek	Century Ballroom A
3:00pm - 3:15pm	Break		Century Ballroom B/C
Aquatic Ecosystem Health Monitoring - Non-Mammalian Research			
Session Chair: Dr. Michael Walsh			
3:15pm- 3:30pm	A Large Scale Chronic Fish Kill In The St. Johns River, Florida: A Complex Consequence Of Cyanobacteria Blooms	Jan Landsberg	Century Ballroom A
3:30pm - 3:45pm	Status, Trends, And Health Of Florida's Coral Reefs	Vanessa Brinkhuis	Century Ballroom A
3:45pm - 4:00pm	Fish Kills And Disease Events In Florida: FWC Role And Resources	Micah Bakenhaster	Century Ballroom A
4:00pm - 4:15pm	Emerging Diseases Of Aquatic Birds	Mark Cunningham	Century Ballroom A
4:15pm - 4:30pm	Anthropogenic Impacts On Florida's Coastal Marine Invertebrates	LeRoy Creswell	Century Ballroom A
4:30pm - 4:45pm	Long-Term Data Provide Insights On The Impact Of Harmful Algal Blooms On Marine Turtles Of The Indian River Lagoon	Christopher Long	Century Ballroom A
4:45pm - 5:00 pm	The role of rehabilitation facilities in sea turtle health assessments	Michael Walsh / Craig Pelton	Century Ballroom A
5:30pm - 7:30pm	Poster Session With Hors D'Oeuvres		Century Ballroom B/C

Wednesday June 3, 2015			
7:00am - 5:00pm	Registration		Registration Desk
7:00am - 8:00am	Morning Refreshments		Century Ballroom B/C
Infectious Diseases - Emerging Infectious Disease Session Chair: Dr. James Wellehan			
8:00am - 8:15am	Pathologic Findings From The 2013 Mid-Atlantic Dolphin Morbillivirus Unusual Mortality Event	David Rotstein	Century Ballroom A
8:15am - 8:30am	Phylogenomics Diversity Of Cetacean Morbilliviruses	Nelmarie Landrau Giovannetti	Century Ballroom A
8:30am - 8:45am	Genomic Characterization Of Two Papillomaviruses In Indian River Lagoon Bottlenose Dolphins	Galaxia Cortes	Century Ballroom A
8:45am - 9:00am	A Viromic Study Of Serum Collected From Wild <i>Tursiops truncatus</i> (Bottlenose Dolphin)	Corey Russo	Century Ballroom A
9:00am - 9:15am	Seroprevalence Of West Nile Virus In Florida Bottlenose Dolphins (<i>Tursiops truncatus</i>) Of The Indian River Lagoon And Proximate Sentinel Birds Following Florida Introduction In 2001	David Kilpatrick	Century Ballroom A
9:15am - 9:30am	Infectious Diseases In The Florida Manatee (<i>Trichechus manatus latirostris</i>)	David Rotstein	Century Ballroom A
9:30am - 9:45am	Fatal Systemic Salmonellosis In An Adult Florida Manatee (<i>Trichechus manatus latirostris</i>)	Bryan Vorbach	Century Ballroom A
9:45am - 10:00am	Characterization Of A Novel Papillomavirus In Florida Manatees, <i>Trichechus manatus Papillomavirus 2</i>	James Wellehan	Century Ballroom A
10:00am - 10:15am	Break		Century Ballroom B/C
Anthropogenic Health Stressors: Emerging Contaminants and Toxicology Session Chair: Dr. Nancy Denslow			
10:15am - 10:30am	Investigating An Increase In Florida Manatee (<i>Trichechus manatus latirostris</i>) Mortalities In 2013 Using Proteomic And Transcriptomic Analyses	Nancy Denslow	Century Ballroom A
10:30am - 10:45am	Trace Metals In Tissues Of Free-Ranging Florida Manatees (<i>Trichechus manatus latirostris</i>) And Its Environment: Thirty Year Perspective	Noel Takeuchi	Century Ballroom A
10:45am - 11:00am	Are Molecular Endpoints Useful For Environmental Monitoring Programs?	Chris Martyniuk	Century Ballroom A
11:00am - 11:15am	Analytical Toxicology Of Inshore Gulf Seafood: Bridging Environmental And Public Health Concerns Post-DWH Oil Spill	Andrew Kane	Century Ballroom A
11:15am - 11:30am	Mercury Concentrations In Atlantic Bottlenose Dolphins (<i>Tursiops truncatus</i>) Inhabiting The Indian River Lagoon, Florida And Local Human Residents: Patterns Of Distribution	Adam Schaefer	Century Ballroom A
11:30am - 11:45am	Investigating The Possibility That Pollution Due To Oil And Gas Activates May Be Affecting Recovery Of Cook Inlet Beluga Whales (<i>Delphinapterus leucas</i>)	Dana Wetzel	Century Ballroom A
11:45am - 12:00pm	Understanding The Use And Limitations Of Clinicopathologic Endpoints In Toxicologic Studies Of Aquatic Animals	Nicole Stacy	Century Ballroom A

Wednesday June 3, 2015 (cont'd)			
12:00pm - 1:30pm	Lunch		Albert's Restaurant
Marine Mammal Molecular Techniques and Immunology Session Chair: Dr. Margaret Hunter			
1:30pm - 1:45pm	Next-generation Sequencing Of Gut Content To Help Inform The Florida Manatee Unusual Mortality Event In The Indian River Lagoon	Margaret Hunter	Century Ballroom A
1:45pm - 2:00pm	Comparative Study Of The Innate Immunity And Clinical Pathology Among Two Populations Of Florida Manatee (<i>Trichechus manatus latirostris</i>) After The 2013 Unusual Mortality Event	Estelle Rousselet	Century Ballroom A
2:00pm - 2:15pm	Sublethal Red Tide Toxin Exposure In Free-Ranging Manatees (<i>Trichechus manatus</i>) Affects The Immune System Through Reduced Lymphocyte Proliferation Responses, Inflammation And Oxidative Stress	Cathy Walsh	Century Ballroom A
2:15pm - 2:30pm	Immune Assessment In Manatees With Cold Stress Syndrome	Tracy Sherwood	Century Ballroom A
2:30pm - 2:45pm	Baseline Leukocyte Cytokine Levels In A Subpopulation Of Healthy Florida Manatees Measured By Quantitative PCR	Jason Ferrente	Century Ballroom A
2:45pm - 3:00pm	A Transcriptomic Analysis Of Skin From The Common Bottlenose Dolphin, <i>Tursiops truncatus</i>	Jeanie Morey	Century Ballroom A
3:00pm - 3:15pm	Kissin' Cousins: A Look At The High Genetic Relatedness And Inbreeding Of The Endangered Florida Manatee (<i>Trichechus manatus</i>)	Michelle Davis	Century Ballroom A
3:15pm - 3:30pm	Getting The Most Information During Health Assessment, Stranding Response And Rehabilitation Studies: Optimizing Sample Collection For Future Molecular Diagnostics And Toxicology Studies	Alissa Deming	Century Ballroom A
3:30pm - 3:45pm	Break		Century Ballroom B/C
Marine Mammal Reproductive Health Session Chair: Dr. Dana Wetzel			
3:45pm - 4:00pm	Defining Baseline Reproduction: The Basis For Reproductive Health Assessment Programs	Todd Robeck	Century Ballroom A
4:00pm - 4:15pm	Reproductive Success Of Bottlenose Dolphins	Randy Wells	Century Ballroom A
4:15pm - 4:30pm	Reproductive Anatomy And Histology Of The Male Florida Manatee (<i>Trichechus manatus latirostris</i>)	Hilda Chaves Perez	Century Ballroom A
4:30pm - 4:45pm	Anthropogenic Health Stressors Potentially Related To Captive Management During Rehabilitation Of Florida Manatees (<i>Trichechus manatus latirostris</i>)	Ray Ball	Century Ballroom A
4:45pm - 5:00 pm	Impaired Reproduction In North Atlantic Right Whales: Assessing Sub-lethal Anthropogenic Influences	Rosalind Rolland / Scott Kraus	Century Ballroom A
5:00pm - 7:00pm	Poster Removal		Century Ballroom B/C

7:00pm - 9:00pm	Reception / Social		Florida Museum of Natural History
Thursday June 4, 2015			
7:00am - 8:00am	Morning Refreshments		Century Ballroom B/C
7:00am - 12:30pm	Poster Removal		Century Ballroom B/C
Moving Towards Solutions For Maintaining Healthy Marine Ecosystems			
Session Chair: Dr. John Reynolds			
8:00am - 8:30am	The Role Of Non Profit Organizations In Aquatic Ecosystem Health	Katie Tripp	Century Ballroom A
8:30am - 8:45am	Role Of Government In Aquatic Ecosystem Health	Larry Williams	Century Ballroom A
8:45am - 9:10am	A Rising Tide: Translational Social Research, Institutional Anthropology And The Policy Sciences As Tools To Navigate The Complex Dynamics Of Social Engagement In Conservation	Adrian Cerezo	Century Ballroom A
9:10am - 9:35am	Applying Economics to Marine Mammal Conservation	Elizabeth Pienarr	Century Ballroom A
9:35am-10:00am	Break		Century Ballroom B/C
10:00am - 12:00pm	Panel: Linking Priorities And Partners For Ecosystem Conservation	Adrian Cerezo, James Lloyd, Elizabeth Pienarr, Katie Tripp & Larry Williams	Century Ballroom A
12:00pm - 12:30pm	Conference Closing Remarks	Michael Walsh / Thomas Waltzek	Century Ballroom A

Keynote Speaker Biographies & Abstracts

Dr. Jan Landsberg

Dr. Jan Landsberg is a Research Scientist with the Florida Fish and Wildlife Conservation Commission (FWC) at the Fish and Wildlife Research Institute, St. Petersburg, Florida. She has been with the agency since 1989. Jan received her Ph.D. in Biology from London University, England in 1981. From 1982–1987, Dr. Landsberg worked at the Fish Disease Laboratory, Nir-David, Israel, and, from 1988–1989, she conducted aquatic animal health research at the College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina. At FWC, Dr. Landsberg has investigated disease and mortality events affecting Florida’s aquatic animals, provided health support to FWC’s aquaculture and stock enhancement operations, and conducted research on the effects of harmful algal blooms (HABs) on aquatic organisms, with potential implications for public health. She has authored or co-authored ~ 75 peer-reviewed papers or book chapters on aquatic animal health/HABs. In 2002, she was the agency lead on the investigation of pufferfish poisoning incidents in the Indian River Lagoon (IRL), Florida, and coordinated a multiagency team that identified saxitoxins in *Pyrodinium bahamense* as the putative toxin source in the USA for the first time. More recently, Jan has focused on cyanobacteria issues, researching the effects of cyanotoxins on aquatic animals in Florida. She also just completed a three-year multi-collaborator synoptic survey on amphibian diseases. From 2005–2008, she served on the U.S. National HAB Committee and from 2008–2014 was a member of NOAA’s National Working Group on Unusual Marine Mammal Mortality Events. She is the agency lead on the investigation of the 2013 UME events in the IRL.



AN OVERVIEW OF AQUATIC ECOSYSTEM HEALTH AND TIPPING POINTS IN FLORIDA

Jan H. Landsberg¹

¹Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL, USA

Florida has a high diversity of habitat and species with hundreds of miles of natural and developed coastline that is particularly at risk from natural and anthropogenic stressors. In this presentation aquatic ecosystem health is viewed in terms of: number and scale of die-offs and disease events; species health; shifts or declines in communities and habitats due to multiple environmental stressors, including poor water quality; and land-based nutrient, pathogen, or contaminant inputs and introductions. Environmental drivers affecting aquatic ecosystem health are significant. Florida has more harmful algae bloom (HABs) species than any other state. HABs can cause acute and chronic effects with lethal to sublethal consequences at the species, habitat, and community levels. Anthropogenic and natural contaminants are diverse and in some cases partially managed or mitigated, but there are new and emerging issues from a range of human-derived products entering aquatic systems. Extreme and fluctuating weather patterns have caused die-offs of tropical species at their maximal tolerance range. In the last two decades, epizootics have emerged in major communities such as coral reefs and seagrass beds, with widespread incidences in keystone, economic, or endangered species, and there have been multiple wide-scale mass mortality events, affecting hundreds of thousands of animals; benthic community die-offs; and local extirpations. Events are occurring at multiple scales and over different time periods. Water quality is a prime driver of ecosystem health with respect to aquatic organisms, particularly from excess nutrient loading. Sewage spills and microbial inputs are not usually monitored for effects on aquatic animal health yet antibiotic resistance and introduced pathogens are but two potential consequences that need further attention. At the regional level, a wide-scale chronic fish kill in the Lower St. Johns River in 2010 was triggered by a HAB, lasted six weeks, and was then followed by a bottlenose dolphin mortality event. Causal connections, if any, between the two die-offs remain unknown. In the Indian River Lagoon, the 2011 widespread die off of seagrass triggered by a superbloom lead to manatee, brown pelican, and bottlenose dolphin mortalities with undetermined connections. The cascading consequences of these multifactorial, multispecies events in aquatic systems demonstrate the continued need for multidisciplinary partnerships and diverse diagnostic approaches at all functional levels, biochemical to ecosystem. Synergistic effects of multiple stressors should also be anticipated. To address these issues in a holistic manner, scientists, veterinarians, and managers should ideally assess commonalities and differences between species and habitats with an overview of disease and mortality occurrences and environmental stressors in the same spatial domain. There is a tendency to address health at the species level, rather than holistically, as proposed for the one health approach in human medicine. Our assumptions are that mortality events and disease outbreaks are consequences of environmental degradation and ineffective stewardship, but it is also necessary to assess if such negative feedback mechanisms are a consequence of healthy and robust population controls at the ecosystem level.

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Dr. John Reynolds

John Reynolds graduated Cum Laude with Departmental Honors in Biology from Western Maryland College (now McDaniel College) in 1974. He received his M.S. and Ph. D. degrees in Biological Oceanography from University of Miami's Rosenstiel School of Marine and Atmospheric Sciences in 1977 and 1980, respectively. He was employed at Eckerd College, St. Petersburg, FL from 1980-2001, where he served as Professor of Marine Science and Biology and Chairman of the Natural Sciences Collegium. In 1989, Reynolds became a member of the Committee of Scientific Advisors on Marine Mammals for the U.S. Marine Mammal Commission, the federal agency with oversight for all research

and management of marine mammals in the United States. In 1990, he became Chairman of the Committee of Scientific Advisors, and in 1991, he was appointed by President George H. W. Bush to serve as Chairman of the Marine Mammal Commission. He led that agency through mid-2010 under four different administrations, and in 2010, the agency's accomplishments were recognized by a distinguished service award by the international Society for Conservation Biology. Since 2001, Reynolds has been a Senior Scientist for Mote Marine Laboratory, Sarasota, FL. From 2001-2008, he was co-Chair of the IUCN Sirenian Specialist Group and from 2006-2008, he served as President of the international Society for Marine Mammalogy. Recently he has worked with the United Nations Environment Programme to develop and implement a Caribbean-wide Marine Mammal Action Plan. Reynolds is a member of Phi Beta Kappa; has been nominated for international awards for his accomplishments in conservation and science; and has published more than 300 books, papers, and abstracts.



OPTIMIZING CONSERVATION SOLUTIONS THROUGH HEALTH PROFESSIONALS

John E. Reynolds, III

Mote Marine Laboratory, Sarasota, FL 34236 USA

In 2003, the Pew Oceans Commission issued a diagnosis and challenge: "...oceans are in crisis and the stakes could not be higher...without reform our daily actions will increasingly jeopardize a valuable natural resource..." A polarized response ensued, with some groups and individuals claiming that Pew was misinformed and alarmist, but others indicating that a clarion call to change the way humans go about their lives and business was overdue and liberating.

Aquatic animal health professionals are already noting multiple manifestations, including changing incidence of diseases in wildlife populations, of our rapidly changing world. Even if exposed animals survive an insult or stressor, diminution of immune function and reproductive potential can occur as sublethal impacts that compromise sustainability of populations and species. Professionals in animal health care can build long careers around monitoring changes in health status of particular groups, but monitoring and science are different from conservation and will not necessarily, of themselves, do anything to "reform our daily actions" for the good of wild living resources, ecosystems, and humans.

The field of conservation medicine and the "one health" approach focus on the intersection of ecosystem, animal, and human health. I challenge health care professionals to consider not simply measuring and monitoring conditions at such critical intersections, but expanding their role to promote conservation through actively seeking *solutions*. The prescription for success in conservation involves being proactive; having some guts; being creative, imaginative, and opportunistic; communicating broadly to address human values and behaviors; working effectively as a part of an interdisciplinary team; and going far beyond simply seeking more information. The crisis for wildlife and aquatic habitats was not created overnight; nor will it be solved immediately. However, an array of emerging tools and unprecedented partnerships can allow dedicated, knowledgeable and passionate individuals to make an enormous difference in the quality of our world and its inhabitants.

The Convention on Biological Diversity (CBD) emerged at the end of the 20th century in response to increasing worldwide biodiversity loss. The year 2010 was declared as the International Year of Biodiversity, reflecting an international commitment to achieve a significant reduction in the current rate of biodiversity loss. However noble the commitment was, it failed to achieve its goal. To be sure, not all biodiversity loss and other conservation issues stem from factors that affect animal health. But some do, either individually or cumulatively and synergistically. Health professionals can and should play a crucial role as parts of interdisciplinary teams that attempt not just to define or monitor conditions, but to mitigate and solve problems. The obstacles to success can be immense; the challenges are unprecedented by professionals who have confined their past work to defining and monitoring. But the need for such teams is urgent and the rewards for even modest success exhilarating.

Contact Information: John E. Reynolds, Mote Marine Laboratory, Manatee Research, 1600 Ken Thompson Parkway, Sarasota, FL 34236 USA, Phone: 941-388-4441, Email: reynolds@mote.org

Oral Presentation Abstracts

Listed alphabetically by presenting author last name.
Presenting author names appear in **bold**.

FISH KILLS AND DISEASE EVENTS IN FLORIDA: FWC ROLE AND RESOURCES

Micah D. Bakenhaster¹, Jan H. Landsberg¹, Theresa T. Cody¹, and Yasunari Kiryu¹

¹Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, Saint Petersburg, FL, USA

The Florida Fish & Wildlife Conservation Commission's (FWC) Fish and Wildlife Research Institute's (FWRI) Fish and Wildlife Health group (FWH) is responsible in part for monitoring fish health, tracking and investigating fish disease and mortality events in state waters, operating the statewide Marine Fish Kill hotline, maintaining an integrated multidisciplinary network of expert partners and collaborators, and providing relevant data, analyses, and information that are useful to resource managers and stakeholders or otherwise serve the public interest. The FWC/FWRI Fisheries Independent Monitoring program and Fish Kill Hotline provide the agency with two complementary means to identify and track fish kills and disease in Florida's coastal waters. During fish population surveys, FWC monitoring crews use various net gears to collect fish, and in addition to obtaining species counts and specimen measurements for population modeling, they check for specimens exhibiting any grossly visible abnormalities. Whenever practical, lesioned fish are returned to the laboratory for full necropsy. Because monitoring is conducted consistently each year and sites are selected with a stratified-random sampling design, prevalence of particular lesions in samples can be considered statistically representative of true prevalence in wild fish populations. This is not necessarily the case with public, partner, or agency reports of lesioned fish or fish kills made to the Hotline; however, this tool increases the likelihood of our detecting fish kills and epizootics over a very broad geographic area. The Hotline additionally provides the opportunity to address public concerns through provision of information and, when appropriate, initiation of investigation and follow through with applied research.

FWC (or predecessor agencies) has been documenting fish kills and disease incidents since the 1950s, initially to address wide-scale die-offs caused by red tide events. Kills can be attributed to natural and anthropogenic stressors including a range of ichthyotoxic algal bloom species, poor water quality, pollution, pathogens, extreme water temperatures, and hurricanes. Here we present a few illustrative case examples of events investigated by FWH that demonstrate diverse application of our resources. These include: 1) the use of long term monitoring data to identify spatial patterns in the prevalence of a hematopoietic neoplasm affecting redbfin needlefish (*Strongylura notata*) in three estuaries, primarily in the northern Indian River Lagoon, 2) investigation of various fish lesions reported by the public in the wake of the Deepwater Horizon disaster, 3) field and experimental identification of the oomycete, *Aphanomyces invadans*, as the causative agent of a major epizootic in striped mullet (*Mugil cephalus*) and other fishes of the St. Lucie River with associated management strategies, and 4) tracking and opportune sampling of beached piscine megafauna.

Contact Information: Micah D. Bakenhaster, Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, 100 8th Ave SE, St. Petersburg, FL 33701 USA, Phone: 727-896-8626, Email: Micah.Bakenhaster@MyFWC.com

ANTHROPOGENIC HEALTH STRESSORS POTENTIALLY RELATED TO CAPTIVE MANAGEMENT DURING REHABILITATION OF FLORIDA MANATEES (*TRICHECHUS MANATUS LATIROSTRIS*).

Ray L Ball¹, Lauren N. Smith², Ashley Barratclough²

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Rehabilitation is an important component to the recovery plan for the Florida manatee (*Trichechus manatus latirostris*). Providing individualized health care yet avoiding the complications associated with the captive care of wild animals have challenges. Issues revolving around nutrition are common in managed wildlife and may also arise in manatee rehabilitation. The highly efficient digestive strategies that manatees have pose unique challenges and problems related to obesity that are now being recognized. Social issues, especially in orphans, can lead to social maladjustments once these manatees are released. Problems attributable to long-term captivity, where the animal may experience non-reproductive periods of 10–15 years, have been identified in several mammal species. Evidence suggests that prolonged exposure to endogenous sex steroids or extended periods of reproductive quiescence may induce “asymmetric reproductive aging” in captive animals. Steroid hormone-dependent tumor development and growth by estrogen and progesterone have been well established and may explain some of the findings in captive, non-reproducing yet continually cycling mammals. Leiomyomas are known to be estrogen induced, and the cumulative effects of constant estrus activity are the basis for this tumor. A reproducing wild manatee reaching sexual maturity at 5 years of age, with 12-14 months gestation, a 3 year inter-calf interval, and a reproductive life of 35 years will have approximately 12 estrus cycles in her reproductive life. A captive manatee will have an estrus cycle once every 28-42 days and thus approximately 8-13 cycles per year. Within 5 years of captivity, a female manatee will have between 40-65 cycles, and well exceed the normal endogenous exposure to estrogen than she would have in a wild setting. It is important to note long-term captive manatees have been excluded from hormone studies in manatees due to concerns about asymmetric aging. Reproductive neoplasms and pathologies in wild and long-term captive female Florida manatees obtained through a carcass recovery program between April 2009 and May 2014 has brought a new awareness to reproductive health of female manatees. Management changes may include: review of feeding strategies to mimic wild foraging manatees; developing a fitness criteria for release instead of weight and length criteria; explore options for releasing orphans earlier; behavioral training when needed to help orphans re-adjust; minimizing human contact during the rehabilitation process; re-enforcing the existing criteria that does not allow rehabilitated manatees to be held long term; and the re-classification of several existing long-term captive female manatees as conditionally non-releasable.

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ESTABLISHING THROMBOELASTOGRAPHY REFERENCE INTERVALS IN WILD FLORIDA MANATEES (*TRICHECHUS MANATUS LATIROSTRIS*) TO ENABLE FURTHER UNDERSTANDING OF THE PATHOPHYSIOLOGY OF COLD STRESS SYNDROME

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Thromboelastography provides a comprehensive evaluation of clot formation. This diagnostic test can be used to identify abnormalities in coagulation by assessing all aspects of the clotting cascade from clot initiation, to formation and ultimately fibrinolysis. The Florida manatee (*Trichechus manatus latirostris*) is an endangered species which is frequently affected by cold stress syndrome. The pathophysiology of this condition is still not understood however thromboembolic disease is believed to contribute. By establishing the normal reference values for thromboelastography on healthy wild manatees we will have a reference standard for future clinical analysis of rehabilitation manatees suffering from cold stress syndrome. This will potentially enable us to diagnose coagulation abnormalities and improve clinical outcomes.

During the Crystal River Health Assessments twenty nine wild manatees were sampled for use in this study to identify the following mean (SD) normal TEG parameters: reaction time R = 2.1(0.77) minutes, clotting time K = 0.8 (0.0) minutes, α angle 83.1° (2.0), maximum amplitude MA = 75mm (7.6) and clotting lysis LY30 = 0.41% (0.68). No significant differences were found between size, sex or time frame after sampling to running the test within 12hours. In addition to thromboelastography a coagulation panel was performed to assess the normality of the clotting cascade in these wild manatees. This involves testing platelet count, D-dimers, fibrinogen, PT and PTT. This was performed on all 29 Crystal River manatees and on a further 10 manatees in Brevard county to establish normal wild reference ranges.

Preliminary thromboelastography on recently admitted cold stress syndrome cases to Tampa's Lowry Park Zoo have supported the hypothesis of a hypercoagulable state in cold stress syndrome. This has been demonstrated by a reduced R time 1.5min (0.4) which is statistically significantly different ($p=0.03$) from that established in the wild manatee health assessments. LY30 an indication of the stability of the clot was also statistically significant 1.3% (1.7) $p = 0.03$. The other parameters showed variation from normal however were not deemed statistically significant with the current sample size of $n=8$. Additional samples are required to determine if there are further significant differences between healthy wild manatees and those suffering from cold stress syndrome, however with the available data, the trends support the hypothesis.

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STATUS, TRENDS, AND HEALTH OF FLORIDA'S CORAL REEFS

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Coral reef ecosystems worldwide are threatened by increasing environmental and anthropogenic disturbances, and consequently, changes in community composition have been documented. Benthic communities of the Florida Keys National Marine Sanctuary are currently undergoing a phase shift in dominance from stony corals to soft corals. The documented decline of stony coral dominance during the last 15 years has been attributed to acute and chronic stressors of both anthropogenic and natural origin. Ranging from the broad effects of global climate change to local watershed pollution (e.g. nutrients, contaminants, and pathogen inputs) and harmful algal blooms (e.g. macroalgae, microalgae, and cyanobacteria), conditions have developed to facilitate the exacerbation of previous background disease, and the introduction of novel pathogens. Notable benthic groups affected include stony corals, which have been the subject of the majority of recent research, as well as echinoderms, sponges, and soft corals. Despite disease being a prominent cause of colony mortality, direct causal relationships are difficult to determine. The challenges associated with understanding complex multi-factorial disease etiologies and the often alarming consequences from outbreaks have increased efforts in health research and monitoring, the need for obtaining baseline data, and active reef restoration. As multi-partner, multi-collaborative efforts rally to restore and enhance degraded coral reefs employing nursery-reared benthic organisms, FWC scientists and managers are also working with partners to develop health assessment and stocking protocols to minimize or mitigate the spread of detrimental health conditions.

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A RISING TIDE: TRANSLATIONAL SOCIAL RESEARCH, INSTITUTIONAL ANTHROPOLOGY AND THE POLICY SCIENCES AS TOOLS TO NAVIGATE THE COMPLEX DYNAMICS OF SOCIAL ENGAGEMENT IN CONSERVATION

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“A Rising tide lifts all boats” - traditional proverb from New England

This proverb rings self-evident and true. But tropical marine scientists and coastal residents know that the world is much more dynamic and complex. Beyond the tide, a host of other elements determine whether a boat will rise, founder or sink. As part of the session entitled “Solutions for Maintaining Healthy Ecosystems”, this presentation explores the role of social elements as fundamental elements in increasing and sustaining the health of marine ecosystems. When applied as part of a wide, interdisciplinary, multidimensional process, these approaches have proven effective in gaining a deeper, more complex understanding of social aspects of conservation, as well as supporting the development of sustainable and inclusive solutions.

The goal of this presentation is to provide the audience with information about three social ecology tools and discuss their value in improving our understanding, documenting, planning and implementing effective and sustainable marine conservation policies. These tools are: translational social research (with an emphasis on information visualization), institutional anthropology and policy sciences. Each tool will be described briefly and examples will be presented on how they have been applied in the context of tropical marine ecosystems. Working in collaboration with marine scientists, social ecologists can help the tide of healthy marine ecosystems rise higher, and make sure that more boats rise with it.

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ONE HEALTH AT THE TERRESTRIAL – AQUATIC INTERFACE

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As early as the 1800's, the concept of zoonoses was understood by many scientists. From Virchow's early discoveries of *Trichinella sp.* to our current understanding of viruses such as ebola and influenza, the link between human and animal health has long been recognized. However, today's one health philosophy has a third field to include: the environment. Nowhere is the importance of the inclusion of this third leg of the one health stool more readily apparent than in aquatic habitats specifically at their intersection with the terrestrial world.

It could be as straightforward as nitrogen-containing fertilizer running into the Mississippi river and into the Gulf of Mexico to create a massive "dead zone." Or, as complex as abalone withering syndrome and its relationship to apparent increases in terrestrial parasites Toxoplasmosis and Sarcocystis among sea otters. It is certainly easier to understand how carnivorous marine animals could be readily exposed to terrestrial pathogens secondary to prey items, but what about herbivorous marine inhabitants? The plant life itself is certainly susceptible to changes in the environment and can wreak some havoc on the animal and human population secondarily as happens in the cases of harmful algal blooms. Specifically, what about the manatee? Just as vegetarian humans are not immune from all parasites found in muscle meat, neither are the sea cows. *Toxoplasma gondii* and *Leptospira sp.* are two pathogens readily found in Florida and documented in manatees elsewhere. *Salmonella sp.* have also been found to be pathogenic in dugongs, close relatives of manatees and *Brucella sp.* are becoming more and more common among other related marine mammals. It remains unclear if manatees have a role in higher profile disease transmission such as influenzas, but it seems clear that manatees and their ever responsive aquatic environment should certainly be respected as possibly sentinels and perhaps partners in global one health efforts.

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REPRODUCTIVE ANATOMY AND HISTOLOGY OF THE MALE FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*)

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Information about genital anatomy of the male Florida manatee (*Trichechus manatus latirostris*), is limited. Studies have been conducted on the general and histological examination of the testis and epididymis, but a detailed review of the accessory sex glands has not been performed. The objective of this study is to conduct an anatomical and histological description of the entire reproductive tract of male Florida manatee, including the testes, present accessory glands and penis.

Three reproductive tracts of adult males were collected for histological analysis, and three additional tracts (two adults and one juvenile) for the gross anatomical analysis, from the Marine Mammal Pathobiology Laboratory, in St. Petersburg, Tampa. Four archived tracts (two adults, a juvenile and an infant) were included for histological analysis. The general description of the tracts was determined by dissection. Histological analysis used the following stains: haematoxylin and eosin technique, PAS, Gomori's one step trichrome and immunohistochemistry specific for smooth muscle actin.

The accessory sex glands found on the manatee are the vesicular glands and prostate. They lack ampullary and bulbourethral glands. A prominent colliculus seminalis was found inside of the urethralis muscle. Ductus deferens, vesicular glands and prostate had opening ducts in the colliculus seminalis. The penis of the manatee was approximately 39cm long and 3.75cm width, the os penis is absent in this species. The manatee penis arrangement is similar to the stallion (*Equus ferus*) with a corpus cavernosum (dorsally located) and poor corpus spongiosum nearby the urethra (ventro-medially located), all surrounding by a thick tunica albuginea layer.

The male reproductive tract of the Florida manatee presents gross and histological similarities with the dugong (*Dugong dugon*), another member of the order Sirenia, and with a close terrestrial relative of the manatee, the elephant (*Loxodonta africana*). Further description of the accessory sex glands' contents would help to define the roll of these structures. Along with this information, the phylogenetic history and the mating strategy could explain the presence/absence of the accessory sex glands found in Florida manatee.

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HARMFUL ALGAL BLOOM MONITORING TO PROTECT PUBLIC AND ANIMAL HEALTH IN FLORIDA

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Harmful Algal Blooms (HABs) negatively influence coastal water quality, fish and marine mammal populations, public health, and recreation globally. In marine and estuarine waters of Florida, more than 80 HAB species, including those that produce toxins and diverse bioactive compounds - with a range of effects - have been documented. This presentation reviews HAB monitoring in Florida and its application to public health and resource management. Statewide coordination between monitoring and regulatory agencies has generated effective management strategies that have partially mitigated the negative consequences of HABs. Specifically, monitoring has provided information on bloom formation and progression to (1) support public and environmental health decision making; (2) advise managers about potential risks to aquatic animals; and (3) educate the public. HAB monitoring has largely focused on algal species on which shellfish harvesting areas are regulated, including the toxic dinoflagellates *Karenia brevis* and *Pyrodinium bahamense* and diatoms in the genus *Pseudo-nitzschia*. However, other species are tracked as they are detected. Monitoring, tracking, and information dissemination is accomplished through a unique collaboration between the Florida Fish and Wildlife Conservation Commission, the Florida Department of Agriculture and Consumer Services, Mote Marine Laboratory, University of South Florida, other state and county agencies, other private non-profit agencies, and citizen volunteers. Productive interdisciplinary and interagency collaborations as well as new technologies have increased our ability to track blooms and mitigate associated negative effects.

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GENOMIC CHARACTERIZATION OF TWO PAPILOMAVIRUSES IN INDIAN RIVER LAGOON BOTTLENOSE DOLPHINS

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The Atlantic bottlenose dolphin (*Tursiops truncatus*) Health and Risk Assessment (HERA) project is a comprehensive research program designed to assess environmental and anthropogenic stressors, as well as the health and long-term viability of Atlantic bottlenose dolphins. Members of the Harbor Branch/FAU Marine Mammal Research and Conservation program, the University of Florida's Wildlife and Aquatic Veterinary Disease Laboratory, and the Blood Systems Research Institute teamed up to conduct a viral survey of Indian River bottlenose dolphins sampled during July 2012 health assessments. Respiratory, fecal, and buffy coat samples were initially tested using polymerase chain reaction assays for a wide variety of known marine mammal viruses. Additionally, the first unbiased metagenomic effort to discover novel viruses in bottlenose dolphins using a Next Generation Sequencing approach was performed. This resulted in the detection of previously described agents such as Delphinid herpesvirus 4 and new viruses belonging to the families *Papillomaviridae*, *Parvoviridae*, *Circoviridae*, and *Anelloviridae*. Interestingly, one of two novel dolphin papillomaviruses displayed 100% nucleotide sequence identity for a portion of the viral L1 gene previously reported from a wild orca (*Orcinus orcas*). Papillomaviruses are a diverse group of small double-stranded DNA viruses that have been described from a wide range of marine mammal species. They display host fidelity and induce benign proliferative skin lesions that can progress to neoplasms. Papillomaviruses occasionally cross species boundaries as illustrated by the transmission of *Bovine papillomavirus 1* into horses resulting in equine sarcoids. The discovery of the same strain of papillomavirus in two separate delphinid hosts suggests another potential host jump, the first such case described in marine mammals. Although the health implications of this papillomavirus to its delphinid hosts remain to be characterized, the potential of the virus to spread among dolphin species should be considered in management.

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ANTHROPOGENIC IMPACTS ON FLORIDA'S COASTAL MARINE INVERTEBRATES

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In recent years, Florida's coastal waters have experienced a host of anthropogenic impacts through coastal urbanization, hydrological modifications and canalization, and increasing nutrient loading. The resulting eutrophication of these estuaries has resulted in major phytoplankton blooms that are increasing in longevity and intensity. Those involving the dinoflagellate, *P. bahamense* (red tide), cyanobacteria, and pelagophytes (green tide) are particularly noteworthy because of their magnitude, duration, and/or potential harmful effects. Toxic blooms of dinoflagellates (saxitoxin) and cyanobacteria (cyanotoxin) can be toxic to some crustaceans, and they are bioaccumulated by filter-feeding bivalves (e.g. clams and oysters), vectors for Paralytic Shellfish Poisoning, and a significant public health risk if consumed.

Bacterial decomposition of phytoplankton, grasses, and macroalgae creates a *Biological Oxygen Demand* (BOD) through respiration and results in a dramatic decline in dissolved oxygen (DO). Under these circumstances, the DO throughout the water column may drop to below 3 mg/L resulting in fish mortality, bottom water can become hypoxic (< 2 mg/L) and the sediment (with the greatest BOD) can quickly become anoxic (0 mg/L oxygen). Adult hard clams can revert to anaerobic respiration when DO drops below 4.8 mg/L, but at DO levels lower than 1.4 mg/L clams become extremely stressed and succumb in a few days. Juvenile (seed) clams are more vulnerable to hypoxic conditions than adults.

An emerging issue for estuarine invertebrates is acidification. Mixing with acidified ocean water, and the lower pH of brackish water, the organically rich, anoxic, sulfidic sediments (muck) becomes re-suspended during storm events, reducing carbonate saturation, which results in dissolution of calcium carbonate shells (mollusks and echinoderms) and larval mortality (recruitment limitation). It has also been implicated in mortality in some larval crustaceans.

Vibrio, a genus of gram-negative bacteria, includes many species that are highly pathogenic are common in warm water causing gastroenteritis (shellfish poisoning) and septicemia, with contaminated levels that have been recorded at alarmingly high levels in the Indian River Lagoon. These can cause stress, decreasing feeding and growth in bivalve mollusks and can result in high larval mortality. A strain of *V. parahaemolyticus* has caused a recent epidemic of *Early Mortality Syndrome* in penaeid shrimp. Black gill or brown gill disease is a disease of penaeid shrimp caused by another single celled protozoan known as apostome, which attaches to and penetrates the shrimp's gills, turning them brown or black. Secondary in nature, they take advantage of existing gill damage due to stress from another pathogen (such as *Vibrio* spp.) and perhaps an association with degraded water quality.

Methoprene, a mosquito larvicide, Malathion, and other commercial insecticides have been implicated in mortality of crustacean zooplankters (copepods), as well as callinectid crabs (blue crabs) and palinurid lobsters.

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EMERGING DISEASES OF AQUATIC BIRDS

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Florida and its adjacent waters are home, for at least part of the year, to over 500 species of birds. Of these, >150 are considered aquatic utilizing Florida's numerous lakes and wetlands as well as its extensive coastline, the Gulf of Mexico and Atlantic Ocean. Migratory birds winter in Florida or travel through the state to wintering areas further south utilizing both the Atlantic and Mississippi flyways. Anthropogenic changes to the environment are having an increasing impact on aquatic species and this is especially apparent in Florida. Direct and indirect effects of humans on bird populations include exploitation, physical hazards, habitat loss, alteration, fragmentation, exotic species, pollution and environmental contaminants, artificial feeding, resource depletion, artificial contact among wildlife, exotic, and/or domestic species, and climate change. These anthropogenic stressors have a variety of impacts on birds ranging from direct mortality to effects on reproduction, immunocompetence, and other fitness parameters.

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KISSIN' COUSINS: A LOOK AT THE HIGH GENETIC RELATEDNESS AND INBREEDING OF THE ENDANGERED FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*)

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The endangered Florida manatee has a single mitochondrial haplotype, little genetic divergence between subpopulations, and low nuclear genetic diversity, suggesting a population vulnerable to natural and anthropogenic effects. Small isolated populations tend to be at increased risk of decreased reproductive fitness and population sizes, as well as higher relatedness and inbreeding levels. Given the lack of diversity reported in previous studies, other genetic analyses were needed to better understand the genetic health of the population. Analyses included estimation of population-level and individual relatedness and individual inbreeding coefficients, based on 36 polymorphic microsatellite loci. This marks the first time that individual inbreeding metrics have been used to compare groups of Florida manatees. Simulations indicated that higher order pairwise relationships could be distinguished from distant relationships with sufficient statistical power. The number of loci genotyped was critical; use of fewer loci typically led to upwardly biased estimates of inbreeding and relatedness, and during discrete relationship classification, (e.g., misclassifying first cousins as half siblings). On average, estimated coefficients of relatedness ($r_{xy}=0.115$) and inbreeding ($F_x=0.108$) for pairs of individuals within the Florida manatee population were consistent with levels expected for first cousins. This high degree of 'latent coancestry' – the average underlying amount of shared genetic information between any two individuals in a population – was incorporated into a new inbreeding calculation and used to improve relatedness and inbreeding estimates for both captive and wild populations. Pairwise comparisons indicated that 20-26% of pairs in the population were at least 0.25 related; substantially higher than expected in a healthy, randomly mating population (0.01-0.10). Higher relatedness levels in females supported observations of female philopatry and male dispersal. The significance of higher inbreeding levels in females was less clear, with many hypothetical causes including increased female survivability or an x-linked lethal recessive gene. The Florida manatee is a good example of a species that has an increasing population growth rate, yet the genetic fitness and viability may be reduced by the limited genetic diversity. With the knowledge of a highly related and inbred population, classifying the health, fitness and contribution of individuals to the overall population can now be accomplished. This new knowledge will help inform ongoing management actions and promote the recovery of this endangered species.

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GETTING THE MOST INFORMATION DURING HEALTH ASSESSMENT, STRANDING RESPONSE AND REHABILITATION STUDIES: OPTIMIZING SAMPLE COLLECTION FOR FUTURE MOLECULAR DIAGNOSTICS AND TOXICOLOGY STUDIES

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Anthropogenic impacts and global climate change have raised significant concern about public and ecosystem health. Utilization of sentinel species can contribute to the complex impact these changes have on individuals, populations, and the environment. Marine mammals are an ideal sentinel species because they are long lived, have significant public interest, and often share coastal environments and food sources with humans. Appropriate sample collection and processing during health assessments, stranding events, and rehabilitation are crucial in gathering as much information as possible. With rapidly developing molecular diagnostic methods including PCR, quantitative PCR, next generation sequencing, mass spectrometry, and enzyme linked immunosorbent assays (ELISA), routine sample collection should go beyond basic health parameters when possible. Molecular samples can now efficiently and cost effectively be assessed using population genetics, transcriptomics, metagenomics, proteomics, and toxicological analyses, etc.

Many molecular tests can be run from specimens already collected during routine diagnostic sampling. The critical step is rapid processing and storing these samples appropriately to allow for reliable molecular and toxicology testing. Individual project goals will dictate the type of samples collected, however, standardized collection, processing, and archival DNA, RNA, serum, and toxicological samples can prove invaluable for tests not initially anticipated in the research plan or for additional use of samples for future studies. Sample archives can also prove very useful for comparative baselines in future disease outbreaks, unusual mortality events (UMEs) or environmental disasters.

Special considerations are necessary for collection, processing, and sample storage, as molecular samples are most useful if processed immediately (particularly with RNA studies) and preserved in appropriate sample tubes and storage reagents (i.e. RNA later). Care must be taken that appropriate permits are in place for all samples collected from protected species. Essential field equipment includes a reliable centrifuge that can be run off a portable battery pack (multiple units useful), dry ice, liquid nitrogen, DNA/RNA free storage containers and appropriate reagents and collection/processing materials. Sterile technique for sample processing is critical, especially in variable field conditions. Lastly, appropriate long-term sample storage is necessary. Any samples anticipated for use in molecular and toxicological studies should be stored in an ultra-low, laboratory grade freezer at -80°C or liquid nitrogen dewar designed for long term storage. For rare or important samples, storage in separate freezers, buildings, or institutions can ensure sample preservation for future research opportunities.

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INVESTIGATING AN INCREASE IN FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*) MORTALITIES IN 2013 USING PROTEOMIC AND TRANSCRIPTOMIC ANALYSES

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In 2013, two large-scale mortality episodes reported in Florida manatees (*Trichechus manatus latirostris*) were associated with 830 deaths and a 17% single-year population loss. The mortality episodes occurred on separate coasts of Florida-near Fort Myers on the west coast and in the Indian River Lagoon (IRL) on the east coast. The IRL mortality episode was associated with an unknown etiology and the Southwest Florida episode was attributed to a persistent bloom of red tide (*K. brevis*) algae in the region. In an effort to investigate the cause of the unknown mortality episode and obtain more information on the cellular effects of red tide brevetoxins on manatees, several proteomic and transcriptomic experiments were conducted. To determine the differences in protein concentration in three manatee populations, serum samples from manatees from Brevard County/IRL (n=4) and the Lowry Park Zoo (n=4) were compared to a control population of healthy manatees from Crystal River, Florida (n=4) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). A gel-based approach using two-dimensional difference gel electrophoresis (2D-DIGE) was applied in the first experiment and a gel-free analysis using isobaric tags for relative and absolute quantification (iTRAQ) was conducted in the second experiment. In the Brevard group, several proteins showed significant differential expression including complement C4-a isoform 1 (average ratio 1.25), histidine-rich glycoprotein (1.34), kininogen-1 isoform 1 (1.38), properdin (1.30), and protein AMBP (1.38). In the Lowry group, complement C3 (1.42), complement C4-A isoform 1 (1.83), ceruloplasmin-like (2.32), angiotensinogen (2.08), and pyruvate kinase isozymes M1/M2 isoform 3 (2.29) displayed differential expression above a threshold applied during the data analysis. These proteins are associated with acute-phase response, amyloid formation and accumulation, copper and iron homeostasis, the complement cascade pathway, and other important cellular functions and will be evaluated as potential biomarker proteins for manatees.

To investigate the differences in gene expression, a transcriptomics survey was completed to detect aberrations in gene expression using RNA-Seq on the Illumina NextSeq[®] 500 platform. The transcriptomics dataset was constructed by sequencing manatee white blood cell or 'buffy coat' samples (n=12) from the Lowry, Brevard, and Mote Marine Aquarium (red tide) study groups compared to a healthy 'control' manatee population in a case-control study design. By determining variations in gene expression, the differential expression patterns were assessed and compiled for each of the study groups. The genes with the highest fold changes (FCs) were the RAD9-HUS1-RAD1 interacting nuclear orphan 1, transcript variant 1 (RHNO1) gene (FC 5.06) (Brevard group), putative serine protease K12H4.7-like gene (6.66) (Lowry group), and interleukin 6, interferon, beta 2 (acute and post groups) (10.05 and 10.64). Gene activities related to innate immune response, copper binding and transport, anticoagulation, ceruloplasmin production, and neuronal connectivity were the most impacted.

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BASELINE LEUKOCYTE CYTOKINE LEVELS IN A SUBPOPULATION OF HEALTHY FLORIDA MANATEES MEASURED BY QUANTITATIVE PCR

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Cytokines function in the body as signaling molecules used to mediate an immune response. These proteins activate, recruit, and regulate cells which are responsible for immune, inflammatory, and subsequent repair processes. This study addressed the need for sensitive, species specific assays to measure cytokines in Florida manatees. Whole blood samples were collected from 42 free-ranging Florida manatees. RNA was extracted from the peripheral blood mononuclear cells for use in quantitative, real-time PCR (qPCR) assays. Primer/probe sets were designed for analysis of IFN- γ , IL-2, and IL-10, as well as GAPDH and β -actin as housekeeping targets. Using these assays, preliminary baseline PBMC ranges of β -actin, GAPDH, IFN- γ , IL-2, and IL-10 levels in healthy, free-ranging Florida manatees in the Crystal River and Brevard overwintering sites were established.

The average β -actin value for the healthy Florida manatee population sampled was 1.44×10^5 copies/100 ng cDNA, and for GAPDH the value was 2.08×10^4 copies/100 ng cDNA. Copy values of IFN- γ , IL-2, and IL-10 were normalized to GAPDH values for the same manatee sample. The final normalized count value averages for each cytokine were as follows: IFN- γ average = 0.055 copies/100 ng cDNA; IL-2 average = 0.126 copies/100 ng cDNA; and IL-10 average = 0.031 copies/100 ng cDNA.

These are the first assessments of IFN- γ , IL-2, and IL-10 levels in Florida manatee PBMCs using qPCR. Future investigations of manatees from the Brevard and Crystal River overwintering sites undergoing a physiologic stress response now have a baseline value for comparison to a healthy counterpart.

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BREVETOXIN-ASSOCIATED MASS MORTALITY EVENT OF BOTTLENOSE DOLPHINS AND MANATEES ALONG THE EAST COAST OF FLORIDA

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A mass mortality of bottlenose dolphins (*Tursiops truncatus*) and Florida manatees (*Trichechus manatus latirostris*) co-occurred with a severe bloom of the toxic algal species *Karenia brevis* along the eastern coast of Florida, USA between October 2007 and January 2008. Brevetoxin (PbTx), a potent neurotoxin produced by this marine alga, was detected in 69% and 92% of tested carcasses of manatees and dolphins, respectively, at concentrations similar to those reported for earlier mortality events along the west coast of Florida. Brevetoxin was also detected in fetal and neonate dolphins, providing evidence of maternal transfer of the toxin in wild populations. This study is the first to document a brevetoxin-associated marine mammal mortality event along the Atlantic coast of Florida. It also demonstrates that, despite the rarity of *K. brevis* blooms in this region, significant negative impacts to marine mammals inhabiting this region can occur.

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PHYLOGENOMIC DIVERSITY OF CETACEAN MORBILLIVIRUSES

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Cetacean morbillivirus (CeMV) is a member of the genus *Morbillivirus* in the family *Paramyxoviridae* that include enveloped negative-sense RNA viruses of importance in both human and veterinary medicine. Over the past 25 years, CeMV has emerged as the most significant pathogen of dolphins. We describe the phylogenomic diversity among four CeMV strains: dolphin morbillivirus (DMV-M) isolated from a Mediterranean striped dolphin (*Stenella coeruleoalba*), dolphin morbillivirus (DMV-G) from a bottlenose dolphin (*Tursiops truncatus*) in the Gulf of Mexico, porpoise morbillivirus (PMV) isolated from a harbor porpoise (*Phocoena phocoena*), and beaked whale morbillivirus (BWMV) from Longman's beaked whale (*Indopacetus pacificus*). Full CeMV genomes were sequenced by performing overlapping reverse transcription PCR. The phylogenomic diversity of the CeMV strains were compared to the six other recognized morbillivirus species including: *Measles virus*, *Rinderpest virus*, *Peste-des-petits-ruminants*, *Phocine distemper virus*, *Canine distemper virus*, and *Feline morbillivirus*. Sequences were aligned in MAFFT 7.0 followed by evolutionary model optimization and Maximum Likelihood analysis in MEGA 6.0. The four CeMV strains formed a well-supported clade. BWMV was the most divergent and formed the sister taxon to the rest of the CeMVs. Although considerable sequence variation was detected among the four, the magnitude of the difference was suggestive of separate CeMV strains (i.e. DMV, PMV, and BWMV) rather than separate morbillivirus species. Although preliminary, recent detections of a high divergent morbillivirus in Indo-Pacific bottlenose dolphins (*T.aduncus*) from Western Australia and a Guiana dolphin (*Sotalia guianensis*) from the South Atlantic suggests the creation of a new morbillivirus species may be warranted. This study provides a much needed update to morbillivirus taxonomy, a foundation for future efforts aimed at developing improved CeMV molecular diagnostics, and a better understanding of the temporospatial dynamics of these emerging marine mammal pathogens.

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NEXT GENERATION SEQUENCING OF GUT CONTENT TO HELP INFORM THE FLORIDA MANATEE UNUSUAL MORTALITY EVENT IN THE INDIAN RIVER LAGOON

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A dramatic reduction of seagrass due to long-term, non-toxic phytoplankton blooms in the Indian River Lagoon preceded a Florida manatee unusual mortality event (UME) in Brevard County, Florida. This UME has been ongoing since the late summer of 2012, and a dietary change due to little or no seagrass availability is a hypothesized contributor. To further investigate the gut microbiota and diet consumed by manatees in the area affected by the UME, a high-throughput metagenomic barcoding approach was evaluated. The Illumina MiSeq next-generation platform was used to generate non-overlapping 2x300 bp paired-end DNA barcode sequences to provide comprehensive and rapid indexing of plant and animal species. A targeted sequencing approach was employed to PCR amplify targeted loci of available floral and bacterial species present in the gut instead of sequencing all of the genomic DNA available in the samples. Unusual mortality event manatee fecal material (N=20) was compared to 'control' samples (N=10) from the same area. The MiSeq runs produced millions of sequences with a minimum Phred score of 20 on trimmed reads. This represents a minimum base call accuracy of approximately 99%, providing high confidence in the quality of DNA submitted. Operational taxon units (OTUs), observed richness of species diversity (α -diversity), and differences between control and experimental samples (β -diversity) were assessed to determine differences in species consumed by manatees associated with the control and UME samples. For example, bacteria from over 500 genera have been identified, leading to the identification of nearly 1800 species. This high-throughput approach can help to inform the ingested material and gut microbiota of manatees in the Indian River Lagoon and add to the many tools used to investigate UMEs.

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ANALYTICAL TOXICOLOGY OF INSHORE GULF SEAFOOD: BRIDGING ENVIRONMENTAL AND PUBLIC HEALTH CONCERNS POST-DWH OIL SPILL

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This study focused on the analytical toxicology of inshore-harvested seafood to address public health and coastal community concerns regarding seafood safety in the Gulf of Mexico following the Deepwater Horizon oil spill. Over 1,000 fish, shrimp, blue crab and oyster samples were homogenized and prepared using dispersive solid phase extraction, and analyzed using GC/MS-SIM. Preliminary data with the sum of parent polycyclic aromatic hydrocarbons (PAHs) and PAH alkyl homologs for all analyses to date revealed that 74% of samples were below detection limits; 23% were between 0.1-0.9 ppb; and 3% were between 1.0 and 48.0 ppb. Based on PAHs measured in Gulf seafood thus far, contaminant levels are remarkably low based on FDA levels of concern, and indicate that edible portions of inshore-sampled seafood species do not have elevated contaminant body burdens. These data do not necessarily reflect hepatic or biliary burdens of contaminants that were not analyzed in this project. To provide perspective and risk communication to coastal communities regarding oil spill contaminants in seafood, risk assessments are being developed that will incorporate (1) contaminant levels in seafood, and (2) potential exposure risk based on the type, quantity and frequency of seafood products consumed in Gulf communities (i.e., humans are considered as part of this transdisciplinary ecosystem health assessment). To accomplish the latter, an in-person food frequency questionnaire (FFQ) was developed and implemented to analyze household seafood consumption patterns and body weights from five Gulf coast communities in Florida and Alabama. Initial FFQ data from over 900 individuals indicate that seafood consumption in Gulf coast communities is higher than national estimates derived from 2003-2010 NHANES studies (from which limits of concern were derived for this oil spill). Upper percentile seafood consumption for Gulf coast survey participants was 231% (adults) and 298% (>21yo) higher for finfish, and 536% (adult) and 984% (>21yo) higher for shellfish (shrimp + crab + oyster), than upper percentile national estimates. Further, seafood consumption patterns varied substantially between communities. Analytical toxicology data, combined with consumption patterns of coastal high-end consumers of Gulf seafood, are being used to refine risk communication and resiliency programs, and develop probabilistic community-based risk assessments.

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SEROPREVALENCE OF WEST NILE VIRUS IN FLORIDA BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) OF THE INDIAN RIVER LAGOON AND PROXIMATE SENTINEL BIRDS FOLLOWING FLORIDA INTRODUCTION IN 2001.

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Bottlenose dolphins (*Tursiops truncatus*) of a lagoon estuary in southeastern coastal Florida underwent health evaluations as part of a multidisciplinary, collaborative, cross-sectional research project in the summers of years 2003-2007. Sampled dolphins were tested for serologic evidence of West Nile Virus (WNV). Dolphins with evidence of WNV infection were compared spatially and temporally with seroprevalence of WNV among proximate, terrestrial sentinel birds by year. WNV-positive dolphins exhibited evidence of viral infection in year 2003 only which correlated with increased sero-positivity among proximate sentinel birds and increased disease rates among humans state and nationwide. All WNV infected dolphins were members of a serially observed group of individuals found to occupy a distinct, southern home range water within the estuary. Among the affected group of animals (n=125) there were 5 (4 males, 1 female) individuals demonstrating serologic evidence of WNV infection. Seroprevalence in the affected animal group was 4.0%. The overall mean age of the sampled population was 11.37 years (SD 5.62) and the mean age among WNV infected individuals 14.00 years (SD 6.35). Further research is needed to evaluate evidence of WNV persistent infection among these dolphins and anthropogenic and environmental relationships to WNV seropositivity.

(All methods used in the dolphin health assessment project for capture and sample collection were approved under National Marine Fisheries Service Scientific Research Permit Numbers 9981678 and 14352-02 issued to Greg Bossart and Florida Atlantic University IACUC Protocol Number A10-13.)

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GLACIALIS): FROM INDIVIDUALS TO POPULATION LEVEL PROJECTIONS

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Marine mammals are faced with increasing challenges from environmental fluctuation, climate change, and disturbance from human activities. Anthropogenic mortalities have been well documented, but it is difficult to assess the sub-lethal impacts of disturbance on fitness of marine wildlife. Monitoring marine mammal health is uniquely challenging, as sightings of individuals are sporadic and data are difficult to obtain. Further, it is difficult to use records of health and disturbance events from single animals to understand how those impacts may affect survival, reproduction and population growth. Here, we used photographic data on body condition, skin condition, presence of cyamids on the blowholes, and the presence of rake marks to estimate health of North Atlantic right whale (*Eubalaena glacialis*) individuals. To this data, we applied a Hierarchical Bayesian Model to assess the underlying continuous health of individuals demographic sub-groups, and the population, from 1980-2008. Modeled health estimates varied by sex, age and reproductive status, with the greatest annual variability in actively reproducing females. Calving females had significantly higher health scores than non-calving females that were available to reproduce in a given year. A steep deterioration in population health from 1998-2000 coincided with a dramatic decline in calving, demonstrating a link between population-wide fitness and reproduction. Modeled health profiles show fluctuations in whale condition caused by natural factors (i.e. environmental conditions, lactation), as well as the effects of anthropogenic factors (e.g. fishing gear entanglement). For marine mammals with extended health data sets, this model offers a promising approach to link changes in health to reproductive success and population vital rates, and to assess the effects of anthropogenic stressors on both individuals and populations.

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A LARGE SCALE CHRONIC FISH KILL IN THE ST. JOHNS RIVER, FLORIDA: A COMPLEX CONSEQUENCE OF CYANOBACTERIA BLOOMS

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In the summer of 2010, a chronic multi-species fish kill, affecting primarily adult red drum (*Sciaenops ocellatus*), was documented for six weeks along 30 miles of the Lower St. Johns River (LSJR), Florida. A co-occurring *Aphanizomenon flos-aquae* cyanobacterial bloom was present in the freshwater reaches prior to the fish kill. The kill was triggered by a significant reverse flow event and sudden influx of high salinity water in late May which contributed to the crash of the bloom further upstream, and brought euryhaline fish into the vicinity of the senescing bloom or its associated by-products downstream. The decomposing bloom led to a cascade of events, including the release of low concentrations of cyanotoxins, bacterial lysis of cyanobacteria cells, high organic loading, and changes in the diversity and dominance of the plankton community to include the potentially toxic cyanobacteria *Microcystis*, *Leptolyngbya*, *Planktolyngbya*, and low concentrations of the harmful raphidophyte *Heterosigma akashiwo*. Dissolved oxygen and other water quality parameters were within normal ranges in the reach of the fish kill, although high ammonia and pH levels were present further upstream. These consequences resulted in complex pathological changes in fish that were not consistent with acute cyanotoxin exposure or with poor water quality, but were attributable to chronic lethal hemolysis.

This long term effect on aquatic organisms has not previously been documented during harmful algal bloom (HAB) events. Potential sources of hemolytic activity included *Heterosigma akashiwo*, *Microcystis* spp., and *Bacillus cereus*, a hemolytic bacterium known to cause animal disease and human food poisoning. Notably, the fish kill was followed by an extensive foam event across miles of the LSJR, and an unexplained bottlenose dolphin (*Tursiops truncatus*) die off that was designated by NOAA as an Unusual Mortality Event. The linkage, if any, between the two mortality events remains unknown. The presence of *A. flos aquae* in the LSJR has the potential for significant environmental repercussions if the causal factors contributing to bloom growth and maintenance are not fully understood and managed. This sequence of events in the LSJR provides a compelling reason to better understand the interplay between bacterial and HAB populations, the function of HABs as pathogen vectors, and the link between estuarine HAB events and changes in coastal ecosystems.

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LONG-TERM DATA PROVIDE INSIGHTS ON THE IMPACT OF HARMFUL ALGAL BLOOMS ON MARINE TURTLES OF THE INDIAN RIVER LAGOON

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From 2011-2013, several harmful algal bloom (HAB) events affected the Indian River Lagoon (IRL), a hyper-diverse estuary in east central Florida that is economically and ecologically important to the region. These HABs led to dramatic reductions seagrass and macroalgae biomass, and unusual mortality events of bottlenose dolphins (*Tursiops truncatus*) and West Indian manatees (*Trichechus manatus*). However, the impacts of the “superbloom” and associated ecosystem changes on marine turtles inhabiting the IRL were unknown. In this study, we used the long-term data on marine turtles collected by the University of Central Florida Marine Turtle Research Group (hereafter UCF) to evaluate the effects of HABs on capture rates and body condition of two species of marine turtle: green turtles (*Chelonia mydas*) and loggerhead (*Caretta caretta*).

UCF has conducted capture sessions on a bi-monthly basis in the IRL since 1982, using large tangle nets (greater than >450m in length) to capture the juvenile green turtles and sub-adult loggerheads that forage and grow in the lagoon. Each turtle that is captured as a part of this study is tagged, measured, biopsied, and weighed using standard techniques. For this study, we limited our scope of study to 2005-2013 in order to provide a more reasonable comparison of capture rates before and during the bloom impacts. We defined the “before bloom” time period as January 2005-March 2011, and “during the bloom” as April 2011-August 2013, following recent literature documenting the impacts of HABs on primary producers in the IRL. We used analysis of variance to evaluate changes in the body condition (mass/carapace length³) and abundance (evaluated using a catch-per-unit-effort metric: the number of captures per net kilometer per hour) of both green turtles and loggerheads among these time periods.

Our results indicate that green turtle body condition and abundance decreased during the bloom, while loggerhead body condition and abundance did not change. We hypothesize that the difference among species is primarily a result of differences in foraging strategies: green turtles are herbivorous, and in the IRL forage almost exclusively on the same macroalgae and seagrasses that were drastically impacted by the blooms. In contrast, loggerheads are benthic carnivores and less likely to be affected, at least immediately, by the changes in ecosystem structure and health brought on by the HABs. These results stress the importance of longitudinal data in evaluating the effects of HABs on ecosystem function, and in particular on long-lived species.

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ARE MOLECULAR ENDPOINTS USEFUL FOR ENVIRONMENTAL MONITORING PROGRAMS?

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Molecular endpoints are measured in both freshwater and marine organisms to assess biological responses to stressors such as aquatic pollutants. While these data provide important information on how a chemical affects an individual (i.e. mechanisms of action), governmental regulators have yet to embrace molecular endpoints for long term monitoring of healthy fish populations. Immediate steps to advance omics technology in risk assessment include baseline collection of omics endpoints across different species and sites to generate a range of what is biologically normal within a given ecosystem. Natural individual variability in the omes is not adequately characterized and is not measured in the field, but it is central to an environmental monitoring program; estimates are needed to determine the critical effect size or a threshold of when action should be taken. Reproductive transcripts, for example are often measured in fish from the environment and data suggest that transcripts related to reproduction show wide variability across individuals, with some transcripts being very stable (estrogen and androgen receptors), while others showing 10 times higher variability (membrane progesterin receptors, reductase enzymes). Sample size estimates needed to capture a two-fold difference among groups with sufficient experimental power (0.8) was >20 samples for ~50% transcripts investigated. Moreover, while many biotic and abiotic variables are recorded in field studies (sex, size, temperature, dissolved oxygen), many variables are simply not measured and it is unknown how the omes are influenced by variables such as photoperiod, fish age, collection methods, and capture stress, among others. Our group is currently conducting studies to determine how breeding strategy is related to transcriptome variability. This presentation introduces these concepts and discusses the steps required before omics approaches can be implemented into a nationally recognized environmental monitoring program. Omics endpoints must develop a level of standardization, consistency, and rigor that will allow interpretation of the relevance of changes across broader scales. A consensus as to the significance of genome-wide molecular responses in an organisms or population health has not yet been determined.

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COST, BENEFIT AND SOLUTIONS: AN OVERVIEW OF PLANNING AND EXECUTING MARINE MAMMAL HEALTH AND ENVIRONMENTAL RISK ASSESSMENTS AND INTERVENTIONS

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Marine mammal health and environmental risk assessment (HERA) programs have been ongoing for more than two decades. Primary goals of multi-disciplinary, comprehensive HERA research platforms are to measure overall health and identify potential health hazards for marine mammal populations. Dolphin health and population status in coastal regions reflect the effects of natural and anthropogenic stressors and serve as a sentinel for ecosystem health. Therefore, defining health status of bottlenose dolphins is important for future management of this species and provides insights into our shared ecosystems.

Marine mammal entanglement in fishing gear is a global problem that impacts more than 260 species including marine mammals, sea turtles and seabirds. More whales, dolphins and porpoises die every year by getting entangled in fishing gear than from any other threat. Effects of fishing gear interactions are among the most pressing issues currently being addressed by marine mammal management agencies in the United States.

In the Southeastern United States (SEUS) region, between (2003-2010) there were a total of (33) marine mammal entanglements involving bottlenose dolphins (*Tursiops truncatus*) that were identified by the National Marine Fisheries Service (NMFS) as having life threatening entanglements. In such cases where life-threatening anthropogenic stressors are identified, efforts to intervene are made by NMFS.

Field operations for both operations are similar. Both HERA and Interventions are logistically complex and high risk. Operations require specialized equipment, resources and teams of 20-60 individuals who possess a broad range of capabilities.

This presentation highlights the planning, execution, cost and benefits associated with these operations, and explores opportunities to teach, train and learn from others in the improving the process.

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A TRANSCRIPTOMIC ANALYSIS OF SKIN FROM THE COMMON BOTTLENOSE DOLPHIN, *TURSIOPS TRUNCATUS*

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The common bottlenose dolphin, *Tursiops truncatus*, is a long-lived apex predator in the inshore waters of the southeast US. As such, their health is endangered by anthropogenic inputs to the marine environment by both direct exposure and food web biomagnification. Remote biopsy sampling of skin and blubber has been an effective method for monitoring of free ranging or difficult to access animals and threatened/endangered species. In cetaceans, remote biopsies are frequently used to assess contaminant levels stored in the lipid-rich blubber layer. However, this does not address the resultant effects of contaminant exposure on marine mammal health. To that end, recent studies have focused on applying genomic or proteomic analyses to remote biopsy samples to more directly describe animal health status via alterations in well-described detoxification pathways or proteins and receptors related to alterations in health endpoints.

A recent study in our laboratory utilized a dolphin-specific microarray to investigate global gene expression in skin from 96 bottlenose dolphin remote biopsies. While the microarray was the best tool available at the time, it contained just over 24,000 probes, of which only approximately 7000 were annotated, and lacked probes for certain genes of interest (cytochrome p450s, aryl hydrocarbon receptor, etc.). Therefore, skin samples from a subset of 65 animals were transcriptionally profiled using RNA-seq, which allows deeper analysis and reveals both novel and low-abundance transcripts. In this study, we are comparing RNA-seq analysis using two parallel approaches, one utilizing a low-coverage dolphin genome, available through ENSEMBL, and the other utilizing a *de novo* transcript assembly generated in Velvet/Oases.

For each of the 65 animals, approximately 30 million 100 base single-end reads were generated from a directional library on an Illumina HiSeq 2000 sequencer. Using the Tuxedo suite of tools in Galaxy, 100% of reads mapped to the ENSEMBL dolphin genome (turTru1 release-78). Sequence annotation from genome mapping is being used for all downstream analyses of this data set. In parallel, sequence data from 12 dolphins, representing all locations, sexes, and seasons in the dataset, were used to create a *de novo* transcriptome in Velvet/Oases. Following transcript length and coverage filtering, the single k-mer assembly resulted in 64,368 transcripts to which 75.6% of raw sequences mapped. Annotation of these sequences is currently underway using BLASTx searches against NCBI's non-redundant protein database. Together, the results from these analyses will provide an in depth characterization of the dolphin skin transcriptome, augmentation of the ENSEMBL dolphin genome annotation, and a comparative analysis with other mammalian skin transcriptomes. The availability of the dolphin skin transcriptome will provide insights into the genomic adaptation to aquatic life and will be a valuable resource to researchers seeking to expand the genomic and proteomic component of marine mammal health assessments.

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THE RISE AND FALL...AND RISE AGAIN OF THE BENTHIC MACROPHYTE COMMUNITIES IN THE INDIAN RIVER LAGOON

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The Indian River Lagoon (IRL) was been hit by two years of consecutive “super blooms.” The first bloom began in March 2011 and consisted of a marine chlorophyte from the Pedinophyceae sp. The chlorophyll *a* concentrations reached 120 µg/L, categorizing it as a “superbloom.” The next bloom began in July 2012 and was composed of a Pelagophyte species, *Aureoumbra*, commonly called brown tide. This time, chlorophyll *a* concentrations reached 200 µg/L and cell counts approached 3 billion cells/L. Since both of these blooms occurred during the peak growing season for seagrasses, there have been large reductions in total seagrass coverage. Average losses lagoonwide exceed 33,000 acres, approximately 42%, but some areas, such as the central IRL, lost as much as 99%.

Seagrasses were not the only macrophyte impacted during the superbloom events. Drift macroalgae (DMA), a predominant benthic community in the IRL, typically contributes more biomass than seagrass and epiphytes combined. DMA actually extends the benthic habitat and vital nutrient cycling functions into deeper water, beyond the seagrass beds. The amount of stored nutrients in DMA may be equivalent to that received annually from external sources. Therefore, it is hypothesized that the unusually high amount of DMA lost and subsequent lack of nutrient uptake helped initiate and prolong the unprecedented phytoplankton (super) blooms.

Following these massive losses, the IRL system is showing signs of recovery. There was a 12% gain in total seagrass acreage between the 2011 and 2013 mapping years. Further gains in 2014 were documented at 52 of the 69 fixed seagrass transects, which either lengthened or remained the same. Even the DMA coverage has started to recover to its pre-bloom biomass in most segments. However, recovery has not been uniform throughout and efforts continue to evaluate spatial variation and drivers of these discrepancies.

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EVALUATING ECOSYSTEM IMPACTS ON THE HEALTH OF INDIAN RIVER LAGOON BOTTLENOSE DOLPHINS DURING THE 2013 UNUSUAL MORTALITY EVENT

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A cascade of large scale ecosystem changes preceded the largest Unusual Mortality Event (UME) to impact Indian River Lagoon (IRL) bottlenose dolphins. Following a catastrophic loss of seagrass in the Indian River Lagoon, significant increases in manatee and pelican mortality occurred. Subsequently, bottlenose dolphin mortalities increased in 2013 the northern IRL and the event was declared an Unusual Mortality Event.

The UME resulted in 78 documented bottlenose dolphin mortalities, the majority of which occurred in the Banana River and the Northern Indian River. Adults represented the majority of the animals, followed by juveniles and calves, while adult females were the most impacted demographic. Emaciation was the only consistent gross finding. Histological results did not indicate toxic or infectious disease. Boat-based surveys were conducted in the UME area during the event to determine the body condition of free-swimming dolphins during that time period. Based on survey work, the percent of emaciated animals declined between Aug – Dec 2013, corresponding with decreased mortality in the area. Furthermore, emaciation was a more common finding in free-swimming females with calves. Stomach content analyses indicated a potential change in prey consumption and changes in foraging habits were further evaluated with stable isotope analyses.

The consistent findings of emaciation with no concurrent health conditions and the corresponding mortality of other species, led to the hypothesis that ecosystem changes contributed to the dolphin UME. Likewise, the increase in female dolphin strandings in this historically male biased population further supports the hypothesis that additional nutritional demands placed on females with dependent calves may have influenced stranding events. Future efforts to enhance ecosystem health of the Indian River Lagoon estuary may help decrease the reoccurrence of large scale multi-species mortality events in the future.

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SUPPLEMENTING HEALTH ASSESSMENTS – OPPORTUNITIES AND POTENTIAL VALUE***Craig A. Pelton¹ and Michael T. Walsh¹***¹Aquatic Animal Health, College of Veterinary Medicine, University of Florida

Current organized, regular dolphin health assessments from each coast provide the best opportunities to collect in depth samples and data from two Florida dolphin populations. Scheduled catches are not the only opportunities available to assess dolphin health. Additional opportunities include disentanglement efforts, stranding events, and observational health assessments. Taking a more complete set of predetermined health assessment samples, under the direction of experienced veterinarians, during disentanglements with stable animals and stranded dolphins provides samples from animals in a different state of health, geographic locations, and time of the year and in between the organized events. These individuals could potentially forewarn of impending environmental or infectious disease issues prior to large scale organized UME responses. Adding health assessment data collection methods, such as on site centrifuges, training for additional testing such as collection of buffy coats and proper storage on dry ice will add these animals to a more complete picture of the challenges faced by individuals and populations prior to increases in population mortality. It will also provide properly banked materials for new diagnostic techniques and allow retrospective evaluations for clarification with the onset of major or chronic population illness or intoxication. These techniques will be further discussed in the Molecular techniques session. Additional documentation of categories such as entanglements with accurate drawings of the conditions related to fishing gear and unique objects may give a better understanding of different presentations and how animals become entangled. Coupling this data with the type of entanglement material and a more robust health assessment, including imaging with a portable radiographic system, creates a more complete picture of morbidity. The development and incorporation of observational health assessments, including activity level, body condition score, respiration rate, and respiration character, to be used by photo identification teams would provide basic visual health information on animals from all populations studied and throughout the year. By keeping individual medical records, to be reviewed periodically by veterinarians, on known photo identification animals we can combine observational health assessments with scheduled and intervention capture assessments and, in the unfortunate times, necropsy findings to develop more complete pictures of the health of the individual animals, their populations, and the ecosystems in which they live.

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APPLYING ECONOMICS TO MARINE MAMMAL CONSERVATION

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Increasingly, debates about conservation issues focus on the economic impacts of conservation actions. Proponents of conservation argue that their actions provide various benefits to the public, including: the continued existence of species, ecosystem services, tourism or recreation benefits, and improved quality of life. In contrast, opponents of conservation actions argue that these actions prevent the creation of jobs and economic growth. In reality, conservation generates both benefits and costs. Understanding how to identify and quantify both the benefits and costs of conservation improves decision-making, and ensures that conservation actions are economically sound. Demonstrating that a conservation program generates net economic benefits to the public is important for building political support for the program.

This presentation will provide an overview of the principals of environmental economics as they relate to marine mammal conservation. Case studies will be used to demonstrate how both the benefits and costs of marine mammal conservation can be quantified. In particular, the appropriate use of the surveys and market data will be addressed. These methodologies may be used in both developed and developing countries to quantify the net benefits of marine mammal conservation.

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DEFINING BASELINE REPRODUCTION: THE BASIS FOR REPRODUCTIVE HEALTH ASSESSMENT PROGRAMS

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The sustainability of any population is dependent upon its reproductive health, yet there are few tests which can be used to determine this parameter in individual free-ranging cetaceans. Longitudinal endocrine monitoring of *ex situ* populations has permitted characterization of a female's reproductive status (e.g. acyclic, cyclic, pregnant, senescent) and of the different phases within a physiological state (e.g. follicular phase, luteal phase, mid, early and late pregnancy) including its perturbations. These data from zoological populations, which contribute to baseline reproductive health databases for each species, are invaluable for conducting reproductive assessments of wild animals on an individual- and population-level. Zoological research efforts are also underway to develop and validate assays for evaluation of pregnancy stage and fetal health in free-ranging females, where only a single or very few biological samples (serum, urine, feces) are available. However, similar patterns of reproductive hormone production during different physiological states can reduce the accuracy of these tests. A notable example is observed with the steroid progesterone, which can exhibit similar concentrations during the luteal phase of non-conceptive cycles as well as during pseudopregnancy and normal pregnancy. To combat this issue, researchers have suggested combining results from multi pregnancy related hormone tests (e.g. relaxin and progesterone) to decrease the risk of identifying non-pregnant animals as pregnant. Further inaccuracies can result when evaluating age-related fertility changes. For example in bottlenose dolphins, acyclicity in females can result from documented physiologic states (season, lactation) or hypothesized influences (nutritional deficiencies, senescence, anthropogenic). In the absence of an obvious cause(s), it is not possible to currently classify an acyclic female bottlenose dolphin as senescent or as one undergoing normal temporary anestrus. Recent work with anti-Müllerian hormone (AMH) in beluga has shown promise as an indicator of reproductive senescence but further research is needed to determine the utility of AMH and other hormones as a predictor of fertility potential. For males, hormonal assessment (e.g. testosterone) can aid in the diagnosis of seasonal, social and maturational changes in fertility. While AMH decreases with age in male beluga and bottlenose dolphins, age related changes in fertility are yet to be correlated or described in these species. If non-voluntary semen collection methods become available for use in free-ranging cetaceans, sperm function tests hold promise as another tool for evaluating reproductive health. A panel of sperm function assays (e.g. sperm DNA integrity, mitochondrial potential, motion parameters) which are currently being validated in *ex situ* populations may one day be useful for detecting both age- and anthropogenic-related changes in male fertility in their free-ranging counterparts.

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IMPAIRED REPRODUCTION IN NORTH ATLANTIC RIGHT WHALES: ASSESSING SUB-LETHAL ANTHROPOGENIC INFLUENCES

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The western North Atlantic right whale (*Eubalaena glacialis*) is one of the most critically endangered whales worldwide, with ca. 522 individuals surviving. Because these whales range along the heavily industrialized coastline of eastern North America from Florida to the Atlantic Maritimes, they are exposed to multiple anthropogenic stressors. As a result, recovery of this population has been severely impacted by mortalities from vessel collisions and fishing gear entanglements. Additionally, 35 years of surveys coupled with photo-identification data have shown that impaired reproduction is also contributing to slow population growth rates. Evidence for this includes reduced and variable calving success over three decades, lengthened inter-calving intervals, a high proportion of non-calving mature females, and signs of compromised health. Hypotheses about factors contributing to impaired reproduction in right whales include; inbreeding depression, nutritional stress, marine biotoxins, pollutants, infectious diseases, and chronic stress from underwater noise and habitat disturbance.

Since 1999, we have applied photographic methods, fecal analyses, and endocrinology assays to study health and reproduction in living right whales. First, using a photographic health assessment method, we found a relationship between poor body condition, skin lesions and reproductive failure in right whales. Second, fecal analyses showed that right whales are exposed annually to marine biotoxins (saxitoxins and domoic acid) through their zooplankton prey. These biotoxins have caused morbidity and mortality in marine mammals, including reproductive failure. Five years of data also showed that 68% of sampled right whales were positive for *Giardia* and 14% for *Cryptosporidium*, which are both important enteric pathogens. Finally, we validated radioimmunoassays to measure fecal metabolites of the major reproductive hormones (estrogens, androgens, progestins), which are reliable predictors of gender, pregnancy and lactation in females and sexual maturity (in males). We also validated assays for adrenal glucocorticoids, which reflect adrenal activation, and relative stress levels. Elevated glucocorticoids occurred in whales entangled in fishing gear, and in response to high levels of ambient underwater noise from ship traffic. New approaches to study right whale physiology include analysis of fecal thyroid hormone, and assays of steroid hormones in respiratory vapor.

All of these studies represent the foundation of individual-based profiles of health and reproductive status in right whales, and can be used to determine factors that are negatively impacting the health of the population.

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PATHOLOGIC FINDINGS FROM THE 2013 MID-ATLANTIC DOLPHIN MORBILLIVIRUS UNUSUAL MORTALITY EVENT

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Outbreaks of morbillivirus have caused dies-offs of pinnipeds and cetaceans in the US and Europe including the Mid-Atlantic (1987-88) and Gulf of Mexico (1992, 1994). On August 8, 2013, the National Marine Fisheries Service declared an Unusual Mortality Event (UME) for increased bottlenose dolphins (*Tursiops truncatus*) from NY to VA which spread southward from NC to FL. The investigation has focused on response mobilization, morphometrics, epidemiologic data, virology, parasitology, microbiology, histopathology, serology, and molecular diagnostics for surveillance, lesion identification, and research. Histopathologic evaluation has been completed on ~120 bottlenose dolphins and 18 non-bottlenose dolphins, whales, and pinnipeds. Gross necropsy findings included ulcerative stomatitis/glossitis/dermatitis, fibrinous osteoarthritis, and pulmonary congestion. Histopathologic findings included morbillivirus-associated lesions including bronchointerstitial pneumonia and/or pulmonary fibrosis, lymphoid depletion, syncytial cells, and less commonly intranuclear and intracytoplasmic viral inclusions. Secondary bacterial, fungal, and protozoal infections were observed. Confirmatory testing of bottlenose dolphins (n = 292) included PCR, sequencing, and immunohistochemistry. Based on the case definition, 93% (272/292) of bottlenose dolphins were positive. In other species, 9% (10/115) were positive (1 confirmed striped dolphin (*Stenella attenuata*; 9 suspect). The investigation continues to better understand the impact on the bottlenose dolphins populations involved.

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INFECTIOUS DISEASES IN THE FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*)

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The main causes of mortality in the Florida manatee (*Trichechus manatus latirostris*) include brevetoxicosis, cold stress, human interactions (trauma), orphaned calves, gastrointestinal conditions, and various primary or secondary infections. In a small population such as the Florida manatee, this could be of significant importance if sensitive age classes such as calves or reproductively active females are affected. Infectious diseases documented in manatees include viral, bacterial, and parasitic etiologies, all of which are mainly associated with individual animals or less commonly with geographical focal groups. Although exposure to infectious agents frequently occurs in the wild, a number of factors may be necessary to enable manifestation of clinical disease. Continued monitoring will provide the basis of understanding the significance of various stressors on the manatee population in Florida.

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COMPARATIVE STUDY OF THE INNATE IMMUNITY AND CLINICAL PATHOLOGY AMONG TWO POPULATIONS OF FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*) AFTER THE 2013 UNUSUAL MORTALITY

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The Florida manatee is endangered subspecies of the West Indian manatee and faces numerous environmental threats including anthropogenic chemical pollutants, watercraft collisions, and loss of warm water habitat. Despite consistent monitoring through manatee carcass recovery and rescue, certain aspects of manatee health remain unknown, such as undetermined cause for a large number of mortalities each year, and unknown effects of repeat exposure to red tide. To better understand manatee health, conservation agencies examined free-ranging manatees captured at two different habitats, Crystal River (CR, Gulf coast) and Brevard County (BC, Atlantic coast). Eighteen hematological and 20 biochemical analytes were analyzed in manatees from CR (n=30) and BC (n=12). Although from a population standpoint these analytes were within normal limits for wild manatees, some significant differences were observed. Total solids, total proteins, total bilirubin, glucose, triglyceride, magnesium, and chloride were significantly higher in BC manatees, compared to CR. In contrast, calcium, creatinine, and blood urea nitrogen were significantly higher in CR manatees. Phagocytosis and respiratory burst, two important innate immune functions, were measured. Neutrophil and monocyte phagocytosis were not significantly different between sites ($p=0.11$ and $p=0.27$). Both neutrophil and monocyte respiratory burst were significantly higher in manatees from BC compared to CR ($p=2.10^{-7}$ and $p=0.01$ resp.). Observed differences in clinical pathology parameters and innate immune functions could be related to different environmental conditions and/or pressures between the two sites. Further analysis of other biomarkers such as cytokines as well as contaminant levels can provide more information on possible causes. This research will help to assess manatee health and integrity of immune defense for future comparison and correlation with injury and illness contributing to the development and application of future tools in caring for and treating compromised animals

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A VIROMIC STUDY OF SERUM COLLECTED FROM WILD *TRUSIOPS TRUNCATUS* (BOTTLENOSE DOLPHINS)

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Viral particles constitute 10^6 - 10^9 /mL of oceanic waters. Viral infections in marine mammals are responsible for mass stranding events, unusual mortality events, chronic infection, clinically expressed disease, and inapparent/sustained infections. Virology studies on *Tursiops truncatus* (bottlenose dolphin) are of particular importance as the bottlenose dolphin is a sentinel species. Specific primer approaches have identified viral particles representative of 8 viral families in the bottlenose dolphin. We have implemented a random primer approach, followed by full genomic sequencing of purified viral RNA and DNA from bottlenose dolphin samples. The purpose of this study is to identify and characterize viromes of bottlenose dolphins.

RNA and DNA has been purified and confirmed to be viral nucleic acid via 16sPCR. Viral nucleic acid was randomly amplified via a multiple displacement amplification procedure and sequencing was accomplished via the MiSeq sequencing platform. In total 8 families, 13 genera and a total of 68 viral species are reported in this study as being recovered from bottlenose dolphin serum. The specific primer approach falls short in characterizing the viral ecology of a sample. A better understanding of viruses capable of infecting bottlenose dolphins is important in disease diagnosis, etiology, treatment, and infectivity and evolutionary relationships amongst viruses. As sentinel species, studies involving virus exposure and the health of the bottlenose dolphin are applicable to both ocean and human health.

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MERCURY CONCENTRATIONS IN ATLANTIC BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) INHABITING THE INDIAN RIVER LAGOON, FLORIDA AND LOCAL HUMAN RESIDENTS; PATTERNS OF DISTRIBUTION

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Bottlenose dolphins (*Tursiops truncatus*) inhabiting the Indian River Lagoon (IRL) have total mercury (THg) concentrations in blood and skin that are among the highest reported for the species. As an apex predator, dolphins serve as indicators of THg contamination in the environment. Due to the high degree of site fidelity of the IRL population, concentrations observed in these dolphins may reflect regional differences in THg exposure. These findings also have implications for public health in the same geographical areas since mercury is a well-known neurotoxicant, especially for the fetus.

Analysis of total mercury (THg) concentrations in blood collected between 2003 and 2012 showed a significant linear decrease over time ($p=0.04$). Significant differences in the spatial distribution of THg in resident IRL dolphins were also observed with a general gradient in concentration from north to south. Strong social affiliation and clustering of individuals within the highest category of THg concentration (990.5 $\mu\text{g/L}$ wet weight) suggested shared exposures of dolphins inhabiting specific areas of the estuary. Evaluation of local biogeochemistry and accumulation of mercury in prey species is needed to better understand factors influencing the distribution of Hg in the apex predator. Analyses of temporal and spatial patterns of exposure to THg in this sentinel species have implications for both ecosystem and public health in the region.

Therefore, we also conducted a cross sectional study among human residents of the counties bordering the IRL to test the hypothesis that consumption of local seafood was associated with hair THg concentration. The total mean hair mercury concentration for 135 residents was $1.53 + 1.89$ ppm, well above the U.S. EPA's equivalent reference dose of 1.0 ppm. Hair mercury concentration was significantly higher among those who consumed seafood \geq once/day and among individuals who obtained all or most of their seafood from recreational sources. This study is one of the first of its kind to apply the sentinel concept to a contiguous human population by providing a quantitative comparison of a shared environmental exposure and provides a validated model of the concept currently known as "One Health".

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IMMUNE ASSESSMENT IN MANATEES WITH COLD STRESS SYNDROME

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Each year during the winter months manatees can be exposed to water temperatures at or below 68°F, which in turn can cause a life threatening syndrome known as cold stress. Manatees experiencing cold stress syndrome are emaciated, have skin lesions and are susceptible to infection. In addition, cold stress syndrome can have long term pathological effects in the rehabilitated animals such as, but not limited to, decrease in immune white blood cells and increase heart degeneration. In the past several years the Environment Forensics Program at Mote Marine Laboratory has been monitoring cold stress manatees and progressing in the development of a multi-plex bioassay to measure multiple biomarkers potentially related to cold stress syndrome. Biomarkers are measurable substances (i.e. cytokines, reproductive hormones) in an organism that can be related to disease, infection and environmental exposure. Cell culture analysis of manatee PBMC was performed to validate our finding of IL-1ra as a potential biomarker for cold stress. RNA was extracted from the cells for RT-PCR analysis of gene expression. Initial results confirm antibody cross-reactivity of IL-1ra, GM-CSF, IL-4 and IL-10, thereby validating the identity of these particular manatee cytokines.

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DISSEMINATED TOXOPLASMOSIS AND SEROLOGIC SURVEY FOR TOXOPLASMA GONDII IN THE WILD FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*)

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Marine mammals are important indicators for ecosystem health and serve as sentinel species for infectious and zoonotic agents. Cerebral protozoal cysts and intrahistiocytic tachyzoites were found in the caudal mesenteric lymph node of one Florida manatee (*Trichechus manatus latirostris*), and was confirmed as *T. gondii* by immunohistochemistry and sequencing of the nuclear ribosomal internal transcribed spacer region. This raised the concern of a lack of baseline information on this pathogen in these herbivores and prompted a study into the seroprevalence of *T. gondii*. Serum was collected during routine health assessments of 44 apparently healthy manatees from 2011-2014 in two different winter habitats, Crystal River (n=26) and Brevard County (n=18). Serum was screened for detection of *T. gondii* IgG antibodies via the Modified Agglutination Test (MAT). Two animals from Crystal River from years 2011 and 2012 (7.7%) and one animal from Brevard County from 2011 (5.6%) tested positive for *T. gondii* antibodies. This study was conducted to understand the role of manatees as a sentinel species for ocean health, as well as further investigate *Toxoplasma* as a potential pathogen in the Florida marine ecosystem.

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THE USE OF HEMATOLOGY AND CHEMISTRY ANALYSES FOR MONITORING HEALTH IN THE FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*) AND ITS CONTRIBUTION TO UNDERSTANDING BIOLOGIC POPULATION DATA

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The endangered Florida manatee (*Trichechus manatus latirostris*) population is under significant pressure from natural disease (cold stress, neonatal mortality, biotoxins) and anthropogenic factors. Collisions with watercraft and loss of warm-water winter habitat have been identified as the greatest threats. For the purpose of better understanding the health of the Florida manatee population and relating this information to biologic data, partners from various conservation groups have performed exams and sample collections on wild manatees during organized and opportunistic health assessments over the past two decades. The physical exam includes body-condition assessment and thorough evaluation of external lesions (e.g., cold-related lesions, boat propeller wounds). In addition to morphometric, life stage, season, and geographic-location data, blood is routinely collected for hematology, chemistry, genetic, toxicologic, and molecular analyses. Additional samples include urine, feces, and skin for cytology, microbiology, biochemistry, genetic, and molecular studies.

Between December 2008 and January 2015, 263 manatees at various life stages were captured for health assessments in two different winter habitats: one a complex of artesian springs on Florida's west central coast (Crystal River), and the other a region along Florida's east central coast where manatees rely on industrial warm-water effluents and a network of secondary warm-water sites (Brevard County). Reference intervals for hematology and chemistry analytes have been previously established, and a large dataset has been assembled. For example, in 2013/2014 during an unusual mortality event (UME) in Brevard County, apparently healthy manatees from the affected area presented with subtle electrolyte abnormalities. These findings in context of clinical and location data were supportive in the ongoing investigation of the underlying cause of the UME.

The available manatee hematology and chemistry databases provide a valuable resource for monitoring spatial and temporal trends in wild manatees and may help in the investigation of various stressors on the population. In addition, detecting blood abnormalities in individual stranded manatees is helpful for diagnosis and treatment at rehabilitation facilities.

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UNDERSTANDING THE USE AND LIMITATIONS OF CLINICOPATHOLOGIC ENDPOINTS IN TOXICOLOGIC STUDIES OF AQUATIC ANIMALS

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Blood collection provides a non-invasive method with rapid access to a body fluid of any species with various options for different types of analysis, one of them including hematology and chemistry analysis. The latter is readily available and affordable, and offers a valuable resource in answering specific questions that can be asked when choosing appropriate laboratory tests. The hematologic profile includes a number of tests that characterize the quantity and morphology of cellular components in the blood and few analytes in the plasma, and it can detect many abnormalities and disease conditions. Chemistry analysis of serum or plasma, the choice of which is species-dependent, provides information on various body systems. When interpreting laboratory data, physiologic and pathologic conditions or disorders of a certain species, and analytical factors need to be considered. Across the species that live in the aquatic ecosystem, mammals and non-mammalian vertebrates are notably different in various aspects of laboratory testing. Some the major differences include choice of anticoagulant, automated hematology analysis in mammals versus manual methods in non-mammalian vertebrates, unique blood cell types and blood cell responses in disease, the lack or presence of tissue specificity of various chemistry analytes, and extrinsic and intrinsic factors related to the aquatic environment or that are species-specific. Additional factors are essential to consider in wild animals, as biologic and geographic data can have major effects on laboratory results. A number of reported environmental or experimental toxicologic studies in aquatic animals use blood work evaluation as an adjunct tool and apply various methods of statistical data analysis and interpretation to describe and interpret laboratory abnormalities in individuals and/or a group of animals. The use of various interpretative approaches of laboratory and toxicologic data in aquatic animals can be very helpful in gaining the most useful diagnostic information.

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TRACE METALS IN TISSUES OF FREE-RANGING FLORIDA MANATEES (*TRICHECHUS MANATUS LATIROSTRIS*) AND THEIR ENVIRONMENT: A THIRTY YEAR PERSPECTIVE

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Trace metals in the tissues and organs of the Florida manatee were first reported in the 1980s. This study examined trace metal burdens in organs of 13 manatee carcasses from 2008-2011 and compared levels to those of manatee carcasses from 1978-1979. Forage vegetation from Kings Bay, Crystal River, Florida, a potential source of metal accumulation in the Florida manatee, also was analyzed and metal concentrations were compared to metal concentrations from 1979-1980. Cu, Zn, As, Se and Pb were analyzed in various tissues with highest metal levels found in the liver and kidney, similar to other mammalian species. However, high levels of zinc in the liver were found to be unique to sirenians (32.4-578.1ppm). Liver and kidney levels of lead were significantly higher in 1978-1979 (1 ± 0.08 ppm, 0.7 ± 0.03 ppm, respectively) than 2008-2011 (0.05 ± 0.02 ppm, 0.02 ± 0.01 ppm, respectively). Manatee diet may be the primary source of metal exposure, as displayed in other marine mammal species. In this study, metal levels in freshwater vegetation from Kings Bay varied with location, plant species, season and element. However, levels were higher in one of the most favorable species consumed by manatees, *Vallisneria americana*. Moreover, copper concentrations in *Ceratophyllum demersum* were significantly higher in 1979-1980 (13.5 ppm) compared to levels in 2008-2011 (6.9 ppm). A ban on the use of copper-based herbicides in Crystal River since the 1980s may be reflected in this finding. Continuous monitoring of metals in the tissues of this endangered species and the surrounding environment is imperative for the health and survival of the ecosystem.

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THE ROLE OF NON PROFIT ORGANIZATIONS IN AQUATIC ECOSYSTEM HEALTH

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The role of Non Profit Organizations (NGO), as it pertains to aquatic ecosystem health, is as diverse as there are problems to be solved and needs to be met. It can be as simple as providing vital funding to implement a meticulously planned series of actions to preserve and sustain aquatic health or as complex as compelling those charged with guarding the ecosystem's health to take the necessary steps to fulfill their legal and ethical responsibilities in the face of inaction or incompetence.

Although many NGO's have scientists, economists, lawyers and political science experts working within their organizations, they must work with a host of other professionals and lay persons alike, towards achieving consensus within a political environment filled with formidable special interest influence - often with competing ideologies.

It is my objective to share examples of both past failures and successes to highlight the essential role of NGO's in achieving sustainable solutions to many of the present and future challenges requiring action in order to ensure healthy aquatic ecosystems going forward.

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FLORIDA DEPT. OF HEALTH WATER QUALITY MONITORING

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The FDOH conducts, and has funded, ambient water quality monitoring programs to protect human public health from recreating in potentially pathogen contaminated surface water bodies. This paper reviews of summary of those program findings and procedures. A US EPA grant for Healthy Marine Beaches monitoring is presented. The agency participates in water quality monitoring and planning with other state and local entities, and these are summarized.

Environmental factors are suspected but not proven to play a role in prevalence of some pathogens that can be shared between marine mammals and humans. The FDOH has been consulted related to the handling of marine mammals infected with two such agents, *Brucella ceti* and methicillin-resistant *Staphylococcus aureus* (MRSA). Monitoring for these types of pathogens in marine mammals, as well as tracking of key environmental conditions should be continued or expanded to more fully understand how environmental factors impact the health of the inhabitants of marine environments.

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FATAL SYSTEMIC SALMONELLOSIS IN AN ADULT FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*)

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An adult Florida manatee (*Trichechus manatus latirostris*) stranded dead in Hobe Sound on the Atlantic Coast of Florida. Necropsy and histopathologic findings included emaciation, lymphadenitis, acute necrotizing enteritis and splenitis, pyogranulomatous nephritis, bacterial alveolitis, septic arthritis of the elbow joints, and discospondylitis with secondary scoliosis. There was a draining tract from the lateral aspect of the pectoral flipper to the infected right elbow joint. Culture of the affected kidneys and lymph nodes grew a *Salmonella sp.*, which was consistent with the coccobacilli observed on histopathology and cytology. Due to the chronic nature of the skeletal lesions in this emaciated manatee, the infection presumably had been ongoing for months before leading to compromise and death. Considerations for possible sources of the systemic infection include entry from an external wound, an ascending urinary tract infection, or through bacterial translocation from the gastrointestinal tract. To our knowledge, this is the first documentation of salmonellosis associated with mortality in a Florida manatee. In addition to the importance from a marine mammal infectious disease standpoint, this *Salmonella sp.* case demonstrates the importance of health safety precautions by members of the stranding response network.

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SUBLETHAL RED TIDE TOXIN EXPOSURE IN FREE-RANGING MANATEES (*TRICHECHUS MANATUS*) AFFECTS THE IMMUNE SYSTEM THROUGH REDUCED LYMPHOCYTE PROLIFERATION RESPONSES, INFLAMMATION, AND OXIDATIVE STRESS

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The health of many Florida manatees (*Trichechus manatus latirostris*) is adversely affected by exposure to blooms of the toxic dinoflagellate, *Karenia brevis*. *K. brevis* blooms are common in manatee habitats off Florida's southwestern coast and produce a group of cyclic polyether toxins collectively referred to as red tide toxins, or brevetoxins. Although a large number of manatees exposed to significant levels of red tide toxins die, several manatees are rescued from sublethal exposure and are successfully treated and returned to the wild. Sublethal brevetoxin exposure may potentially impact the manatee immune system. Lymphocyte proliferative responses and a suite of immune function parameters in the plasma were used to evaluate effects of brevetoxin exposure on health of manatees rescued from natural exposure to red tide toxins in their habitat. Blood samples were collected from rescued manatees at Lowry Park Zoo in Tampa, FL and from healthy, unexposed manatees in Crystal River, FL. Peripheral blood leukocytes (PBL) isolated from whole blood were stimulated with T-cell mitogens, ConA and PHA. A suite of plasma parameters, including plasma protein electrophoresis profiles, lysozyme activity, superoxide dismutase (SOD) activity, and reactive oxygen/nitrogen (ROS/RNS) species, was also used to assess manatee health. Significant decreases ($P < 0.05$) in lymphocyte proliferation were observed in ConA and PHA stimulated lymphocytes from rescued animals compared to non-exposed animals. Significant correlations were observed between oxidative stress markers (SOD, ROS/RNS) and plasma brevetoxin concentrations. Sublethal exposure to brevetoxins in the wild impacts some immune function components, and thus, overall health, in the Florida manatee.

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THE ROLE OF REHABILITATION FACILITIES IN SEA TURTLE HEALTH ASSESSMENTS

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Florida is home to 5 of the 7 species of sea turtles, *Caretta*, *Chelonia mydas*, *Lepidochelys kempii*, *Eretmochelys imbricata*, *Dermochelys coriacea* that nest on the surrounding beaches. Since they are all either threatened or endangered they are the subject of intense efforts to mitigate mortality factors through preservation efforts, research, rescue and rehabilitation, and examination of animals found dead or that die in facilities. There are currently 16 facilities that are involved in rescue and rehabilitation including small privately funded groups to larger facilities such as Sea World and Disney. The goal for these facilities is to return animals back to the wild after rehabilitation. The facilities are involved in clinical research toward the development of improved care techniques as well as investigations into mortality factors responsible for animal presentation which include numerous disease and environmental factors both natural and related to human involvement. Wild health assessments include open water work with 13 major projects ongoing with multiagency teams including FWC, NMFS, FWS, USGS, IHA, Fla Co-op, SC DNR, NWR, East Coast Biologist as well as university programs such as UF's Archie Carr center for sea turtle research and UCF's Indian River Lagoon and Trident submarine basin projects. While regular wild health assessment play a central role in sea turtle health assessments, rehabilitation facilities see turtles year round and are sometimes on the leading edge of disease indicators which may trickle into facilities over time. The emphasis on health support can influence disease testing and natural illness investigations. Individual health status recovery efforts may prioritize additional testing and interest on group related illness. Newer molecular techniques that are available as a limited research platform have additional sampling and storage requirements including the need for rapidly frozen materials (plasma, serum) at -80°C, buffy coats that are separated and frozen quickly and storage of samples in preservation media such as RNA later. While additional effort is needed for these samples they can be used for numerous tests including proteomics, metabolomics, transcriptome, and metagenomics. They are also then stored for future comparison of changes in the case of species mortality events or unknown illness investigations. Rescue and rehabilitation facilities, in water research groups and universities should further coordinate on long term research goals for future testing to take advantage of opportunities that may reflect once in a lifetime events and establish a backup of a limited number of samples for archiving for the potential wide adoption of these or other technologies for future use.

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CHARACTERIZATION OF A NOVEL PAPILOMAVIRUS OF FLORIDA MANATEE, *TRICHECHUS MANATUS PAPILOMAVIRUS 2*

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Manatees represent the only extant North American Afrotheria, an early diverging superorder of placental mammals. Knowledge of the endemic viruses of manatees has been limited to one papillomavirus and one herpesvirus. Skin samples from a Florida manatee (*Trichechus manatus latirostris*) in rehabilitation were tested for papillomaviruses as part of a health evaluation. Degenerate primers were used to amplify segments of the E1 gene of papillomaviruses, which was then sequenced. A novel papillomavirus was identified. To obtain the complete genomic sequence, rolling circle amplification was performed. Open reading frame (ORF) analysis of the *Trichechus manatus* Papillomavirus 2 (TmPV2) genome revealed the presence of eight ORFs encoding five early (E) proteins (E6, E7, E1, E2 and E4) and three late (L) proteins (L2, unknown, and L1), organized in this order. Bayesian and Maximum Likelihood phylogenetic analysis found that this virus clustered significantly with TmPV1, but not with other papillomaviruses of non-Boreoeutherian hosts, arguing against long-term codivergence of papillomaviruses and their hosts. Given the precedent of diverse papillomaviruses found in other better investigated hosts, it is probable that significant diversity of manatee papillomaviruses remains to be characterized. The clinical significance of this virus remains to be determined, but different human papillomaviruses differ in clinical significance, and the presence of multiple papillomaviruses has significant implications for the specificity of serodiagnostics and other testing in manatees.

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APPLYING DOLPHIN POPULATION HEALTH TO CONSERVATION

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Long-term research in Sarasota Bay, Florida, has established a “natural laboratory” situation for learning about the health of bottlenose dolphins in the wild. Research conducted since 1970 on individually identifiable dolphins has found multi-generational, multi-decadal residency by a community currently consisting of about 160 dolphins, spanning up to five concurrent generations, and including individuals up to 65 years of age. Data on Sarasota Bay dolphins are obtained from regular systematic photographic identification studies, behavioral observations, and brief capture-release for tagging, life history studies, and/or health assessments.

Tagging research in the 1970’s defined the local residency patterns; photo-ID since the late 1970s has confirmed the long-term residency and provides information on presence, reproductive patterns, condition, habitat use, social interactions, population dynamics, and behavior. Periodic capture-release research beginning in 1984 has provided data on ages, reproductive hormones, genetics, morphometrics, and body condition, ultrasound has provided measures of blubber thickness since 1986, and systematic veterinary exams including blood sampling for chemistry, hematology, endocrinology, and serology have been performed since 1988. Since 1985, Mote Marine Laboratory’s Stranding Investigation Program (SIP) has recovered and examined carcasses of resident dolphins, providing opportunities to better understand natural and anthropogenic threats to local dolphins observed frequently, and occasionally examined medically, and track the course of health and other issues through the residents’ lives.

The Sarasota Dolphin Research Program works closely with colleagues from SIP and around the world to better understand the lives of these animals, from birth to death. The resulting, integrated data from long-term observations, health assessments, and stranding response have established the Sarasota Bay resident dolphins as a reference population, serving as a model for NOAA and others for establishing similar programs at other sites, providing data for comparison to other sites to identify the potential impacts of pollution such as PCBs and oil spills, biotoxins, and other causes of unusual mortality events. The combined research approaches lead to a better understanding of the relationships between ecology and health, and adverse human interactions. The Sarasota Bay dolphin baseline data also facilitate evaluation and interpretation of the health and body condition of dolphins managed under human care. The Sarasota Bay natural laboratory provides opportunities to test new diagnostic and research techniques (e.g., body condition from overhead UAV images, breath analysis, chest X-rays), ground-truth them against other methods, and train researchers from around the world, thereby building conservation capacity.

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REPRODUCTIVE SUCCESS OF BOTTLENOSE DOLPHINS

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Long-term research in Sarasota Bay, Florida, has provided opportunities for measuring reproductive success for a population of bottlenose dolphins. Research conducted since 1970 has found multi-generational, multi-decadal residency by a community of bottlenose dolphins, spanning up to five concurrent generations and including individuals up to 65 years of age. Data on Sarasota Bay dolphins were obtained from behavioral observations, photographic identification studies, and brief capture-release for tagging, life history studies, and/or health assessments. Health assessments include diagnostic ultrasound examinations and measurements of reproductive hormones. The primary dataset used to establish reproductive parameters reported here for Sarasota Bay dolphins included 128 identifiable mothers observed over as many as 39 years, and 382 of their calves. Reproductive histories since the presumed first calf have been compiled for 44 of these mothers.

Bottlenose dolphins in Sarasota Bay are capable of producing many calves over a long reproductive lifespan. Calves have been born to females as young as six years of age and as old as 48 years, yielding a potential 43 year reproductive lifespan before reaching reproductive senescence. Individuals have been observed with as many as 10 different calves over the course of their life, without having yet reached the average age of reproductive senescence. Fetal survival, from late second term to being observed swimming alongside mother, is 83%. Calf survival varies with mother's age, experience, and mother's tissue concentrations of environmental contaminants. First time mothers are typically less successful with their calves than are more experienced mothers. About 62% of first-born calves survive their first year of life as compared to more than 70% of later calves. Successful mothers typically rear their calves for 3-6 years. The primary threats to calf survival include predation, disease, biotoxins, environmental contaminants, and commercial and recreational fishing gear.

Male reproductive success, as measured through genetic paternity testing, appears to be related to social patterns. Many males form strong pair bonds after they reach sexual maturity, and these alliances may remain intact for years to decades. Males in alliances have sired disproportionately higher numbers of calves as compared to unpaired males.

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INVESTIGATING THE POSSIBILITY THAT POLLUTION DUE TO OIL AND GAS ACTIVITIES MAY BE AFFECTING RECOVERY OF COOK INLET BELUGA WHALES (*DELPHINAPTERUS LEUCAS*)

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Despite protective legislation and certain regulations of human activities including subsistence hunting, the population of belugas occupying Cook Inlet has not shown signs of recovery for more than a decade. A Conservation Plan prepared by the National Marine Fisheries Service identified potential threats that might impede recovery. Our study addressed one concern identified in the Plan: pollution associated with oil and gas development and other activities that could introduce polycyclic aromatic hydrocarbons (PAHs) into the habitat and wildlife of Cook Inlet. Although levels and effects of PAHs have received little attention among marine mammal scientists and managers, PAHs represent the most toxic components of oil, and include 16 compounds considered priority pollutants by the World Health Organization and the US Environmental Protection Agency. One PAH (benzo-a-pyrene) has been identified as the cause of high numbers of cancers in belugas from the St. Lawrence Seaway, and PAHs have numerous known effects besides carcinogenesis in mammals; these include effects on reproduction and survival of offspring. Our study assessed PAH levels in sediments and beluga prey from four locations where belugas are regularly observed in Cook Inlet; we also assessed PAH levels in archived liver and blubber samples from deceased Cook Inlet belugas. Highest PAH levels in sediments were found in Eagle Bay. Although naphthalenes, anthracenes, and phenanthrenes were the most ubiquitous/abundant classes of PAHs found, benzo-a-pyrene was also detected in all sediment samples. The data suggested inputs from both combustion and fresh oil. Total PAH levels were moderate, relative to those found in other locations known to have environmental problems with PAH contamination. The same general patterns occurred in the salmon, hooligan and saffron cod but the fish contained slightly higher amounts of pyrene and fluorene constituents than sediments. The highest PAH values were in hooligan taken from the Little Susitna River. Some king salmon from Ship Creek contained notable levels of total PAHs in their meat; roe from some sockeye salmon was also notably high in total PAHs. The Cook Inlet belugas appear to be bioaccumulating PAHs from the environment and prey. These animals have much higher PAH levels than do subsistence-harvested belugas from MacKenzie River delta. Highest PAH levels in Cook Inlet beluga livers were found in 3 adult males and a female fetus; the highest levels in blubber were from adult females and fetuses. Our study confirmed that concerns about PAH levels and effects on Cook Inlet beluga reproductive success and recovery are justifiable and warrant further study and, likely, mitigation.

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THE ROLE OF GOVERNMENT IN AQUATIC ECOSYSTEM HEALTH

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Government agencies are empowered by citizens to protect unalienable rights and maintain social standards that are beneficial to society as a whole. In the context of aquatic ecosystems this takes many forms like the right to clean water, healthy recreation, and abundant fish and wildlife. Often these rights and social standards come into conflict with private property rights and the rights of individuals and corporations. Florida's history of conserving aquatic ecosystems provides many case studies of successes and failures in conserving aquatic ecosystem health. This presentation will review some of the most illustrative case studies and discuss the roles that different levels of government (federal, state, and local) had in each scenario. The presentation will also discuss how to effectively deliver ecosystem health in a democratic society.

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PATTERNS IN SEA TURTLE STRANDINGS IN PINELLAS COUNTY IN THE WINTER OF 2015

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Stranding probability can be highly variable amongst the green sea turtle, *Chelonia mydas*, population during the winter months throughout Florida. Winter events are normally associated with marine cold snaps and record low temperatures, where the water temps drop to 50°F. These events tend to lead to cold stun sea turtles. In 2015 between the months of January and February a total of 26 live stranding and 38 dead stranding were reported by Clearwater Marine Aquarium in the Pinellas county and surrounding areas. This was an increase in frequency considering the normal weather conditions that would classify as a cold stun event. Cold stun events are classified by a body temperature at or below 55°F which causes a loss of normal systematic body functions in reptiles. We examined our stranding data and found key trends: 87.8% of the stranding's were green sea turtles, 71.2 % of these sea turtles had fibropapillomatosis (FP) with an average body score of 4. Of the green sea turtles that made it to rehabilitation the average body temperature registered at 64.1°F. WBC counts as high as 58,000 were documented. Looking at the data from this event it could be better cataloged as a die off verse cold stun event. Our findings suggest that the offshore ecosystem where these sea turtles live may be declining causing an increase in stranding's and mortality.

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CO-INFECTION WITH CALIFORNIA SEA LION ADENOVIRUS 1 AND A NOVEL POLYOMAVIRUS IN A HAWAIIAN MONK SEAL (*NEOMONACHUS SCHAUINSLANDI*)

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The Hawaiian monk seal (HMS, *Neomonachus schauinslandi*) is a critically endangered species with less than 1,200 individuals left. Here we present a clinical case of a 26 year old male Hawaiian Monk seal with history of poor appetite followed by the development of renal and heart disease, but no clinical evidence of hepatic disease. Histologic examination found eosinophilic intranuclear inclusions in the liver, compatible with a herpesvirus, adenovirus and/or polyomavirus. We used consensus nested PCR protocols to test for these viruses. Icosahedral virions of 70-80 nm, compatible with adenoviruses, were seen using electron microscopy. Cell culture cytopathic effects were compatible with an adenoviral infection. Finally, the sample was positive for adenovirus and polyomavirus via PCR/sequencing. The adenoviral polymerase sequence obtained was 100% homologous to California sea lion adenovirus-1 (CSLAdV-1). CSLAdV-1 is associated with viral hepatitis in California sea lions; there have been recent reports of fulminant hepatitis in other species of otariids in an aquarium in Japan (*Otaria flavescens* and *Arctocephalus pusillus*) and sequence has been submitted in GenBank as *Otaria flavescens* adenovirus-1 in Spain. This is the first report of CSLAdV-1 infection in a phocid, and suggests that this virus may be a concern in diverse pinniped collections. The polyomavirus is novel and is the first polyomavirus found in Hawaiian monk seals. This new virus is 83% homologous to California sea lion polyomavirus-1. This is the first report of viral co-infection in a Hawaiian monk seal. The clinical significance of both viruses in the overall clinical case remains unclear.

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A NOVEL GAMMAHERPESVIRUS DISCOVERED IN NORTHERN FUR SEALS (*CALLORHINUS URSINUS*) RELATED TO THE CARCINOMA-ASSOCIATED OTARINE HV1, PHYLOGENETIC ANALYSIS, AND QUANTITATIVE PCR SURVEILLANCE

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Herpesviruses are enveloped large double stranded DNA viruses with high host fidelity. In marine mammals, at least 19 herpesviruses have been characterized with partial polymerase sequences. Three have been reported from otariids; Otarine Herpesvirus 1 (OtHV1) is strongly associated with urogenital carcinoma in California sea lions (CSL, *Zalophus californianus*), a major cause of mortality in some populations. OtHV2 was identified from an ocular swab of a CSL with conjunctivitis, and OtHV3 was found in 30.7% of CSL samples surveyed.

In this work we report a new Otarine herpesvirus found in Northern Fur seals (NFS, *Callorhinus ursinus*), here termed OtHV4. This virus was initially found in a vaginal swab of a female NFS in 2011. We obtained partial sequences of the polymerase gene and the glycoprotein B gene using established protocols. OtHV4 is closely related to OtHV1. There are reports of breeding between CSLs and NFSs, and this may represent a host jump. Alternatively, these viruses may have codiverged with their hosts. We developed qPCR assays to determine the prevalence of OtHV4 in NFSs. OtHV4 has not been associated with urogenital carcinoma. Comparative genomics of OtHV1 and OtHV4 may identify candidate oncogenic genes. OtHV4 may have utility as a potential vaccine to prevent OtHV1 infection.

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INCREASES IN MANATEE COLD STRESS RESCUES IN FLORIDA AND ASSOCIATED WEATHER PATTERNS

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The endangered Florida manatee (*Trichechus manatus latirostris*) is at risk from a variety of natural and anthropogenic causes. Manatee rescues have been increasing approximately 6% a year. Manatee cold stress rescues have been increasing in frequency compared to other rescue types. In 1996, cold stress accounted for 25% of rescued manatees. This percentage rose to approximately 50% in 2014 (n=29 out of 64 total rescues), which has a direct impact on managing manatee rehabilitation capacity.

Manatees are susceptible to cold stress syndrome during winter months when water temperatures drop below 20 C. Prolonged exposure to water below this threshold can result in sickness and death. Over 200 cases of manatee cold stress rescue were documented within Florida from 1996-2014. With the increasing rate of cold stress rescues, it is necessary for contingency planning and facility capacity management purposes to understand weather patterns that lead to cold stress. Analysis has found broad scale weather indices, such as the Arctic Oscillation, as well as synoptic scale patterns associated with Arctic air masses and resulting location conditions where manatees were found to be associated with cold stress rescues.

Sick and injured manatees are rescued and taken to a network of regionally based acute care treatment facilities for rehabilitation. The number of spaces available in these facilities has remained relatively static since 1991. The capacity of the system is particularly stressed during cold winters because space becomes further limited due to individual facility constraints. A log-linear regression model of cold stress rescues predicts that at the current rate of increase projected by the available data, the number of cold stress rescues brought into facilities by 2025 in a typical year would be 81 and in a year comparable to the cold unusual mortality event of 2010 could reach 244, depending on the degree of severity of winter weather. This greatly exceeds current rehabilitation capacity. Therefore, better understanding of weather patterns associated with severe and prolonged cold weather in Florida has broad implications for the future planning and management of manatee rehabilitation capacity.

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HEMATOLOGIC, BIOCHEMICAL, AND CYTOLOGIC FINDINGS FROM TWO REHABILITATED ATLANTIC SPOTTED DOLPHINS CALVES (*STENELLA FRONTALIS*)

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A benefit to cetacean rescue and rehabilitation efforts is the ability to conduct research, which often times may be difficult to conduct in the wild. For instance, reference ranges for hematology and biochemistry parameters have been previously established from stranded species, including common dolphins (*Delphinus delphis*) and gray whales (*Eschrichtius robustus*). However to date, reference ranges for most cetacean species are limited, including the Atlantic spotted dolphin (*Stenella frontalis*). The purpose of this study was to establish preliminary reference ranges for hematology and biochemistry values, as well as to analyze cytological findings, for two rehabilitated Atlantic spotted dolphin calves. Reference ranges were calculated as ± 2 standard deviations around the median. Furthermore, cytological findings for fecal, urine, and blow samples were qualitatively described. The results of this study can aid in the clinical evaluation and management of Atlantic spotted dolphin calves in rehabilitation and in managed-care settings. Moreover, through future collaborative efforts with other stranding organizations and managed-care facilities, the sample size can be increased, providing an enhanced baseline reference range for Atlantic spotted dolphin calves.

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CONSUMPTION PATTERNS AND ANALYTICAL TOXICOLOGY OF INSHORE-CAUGHT GULF SEAFOOD: BRIDGING ENVIRONMENTAL AND PUBLIC HEALTH CONCERNS POST-DWH OIL SPILL

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This study discerns seafood consumption patterns and analytical toxicology of inshore-harvested seafood species to address public health and community concerns regarding seafood safety in the Gulf of Mexico following the Deepwater Horizon oil spill. An in-person food frequency questionnaire (FFQ) was developed and implemented to analyze participant and household member seafood consumption patterns and body weight from five Gulf coast communities in Florida and Alabama. Initial FFQ data from over 900 individuals in Florida and Alabama indicate that seafood consumption in Gulf coast communities is higher than national estimates derived from 2003-2010 NHANES studies. Upper percentile seafood consumption for Gulf coast survey participants was 231% (adults) and 298% (>21yo) higher for finfish, and 536% (adult) and 984% (>21yo) higher for shellfish (shrimp + crab + oyster), than upper percentile national estimates. Further, seafood consumption patterns vary substantially between communities. Over 1,000 finfish, shrimp, oyster and blue crab samples have been processed, homogenized and prepared using dispersive solid phase extraction, and analyzed using GC/MS-SIM. The sum of parent polycyclic aromatic hydrocarbons (PAHs) and PAH alkyl homologs for all samples analyzed to date revealed that 74% of samples were below detection limits; 23% were between 0.1-0.9 ppb; and 3% were between 1.0 and 48.0 ppb. Based on PAHs measured in Gulf seafood thus far, contaminant levels are remarkably low based on FDA levels of concern, and indicate that edible portions of inshore-sampled seafood species do not have elevated contaminant body burdens. These data, combined with consumption patterns of coastal high-end consumers of Gulf seafood, are being used to refine outreach and resiliency programs, and develop probabilistic, community-based risk assessments.

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LOOK DEEP INTO MY SHELL: SHELL PARASITISM AND RADIOGRAPHY OF APALACHICOLA BAY OYSTERS

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Oyster health and condition assessments are part of ongoing, community-based efforts focusing on Apalachicola Bay, a heritage oyster fishery along Florida's northern Gulf coast. Baseline and restoration monitoring research, to better understand environmental and management factors, has been in effect since the fishery decline in 2012. Assessments included observations of shell parasitism by boring clams (*Diplothyra*), polychaete worms (*Polydora*) and sponge (*Cliona*). Radiographic visualization provided a far more accurate assessment of prevalence and ranked severity, than direct visual observations (that typically underestimated shell parasite severity up to 10-fold). Radiography also revealed that *Polydora* infection occurs primary from the shell edge, not from other external aspects of the shell. Further, size bin analyses indicated that colonization of live oysters with *Polydora* preceded colonization by *Cliona* and *Diplothyra*. Elevated salinity conditions, associated with drought and reduced water flow into the Bay, favor the presence of these shell parasites, weakening the live shell and making the oyster host more susceptible to predation by drills and crabs. A parasitized shell that remains on the reefs as cultch material will likely degrade more quickly due to enhanced surface area. Shell parasitism and *Perkinsus marinus* histological observations from stained mantle tissue were positively associated with oyster height ($p < 0.05$) from different sample locations within the bay. Health and condition indices, and size class-related recruitment and mortality, are discussed relative to management considerations. Support for these studies was provided, in part, through the National Institute for Environmental Health Science (U19 ES020683), NOAA/Florida Sea Grant Program (NA10OAR4170079), and UF IFAS.

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INFUSING ONE HEALTH AND INTERDISCIPLINARY VIEWS WITHIN AQUATIC ANIMAL HEALTH COURSES ONLINE

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The majority of Chief Academic Officers consider distance learning fundamental in long-term strategic planning. However, significant concern exists about educational quality, integrity, and effectiveness in online classrooms. This has initiated a nationwide (and global) conversation that is likely to improve all classroom settings by emphasizing instructional design based upon pedagogical theory. With this in mind, the Aquatic Animal Health Program at the University of Florida cautiously entered into online education. In our courses we utilized technology (recorded lectures, online videoconferencing/discussion, and survey tools) to: 1) increase participation by expert lecturers (biologists, veterinarians, and government employees to provide content), 2) diversify students in the course enhancing student-student interactions (undergraduate, veterinary, graduate students, and professionals, benefitting from perspectives across the country and globally), and 3) evaluate student satisfaction.

Students enrolled in our online courses provided feedback on each format/tool (responses to individual questions ranged from 77 to 158 students). Students responded favorably to the technology with the majority noting a similar level of learning as a classroom-based course for the recorded lectures (54%) and online discussion sessions (59%). Furthermore, a strong proportion responded that they learned more or significantly more with recorded lectures (31%). Overall, the technologies used are viewed as successful methodologies for learning. The vast majority of students (74%) felt the technology allowed them to understand the material better and half felt it increased their interest in the subject matter. Thus, program resources will continue to be allocated for further development of distance education curricula and additional technologies will be explored for continued improvements in education within this specialized field.

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ANATOMICAL AND ECOLOGICAL PATTERNS IN CARCASS FAT DEPOTS COULD PROVIDE INSIGHT INTO FLORIDA MANATEE HEALTH

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Fat depots play a critical role in the physiological ecology of mammals in fluctuating environments. Specifically, lipid metabolism can provide energy, heat and metabolic water during stressful life history stages. Therefore, many mammals experience cyclical periods of fat deposition, followed by periods of weight loss in response to limited food availability, dehydration, temperature change, migration, hibernation or mating and reproduction. In marine mammals, fats often have additional functions, such as streamlining the body, providing thermal insulation and affecting buoyancy.

Florida manatees (*Trichechus manatus latirostris*) live in variable coastal habitats and have many fat depots, including peripheral (or subcutaneous) and internal stores. However, it is uncertain how they utilize these fats in response to ecological stressors, particularly food shortages, cold weather events and reproductive demands. Knowledge of how manatee fat reserves are compartmentalized and the order in which they are utilized during times of nutritional or physiological stress would help assess the relative contributions of fat depots. Internal stores surrounding the viscera may be metabolically active, but subcutaneous stores may assist with thermoregulation and/or buoyancy, based on prior density and biochemical studies. Additionally, emaciated manatee carcasses recovered by the Florida Fish and Wildlife Conservation Commission often exhibit atrophied or depleted fats upon necropsy. It remains unclear how atrophy affects the distribution and qualities of manatee adipose and if it results in additional physiological consequences for nutritionally compromised animals.

Our goal is to consider manatee carcass fats in the context of manatee ecology to determine the importance of adipose in these marine mammals. If anatomical and ecological patterns exist, we could compare these patterns to those observed in other mammals exposed to fluctuating environments. This will improve our understanding of manatee health and our evaluations of body condition during live animal assessments and necropsies.

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USE OF ENVIRONMENTAL DNA (EDNA) TO IMPROVE DETECTION OF CRYPTIC AND LOW DENSITY MANATEE POPULATIONS

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Detection of environmental DNA (eDNA) can be used to determine invasive and imperiled species' distribution and estimate occupancy and detection probabilities to inform management actions. DNA is shed into the environment by feces, mucus, saliva, skin cells, or carcass decomposition, and is typically collected using water samples. This method is particularly helpful for detecting species which are rare, cryptic, or otherwise difficult to monitor through traditional capture or sighting methods, and can inform resource management efforts. Manatees are an example of one such imperiled species for which eDNA methods can be particularly helpful, as they are often found in low densities, are elusive, and inhabit large bodies of water. Use of eDNA technology would be beneficial in determining the range and distribution of newly expanding manatee populations along the northern Gulf Coast, as well as those found in remote bodies of water such as the Everglades. Additionally, it would be helpful internationally in remote areas of the Amazonian rainforest where visual identification is difficult, and regions of the Caribbean, Central America, and Africa, where manatees are wary from frequent hunting and present in a patchy distribution. To this end, we have developed new species-specific primers and probes for a manatee droplet digital PCR (ddPCR) assay to detect the presence of all three manatee species from water eDNA samples. Droplet digital PCR can detect a single molecule of DNA from an environmental sample and has been shown to enhance accuracy and precision over previously used, traditional PCR detection methods. Using this technology, we can detect the presence of otherwise unseen manatees from just a few copies of genetic material filtered from the environment. Our ddPCR assay was tested on West Coast Florida manatee populations and also elusive populations of Antillean manatees. The assay will be useful for delineation of habitat or conservation areas for manatees in logistically difficult study locations or regions where manatees are cryptic and hard to detect.

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CASE STUDY: CATFISH SPINE MORTALITY IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) FROM THE CENTRAL WEST COAST OF FLORIDA

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While mortality from injuries associated with stingray barbs has been well documented, fish spine interactions have not and may cause similar pathology and mortality. There are two saltwater catfish species in the Gulf of Mexico, the gafftopsail catfish (*Bagre marinus*) and the hardhead catfish (*Ariopsis felis*). Both species have bony serrated spines on their dorsal and pectoral fins. If the catfish are ingested whole these spines can penetrate through the gastrointestinal tract and migrate through the abdominal and pleural cavities. From 2003 through 2014 the Florida Fish and Wildlife Conservation Commission's Marine Mammal Pathobiology Lab (MMPL) necropsied 160 bottlenose dolphins (*Tursiops truncatus*) that stranded along the central west coast of Florida encompassing Dixie, Levy, Citrus, Hernando, Pasco, Pinellas, Hillsborough, Manatee, Sarasota, Charlotte, Lee, and Collier counties. Twelve dolphins were documented with injuries associated with fish spines, and in eight of these cases (67%), these injuries were determined as the cause of death. Only two carcasses were fresh at the time of recovery, the remaining nine were moderately to badly decomposed. In a majority of the strandings (n=8) a single spine was observed, three carcasses had two spines, and one carcass had seventeen (MMPL1312). Here we describe the findings associated with catfish spine mortality, with a specific focus on MMPL1312. The carcass was too decomposed to collect histology samples, but in similar cases where histology has been collected bacterial pleuritis and regional peritonitis have been observed (Gretchen Lovewell, personal communication). This study underscores the importance of accurate documentation of any fish spine observed during necropsy, in order to monitor trends in mortality associated with prey availability and/or selection over time.

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CASE STUDY: UNUSUAL FOREIGN BODIES IN THE STOMACH OF A WILD BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*)

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Documentation of fishery gear and marine debris interaction is critical in conservation efforts to mitigate these specific threats in wild cetaceans. Approximately 8% of bottlenose dolphin carcasses necropsied by the Florida Fish and Wildlife Conservation Commission's Marine Mammal Pathobiology Lab from 2005 through 2014 (n=172) were found to have instances of fisheries interaction, with 50% of those having evidence of foreign body ingestion. The predominant instances of foreign body ingestion were hook/monofilament related; however, other objects were documented as well. Here we present one of those cases involving unusual foreign body ingestion. In 2007, a 244cm female bottlenose dolphin (*Tursiops truncatus*) stranded in Clearwater, Florida. External findings showed moderate to severe emaciation, prominent abrasions on the mandible and a large plastic lure partially protruding from the left side of the mouth. Internal findings presented a large, (~100cm) hard plastic snake folded within the forestomach, combined with a rat's nest of monofilament line, sinkers and hooks. The main and pyloric stomachs were empty, indicating that the presence of the snake could have caused a mechanical obstruction and/or anorexia secondary to gastric irritation. Stomach lining ulcerations, substantial emaciation, and enlarged adrenal cortical regions were all indicative of chronic debilitation. Different types of monofilament and fishing gear were found in the stomach, which may suggest the dolphin had a long term history of unnatural foraging behavior such as depredation and/or scavenging behavior, and had ingested the debris during different occasions. This case study illustrates that not only marine pollution but also human impacts altering wild dolphin behavior need to be considered in conservation measures for cetaceans.

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“TIGER STRIPE” PHENOMENA IN INDIAN RIVER LAGOON DOLPHINS

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A previously undescribed skin abnormality, which we refer to as “tiger stripes,” has been identified in Indian River Lagoon (IRL) bottlenose dolphins (*Tursiops truncatus*) through photo-identification surveys. The condition presents as parallel stripes on the ventral and lateral aspects on the torso of the dolphin that vary in length and depth. We suspect that the condition may be indicative of rapid weight loss, similar to human “stretch marks”, debilitating illnesses resulting in emaciation or as a result of starvation due to lack of prey. The goals of this study were to determine if tiger stripes are: (1) a marker of rapid weight loss using sighting histories of dolphin body conditions, (2) more prevalent in post-partum females, and to (3) examine the spatial and temporal distribution in the IRL.

Among the 1,434 identified animals observed from 1996 to 2013, 61 (4.3%) were documented with tiger stripes. The majority of cases (55.7%) were seen in females, and 91.2% of those females had given birth to one or more calves. There was an increase in the number of cases after 2002, with peaks in 2005 and 2010, with the majority of cases in the North and Central lagoon.

Preliminary findings indicate that tiger striping is a useful marker suggestive of rapid weight loss. The next phase of the research is to determine if the condition can be utilized as a pre-mortem marker of emaciation in IRL dolphins by examination of live body condition histories matched to recovered dead dolphins. If so, tiger stripes may serve as a precursor to morbidity and mortality and be helpful in identifying precursors to Unusual Mortality Events and others with similar etiology, which are often distinguished by emaciated animals.

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