Florida Marine Mammal Health Conference VI

Abstract Compilation

SIXTH FLORIDA MARINE MAMMAL HEALTH CONFERENCE • MARCH 2018 •

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Defining and Maintaining Healthy Aquatic Ecosystems

March 28-30, 2018

SeaWorld, Orlando, FL

Florida Marine Mammal Health Conference VI

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Conference Organizers

Iske Larkin
University of Florida
College of Veterinary Medicine

Craig Pelton
University of Florida
College of Veterinary Medicine

Carlos Risco
University of Florida
College of Veterinary Medicine

Mike Walsh
University of Florida
College of Veterinary Medicine

Thomas Waltzek
University of Florida
College of Veterinary Medicine

Session Chairs

Biosurveillance and Molecular Techniques

Nancy Denslow

Professor, College of Veterinary Medicine
University of Florida

Nancy Denslow's research involves developing and using molecular biomarkers to evaluate changes in gene expression depending on stress or exposure to contaminants. For environmental species, molecular approaches, including microarray analysis and proteomics, were developed for several non-model species, including fish, gastropods, and coral. Fish swim in waters that are contaminated by superfund chemicals and emerging contaminants of concern and, thus, provide a direct measure of effects of the contaminants in vertebrates. Nancy recently served on a Blue Ribbon Panel to assess contaminants found in reused water in California.

Margaret (Maggie) Hunter

Research Geneticist, Wetland and Aquatic Research Center
U.S. Geological Survey

Margaret Hunter attended the University of Florida where she received a B.Sc. in Microbiology and Cellular Sciences and minored in Chemistry and Plant Molecular and Cellular Biology. Following her bachelor degree, she received a PhD in Veterinary Medical Sciences researching Sirenia (manatee and dugong) cytogenetics and conservation genetics. Margaret's affiliations consists of USGS Sirenia Project USGS Genetics and Genomics Research Courtesy Assistant Professor, University of Florida, College of Veterinary Medicine, Department of Large Animal Clinical Sciences Affiliated faculty member, University of Florida Aquatic Animal Health Program Affiliated faculty member, University of Florida Genetics Institute.

Education

Gordon Bauer

Experimental Psychologist
New College of Florida

Gordon is an experimental psychologist at New College of Florida. During his time at New College, Gordon established a manatee research project at Mote Marine Laboratory in 1998. The Mote Marine Laboratory and New College of Florida continues to conduct research about manatees and their habitat until this day.

Iske Larkin

Lecturer, Education Coordinator, and Interim Director, Aquatic Animal Health Program
University of Florida

Iske Larkin's position focuses on coordinating and developing new educational opportunities within the Aquatic Animal Health Program and the College of Veterinary Medicine. Her research activities are only a small portion of her efforts but include monitoring the effectiveness of online learning strategies ranging from upper level undergrads, graduate students, veterinary students and professionals. Iske's scientific research includes the study of manatee reproduction, physiology and behavior. Her previous work consists of developing and validating steroid hormone assays for various species, including mammals and birds. Currently, Iske studies male manatee reproductive anatomy at the gross and histological level.

Entanglement and Rescue – Rehabilitation: Program Techniques and Response

Claire Erlacher-Reid

Veterinarian
SeaWorld Orlando

Dr. Claire Erlacher-Reid graduated veterinary school from the University of Georgia. Since graduation she has completed a one year small animal medicine internship at Hollywood Animal Hospital in Florida, a one year aquatic animal medicine internship at Mystic Aquarium in Connecticut, and a three year residency program in aquatic animal medicine at the University of Florida. Dr. Erlacher-Reid became a Diplomate of the American College of Zoological Medicine in 2014. She began working as a veterinarian for SeaWorld Parks in 2014, first in San Diego, and now in Orlando where she currently resides and serves as co-director for the University of Florida Aquatic Animal Health Residency Program. She has been involved in marine mammal health assessments, rescue, and rehabilitation efforts in Florida since 2004.

Michael Walsh

Clinical Coordinator and Clinical Associate Professor, Aquatic Animal Health Program
University of Florida

Mike Walsh is a former veterinarian at SeaWorld in Florida. Mike currently works at the University of Florida as a Clinical Associate Professor and a Clinical Coordinator for the Aquatic Animal Health Program. His research interest varies from preventive medicine program development, conservation and endangered species research in sirenians, cetaceans, and sea turtles. Mike's current research projects include nutritional evaluation and components in cetaceans, manatees and sharks, whole blood element analysis in marine animals as a diagnostic tool, manatee milk analysis, and use of thermography as a diagnostic tool.

Health Assessments

Martine deWit

Veterinarian
Florida Fish and Wildlife Conservation Commission

Martine de Wit graduated from Utrecht University in the Netherlands, where she started her career in exotic animal medicine. Since 2004, she has been working with the Florida Fish and Wildlife Conservation Commission in the Marine Mammal Pathology Lab that responds to calls of manatees in distress and researches manatee health through necropsy and live animal health assessments.

Craig Pelton

Adjunct Clinical Assistant Professor, Aquatic Animal Health Program
University of Florida

Dr. Pelton has been working with marine mammals for 30 years as a trainer, researcher, stranding response coordinator/ rehabilitation director. For the past 11 years, Dr. Pelton worked as veterinarian for several marine parks, stranding response organizations, and the University of Florida. His seven-year involvement with dolphin and Sirenian health assessments include the Health and Environmental Risk Assessment program in the Indian River Lagoon and Charleston Harbor, and the Sarasota Dolphin Research Program in Sarasota Bay. He also was involved with manatee health assessments throughout Florida and Alabama, and dugong work in Moreton bay, Australia.

Infectious Diseases, Toxoplasmosis and Zoonotic Diseases

Thaís C. S. Rodrigues

Postdoctoral Researcher, Wildlife and Aquatic Veterinary Diseases Laboratory
University of Florida

Thaís C S Rodrigues is a DVM, Masters in Veterinary Sciences and PhD candidate in Veterinary Sciences at the Federal University of Uberlândia (UFU - Brazil). She has a background working on wildlife infectious diseases, focusing mostly on zoonosis. She was a research scholar at the Amazonian Aquatic Mammal Research Group, Mamirauá Institute for Sustainable Development (Mamaq/ISDM - Brazil), researching on Amazonian river dolphins and Amazonian manatee's infectious diseases. Currently she is a Postdoc associate at the Wildlife and Aquatic Veterinary Diseases Laboratory, University of Florida (WAVDL - UF), supporting the WAVDL and the UF Aquatic Animal Health Program in the discovery and characterization of emerging aquatic animal pathogens.

Thomas Waltzek

Research Coordinator and Assistant Professor, Aquatic Animal Health Program
University of Florida

Thomas Waltzek is a current Assistant Professor at the University of Florida and a Research Coordinator for the Aquatic Animal Health Program. His interests include the characterization of emerging aquatic animal viruses (EAAVs), the development of diagnostic methodologies to track EAAVs, and aquatic animal zoonoses. Thomas enjoys fishing, hiking, traveling, snowboarding and racquetball.

Water Quality and Indian River Lagoon

Duane De Freese

Executive Director
Indian River Lagoon Council

Duane DeFreese is the Executive Director for the Indian River Lagoon Council. DeFreese has worked for over three decades in Florida to educate people about economic and environmental values associated with the protection of Florida's land, freshwater and marine natural assets. Nationally recognized, DeFreese puts forth his efforts to promote ocean and coastal scientific research, conservation and technology development in Florida.

Keynote Speaker

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Dr. Jack M. Payne

Senior Vice President for Agriculture and Natural Resources
University of Florida
Institute of Food and Agricultural Sciences (IFAS)
Gainesville, Florida

Dr. Payne is the Senior Vice President for Agriculture and Natural Resources at the University of Florida and the Administrative Head for the Institute of Food and Agricultural Sciences. Prior to his current position he served as a Vice President at Iowa State University, and, previous to Iowa State, he was a Vice President and Dean at Utah State University. Dr. Payne also has experience at two other land-grant institutions: Pennsylvania State University, where he served on the faculty of the School of Forest Resources, and, later, at Texas A&M University, where he served as a faculty member in the Fisheries and Wildlife Department.

After leaving Texas A&M University, Dr. Payne had a long career with Ducks Unlimited (DU), as their National Director of Conservation. While at Ducks Unlimited, some of his successes included the development of DU's private lands program with agriculture, the development of a national conservation easement program and the expansion of their Mexican program to Central and South America.

Dr. Payne received his M.S. in Aquatic Ecology and his Ph.D. in Wildlife Ecology from Utah State University and is a graduate of the Institute for Educational Management at Harvard University. He is a tenured professor in the Department of Wildlife Ecology and Conservation at the University of Florida.

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Abstract Compilation

The following abstracts are listed alphabetically by presenting author's last name.
Presenting author names appear in **bold.**

CLIMATE CHANGE: WORLD VIEWS, POLITICAL ATTITUDES AND IMPLICATIONS FOR ENVIRONMENTAL EDUCATION

Frank Alcock
Division of Social Sciences, Sarasota FL, USA

Climate activists often lament a lack of scientific and/or environmental literacy as major factor that weakens support for conservation policies. These sentiments reinforce what has been referred to as a "pipeline" approach to educating youth, citizens and government officials. The sentiment is the idea that if we provide our target audiences with adequate, accurate information then our target audiences will or shoud respond accordingly and in a rational manner. Climate change activists and, more broadly, conservation advocates are increasingly flustered when what they perceive to be objective scientific claims fall upon deaf ears or are simply ignored by targeted audiences.

This presentation will provide an overview of relevant research into climate change attitudes in the American public. The research will include a broad range primary literature along with some insights from the authors own research and professional experience. A key point will be that attitudes toward climate change science and policy are not objectively formed in a vacuum but rather subjectively constructed within a more fundamental worldview structure. Core values and political principles shape perspectives on climate change far more than knowledge about climate change shapes policy preferences and more fundamental worldviews.

Revolutionary advances in information technology and social media trends are likely contributing to acute, contemporary political polarization. Regardless of the reason, political principles are seemingly more sacrosanct than ever before. When new information threatens core political principles the response is likely to ignore or dismiss the information and/or reframe the information in a manner that reinforces rather than repudiates core values.

These insights do not bode well for traditional, pipeline approaches to environmental education in the classroom and broader community. Simply providing information consumers with more and better information is unlikely to trigger a behavioral response. Careful attention needs to be paid to tailoring messages to audiences that are explicit with respect to their desired behavioral response while remaining sensitive to diverse experiences and perspectives.

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EXPLORING PATTERNS IN MORTALITY OF ANTILLEAN MANATEES (*TRICHECHUS MANATUS MANATUS*, LINNAEUS 1758) IN CUBA

Anmari Alvarez Aleman*[1,2]*, Eddy Garcia Alfonso*[3]*, Yanet Forneiro Martin-Viana*[3]*, James Powell*[4]*, Andrew J. Read*[5] *and Thomas K. Frazer*[1,6]*.

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[4]Sea to Shore Alliance, USA
[5]Nicholas School of the Environment, Duke University, USA
[6]Fisheries and Aquatic Sciences Program, University of Florida, USA

The Antillean manatee is categorized as Endangered by the IUCN due to reduction in abundance of animals and habitat loss. In coastal waters surrounding Cuba, the proximate causes of manatee mortalities are not well documented or evaluated in a systematic manner. As a consequence, appropriate conservation oriented management plans have not been implemented. To address this information void, we reviewed reports (some historical and others consistently available since 2001) of dead, stranded or captured manatees to assess patterns in the mortality. In 90% of the cases, carcasses of dead animals were reported; in only 5% of the cases were stranded manatees reported and subsequent rescue and rehabilitation efforts attempted. Documented sources of mortality, and alive strandings, included poaching (36%); entanglement in fishing gear (14%); orphaned/dependent calves (5%); and boat collisions (3%). In nearly half of the reports, i.e. 43%, the cause of death could not be determined, though reported mortalities were highest in areas with intensive commercial fishing effort. The presence of marine protected areas afforded no apparent protection for manatees, likely due to a lack of enforcement effort. These initial findings provide the first estimates of the magnitude of human-induced mortality of manatees in Cuba, and yield valuable insight into the causes of manatee mortality. The existence of intense and poorly regulated fishing in certain areas represents a significant threat to manatees. Further evaluation and regulation of this activity, especially in manatee critical habitats, merits further attention.

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EXTERNAL WATERCRAFT-RELATED WOUND TYPES IN THE FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*)

Brandon L. Bassett

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

The Florida manatee (*Trichechus manatus latirostris*) is a herbivorous marine mammal that inhabits coastal, estuarine, and inland waters of the Southeast U.S. sometimes leading to human interactions that can be injurious or fatal. The number of watercraft-related deaths, based on public reports of carcasses, has been well documented for decades by the Florida Fish and Wildlife Conservation Commission (FWC) (http://www.myfwc.com/research/manatee/). Although watercraft-related scar patterns from carcasses recovered in Florida are recorded, defining common characteristics as they relate to fresh wounds will help to better assess trends of acquired injuries, thus adding to our understanding of human-related threats to the species as well as potential conservation actions to abate impacts. Currently, FWC reports watercraft-related trauma using broad categories including Impact, Propeller, Both Impact and Propeller, and (more recently) Sharp Trauma. These categories help to shed light on the nature of the trauma, but do not necessarily describe the types of wound patterns. For instance, propellers can cause impact-related trauma and be reported as such. To enhance our understanding of non-lethal impacts, we initiated an investigation using 10 years of acute watercraft-related cases. Nine standardized categories of wound types were defined based on external appearance. We were able to categorize the majority of observed external wounds from 387 carcasses that exhibited evidence of acute injuries from collision (s) with watercraft. A preliminary summary of this approach shows promise as an additional metric to assess long-term human-impacts to the species.

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MARINE MAMMAL EDUCATION: OVERCOMING COGNITIVE BIAS

Gordon B. Bauer[1,2]

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Prominent marine mammal researchers have recognized the need to collaborate with social scientists (anthropologists, economists, political scientists, psychologists, and sociologists) and educators to effectively address important issues in wildlife protection that lie outside of the traditional training for conservation biologists, such as modification of human behavior and development of policy. Overcoming cognitive biases is one of the critical areas addressed by social scientists. A variety of cognitive biases (e.g., confirmation bias, motivational effects, and illusory correlations), as well as challenges to personal beliefs may lead educated people to ignore or deny factual evidence. Denial of anthropogenic contributions to climate change, rejection of biological evolution, and resistance to use of vaccines provide a few notable examples of the dismissal of empirical findings. Even a good science education does not necessarily act as a prophylaxis against cognitive bias. Hornsey observed that, "It's almost as though the sophisticated approach to science gives people more tools to curate their own sense of reality."

Fortunately, social scientists are developing methods to address cognitive biases. Effective dissemination of a marine mammal conservation message requires an array of techniques to educate the public as well as to educate professionals in effective utilization of approaches for generating attitude and behavior change. How effects are framed has substantive effects on outcomes. For example, aligning arguments with pre-existing beliefs, highlighting positive outcomes of environmental mitigation, and emphasizing proximate consequences vs distal ones can enhance realization of environmental goals. Much work remains to be done in investigating the transfer of laboratory principals of attitude and behavior change to people who can effect real world marine mammal conservation. In this presentation, I will discuss various approaches to changing attitudes and behavior.

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PRE-DEPARTURE TECHNOLOGY ENHANCED INSTRUCTION FOSTERS DEEP LEARNING DURING AN INTERNATIONAL STUDY ABROAD COURSE AND SERVICE LEARNING INTERNSHIPS AT THE ROATAN INSTITUTE FOR MARINE SCIENCES, HONDURAS

*Elizabeth A Balko,[1,2] and **Teri T Bolton**[3]*

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[3]Roatan Institute of Marine Sciences, Roatan, Honduras

Education programs are powerful tools for engaging the next generation of global citizens in the conservation of marine mammals and their ocean habitats. Study abroad courses and international service-learning internships have tremendous transformative and empowering potential for students. Participation in our overseas programs increases students' intercultural competence, tolerance for ambiguity, and experiential understanding of marine mammal husbandry, training, and behavior. Self-assessment and reflection, important components of the transformational learning process, journaling, peer assessment, rubric analysis of quizzes, research poster, and an oral presentation help to achieve desired learning outcomes and foster deep learning. Pre-departure instruction includes a series of lesson modules designed to prepare students for field research at the Roatan Institute for Marine Sciences (RIMS). Backwards course design leverages the use of desired learning outcomes to first design assessment activities then to create supporting lesson module content. The resulting course design engages students in critical thinking and collaborative skill building while fostering a community of inquiry to help them better navigate cognitive dissonance encountered both while working overseas and when challenged with studying a dolphin social network.

Interactive learning modules help students to master the identification of dolphins swimming together in social groups at RIMS. “Smart videos” featuring RIMS dolphins are leveraged to take identification and behavioral observation training to the next level. Video footage of the RIMS dolphins is used to create identification and behavioral scoring exercises. First, video footage is used to augment static photo-identification activities by introducing students to the challenges of identifying dolphins swimming together in a lagoon. Next, students are instructed to watch the video for a second time and reference the photo-ID log to help assign IDs for individuals at given time stamps. Students are then assigned a set amount of time (e.g., week) to engage in an online class discussion to compare how they scored individuals featured in the video and how they might resolve unknown identities and behaviors. The training module culminates with a synchronous videoconferencing event attended by the course instructor(s) and students. The online event begins with a showing of an annotated version of the video footage they have been watching. Annotations correctly identify individual dolphins for the assigned time stamps. Students discuss what they have learned with the instructor before moving onto the practice modules. Interactive practice modules include presentation of video footage featuring unidentified dolphins swimming together, followed by an annotated version identifying the dolphins at given time stamps. Completion of the module includes a series of low stakes assessment activities requiring students to identify individuals in both static photos and time stamped video-clips.

Upon successful completion of both the pre-departure course and field work at RIMS students are able to collect and interpret data on behavioral signaling and social alliances among dolphins, demonstrate an ability to employ critical thinking, collect and analyze quantitative data, and present conclusions derived from scientific investigation. Benefits of the online instruction include affordance of pre-departure training, a working resource supporting fieldwork, and a resource to facilitate post program activities including conference presentations.

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HEALTH ASSESSMENT ON THE FLORIDA MANATEE: WHAT HAVE WE LEARNED AND WHERE ARE WE GOING?

Robert K. Bonde
U.S. Geological Survey, Wetland and Aquatic Research Center, Gainesville, FL, USA

In the history of health assessments (HA) on manatees, more than 1000 examinations have been performed to date. Additionally, more than 1500 injured or stranded manatees have been rescued. Since 2006 there have been 45 published accounts in peer-reviewed journals utilizing HA obtained data. More than 45 graduate level studies have been completed, increasing our knowledge on the baseline biological condition of the manatees handled. Outside of the southeastern US, HA exams are being conducted throughout the range of manatees, with paramount efforts occurring in Mexico, Belize, Brazil, Puerto Rico, and Cuba. Information obtained from rescued manatees and carcasses has been utilized throughout the range as well. Carcass examination is the first level surveillance system for detection of anomalies in manatee biology and through informed direction HA can assist in determining causes and effects of mortality events.

Our efforts for HA have been focused on addressing the physical condition of manatees to help us better understand their biological potential. We address issues using HA data and sort out the factors that are not biologically significant. HA is employed to specifically address factors that impact the population, such as emerging diseases, causes of unusual mortality events, limits on habitat that influence carrying capacity, environmental health, and anthropogenic changes that are detrimental to sustaining manatee populations. All these factors influence manatee fitness, reproductive potential, health, nutrition, and sustainability of the population. Highlights in manatee research through HA have been observed in diverse areas such as, novel research on genetics, endocrinology, osteology, virology, physiology, contaminants, health monitoring, blood chemistry, and behavior.

As recently as 40 years ago, the Florida manatee population was estimated at 800 individuals. Today, the number is over 6,000. That growth and trend in increasing their numbers follows our projections for their biological potential, despite increasing numbers of annual deaths along with unprecedented growth in the human population. However, births in the Florida manatee population have surpassed the annual mortality, leading to a steady increase in the population. That increase is tantamount to our courageous efforts to protect manatees through regulatory actions. This conservation will continue to be important and data derived from HA will help document those efforts.

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USGS-MANATEE CONSERVATION-GOALS FOR THE FUTURE

Robert K. Bonde
U.S. Geological Survey, Wetland and Aquatic Research Center, Gainesville, FL, USA

The U.S. Geological Survey-Sirenia Project has a proud history of manatee research. That research has helped lay the foundation for better understanding manatee recovery in Florida and Puerto Rico. Specialization has focused on aerial surveys, photo identification, salvage, radio telemetry, molecular and physiological studies, as well as life history and behavioral research. Over the years, the Project has focused our efforts into four general areas: photo identification, population modeling, radio telemetry, and health assessment. Through these programs, we have documented information on annual survival estimates, movements and habitat utilization, foraging, reproduction, nutrition, overall health, disease, and habitat assessment. Our efforts have been multipronged and we have worked with several agencies, organizations, and academic institutions. Over the last 40 years, we have produced 241 peer-reviewed publications advancing our knowledge of manatee biology and conservation. Our efforts have been realized and utilized by scientists throughout the range of all sirenians worldwide.

Goals for the future of manatee research should include: (1) examination of manatee productivity related to annual mortality; (2) development of robust survival rate estimates; (3) monitoring individuals through telemetry; (4) examining manatee health-related condition and fitness of the population; and (5) conducting habitat assessment to predict carrying capacity potential. Despite our efforts to understand manatee population biology, the population by and large has done much better than we would have guessed. Manatees in Florida have out-performed our conservative expectations, and recent high counts have led to reclassification of the manatee under the Endangered Species Act. However, research must continue into the next couple of decades before we truly understand the biologically significant issues facing the survival of manatees, especially in Puerto Rico and elsewhere throughout the range of the West Indian manatee.

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PERFLUOROALKYL ACIDS (PFAAS) IN PLASMA OF THE WEST INDIAN MANATEE (*TRICHECHUS MANATUS*)

Kady Palmer[1], Jacqueline T. Bangma[2], Jessica L. Reiner[3], Robert Bonde[4], Jeffrey E. Korte[2], Ashley S. P. Boggs[3], ***John A. Bowden[3]***

[1]Grice Marine Laboratory, College of Charleston, Charleston, SC, USA
[2]Medical University of South Carolina, Charleston, SC, USA
[3]National Institute of Standards and Technology, Hollings Marine Laboratory, Charleston, SC, USA
[4]U.S. Geological Survey, Wetland and Aquatic Research Center, Gainesville, FL, USA

Perfluoroalkyl acids (PFAAs) are ubiquitous anthropogenic chemicals which have recently received significant media and research attention due to their increased presence in the environment, wildlife, and humans. These commonly utilized synthetic and toxic chemicals are noted to have long half-lives, are resistant to environmental degradation, and bioaccumulate. New strategies are needed to fully characterize the presence of these chemicals in the environment and the consequential health effects of exposure. Here, we investigated the presence of 15 PFAAs in a threatened herbivore, the West Indian manatee (Trichechus manatus). Seven of the 15 PFAAs examined were detected in manatee plasma via liquid chromatography tandem mass spectrometry (LC-MS/MS). Perfluorooctanesulfonic acid (PFOS) and perfluorononanoic acid (PFNA) were detected in every manatee plasma sample examined (n = 69), with differing medians across sampling sites in Florida, Crystal River (n = 39), Brevard County (n = 18), Everglades National Park (n = 8), and four samples (n = 4) from Puerto Rico.

A primary focus of PFAA analyses on marine wildlife has been placed on examining predatory species, due to the route of exposure (fish consumption) and concern over bioaccumulation. However, to date, no studies have examined the PFAA burden in large herbivorous marine mammals, which represents a current gap in current PFAA exposure research. Surprisingly, predator-like levels of PFAAs were determined in manatees from Brevard County (maximum of 166 ng/g wet mass), a site in close proximity to Merritt Island National Wildlife Refuge, where we have previously found high levels of PFAAs in other species (e.g., the American alligator).

To determine the potential health effects of PFAA exposure, we compared PFAA burden to traditional (e.g., cholesterol) and emerging health markers (e.g., lipidomics), resulting in tentative correlations that warrant further investigations with larger cohorts. With a herbivorous diet and long life span, the manatee provides a new model to monitoring PFAA contamination and alternative routes of exposure.

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THE FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS*) T CELL RECEPTOR LOCI EXHIBIT V SEGMENT LOCUS SYNTENY AND CHAIN-SPECIFIC EVOLUTION

Michael F. Criscitiello[1,2], ***Breanna L. Breaux***[1], *Margaret E. Hunter*[3,4], *Maria Paula Cruz-Schneider*[5], *Leonardo Sena*[6], and *Robert K. Bonde*[2,3]

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The Florida manatee (*Trichechus manatus latirostris*) is not only important for aquatic ecology, but for understanding the evolution of the immune genes as well. The T cell receptor (TR) is a key stone for the adaptive immune response in jawed vertebrates. It is responsible for recognizing specific foreign antigens, which initiates both cytotoxic and antibody-driven immune responses. The T cell receptor has two heterodimer forms made from four receptor chains (TRA, TRB, TRD, and TRG) encoded in three distinct genomic loci (TRAD, TRB, and TRG). These loci utilize a complex gene rearrangement mechanism called V (D) J recombination to create antigen binding diversity. Each locus has several Variable (V) segments, Diversity (D) segments, and Joining (J) segments, and each T cell rearranges the genome to bring together one of each segment to create the binding region of the T cell receptor. These loci have been studied in several mammalian species, yet one evolutionary radiation has yet to be studied: Afrotherians. This clade is part of the basal split of eutherian mammals, so it is a stepping-stone between marsupials and boreoeutheria (including human, mouse, bovids, etc.). The Florida manatee is the only member of Afrotheria that inhabits North America. Therefore, we characterized the four T cell receptor chains in the Florida manatee genome to identify evolutionary patterns within eutherian mammals. We manually annotated the three TR loci on the *T. m. latirostris* genomic scaffolds using BLAST and RSSsite, and compared the segments and overall genomic organization to the human TR loci. We identified the human V segment subgroups that are conserved in *T. m. latirostris* and found that TRADV are most conserved, TRBV are moderately conserved, and TRGV are the least conserved. The V segment order in the TRAD and TRB loci also showed synteny between the two species that had not been identified in other eutherian mammals previously. The TRAD locus had five V segment subgroup synteny blocks that showed long-range duplications in the *T. m. latirostris* locus. The TRB locus had three V segment subgroup synteny blocks that showed short-range duplications. No locus synteny was identified in the TRG locus. The conservation of V segment sequence and order correlated to the locus complexity, emphasizing the role of genomic organization on gene evolution. We also identified evidence for a TRD-VH pseudogene for the first time in a eutherian mammal. These novel findings underline the value of including the species within the basal radiation of eutherian evolution in comparative studies. Therefore, the Florida manatee is a strong candidate for future genome evolution studies.

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DEVELOPING NON-INVASIVE BIOMARKERS TO MONITOR THREATS TO FLORIDA MANATEES

Elizabeth Burgess[1]*, Robert Bonde*[2]*, Martine de Wit*[3]*, Charles Deutsch*[3]*, Margaret Hunter*[2] *and Rosalind Rolland*[1]

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As a coastal species, Florida manatees (*Trichechus manatus latirostris*) are vulnerable to anthropogenic and environmental pressures that are increasing in frequency and often challenging to investigate. Here, we validated and applied enzyme immunoassay techniques to monitor manatee health by measuring glucocorticoids (e.g., cortisol; adrenal stress hormones) and triiodothyronine (T3; a thyroid metabolic hormone) in fecal samples collected during live health assessments (total n = 90). Excretion of hormone metabolites in feces provides a highly accessible, noninvasive approach with enormous value for understanding health. Cortisol is a biomarker of physiological stress that helps to mobilize energy reserves to respond to stressful situations, and T3 functions to regulate metabolism. Results showed that fecal cortisol and T3 levels in manatees varied across seasonal habitats (GLM: $P = 0.004$ and $P = 0.004$, respectively). During winter, manatees in an artesian spring complex (n = 40 individuals) had lower concentrations of both cortisol (mean 7.5 ± SEM 0.4 ng/g) and T3 (199.8 ± 28.6 ng/g) than manatees in a winter habitat of industrial warm water and secondary warm water sites (9.6 ± 0.5 ng/g and 277.0 ± 23.1 ng/g; n = 36). These differences could be reflective of water temperature gradients or habitat quality of overwintering areas. In this study, the highest concentrations of both hormones were measured in manatees sampled during non-winter seasons (11.2 ± 1.1 ng/g and 453.0 ± 99.0 ng/g; n = 14), which may suggest heightened metabolic demands due to increased breeding activity and/or food availability over summer-spring. Among reproductive cohorts, adult male manatees (n = 36) showed greater variation in T3 levels (2–fold increase) between winter and non-winter seasons ($P = 0.03$). Given the vital processes reflected by adrenal and thyroid activity, we plan to investigate fecal cortisol and T3 in cases of cold-stress, and apply these techniques to carcasses to aid in mortality investigations. This study will establish critical reference ranges and provide a noninvasive tool to evaluate manatee health – enhancing the opportunity for improved monitoring and potential timely intervention.

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TEMPORAL AND SPATIAL DISTRIBUTION OF CETACEAN STRANDINGS FOCUSING ON THE BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) WITH A SYNTHESIS OF POTENTIAL CAUSES

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The reasons why marine mammals strand is still not well documented. A comprehensive review of the stranding literature and a quantitative statistical analysis of that information are presented. A total of 6,015 cetaceans stranded at 36 locations from 24 peer-reviewed articles published between 1999 and 2014 are documented. The documented cetacean stranding events included 805 for bottlenose dolphins (*Tursiops truncatus*) at a total of ten locations from 11 peer-reviewed articles. When all single stranding events are eliminated and the collected data is analyzed, there are more documented stranding events of bottlenose dolphins than of any other cetacean species in the peer-reviewed literature.

Using data collected from the Marine Mammal Stranding Network for the Southeast United States, a Chi-Square goodness of fit test was used to determine whether the number of bottlenose dolphin strandings is significantly different from the causes of strandings or causes of death categories. The analyses concluded that there is no significant difference among the categories of strandings or death. The general life history of the bottlenose dolphin is presented with special emphasis on its behavior and social structure, which are thought to affect stranding event frequencies.

A review of the factors in or causes of dolphin strandings is discussed, and includes information on diseases and parasites, persistent organic pollutants (POPs) such as organochlorines, heavy metals and trace elements, marine debris, and harmful algal blooms (HABs).The conclusion includes a synopsis of speculated causes for stranding events and a synthesis discussion of stranding causes. It is concluded based off of the literature and data collected and analyzed that the majority of all bottlenose dolphin strandings are due to an unknown cause. A great deal of those strandings that have a known cause are due to diseases, especially morbillivirus. However, a commonality among all categories of strandings researched is the presence of immunosuppressants such as POPs, heavy metals, and HABs. Many diseases have been known to suppress the immune system as well, allowing for more diseases to enter the body or allowing for parasites to take over the body. Immunosuppressants that can lead to diseases or lead to more diseases tend to be the most common reason or at least a factor for why bottlenose dolphins strand. Therefore, the belief is that immunosuppressants affecting these species should be further studied and thought of as one of the top reasons for strandings.

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ANCIENT CONVERGENT LOSSES OF THE *PARAOXONASE 1* GENE COULD RENDER MARINE MAMMALS SUSCEPTIBLE TO ORGANOPHOSPHATE PESTICIDES

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A major goal of evolutionary genetics is to characterize how organisms adapt to a particular environment. Toward this goal, we bioinformatically screened 58 mammal genomes for genes that have preferentially lost function in 3 aquatic marine mammal lineages: cetaceans, pinnipeds, and sirenians. Evolutionary theory guides us to expect such gene loss when a gene is no longer needed or has become disadvantageous. Our results showed a statistical excess of genes lost in aquatic marine species are involved in olfaction, taste, and blood clotting, but the most striking case of marine-specific loss was for the *Paraoxonase 1* gene (*PON1*). It was lost in 4 of the 5 marine species screened, but was intact in all 53 terrestrial mammals. The PON1 protein is a bloodstream enzyme that is thought to mitigate the effects of oxidative damage to lipids, but it is also the major enzyme that detoxifies some organophostate pesticides, such as the controversial chlorpyrifos.

Multiple lines of evidence point to 4 independent deactivations of PON1 in these 3 marine mammal lineages. All extant cetaceans, for example, share a set of 6 genetic lesions, i.e., premature stop codons and frameshifts, so PON1 function was already lost in their last common ancestor. Consistent with this observation, we found that blood plasma from bottlenose dolphins has almost no biochemical activity against PON1 substrates, including the pesticides chlorpyrifos and diazonon, in contrast to high activity observed in outgroup species like the goat and cow. In sirenians, manatees and dugongs share a common genetic lesion, and have accrued additional lesions since their divergence. Biochemical assays of plasma from 7 manatees found almost no activity against PON1 substrates. Pinnipeds experienced a more complicated pattern of loss, with retention of activity in the walrus but evidence of independent losses in some clades of the Otariidae (fur seals, sea lions) and Phocidae (true seals).

The reason for PON1 loss in aquatic marine species is not clear. One major hypothesis is related to PON1's physiological role in mitigating oxidative damage, because long-duration dives cause oxidative stress in diving animals. In support of this hypothesis, we have discovered that the semi-aquatic beaver also lost PON1 biochemical activity, and the sea otter *PON1* gene has a frameshift that would render the protein nonfunctional. Another hypothesis is that PON1 loss is related to diet. PON1's natural substrates are thought to be oxidized lipids, and marine and terrestrial ecosystems differ in their lipid profiles.

The health concern raised by PON1 loss is that many aquatic species may be vulnerable to organophosphates. Mice lacking PON1 are highly susceptible to organophosphate toxicity, and indeed the reason that organophophates are effective insecticides is that insects lack PON1. Organophosphates have been detected in coastal waterways downstream of agricultural land, and could cause neurological and developmental problems after pesticide applications or chronic problems through bioloading. We recommend monitoring and limiting organophosphates near marine mammal habitat and captive animals, and encourage further research into the effects of organophosphates in coastal habitats.

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WHAT IS NORMAL? ESTABLISHING BASELINE DATA FOR REPRODUCTIVE PARAMETERS IN MALE FLORIDA MANATEES

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Few studies have focused on the reproductive physiology of male Florida manatees (*Trichechus manatus latirostris*). Therefore, evident gaps in the fundamental knowledge of male manatee reproduction still remain. The purpose of this research is to study multiple reproductive parameters, including cellular proliferation within the seminiferous epithelium and semen parameters in order to both establish baseline data for reproduction and study normal gonadal function in male Florida manatees. Multiple methodologies were utilized to study each reproductive parameter. First, male gonadal tissues (n=68) were collected from salvaged carcasses, within 24-48 hours of death, from the Florida Fish and Wildlife Conservation Commission Marine Mammal Pathobiology Laboratory. Testes were fixed, sectioned, and immunostained with an anti-human rabbit monoclonal antibody to proliferating cell nuclear antigen (PCNA). Second, whole semen samples (n=3) were collected from a captive male at the Puerto Rico Manatee Conservation Center. Sperm was also collected post-mortem from salvaged carcasses (n=5). Basic semen parameters including volume, concentration, total sperm number, motility, vitality, morphometry, and morphology were analyzed using both conventional methods and a Microptic SCA® computer-aided sperm analysis system (CASA). For cellular proliferation, our results showed developmental differences with adult testes exhibiting higher germ cell proliferation than both juveniles, which exhibited moderate proliferation, and calves, which had low to absent proliferation. Additionally, there was a marked seasonality in germ cell proliferation. Testes from sexually mature males collected during the summer breeding season exhibited extensive proliferation of basally located spermatogonia and all stages of spermatogenesis were present. Spermatogenesis was repressed in adult and juvenile testes collected in the winter non-breeding season but proliferation of spermatogonia was still seen in basal layers of seminiferous tubules. For whole semen samples, both total and progressive motility exceeded 80% and vitality exceeded 55% for each sample. Each sample contained multiple morphological defects with distal droplets and midpiece defects accounting for the highest proportion of defects. Spermatozoa head morphometry differed significantly from previously published literature but no differences were observed between sperm from whole semen samples and sperm collected post-mortem. Although the results are preliminary, they begin to address the lack of basic knowledge of male manatee reproduction by defining baseline values for each reproductive parameter and help to establish reference points for continued reproductive research in this species. The results of this research become more applicable when correlated with the effects of varying environmental conditions and health-related factors, providing a broader scope for determining how each factor affects reproduction. Ultimately, males represent the second half of the equation for successful reproduction and studying each of these parameters and how they relate to reproduction is critically important for the long-term management and conservation of this species.

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CETACEAN RESCUE AND REHABILITATION AT SEAWORLD

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The rescue and rehabilitation program at SeaWorld dates as far back as 1965 in San Diego, and SeaWorld of Florida has participated in the rescue of 334 cetaceans to date with an average of approximately 8 animals each year. Since re-opening the rehabilitation facility on site in Orlando, Florida in 2012, 18 cetaceans have been admitted and cared for by the rescue team. Cetacean rescue and rehabilitation efforts are coordinated by the national stranding network under the supervision of the National Marine Fisheries Service (NMFS).

Clinical veterinarians are intimately involved in the rescue and rehabilitation program in order to help make appropriate medical decisions and restore the health of animals admitted into rehabilitation with the ultimate goal of return to their natural habitats. Involvement of veterinarians in the rescue program helps to ensure a humane response to stranded marine mammals and maximize specimen and data collection pertinent to the health of the population and species as well as the individual animal. Veterinarians often work in the field alongside first responders and the local stranding coordinator to provide immediate medical attention, collect biological samples and guide decisions regarding whether an animal can be treated and released on site, should be admitted for continued care or if humane euthanasia is warranted. Common scenarios for assessments and response in the field are those involving mass strandings, disentanglement or assistance with unusual mortality events.

Cetaceans that are admitted to SeaWorld for rehabilitation are monitored and cared for around the clock and are immediately evaluated by the attending veterinarian to establish a medical plan for each case, utilizing continued advancements in marine mammal medical diagnostics and therapeutics. Animals are screened for bacterial, viral and parasitic diseases and efforts are made to address possible myopathy which can be a common sequela of stranding. Following successful rehabilitation efforts, the attending veterinarian helps to determine when an animal is ready for return and conveys recommendations regarding the animal's final disposition in accordance with the best practices and policies regarding standards for release established by the National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Services (NMFS).

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WATER QUALITY MONITORING FOR THE INDIAN RIVER LAGOON – CHALLENGES AND OPPORTUNITIES AHEAD

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The Indian River Lagoon experienced a dramatic ecological state-shift from a benthic aquatic vegetation system to one dominated by planktonic microalgae in 2011 with an unprecedented pico- cyanobacterial bloom (now referred to as the "2011 superbloom"). The post-2011 IRL is now characterized by intense, recurring and long-lasting algal bloom conditions, widespread loss of seagrass habitat, and episodic wildlife mortality events. On-going blooms of pico-cyanobacteria, dinoflagellates and the Texas brown tide species, *Aureoumbra lagunensis*, now appear to be the "new normal" for the central and northern IRL. These ecosystem shifts challenge our scientific understanding of nutrient enrichment thresholds, nutrient cycling and tipping points for the IRL. Concurrent with these ecosystem-wide stress-response issues, the southern IRL is severely impacted by massive seasonal freshwater release events from Lake Okeechobee during times of high water. In 2016, large-volume, high-velocity freshwater releases from Lake Okeechobee from February through November ("the Lost Year") fueled an intense *Microcystis* cyanoHAB with reported microcystin toxin concentrations that greatly exceeded World Health Organization standards. These changes in IRL water quality, in conjunction with potential long-term water quality trends associated with climate change, suggest that the IRL science and resource management community need to rethink IRL water quality monitoring activities to provide reliable, continuous environmental data in cost-effective ways to better quantify, model and predict the complex relationships between water quality, environmental factors and biological processes. This presentation will discuss challenges and opportunities ahead for implementing a comprehensive, coordinated and integrated water quality-monitoring network for the IRL.

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BIOMARKER DEVELOPMENT FOR MANATEE HEALTH ASSESSMENTS

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Florida's manatee population has recovered mainly due to the effort to reduce watercraft collisions. However, the human population in the area is increasing and the aquatic environment is considered the ultimate sink for anthropogenic chemicals, resulting in continued threats to their populations, for example increasing the danger of developing chronic diseases. The enormous development in molecular biology, proteomics, and lipidomics provides numerous possibilities to incorporate new biomarkers in manatee health assessments. A previous study has shown differential plasma protein expression between manatees affected by red tide compared to controls, making these biomarkers useful to diagnose this condition and distinguish it from other illnesses. Currently, we are working on the possible exposure of manatees to glyphosate, an herbicide heavily used in Florida. Glyphosate is sprayed directly in the water or it may run off from crops, like sugar cane. Chronic exposure can produce kidney disease as has been demonstrated for experimental animals such as rodents and fish. We are working on largemouth bass as a model for chronic exposure through the water to two concentrations of glyphosate and its commercial product, Rodeo (10 and 0.5 mg L^{-1}). We will analyze changes in gene expression associated with tissue damage in kidney through RNA sequencing and qPCR. We will also isolate exosomes from plasma to look for possible lipid biomarkers of glyphosate exposure and immune dysfunction. The isolated biomarkers may provide a better method for assessing health issues in fish and manatees that are exposed to contaminants.

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EVALUATING INTERVENTION SUCCESS AND SURVIVAL OF FREE-SWIMMING DOLPHINS ALONG THE EAST CENTRAL COAST OF FLORIDA (2007-2017)

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The survival of free-swimming bottlenose dolphins (*Tursiops truncatus*) in life threatening situations is often dependent on human intervention. Frequently, intervention success is correlated with injury severity, health, age class, and time prior to discovery/intervention. The evaluation of each case is dependent on data collected prior to and following intervention. The objectives of the study were to compile cases warranting intervention along the east coast of central Florida (2007-2017) to: examine conditions leading to intervention, evaluate trends between case types, and to evaluate success/failure and associated factors. Intervention success was determined based on direct observation of normal behavior (foraging/traveling) for a minimum of six weeks post-intervention and not restranding within 12 weeks post-release. In total, 21 interventions occurred including four dolphins entrapped by natural/manmade barriers, two out-of-habitat animals, and 15 entanglement cases. Two additional entanglement interventions were planned but the animals perished prior to intervention. Post-release monitoring was enabled through photo-identification (52.6%) and radio telemetry (42.1 %). Out-of-habitat animals remained in low salinity habitats for at least 24.0 ± 22.63 d, and presented lesions; these interventions were unsuccessful despite relocation. Entrapped animals were relocated within 6.75 ± 4.5 d and exhibited a success rate of 75%, with one animal stranding after 25 d from natural causes (prey-induced asphyxiation). A total of 66.7% of disentanglement efforts were successful, while 20% failed and two cases lacked sufficient information. Animals were entangled for at least 24.59 ± 30.05 d and ranged in age from nine months to adults of advanced age. Failed entanglement interventions cases involved: dolphins in decreased nutritional condition (very thin or emaciated), 83% were calves, 3 cases involved substantial injuries to the flukes and 2 cases involved the gear circumscribing the animal with wounds to the pectoral flippers and gape. The relocation of out-of-habitat animals was not successful, suggesting a more rapid response and/or further medical attention may be required. Entangled animals in poor body condition had a 50% survival rate; therefore, intervening prior to decline in body condition may aid success. Injuries to the gape and flukes present substantial risks to survival and unfortunately the severity of these injuries often cannot be evaluated prior to intervention, therefore, prompt interventions are warranted. Likewise, foraging behavior prior to intervention may not be an indicator of health as this behavior was observed just prior to mortality in one case. Since rehabilitation is frequently not an option, outreach to enhance public reporting and decrease close human interactions is essential. Lastly, timely response depends on the proximity of trained responders; therefore, emphasis should be placed on increasing network capacity in the future.

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NOSTRIL TEMPERATURE AS INDICATOR FOR BODY TEMPERATURE – USING INFRARED THERMOGRAPHY AS A DIAGNOSTIC TOOL IN FLORIDA MANATEES

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Assessing the body temperature is an essential part of diagnosis and health assessment in mammals. However, in particular in fully aquatic mammals, such as sirenians, this can be challenging. So far, there is no reliable non-invasive method to measure body temperature in manatees. Oral temperature has been used often as an indicator for manatee body temperature. However, besides the unaccuracry of orally assessed body temperature in general, manatees often resist the placing of the sensor, and the actual location of the sensor in the oral cavity is hardly manageable. Since thermal stress is a severe threat to Florida manatees (*Trichechus manatus latirostris*) a non-invasive way to assess the thermal state of an individual is beneficial. Therefore our goal was to develop a simple, undisturbing method to assess and monitor the thermal state of a manatee. Infrared thermography (IRT) suits this purpose pretty well as it is non-invasive, contact-free and easy to assess.

We used IRT video recording to measure and record the temperature within the open nostrils of a manatee during a breath. This way we recorded nostril temperature in five Florida manatees continuously before and during sedation. All animals were in a healthy state and were sedated prior to transportation to release or to a different holding facility. Recording started pre sedation when the animal was still in the water and continued as soon as the animal was loaded into the truck.

After an initial increase, temperature within the nostrils decreased by up to 6°C over a time period of up to 70 minutes post injection of the sedative. Abation of the sedative, i. e. the manatee became active and started moving, was accompanied by an increase of nostril temperature of up to 6°C within as short as 10 minutes.

The decrease of body temperature, as found in our results, is a common side effect of anaestesia and sedation, which supports the reliability of our approach. Although the measured temperatures may not display the actual core body temperature, the values serve as relative values displaying temperature progression in a manatee during handling situations. For example the necessity for administring further sedation could be early indicated. Furthermore, the application of this method in other species, difficult to handle, such as predators, is conceivable.

Further measurements are being conducted as discribed above, with and without sedation and under variable conditions, in order to varify measurements and optimize the method. In any case, our approach provides a simple method to monitor the thermal state of a manatee during sedation, transportation, health assessments and other potentially stressful situations.

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MANATEE ENTANGLEMENT

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Derelict fishing gear, and other forms of aquatic debris, can adversely affect aquatic animals and underwater habitats, result in economic loss, and negatively impact human health and safety. Recent research has demonstrated that entanglement in aquatic debris was the primary anthropogenic cause for live manatee rescues (25%) between the years 1993 and 2012. Trap lines and monofilament fishing lines were the most commonly documented materials involved in these entanglements.[1] Entanglements may negatively impact manatee reproduction and nursing, reduce or prevent mobility and foraging, predispose to secondary infections or additional entanglements, and/or result in death. Of the manatees that were necropsied between the years 1993 and 2012, over 11% demonstrated evidence of entanglement or ingestion of foreign debris.[1]

Once an entangled manatee is identified in the field, intervention and rescue efforts are planned accordingly by the Florida Fish and Wildlife Conservation Commission based on the geographical location of the animal, the anatomical location of the entanglement, and the perceived severity of entanglement based upon a brief examination in the field. Disentanglement on site may be possible; however, in some cases it may be necessary for the animal to be transported to a rehabilitation facility for disentanglement, limb amputation, continued wound therapy, systemic medications, and/or supportive care.

Continued efforts to rescue entangled manatees and remove debris from aquatic environments are paramount for the conservation of this threatened species. Existing strategies to prevent and remove aquatic debris have been established including the Monofilament Recovery and Recycling Program, derelict trap removal programs, and beach clean-up initiatives. A wildlife alert hotline (888-404-3922) was also established for the public to report injured, orphaned, entangled, distressed, or deceased manatees to the Florida Fish and Wildlife Conservation Commission to help facilitate timely manatee rescue efforts.

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MANATEE MEDICINE IN A REHABILITATION SETTING AT SEAWORLD

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SeaWorld of Florida has been involved in the rescue, rehabilitation and return of manatees (*Trichechus manatus*) since 1976. Since the inception of the rescue program, a total of 626 manatees have been rescued to date, averaging approximately 15 animals each year. SeaWorld remains active in and the Manatee Rescue and Rehabilitation Partnership (MRP), and rescues and returns are coordinated by the Florida Fish and Wildlife Conservation Commission (FWC). The SeaWorld rescue team, which includes the clinical veterinary team, responds to rescue efforts throughout the state of Florida, as well as in numerous surrounding states whenever intervention is needed.

Florida manatees face both anthropogenic and natural causes of threats to their health and survival. The most common health threats resulting in admission for rehabilitation to SeaWorld are watercraft injuries, entanglement, cold stress syndrome and orphaned calves. Veterinarians triage rescued animals immediately, oversee their medical care and determine when an animal is medically cleared to be returned to its natural habitat. Most diagnostic tests, such as analyses of biologic samples, radiographic studies and ultrasonography are performed on site, but more advanced diagnostics, such as computed tomography, may also be available for some cases. Continued advancements in marine mammal medicine are employed in diagnostic techniques, anesthesia and therapeutics used to treat these cases with a goal of full recovery and return.

In addition to providing medical care to animals admitted for rehabilitation, SeaWorld veterinarians may also provide medical expertise in the field through participation in health assessments, triage in the field to determine whether or not an ill or injured manatee needs to be admitted to a rehabilitation facility at the time of intervention and critical care and observation during transport.

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USE OF QUANTITATIVE PCR ASSAYS TO MEASURE IMMUNE FUNCTION GENE EXPRESSION IN COLD STRESSED FLORIDA MANATEES

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Manatees with cold stress syndrome (CSS) exhibit associated suppressed immunity which leaves them susceptible to many secondary opportunistic infections including viral, bacterial, and fungal infections. Specific knowledge as to whether cold stress directly or indirectly suppresses immune response is necessary for the improved treatment of manatees rescued with CSS and the understanding of the cold stress response. Cold stress represents on average 10% of mortality identified in stranded carcasses annually, but can vary greatly. In 2010, during an early, cold winter, CSS was implicated as the cause of mortality for 36.8% of strandings. During a mild winter, such as in 2013, as low as 5% mortality from CSS was observed.

This study utilizes species-specific quantitative, real-time PCR (qPCR) assays to measure mRNA expression of genes associated with immune function in Florida manatees. Blood samples were obtained from adult to subadult manatees during health assessments in Brevard county, FL during December of 2010 (N=10) and of 2013 (N=10). Manatees samples from 2010 were from animals confirmed for CSS and 2013 samples were from confirmed healthy manatees (no signs of CSS). Preliminary data show higher expression of interleukin-6 in CSS manatees versus healthy (x=57 vs 30 copies/100 ng cDNA) and lower interleukin-2 (x=237 vs 379 copies/100 ng cDNA), with similar interleukin-10 values (x=115 vs 137 copies/100 ng cDNA) although these results are not significantly different when assessed using the Wilcoxon-Mann-Whitney test. Intriguingly, the housekeeping gene GAPDH was significantly lower in CSS manatees versus healthy (x=377 vs 32,915 copies/100 ng cDNA). Verification of this reduced expression is ongoing. Further analysis will include investigating relative levels of IFN-γ and TNF-α, as well as the housekeeping gene β-actin.

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THAT TINGLING SENSATION MEANS IT'S WORKING: SAXITOXIN IN INDIAN RIVER LAGOON DOLPHINS.

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Exposure to harmful algal bloom (HAB) toxins is the most common cause of large-scale marine mammal mortality events, but sublethal impacts are poorly understood due to a lack of baseline toxin data for marine mammals in most regions. One such region, Florida's Indian River Lagoon (IRL), is home to HAB species that produce saxitoxin, a neurotoxin which causes paralytic shellfish poisoning in humans. The IRL is also home to a resident population of bottlenose dolphins (*Tursiops truncatus*), an important indicator species for marine ecosystem health. Since the presence of saxitoxins in Florida waters has only recently been discovered, we conducted the first assessment of saxitoxin exposure in IRL dolphins as an initial step in assessing the emerging health risk to this population. Here we report the first detection of saxitoxin in IRL dolphins, during the period 2002-2016. We compare levels of saxitoxins and other HAB toxins in dolphins sampled during various IRL HABs to levels detected during non-bloom conditions, and propose the first reference values for HAB toxin accumulation in IRL dolphins. We anticipate these data to be useful for evaluating future HAB impacts on marine mammals in the IRL.

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AN OVERVIEW OF THE MARINE MAMMAL STRANDING RESPONSE PROGRAM IN THE SOUTHEAST U.S.

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All marine mammals in the United States are protected under the Marine Mammal Protection Act (MMPA). Under the MMPA, the National Marine Fisheries Service (NMFS) is responsible for managing cetaceans (whales, dolphins and porpoises) and pinnipeds (seals, sea lions, and walruses). The MMPA establishes the Marine Mammal Health and Stranding Response Program with the goal of collecting and disseminating data on health and health trends, correlating those data with biological, physical, and chemical environmental parameters, and coordinating effective responses to unusual mortality and morbidity events. The volunteer Marine Mammal Stranding Network (MMSN) is comprised of trained responders and veterinarians who are authorized under the MMPA to respond to and rehabilitate live stranded marine mammals and investigate dead stranded marine mammals. Increasingly, the MMSN is also called upon to assist NMFS with rescuing entangled, free-swimming small cetaceans ("interventions") and performing follow-up monitoring. The MMSN plays a critical role in helping NMFS to meet its mandates under the MMPA by identifying natural and anthropogenic threats to marine mammals, increasing understanding of the basic biology and distribution of marine mammals, and helping to determine the efficacy of management measures. This presentation will give an overview of the MMSN in the Southeast U.S., with a focus on the importance of the MMSN to management of marine mammals in the Region. Case studies of cetacean rehabilitation and release and bottlenose dolphin interventions will be presented. Challenges, emerging issues, and future directions for the MMSN will be discussed.

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LARGE WHALE DISENTANGLEMENT: SAVING NORTH ATLANTIC RIGHT WHALES, ONE ENTANGLED WHALE AT A TIME

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Bycatch in commercial fishing gear is a worldwide problem affecting large whales and other marine wildlife. Entanglement in fixed, unattended fishing rope is particularly problematic for North Atlantic right whales, which number fewer than 450 individuals and are currently declining. Over 80% of living North Atlantic right whales have scars from previous rope entanglements, and nearly 5 whales per year are observed dead or seriously injured from new entanglements. Additional whales likely die each year from entanglements that go undetected. The rate of serious entanglements has risen over the past 30 years, coincident with the use of stronger synthetic ropes by the fishing industry. The origin and source of most entanglements cannot be determined, but of those that can, most have been attributed to gillnet and trap/pot fisheries (e.g., lobster, snow crab). To address this issue, U.S. regulators have required a variety of fishing gear modifications, gear marking requirements, seasonal closures and other measures. Despite these efforts, no improvements in whale entanglement or whale survival rates have been observed. Consequently, whale disentanglement, once considered a stopgap measure, is increasingly being seen as a mitigation tool.

The Atlantic Large Whale Disentanglement Network was created in the 1990s by NOAA and other partners with the goals of (1) reducing whale injury and mortality, (2) documenting sources of entanglement, (3) ensuring responder safety through training and protocols and (4) increasing public awareness. Today the Network consists of 16 organizations operating from Quebec to Florida. At its most basic, disentanglement methods involve approaching whales by boat and cutting whales free of rope. Buoys are often added to the rope to slow the whales and keep them near the surface, thereby making it easier for responders to approach evasive whales. Advances in cutting tools, satellite tracking, digital imaging and other technology have improved success over time. Recent methods to deliver sedatives remotely and reduce whale evasiveness are also promising. Two case studies from the Southeast U.S. will be presented that illustrate these methods and the logistical challenges inherent in this work. Despite the numerous advances in disentanglement methods, almost half of entangled right whale cases do not end successfully. Disentanglement response, while valuable, is therefore no substitute for prevention. Efforts must focus on removing ropes with high breaking strength from North Atlantic right whale habitat.

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EFFECT OF ANTHROPOGENIC INJURIES ON THE SOCIAL ASSOCIATIONS OF BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) IN SARASOTA BAY, FLORIDA

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Social connectivity may be an important factor influencing the survivability of bottlenose dolphins (*Tursiops truncatus*). Dolphins rely upon other individuals for information transfer, protection from predators, and feeding success. When animals become injured, there is the potential for this connection to diminish, thereby threatening the survivability of the animal. In Sarasota Bay, recreational boating and fishing have led to increases in the number of boat strikes and entanglements impacting long-term resident dolphins. These anthropogenic factors can cause debilitating injuries to the animals and disrupt their group associations and social networks. Using long-term data sets available for the dolphin community in Sarasota Bay, we investigated how these injuries affect the social associations of the dolphins by (1) examining changes in their group size before and after injury; (2) evaluating differences in their networks of close associates before and after injury. We found that after injury, dolphins do not have a significantly different group size than control animals. However, injured dolphins do have lower connectivity to the other animals within their individualized networks. Our findings support the need to consider negative impacts of ecotourism on wild animal populations.

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EDUCATING THE PUBLIC ABOUT OTHER MINDS: DOLPHIN COGNITION RESEARCH AS A WINDOW TO SCIENCE AND OTHER ANIMALS

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As a species, humans have had an unprecedented impact on the other species with whom we share the planet. Understanding other animals' perspectives is one-step towards making better decisions about humans' effects on them. Comparative psychologists study the minds of other animals through methods that help identify their perspectives, thereby providing an alternative to anthropomorphic approaches. Here we describe a case study in which the public can learn about the scientific method and the dolphin mind through watching trial-by-trial scientifically valid data collection. Our tasks focus mostly on questions related to dolphin echolocation, communication, and social learning and dynamics. This approach has led to multiple benefits for marine mammals including: (1) offering educational opportunities to hundreds of thousands of people, (2) enhancing the lives of the dolphins in our facility and those at other oceanaria, (3) producing reputable science published in peer-reviewed journals, , and (4) promoting the welfare of wild dolphins. This model might be useful for other public facilities housing marine mammals.

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INVESTIGATING MANATEE TAXONOMIC RELATIONSHIPS USING HIGH-THROUGHPUT GENOTYPING-BY-SEQUENCING SNP DATA

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The evolution and taxonomy of the three extant manatee species is still under investigation. Previous mitochondrial DNA control region analyses indicated that the Amazonian manatee, *Trichechus inunguis*, is a sister group to the West Indian manatee, *T. manatus,* and is not more genetically different from the three classified *T. manatus* clades than the three clades are from each other. Additionally, *T. manatus* likely dispersed from two separate regions forming three deeply divergent lineages. However, *Cytochrome b*, which is less prone to homoplasy, suggests that *T. inunguis* is basal to *T. manatus* and the African manatee, *T. senegalensis*, which were later derived from a similar marine ancestor. Mitochondrial and nuclear genetic evidence also indicate recent hybridization events between *T. inunguis* and the Antillean subspecies of *T. manatus*. To provide a nuclear perspective on Trichechid divergence and genetic structure, we employed genotyping-by-sequencing (GBS), similar to RADseq, using next generation sequencing to assess thousands of nuclear loci. For this study, 114 manatee samples were sequenced from the three manatee species and two subspecies at 19,908 GBS loci. Bayesian clustering indicated four groups initially, which separated out further when assessed independently in hierarchical analysis. The African species formed a single cluster, and separated geographically north and south when analyzed alone. The Florida and Belize genotypes grouped together initially as a northern West Indian clade. Florida further broke down into East and West coast populations, as supported by the microsatellite data. Coastal Brazil and South American samples clustered independently from all other samples. The Amazonian species had some similarity with the other West Indian samples, potentially related to it a common ancestral colonizing Central America and/or to a hybrid zone at the mouth of the Amazon River. In phylogenetic tree analyses, two putative hybrid samples branched between the Brazil and Amazon samples. Further statistical investigation of these loci, and the development of SNP panels, will also be used to identify evolutionary patterns and shed light on speciation, hybridization potential, population structure and paternity assessments.

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INDIAN RIVER LAGOON: PERFECT STORM OR NEW NORM

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The highly diverse Indian River Lagoon extends for 156 miles along Florida's central east coast, and it provides billions of dollars in annual revenue to the region. The system is shallow and microtidal, without flow from a major river. The nature of this barrier island lagoon both supports its diversity and makes it vulnerable to detrimental impacts from people's activities. In fact, people have altered the system since the mid-1700s, with significant changes in recent decades. As a result, major portions of the system are impaired by excess loads of nutrients, with reductions to meet total maximum daily loads being applied over the next 12 years. Recently, stakeholders became concerned about a shift in the ability of the lagoon to cope with loads of nutrients. For example, the intensity, duration and composition of phytoplankton blooms has changed since 2011. Blooms of greater intensity and longer duration have led to substantial losses of seagrass, which translates into reduced habitat and food for invertebrates, fish and marine mammals. In addition, increased biomass of phytoplankton has led to fish kills, including a large-scale event in 2016. Although the exact causes remain uncertain, the lagoon also experienced an unusual mortality event for manatees that began in 2012 and another event for bottlenose dolphin in 2013. Therefore, as we implement projects that reduce nutrient loads, restore natural cycling of nutrients and increase consumption of phytoplankton, we also are improving our understanding of the lagoon's nutrient budget and the factors that initiate and control phytoplankton blooms. Evidence suggests that meteorological events influence the initiation and composition of blooms. Bottom-up drivers of increased phytoplankton production include nutrients delivered in surface water runoff, injected via submarine groundwater and released from accumulated "muck." Changes in the abundance of macroalgae and seagrasses alter storage and cycling of nutrients, which affects the availability of nutrients for phytoplankton. Furthermore, blooms of certain taxa may escape top-down control by filter feeders. Overall, the situation points to a need to consider the effects of events on ecological systems when managing nutrient inputs.

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CHARACTERIZATION OF A NOVEL CIRCOVIRUS FROM A STRANDED LONGMAN'S BEAKED WHALE (*INDOPACETUS PACIFICUS*)

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Members of the family *Circoviridae* are small non-enveloped viruses with icosahedral nucleocapsids that contain the single-stranded circular DNA genome ranging in size between 1.8-2 kbp. The family is divided into the genera *Circovirus* and *Cyclovirus*. They infect a wide range of vertebrates (mammals including humans, birds, and fish), and have been detected in invertebrates (e.g. dragonflies). Tissues from a juvenile Longman's beaked whale (*Indopacetus pacificus*) that stranded in Hawaii were screened for viruses using an Illumina MiSeq Next-Generation Sequencer (NGS). The full genome (1,894 bp) of a novel circovirus was recovered from the NGS data (hereafter referred to as the beaked whale circovirus; BWCV). Two open reading frames (ORF) were annotated including ORF1 that encodes the capsid gene and ORF2 that encodes the replication gene. Endpoint PCR screening using primers specific to BWCV of all tissue DNA (lung, cerebrum, cerebellum, scapular and mediastinal lymph nodes) were positive, suggesting a systematic infection or circulation in the blood. The phylogenetic analysis based on the deduced amino acid sequences of the capsid and replication-associated proteins revealed BWCV is a member of the genus *Circovirus* branching as the sister species to the recently discovered dog circovirus (DogCV). Sequence identity matrices generated from the genomic alignments between BWCV and other circoviruses were found to be <80% pairwise identity (48.7 – 55.1%) used to demarcate new species in the family. Similar to other members of the genus *Circovirus*, BWCV displayed the conserved nanomer within the stem-loop.

Although the pathogenicity of the BWCV is unclear, other circoviruses such as Porcine circovirus 2 (PCV2) induce host immunosuppression (lymphoid depletion) promoting secondary bacterial, viral, fungal, and parasitic infections. Lymphoid depletion was described in the same beaked whale that was attributed to a morbillivirus infection. Similar to West and colleagues, we also detected a novel alphaherpesvirus that may have reactivated given the immunocompromised state of the juvenile beaked whale. This report provides the first description of a circovirus in a marine mammal and we propose the name Beaked whale circovirus for consideration by the International Committee on the Taxonomy of Viruses.

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CHARACTERIZING SUCCESS OF THE UF AQUATIC ANIMAL MEDICINE CERTIFICATE

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Aquatic animal medicine is a specialty of veterinary medicine and students may become boarded within the American College of Zoological Medicine. The Certificate in Aquatic Animal Medicine (AAM) was developed for students to receive specialized and directed training within the University of Florida (UF) Doctor of Veterinary Medicine (DVM) curriculum. This elective certificate program is the first of its kind. Students must complete 15 academic credits and maintain a 3.0 GPA. Core requirements include successful completion of Diseases of Warmwater Fish, SeaVet Clinical Training, Topics in Aquatic Animal Health, and a choice between an aquatic-related research project or externship.

The goal of the program is to provide sufficient training and mentorship so that newly graduated veterinarians are ready for aquatic-related post-DVM training, an entry-level position in aquaculture, or could provide basic medical care to aquatic animals as part of a companion animal practice. Post-DVM training may include continued graduate education (master's or PhD degree), internships, residencies, or specialized clinical training in zoological medicine. Characteristics we evaluated to define success include approval by accreditation bodies, awareness of the program by pre-veterinary students, certificate completion rates, and placement of graduates in an aquatic or zoological veterinary position.

The AAM Certificate program has been running for 10 years, with the first student completing all requirements graduating in 2008. To date, we have had a total of 113 students participate in the program, averaging 8% of each DVM class, including 31 students currently working on the certificate. To date, 57 students have successfully completed the AAM Certificate and it has had a mean completion rate of 82%. Data collection post-graduation is challenging especially when we often recommend that they minimally spend 1-2 years within a small animal clinical setting to solidify their training. Of those students who graduated in 2015 or earlier (32 students), we have been able to confirm 38% (12 students) were successful in securing a position in a zoo or aquatic-related field.

In 2012, we added new questions to our application process. From this we have learned that 41% of our students were 'out of state', 89% of our students knew about our aquatics program before they applied to the UF veterinary program, and 76% indicated that our Aquatic Animal Health program played a significant impact in their decision to attend UF. The AAM Certificate is approved through the UF College of Veterinary Medicine (CVM) and Florida Board of Governors to be formally recognized, providing student recognition on their transcripts and an official state certificate mailed along with their diploma upon graduation. This includes recognition through the Southern Association of Colleges and Schools through UF and the American Veterinary Medical Association through the CVM.

We have worked towards improving our courses and our certificate over the years by adding mentorship from our faculty, more critical thinking aspects to our core required courses, and greater variety in assessment (including hands-on and experiential opportunities, compared to an exam only format). Moving forward we will work to improve our mentoring relationships, completion rates, and placement within aquatic and zoological fields.

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ANTHROPOGENIC CONTAMINANTS IN STRANDED CETACEANS IN THE SOUTHEASTERN UNITED STATES, 2012 – 2017

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A wide variety of contaminants are released into coastal marine environments from human activities, including manufacturing, pesticides, mining, smelting, waste incineration, and burning of fossil fuels in addition to their uses in plastics, detergents, paints, and antibacterial soaps. These anthropogenic contaminants in the marine environment biodegrade slowly, can bioaccumulate in organisms, and are known to have a number of reproductive, immune, and developmental effects on wildlife; however, the effects of some of these contaminants have not been extensively studied in cetaceans. This study aims to fill knowledge gaps about the quantities and biological effects of anthropogenic contaminants in stranded cetaceans in the southeastern United States during 2012–2017. Concentrations of toxic, trace, and necessary elements along with endocrine disrupting contaminants in blubber and liver samples of stranded cetaceans were measured, and gross and microscopic pathology data were retrospectively evaluated to identify demographic and/or pathologic data that may be linked to those contaminants. To accomplish this, we analyzed 64 cases of stranded cetaceans, including mostly odontocetes (e.g., *Tursiops truncatus*, *Mesoplodon europaeus, Kogia breviceps, Lagenorhynchus albirostris, Stenella frontalis, Grampus griseus*) and one mysticete (*Megaptera novaeangeliae*). Liver samples were evaluated for 19 toxic, trace, and necessary elements (Al, As, Ba, Be, Cd, Cs, Co, Cu, Fe, Pb, Li, Mg, Mn, Hg, Mb, Ni, Se, Tl, Zn) using inductively coupled plasma-mass spectrometry. Blubber samples were analyzed for five known endocrine disrupters (atrazine, bisphenol A [BPA], diethylphthalates, nonylphenol ethoxylates, triclosan) using gas chromatography-mass spectrometry. Complete reviews of demographic, pathologic, and toxicologic reports for each individual animal were performed, and compiled data were analyzed using basic statistics and multivariate logistic regression. In cases with relatively high concentrations of endocrine disrupting contaminants, we expect to see related pathology in targeted endocrine organs such as thyroid and reproductive tissues. In cases with relatively high concentrations of toxic, trace and necessary elements, we expect to see pathologies related to the toxicant of consideration (e.g., liver and kidney pathology). Initial analyses revealed positive correlations (≥ 0.7) between concentrations of mercury and selenium; mercury and cadmium; selenium and cadmium; and BPA and rubidium. The results of this study will provide information on lesions as a result of chronic exposure to these contaminants, which have not been thoroughly characterized to date.

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ASSESSMENT OF THE PREPARATION OF AQUATIC ANIMAL HEALTH VETERINARIANS FOR ENTRY-LEVEL POSITIONS

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Recent graduates (those who graduated between 2009 and 2014) from veterinary school in aquatic animal health (AAH) careers and their employers were surveyed regarding the preparation of these graduates for entry-level veterinarian positions. Technical and non-technical skills were both investigated. A 43-item novel survey instrument was developed and sent to 93 employers. The response rate was 72% and the respondents (n = 67) have been working in the AAH field for a mean of 19.5 years. The participating employers were then asked to provide contact information for their recent graduate hires over the past five years (including interns and residents) or forward a 36-item survey instrument (similar to the one they took) to them. At least 29 recent graduates received the survey and 86% of them responded (n = 25). Respondents in both groups represented government, universities, small business (including private practice and independent contractors), corporations and non-profits (including zoos and aquariums).

The majority of employers responded that the average recent veterinary graduate does not have the knowledge and experience needed in any of the aquatic taxon categories (mammals, fish, birds, invertebrates, reptiles, and amphibians) to meet their organization's needs. The largest deficiency is in fish medicine (both fresh and saltwater species). Alternatively, the majority of recent graduates felt they met the needs of their first position in all taxa except amphibians and invertebrates. Effective use of educational resources, including critically evaluating scientific literature, was viewed as more valuable by employers than an additional year of work experience in small animal medicine. This was not true for recent graduates. However, both groups agreed that effective use of resources is expected for most problem solving (58%) whereas the ideal percentage of memorized aquatics knowledge that a recent graduate would possess for problem solving is 42%.

Employers and recent graduates are mostly in agreement about the importance of non-technical skills. One notable difference was that Honesty/Integrity was most important to employers whereas Work Ethic was most important to recent graduates (both were viewed by employers as commonly possessed by veterinary colleagues). Teamwork/Interpersonal Skills was valued second most by employers yet listed first for commonly needing improvement. This was closely followed by Communication and then Time Management as areas in most need of improvement amongst DVM employees and colleagues. Yet, recent graduates mostly felt that they met or exceeded the need for all non-technical skills except Business Savvy.

This study not only investigated need-at-entry knowledge and skills for AAH veterinarians but also the elective experiences of those seeking AAH careers, job market outlook, and career satisfaction. Both consensus and divergences in the employer's and alumni perspectives are important to consider during veterinary medicine or continuing education curricula modification. Findings from this study could also be used to inform students' elective choices and career path decisions.

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GEOGRAPHICAL, ENVIRONMENTAL AND TEMPORAL DISTRIBUTION PATTERNS BETWEEN BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) AND PYGMY AND DWARF SPERM WHALE (*KOGIA* SPP.) STRANDINGS ALONG THE CENTRAL EAST COAST OF FLORIDA

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Marine mammal stranding events are important sources of data on cetacean biology, ecology, disease and mortality that are otherwise logistically challenging to obtain. Environmental conditions are considered important contributors to cetacean stranding events worldwide; however, few studies have examined how environmental factors affect stranding patterns for cetacean species. The objective of this study was to assess temporal, spatial, meteorological and hydrological correlates of *Tursiops truncatus* (bottlenose dolphin) and *Kogia* spp. (dwarf and pygmy sperm whales) strandings along the east coast of Florida between 1998- 2014. Over half of the *T. truncatus* stranding events occurred in regions with a shore to shelf distance of 32.95-43.25 km. Low sea surface temperature and high barometric pressure, which are common in Florida winters, significantly increased the likelihood of a *T. truncatus* stranding event when compared to a *Kogia* spp. stranding. In contrast, *Kogia* spp. stranding frequency was lowest in areas with the widest shelf and relatively evenly distributed across shelf to shore regions between 2.04 km and 43.25 km. *Kogia* spp. were more likely to strand during high sea surface temperature and low barometric pressure, common in Florida summers. Wind speed and direction were not related to *Kogia* spp. or *T. truncatus* stranding events. Understanding the predictors of strandings may lead to improved management and allocation of resources for these scientifically valuable events. Additionally, the data provide a baseline to evaluate the potential impacts of a changing environment on the morbidity and mortality of cetacean populations.

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JUVENILE *TURSIOPS TRUNCATUS* CASE STUDY: MONOFILAMENT ENTANGLEMENT RECOVERY AND DAMAGE

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Florida Atlantic University Harbor Branch staff received a report of a juvenile *Tursiops truncatus* in the Indian River Lagoon, FL, entangled with monofilament fishing line wrapped around the maxilla. National Marine Fisheries Service and several veterinarians deemed this entanglement life threatening and a multi-agency disentanglement effort was successful. Over two years later, this dolphin's freshly dead carcass was recovered. The carcass was taken for immediate radiographic imaging. Photos and measurements of the scarring left from the entanglement were taken prior to necropsy. Bilateral maxillary dental arcade deformation was noted. The cleaned skull revealed a 2.5 cm long by 0.5 cm deep groove from the entanglement damaging the lateral edges of the maxilla. This case provides learning opportunities for the effects of monofilament line in future live animal entanglement cases.

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PATHOLOGICAL SURVEY OF PULMONARY DISEASE IN FREE-RANGING BRAZILIAN RIVER DOLPHINS

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The Amazon River dolphin (*Inia geoffrensis*) and tucuxi (*Sotalia fluviatilis*) are data deficient species. Despite very limited knowledge on health and disease aspects of these species, main threats to their conservation include incidental mortality in fishing gear, population fragmentation, habitat loss and environmental pollution. It is also suggested that underlying diseases may contribute towards their mortality rates. Pneumonia and other respiratory diseases have been recognized as important causes of death in cetaceans, but very limited and fragmented information regarding pathology of *I. geoffrensis* and *S. fluviatilis* is available in the literature. Aiming to partially fill in this gap of knowledge, we retrospectively analyzed gross and microscopic pulmonary lesions in free-ranging *I. geoffrensis* (n= 24) and *S. fluviatilis* (n= 28) carcasses collected in the areas of Amanã and Mamirauá Sustainable Development Reserves and Lake Tefé, Amazonas state, Brazil, from 1995 to 2016. Nearly 85% of the examined animals presented some kind of primary lung disease, wherein main etiological diagnoses were verminous pneumonia (25%), bacterial pneumonia (25%), and others (32.7%), including the first case of meconium aspiration syndrome reported in *S. fluviatilis* (1.9%). All verminous pneumonia cases in which it was possible to identify the nematode, were caused by *Halocercus brasiliensis* and we report the first case of *H. brasiliensis* infection in *I. geoffrensis*. We also observed pulmonary nematodiasis by *H. brasiliensis* in a neonate *S. fluviatilis,* further supporting the theory of transplacental infestation and widening the host record where this route of transmission appears to occur. Bacterial pneumonia was chiefly represented as suppurative bronchopneumonia and Gram-positive and Gram-negative coccoid and bacillary bacteria were noted within the inflammatory exudate. Although bacteriological analyses were not performed, in suspected diseased lungs with suppurative bronchopneumonia and/or pleuropneumonia we detected Gram-positive cocci, *S. iniae* infection was considered a highly probable etiology. Due to its zoonotic importance and highly prevalent artisanal fishery in the studied area, along with fresh fish consumption, public health concern is raised. Future studies may try to assess presence of this zoonotic bacterium in these areas and human risk exposure. Evidences of anthropogenic interactions, compatible with fishing activities, were observed in a remarkable number of carcasses, confirming reports on the high incidence of these interactions in the area. We also observed that for some of these human-caused injuries, there was a clear-cut link with lung disease. Further research is needed to better delineate the impact of lung disease in these riverine species. Our findings may provide scientific basis for future medical and conservation efforts on these Amazonian dolphins.

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THE ATLANTIC BOTTLENOSE DOLPHIN HEALTH AND ENVIRONMENTAL RISK ASSESSMENT PROJECT FROM THE SOUTHEASTERN USA: 2003-2015

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From 2003 to 2015, 360 free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus)* inhabiting the Indian River Lagoon (IRL, n = 246), Florida, and coastal waters of Charleston (CHS, n = 114), South Carolina, USA, were captured, given comprehensive health examinations and released as part of a multidisciplinary and multi-institutional study of individual and population health. The aim of this review is to summarize the substantial health data generated by this study which has resulted in over 95 scientific publications. The IRL and CHS dolphin populations are affected by complex infectious and neoplastic diseases often associated with immunologic disturbances and environmental exposure to anthropogenic contaminants. We found evidence of infection with cetacean morbillivirus, dolphin papilloma and herpes viruses, *Chlamydiaceae*, a novel uncultivated strain of *Paracoccidioides brasiliensis* (recently identified as the causal agent of dolphin lobomycosis/lacaziasis) and other pathogens. High concentrations of persistent organic pollutants including legacy contaminants (DDT, other pesticides, polychlorinated biphenyl compounds) as well as 'emerging' contaminants (polybrominated diphenyl ethers, perfluorinated compounds) were detected in dolphins from CHS, with lower concentrations in the IRL. Conversely, the concentrations of mercury in the blood and skin of IRL dolphins were among the highest reported worldwide and approximately 5 times as high as those found in CHS dolphins. A high prevalence of resistance to antibiotics commonly used in humans and animals was detected in bacteria isolated from fecal, blowhole and/or gastric samples at both sites, including methicillin-resistant *Staphylococcus aureus* (MRSA) at CHS. This is the first long-term study documenting the various types, progression, seroprevalence and pathologic interrelationships of infectious diseases and environmental exposures in dolphins from the southeastern USA. Collectively, these studies illustrate the importance of long-term surveillance of estuarine populations of bottlenose dolphins and demonstrate that the bottlenose dolphin is a valuable sentinel animal that may reflect environmental health concerns and parallel emerging public health issues. As a sentinel species, dolphins become an important ocean component of the emerging 'One Health' initiative.

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SMALL CETACEAN ENTANGLEMENT

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Current small cetacean interventions are conducted, under National Marine Fisheries Service (NMFS) permit, for 4 reasons: beaching, out of habitat, entanglement, and anthropogenic injury. As photo identification programs and boater awareness increases, the number of small cetaceans documented and reported in distress, either from entanglements or other reasons, increases. Besides out of habitat, non-beached interventions permitted are for obvious anthropogenic causes, entanglements and occasionally boat strikes.

Most interventions are multi-organizational responses conducted after NMFS has deemed that the animal's life is in danger. The process is initiated with photo documentation of a distressed animal submitted to the NMFS regional stranding coordinator who then consults experienced marine mammal veterinarians and biologists seeking advice on the risk to the animal's life. If, based upon these recommendations, NMFS deems the animal's entanglement or injury is life threatening, approval for an intervention is given and the regional stranding coordinator initiates a planning call. The date of the intervention is determined by personnel availability, weather conditions, the animal's condition, and animal sightings. Current interventions entail capturing the animal, photo documentation of the entanglement and injuries, disentanglement, medical evaluation, limited diagnostic sampling, possible administration of drugs, possible tagging, and release. Other uncommon but possible procedures and outcomes include more advanced diagnostics, such as radiographs, transporting the animal to a rehabilitation facility for long-term care or euthanasia.

Considerations for future interventions include expanded diagnostics to improve the understanding of the extent of morbidity and as an opportunity to collect a modified set of health assessment samples for immediate analysis and to bank for future use. In addition, thought must be given to interventions with other distressed animals, such as, pneumonia or emaciation, as there could be underlying, hidden anthropogenic causes of these conditions.

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CHARACTERIZATION OF A NOVEL *PEGIVIRUS* 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, integrated, multi-disciplinary research program designed to assess environmental and anthropogenic stressors, as well as the health and long-term viability of Atlantic bottlenose dolphins (BD). Health and risk assessments of dolphin populations are especially critical in areas where stocks are depleted or show signs of epidemic disease and/or high mortality, and in areas where the habitat is being intensely altered or impacted by human influences. Toward this goal, the HERA program and the University of Florida's Wildlife and Aquatic Veterinary Disease Laboratory (WAVDL) teamed up to conduct a viral survey of Indian River Lagoon bottlenose dolphins. As part of the HERA project, Indian River BD were sampled during health assessments from 2015. Serum, gastric, and urine samples were shipped to WAVDL where validated conventional and quantitative polymerase chain reaction assays were used to survey samples for a wide variety of known marine mammal viruses. Additionally, a viral metagenomic approach using an Illumina MiSeq platform resulted in the discovery of a novel RNA virus within the family *Flaviviridae* in serum samples. The nearly complete genome (9,649 bp) was recovered and phylogenetic and phenetic analyses based on the deduced amino acid polyprotein sequences revealed the BD virus is a novel *Pegivirus* branching near equine and porcine pegiviruses (hereafter referred to as the Bottlenose Dolphin Pegivirus; BDPgV). The amino acid sequence similarity of the BDPgV ranged from 50.3-53.8%, lower than the recently proposed species demarcation threshold (69%). Thus, we propose the BDPgV for consideration as a new species, to be known formally as Pegivirus L, pending acceptance by the International Committee on Taxonomy of Viruses. We are currently developing BDPgV specific assays to determine the prevalence (quantitative PCR and Enzyme Linked Immunosorbent Assay) and any association with disease (*in situ* hybridization) in wild and managed BD populations.

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CHARACTERIZATION OF POTENTIALLY ONCOGENIC VIRUSES IN BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) TUMOR TISSUES

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Oncoviruses have been characterized from neoplastic tissues in an increasing number of marine mammals. In bottlenose dolphins (BDs; *Tursiops truncatus*), herpesviruses and papillomaviruses have been detected in lingual, esophageal, genital, rectal and skin tumors. Aiming to improve our understanding of oncoviruses associated with tumor tissues in BDs, archived tumor samples were screened for viruses using a metagenomic approach. Tumor tissue homogenates were first centrifuged and filtered to remove bacteria and cellular debris. The filtrates were then digested with a mixture of DNases and RNases to remove nucleic acid not present in viral capsids. Total viral nucleic acid was then extracted using a commercial extraction kit. DNA and cDNA libraries were prepared using a previously published sequence-independent, single-primer amplification protocol and a Nextera XT DNA Library Prep Kit. The libraries were then sequenced on an Illumina MiSeq platform. The sequence data was assembled and the resulting contigs were screened against the GenBank non-redundant database. The full genomes of several novel herpesviruses and papillomaviruses were recovered, expanding the number of recognized BD viruses. The sequence data will be used in future studies to develop molecular diagnostic assays capable of rapidly detecting these novel papillomaviruses and herpesviruses in BD samples. Further research is needed to confirm casual links between the novel viruses discovered in this study and the BD tumor tissues they were recovered from. This study raises important questions about the prevalence, routes of transmission and potential health impacts of these novel viruses in free-ranging BD populations.

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HISTORICAL ECOLOGICAL CONTEXT REVEALS INSIGHTS INTO THE 2013 BOTTLENOSE DOLPHIN UNUSUAL MORTALITY EVENT AND LEGACY OF NUTRIENT LOADING

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The Indian River Lagoon (IRL) ecosystem has experienced three unusual mortality events (UME) in the last two decades, more than any other estuarine system in the United States. This is concerning not only for the bottlenose dolphin population resident to the IRL but also suggests larger ecosystem level concerns as well as a potential risk to human health in the region. To date the UMEs remain largely unsolved. In the most recent UME, 2013, the catastrophic loss of seagrass habitat in 2011-2012 and associated loss of prey was implicated as a possible causal factor. To test if the loss of seagrass habitat was associated with the 2013 UME and to provide historical ecological context for bottlenose dolphins foraging habits we performed stable isotope analysis on archived samples from the IRL from the early 1990s to present.

Samples were collected from stranded deceased bottlenose dolphins predominately in the northern Indian River and Banana River from 1993 to 2013. Muscle tissue was freeze-dried, lipid extracted, and homogenized then analyzed on an elemental analyzer interfaced to a rare isotope mass spectrometer.

Stable carbon isotope values differentiate benthic seagrass primary production from that of more pelagic phytoplankton sources. These differences are passed up the food web to top predators such as bottlenose dolphins. The stable carbon isotope values of bottlenose dolphins which stranded as part of the 2013 UME (2013: -16.2±1.0, n=51) were very similar to those which stranded directly before the UME (2009-2012: -16.0±1.2, n=60) showing no significant difference. While carbon isotope values tended to be slightly higher in the early 90s, there was no biologically significant trend from 1993 to 2013 (slope = -0.03 per year, n = 119, $p = 0.05$).

Stable nitrogen isotope values increase with each trophic step thus a top predator will have a higher nitrogen isotope value compared to that of lower consumers. Nitrogen isotope values of bottlenose dolphins which stranded during the 2013 UME (2013: 12.2±1.3, n=51) were like those which stranded directly before the UME (2009-2012: 11.9±1.1, n=60). Interestingly, over the study period nitrogen isotope values significantly declined (slope = -0.1 per year, n = 119, $p < 0.001$).

Results indicate dolphins that died during the 2013 UME had varied foraging habits using both seagrass and phytoplankton based food webs and were similar in foraging habits to dolphins who stranded prior to the UME. Based on this finding we conclude that the loss of seagrass habitat was unlikely to be a predominant casual factor in the 2013 UME as dolphin mortality was not segregated by foraging habitat. Thus, the 2013 UME remains unexplained and is deserving of further investigation. The decline in nitrogen isotope values across the study period may signify a positive step in the effort to remediate nitrogen pollution in the IRL ecosystem. Wastewater nitrogen is extremely high in its nitrogen isotope value. The decline from 1993-2013 corresponds to the remediation of wastewater treatment in 1996 and the elimination of direct discharge of wastewater into the IRL. The elimination of direct discharge was effective in removing this source of pollution throughout the food web although the amount of anthropogenic nitrogen in the IRL remains concerning and other form of nitrogen pollution are likely negatively impacting the ecosystem.

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TEMPORAL EVALUATION OF ANTIBIOTIC RESISTANCE FROM COMMON BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*), A SENTINEL SPECIES

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The increase in resistance to antibiotics among common bacterial pathogens is one of the most significant threats to public health. Increases in antibiotic resistance (AR) has been documented globally in isolates from humans, wildlife and the environment. As a result, a One Health approach to surveillance is essential. To date, few studies have examined long-term trends in AR in organisms isolated from marine mammal populations. The objective of this study was to examine temporal trends in resistance to antibiotics among pathogens isolated from common bottlenose dolphins (*Tursiops truncatus)* inhabiting the Indian River Lagoon, FL between 2003 and 2015. A total of733 isolates was obtained from the blowhole, gastric fluid and feces of 171 individual dolphins. The most commonly cultured pathogens included *Aeromonas hydrophila* (17.6%), *Plesiomonas shigelloides* (12.3%), *Pseudomonas fluorescens* (12.3%), and *Escherichia coli* (8.2%). The multiple antibiotic resistance index (MAR) was compared between two sampling periods for each organism and was significantly higher among *Pseudomonas aeruginosa* and *Vibrio alginolyticus during* 2010-2015 compared to 2003-2007. For all bacterial isolates, resistance to cefotaxime, ceftazidime and gentamicin increased significantly between sampling periods. The significant increases in resistance among some dolphin bacterial isolates observed in this study likely reflect shared environmental exposures to antibiotics and antibiotic resistant bacteria from terrestrial sources between dolphin and human populations. The study supports a recent recommendation by the World Health Organization to evaluate antibiotic resistance in key ecosystems that may represent a risk to health.

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MANATEE HEALTH ASSESSMENT BLOOD SAMPLES: KEY INFORMATION FOR LONG-TERM MONITORING AND UNDERSTANDING POPULATION HEALTH

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Health assessment studies of free-ranging wildlife allow for unique opportunities to collect comprehensive biological information using minimally invasive techniques such as blood, urine, feces, and skin sampling. The obtained data from targeted analyses provide a snapshot of representatives of a population in a geographical area under given environmental conditions. Florida manatees are exposed to a number of stressors, with the greatest threats to the population including watercraft collisions and loss of warm-water habitat. In addition, eleven mass mortality events have occurred in Florida since 1996. For the purpose of establishing baseline health data, understanding various stressors, and developing a long-term monitoring program for the Florida manatee population, conservation partners joined resources in 2008 to initiate live capture health assessments of free-ranging manatees in three different habitats in Florida. Ten years later, samples and biological data from 270 manatees have been collected, analyzed, and archived for future studies. Many publications have resulted from various sample analyses by investigators, including hematology, blood chemistry, hormones, nutrition, pathogens, epibiota, urinalysis, and toxicology. The initial establishment of baseline data of all these different analyses were crucial in detecting abnormalities, either in individuals or between groups of animals. Hematology and blood chemistry data is a widely used diagnostic tool in health assessments from any species. The data is useful in the evaluation of most organ systems through a minimally invasive and cost-effective method. Through the establishment of baseline data, it was possible to identify various findings in the Florida manatee population: methodology challenges in hematology, unique hematology and chemistry in nursing calves, blood work abnormalities in manatees affected by hypothermia, inflammatory markers in response to various underlying conditions, electrolyte differences between habitats that indicated changes in the environment, and the observation that reference intervals for some analytes have to be re-defined. The objectives of this presentation are to 1) provide an overview of what we have learned from manatee hematology and chemistry data so far and 2) discuss the use of this data in context of long-term monitoring of the health status in the Florida manatee population, including the identification of various stressors to individuals and the population. Using basic logical and statistical principles, long-term monitoring data allows for cautious extrapolation of data from individuals to the population level and can be used to support decision-making in conservation efforts and future needs for surveillance in changing environments.

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NOVEL "TIGER STRIPE" CONDITION IN FREE-RANGING BOTTLENOSE DOLPHINS

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A previously undescribed skin abnormality, referred to as "tiger stripes," has been identified in free-ranging bottlenose dolphins in the Indian River Lagoon, FL (IRL). The condition presents as parallel lines running dorsoventrally on the torso of the dolphin, which vary in length, width, and depth. The goals of this study were to determine the: 1) prevalence of the condition in IRL dolphins, 2) spatial and temporal distribution patterns, 3) age and sex and 4) duration of the condition.

The IRL is a 251 km brackish estuary, comprising 40% of Florida's eastern seaboard from Ponce to Jupiter Inlets. Images of individual dolphins, obtained during boat-based surveys from 1996-2015, were systematically checked for presence of tiger stripes and life history data (age, sex, reproductive status and home range) derived from photo-identification and stranding records.

Among the 1,380 non-calf individual dolphins identified photographically during the study period, 93 (6.7%) were documented with tiger stripes. The largest number of cases (37/93 or 39.5%) were observed from 2002-2003, concurrent with expanded survey coverage and the implementation of digital technologies. Prior to and thereafter, the number of animals that presented with these stripes remained stable at approximately four new cases annually. A preponderance of cases, 52/93 (56%), occurred in dolphins with home ranges in the northern and central lagoon. The majority of known sex cases were females, 48/58 (83%) of which 47/48 (98%) had given birth to one or more observed calves. Seven dolphins developed the condition during the course of the study and age of onset ranged from 3-17 (σ4.7) years. Eight dolphins were recovered dead in which the duration of the condition ranged from 1-12 (σ4.2) years prior to mortality. The precise etiology of this condition has not been established.

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UNDERSTANDING THE HEALTH AND POPULATION STATUS OF NORTH AMERICAN RIVER OTTERS: THE OTHER IRL "MARINE MAMMAL"

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The North American river otter (*Lontra canadensis*) is widely distributed throughout Florida but the population and health status of this species is not well studied in the state. As one of two top mammalian predators in the Indian River Lagoon, we propose that river otters may be an important sentinel species in the lagoon watershed as it epitomizes the land/water interface. Although river otters are not defined as a marine mammal under the MMPA, IRL otters should be considered along with manatees and dolphins as they all occupy similar habitats and rely on a healthy estuary and prey populations. To better understand the threats and status of this species, we have initiated a two-pronged study: 1) a citizen science program to collect otter sighting information throughout the area and 2) a collection program for dead otters modelled after stranded marine mammal programs. To date, we have collected 106 reports of live otters from 6 counties. We have synthesized data from 35 dead otters (33 vehicle strikes, one non-traumatic death and one orphaned animal that died in rehabilitation). Vehicular trauma cases were distributed widely throughout Brevard County and were less prevalent during the summer months (12%). Twenty-nine animals were examined for life history and health. The sex ratio for dead animals was essentially equal (14 M: 15 F, with 6 unknown). Juvenile and adult age classes were included with total length ranging from 79-125 cm for males and 87-114 cm for females. Samples were collected for histopathology, virology, parasitology, protozoal serology (*Toxoplasma*, *Sarcocystis*, *Neospora),* stable isotopes, stomach content analysis, cytology and viral serology. Gross necropsy results were consistent with vehicle strikes and included blunt force trauma with associated multi-organ rupture, fractured long bones, ribs, and skulls, proptosis, and associated soft tissue hemorrhage. Other signs of chronic and sub-lethal disease were apparent from histopathology and included: hepatitis (2/14), pneumonia (5/14), lymphadenitis (4/14), myocarditis (1/14), and parasitic associated pathologies (10/14). Sarcocysts were observed in myofibers without associated inflammation or myofiber degeneration.

These preliminary results represent the first study of the health and status of river otters in east central Florida and the most comprehensive study of Florida otters in recent years. These data will be important as threats to the Indian River Lagoon persist and as the human population modifies the landscape of Florida. Increased pressure from road construction, contaminant accumulation, loss of wetlands and pathogen pollution poses a significant threat to river otters. This study represents an attempt to understand these chronic and acute threats before the population is depleted and to understand how anthropogenic changes in the lagoon may result in changes in the health of predator and prey species.

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NEXT GENERATION RESTORATION OF THE ROSE BAY WATERSHED IN VOLUSIA COUNTY, FLORIDA

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More than half of the 5,800-acre urban Rose Bay watershed in East Volusia County, Florida is developed. Rose Bay benefitted from two decades of restoration activities that removed septic tanks and connected the adjacent Harbor Oaks community to sewer; replaced causeways with full span bridges to increase flow; and removed muck. As a result of that $50 million project, which ended in 2012, Rose Bay water quality improved and shoreline habitats began to recover.

However, intertidal areas remain degraded along the hard armored northern shoreline. Like many coastal communities, Harbor Oaks is grappling with sea level rise. The purpose of the subject restoration project is to provide this community's residents the opportunity to continue a legacy of supporting the Bay by moving restoration effort onto their own properties. Specifically, the methods to be used are exotic vegetation control; debris removal; storm water conveyance retrofit (achieved via installation of rain barrels, rain gardens, and/or backyard buffer areas); living shoreline creation in the intertidal zone water ward of any existing hard armoring; and long-term community-led monitoring and maintenance. The project scope for the initial phase spans 1.6 miles of Rose Bay's shoreline and consists of 52 privately held properties. Fifty of these are single-family homes, one is a trailer park, and one is a restaurant. There are also two city-owned parks that will receive improvements to enhance their shorelines and protect vegetation by creating designated walkways and/or dockage. Individualized plans have been created for each property.

All property owners have been contacted and given project information as well as a contract. Permitting and fundraising are in progress. The project will have rolling enrollment through the life of the permit so that homeowners may join at any time during the life of the project. Removal of debris and Brazilian pepper has begun on properties whose homeowners have joined the project. Mangroves are being grown from seed collected along neighborhood shorelines beginning in autumn 2017.

Multiple agency and NGO partners are engaged, but the support and involvement of the local community is paramount to project success. Education regarding living shorelines and their benefits to both ecology and property protection are essential for early participation. Sustaining project interest and commitment over a number of years is expected to be challenging but surmountable. Identifying residents or other community members to conduct monitoring and long-term maintenance will require ongoing effort. Lessons learned from this project can be used to create a template for other coastal communities interested in community-scale estuarine restoration and management.

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PROTEIN ANALYSIS OF NON-SPECIFIC GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR TO PROGRESS *IN VITRO* RESEARCH STUDIES IN FLORIDA MANATEE (*TRICHECHUS MANATUS LATIROSTRIS)* IMMUNOLOGY

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Plasma cytokine expression profiles and cytokine gene expression in tissues can be important implements in the evaluation of the status of Florida manatee (*Trichechus manatus latirostris*) immune systems. Among the cytokines is the granulocyte-macrophage colony-stimulating factor (GM-CSF), which influences a variety of immune cells. Granulocyte-macrophage colony-stimulating factor is of great relevance as it can be used for the *in vitro* production of dendritic cells (DC) for future research in therapies, clinical applications and diagnosis.

Modulating immune responses and bone marrow cell differentiation can be achieved with GM-CSF. Granulocyte-macrophage colony-stimulating factor has been shown to be fundamental for the generation of human and mouse DC. Dendritic cells are necessary immune system cells that can coordinate immune response and preserve host homeostasis. Despite these important functions, GM-CSF has received minimal attention in Florida manatee (*Trichechus manatus latirostris)* immunology field studies.

In Florida manatee (*Trichechus manatus latirostris*), the presence of GM-CSF has been predicted, but there are no reports of it being synthesized or made available *for in vitro* studies. Protein evaluation of non-specific GM-CSF should be considered to further progress manatee hematopoietic *in vitro* growth and studies in the field of immunology. Since specific GM-CSF is not available on the market, obtainable non-specific GM-CSF should be evaluated before considering it as a possible substitute. Therefore, protein analysis of non-specific GM-CSF related to Florida manatee (*Trichechus manatus latirostris)* was addressed.

Predicted GM-CSF sequences are available. Information on sequences producing significant alignments, determined with the BLAST program, was obtained from the National Center of Biological Information. These alignments show that, of all the species, the highest percentage peptide identity, compared to the Florida manatee (*Trichechus manatus latirostris)*, is the African Elephant *(Loxodonta Africana)* with 74%. This is a predicted sequence; therefore, the protein is not available. Granulocyte-macrophage colony-stimulating factor in wild boar *(Sus scrofa)* present*s 64%* identities with the predicted Florida manatee GM-CSF. Human GM-CSF shows 64% peptide percentage identity; while the mouse *(Mus musculus)* has 50% identity with Florida manatee (*Trichechus manatus latirostris)* predicted GM-CSF. Recombinant human and mouse GM-CSFs are available.

Interaction between these non-specific GM-CSFs and the Florida manatee (*Trichechus manatus latirostris*) GM-CSF receptor is a possibility. Due to the lower percentage identities of human and mouse GM-CSF, *in vitro* studies on the functionality of non-specific GM-CSF need to be considered and evaluated. Granulocyte-macrophage colony-stimulating factor *in vitro* studies will increase understanding of cell immunology and improve health assessments of Florida manatee.

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CETACEAN FIELD IMAGING ENHANCEMENT FOR HEALTH ASSESSMENT OF WILD SMALL CETACEANS UTILIZING A PORTABLE WIRELESS RADIOGRAPHIC SYSTEM, MULTIUSE WORK STATION PAD AND UNIQUE BUCKY SYSTEM

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The determination of "healthy" in all animals, including those under human care and wild cetaceans is dependent on a number of associated factors. These include expectations of the medical evaluator, standardization of diagnostic parameters, recognized common components of a health evaluation, equipment available for use, and animal handling access including transportation limitations. The foundation of the expansion of applications is a collaborative approach between medical and non-medical personnel. Field adaptation of equipment such as portable anesthesia machines, monitoring equipment and imaging technology such as ultrasound has widened the definition dramatically. Improvements in miniaturization and capability of machines, portability and less reliance on focal power sources now allows expanded field applications of high detail radiographic systems. The VetRocket X1 system was adapted to boat-based health evaluations with the development of a combination pad and bucky tray to facilitate its use within the physical confines of the boat and time limits on health assessments. The work station/bucky tray includes a pad for the animal's workup prior to radiographic application and a built-in unique bucky tray to allow movement of the plate under the animal, allowing the operator to image 4 quadrants of the dolphin in a dorsal-ventral or lateral position without moving the patient. The first phase required innovations in engineering to allow maximization of detail, tray and plate relocation for each image, and a decreased handling time with the patient. During Sarasota Dolphin Research Program health assessments of bottlenose dolphins, animals up to 240 kilograms have been successfully imaged and the technique has shown the value of radiographic evaluation in the determination of health of wild dolphins.

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INTEGRATION, COLLABORATION, PARTNERING AND SYNERGY: BIOLOGIST, FIELD SCIENTIST AND WILDLIFE VETERINARIANS WORKING TOWARD LONG TERM CONSERVATION PROGRAMS

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Aquatic environmental and animal research programs, concerned with the state of the wild environments, and emanating from a multitude of agencies and facilities, have historically been pursued as more organizational centric endeavors. Research "partners" have varied from providers of data or samples to full coordinated equal participants.

While there is a higher level of collaboration of field biologist/researchers/veterinary/agency partnerships, they are still impacted by common human and organizational characteristics including internal and external competition, and ambition. These characteristics are fostered by principles and organizational pressures that do not fully recognize the value of, or need for, interdisciplinary multi career programs and their positive effects on long-term conservation needs of the environment and its inhabitants. Mitigation of human impediments to long term eco-animal-human programs while daunting and widespread, should be addressed and evolving remedies discussed, evaluated, and acted on to infuse continual change into the scientific, government, educational and public cultures. To change our habits and ideas that are supported by our professions or inhibited by policies requires understanding the tendency to think in the short term to the next paper and not be more concerned about the future of the subjects being studied. Challenges to long-term thinking include cognitive bias traits that humans and organizations develop over time and may fail to recognize. Confirmation bias and in-group bias, the latter which leads us to overestimate the abilities and value of our immediate group impair our ability to look at opportunities with ideas or groups outside of our profession or research area that may be even more valuable. As an example, we may be dedicated to the need to appropriately keep biologic samples in a -80 freezer for analysis for a project, but not to maintain those samples beyond allowable time limits imposed by systems where priorities may be geared to availability of space or immediate maintenance costs. With never before seen levels of loss and change of the natural world, comparative environmental or animal biological sample retention should be viewed on a multigenerational approach and not limited to a personal career. With changes in personnel or retirement, irreplaceable samples are lost to short-term thinking or used for low yield bursts of productivity. Centerpiece biological samples should be maintained for multi 100 year timelines so new technologies and shifting priorities can directly compare samples in succeeding generations and centuries. Regional sample repositories or archives should be assembled like the Norwegian Svalbard Seed vault and the basic concepts utilized by NIST that include animal and ecological samples to evaluate disease, intoxication and genetic materials. This approach would require an unparalleled collaborative effort between and across all parties.

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PHYLOGENOMIC DIVERSITY OF NORTHERN HEMISPHERE 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 six CeMV strains: dolphin morbillivirus isolated from a Mediterranean striped dolphin (*Stenella coeruleoalba)*, dolphin morbillivirus from a bottlenose dolphin (*Tursiops truncatus*) in the Gulf of Mexico, porpoise morbillivirus isolated from a harbor porpoise (*Phocoena phocoena*), and beaked whale morbillivirus from Longman's beaked whale (*Indopacetus pacificus*). The phylogenomic diversity of the CeMV strains were compared to the six other morbillivirus species: *Measles virus*, *Rinderpest virus*, *Peste-des-petits-ruminants, Phocine distemper virus, Canine distemper virus, and Feline morbillivirus.* Phylogenetic trees were constructed in IQ-Tree and nucleotide sequence identity matrixes were constructed using Sequence Demarcation Tool. The six CeMV strains formed a well-supported clade. Beaked whale morbillivrus was the most divergent and formed the sister taxon to the rest of the CeMVs. Although considerable sequence variation was detected among the six, the magnitude of the difference was suggestive of separate CeMV strains rather than separate morbillivirus species. Although preliminary, recent detections of a high divergent morbillivirus from the Southern Hemisphere 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 in developing improved CeMV molecular diagnostics, and a better understanding of the temporospatial dynamics of these emerging marine mammal pathogens.

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LONGITUDINAL BOTTLENOSE DOLPHIN HEALTH ASSESSMENT AND POPULATION MONITORING IN SARASOTA BAY, FLORIDA

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As top predators in estuarine ecosystems, common bottlenose dolphins (*Tursiops truncatus*) integrate across the entire range of trophic levels as they respond to changes in the environment. Coastal bottlenose dolphins face large scale impacts from natural and anthropogenic threats, including harmful algal blooms, fisheries, environmental contaminants, oil spills, and climate disruption. A "natural laboratory" situation established through long-term research in Sarasota Bay, Florida, provides unparalleled opportunities to investigate potential impacts of environmental changes on health and reproductive parameters in bottlenose dolphins. Research initiated in 1970 identified a resident community of about 130-160 dolphins, spanning up to five concurrent generations, inhabiting the area year-round and across decades. Most of the individuals are identifiable, and many are of known age (up to 67 years old), sex, maternal lineage, and in many cases, paternity. Sheltered waters facilitate monitoring of dolphin occurrence, ranging patterns, interactions with humans, and births and fates of calves, through regular photographic identification surveys. Shallow, calm waters and a highly experienced team (and many highly experienced dolphins) allow for safe capture-release operations for veterinary examinations and to obtain biological samples for life history and genetic analyses, health assessment, and contaminant concentration measurement. Health assessments occur as warranted by research questions or conservation needs. Health assessments conducted in most years during 1988-2017 have established Sarasota Bay resident dolphins as a reference population for comparisons in investigations of populations that are at-risk or that have been impacted by large-scale anthropogenic events or unusual mortality events. In additional to providing basic health and life history data, Sarasota capture-release efforts also provide opportunities for: 1) testing new research approaches, tools, and techniques, in some cases in advance of application to at-risk populations, 2) perform follow-up assessments of dolphins for which interventions have been conducted previously, 3) conduct studies of acoustic communication and responses to disturbance, 4) training of veterinary, stranding response, researchers from other countries, and law enforcement personnel, 5) marking of individuals for long-term monitoring, and 6) archiving samples for future analyses. Recent health assessments have included more than 40 projects, with more than 90 participants in the field each day.

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LONG-TERM RESEARCH AND CONSERVATION PROGRAMS – VALUE AND CHALLENGES

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The fields of marine mammal science and stranding response, rescues, and rehabilitation are maturing, with many programs having been in existence for decades. Over time, they have developed expertise, collaborations, and resources that have increased their research and/or response capacities, thanks in part to support from sources such as NOAA Prescott grants and philanthropic recognition of success. Without compromising the core and vital missions that led to the initial establishment of the programs, some are also able to now look beyond the next funded research project or rescue, to the broader, emerging and longer-term issues facing marine mammals, and make contributions toward conservation at larger scales, through long-term research and/or education and outreach. As one example, the Sarasota Dolphin Research Program (SDRP) conducts the world's longest-running study of a wild dolphin population, and works in partnership with Mote Marine Laboratory's (MML) Stranding Investigations Program to provide "cradle-to-grave" service to local dolphin populations, as well as developing knowledge and expertise that benefits dolphin populations elsewhere. The SDRP conducts monthly photo-ID surveys and seasonal fish surveys to monitor the long-term resident bottlenose dolphin community and its prey base. These are supplemented by health assessments, biopsy sampling, tagging and tracking, focal animal behavioral observations, an acoustic recording station network, disentanglements, and rescue captures, as warranted. Combined with MML's stranding response and rehabilitation operations, and HAB monitoring, these efforts have the goal of enhancing protection of dolphin populations, and involve: 1) the establishment of a reference population for comparative investigations of at-risk populations elsewhere, 2) development of reference health parameter values to benefit dolphins under human care, 3) surveillance, facilitating discovery of disease, as well as timely detection of injuries and entanglements that would benefit from interventions, and as the basis for follow-up monitoring, 4) compilation of long-term datasets on dolphins, prey, and environmental variables for trend detection, 5) archiving of biological samples for subsequent analyses including retrospective studies as new concerns emerge and new assays are developed, 6) opportunities to test or refine new approaches, techniques and tools, 7) maintaining readiness with a trained team and field equipment, 8) opportunities to train personnel to build capacity in other regions/countries, and 9) understanding the relative contributions of different sources of mortality and serious injury within the resident population. Findings from these field and laboratory efforts feed into education and outreach programs targeted to mitigating anthropogenic threats, and are used by wildlife management agencies. Long-term datasets, surveillance, and response readiness will be of increasing importance for dolphin conservation throughout Florida and beyond, especially in light of increasing impacts from humans on coastal environments, and resistance by segments of the public and government to acknowledge these linkages. Non-governmental research and stranding response programs are in a unique position to leverage their established presence and capabilities to make a difference for these animals through continuing and expanding research and response efforts, developing the collaborations necessary to optimize the information resulting from these efforts, and disseminating the information necessary for evidence-based conservation decisions.

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MOLECULAR CHARACTERIZATION OF CETACEAN SKIN – LAYING THE FOUNDATION TO DEVELOP CUTANEOUS BIOMARKERS

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The integument is a critical organ in immune surveillance and response to environmental changes. As such, cellular and molecular changes in the skin can provide early markers of environmental or pathogenic insults to animals and human populations. Studies of wound healing in humans have discovered predictable biomarkers of injury, tissue repair, and healing outcomes that can be applied to marine mammals. The rapid epidermal turnover rate in cetacean results in the superficial appearance of a healed wound; however, the basal epidermal and dermal layers may not be fully repaired. Cellular and molecular features of these wounds can potentially be used as diagnostic markers of injury etiology that can ultimately provide information on environmental or pathogenic changes in the oceans to which cetaceans are exposed. Using comparative genomics, we identified cetacean genes homologous to human wound healing markers including epidermal keratins and components of the Wnt signaling pathway. To establish foundation for cetacean cutaneous biomarkers we have performed cellular and molecular characterization of archived tissue from normal and wounded skin of the beaked whale (*Mesoplodon spp*). Marker for basal cells keratin 5 (K5) and keratinocyte activation marker K17 were found induced in wounded skin. To further assess the status of the Wnt signaling pathway, which has been shown to correlate with healing outcomes and stem cell activation, we performed immunofluorescent staining using an antibody that recognizes the phosphorylated, activated form of β-catenin. We observed prominent nuclear localization of phosphorylated β-catenin throughout parakeratotic epidermis of non-wounded skin, and further induction in wounds. These data indicate activation of the keratinocytes and epidermal stem cells in cetacean skin in response to wounding and stress. Our data provide foundation for further application of the cutaneous markers for detection of pathogenic exposure or marine mammals.

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