



*The 2009 International*  
**SIRENIAN CONSERVATION**  
*Conference*

March 23-24, 2009

**HARBOR BRANCH**

FLORIDA ATLANTIC UNIVERSITY



GEORGIA AQUARIUM

# WELCOME

Welcome to Atlanta! On behalf the Georgia Aquarium and Harbor Branch Oceanographic Institute at Florida Atlantic University, We would like to welcome all of you to the 2009 International Sirenian Conservation Conference.

This meeting is the next progression from the First International Manatee and Dugong Research Conference held in Gainesville Florida, March 11-13, 1994. It has been a long 15 years since our first meeting and an abundance of new information on sirenian natural history, anatomy, physiology, genetics, medicine and conservation is now available.

Specifically, this two-day international conference will combine a variety internationally known experts to share a broad spectrum of manatee conservation issues from a global perspective.

The conference will be comprised of three moderated sessions that include Current Field and Laboratory Research, Management Perspectives and Veterinary Aspects of Rehabilitation and Release.

Additionally, four student travel awards have been made to encourage and expose young researchers to a wide variety of sirenian experts.

Special thanks go to Juli Goldstein, DVM and Mr. Stephen D. McCulloch of Harbor Branch Oceanographic Institute who have organized an exceptional program for us, and to the Georgia Aquarium who have provided such a beautiful environment for this conference. We also wish to acknowledge and thank the Mathews Estate for providing funding assistance for this meeting.

Finally, thank you to the manatees and dugongs we have known over the years that have made our lives so much better.

Gregory D. Bossart, V.M.D., Ph.D.  
Senior Vice President and Chief Veterinary Officer  
Georgia Aquarium  
Affiliate Professor-Marine Mammal Research and Conservation Program  
Harbor Branch Oceanographic Institute at Florida Atlantic University

Juli D. Goldstein, D.V.M.  
Staff Veterinarian – Marine Mammal Research and Conservation Program  
Harbor Branch Oceanographic Institute at Florida Atlantic University

Stephen. D. McCulloch  
Founder / Program Manager - Marine Mammal Research and Conservation Program  
Harbor Branch Oceanographic Institute at Florida Atlantic University

## **About the Conference Organizers:**

### **Juli D. Goldstein, DVM**

Dr. Juli D. Goldstein has been actively involved in the marine mammal field for the last 7 years. In 1999 she graduated from Auburn University's College of Science and Mathematics with a Bachelor of Science Degree in Zoology and pre-veterinary medicine. She was then accepted to the Auburn University College of Veterinary medicine where she received her Doctor of Veterinary Medicine degree in 2003. Dr. Goldstein has completed extensive training courses and veterinary externships that focused exclusively on the husbandry, care, assessment, and medical treatment of stranded and captive marine mammals. She has been an active member of the Harbor Branch Oceanographic Marine Mammal Research and Conservation group since 2003. Dr. Goldstein has also provided specialized veterinary services for the last 5 years of the Indian River Lagoon Live Dolphin Health and Risk Assessment Project (2003-2007), and has published in several peer-reviewed journals on research data related to the health of marine mammals. She currently serves as the HBOI Staff Veterinarian and attending clinical veterinarian for the HBOI/FAU Institutional Animal Care and Use Committee.

### **Stephen D. McCulloch**

Steve began his career collecting and training marine mammals in the Florida Keys in 1971, and has more than 30 years experience working within the marine mammal industry and research community. During this time, he has contributed to dozens of special projects, films, research and health assessment programs and several journal publications. In 1997, Steve founded the division of Marine Mammal Research and Conservation at Harbor Branch Oceanographic Institute and worked to establish programs that support several multi-disciplinary scientific and conservation initiatives. In order to fund such initiatives, Steve created and passed three Florida State Laws and worked to create the Florida Oceans Initiative. Since 1999, Steve has raised more than \$30 million dollars to support various marine mammal research and related conservation programs throughout the State of Florida. As a scientific investigator, Steve has been conducting monthly photo-id surveys along 40% of Florida's East coast and has served in a primary role to provide regional stranding response since 1997. During this time, Steve has responded to more than 300 stranding events, has rehabilitated and released more than a dozen dolphins back into their native environment, and is often called upon by the National Marine Fisheries Service to lead open-water interventions of entangled, injured or out-of-habitat marine mammals. Steve also oversees and manages the planning and complex logistics of annual live dolphin health and risk assessments involving the safe capture, sampling and release of more than (240) dolphins, and presently, serves as the Program Manager for the Harbor Branch Marine Mammal Research and Conservation Program, the Protect Wild Dolphins and the Protect Florida Whales specialty license plate programs in the State of Florida. In 2008, Steve was officially recognized by the Wyland Foundation as an "Environmental Ambassador", was appointed to serve as a Scientific Advisor to the Guy Harvey Ocean Foundation, and was also one of (29) conservationists worldwide to be nominated for the prestigious Indianapolis Prize for Global Conservation initiatives.

**Gregory D. Bossart, V.M.D., Ph.D.**

Dr. Gregory Bossart has spent the last 29 years working in clinical domestic, marine mammal and avian medicine and wildlife pathology on a national and international basis. He has over 100 publications focused primarily on the pathologic basis of disease in wild animals. His undergraduate degrees in biology and physical geography are from the University of Pittsburgh. He received his doctorate in veterinary medicine from the University of Pennsylvania. From 1981-1985, he was a comparative pathology resident and NIH fellow in the Department of Pathology at the University of Miami School of Medicine. In 1995, he completed his Ph.D. in immunology at Florida International University. He has been in private veterinary practice and presently is a clinical veterinary consultant at facilities in the US, Latin America and Asia. Since 1981, he has been the Medical Director at the Falcon Batchelor Bird of Prey Center at the Miami Museum of Science. Presently, he is Senior Vice President and Chief Veterinary Officer at the Georgia Aquarium in Atlanta Georgia where he oversees the animal care, research and conservation programs. He is an Adjunct Professor in the Department of Pathology at the University of Miami School of Medicine, Research Professor at Harbor Branch Oceanographic Institute at Florida Atlantic University and on the graduate faculty at the Medical University of South Carolina. His recent published research has documented re-surfing and emerging diseases in manatees, whales, dolphins, and birds. He has helped characterize the first viral disease in manatees and was responsible for developing the first immunohistochemical technique for diagnosing brevetoxicosis (red tide poisoning) in marine mammals and birds. He is particularly interested in the application of aquatic species as sentinels for the effects of global climate change, ecosystem and human health. Dr. Bossart was awarded the Dean's Clinical Research Award for his work at the University of Miami School of Medicine. He has collaborative research projects with the National Marine Fisheries Service, NOS, Florida Wildlife Research Institute, National Institute of Environmental Health Sciences and the Miami Museum of Science. Examples of his research can be found in the *Journal of the American Veterinary Medical Association*, *Veterinary Pathology*, *Journal of Zoo and Wildlife Medicine*, *Veterinary Record*, *Journal of Avian Medicine and Surgery*, *Toxicologic Pathology*, *Marine Mammal Science*, *Experimental and Molecular Pathology*, *Aquatic Mammals*, *Florida Scientist*, *Journal of Raptor Research*, *Journal of Veterinary Diagnostic Investigation*, *Oceanography*, *Journal of Wildlife Diseases*, *Environmental Science & Technology*, *Journal of Parasitology*, *Aquatic Toxicology* and *Nature*.

## **About the Sponsors:**

### **Manatee Mathews Fund**

Primary funding for this conference was acquired by the generosity of two benefactors: The late Mrs. Norma Mathews and The Marine Mammal Center of California. Mrs. Mathews lived in Ohio and she loved animals. Her will designated the following beneficiaries to receive the proceeds from her estate:

Best Friends Animal Society in Kanab, Utah  
Dedication and Everlasting Love to Animals (DELTA) in Glendale, CA  
The Marine Mammal Center

She specified that the gift to The Marine Mammal Center was to be used for the benefit and preservation of manatees. Since manatees are not in the geographic area of The Marine Center, Dr. Frances Gulland generously recommend that the funds be split between Harbor Branch Oceanographic Institute's Marine Mammal Research and Conservation Program and the Mote Marine Laboratory. We at HBOI MMRC felt that a meeting of international conservationists was the perfect way to honor her wishes and advance the treatment and protection of Sirenians.

### **About the Marine Mammal Center:**



The Marine  
Mammal Center

The Marine Mammal Center has been in operation since 1975 and it is the only facility between Mendocino and San Luis Obispo counties (600 miles of California coast line) that is federally authorized to rescue and rehabilitate marine mammals. The Center's physical operations began with a handful of pens and pools for its patients, and freight containers used as make-shift offices for staff. Many thousands of animal rescues later, it has grown into a veterinary teaching hospital that integrates day-to-day rescue and rehabilitation work with that of scientific research and marine science education.

The Center's facilities include a rehabilitation hospital, lab and administrative offices in the Marin Headlands in Sausalito (Marin County); field offices at Moss Landing (Monterey County), Morro Bay (San Luis Obispo County) and Anchor Bay (Mendocino County); and a retail store and educational kiosk at San Francisco's PIER 39.

# HARBOR BRANCH

FLORIDA ATLANTIC UNIVERSITY

*Ocean Science for a Better World™*

## **Marine Mammal Research and Conservation Program Harbor Branch Oceanographic Institute at Florida Atlantic University**

The Marine Mammal Research and Conservation Program (MMRC) has multifaceted veterinary medicine, research, education, and conservation programs involving marine mammal species from around the world. Current research programs are documenting causes of marine mammal strandings; health status of bottlenose dolphins (*Tursiops truncatus*) inhabiting the Indian River Lagoon, Florida (IRL); effects of harmful algal bloom biotoxins; population ecology of the IRL bottlenose dolphin population through a photo-identification study; and identification of emerging diseases in various marine mammal species. MMRC's educational program includes M.S. and Ph.D. marine mammal studies in the areas of molecular biology, pathology, and epidemiology. MMRC is an educational component of the internationally recognized Envirovet program.

The MMRC has conservation outreach programs established in Latin America that provide veterinary care to aquatic mammal species while at the same time training local caregivers to provide future care on their own. Past conservation outreach programs involving Antillean manatees (*Trichechus manatus manatus*) and Amazonian manatees (*Trichechus inunguis*) were conducted in Veracruz and Villahermosa, Mexico; Georgetown, Guyana; Leticia, Colombia; and Tefe, Brazil.

## **Harbor Branch Oceanographic Institute at Florida Atlantic University**

For over four decades the shared vision of founder J. Seward Johnson, Sr. and inventor Edwin Link to explore, protect and wisely use the oceans' resources shaped the work at Harbor Branch Oceanographic Institute. Today, articulated as Ocean Science for a Better World™, this same vision drives over 140 Harbor Branch Oceanographic scientists, engineers and support staff to be leaders in ocean-related innovation, exploration, research, education and conservation. In the deep sea, our ship and research submersibles enable us to explore and work in depths as great as 3,000 ft. Our scientists study and unravel the oceans' mysteries and identify unique organisms that are evaluated for their disease-fighting potential. Closer to our coastline, we study deep and shallow coral reefs, sea grasses and marine mammals and how these communities are affected by human impacts. On land, our aquaculture team works on new ways to farm seafood, and our engineers support research and exploration missions with custom-designed platforms, vehicles, tools and instruments. In the classroom, our specialized graduate and undergraduate programs in marine science and biotechnology give students a chance to augment textbook learning with the resources of a leading oceanographic institute. In 2007 Harbor Branch became a research institute of Florida Atlantic University, paving the way to expand opportunities in ocean technology, coastal and deep sea exploration and research, marine biotechnology, aquaculture, ocean and human health, and marine science education.



## ABOUT GEORGIA AQUARIUM

**Georgia Aquarium** opened in November 2005 as the world's largest aquarium with more than eight million gallons of water and the largest collection of aquatic animals. The Aquarium, a 501(c)3 nonprofit organization, is a \$250+ million gift to the Atlanta community and the people of the state of Georgia from Bernie Marcus, co-founder of The Home Depot, and his wife Billi, through the Marcus Foundation. The Aquarium in total is a \$320+ million facility. As a result of Marcus' generous gift, the Aquarium opened debt-free.

**The Georgia Aquarium** houses the world's largest aquarium exhibit, Ocean Voyager, built by The Home Depot. Ocean Voyager houses the world's largest fish, the whale shark, along with a manta ray, the only one in the United States. The Aquarium is the only one outside of Asia to house whale sharks. The Aquarium is also home to beluga whales, southern sea otters, a loggerhead sea turtle, hammerhead sharks, sand tiger sharks, giant grouper, bowmouth guitarfish, jellies and tens of thousands of other aquatic animals. In 2008, the Aquarium announced the expansion of Dolphin Lagoon, a 1.8 million gallon exhibit featuring bottlenose dolphins, scheduled to open in late 2010.

### **Research**

The Correll Center for Aquatic Animal Health is a state-of-the-art animal health facility with more than 10,500 square feet and was designed by world-class veterinary professionals and conservation organizations. Currently, The Georgia Aquarium is the only facility that has opened with a program which is an integration of an aquarium and veterinarian teaching hospital. The partnership with the University of Georgia (UGA) Veterinary Teaching Hospital will allow the Aquarium to provide complete aquatic animal pathology program while training veterinary residents, interns and veterinary students. The Aquarium provides the same veterinary diagnostic, medical treatment and surgical services that you would find at the best veterinary teaching hospitals.

**The 4R Program** (Rehabilitation, Responsibility, Rescue and Research) is designed to give people the unique opportunity to make a positive difference in the health and well-being of aquatic life around the world. Donations made to the 4R Program fund programs like the relocation and rehabilitation of a beluga whale and California sea lions.

### **Educational Programs**

The educational experience at the Georgia Aquarium is an innovative approach to the traditional field trip. In addition to exploring the main floor of the Aquarium, The Learning Loop gives students their own level of the Georgia Aquarium, and the Georgia Aquarium is the only aquarium in the U.S. to dedicate this type of space solely to the educational benefit of school-aged students and teachers. As part of the education program, students are engaged by animal encounters, interactive activities and research applicable to real-world situations. Aquarium programs are also tailored to meet Georgia Performance Standards and national curriculum standards.

### **Visitor and Economic Facts**

To date, more than eight million guests have visited the Georgia Aquarium. A Georgia State study estimated that the Aquarium will have an impact of between \$1 billion and \$1.5 billion on the state of Georgia in its first five years of operation. Tom Bell of Cousins properties estimates \$3 billion in construction development in the immediate vicinity of the Aquarium since 2005. Additionally, the number of nights visitors choose to stay in Atlanta has increased, according to the Atlanta Convention & Visitors Bureau.

### **On-site Facilities**

The Coca-Cola Company donated the 9 acres the Aquarium currently sits on. The Aquarium has more than 25,000 square feet of dedicated event space including a 16,400 square foot ballroom with windows in the Ocean Voyager and beluga whale exhibits. Wolfgang Puck Catering serves as the “dining dream team” for special events at the Aquarium.



## **Dolphin Discovery**

Dolphin Discovery began in 1990 with the dream of creating unique interactive programs that would allow our guests to get closer to dolphins than they ever dreamed possible.

But we're no longer just about dolphins – with the best facilities, and offering you the most dynamic programs in the Caribbean, we want you to have The Experience of a Lifetime! while you interact with manatees, sea lions, stingrays and sharks too. Come and share in our love and fascination for these creatures and enjoy them as much as we do.

With locations in Mexico, on Cozumel, Isla Mujeres and at Puerto Aventuras, as well as Grand Cayman and Tortola, BVI we are ranked among the top dolphinariums in the world. Also our locations in Vallarta, Mexico (Sealife Park Vallarta)

We take our responsibilities seriously - Dolphin Discovery is an organizational member of the International Marine Animal Trainers Association, and our Cozumel facility is also a member of the Alliance of Marine Mammal Parks and Aquariums.



## ***Friends of Swallow Caye: A Note From Hayley Thomson***

*“Friends of Swallow Caye (FOSC) is a community based non-profit conservation association focused on the protection of manatees in the Swallow Caye area. The group was organized in 1996 to promote the designation of this area as a Wildlife Sanctuary.*

*The mission of FOSC is "to safe guard the future of manatees by reducing threats to their health and their habitat." FOSC has active members from Belize City, Caye Caulker and Ambergris Caye and its office is located on Caye Caulker. These members lobbied for the establishment of the Sanctuary. Success came in July 2002, when the Hon. Minister of Natural Resources signed the statutory instrument declaring the Swallow Caye Wildlife Sanctuary” (FOSC website”*

I visited Caye Caulker the summer 2008 and the winter 2009 and met with Chocolate Heredia - who I like to call the ‘manatee legend’ – to find out about Swallow Caye and interview him about his experiences working to conserve the Antillean manatee in Belize. They are working hard to manage the sanctuary and Swallow Caye and have patrols going out daily. One way they said I could help them at this time is to work on providing professional-looking t-shirts and hats for the patrol staff to wear so they have the same ‘official look’ and gain more respect in their work. I agree that such a step would provide them with a more official presence and allow them to be more easily identified. Thus, I encourage you to donate as much as is possible to help them on their way to achieving this goal – please keep your eyes open for the collection box during the conference (any questions, please email: [thompson.hayley@gmail.com](mailto:thompson.hayley@gmail.com)) Thanks! Hayley Thomson  
Current T-Shirt Designs:





**Keynote Speaker: Dr. Tom O'Shea**

*Fifteen Years Further Down the Road: An Arm-Chair Perspective on Advances and Challenges in Sirenian Biology and Conservation*

Tom O'Shea is a mammalogist who has conducted studies on sirenians, bats, and other groups of mammals. He has been involved in research in the United States, as well as in Africa, South and Central America, and Micronesia. He is the author or co-author of about 130 publications in scientific journals and books. Tom is currently a research wildlife biologist with the United States Geological Survey, Fort Collins Science Center in Colorado. His recent research emphasis is on bats, but from 1979 to 1992 he was a research biologist and Sirenia Project Leader in Florida for the U.S. Fish and Wildlife Service. Tom has held other federal positions as Assistant Director of the National Ecology Research Center, Fort Collins, Colorado (1992-1996) and Research Biologist at the Patuxent Wildlife Research Center, Laurel, Maryland (1977-1979). Tom has also held courtesy appointments at various universities and museums, and served on a number of advisory committees and commissions, including the Committee of Scientific Advisors to the U.S. Marine Mammal Commission, the federal Working Group on Marine Mammal Unusual Mortality Events, the Florida Manatee Technical Advisory Committee and the Florida Manatee Recovery Team. He has been an Associate Editor for the *Journal of Mammalogy* and chaired the standing Committee on Marine Mammals for the American Society of Mammalogists. He has a B.S. in Zoology from Colorado State University and M.S. and PhD. degrees in Zoology from Northern Arizona University, where he studied bats in Arizona and Kenya under Professor Terry Vaughan.

## March 23, 2009 Agenda

(\*\*) denotes student travel award winner

**8:00 am- 9:00 am: Breakfast Provided: Georgia Aquarium Arctic and Indian Room**

**9:00 am-9:15 am: Welcome and Opening Remarks: Arctic Room**

Dr. Juli Goldstein, Stephen McCulloch and Dr. Gregory Bossart

**9:15 am-10:00 am: Keynote Speaker**

**Dr. Tom O'Shea**- *Fifteen years further down the road: An arm-chair perspective on advances and challenges in sirenian biology and conservation.*

**10:00 am-10:20 am**

**John Reynolds** Dana L. Wetzel and W. E. Roudebush - *Manatee research and conservation at Mote Marine Laboratory: Same vision, new directions*

**10:20 am- 10:40 am**

**Martin de Wit** and Leslie I. Ward - *An update from the FWC Florida manatee necropsy and rescue program*

**10:40 am-11:00 am**

**Roberto Sanchez** -*The history of rescued manatees in Mexico where we came from and where we are*

**11:00 am- 11:20 am: Coffee Break**

**11:20 am- 11:40 am**

**James Powell**, Anmari Alvarez and Nicole Auil - *Manatee research and conservation in Cuba*

**11:40 am -12:00 pm**

**Camile Lugarini**, Camila R.G.C. Aerts, Carolina M. C. Alvite and Iran C. Normande  
*Analysis of ten years of monitoring Antillean manatee in Barra de Mamanguape Protected Area, Paraiba State, Brazil*

**12:00 pm-1:30 pm LUNCH**

**1:30 pm- 1:50 pm**

**Caryn Self-Sullivan** and Katherine S. LaCommare - *Conservation of Antillean manatees in the Drowned Cayes Area of Belize*

**1:50 pm-2:10 pm**

**Blanca Elizabeth Cortina-Julio**, Ibiza Martínez-Serrano, Domingo Canales-Espinosa and Enrique Portilla-Ochoa  
*Environmental education and participative planning for manatee conservation at the Alvarado Lagoon System, Veracruz, Mexico (1999-2008)*

**2:10 pm-2:30 pm**

**Arturo Serrano**, Iliana del Carmen Daniel-Rentería, Laura Vázquez-Castan and Miriam Ramos-Ramos - *Density, distribution, and manatee health status in the Alvarado Lagoon System, Veracruz state, Mexico*

**2:30 pm – 2:50 pm**

**Edward Keith**, Enrique Portilla-Ochoa, Alejandro Ortega-Argueta, Blanca Cortina-Julio, Crystell Contreras-Torres and Jesus Hernandez-Montero - *Status and recovery of the Antillean manatee in the Alvarado Lagoon System, Veracruz Mexico*

**2:50 pm – 3:10 pm**

**Hayley Thompson (\*\*)** - *Ongoing investigation looking at community involvement in the efforts to conserve Antillean manatee in Central America*

**3:10 pm- 3:30 pm: Coffee Break**

**3:30 pm- 3:50 pm**

**Jaymee Silva** - *The situation of Amazonian manatee in Iquitos-Peru and efforts for environmental education*

**3:50 pm- 4:10 pm**

**Hervé Magnin**, Sandra Pédurthe, Claude Bouchon, Dana Wetzel and John E. Reynolds  
*Possible reintroduction of the Antillean manatee (*Trichechus manatus manatus*) in the Grand Cul-de-Sac Marin (Guadeloupe, Lesser Antilles)*

**4:10 pm- 4:30 pm**

**Nataly Castelblanco-Martínez\*\***, Benjamín Morales-Vela, Héctor Abuid Hernández-Arana; Everardo Barba and Janneth Padilla-Saldivar - *Feeding ecology of manatees in Chetumal Bay, México*

**4:30 pm- 4:50 pm**

**Miriam Marmontel** - *Manatee research and conservation in the western Brazilian Amazon*

**4:50-5:00 pm Closing Remarks**

**6:00 pm- 8:00 pm: Atlantic Room**

**Wine and Cheese Ice Breaker Sponsored by Dolphin Discovery and Aquarium Tours**

The Georgia Aquarium will be offering special behind the scenes tours for participants. The tours will take you above the exhibits to see the working areas and the education facilities, and to the under-story life support systems, veterinary clinic, laboratory, and animal commissary. Groups of 15 can be accommodated and a sign up system will be available at the conference. Any children accompanying adults must be 7-years old or older. Photography is permitted.

## March 24, 2009 Agenda

**8:00 am – 9:00 am: Breakfast Provided: Georgia Aquarium Arctic and Indian Room**

**9:00 am-9:15 am: Intro and house keeping**

**9:15 am-9:35 am**

**Robert Bonde**, Margaret Kellogg, Peter McGuire, James Reid, Andrew Garrett, Martine deWit, Michael Walsh, Antonio Mignucci-Giannoni, Nicole Auil Gomez, James Powell Benjamin Morales and Janet Lanyon - *Manatee biogeography and sirenian health assessment*

**9:35 am-9:55 am**

**Arthur W. Wong**, Robert K. Bonde Jessica Siegal-Willott, M. Andrew Stamper, James Colee, James Powell, James P. Reid, Chip Deutsch and Kendal Harr - *Monitoring oral temperature, heart rate, and respiration rate of manatees exposed to capture and handling in the field*

**9:55 am-10:15 am**

**Benjamin Morales Vela** and Janneth Padilla-Saldivar - *Demography, ecology and health assessment of manatee population in Quintana Roo, Mexico*

**10:15 am – 10:35 am**

**Haydée M. Domínguez Tejo (\*\*)** - *Field research on Antillean Manatees in marine mammal sanctuaries of Dominican Republic*

**10:35 am- 10:55 am: Coffee Break**

**10:55 am-11:15 am**

**Pat Rose** - *A recipe for saving manatees and more*

**11:15 am- 11:35 am**

**James Reid**, Nilda Jiménez, Susan Butler, José Montalvo, Dan Slone, Jorge Saliva and Brad Stith - *Use of Argos-linked GPS tags to document specific habitat use patterns of manatees in eastern Puerto Rico*

**11:35 am-11:55 am**

**Dalila Caicedo-Herrera**, Jhoana M. Barbosa-Cabanzo, Yenyfer Moná-Sanabria, Natalia Gongora-Correa, Vivian Ochoa-Cardona, Rafael Espinosa-Forero and Antonio A. Mignucci-Giannoni - *Release and radiotracking of long-term semicaptive West Indian manatees (*Trichechus manatus*) in the Sinú River of Colombia*

**11:55 am-12:15 pm**

**Antonio A. Mignucci-Giannoni**, Raúl J. Rosario-Delestre, Mayela M. Alsina-Guerrero, Vera M. Rosado-Odom and Ivette E. Laborde =*Synoptic aerial surveys for West Indian manatees (*Trichechus manatus*) off the south coast of Puerto Rico—2001-2008*

**12:15 pm-1:45 pm: LUNCH**

**1:45 pm-2:05 pm**

**Margaret E. Kellogg**, Robert K. Bonde, and Peter M. McGuire - *Reduced genetic diversity and delineation of management units in *Trichechus manatus* populations*

**2:05 pm-2:25 pm**

**Coralie Nourisson**, Benjamin Morales-Vela, Janneth Padilla-Saldivar, Kimberly Pause, AnnMarie Clark, Robert Bonde and Peter McGuire - *Genetic studies and population structure of the West Indian Manatee (*Trichechus manatus manatus*) in Mexico*

**2:25 pm- 2:45 pm**

**Joseph C. Gaspard III**, Gordon B. Bauer, David A. Mann, Kimberly Dziuk, LaToshia Read, Adrienne Cardwell and Roger L. Reep = *Auditory and tactile detection by Florida manatees*

**2:45 pm -3:05 pm**

**Roger L. Reep**, Diana K. Sarko - *The neural basis for tactile hair sensation in manatees*

**3:05 pm-3:25 pm: Coffee Break**

**3:25 pm-3:45 pm**

**Michael Walsh** and Martine de Wit - *The manatee respiratory system in health, rehabilitation, anesthesia, and disease*

**3:45 pm -4:05 pm**

**Gregory Bossart** - *Manatee brevetoxicosis and cold stress syndrome: pathologic features of 'natural disease'*

**4:05 pm-4:25 pm**

**Shin-je Ghim**, Marie Gabriella Dona, Manuela Rehtanz, Annabel Rector, Marc van Ranst, Nicole Adimey, Robert K. Bonde, and Gregory D. Bossart and A.B. Jenson *Manatee papillomavirus (*TmPV-1*) infection among captive and free-ranging manatees.*

**4:25 pm-4:45 pm**

**Noel Y. Takeuchi**, Michael T. Walsh, Martine de Wit, Robert K. Bonde, and David S. Barber - *Evaluation of trace metals in the Florida manatee (*Trichechus manatus latirostris*) from the Gulf Coast*

**4:45 pm- 5:00 pm: Closing remarks**

Fifteen Years Further Down the Road: An Arm-Chair Perspective on Advances and Challenges in Sirenian Biology and Conservation

**Thomas J. O'Shea**, U.S. Geological Survey, Fort Collins Science Center, Colorado 80526-8118

In 1994 I delivered a presentation at the International Manatee and Dugong Conference held at the University of Florida. The talk was titled: "The road of the manatee: where has science taken us, and where will it lead?" The road of the manatee was a translation for the name given to the Milky Way Galaxy by the Warauno Indians of the Orinoco Delta, an analogy to its resemblance to the path of the nearly effervescent sediment plumes and boils that rise to the surface as manatees move surreptitiously across the bottom in still black-water lagoons. In that talk I gave a history of knowledge of manatee biology and the changing nature of threats to their populations from prior to European contact to the early 1990s. In the present talk I revisit some of those points, and expand the subject to include dugongs and extinct sirenians. I examine the literature that has appeared in primary scientific journals over the past 15 years (1994-2009) and characterize these advances in knowledge by broad fields of study. I compare published findings of the last 15 years with those that were published in the prior 15 years (1979-1993).

There has been an explosion in publication of results of sirenian research in recent years. Sirenian science involves increasingly specialized subdisciplines and increased collaboration. I give brief summaries of some examples of areas of research that seem especially noteworthy from the point of view of an arm-chair sirenologist. Examples include true breakthroughs in understanding the evolution of the Sirenia, an awakening to the importance of the influences of habitat and predation on dugong ecology and behavior, and a deeper understanding of the interactions between manatees and red tide at scales ranging from the molecular to the global. Nonetheless, I found disappointments in that knowledge of the extant sirenians remains highly biased by results from studies of the dugong (*Dugong dugon*) and the Florida manatee (*Trichechus manatus latirostris*). West African (*Trichechus senegalensis*) and Amazonian (*Trichechus inunguis*) manatees undoubtedly have much to tell us about sirenian biology and conservation and deserve more attention.

Despite this explosion of findings and increasing sophistication and collaboration in sirenian research, it is my opinion that the most stunning discovery in sirenian biology remains that of one person prepared with no specialized credentials, Georg Wilhelm Steller, who published his findings over 250 years ago. His discovery also provides a quintessential lesson in sirenian conservation. Therefore I describe the background, hardships endured, and significance of this event for those unfamiliar with its details. I close with speculation about the future challenges to sirenian conservation posed by climate change and an increasingly degraded and overpopulated world.

Contact information: Dr. Thomas J. O'Shea, U.S. Geological Survey, Fort Collins Science Center, 2150 Centre Avenue Building C, Fort Collins, Colorado 80526-8118. Phone: 970-226-9397. Fax: 970-226-9230. Email: tom\_o'shea@usgs.gov.

## Manatee research and conservation at Mote Marine Laboratory: Same vision, new directions

**John E. Reynolds, III**<sup>1</sup>, Dana L. Wetzel<sup>1</sup>, and W.E. Roudebush<sup>2</sup>

<sup>1</sup>Mote Marine Laboratory, Sarasota, FL

<sup>2</sup>Beckman Coulter, Inc., Chaska, MN

We live in an exciting time for research and conservation. Reports from groups such as the Pew Foundation and the President's Ocean Policy Commission underscore that the ways in which people have been doing marine science and management simply have not worked well. Those reports called for new partnerships, new approaches, and even whole new paradigms if people truly wish to ensure the viability and persistence of various taxa for future generations. More specifically, Ragen et al. (2002) noted that for manatees and other marine mammals, traditional approaches to assessing status provide an incomplete picture of the present or future prospects of species and populations.

At Mote Marine Laboratory, we are developing a three-pronged approach to maintain our commitment to provide enhanced science and conservation of manatees and other marine mammals. *Approach #1* represents traditional, long term research that a number of entities in Florida have done for decades (photo-identification, aerial surveys, behavioral ecology) or more recently (genetics). *Approach #3* represents an expansion of the policy and conservation efforts in which Reynolds, in particular, has been actively engaged through the Marine Mammal Commission, IUCN Sirenian Specialist Group, and science advisor to the UNEP Caribbean Marine Mammal Action Plan. Mote continues to actively support State and Federal partners by testifying at hearings and formal meetings, and continues to provide input to assist real-time enforcement efforts and permitting issues. Two projects of particular note with regard to novel conservation actions are: a) promoting acquisition of Warm Mineral Springs as a manatee sanctuary in perpetuity, and b) the innovative re-introduction project in Guadeloupe (Magnin et al., this conference). Mote/Reynolds are also intimately involved in helping to ensure that the imminent shutdown and repowering of critical power plants in Florida has no negative effects on manatees. *Approach #2* involves novel research and partnerships.

Although the conservation actions mentioned above will likely have more important long-term consequences than almost any particular research approaches, there may be exceptions. We have developed or are developing partnerships throughout the world to promote the use of biomarkers to demonstrate *effects* of human-related or natural stressors on sirenians and other marine mammals. For example a pair of markers developed to quantify fertility status of male and female humans works very well with manatees too. Preliminary assays, using serum generously provided by R. Bonde, show differential responses of males and females, large and small individuals, and individuals sampled at different seasons. Soon, we expect to be able to state what fertility potential of manatees is, as a function of location, contaminant burdens, nutritional status and other variables. More remarkably, we are setting up the only reference lab for these assays in the US, and we expect them to work equally well on other endangered mammals. Exciting times are truly here as we help promote enhanced ways to clarify species status and to promote informed conservation actions.

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An update from the FWC Florida manatee necropsy and rescue program

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The Florida Fish and Wildlife Conservation Commission (FWC) assumed responsibility for the State's manatee mortality and rescue program in 1985. Since then, the average number of reported manatee carcasses has tripled, with recent carcass counts ranging between 300 and 400 annually. The program provides important information for managers in their efforts to mitigate human-related causes of manatee death as well as a valuable tool to monitor the health of the population. The fraction of mortality due to watercraft represents a critical and long-term threat to the species that requires ongoing monitoring and continued development of forensic methods for improved wound diagnostics. Naturally occurring threats to manatee health include exposure to cold water and harmful algal blooms (i.e. red tide). While red tide has been recognized as one of the major natural sources of manatee mortality in the southwest region of Florida, the stability and longevity of warm-water refuges is one of the greatest threats statewide. Over the years, FWC's necropsy program has identified numerous appearances of cold stress disease, most of which are related to chronic exposure to cold, but some of which are more acute or possibly even peracute. An additional health concern during the cold season may involve the spread of pathogens when manatees are aggregated in warm water; however, until today there is no evidence of pathogens that have an impact on the manatee population. Numbers of perinatal mortality have followed the overall increase in carcass numbers. "Perinatal" deaths include aborted fetuses, orphaned or abandoned calves, or other natural causes. Over the last few years, several congenital defects have been documented, including umbilical hernia, skeletal defects, and atresia ani. Along with the carcass salvage, the number of rescued manatees has increased over the years. Both the rescue and mortality program have provided valuable insight in the pathophysiology of manatee diseases and continue to be an important monitoring tool for possible emergent health threats to the population.

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The History of Rescued Manatees in Mexico: Where we came from and where we are

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The modern history of manatees living under human care in Mexico goes back to the mid 1970s. During this time Lake Xochimilco in the urban area of Mexico City, received 2 or 3 animals with the idea of controlling the development of water lilies in this important aquifer. The manatees died quickly due to the low water temperatures in the aquifer. In 1978 the Mexican government authorized the capture and transport of 2 animals to the aquarium in Okinawa in Japan. This capture was repeated again 11 years later in 1997. In 1988 the Payo Obispo Zoo in the city of Chetumal received two young manatees. One manatee died shortly after arrival to the zoo. Local biologists and conservationists pressured the zoo to release the other surviving manatee. It was subsequently released to the Bay of Chetumal. A few months later in Zapata, Tabasco 2 sub-adult males were found stranded in a dry area. They were captured and transported to a lagoon in the Yumka Park Zoo in Villahermosa. Under the same conditions a year later 2 more manatees were stranded and relocated to the Yumka Zoo. Again in 1988 the Aquarium of Veracruz rescued 1.1 manatee orphaned calves. Pablo and Silvia become the first of this species that successfully lived in captivity in Mexico.

In 1999 the Marine Coastal Zone Institute in Belize rescued an orphan calf. A few days later it was transported to Xcaret Park in Quintana Roo, Mexico. 2 years later in 2001 the calf was rehabilitated and returned to Belize it was release and monitored since then.

In 2000 a young injured manatee arrived to Yumka zoological park but he died a few months later. In Jonuta, Tabasco, a small village captured 4 manatees and kept them in a lagoon. There was insufficient food source for these manatees. In 2001, after several years of negotiations, studies and various meetings between the state government, community college, research centers and companies specializing in management of marine mammals 2 manatees were transported to Dolphin Discovery and 2 manatees to Via Delphi for rehabilitation and care.

In 2003 the first calf was born in captivity at the Veracruz Aquarium and the second calf was born at Dolphin Discovery. Since this year other calves have been born and survived at Veracruz Aquarium, Dolphin Discovery and at Via Delphi. In September of 2003 a male calf 1 week old is rescued and housed in a temporary location in the Bay of Chetumal. The calf, named Daniel, survives but is determined that it cannot be released. Nowadays, Daniel remains under human care- living in a pen in the Chetumal Lagoon. The pen remains open and he is free to come and go. Daniel takes part in education, conservation and research programs.

Since the mid 1970's we have learned how to successfully care for manatees living in captivity. We now have successful reproduction programs, preventive medicine care, and can provide excellent nutrition. We have also learned about the cognitive abilities of the manatee due to the training for interactive programs as well as husbandry behaviors. Dolphin Discovery has been a pioneer in many of these advances. Husbandry behaviors include blood sampling, stool, urine, DPE, ultrasound. All these behaviors are

voluntary and performed on a routine basis. Additionally we have made substantial progress in the nasogastric gastroscopy and exposure of the penis to take samples of semen for possible use in artificial insemination.  
Conservation of this species and its habitat as been greatly enhanced due our failures and success of manatees living in captivity in Mexico.

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## Manatee Research and Conservation in Cuba

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There is little information on the status of manatees in Cuba. The Institute for Marine Investigations has been collecting data on the status of manatees for nearly a decade under the guidance of Dra. Maria Elena Ibara. The Enterprise for Flora and Fauna along with other Cuban agencies have been developing manatee conservation actions including a country-wide manatee conservation plan and interagency manatee conservation team. In 2001, Wildlife Trust began working in Cuba to strengthen and facilitate manatee research and conservation. Joint activities have included interviews with coastal residents and boat surveys, a marine mammal necropsy workshop, in-depth manatee study in the Isle of Youth and participation by Cuban biologists in manatee research activities in Belize.

Cuba contains extensive coastal manatee habitat on both northern and southern coasts. Additionally, expansive riverine and estuarine habitats are available along the southern coasts particularly in Zapata Swamp and around the Guacanayabo Gulf. Manatees are reported from around the entire coast and also the Isle of Youth off the southwest coast of the Island. It is likely that Cuba contains the largest population of manatees in the Greater Antilles. Manatees are attracted to sources of freshwater and sheltered lagoons dominated by *Halodule* sp. There is evidence of Florida manatee movement to Cuba. In January 2007 a female manatee with calf, first sighted in Crystal River 30 years ago, was photo-documented in the intake canal of a power plant located about 60km east of Havana (Alvarez-Aleman, Beck & Powell. in prep).

Threats to manatees in Cuba include hunting, drowning and entanglement in fishing gear and modification of habitat. Increased awareness as a consequence of the manatee necropsy workshop resulted in reports of manatee deaths in several areas and better capacity to determine causes of mortality. Manatee deaths led to a moratorium on inshore trawling in that particular area. Genetic samples are being collected and awaiting analysis from carcasses and existing specimens from around the country.

An in-depth manatee study is being conducted along the western coast of the Isle of Youth by the Institute for Marine Investigations. The study includes distribution and abundance surveys, habitat analysis and fecal analysis. The Enterprise for Flora and Fauna, Villa Clara provincial office is taking a lead in manatee conservation, through the focused and dedicated efforts of Jose Antonio "Pepe Tony" Santos. Initiatives include changes in fisheries practices, establishment of coastal biological stations, training of biologists in manatee research and conservation techniques, establishment of sanctuaries, manatee sighting and carcass recovery network, public education and awareness activities, and linkages and student participation from the Universidad Central Marta Abreu de Las Villas.

This work is providing a foundation for future manatee research and conservation activities throughout the country. The relationship and status of the manatee population in Cuba to Florida and Antillean populations are of particular interest and has implications for manatee conservation in the Caribbean region.

Analysis of ten years of monitoring Antillean manatee (*Trichechus manatus manatus* Linnaeus, 1758) in Barra de Mamanguape Protected Area, Paraíba state, Brazil

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The Antillean manatee (*Trichechus manatus manatus*) is classified as vulnerable by IUCN (IUCN, 2008), and considered the most endangered aquatic mammal in Brazil (IBAMA, 2001), although the present status of Brazilian populations remain poorly understood. The estimated population is about 500 individuals, non-continuously distributed in North and Northeast Brazil coast, from Amapa to Alagoas states (Lima, 1997; Luna *et al.*, 2008). Manatee Project from National Centre for Research, Conservation and Handling of Aquatic Mammals and Aquatic Mammal Foundation is accompanying wild manatee populations with the purpose of monitor the species conservation status. Seven points in the North and Northeast coast has being monitored since 1987. These points were determined based in concentration areas of manatees, according status conservation evaluation realized in 1990 to 1993 (Lima, 1997). The aim of this study was analyze ten years of monitoring in the point build in Barra de Mamanguape Protected Area to characterize the utilization of this specific area for manatee groups, defining occurrence pattern and seasonal preference.

Dates were colleted from direct observation in four squares, totalizing 0,25 km<sup>2</sup>, in a fixed station build in Barra de Mamanguape Protected Area, Paraíba State, Brazil (06°46'22"S, 034°55'10"W). Barra de Mamanguape estuary is protected by a reef line and sand banks, turning the water calm, with 1-5 m deep and maximum tide amplitude of 2.8 m. Tide is semidiurnal. Population indicators established were: (1) Occurrence Frequency (OF): number of days of watching manatees (DW) divided by total of monitoring days (MD) ( $OF = DW/MD \times 100$ ) and (2) Median estimated density (D): total number manatee watched (MW) by total of monitoring days (MD) ( $D = MW/MD$ ). To trace the relation between manatee occurrence and moon, tide and month, Pearson Chi-square test was applied.

Manatee monitoring was made three times per week (Monday, Wednesday and Friday) at 6 to 10 a.m., totalizing effort of 6.004 hours, since April 1999 to December 2008. OF was 22.32%, representing use of this area by manatees. Median estimated density was 0.46, a density relatively low. Seasonality was verified: percentage of manatees was statistically lower (13.4%) in June and higher (33.8%) in October ( $p = 0.046$ ). Chi-square showed association between manatee presence and tide/moon variation. Manatee presence were more common in full moon (33.4%) and less common in first quarter (10.7%). In relation to tide, in high water the presence of manatees was higher (45.4%), followed by turning tide (24.9%). The median permanence in the area was 35 min and 30 sec. Solitary individuals were seen more common, registered in 47.8% of observations. Groups varied from two to ten individuals (mean  $\pm$  SD:  $3.03 \pm 1.49$ ). Groups of ten individuals were watched once (0.57%). Groups with two individuals were more commonly observed (53.14%), followed by three (20%), four (12.57%), six (6.86%), five (4.57%), seven (1.14%) and eight (1.14%) individuals. Observation of calves represented 15.43% of group watched and 1.80% overall monitored days. Only in December 2008 was observed two calves in the groups. This observation

coincides with a calf stranding and immediate release. The other observations were correspondent to one calf. Calves were seen more commonly in December (33.33%), followed by November, October and February (11.11%) and were not seen in June and August.

These results showed that Barra de Mamanguape still being used as rest, reproductive and alimentary place for manatees, in spite of the low density observed in this area. Maranhão and Ceara states seem have higher medium density and abundance (Alvite, 2007). Manatees have preference in use this habitat, being more common in high tide and in Spring and Summer months. Calves also are more common in this period. The strategy of creating a Protected Area by Government in 1993 is the major help to protect this population, since motorboat traffic and coastal occupation is illegal. Antillean manatees require continued research efforts to improve conservation status in Brazil.

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## Conservation of Antillean Manatees in the Drowned Cayes Area of Belize

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For the past decade, we have investigated the behavior and ecology of West Indian manatees using Swallow Caye, the Drowned Cayes, and Gallows Reef, three distinct habitat types within the Belize Barrier Reef lagoon system near Belize City. Data were collected during two 3-month field seasons/year, using boat-based point scan methods with the assistance of volunteers. Presence/absence and photo ID methods ensured consistency of data collection despite a changing pool of volunteer researchers.

Results confirmed the mangrove and seagrass ecosystem between the Belize Barrier Reef and Belize City as important manatee habitat. Inconsistent with the prevailing "seasonal distribution hypothesis" for manatees in Belize (Auil 2004), the probability of encountering manatees at Swallow Caye and in the Drowned Cayes was equal between dry and wet seasons. However, manatees were only observed at Gallows Reef during the wet season. Swallow Caye had the highest probability of encountering manatees, confirming traditional knowledge held by local tour operators, which led to the establishment of Swallow Caye Wildlife Sanctuary in 2002.

In contrast to previous studies (O'Shea and Salisbury 1991), our data suggest that at least 44% of the manatee population carry scars from non-lethal boat strikes. The proportion of scarred animals did not vary as a function of habitat type, season, or year. The probability of encountering manatees did not change between years, despite an exponential increase in cruise ship tourism. However, there is still cause for concern. Manatees do not remain inside designated refuge boundaries; governmental agencies depend on co-management agreements with local non-governmental organizations for enforcement of rules inside MPAs; regulations governing human behavior outside MPAs are lacking; funds for monitoring and evaluation of MPAs are lacking.

We recommend that manatee conservation strategies be integrated into a system of riverine, coastal, and marine protected areas supported by additional tactics such as required manatee training for boat captains, slow zones at hot spots outside MPAs, and continued educational outreach. With few modifications and increased enforcement and monitoring, the Belize model for manatee conservation could lead to a shared "triumph on the commons" for the manatees and the user groups that share their habitat.

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Environmental Education and Participative Planning for Manatee (*Trichechus manatus manatus*)  
Conservation at the Alvarado Lagoon System, Veracruz, Mexico (1999 – 2008)

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The Alvarado Lagoon System (ALS) is one of the most productive wetlands in Veracruz, and the third largest ecosystem of this type in Mexico. Here occurs an Antillean manatee (*Trichechus manatus manatus*) population, highly endangered by hunting, pollution and habitat loss (Ortega-Argueta, 2002). In 1999 it was established a strategy focused on Environmental Education (EE) under the vision of developing integral conservation actions to catch the local people attention.

Two phases were set: the first one under the anthropological perspective consisted in personal interviews to local fishermen in order to know their perception about the presence and importance of the manatee at economical, social and cultural levels. We were able to record uses, ways of consumption, hunting techniques, beliefs, myths and customs; and after we built a complete diagram of the relationships between the coastal communities and manatees.

The second phase consisted in the application of EE workshops based mainly in the environmental problematic showed previously with the interviews. Workshops were applied to fishermen, children, clam catchers, and women. Until date, we applied 245 workshops at 10 municipalities, with the participation of 2,200 people. Also, 102 courses about the ecological importance of manatee had been taught. Thanks to these efforts, through the participative planning three cooperatives were formed, bringing to local fishermen (women and men) alternatives of development and an attitude change about conservation of manatees. This fact produced twelve experience interchanges in other parts of the country, where fisherwomen exposed their conservation efforts towards manatee and their habitat. All these 10-year activities had been published in media such as radio, television, newspaper, videos, fliers and posters.

With these efforts, manatee mortality has decreased and instead more sightings have been recorded. Seven animals have been rescued from nets, being the fishermen the main informers and rescuers. In general, people who were hunters years ago now are protectors thanks to the intense campaign through 10 years of work promoting sustainable projects to both improve life quality without impact the manatee habitat and contribute to its conservation.

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Density, Abundance, Distribution and Manatee (*Trichechus manatus manatus*) Health Status in the Alvarado Lagoon System, Veracruz State, Mexico

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The Alvarado Lagoon System (ALS) has an extremely high biodiversity so it was declared a Ramsar site in 2004 with an extension of 267,000 hectares. . The ALS has over 100 lagoons and several rivers. The most emblematic mammal in the system is the manatee (*Trichechus manatus manatus*). Since 1975, the manatee is considered as threatened with extinction by CITES; in 1982 was included in the Red List of the IUCN as vulnerable; and is considered as threatened with extinction by the Mexican government.

Knowledge about the distribution and abundance of the manatee along the coast of the Mexican state of Veracruz is scarce, but it has been reported as distributed along the entire state (Colmenero and Hoz, 1986). However, recent studies proved that the manatee has disappeared along the northern region of Veracruz (Serrano *et al.*, 2007). It is believed that the manatee population that inhabits the ALS is one of the few populations remaining in the state. Therefore, the goal of our study is to determine the density, abundance, distribution and manatee health status in the ALS. From October to November we carried out over 34 line transects with a total survey effort of 72.8 hours covering about 10,000 hectares. We have detected 8 animals: three were observed during the surveys, four were detected by means of passive acoustics, and one was detected with sonar. We used the distance sampling methodology and the software Distance to estimate the manatee density and abundance. The estimated density was 0.93 animals/hectare (C.V. 48%), and it was not possible to estimate the abundance due to our sampling effort.

Currently, we are expanding this study by including an evaluation of the manatees' health status. Also, we will increase our sampling effort in order to have enough data to estimate the abundance and in order to reduce our data C.V.

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Status and recovery of the Antillean manatee (*Trichechus manatus manatus*) in the Alvarado Lagoon System, Veracruz, Mexico.

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Since 1999 we have developed a research and conservation program for the Antillean manatee (*Trichechus manatus manatus*) in the Alvarado Lagoon System (ALS), in central Veracruz State. We focused on delivering educational courses and workshops to fishers and local communities with the objective of reducing poaching and habitat loss, the principal reasons this species is endangered. Historically, manatees were relatively common in the ALS, but studies in the 1980s documented their abundance reduction in this region. Nevertheless, recent rescues of three calves (between 2000 and 2004) and the continuous reporting of sightings have reemphasized the ALS as an important manatee area. Manatee and habitat surveys from 2000-2003 along the entire coast of Veracruz corroborated ALS as a critical wetland site for the conservation and recovery of the species. Potential manatee habitat comprises 315,000 ha of low human-development areas including coastal lagoons, and interior lagoons, estuaries, mangrove wetlands, rivers, and canals.

We focused on delivering educational courses and workshops to fishers and local communities with the objective of reducing poaching and habitat loss, the principal reasons this species is endangered. We also conducted interviews of the inhabitants of the ALS to determine their knowledge of manatee biology and the cultural and historical importance of manatees. We have found that clam divers and river and lagoon fishers possess a traditional knowledge of manatees in the ALS because they are continuously working in manatee habitats. In the ALS manatees are most commonly sighted in the Limon River and adjacent lagoons, and are rarely sighted in the Acula River and adjacent lagoons; the marine zone appears not to be utilized by manatees, except when moving locally between rivers along the coast.

Manatee hunting was still common in the 1970s. Locals described uses of manatee as food and medicine, and for the elaboration of artifacts. One of our most significant achievements in conservation was the designation of the ALS as Ramsar Site No. 1355, encompassing 267,010 ha of wetlands, including critical manatee habitats. With the potential for an increase in the population of manatees in the ALS, continued educational and informational campaigns are essential to educate the local communities about the need to protect and conserve manatees and their habitats.

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Ongoing investigation looking at community involvement in the efforts to conserve Antillean manatees (*Trichechus manatus manatus*) in Central America

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The Antillean manatee (*Trichechus manatus*) is classified as endangered under Belize's Wildlife protection act of 1981. Belize is considered the last stronghold for these vanishing mermaids in Central America. As a result, current conservation work in Belize is focused on national efforts to preserve the species and its habitat, with a recognition that efforts also need to go beyond the country's borders. As such, Belize is an ideal place to study the human aspect of conservation, exploring involvement at the community level in conservation and research initiatives, relating to Antillean manatees. Similarly, in Mexico, research and conservation work is ongoing to ensure the survival of the Antillean manatee in Mexican territory. Known for being one of the remaining areas with a concentrated population of Antillean manatees in Mexico, Bahía de Chetumal has been designated a natural manatee protected area (1999). This area is invariably linked to Belize and the Corozal Bay Wildlife Sanctuary for manatees (1998) and is important to explore in relation to inter-country collaboration efforts.

With the aim of understanding community experiences, opinions and participation in the manatee conservation effort in Mexico and Belize, a total of sixteen extended interviews and 146 questionnaires were administered. Fieldwork was carried out in Chetumal in Mexico, as well as Sarteneja, Caye Caulker and Placencia in Belize. In this case study approach, surveys and interviews with locals and others involved in manatee conservation projects in Belize and Mexico have reiterated the need do more to save the Antillean manatee. Initiatives are varied and inconsistent between the fieldwork sites I explored suggesting the need for a more integrated conservation approach. Additionally, community outreach and education needs to be further enacted; many individuals reported knowing the name of a person and/or organization that is involved in the protection of manatees in their community, but are not informed as to what they do or how they can get involved, if at all.

Preliminary analysis of the data shows that in Chetumal 72% of people surveyed had seen at least one manatee in their lifetime, compared to 84% of respondents in Sarteneja, 89% in Caye Caulker and 78% in Placencia. That so many people have had personal experiences with manatees is promising. However, compared with data showing self-indicated participation in manatee conservation or education (administering or participating), numbers are much lower with only 13% of those surveyed involved in Chetumal, 39% in Sarteneja, 29% in Caye Caulker and 18% in Placencia. Interestingly, tour guides, who are leading 'manatee tours,' did not always rate themselves as being involved in conservation or education projects, even though they were presenting information about manatees and taking people to see them. The results presented here, provide significant insight into what changes need to be made to better protect the manatee populations in Belize and Mexico. That, said, further analysis of qualitative data

from the open ended survey questions and interviews using ATLAS.ti will seek to identify additional relationships between the data and further explore people's relations with manatees and current research and conservation work. Further research will hope to incorporate GPS and GIS technology, to map manatee population distribution against 'pockets' of community involvement, to assess whether focused conservation and education work is taking place in the appropriate areas where manatee populations are most dense and where the risk of injury or death to manatees is greatest.

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## The situation of Amazonian manatee in Iquitos-Peru and efforts for environmental education

**Jaymee D. Silva**

The Amazonian Manatee (*Trichechus inunguis*) is the smallest sirenian and the only one restricted to freshwater habitats. This species is endemic to the Amazon river basin, founding them in Brazil, Peru, Colombia, Ecuador, Guyana and Venezuela.

In Peru they have been reported mostly in the region of Loreto, in rivers such as Ucayali, Huallaga (Grimwood, 1968), Napo, Tigre, Marañón, Pastaza, Samiria, Pacaya (Soini et al., 1996 y Álvarez, 1996) Amazonas and others. In this country their biggest threat are the hunters and opportunist fisherman. As they are a great source of protein and fat people have been hunting them for their meat hundreds of years. A more recent threat is the illegal pet trade of baby manatees.

The 2007 IUCN Red List of Threatened Species categorizes *T.inunguis* as vulnerable.

This species is protected by law in most countries. Hunting them for meat or any type of commercialization, dead or alive, is forbidden. In Peru, since 1973 these animals have been protected by laws; however hunting kept going as always (Soto, 2007). A recent regulation for manatees and other aquatic mammals in Loreto-Peru have been approved in June 2008.

Environmental education needs to be done in order to stop the hunting and the illegal traffic of manatees. Some education projects are currently ongoing in Loreto. The principal target is the children, as they influence directly on their parents. Work with communities living next to rivers seems to be the more significant as they interact more with wildlife than the population of the city. However education in the city is very important as the pet trade occurs with wealthier people.

A survey was done in the city of Iquitos-Loreto in order to explore the knowledge of the people towards the manatee. This research was done by interviewing people of all ages and social status. 316 persons were interviewed with the help of questionnaires. On this sample population, only 56% affirmed they knew what a manatee was. When asked what other names they know for the manatee, many answered sea lion, otter, dolphin and many others. This suggests that a significant number of persons that affirmed to know what a manatee was were actually thinking of another animal.

This survey shows also how only 31.6% have seen live manatees; however 14.6% referred to the captive animals being rehabilitated by ACOBIA (Association for the Conservation of the Amazonian Biodiversity) in the last year. It is a fact that manatees are no longer seen easily in the wild.

Even a lower percentage (10.8%) of people knew about laws that protect this animal, and none knew exactly the regulations. However many did know about the threats that are lowering their populations. The majority agree that fishermen are their biggest threat, as well as meat hunting, pet trade, commercialization of sub products and pollution of their habitats.

The research objectives were not only statistics, but to gather information that could guide us deeper into the actual situation of this aquatic mammal in the region. Information about manatee mythology, folklore and tradition, as well as places where they can be found was collected. The results support the idea that environmental education is the key for the conservation of *Trichechus inunguis* together with reinforcement of law, rescue and rehabilitation projects and deep research of this unique species.

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Possible reintroduction of the Antillean manatee (*Trichechus manatus manatus*) in the Grand Cul-de-Sac Marin (Guadeloupe, Lesser Antilles)

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The National Park of Guadeloupe has undertaken a project of noble intent : re-establishing the Antillean manatee, an endangered species according to the IUCN Red List of Threatened Species (Self-Sullivan and Mignucci-Giannoni, 2008), in the waters of the “Grand Cul-de-Sac Marin.” This large (37,070 acre) protected area is currently managed by the National Park. Manatees were extirpated from the waters of Guadeloupe several decades ago by hunters for food, but currently the Grand Cul-de-Sac Marin represents a well-managed area with relatively few, minor threats to manatees, compared to many other locations in the wider Caribbean.

The project is part of a larger initiative that seeks to: a) overcome and reverse loss of biodiversity in Guadeloupe; b) improve the global conservation status of the species and subspecies; and c) to provide a transferable model for other conservation projects.

A feasibility study (Lartiges et al., 2002) concluded that the reintroduction of manatees had merit, even if hurdles needed to be overcome to ensure success. The conclusion was echoed by the assessment of the Mote Marine Laboratory (Reynolds and Wetzel, 2008) during a workshop in April, 2008 in Guadeloupe. Factors that will contribute to the possible success of the project include: the large area of seagrasses (about 13 700 acres [Chauvaud et al., 2005]) within a protected marine park; presence of little boat traffic and relatively few other apparent threats; and general acceptance (and even some enthusiastic endorsement) of agency scientists and managers, politicians, and local citizens around the Grand Cul-de-Sac Marin.

The first part of the project is the preparation phase (3 years), during which studies of environmental contaminants of seagrasses and sediments, seagrass productivity, and socio-economic factors will be done. In addition, there will be discussions with regard to the optimal location(s) from which manatees might be taken to populate the new area, and to organize a cooperative network of scientists and managers from various Caribbean countries to advise the project. The second part will last about 5 years, and involves the actual reintroduction of selected manatees. It will begin once everyone is assured that environmental and other possible threats to manatees have been identified and are under control. The reintroduction will involve soft releases and VHF and satellite monitoring of the animals.

The success of the project depends to a large extent on its endorsement by the people of Guadeloupe. Therefore, throughout the project, it is essential to develop an education and awareness program for the island.

The project has the support of the French Ministry of Ecology which has provided funding since 2008. In Europe, governmental agencies and private firms will be approached in 2009 to solicit additional financial support. The National Park has also established partnerships with the Mote Marine Laboratory for scientific guidance and with the Aquarium of Gosier for a future care center dedicated to manatees.

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## Feeding Ecology of Manatees in Chetumal Bay, México

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Chetumal Bay (BCH) supports one of the largest populations of manatees (*Trichechus manatus manatus*) in the Caribbean. In order to understand the habitat requirements and the role of manatees in the structure and functioning of BCH, we estimated the Centers of Activity (COA) defined as the geographical locations of the point of greatest activity within the home range. Nine areas were selected on the basis of the preliminary COAs. Additionally, 3 areas were randomly created where manatee's COAs were not registered. Plant cover and soil type were determined for each climatic season (Northern winds, dry and rainy season) and for each area. Besides, manatee fecal samples were analyzed by comparing the vegetal fragments in the samples with voucher microscope slides or illustrations. Using vegetation data, and other ecological information of the BCh, we built a trophic model of the system using the Ecopath software. The main submersed aquatic vegetation in the bay includes *Bathopora* sp., *Halodule wrightii*, *Thalassia testudinum*, *Chara* sp., *Najas marina* and *Ruppia maritima*. All the species excepting *Bathopora* sp. were found in the fecal samples. However, local people saw manatees consuming *Bathopora* sp., and it's possible that this procedure was not appropriate to detect this algae in the feces samples. We did not find *Syringodium filiforme*, a seagrass distributed in the neighboring reef lagoon system but not in BCH, suggesting that manatees spend an important part of their time in estuarine and freshwater areas. More than 80% of samples had *Rhizophora mangle* tissues, enforcing the need of mangrove conservation as an important source of food for manatees. The Ecopath model showed that manatees play a decisive role in the trophic dynamic of the BCH, due to their abundance, biomass and function in the detritus cycle.

## Manatee research and conservation in western Brazilian Amazon

### **Miriam Marmontel**

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Amazonian manatees, *Trichechus inunguis*, are considered vulnerable, both under the IUCN Red Data List and the Brazilian list of threatened species. Although commercial hunting has been terminated, hunting at subsistence levels and catches of young manatees, associated with lack of information on reproductive parameters and abundance estimates, continue to worry scientists about the future of the species. Recent efforts to improve chances of recovery include the establishment of protected areas, rescue and recovery of accidentally-captured calves or illegally-held specimens, and research initiatives. The Mamirauá Institute for Sustainable Development has worked with manatees in two large sustainable development reserves (Mamirauá and Amanã) in western Brazilian Amazon since 1993, with emphasis on the natural setting, working both with free-ranging animals and biological material from carcasses.

Over 20 manatees have been captured in the wild with the main purpose of fitting belt-mounted transmitters operating at unique VHF frequencies. Monitoring efforts resulted in documentation of annual migratory routes: manatees move from the aquatic plant-rich floodplain areas they use during the wet season, to deep, blackwater lakes during the dry season (Marmontel *et al.*, in prep). A habitat study based on satellite images and modeling mathematically confirmed the importance of the water level - and its associated plant growth - to manatee dynamics (Arraut 2008). Hunting is also related to water-level changes and is almost exclusively done by older men, with the use of harpoons and mostly geared to subsistence; unfortunately, culling numbers do not seem to have decreased in the last decade (Calvimontes and Marmontel 2008). Animals of all ages are being taken, the oldest documented so far having died at 36 (Vergara-Parente 2009).

Fifty plants mentioned by local people as eaten by manatees were confirmed through the examination of feces samples (Guterres and Marmontel 2008, Guterres *et al.* 2008). Parasitological analyses identified the presence of *Cryptosporidium* (Borges *et al.* 2007) in manatee feces and *Chiorchis* in the intestines. Levels of steroid hormone metabolites present in over 200 free-floating feces are being analysed to examine reproductive function in wild manatees. Mitochondrial and microsatellite DNA research showed manatees in the Amazon still present a high genetic diversity (Vianna *et al.* 2006), but confirmed the presence of hybridization between Amazonian and Antillean manatees around the mouth of the Amazon.

Every year, a considerable number of orphaned calves is rescued, mainly victims of the extensive use of fishing nets throughout the region. At the end of 2008, there were over 60 animals in legal captivity. As an alternative to urban captive settings, the Mamirauá Institute has been approved as a community-level conservation and rehabilitation center, working in a semi-natural setting, and with the collaboration of local people. The center presently holds two calves, planned to be released during the wet season, in May 2009. Meanwhile, a very intense awareness program is conducted with the local communities.

The scientific aspect of the program is expanded and enhanced by the participation of co-investigators from the local communities, in a rich atmosphere of knowledge exchange and experiences sharing.

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## Manatee biogeography and sirenian health assessment

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### Biogeography

To date, thousands of manatee tissue samples have been collected from throughout the Florida and Caribbean populations, providing a good foundation for future genetic assessments and comparisons between population units. This massive effort will allow for inferences about manatee life history and population structure, including reproductive potential, movements, and overall population size.

Manatees have had an uncanny ability to establish new populations within their subtropical range, as evidenced by their evolutionary history and genetic traits (Domning, 2005). Although vicariance separated the taxa over time, the vagility of this unique group of aquatic mammals enabled populations to disperse through deliberate migration or stochastic events. This phenomenon of population expansion is characteristic when the existing population is large enough to act as a source to populate new areas. Although novel habitats are not always suitable, the trichechines exhibit adaptive plasticity as evidenced by behavioral modification and distinct, subtle morphological characteristics among populations (Domning and Hayek, 1986). The Florida population is recently established within the last 20,000 years and contains a newly differentiated population unit in the northwest part of the state near Crystal River. Thus, within peninsular Florida, where populations are more established, distinct habitat types require different survival strategies by the resident manatees.

Previous studies have examined the genetic diversity of the Florida population in detail (Garcia-Rodriguez et al., 1998; Vianna et al., 2006). Early studies using allozymes and nuclear microsatellites determined that Florida manatees have low to average genetic variation (McClenaghan and O'Shea, 1988; Bradley et al., 1993; Pause et al., 2007), which could be explained by panmictic breeding and the absence of barriers to gene flow between contiguous areas. Nevertheless, questions arise as to the fitness and health of this population. The genetic data suggest that even with adequate gene flow, there may be issues regarding low allelic diversity among Florida manatee population units due to inbreeding, bottleneck events, and

founder effects. The best remedy for this low genetic diversity in the population would be to encourage growth and address conservation practices in order to promote breeding between population units throughout all regions of Florida.

Although there has been an increase in the population size of Florida manatees in recent decades, genetic evidence of prior founder events are still evident in the population. Manatees were subject to anthropogenic take for centuries, and it will likely take many generations to resolve the genetic consequences. Genetic connectivity and pedigree studies can give us information on breeding among different population units. Knowledge of the genetic composition of the Crystal River group will determine whether, and to what extent, breeding with parapatric populations is occurring, and may play a role in understanding the population structure by complementing efforts to model various manatee life history strategies.

### **Health Assessment**

The concept of using marine mammals as ecosystem sentinels has been proposed (Bonde et al., 2004; Wells et al., 2004; Bossart, 2006; Moore, 2008). Detailed health assessment studies in sirenians have provided information on the health of the individuals and populations. These assessments have been conducted in Florida, Puerto Rico, Belize, Mexico, and recently in Australia. The information has provided data on basic biology and allowed researchers to establish normal baseline criteria for comparing the health status of individuals within populations. These studies are akin to recent studies underway on other marine mammals (Wells et al., 2004; Fair et al., 2006). Various capture and restraint strategies have been employed to evaluate and sample individuals. Currently, data are collected and basic health status is assessed through physical examination, medical evaluation and intervention as necessary, morphometrics, photo-documentation, ultrasound, biopsy, genetics, immunology, endocrinology, microbiology and cytology, virology, hematology and blood chemistry, toxicology, urine, milk and fecal examination, and radio-telemetry follow-up. Capture-release assessment of wild sirenians will continue to expand over time as it attracts more collaborative researchers.

In this presentation we will discuss some of the mechanisms that have enabled sirenians to adapt to different environments and habitats throughout their range. This adaptive resilience has aided their ability for population expansion, but may have an underlying associated cost. Through health assessment studies, researchers are able to gauge some of the limits to adaptive resilience from a population and habitat perspective.

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Monitoring oral temperature, heart rate, and respiration rate of manatees (*Trichechus manatus latirostris*, *T. m. manatus*) exposed to capture and handling in the field.

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In veterinary medicine, the monitoring of oral temperature (OT), heart rate (HR), and respiration rate (RR) is often performed to assist in evaluating the health of an individual animal. Protocols for monitoring these vital signs are well defined in many species of domestic and wild animals, but not with manatees. Normal manatee OT, HR, and RR values have been defined under captive conditions (Scholander and Irving 1941, Bossart 2001, Murphy 2003). However, many manatee research studies require the capture and handling of free ranging manatees to collect data. Understanding how manatee OT, HR, and RR can change during a capture event is important for researchers who want to ensure an animal's well-being. Furthermore, the correlation of manatee OT, HR, and RR with blood chemistry have not been studied in a field setting. To determine the effects of capture on healthy, awake, juvenile/adult manatee vital signs: a total of 38 Florida manatees (*Trichechus manatus latirostris*) and 48 Antillean manatees (*T. m. manatus*) was continuously monitored for OT, HR, and RR during field research captures, for 50 minutes. Creatine kinase (CK), potassium (K<sup>+</sup>), serum amyloid A (SAA), and lactate values were examined for each animal to assess possible systemic inflammation and muscular trauma (Harr et al. 2006, Harvey et al. 2007). Antillean manatees had higher initial OT, HR, and RR than Florida manatees ( $p < 0.0001$ ). Over time OT, HR, and RR were no longer significantly different between the subspecies. Mean OT of Florida and Antillean manatees was initially low then increased to a normal value. Mean HR of Florida and Antillean manatees was initially high then decreased to a normal value.

Mean RR of Florida and Antillean manatees was initially high and decreased over time, but did not reach a normal value. High mean respiratory rate over time was associated with high lactate values ( $p=0.018$ ). Antillean manatees had higher overall lactate values than Florida manatees ( $p<0.001$ ). Monitoring of manatee OT, HR, and RR in the field is recommended as a standard protocol for researchers, to better assess the condition of an animal.

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Demography, ecology and health assessment of manatees in Quintana Roo and its genetic interpretation in Mexico: 2004-2008

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One of the most important manatee populations in Mexico inhabits the Caribbean coast of Quintana Roo (QR). This population is estimated to be from 200 to 250 manatees. In order to know regional manatee movements, health status, habitat use, home range, and genetic structure and variability, an intensive research project was carried out from 2004 to 2008 in QR., with the participation of several research institutes in Mexico and U.S.A. Fifty four manatees (29 males and 25 females) were captured to get morphometric measures, weight, blood, biopsy samples, feces, commensals, photos for ID and documentation, and a general evaluation of body condition was done. Fifteen of them (8 females and 7 males) were radio-tracked with GPS tags in Chetumal Bay to follow its movements. All the manatees were captured with a locally developed technique, and PIT tags were used to identify each manatee. Biological and health data were obtained as normal base line criteria for conservation management and to compare among manatee populations in the world. Data like morphometrics, hematology and blood chemistry, toxicology, nuclear genetics analysis, fecal examination, parasites, movements, home range estimations, habitat use are currently analyzed. In general the males had larger movements than the females. Those movements along the Mexico-Belize coast also show regional connectivity among areas of localized manatee occurrence with movements between distant sites and site fidelity (Morales-Vela et al. 2007). During this project manatee commensals and epibionts were collected from 47 manatees, in eight of them well established epibiotic copepod communities of the tanaidacean *Hexapleomera robusta* (Moore) were found (Morales-Vela et al. 2008). This tanaid formed patches of tubes adhered to the skin surface, the tanaid probably captures suspended particles as the manatee feeds. The international collaboration in this project also gives us the opportunity to determine baseline concentrations of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in nine manatees sampled in Chetumal Bay (Erin L. Pulster et al, 2007). The PCB concentrations ranged from 0.022 to 20.1 mg/g wet weight in blubber. These high levels exceed current thresholds for toxic endpoints and warrant further research. Future plans include expanding the range of contaminant analysis and attempt comparable studies with other sirenian populations. All of this base line biological information will be very useful for implementing better conservation strategies and to encourage developing stronger trans-border manatee conservation strategies between Mexico and Belize.

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## Field research on Antillean Manatees in Marine Mammal Sanctuaries of Dominican Republic

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In Dominican Republic, the general distribution of Antillean manatees (*Trichechus manatus manatus*) has been documented through different country-wide survey efforts (Lefebvre *et al.*, 2001). However, there is a lack of site-specific studies on manatees and their habitat within their three main distribution areas: (1) the northwest coast, from Manzanillo Bay to the Bajabonico River mouth; (2) the northeast coast, along the north coast of the Samana Peninsula, and in the south coast of Samana Bay; and (3) the southwest coast, in the Neiba and Ocoa Bays, and east of the Oviedo Lagoon. Recent research efforts have been made in the northwest and northeast coast to contribute to our knowledge and management of the species. Information was gathered through literature reviews, interviews with locals, and both land and boat based surveys. The latter consisted of non-invasive 30 minute point-scans in selected stations, following the methodology described in LaCommare *et al.*, (2008). In addition, a video camera with underwater housing was used to determine the feasibility of pursuing manatee photo identification studies at both sites.

In the northwest coast, surveys were conducted in the Marine Mammal Sanctuary of Estero Hondo in 2007-2008, to determine manatee distribution, habitat use and threats. A total of 138 interviews were carried out in six nearby communities, where locals proved to be very knowledgeable about manatees. Of 119 sighting reports, 61% occurred in Caño Estero Hondo, a coastal saltwater lagoon surrounded by mangroves located within the Sanctuary. Sampling for environmental variables and manatee surveys were limited to Caño Estero Hondo and its immediate surroundings. Shallow protected warm waters, freshwater sources and abundant seagrasses make this an ideal habitat for manatees. A total of 270 hours of effort resulted in 103 sightings: 27 within the point-scan sampling design and 76 opportunistic. Presence of manatees, including calves, was confirmed throughout the year. Group size varied from one to nine individuals. Manatee distribution was related to the location of seagrasses and resting holes. Although evasive, manatees have been observed feeding, resting, travelling, socializing and milling within Caño Estero Hondo. Tagging and tracking studies are recommended to determine individual movements and residency in this manatee activity center. The principal threat to manatees is the illegal use of fishing nets within the Sanctuary. A conservation action plan for the Sanctuary is currently in progress.

In October 2008, a five day field visit was conducted in the north coast of the Samana Peninsula, which is part of the Marine Mammal Sanctuary of Banco de la Plata y de la Navidad. According to interviewed locals (n=17), sighting reports were most common in El Estillero and Portillo. In the former, land based surveys were conducted for a total of 9.5 hours of effort, resulting in five opportunistic manatee sightings in four consecutive days. Group size varied from one to five individuals, and calves were present. Shallow protected waters and abundant seagrasses favor manatee presence in this bay. Animals were mostly milling during observations, but feeding evidence was found. Coastal development and watercraft use were identified as the principal threats to manatees. Continued research effort is recommended to determine manatee distribution and habitat use in this study area.

Due to the poor water visibility of Caño Estero Hondo, capturing underwater images for manatee identification purposes is only recommended for the north coast of the Samana Peninsula. This study was made possible with the support of the Secretaría de Estado de Educación Superior Ciencia y Tecnología (SEESCyT) and the Fundación Brugal.

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Use of Argos-linked GPS tags to document specific habitat use patterns of manatees in eastern Puerto Rico

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The population of manatees in Puerto Rico is the only group of Antillean manatees (*Trichechus manatus manatus*) under the jurisdiction of the United States. Unlike Florida manatees, which make extensive use of estuarine and freshwater habitats, Antillean manatees in Puerto Rico are found almost exclusively in marine habitats and are dependent on seagrass and other marine plants for food. Increasing human activity and development in the coastal zone threaten the long-term existence of this protected species in Puerto Rico. Federal manatee recovery efforts mandate the need for data on manatee movements and habitat utilization. The U.S. Navy, faced with the pending closure of the former U.S. Naval Station Roosevelt Roads, requested detailed information on manatee activity patterns in the vicinity of the naval base. To address these concerns, the U.S. Geological Survey initiated a study to document the movement and habitat use patterns of manatees in eastern Puerto Rico and assess the resources they depend on. In spring 2005, nine manatees were tagged in eastern Puerto Rico using satellite-linked Global Positioning System (GPS) tags. GPS receivers coupled with Argos satellite transmitters, encased in floating tethered housings, provided accurate locations of the manatees and enabled us to remotely monitor the detailed movements of tagged individuals. GIS analysis of these data with aerial imagery and USGS benthic habitat maps allowed us to correlate their movements with habitat types, and to identify travel corridors, sources of fresh water for drinking, and feeding locations. Tagged manatee movements ranged along the coast of eastern Puerto Rico from Fajardo to Humacao and Vieques Island, with the greatest documented use in shallow areas having extensive seagrass beds. They accessed fresh water at the mouths of rivers (Fajardo, Daguao, Blanco, and Humacao) and from the Naval Station's wastewater treatment plant discharges (Capehart and Bundy). Detailed data from GPS tagged manatees have proven valuable in the development of manatee and habitat protection recommendations related to the transfer of Naval Station lands to public and private ownership.

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Release and radiotracking of long-term semicaptive West Indian manatees (*Trichechus manatus*) in the Sinú River of Colombia

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West Indian manatees (*Trichchus manatus*) in Colombia are protected by national legislation and their conservation is implemented through a National Recovery Plan (Ministerio de Ambiente & Fundación Omacha 2005). As part of these efforts, manatees were rescued from being butchered in the 1990s, and through local NGOs and regional environmental government agencies, 43 manatees were placed in custody in semi-captivity to secure their survival. Two main colonies were established in the Caribbean region to house these animals, one in the Magdalena River (33 animals), and one in the Sinú River (11 animals). Since 2003, we have begun field studies and conservation in the Sinú River swamp, including basic interviews with local residents, public outreach, and field observations and documentation of distribution and life history parameters of manatees in the area. This set up the proper conditions for releasing back to the wild, the previously rescued manatees.

As a first phase of the release project, four manatees were medically examined and cleared for release. They were fitted with belt and floating VHF transmitters following a combination of techniques used in Brazil with Amazonian manatees and in Florida with West Indian manatees. Two of the animals were fitted as well with UHF transmitter to monitor their movements via Argos satellite tracking. Two males and two females were released on 14 February 2009 and are at present being tracked on a daily basis along the Sinú River. Parallel, a public education and outreach campaign is being conducted with local fishermen and schools to protect the released animals and to achieve community involvement in manatee conservation.

This release and monitoring effort, first in Colombia, together with the public outreach campaign, will lead to a second phase of releasing the remaining six animals semi-captive in the Sinú River colony, and a third phase of releasing the 22 manatees semi-captive in the Magdalena River colony. Future telemetry studies using wild manatees off the Sinú, are also planned to understand natural movements and habitat use of West Indian manatees throughout the Sinú River swamp.

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Synoptic aerial surveys for West Indian manatees (*Trichechus manatus*) off the south coast of Puerto Rico—2001-2008

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The West Indian manatee (*Trichechus manatus*) is a protected species in Puerto Rico due to its endangered status. They were hunted for food up to the early 1990s, but nowadays, they face threats from boat collisions, habitat encroachment, and industrial pollution. Manatees have been reported to use the south coast of Puerto Rico, especially the industrialized estuaries of Guayanilla and Jobos bays for daily activities, both also reported to be areas of high manatee mortality. We document the population dynamics and the extent of usage by manatees of these two estuaries and its adjacent coastline through a series of aerial surveys using a helicopter platform.

The study comprised of a series of surveys to document the presence of manatees along Puerto Rico's south coastline between Cabo Rojo and Maunabo. Given the flying capabilities of helicopters versus fix-winged airplanes, a Eurocopter A-Star 350B helicopter was used as the survey platform, flying 500 m parallel to shore at an altitude of 150 m. Smaller cays distant from shore, were surveyed by flying over the cay's land, with observers searching the water over to each side of the aircraft. Twenty-four surveys were flown along the south coast between January 2001 and November 2008. Sixteen of these were conducted for Guayanilla Bay and vicinities, and 8 were conducted for Jobos Bay and vicinities. A total of 77.4 flight hours were conducted, representing 53.9 overall hours of survey effort.

A total of 271 sightings were recorded during the 24 surveys conducted. The number of sightings per survey averaged 11.8 (min 3, max 30). The average sighting per effort hour for manatees was 5.2. The average number of manatees observed per survey was 21.7 (min 6, max 61), higher than that found for the same area by Powell et al. (1981) (0-14), Rathbun et al. (1985) (0-10) and Freeman and Quintero (1990) (0-8). However, there was a slight difference between the average number of manatees observed in the southeast coast (mean 27.9, min 9, max 61) in comparison to those observed in southwest coast (mean 18.4, min 6, max 37). The average number of manatees per effort hour was 9.7, much higher than that found for the area by Powell et al. (1981) (3.1), Rathbun et al (1985) (5.6), and Freeman and Quintero (1990) (2.1), and considerably higher when comparisons are made with other manatee survey studies in the Caribbean.

Manatees utilize the entire south coastline of Puerto Rico, from Cabo Rojo to Patillas. In the southwest coast, manatees are evenly distributed, with patches of higher use in Bahía Montalva (Lajas and Guánica), Ensenada las Pargas, Bahía de Guánica off Río Loco (Guánica), Punta Verraco, on both sides of Punta Guayanilla, cayos Caribe, Palomas, Río and María Langa in Peñuelas, Isla del Frío in Ponce. Manatees constantly

use the mouth of Río Loco in Guánica, at times venturing within the river, which seems to be one of the most important fresh water sources for manatees in the area. Ensenada las Pardas in Guánica and Punta Verraco in Guayanilla as well as windward side of Punta Guayanilla and the cays at Bahía Tallaboa serve as important feeding areas. The leeward side of Punta Guayanilla seems to provide them with shelter. Manatees were repeatedly observed in La Parguera's Bioluminescent Bay and Parguera's offshore cays, particularly Arrecife Margarita.

On the southeast coast of Puerto Rico, manatees are found from Ponce, east to the mouth of Río Jacabo in Patillas. They are commonly observed in Santa Isabel between Punta Aguila and Isla Puerca, in Sailnas near Cayo de la Mata and Puerto de Salinas, and in Puerto de Patillas. The highest concentration of sightings was recorded in Jobos Bay, specifically within an imaginary triangle consisting of north of Punta Pozuelo, east of Central Aguirre and west of Puerto de Jobos. Of interest, was the use of manatees of the cays off the coast, including Cayos de Barca, Cayos de Ratones in Salinas, and Cayos Cabezasos, Cayo Caracoles and Cayo Barbería off Santa Isabel.

Given that manatee survival in Puerto Rico is threatened by anthropogenic impact, now a days mostly by watercraft collisions, habitat use maps and analysis resulting from these surveys, together with radiotelemetry data, may serve to delineate critical manatee habitat in southern Puerto Rico in order to put in place a buoy system that will warn boaters of probable manatee presence in heavily used areas, establish speed limits in these areas, and thus help protect this species from human-induced mortality.

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## Reduced genetic diversity and delineation of management units in *Trichechus manatus* populations

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Effective management of imperiled species requires detailed genetic and taxonomic information to identify properly species, subspecies, and distinct population segments. The threatened West Indian manatee (*Trichechus manatus*) is a slowly reproducing aquatic mammal, whose small, isolated populations are negatively impacted by habitat destruction and anthropogenic mortality. Relatively little information is available on the amount or distribution of genetic diversity in the West Indian manatee, presenting challenges to conservation biologists seeking to promote healthy ecological and evolutionary processes. Consequently, genetic studies, using microsatellite and mitochondrial DNA, were implemented to quantify the genetic diversity and identify unique populations or regions in need of protection.

The Florida (*T. m. latirostris*) and Puerto Rico (*T. m. manatus*) manatee populations are currently listed together under the U.S. Endangered Species Act. The 2007 species status review suggested the downlisting of the populations from endangered to threatened, primarily due to the recent recovery of the Florida population (USFWS, 2007). Here, a survey of microsatellite DNA variation in the Florida and Puerto Rico populations identified highly significant differentiation ( $F_{ST} = 0.16$ ,  $P < 0.001$ ), which suggests that each population should be considered a unique unit of management. The Puerto Rico population is considerably smaller, occupies a strictly marine habitat, experiences distinctive threats, and would benefit from a separate management plan.

Long-term exploitation and small population sizes can lower genetic diversity, which results in decreased fitness, reduced adaptation to environmental change, and potentially leads to extinction (Frankham et al., 2002). A meta-analysis of population genetic studies determined that disturbed, hunted or fragmented, mammalian populations ( $A_{ave} = 6.9$ ) have appreciably lower genetic diversity than large outbred mammalian populations ( $A_{ave} = 8.8$ ; DiBattista 2002). The Florida, Belize, and Puerto Rico manatee populations have reduced genetic diversity ( $A_{ave} = 5.3, 3.4, \text{ and } 3.9$ , respectively) as compared to the average disturbed mammalian population, emphasizing the need for conservation practices that protect and maximize the existing genetic diversity. Additionally, the Florida population has a smaller effective population size,  $N_e$ , relative to the  $N_e$  and size of the Belize and Puerto Rico populations.  $N_e$  is defined as the number of individuals in an idealized population that would show the same amount of inbreeding or loss of genetic diversity as the population under consideration (Wright 1931, 1938). These data suggest that although Florida is the largest West Indian manatee population, levels of genetic diversity have not yet recovered to allow for long-term sustainability. Small population sizes, reduced genetic diversity, and lack of understanding of evolutionary relationships underscore the need for continued research, monitoring, and conservation of all West Indian manatee populations.

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Genetic Studies and Population Structure of the West Indian Manatee (*Trichechus manatus manatus*) in Mexico.

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The West Indian manatee (*Trichechus manatus manatus*) is an endangered species that inhabits the Atlantic coast from the Gulf of Mexico to Brazil. It is a protected species under international agreements and in each country further protected under national laws. To date there is little known about the population estimate in Mexico. For the state of Quintana Roo there is an estimation of between 200 to 250 manatees, which represent close to the suspected total of individuals occurring in the Yucatan Peninsula. The conservation of the manatee in Mexico could be improved by incorporating genetic data into the management plan. Manatee population analysis, using mtDNA, from different areas of its worldwide distribution, illustrates a low level of genetic variability (Garcia-Rodriguez *et al.*, 1998, Vianna *et al.*, 2006). In Mexico, the Gulf population shows a lower level of genetic diversity compared to the Caribbean population (Castañeda and Morales, 2005). Microsatellites, for their high level of variability between individuals, is used for better evaluation of the level of genetic variability tends to more accurately reflect the genetic differentiation not only of the maternal genes but that of both parents.

We analyzed 98 samples from Quintana Roo (66), Tabasco (18), Chiapas (5) and Veracruz (9) for sixteen microsatellites. We observed high variability among individuals using these polymorphic microsatellites that allows us to identify each individual (Sib P(ID) = 1,65E-05; HW P(ID) = 2,68E-13). Genetics structure results indicate a moderate differentiation ( $R_{ST} = 0.1042$ ) between the Caribbean Coast cluster and the Gulf of Mexico one, as has been previously suggested by mtDNA analysis. The Caribbean Coast appears as a mixture of manatees from the Gulf of Mexico and Florida. The information generated from microsatellites is used to make recommendations to determine management units and for conservation. It will also be used to better estimate the number of individuals, determine the reproductive success of the individual manatees in the population in Mexico as well as propose a baseline for reproduction in captivity to minimize inbreeding.

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## Auditory and Tactile Detection by Florida Manatees

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The turbid waters that manatees inhabit in conjunction with poor visual acuity (Bauer et al., 2003) suggested to researchers that the senses of hearing and touch would play a substantive role. This has been born out for both hearing (Gerstein et al., 1999; Mann et al., 2005; Colbert et al., 2007) and active touch (Batcheler and Dehnhardt, 1999; Bauer et al., 2005). Our recent research further emphasizes the manatees' specializations for detecting high frequency pressure waves using auditory mechanisms and low frequency vibrotactile stimuli.

A behavioral audiogram indicated that manatee auditory frequency detection for tonal stimuli ranged from 0.25 to 90.5 kHz with peak sensitivity extending from 8 to 32 kHz. Critical ratios, thresholds for tone detection in the presence of background masking noise, were determined with one-octave wide noise bands, 7 – 12 dB (spectrum level) above the thresholds determined for the audiogram under quiet conditions. Manatees appear to have quite low critical ratios, especially at 8 kHz. This suggests that manatee hearing is sensitive in the presence of background noise, which also suggests that they have relatively narrow filters in this frequency range. Interestingly, many manatee vocalizations are tonal harmonic complexes that often include a tonal component in the 4-8 kHz range.

Manatees possess specialized sensory hairs (vibrissae) that cover their face and body, a unique attribute among mammals. Reep (2002) has suggested that they use these vibrissae to tactually sense hydrodynamic stimuli. The first phase of a planned, multi-stage investigation of the role of the vibrissae in detecting low frequency vibrations has been completed. Two Florida manatees were tested in a go/no-go paradigm using a modified staircase method to assess their ability to detect water movements created by a sinusoidally oscillating sphere that generated a dipole field at frequencies below the apparent functional hearing limit. The detection data were used to generate a tactogram, a graphic representation of thresholds for particle velocity at various frequencies. The manatees detected stimuli down to a frequency of 5 Hz.

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## The Neural Basis for Tactile Hair Sensation in Manatees

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All sirenian hairs are vibrissae (tactile hairs), distributed over the entire face and postfacial body, in contrast to the restricted distribution seen in most other mammals. This suggests an expanded functional role for vibrissae in sirenians. The facial vibrissae are used most often in direct tactile contact during feeding or investigation of novel objects. The postfacial vibrissae appear to detect hydrodynamic stimuli, potentially including significant environmental stimuli such as approaching animals, water currents and tidal flows, and changes in topographic contours of the shallow water environment. Therefore, this system may be used for “touch at a distance”, analogous to the lateral line system in fish.

As revealed by immunofluorescence, all manatee vibrissae follicles have many types of C, A $\delta$  and A $\beta$  innervation including Merkel, club, and longitudinal lanceolate endings at the level of the ring sinus (Sarko et al., 2007a). Manatee follicles have two unique types of A $\beta$ -fiber endings: exceptionally large-caliber axons that branch to terminate as novel, gigantic spindle-like endings located at the upper ring sinus, and smaller-caliber A $\beta$  fibers that terminate in the trabeculae of the cavernous sinus as an ending that resembles a Golgi tendon organ. Postfacial follicles are markedly smaller than those of the face. Each postfacial follicle is supplied by ~30 axons, compared to ~50 axons innervating follicles of the perioral bristles, and 70-225 axons per oral disk follicle. A total of ~5000 follicles are present on the body, innervated by a total of ~210,00 axons.

The brainstem, thalamus, and cerebral cortex exhibit anatomical specializations likely associated with processing the large amount of information from the vibrissae. In the brainstem these include large, lobulated trigeminal nuclei for inputs from oral disk vibrissae and perioral bristles, an intricately patterned cuneate-gracile complex for vibrissal projections from the forelimb flipper and trunk of the body, and a large Bischoff's nucleus in the caudal brainstem that presumably receives input from the fluke (Sarko et al., 2007b). In the thalamus large, subdivided ventral posterior thalamic nuclei that receive input from these brainstem somatosensory nuclei constitute a disproportionately high volume of the thalamus. The trigeminal-recipient ventral posteromedial thalamic nucleus and the ventral posterolateral nucleus, which receives inputs from the postfacial body, are comparable in size (Sarko et al., 2007b). Within cerebral cortex, the large presumptive somatosensory cortex appears to contain multiple functional representations, including neuron aggregates that may correspond to vibrissal specializations seen in the cortices of other taxa (Sarko and Reep, 2007). The putative primary somatosensory cortex of the manatee is disproportionately large relative to primary auditory and visual cortices, as in other somatosensory specialists like the naked mole-rat, echidna and platypus.

Many of the somatosensory specializations present in manatees are also seen in dugongs, and thus appear to be associated with adaptation to aquatic herbivory. Unusual cases such as these

are important not only because of their relationship to taxon-specific behavioral adaptations, but also because they represent the range of variation present in sensory systems and their central representations, and thus the known extent of evolutionary potential.

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## The Manatee Respiratory System in Health, Rehabilitation, Anesthesia, and Disease

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The Florida Manatee, *Trichechus manatus latirostris*, has a number of interesting and unique anatomic characteristics of its respiratory system that have evolved for success in the aquatic environment (Rommel and Reynolds, 2000). These anatomic differences impact the severity of lesions from traumatic episodes and can affect the level of care given to injured, ill and anesthetized animals.

With the initiation of a new respiratory cycle the nasal flaps fold ventral caudally. Expiration is rapidly initiated and is closely followed by a strong more prolonged inspiration. Air travels the nasal passage divided into channels (meatus) before converging at the caudal pharynx. Here it passes by relaxed pharyngeal tissue that also may serve to protect the animal from accidental inspiration before entering the open glottis. The distance from the glottis to the separation of the trachea into right and left main stem bronchus is very short often less than 10 cm depending on the size of the animal. The manatee lungs are elongate, nonlobed, and relatively flattened structures. The lungs are situated longitudinally along the back with each lung anchored to the vertebrae along the medial border and completely separated from the other by a complete hemidiaphragm. Each hemidiaphragm is attached to the ventral surface of the vertebrae in a horizontal plane.

The clinician involved with manatee anesthesia is aware that intubation through the oral cavity is difficult since the mouth is very narrow, the surface of the tongue is curved and the soft palate extended interfering with access to the glottis. The first attempts at oral intubation were unsuccessful so the nasal cavity was used providing direct access to the glottis. Once intubated the tube placement is checked with a bronchoscope to verify that the endotracheal tube is placed in the short trachea and not extended into one lung.

The upper respiratory system may be affected by parasites (*Cochleotrema cochleotrema*). A severe infection of these trematodes can cause nasopharyngitis. *C. cochleotrema* can also be found in the airways of the lungs. Inhalation of aerosols of brevetoxin can lead to mucosal congestion in the nasopharynx. Cold stress may affect the borders of the nasal flaps resulting in epidermal and dermal necrosis. Fishing hooks have been embedded in the caudal pharyngeal tissue or soft palate resulting in abnormal inspiratory and expiratory noise.

The lower respiratory system of the manatee may be affected by a number of etiologies similar to other species including bacterial (broncho)pneumonia, pleuritis, and abscessation. However, the most common pathologies of the lower respiratory system are trauma-related. Sharp propeller trauma can expose the chest, and in some cases a lung prolapses through the wound. Acute blunt trauma may involve fractured ribs, lung

damage/collapse, pneumothorax, hemothorax, and/or subcutaneous emphysema. Secondary infection of the damaged tissues can lead to pulmonary adhesions, pneumonia, pyothorax, open fistulous tracts and chronic pulmonary consolidation. Other complications from trauma involve diaphragmatic hernia and lung torsion when the caudal loose portion of the lung folds anteriorly and becomes trapped. When large amounts of blood, purulent material, or air accumulate in the damaged hemithorax, the animal will function on only one lung and exhibit buoyancy problems. Bacterial etiologies include aerobic and anaerobic organisms and require therapy geared toward both categories. Knowledge of the common complicating factors in injured animals can allow the clinician to prevent additional deterioration with the proper use of antibiotics, fractured rib management techniques and sedation to minimize additional chest damage during handling and feeding.

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## Manatee Brevetoxicosis and Cold Stress Syndrome: Pathologic Features of 'Natural Disease'

### **Gregory D. Bossart**

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The Florida manatee (*Trichechus manatus latirostris*) is one of four living species in the scientific order Sirenia and is considered endangered due to human-related and perinatal mortality, as well as destruction and degradation of habitat caused by widespread development in Florida. Manatees appear to be remarkably resistant to natural disease although brevetoxicosis (intoxication with Florida red tide toxins) and cold weather conditions have taken a toll during the last decade. The pathologic features of these natural mortality events are unique.

Manatee brevetoxicosis can occur via ingestion and/or inhalation. The inhalational route of brevetoxin exposure appears to be unique in manatees but shared with humans. The gross lesions of brevetoxicosis include severe nasopharyngeal, pulmonary, hepatic, renal and cerebral congestion and nasopharyngeal and pulmonary edema and hemorrhage. Consistent microscopic lesions may consist of catarrhal rhinitis, pulmonary hemorrhage and edema, multiorgan hemosiderosis and nonsuppurative leptomeningitis. Immunohistochemical staining using a polyclonal primary antibody to brevetoxin (GAB) demonstrates intense positive staining of lymphocytes and macrophages in the lung, liver and secondary lymphoid tissues. Additionally, lymphocytes and macrophages associated with inflammatory lesions of the nasal mucosa and meninges also are positive for brevetoxin. The data suggest that manatee mortality resulting from brevetoxicosis may not necessarily be acute but may occur after chronic inhalation and/or ingestion. Immunohistochemical staining with interleukin-1- $\beta$ -converting enzyme shows positive staining with a cellular tropism similar to GAB. This suggests that brevetoxicosis might initiate apoptosis and/or the release of inflammatory mediators that culminate in fatal toxic shock. Manatees from Florida's coastlines have continuous potential brevetoxin exposure because red tide blooms are common in these areas. Therefore mortality-associated brevetoxicosis may be cumulative and the result of high dose or prolonged low dose exposure to these biotoxins. Additionally, prolonged non-lethal toxin exposure may compromise normal immunologic responses predisposing these manatees to opportunistic disease.

Chronic exposure to cold water produces a cascade of clinical signs and disease processes termed the manatee cold stress syndrome (CSS). Emaciation, fat store depletion, serous fat atrophy, lymphoid depletion, epidermal hyperplasia, pustular dermatitis, enterocolitis and myocardial degeneration are consistent lesions of CSS. The data indicate that CSS is a complex multifactorial disease process that involves compromise to metabolic, nutritional and immunologic homeostasis and culminates in secondary opportunistic and idiopathic diseases.

These findings are critical for developing future management strategies for this endangered species due to the apparent increased prevalence of red tides and the disappearance or sporadic availability of man-made sources of warm water that manatees habituate. Additionally, the two diseases may be synergistic further complicating management strategies.

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Manatee papillomavirus (TmPV-1) infection among captive and free-ranging manatees.

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Manatees are among the oldest existing species. During the last decade, we identified and characterized papillomavirus, TmPV-1, from Florida manatees (*Trichechus manatus latirostris*) captive in Homosassa Springs State Wildlife Park (HSSWP) (Rector et al., 2004). This manatee-specific PV causes sessile lesion on the skin of manatees. TmPV is the only PV identified in Florida manatees, but also is one of oldest among almost 250 PV identified so far. Safe and efficacious vaccine against human PVs have been approved by FDA. It is certain that such a vaccine can be produced against TmPV-1 infection, and we generated large quantities of genetically engineered recombinant TmPV VLPs as well as anti-immunoglobulin of manatee. We used these materials to survey for serological evidence of TmPV-1 infections in manatees. Our initial pilot sero-epidemiological study showed that TmPV is highly contagious among captive manatees housed in a pool without clear evidence of the presence of TmPV in free ranging manatees. Later on, however, a large seroepidemiological study carried out on 176 sera collected from 157 different individuals over a period of 11 years at the Florida Integrated Science Center indicated that TmPV infections are common among free-ranging manatees as well as captive ones. 35.0% (14/40) of captive animals were sero-positive for TmPV and 29.1% (34/117) among the free-ranging ones. Close observation showed clinical evidence of papillomas in only the former. This accumulated information can be used in conservation of manatees.

Evaluation of trace metals in the Florida manatee  
(*Trichechus manatus latirostris*) from the Gulf Coast

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The potential impact of anthropogenic contaminants is a growing concern in many aquatic species, as these animals are exposed to a diverse group of toxicants and chemicals, including trace metals, from their surrounding environment. The Florida manatee habitat often coincides with areas of human use, placing manatees in very close proximity to anthropogenic influences by residing in many urban areas. While many studies have examined trace metal concentrations in cetaceans and pinnipeds, only two studies have been reported for metal levels in manatees (O'Shea et al. 1984, Stavros et al. 2008). Under the supervision of USGS Sirenia Project and Florida Fish and Wildlife Conservation Commission researchers, whole blood samples from 42 free-ranging manatees were collected during health assessments at 3 different sites throughout the state of Florida (Crystal River, Lemon Bay, and Everglades). Our results suggest that trace metal concentrations in whole blood of manatees differs from other marine mammals. Zinc was quite elevated at  $14.88 \pm 1.31$  ppm, while selenium was quite low at  $0.22 \pm 0.09$  ppm. However, it is unclear whether the unusual levels of trace metals are due to local environmental influences or to differences in metal metabolic processes. Whole blood metal concentrations were then compared across three sites. For example, copper concentrations in the Everglades ( $0.4 \pm 0.19$  ppm) were significantly lower than those in Crystal River ( $0.82 \pm 0.19$  ppm) or Lemon Bay ( $0.75 \pm 0.11$  ppm). These results demonstrate that the local environment can affect trace metal levels in manatees. To investigate factors that may contribute to site specific differences, plant, sediment and water samples are currently being analyzed, in addition to trace metal distribution. We have obtained various tissues from a number of different age groups and sex combinations to determine distribution of trace metals. Due to the dramatic differences in zinc levels, we are interested in how manatees maintain these levels without apparent adverse effects. Metallothionein (MT) is a small metal binding protein involved in divalent metal homeostasis in most mammals. MT has been characterized in many aquatic animals, however, has yet been examined in the Florida manatee and may play an integral role in trace metal homeostasis. We are currently developing tools to determine expression of metallothionein in tissues from the Florida manatee and the response of MT

to metal exposure. We anticipate that we may be able to develop MT expression assays as a biomarker of metal exposure in the manatee.

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## Manatee Entanglement Cases in The Field: A Team Approach in Southeast Florida

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Entanglement is the second most common traumatic injury in the Florida Manatee (*Trichechus manatus latirostris*) (Walsh and Bossart 1999). These cases present as crab trap, rope, monofilament, plastic straps or other entangled debris on the flippers, head or body. Some of the loose acute entanglements such as crab traps can be removed in the field. Cases with deeply embedded line or severe trauma are taken to a critical care facility for a thorough workup including radiographs, surgery and treatment. Although the most thorough care for any injured manatee is to go into a critical care facility, this approach may sometimes be less desirable. In recent years some unique cases have driven a third approach: aggressively removing deeply embedded line in the field to leave the manatee in the field. Using a systematic approach gives a greater degree of confidence that entangling materials are removed in entirety. Over the last recent years this approach has yielded several successful outcomes. Some of these episodes include pregnant manatees, manatees that repeatedly become entangled, and cow-calf pairs. Three pregnant manatees were disentangled in the field. Two of those were known to have given birth and were sighted with their offspring at a later date. This is important, as there is a risk involved in transporting pregnant animals. Four cow-calf pairs were disentangled in the field (in two cases the offspring was entangled and in the other two the dam was entangled). This allowed the pairs to stay together in the field. The importance is, as females are more likely to become entangled than males. This is believed to be because of the anatomy and physiology of their teats (Reep and Bonde 2006). Two of the manatees that have been known to entangle multiple times have been sighted for several years and have had offspring and even migrated during that time.

When a manatee is observed in the field with an entanglement, a radio-telemetry tag is attached to any hanging line or rope with a hemostat. It is ideal to rescue this manatee within three days of applying the tag due to the tenuous nature of the attachment and minimize further drag trauma to the entanglement site. This urgency mobilizes the rescue team to work rapidly in rescuing the manatee. A veterinarian from the critical care facility systematically removes the line to try to ensure that it is all removed. A lateral window approach utilizing numerous hemostats is used. The subject is given antibiotics, micro tagged, sampled and documented and then released in the immediate area. Adult females are ultrasounded for pregnancy. At any point if the veterinarian feels the trauma is too extensive or is unsatisfied with the amount of line removed the manatee can still be transported to a critical care facility. It is not a novel approach but one that is being utilized more to meet the needs of our manatee-human cohabitation relationship.

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Commission, Dolphin Research Center, Marine Animal Rescue Society, Save The Manatee Club, US Fish and Wildlife Service, and The Entanglement Working Group.

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The Effect of the Visiting Public on the Swimming Behavior of Captive Florida Manatees  
(*Trichechus manatus latirostris*)

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During a study of Captive Florida manatee (*Trichechus manatus latirostris*) vocalizations at the Cincinnati Zoo & Botanical Garden, our group noticed that the swimming behavior of the manatees appeared to increase when there were visitors present in the viewing hall. Because manatees are known to interact with environmental stimuli, this behavioral response was studied over a thirteen month period, during different times of the day, to determine if the swimming activity of the manatees was notably affected when visitors were present for public viewing. The two manatees (Slip and Little Joe) were males of approximately the same age, and were housed in a fresh water exhibit. Swimming activity was monitored for each manatee by counting the number of quadrants they traversed across a grid placed on the outside of the viewing windows of the tank when there were visitors present in the exhibit hall, and this was compared to swimming activity when there were no visitors present. When visitors were present, there was an overall increase in both Slip's and Little Joe's swimming activity by ~ 133% (from 3 to 7 quadrants traversed;  $p=3.3e-7$ ,  $p=3.62e-6$  respectively). Furthermore, when there were no visitors present in the P.M. hours (1201-1800), both manatees' swimming activity decreased between 50 – 60% ( $p=0.00421$ ;  $p=0.0009566$  respectively) from the A.M. hours (0700-1200), suggesting an inclination towards higher A.M. activity. However, when visitors were present, there was no significant difference in Slip's swimming activity between the A.M. of 7 quadrants traversed, and the P.M. of 7.5 quadrants traversed ( $p=0.594$ ), suggesting that the manatee may be hyperstimulated. These findings suggest a relationship between the behavioral responses of captive manatees and the presence of people in the exhibit hall.

We are currently carrying out a follow-up study with the same manatees to determine if their swimming activity is still affected in this way after they have been acclimated for a longer period to their environment. We have also expanded the study to determine if the manatees are occupying the medical pool quadrant, which is out of the view of visitors, more often when visitors are present for viewing. Further investigation may help ascertain the reason that captive manatees interact with visitors in this manner, and may also contribute to a better understanding of the effects of visitors on the swimming activity of wild manatees.

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## Monitoring and Health Assessment of Antillean Manatees (*Trichechus manatus*) in Belize

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The science of health monitoring in the marine environment at the ecosystem level is still evolving. Since we cannot easily monitor the health of all animals in an ecosystem, one method is the identification and study of “sentinel species.” These “sentinels” can provide early warnings of emerging health concerns in the marine environment, like the proverbial “canaries in the coal mine” (Tabor and Aguirre 2004). Within ecosystems the search for “canaries” needs to take into account the delicate ecological balance that is derived from species interactions. An environmental disturbance can ripple through trophic levels as cascade events, altering abundance and distribution of species. Disease outbreaks may only be a minor manifestation of diminished ecosystem health, while large alterations in predator and prey relationships may be more symptomatic. Proactive monitoring can provide sufficient information before it is too late for ecosystem remediation and healing (Aguirre and Tabor 2004).

Until recently, Belizean manatees inhabit relatively undisturbed and clean ecosystems therefore providing a unique opportunity to determine baseline ecological and health parameters (Bonde et al. 2004). Although relatively little information is known about manatees outside Florida, studies in other regions offer opportunities to better understand the behavioral ecology, life history and health of the species where they are less affected by anthropogenic factors and cold weather (Bonde et al 2004). We have studied manatees in Southern Lagoon, Belize since 1997. Manatees were captured using a boat and a net set in 1-1.5 m deep open water. Over 120 individuals were captured in multiple occasions twice a year from 1998 to 2007. Out-of-water holding time ranged from 24 to 140 minutes with an average duration of 90 minutes. Many animals were tagged with VHF or satellite radio tags connected to belts fitted around the tailstock and all were implanted with passive integrated transponder (PIT) chips to facilitate re-identification if handled again. Most of the tagged manatees were recaptured biannually to replace the tags or batteries. Health assessments were conducted based on clinical exams, ultrasonic fat measurements, hematology, blood biochemistry, and urine and fecal analyses. Morphometrics and skin tissue specimens for genetics were also collected. Data were also collected on seagrasses and environmental parameters such as salinity, water turbidity and temperature. Aerial surveys by helicopter were conducted twice a year during several years to monitor population numbers (Powell et al. 2001).

Tagged females and few males stayed in Southern Lagoon, whereas some males roamed along the coast. The calving interval for three females was longer relative to Florida manatees. Results of urine analyses revealed a new species of diplogasterid nematode. Fecal samples were not pathologic and did allow for identification of local vegetation types. Blood values for hematology and serum chemistry profiles were in the range reported as normal for manatees in Florida and Puerto Rico (Walsh and Bossart 1999).

Preliminary results indicate that variables examined to address potential affects of capture stress included the panel of serum enzymes; as well as biochemical indicators such as BUN, creatinine and potassium. Higher-than-normal elevations of serum enzymes were not detected in 15 individuals sampled 20 times. Hematological parameters were also similar when compared to Florida manatees, excepting for eosinophil counts that were higher in manatees from Belize (Table 1).

Table 1. Comparison of blood parameters measured in wild manatees captured in Belize and Florida.

Blood Parameter	Measure	Manatees in Belize n=15*	Manatees in Florida n=12**
RBC	(10 <sup>6</sup> /mm <sup>3</sup> )	2.2-2.9	2.4-3.1
Hemoglobin	(g/dl)	8.2-12.8	10.3-12
Hematocrit	(%)	26-43.4	32-40
MCV	(fl)	123-128	121-135
MCH	(pg)	33.4-44.6	37-43
MCHC	(g/dl)	31.1-42.6	30-33
Platelets	(10 <sup>3</sup> /mm <sup>3</sup> )	166-998	261-634
Leukocytes	(mm <sup>3</sup> )	1,700-7,100	4,000-11,700
Bands	%	0	0
Neutrophils	%	30-78	25-64
Lymphocytes	%	21-74	21-77
Monocytes	%	0-3	0-18
Eosinophils	%	0-55	0
Basophils	%	0-1	0-1
Total protein	(g/dl)	5.4-8.7	6.8-7.3
Albumin	(g/dl)	4.3-6.4	3.8-5.3
Glucose	(mg/dl)	36.5-169	56-117
BUN	(mg/dl)	2.0-11.6	6.4-16
Creatinine	(mg/dl)	0.8-4.4	0.4-2.1
Bilirubin T/D	(mg/dl)	0.1-1.1/0.0-0.3	0-0.1/N.D.
Cholesterol	(mg/dl)	10.8-164	107-328
Alkaline phosphatase	(U/l)	46.1-109	64-183
ALT (SGPT)	(U/l)	6.7-77.1	6-30
AST (SGOT)	(U/l)	4.7-74.7	5-28
GGT	(U/l)	14.6-265	39-64
CK	(U/l)	10.1-287	79-302
LDH	(U/l)	21.8-342	94-372
Calcium	(mg/dl)	8.0-12.2	10.1-12.2
Phosphorous	(mg/dl)	1.9-5.9	3.0-8.0
Sodium	(mEq/l)	138-155.5	142-157
Potassium	(mEq/l)	3.6-6.7	4.2-6.6
Chloride	(mEq/l)	83.3-114	90-103
Iron	(mcg/dl)	78-180	59-199

\* From Aguirre et al. 2003; \*\*From Walsh and Bossart 1999

No adverse effects of capture stress were detected post-release in the manatees analyzed for this preliminary study. Dugongs have been prone to capture myopathy and typically display elevated serum enzymes and biological indicators of tissue damage. Florida manatees have been documented to tolerate capture and handling activities without susceptibility to capture stress. Future studies will include bacteriologic and virologic analysis of samples and hormonal profiles. Complete health assessments are conducted on Florida manatees whenever possible (Walsh and Bossart 1999). We will make comparisons of haematology and serum biochemistry panels recently published for the Florida manatee and determine the health status of populations at both locations. Prospective epidemiological studies comparing condition indices, hematological and serum biochemistry parameters within and among

individuals, seasons and subpopulations will serve as indicators of health of this brackish lagoon ecosystem (Aguirre et al. 2002a, 2002b).

Investigations on the infectious disease of manatees are relatively recent. Antibodies to some infectious agents have been found in the wild and captive Florida manatees, including porpoise and dolphin morbilliviruses, pseudorabies virus, San Miguel sea lion virus type 1 and several equine encephalitis viruses (Duignan et al. 1995). No evidence of clinical disease to any of these agents has been demonstrated. Manatees that are environmentally stressed are known to be immunologically suppressed and susceptible to pathogens, as was recently reported with an outbreak involving papillomavirus in Florida (Bossart et al. 2002).

In collaboration with University of California-Davis, serum specimens from this study will be tested for a variety of agents incriminated as potential pathogens in marine mammals including canine distemper virus, marine morbilliviruses, pseudorabies, bovine herpes mammillitis, bovine coronavirus, San Miguel seal lion virus, marine caliciviruses, bovine adenovirus type 1 and type 5, avian influenza, eastern equine encephalitis, Venezuelan equine encephalitis, western equine encephalitis, calicivirus, adenovirus, *Leptospira* sp., *Brucella* sp., *Chlamydomphila psittaci*, *Toxoplasma* sp., brevetoxins, St. Louis encephalitis virus and West Nile virus, among others. Also, in collaboration with University of Florida and Columbia University we will measure exposure to heavy metals and compare to environmental conditions and potential anthropogenic influences in Belize.

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