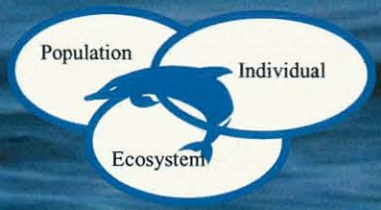
Synopsis of Researcher Meeting
Bottlenose Dolphin Health & Risk Assessment Project
February 22-24, 2005



NOAA Technical Memorandum NOS NCCOS 10

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HBOI for the two insert pictures on dolphin captures in the Indian River Lagoon, FL and NOS/CCEHBR for cover picture on freeze-brand #822 dolphin in Charleston, SC (NMFS Permit No. 998-1678-00)

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Synopsis of Researcher Meeting – Bottlenose Dolphin Health & Risk Assessment Project February 22-24, 2005

Patricia A. Fair, Ph.D., NOAA/ National Ocean Service/Center for Coastal Environmental Health & Biomolecular Research and **Gregory D. Bossart, V.M.D., Ph.D.**, Division of Marine Mammal Research and Conservation, Harbor Branch Oceanographic Institution, Fort Pierce, FL

Center for Coastal Environmental Health and Biomolecular Research
NOAA/NOS/NCCOS
219 Fort Johnson Road
Charleston, South Carolina 29412-9110

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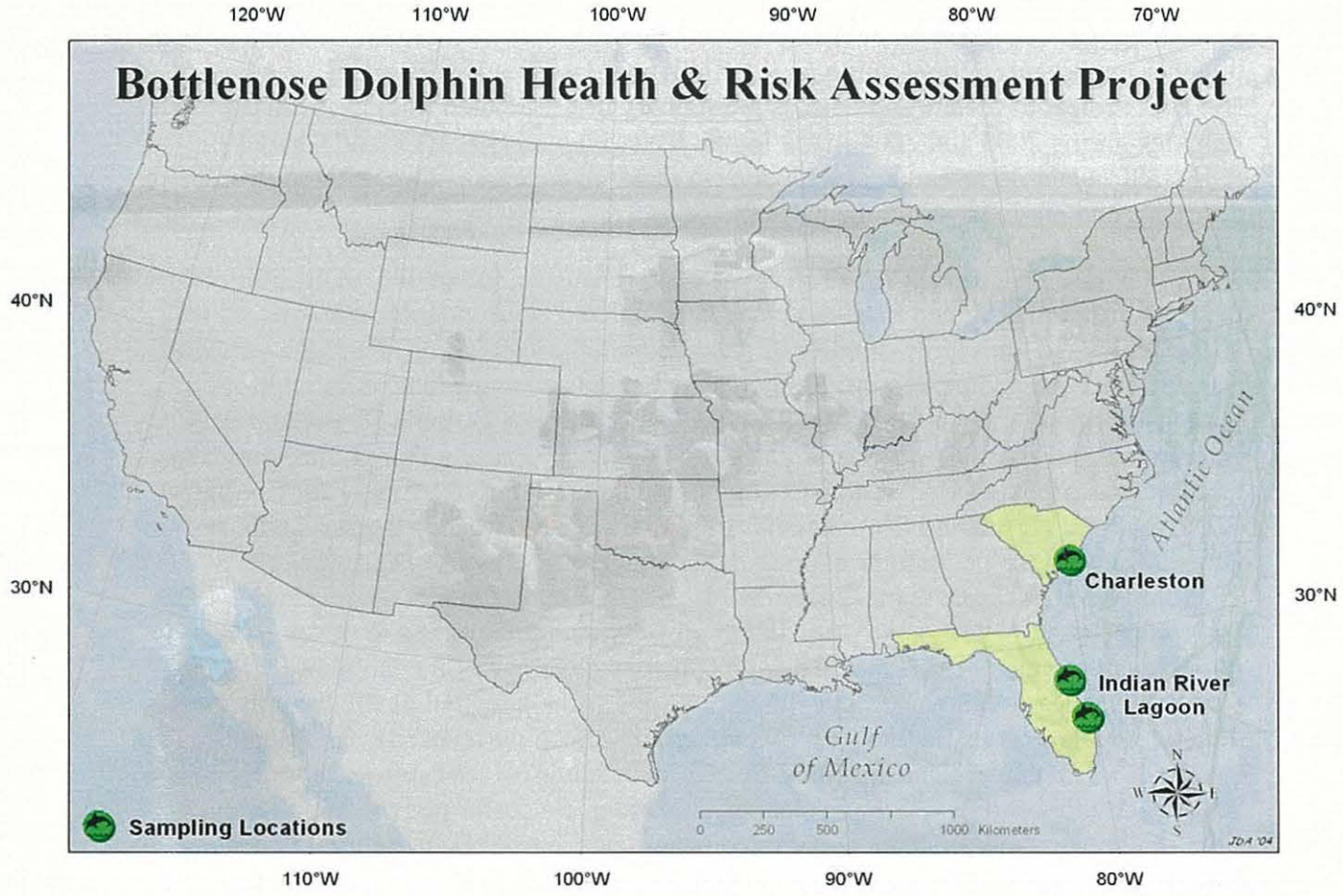


Foreground: Map showing the Charleston South Carolina and Indian River Lagoon (north and south) Dolphin HERA Project study area locations. Background: Collecting and sampling bottlenose dolphins in the Charleston, SC study area.

Executive Summary

A meeting was convened on February 22-24, 2005 in Charleston, South Carolina to bring together researchers collaborating on the Bottlenose Dolphin Health and Risk Assessment (HERA) Project to review and discuss preliminary health-related findings from captured dolphins during 2003 and 2004 in the Indian River Lagoon (IRL), FL and Charleston (CHS), SC. Over 30 researchers with diverse research expertise representing government, academic and marine institutions participated in the 2 ½ day meeting.

The Bottlenose Dolphin HERA Project is a comprehensive, integrated, multi-disciplinary research program designed to assess environmental and anthropogenic stressors, as well as the health and long-term viability of Atlantic bottlenose dolphins (*Tursiops truncatus*). Standardized and comprehensive protocols are being used to evaluate dolphin health in the coastal ecosystems in the IRL and CHS. The Bottlenose Dolphin Health and Risk Assessment (HERA) Project was initiated in 2003 by Dr. Patricia Fair at the National Oceanic and Atmospheric Administration/National Ocean Service/Center for Coastal Environmental Health and Biomolecular Research and Dr. Gregory Bossart at the Harbor Branch Oceanographic Institution under NMFS Scientific Research Permit No. 998-1678-00 issued to Dr. Bossart. Towards this end, this study focuses on developing tools and techniques to better identify health threats to these dolphins, and to develop links to possible environmental stressors. Thus, the primary objective of the Dolphin HERA Project is to measure the overall health and as well as the potential health hazards for dolphin populations in the two sites by performing screening-level risk assessments using standardized methods. The screening-level assessment involves capture, sampling and release activities during which physical examinations are performed on dolphins and a suite of nonlethal morphologic and clinicopathologic parameters, to be used to develop indices of dolphin health, are collected. Thus far, standardized health assessments have been performed on 155 dolphins during capture-release studies conducted in Years 2003 and 2004 at the two sites. A major collaboration has been established involving numerous individuals and institutions, which provide the project with a broad assessment capability toward accomplishing the goals and objectives of this project.

The objectives of the meeting were to:

- review findings to date on the health assessment of IRL and CHS dolphins
- identify meaningful health indices and their implications
- identify existing data gaps and advise on future directions

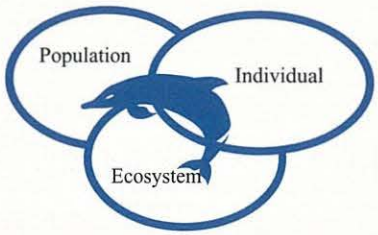
Researchers provided an overview of their subject matter, described those analyses which have been completed, provided a summary of their findings to date, indicated what other potential data might be useful in interpreting and understanding their findings, described the health implications of these correlations, and identified any planned publications. Abstracts of all presentations are included in this report. Breakout groups convened into working groups (I. Pathology/Epidemiology, II. Immunology, III. Molecular, Cellular and Contaminants, IV. Microbiology/Nutrition) on the afternoon of February 23 to discuss finding among the studies and which parameters may be important and sensitive indicators for assessing dolphin health. Discussion topics also addressed what other measurements and research approaches might be considered. Discussion leaders reported

summaries from each of the research areas the during the morning session on February 24. A consensus from the group discussions highlighted the need to integrate and standardize data and discussion centered on ways in which this could be done. In order to conduct assessment on a complex and large data set it was proposed that a tiered approach be developed. The first tier would develop criteria for determining "healthy" animals identified by veterinary observations and body condition (Tier I) and the health status would be further delineated using CBC/serum chemistries (Tier II) followed by other health related data (Tier III). A Data Integration/Standardization Committee was proposed to facilitate data comparisons in all areas both categorically and statistically in a consistent manner among data sets.

Thus far, preliminary findings indicate several differences between dolphins who inhabit the IRL and those that reside along the Charleston coastal regions. Some of these findings reported at the Researcher Meeting are highlighted below:

► Indian River Lagoon dolphins show higher signs of infectious diseases, such as lobomycosis, which was found to occur only in the south IRL at a 30% prevalence. Benign mucocutaneous neoplasia associated with novel papilloma and herpes viruses have been documented in IRL study dolphins (see Reif et al., ABSTRACT).
► Cytological evaluations in 2003 indicate higher occurrence of severe acute gastric inflammations in the IRL dolphins compared to the CHS dolphins, but lower levels for both populations occurred during 2004.
► Immunologic evaluations indicate difference in immune responses with the IRL dolphins having higher innate responses evidenced by higher plasma lysozyme and monocte phagocytosis than animals in Charleston. In contrast, animals in IRL exhibited lower B-cell proliferation than animals in Charleston (see Peden-Adams and Romano ABSTRACT; Romano et al., ABSTRACT).
► Results to date indicate that the Charleston dolphins have a higher body burden of persistent chemicals. Measurements include perfluorinated compounds that have emerged as priority contaminants due to recent reports regarding their persistence in environmental and biological samples as well as concerns regarding their toxicity. Results to date suggest that the Charleston animals have the highest average perfluoroctane sulfonate in plasma of any dolphin population studied (see Magali et al., ABSTRACT) and among the highest of any species (marine and freshwater) surveyed to date. And these results are consistent with the biomarker studies examining cytochrome P4501A1 expression (see Montie et al., ABSTRACT).
► Antibiotic resistance of *Esherichia coli* bacteria in fecal samples was evaluated for resistance to 25 antibiotics during the health assessment studies. Results from 2003 indicated that of the 15 animals captured in the IRL, three (20%) exhibited antibiotic resistance to one or more of the antibiotics tested. Of the 23 animals captured in CHS 16 (70%) exhibited resistance to one or more of the antibiotics tested (see Bemiss et al., ABSTRACT). Data from this study on antibiotic resistant *E. coli* in wild caught dolphins will be included in a larger assessment of health and environmental parameters to further our understanding on the transfer and potential impacts of antibiotic resistance in coastal dolphins.

Early findings from the comprehensive health assessments of dolphins in the IRL and Charleston are revealing differences in several health parameters between these animals, and several new tests and tools have promising applications. Conducting health assessment studies over the 5-year timeframe is critical to identifying trends and addressing variability between years. Comparisons between two distinct populations over time will help researchers identify factors that pose the greatest health risks to these dolphins and determine their relationships to environmental exposures.



Bottlenose Dolphin HERA Project
Feb 22-24, 2005 Researcher Meeting

List of Collaborators and Participants

National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC – Patricia Fair [pat.fair@noaa.gov] Jeff Adams [jeff.adams@noaa.gov] Wayne McFee [wayne.mcfee@noaa.gov] Bobbie Lyon [bobbie.lyon@noaa.gov] Greg Mitchum [greg.mitchum@noaa.gov] Maggie Holbrook [margaret.holbrook@noaa.gov] Gloria Seaborn [gloria.seaborn@noaa.gov] Melissa Recks[Melissa.recks@noaa.gov] Amy Wang [amy.wang@noaa.gov] Eric Zolman [eric.zolman@noaa.gov] Leslie Burdett [leslie.burdett@noaa.gov] Lauren Beddia [lauren.beddia@noaa.gov]

Harbor Branch Oceanographic Institution, Fort Pierce, FL – Gregory Bossart [gbossart@hboi.edu] Rene Varela [RVarela@HBOI.edu] Juli Goldstein [JGoldstein@HBOI.edu] Steve McCulloch [McCulloch@HBOI.edu] Manuela Rehtanz [MRehtanz@HBOI.edu]

Medical University of South Carolina – Margie Peden-Adams [pedenada@musc.edu] Sebastiano Gattoni-Celli [gattoni@radonc.musc.edu] Annalaura Mancia[mancia@musc.edu]
Mats Lundquist [lundquml@musc.edu] Tom Hulsey [hulseytc@musc.edu]

Mystic Aquarium, Mystic, CT – Tracy Romano [tromano@mysticaquarium.org]

Brown Cancer Center, University of Louisville School of Medicine, Louisville, Kentucky – Manuela Rehtanz [MRehtanz@HBOI.edu]

Woods Hole Oceanographic Institution, Woods Hole, MA – Mark Hahn [mhahn@whoi.edu] Eric Montie [emontie@whoi.edu]

University of Connecticut, Storrs, CT – Sylvain Deguise [sdeguise@canr1.cag.uconn.edu]

University of Southern Mississippi, MS – Rhonda Patterson [Rhonda.patterson@usm.edu]

Micrim Labs, Ft. Lauderdale, FL – John Pisani [info@micrimlab.com]

United States Department of Agriculture, Beltsville, MD – Ron Fayer [RFAYER@ANRI.barc.usda.gov]

National Water Research Institute, Environment Canada, Ontario, Canada and University of Guelph, Ontario, Canada – Derek Muir [derek.muir@ec.gc.ca] Magali Houde [mhoude@uoguelph.ca]

Hubbs Sea World Research Institute/University of Central Florida, Orlando, FL – Asha Samuel [asamuel@mail.ucf.edu]

Collaborators/Researchers who could not attend:

National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC – John Bemiss [john.bemiss@noaa.gov]

US Navy Marine Mammal Lab, San Diego, CA – Bill VanBonn [william.vanbonn@navy.mil]; Stephanie Wong [stephanie.wong@navy.mil]

Clemson University, Clemson, SC – Charlie Rice [cdrice@CLEMSON.EDU]

Skidaway Institute of Oceanography, Savannah, GA – Dick Lee [dick@skio.peachnet.edu]

Medical University of SC, Charleston, SC – Greg Warr [warrgw@musc.edu] John Baatz [baatzje@musc.edu] Gary Gilkeson [gilkeson@musc.edu] Pam Morris [morrisp@musc.edu]

Brown Cancer Center, University of Louisville School of Medicine, Louisville, Kentucky - A Bennett Jenson [abjenson@yahoo.com] Shin-je Ghim [shinjeg@yahoo.com]

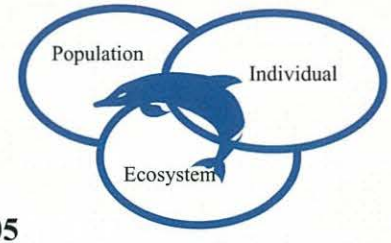
Bayside Hospital for Animals, Fort Walton, FL – Forrest Townsend [bayvet@bha.gccoxmail.com]

University of FL, Gainsville, FL – Lou Guillette [ljg@zoo.ufl.edu]

USDA, Beltsville, MD – J.P. Dubey [JDUBEY@ANRI.barc.usda.gov]

Hubbs Sea World Research Institute/University of Central Florida, Orlando, FL – Graham Worthy [gworthy@mail.ucf.edu>]

Mystic Aquarium, Mystic, CT – Lisa Mazzaro [lmazzaro@MysticAquarium.org]



HERA Bottlenose Dolphin Project
Researcher Meeting – February 22-24, 2005

Agenda

Tuesday - February 22

8:30 am	Welcome and Introductions – Pat Fair and Greg Bossart
8:45 am	Overview, Goals and Objectives of HERA Dolphin Project – Pat Fair
9:25 am	HERA Database – Jeff Adams
9:40 am	HERA Data Analysis/Epidemiology – Tom Hulsey
10:00 am	Epidemiology – John Reif
10:20 am	***Break***
10:35 am	Animal Demographics/Life History – Wayne McFee
10:55 am	Clinicopathologic Data • Clinicopathologic Review – Gregory Bossart • IRL Blood Data – Juli Goldstein, Eric Reese, Rene Varela, Gregory Bossart • Clinical Analytes/Istat – Rene Varela, Juli Goldstein, Gregory Bossart
12:10 - 1:15	***Lunch***
1:30 pm	Clinicopathologic Data – continued- • Ultrasound Analysis – Juli Goldstein, Rene Varela, Woody Hayes, Gregory Bossart • Description of A New Dolphin Papilloma Virus (TmPV-2) – Manuela Rehtanz, Bennett Jenson, Shin-je Ghim, Gregory Bossart
2:30 pm	Immunology – • Development of An Immunology Diagnostic Suite – Margie Peden-Adams • Immunology Assessments - Margie Peden-Adams • Immunology Assessments - Tracy Romano
3:30 pm	***Break***
3:45 pm	Immunology Assessments - Charlie Rice
4:05 pm	Immunology Assessments – Sylvain DeGuise
4:25 pm	Contaminants • Organic Contaminants – PCBs, pesticides, PBDE – Pat Fair, Greg Mitchum • Perfluorinated chemicals – Magali Houde, Derek Muir

Wednesday - February 23

8:30 am Molecular/Cellular Diagnostics

- Cytochrome P450, Other Biomarkers – Eric Montie, Mark Hahn
- Dolphin Microarray – AnnaLaura Mancia, Greg Warr
- Development of Skin Cell Cultures – Sebastiano Gattoni-Celli

9:45am Microbiology/Serology
Serology

- Toxoplasma, Cryptosporidium and Microsporidia – Ron Fayer
- Erysipelothrix – Rhonda Patterson

10:25 am ***Break***

10:40 am Microbiology

- *E.coli* Antibiotic resistance – John Bemiss, Bobbie Lyon,
- Tom Greig, Pat Fair
- Microbial Flora – John Pisani, Pat Fair

11:20 am Nutrition

- Lipids/ Fatty Acids – Gloria Seaborn
- Lipids/ Fatty Acids – Asha Samuel

12:00 - 1:15 ***Lunch***

1:30 pm Breakout Groups

Thursday - February 22

8:00 am - 10:30 am Breakout Group Summary by Leaders
10:30 am Discussion/Closing Comments

Overview of the Bottlenose Dolphin Health & Risk Assessment Project

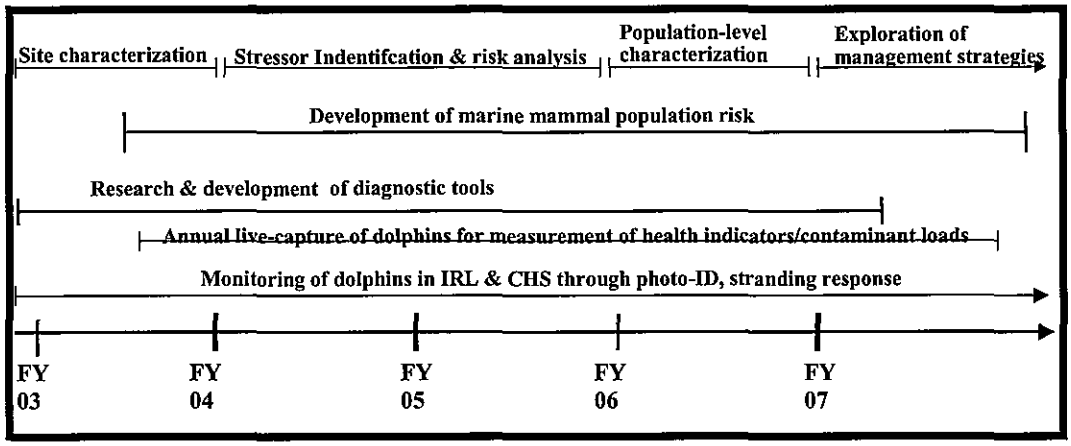
Patricia A. Fair[1] and Gregory D. Bossart[2]

[1]National Oceanic and Atmospheric Administration/National Ocean Service/Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC; [2]Harbor Branch Oceanographic Institution, Ft. Pierce, FL

A multi-disciplinary study initiated in 2003 evaluated the health of bottlenose dolphin *(Tursiops truncatus)* populations at two U.S. sites in the southeastern United States: Charleston, South Carolina (CHS) and the Indian River Lagoon, Florida (IRL) (Figs 1, 2, and 3). A specific goal was to assess anthropogenic and environmental factors that might affect dolphin health. During the summer seasons of Years 2003 and 2004 (June in the IRL and August in CHS) 155 bottlenose dolphins were captured, examined, sampled, marked and safely released. This study focuses on developing tools and techniques to better assess health of bottlenose dolphins, as well as to identify health threats and develop links to possible environmental stressors. Additional research goals were to: 1) evaluate dolphin health from individual, population and comparative perspectives at two distinct geographic locations, 2) apply classical and novel methods and diagnostic tools to detect and assess anthropogenic and environmental factors that affect dolphins, and 3) use these data to develop predictive models which evaluate conservation and management strategies. These comprehensive investigations are aimed at understanding the cumulative effects of multiple stressors and will ultimately provide information critical to the preparation of effective management plans. One of NOS/CCEHBR goals, consistent with NMFS mandates, is to validate and optimize a comprehensive suite of meaningful parameters that can be applied to evaluate health of dolphin populations throughout their geographic range.

The HERA Dolphin Project was designed as a 5-year project as outlined below.

HERA Dolphin Project 5-Year Timeline



Site Investigations

The health assessment of dolphins is especially critical in areas where stocks are depleted or show signs of epidemic disease or high mortality, and in areas where the habitat is being intensely altered or impacted by human influences. Recent studies, as well as observations and expert recommendations, highlight the need for a bottlenose dolphin health assessment at the IRL and CHS sites. The National Coastal Condition Report (2001, 2004) described ecological conditions in southeastern estuaries as "fair", with a high level of contaminants detected over moderate areas and low-level contamination over broader areas, particularly pesticides and metals. Of the Carolinian province, nearly 772 from a total of 4,487 square miles were ecologically degraded in respect to benthos. The bottlenose dolphin represents a key component in both the Indian River Lagoon and Charleston ecosystems. Further, as an apex predator, bottlenose dolphins serve as a sentinel species for monitoring the health of the environment.

Indian River Lagoon, Florida (IRL): The IRL is a shallow water ecosystem that comprises 40% of Florida's central east coast. Human population growth adjacent to the Indian River Lagoon increased from 1970-1990 by 124% and is projected to reach 1.1M by 2010 (Indian River Lagoon National Estuary Program 1990). The IRL extends 250 km from Ponce De Leon Inlet in the north to Jupiter Inlet in the south and consists of three estuarine bodies of water, the Indian River, Banana River and Mosquito Lagoon. For the health assessment capture-release studies, the IRL was divided into two separate areas consisting of a north and south site. Dolphins captured in the IRL during 2003-2004 are shown in Fig. 1 and 2. The IRL is connected to the Atlantic Ocean by six small

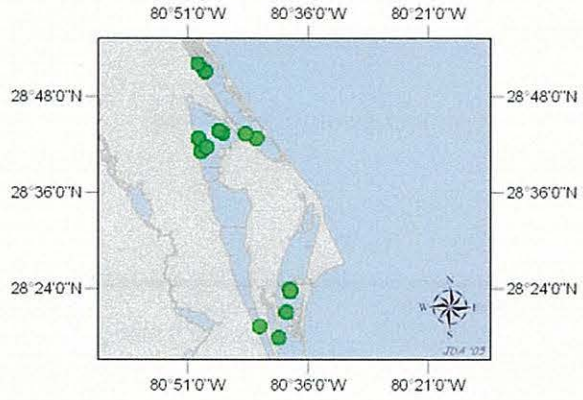


Figure 1. Indian River Lagoon (north site) 2003 and 2004 captured dolphins.

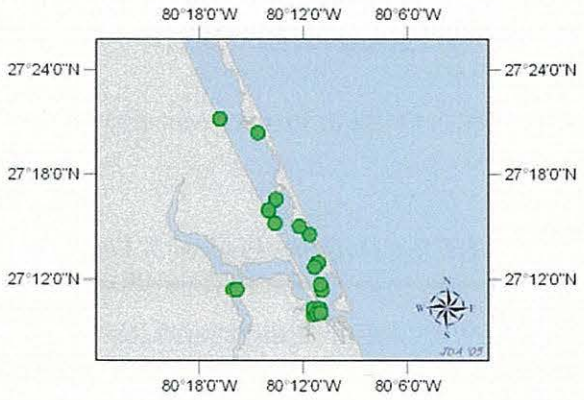


Figure 2. Indian River Lagoon (south site) 2003 and 2004 captured dolphins.

and widely spaced inlets. Exchange of waters between the Lagoon and ocean is limited due to the size of these inlets and the shallow nature of the Lagoon. As a consequence of limited circulation and tidal exchange, the lagoon is particularly vulnerable to the influx of pollutants. Water quality in the IRL has changed significantly over the past five decades due to significant watershed alteration and land drainage patterns. The major cause of water quality decline is fresh and storm water discharges that alter salinity, water

clarity and introduce nutrients and pollutants into the system (Scott et al. 2003). All of Florida's sugarcane, approximately 38 % of the citrus and 42% of the vegetable crops are grown in an area that drains into the IRL (Miles and Pfeuffer 1997).

Presently, there is concern for the overall health of the IRL ecosystem due to destruction of sea grass habitat, alteration of water flow, and declining water quality. Examination of benthic distributions in the southeast U.S. showed that large tidal rivers such as the IRL had the largest proportion of their estuarine bottom area represented by poorer than expected benthic estuaries and that significant sediment toxicity associated with sediment existed at Newfound Harbor in the IRL (National Coastal Condition Report, 2001). Recent data collected by NCCOS/CCMA scientists and others have shown high levels of copper, as well as mercury, in the sediments of the St. Lucie Estuary in the southern part of the IRL. In addition, these same data have shown increasing levels of copper concentration in Indian River Lagoon bivalves.

Dolphins in the IRL have experienced high rates of seasonal strandings since 1996 (Stolen 1998). In 2001, an unusual mortality event of unknown etiology occurred in the northern portion of the IRL, in which at least 30 dolphins died over a two month period (MMC 2002). A substantial proportion of the observed mortality in the IRL is due to infectious diseases, and many have been diagnosed with a variety of skin lesions, including proliferative ulcerative dermatitis due to protozoans and fungi, dolphin pox dermatopathy, and a vesicular dermatopathy of unknown etiology (Bossart et al. 2003). Understanding the pathologic features associated with mortality of stranded dolphins is important, and observed patterns of skin, lymphoid and other lesions suggested a state of altered immunologic homeostasis and subsequent immunologic dysfunction.

Charleston, South Carolina: Charleston Harbor is a 1,939km^2 estuarine environment formed by three river systems, the Cooper, the Ashley, and the Wando. It is located in the central portion of South Carolina and the river systems form the third largest estuarine drainage area in South Carolina, over 26,000 ha of valuable marshland and tidal habitat (Tiner, 1997). The Charleston Harbor estuary has a soft mud bottom. Average depth is 12 m at low tide and it varies in width from 120 to 300 m. The Charleston-Harbor-Cooper River coastal plain measures 66 km^2 with an additional 105 km^2 of intertidal wetlands. The estuary is subject to semidiurnal tides with a mean range of 1.6 m near the ocean.

Long-term trends in water quality monitoring and recent scientific research suggest that waste load assimilation, nonpoint source runoff impacts, contaminated sediments, and toxic pollutants are key issues. In addition, several 'hot spots' showing high levels of heavy metals and organic compounds have been identified with high levels of heavy metals and organic compounds have been identified. High concentrations of anthropogenic trace metals, polychlorinated biphenyls (PCBs) and pesticides have been found in the sediments of Charleston Harbor, as well as the Ashley and Cooper Rivers (Long et al. 1998). Two superfund sites are found in the Charleston Harbor area and its tributaries, with the following key contaminants of concern: petroleum aromatic hydrocarbons (PAH), lead, chromium, copper arsenic, zinc and dioxin. The Charleston Harbor estuary is surrounded by urban development and has the second largest container port on the Atlantic seaboard and is the fifth largest U.S port. Additionally, two sewage

treatment plants also discharge into the basin. Among the three rivers, the Cooper River has the greatest number and density of industrial and port facilities and over 95% of the total pollutant loads are thought to be from point sources. Population from 1970-1990 grew 66% in the Charleston tri-county area with slightly over 500,000 people currently. It is the fastest growing metropolitan South Carolina area and another 600,000 people are projected by 2015 (U.S. Census Bureau, 2000).

In addition to the Charleston Harbor estuarine areas, the study area for the dolphin health assessment study extends to an adjacent area, the Stono River Estuary, approximately 20 km southwest of Charleston Harbor. This area is characterized as a well-mixed, C-type salt marsh estuary, with little freshwater inflow (Day et al. 1989), small tidal inlets with extensive mud banks and marsh vegetation, and is influenced primarily by residential development. Dolphins captured in the Charleston Harbor and Stono River estuarine areas during 2003-2004 are shown in Fig. 3.

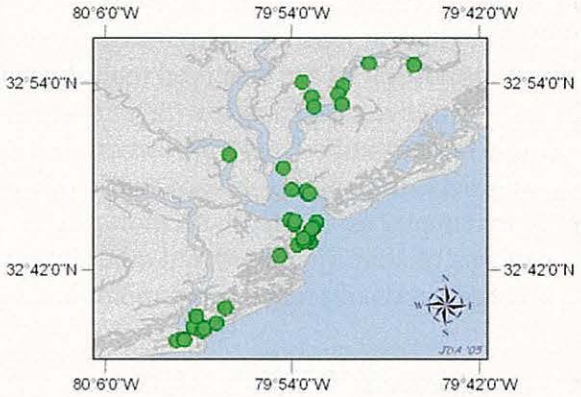


Figure 3. Charleston Harbor and Stono River estuary 2003 and 2004 captured dolphins.

Dolphins inhabiting the Charleston habitat do not show evidence of the skin lesions found in IRL dolphins. However, an increase in commercial development and the proximity of heavy industrial and agricultural influences in the CHS area suggests a potential for detrimental effects on dolphin health. Biopsy samples taken from dolphins in CHS during the last five years reveal higher concentrations of persistent organic contaminants in blubber compared to IRL dolphins, which, in turn, may indicate a greater level of environmental contamination from these compounds.

In order to enhance the validity of comparisons between the two study populations, uniform collection and sampling processing methods used standardized operating protocols (SOPs) with Quality Assurance /Quality Control measures for the capture-release studies, animal handling and sample collection. Included among these comprehensive and standardized health assessment protocols were a complete physical examination, ultrasound examination, measurement of morphometric variables and weight, collection of blood, urine, and various tissues for hematology, serum chemistry, microbiology, immune function, and an extensive suite of biomarkers. A comprehensive array of tests and measures was developed for the Dolphin HERA Project and includes

over 170 physical, clinical and chemical tests/analyses/measurements, in addition to more than 250 traditional and emerging contaminants. Early in 2004 the Dolphin HERA Project developed Safety Standard Operating Protocols for the conduct of dolphin capture-release studies.

Assessment of dolphin health with environmental parameters is a key component of the HERA Project. Environmental characterization efforts are on-going at both sites using current and historical monitoring data. The health assessment component is using an integrated approach by identifying stressors (physical, chemical, environmental) and linking diagnostic observations across levels of biological organization (toxicant, macro-molecular interactions, cellular responses, organ responses, individual and population responses). A proposed concept was to link environmental data, such as those utilized in the National Coastal Condition report, with the HERA dolphin health data as an integrated assessment. Long-term dolphin photo-identification studies at both sites will provide baseline data to compare health parameters of dolphins having known site fidelity and potential environmental exposures.

During 2004, 39 dolphins were captured in the IRL and 24 in CHS bringing the total number of captured animals for the HERA Project to 155 for the first two years. Thus far, preliminary findings indicate several differences between dolphins who inhabit the IRL and those that reside along the Charleston coastal regions. Indian River Lagoon dolphins show higher signs of infectious diseases, such as lobomycosis, which was found to occur only in the south IRL at a 30 % prevalence. Benign mucocutaneous neoplasia associated with novel papilloma and herpes viruses have been documented in IRL study dolphins. Cytological evaluations in 2003 indicate higher occurrence of severe acute gastric inflammations in the IRL dolphins compared to the CHS dolphins, but lower levels for both populations occurred during 2004. Immunologic evaluations indicate difference in immune responses with the IRL dolphins having higher innate responses evidenced by higher plasma lysozyme and monocte phagocytosis than animals in Charleston. In contrast, animals in IRL exhibited lower B-cell proliferation than animals in Charleston. Results to date indicate that the Charleston dolphins have a higher body burden of persistent chemicals. We have expanded our measurements to include perfluorinated compounds which have emerged as priority contaminants due to recent reports regarding their persistence in environmental and biological samples as well as concerns regarding their toxicity. Results to date suggest that the Charleston animals have the highest average perfluoroctane sulfonate in plasma of any dolphin population studied (see Magali et al., ABSTRACT) and among the highest of any species (marine and freshwater) surveyed to date. And these results are consistent with the biomarker studies examining cytochrome P4501A1 expression. Antibiotic resistance of *Escherichia coli* bacteria in fecal samples was evaluated for resistance to 25 antibiotics during the health assessment studies. Results from 2003 indicated that of the 15 animals captured in the IRL, three (20%) exhibited antibiotic resistance to one or more of the antibiotics tested. Of the 23 animals captured in CHS 16 (70%) exhibited resistance to one or more of the antibiotics tested (see Bemiss et al., ABSTRACT). Data from this study on antibiotic resistant *E. coli* in wild caught dolphins will be included in a larger assessment of health

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Acknowledgements

All research in the Bottlenose Dolphin Health and Risk Assessment Project was conducted under National Marine Fisheries Permit No. 998-1678-00, issued to Dr. Gregory Bossart. We would like to thank the numerous researchers who participated in the capture and release of dolphins in Florida and South Carolina and the all of the researchers who conducted sample analyses. We are especially thankful to Dr. Forrest Townsend, Larry Hansen, Eric Zolman, Steve McCulloch, Wayne McFee, the NOS and HBOI staff and all of the veterinarians who provided their expertise, and the volunteers whose help made the health assessment studies possible. We are especially grateful to the many collaborators on this project who bring extraordinary expertise and rewarding partnerships. We would like to also thank the reviewers of this report: Dr. R.H. Defran, Dr. Malcolm Meaburn, and Paul Comar.

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The Bottlenose Dolphin HERA Database

Jeffrey D. Adams

NOAA, National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC

A substantial amount of data from many collaborators has been generated during the first two years of the Dolphin Health and Risk Assessment (HERA) project. The HERA database serves as the repository for these data. The database currently contains 37 data tables (Appendix A), the majority of which are linked using the freezebrand number assigned to each captured individual. These data tables contain approximately 750 fields, the contents of which are described in Appendix B. In addition to the data tables, the database also contains 56 lookup tables (Appendix C). Lookup tables are database conventions used to reduce storage requirements, minimize data entry errors, and speed up data processing routines.

In addition to linking the data tables within the HERA database, the freezebrand number of the captured individuals also serves as a link to the Charleston, SC (CHS) and Indian River Lagoon (IRL) photo-identification databases. The link to the photo-identification databases is of particular importance as one of the primary research goals of the HERA Project is to examine the relationship between the measured dolphin health parameters and the health of the environment. To explore this relationship, it is necessary to examine the sighting histories of the captured individuals to determine the areas they traverse when conducting activities such as feeding, mating, and caring for young. While the capture of an individual may constitute the first known sighting for the individual, many of the captured individuals have sighting histories stored in the photo-identification database prior to capture. In addition, many have been sighted during photo-identification surveys subsequent to capture. These sighting histories will play a vital role in linking the health data back to the environment. Tools are currently being developed to assist researchers with little to no background in geographic information systems (GIS) or relational databases in displaying and analyzing the data contained in the health and photo-identification databases within a GIS environment.

2003-2004 Live Captures: Demographics and Life History Information

Wayne McFee, NOAA, NOS, Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC

General information on bottlenose dolphin demographics and life history was presented at the HERA live capture workshop on February 22, 2005 in Charleston, South Carolina. This information is essential for the interpretation of many of the analyses that are being performed on these wild caught dolphins in the Indian River Lagoon (IRL), Florida and Charleston, South Carolina. Data on age, length, weight, Body Mass Index (BMI), and girths were presented. Stained thin sections of 111 (51 Charleston; 60 IRL) teeth were aged following the methods of Hohn *et al.* (1989). Of these, 74 (33 Charleston; 41 IRL) were male and 37 (18 Charleston; 19 IRL) were female. The mean age of males (CHS – 15.4, range 3.5 – 28; IRL – 12.9, range 4.5-27) was greater than females (CHS - 12.6, range 3.5-33; IRL 9.7, range 3.5-26) at both sites with overall mean ages greater in Charleston than in the IRL. Asymptotic lengths were estimated using the Gompertz Growth Curve. Both sites exhibited sexual dimorphism with males reaching asymptotes at a greater length (CHS – 267.4 cm, r^2= .71; IRL – 276.3 cm, r^2= .79) than females (CHS – 247.0 cm, r^2= .94; IRL – 251.0 cm, r^2= .83). These asymptotes probably do not reflect the actual asymptotic lengths of either population as the data is severely lacking information on animals < 5 and > 20 years of age. The use of ages from stranded dolphins would provide a more accurate representation of asymptotic lengths. For instance, when the Gompertz growth curve was used on South Carolina stranded animals and live capture animals the asymptotic lengths changed considerably, especially for males (male – 248.5 cm, r^2= .92; female – 240.5 cm, r^2= .93). During the process of viewing the growth layers in the teeth, pulp stones were observed from a number of teeth. These globular masses of secondary dentine were more prevalent in dolphins from the IRL (36.7%) than from Charleston (19.6%). Pulp stones were more common between the growth layer groups (GLG's) 2-6 than at any other stage. Further investigation is needed on pulp stones to determine if they are indicative of health.

Dolphins were stratified into one of five age-length classes: 1) juvenile males (<15 GLG's, <240 cm), 2) juvenile females (<7 GLG's, <220 cm), 3) adult males (≥15 GLG's, ≥240 cm), 4) adult females (≥7 GLG's, ≥220 cm), and 5) pregnant females. Adult males were categorized based on testosterone levels of ~3 ng/ml and higher. Caution should be used here, since some males <15 GLG's and <240 cm could be considered adults. However, testosterone generally increased at these ages and lengths. Interestingly, mean testosterone levels reached a peak at approximately 20 years of age (16.7 ng/ml) and then decreased thereafter to 8.9 ng/ml. Charleston dolphins were heavily skewed toward adult males and the IRL was heavily skewed toward juvenile males.

Weights were collected for 104 animals (IRL – 59; CHS – 45). In both the juvenile and adult male categories, IRL animals were longer (juvenile M mean=222.5 cm; adult M mean – 255.4 cm) than their Charleston counterpart (juvenile mean – 209.8 cm; adult mean – 252.5 cm), but adult males in Charleston were heavier (CHS mean – 422.1 lbs.; IRL mean – 395.5 lbs.). Females, although longer and heavier in the IRL as juveniles (IRL mean length – 203.4 cm, IRL mean weight – 215.5 lbs.; CHS mean length – 193.0

cm, CHS mean weight – 203.0 lbs.), were longer and heavier as adults in Charleston (CHS mean length – 242.3 cm, CHS mean weight - 347.0 lbs.; IRL mean length – 235.8 cm, IRL mean weight – 314.6 lbs.).

Body Mass Index (BMI) was calculated (weight/length^2) for 104 dolphins. Mean range BMI scores for Charleston were 24.4 (juvenile females) to 30.2 (adult males) and for the IRL were 22.5 (juvenile females) to 27.5 (pregnant females). With the exception of pregnant females, Charleston dolphins had higher mean BMI scores in each age category. The largest differences between the two sights were in the juvenile female (CHS – 24.4, s.d. – 3.0; IRL – 22.5, s.d. – 2.2) and adult male (CHS – 30.2, s.d. – 2.1; IRL – 26.8, s.d. – 4.0) classes. These differences in weight, length, and BMI between Charleston and IRL dolphins may simply be the occurrence of dolphins at a higher latitude in Charleston. When comparing northern IRL dolphins with southern IRL dolphins, the largest differences in BMI were between pregnant females (North – 23.0, s.d. -0; South – 29.0, s.d. – 2.6) and adult males (North – 25.5, s.d. – 4.2; South – 29.5, s.d. – 1.7). However, the pregnancy status of the one dolphin from the northern IRL is questionable, and therefore, this may not accurately reflect any differences in this age class.

Lobomycosis in Atlantic Bottlenose Dolphins (*Tursiops truncatus*) from the Indian River Lagoon, Florida.

John Reif,[1,2] M. S. Mazzoil,[1] S. D. McCulloch[1], R. A. Varela[1], P. A. Fair[3], and G. D. Bossart[1]

[1]Division of Marine Mammal Research and Conservation, Harbor Branch Oceanographic Institution, Ft. Pierce, Florida, USA; [2]Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, Colorado, USA; [3]National Oceanic and Atmospheric Administration, NOS Center for Coastal Environmental Health and Biomolecular Research, Charleston, South Carolina, USA.

Lobomycosis is a mycotic infection of humans and dolphins caused by a yeast-like organism (*Lacazia loboi*). During a health assessment of bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon (IRL) along Florida's central east coast in 2003-2004, 9 of 75 (12.0%) dolphins sampled had histologically confirmed evidence of lobomycosis. In the southern section of the IRL, an area characterized by freshwater intrusion and lower salinity, the prevalence was 30.0 percent. Cases were not detected in the northern section of the 260 km waterway, nor among 71 dolphins sampled in estuarine waters near Charleston, SC. The reasons for the emergence of this rare infectious disease in the dolphin population of the IRL are unknown. Our findings suggest that exposure to environmental stressors may contribute to the unusually high prevalence of the disease. Alternatively, local environmental perturbations in salinity, temperature or vegetation may play a role in the expression of lobomycosis in this sentinel species.

Clinicopathologic Findings from the Health and Risk Assessment (HERA) Atlantic Bottlenose Dolphin Project: 2003-2004

Gregory D. Bossart, R. Varela, J. Goldstein, D. Kilpatrick, and M. Rehtanz
Division of Marine Mammal Research and Conservation, Harbor Branch Oceanographic Institution, Ft. Pierce, Florida USA

In 2003 and 2004, a total of 155 Atlantic bottlenose dolphins (*Tursiops truncatus*) were collected, examined, sampled, marked and released in the Indian River Lagoon (IRL) FL (n=82) and Charleston (CHS), SC (n=73) under authorization of NMFS Scientific Permit No. 998- 1678- 01. Following morphometric, body weight and ultrasonic blubber measurements, experienced marine mammal veterinarians performed complete hands-on physical examinations. Briefly, physical examination included visual and/or digital evaluation of the eyes, oral cavity, integument, genital slit, and nasal sacs with thoracic and abdominal auscultation and ultrasound exams (blubber, pleural, abdominal organs including reproductive tract). Blood (pre- examination and post-examination samples), cytologic specimens (gastric fluid, nasal sac and fecal), urine, a blubber biopsy and feces were taken via aseptic procedures for a wide range of diagnostic procedures previously outlined (Bossart 2003). Skin lesions were aseptically biopsied for later pathologic examination. A subjective body condition score was designed to indicate overall nutritional status. Along these same lines, an ultrasonic evaluation of postnuchal fat depth and echogenicity was designed in order to characterize this fat which may be more metabolically available than other previously measured fat stores. Goldstein et al. and Varela et al. review the blubber, hematologic and serum analyte data in this volume.

Abnormal physical examination findings were uncommon except for mucocutaneous disease (see below). Abnormal exam findings included mild cachexia, grade 1-3 pansystolic heart murmurs, mild to moderate unilateral corneal edema, mild to moderate gingival hyperplasia, and reduced range of motion of the pectoral fin with palpable callus formation, likely secondary to past skeletal trauma.

Cytopathologic findings from gastric samples in the IRL in 2003 indicated a high number of animals with severe acute inflammatory changes (14% vs. 5% in CHS) (Fig. 1). In 2004, IRL gastric samples with severe acute inflammation had a similar pattern as CHS (4% vs. 6%, respectively). Blowhole and fecal samples from 2003 and 2004 from both sites had no pathologic findings.

Skin lesions typical of recent and healed rake marks were common. Scarring patterns consistent with past shark attacks, as well as entanglement injury, were also common. Mucocutaneous infectious lesions included dolphin pox (Fig. 2), lobomycosis (Fig. 3), and newly documented orogenital sessile papillomas (Fig. 4). A novel papillomavirus was found in one genital papilloma using molecular methodology. A novel herpes-like virus was found in another sessile papilloma, which was demonstrated with transmission electron microscopy. Lobomycosis was found only in southern IRL dolphins and Reif reviews this disease in this volume. One 2004 dolphin with oral papillomas was previously captured in 2003, at which time the oral lesions were not present.

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Acknowledgements

We extend special thanks to Dr. Forrest Townsend and Larry Hansen for their invaluable help with this project as well as the remaining HERA veterinary staff Dr. Al Segars, Dr. Debbie Andrews, Dr. Sylvain DeGuise, Dr. Jean Herman, Dr.Woody Hayes, Dr. Tom Sheridan, Dr.Alfonso Lopez and Dr. Jaime Bernal.

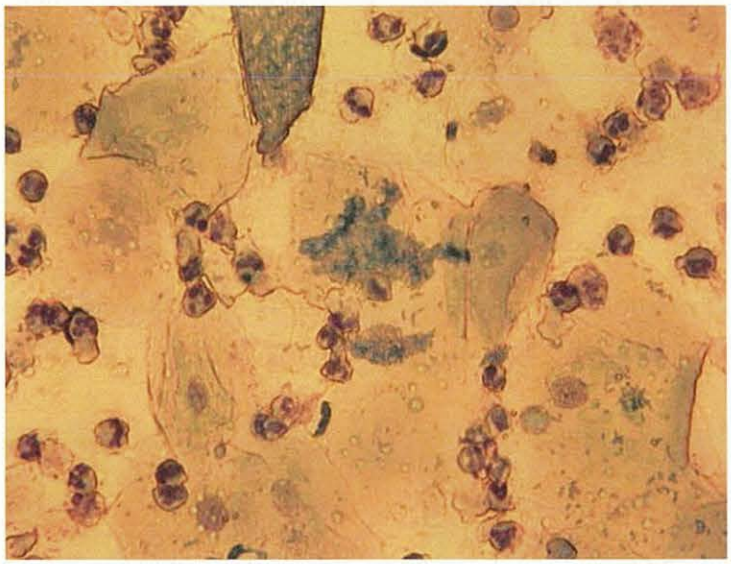

Figure 1- Gastric cytology from an IRL dolphin with abundant neutrophils consistent with severe acute inflammation.

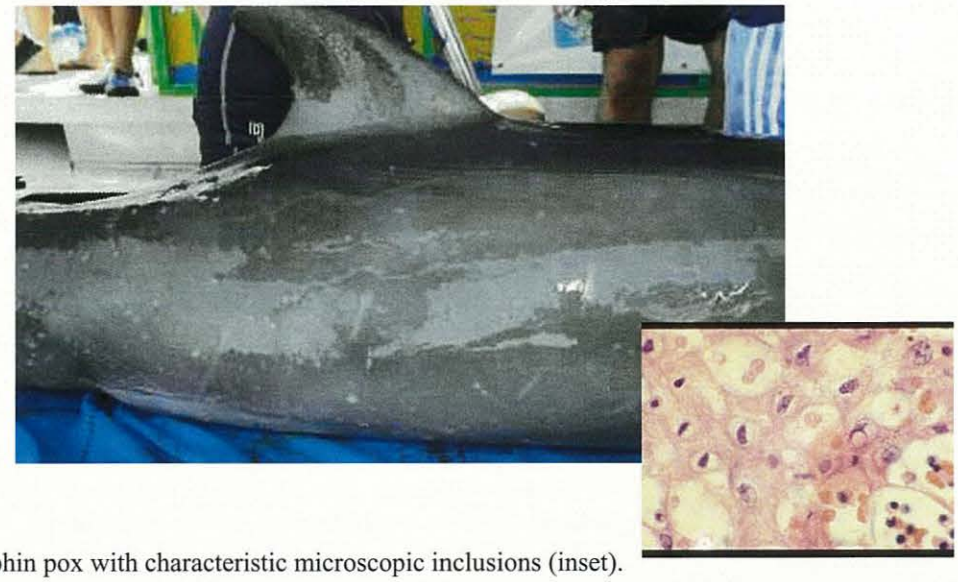

Figure 2 - Dolphin pox with characteristic microscopic inclusions (inset).

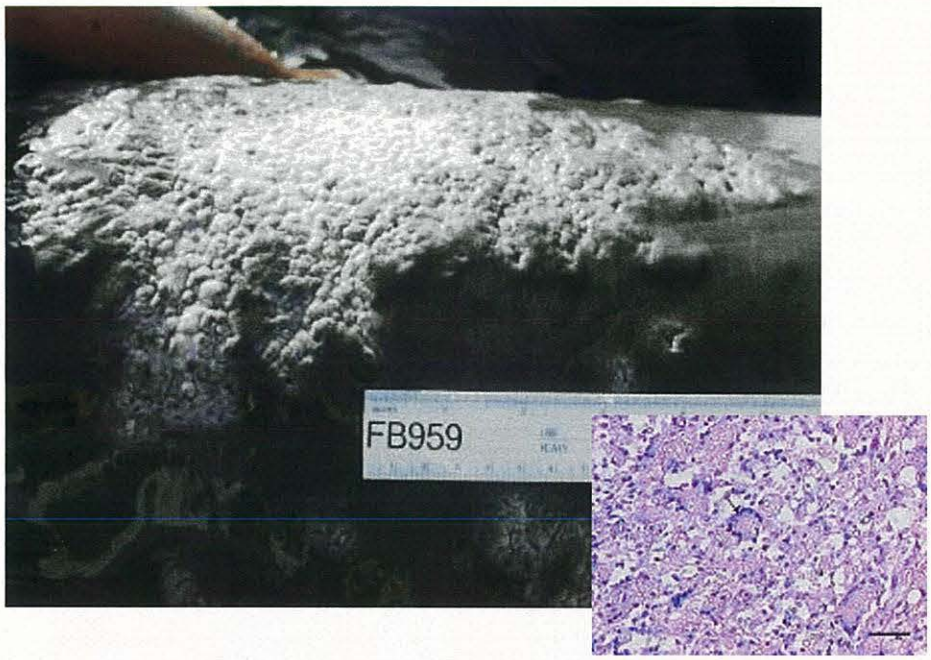

Figure 3 - Lobomycosis with microscopic appearance (inset, arrow indicates multinucleate cell with cytoplasmic fungi).

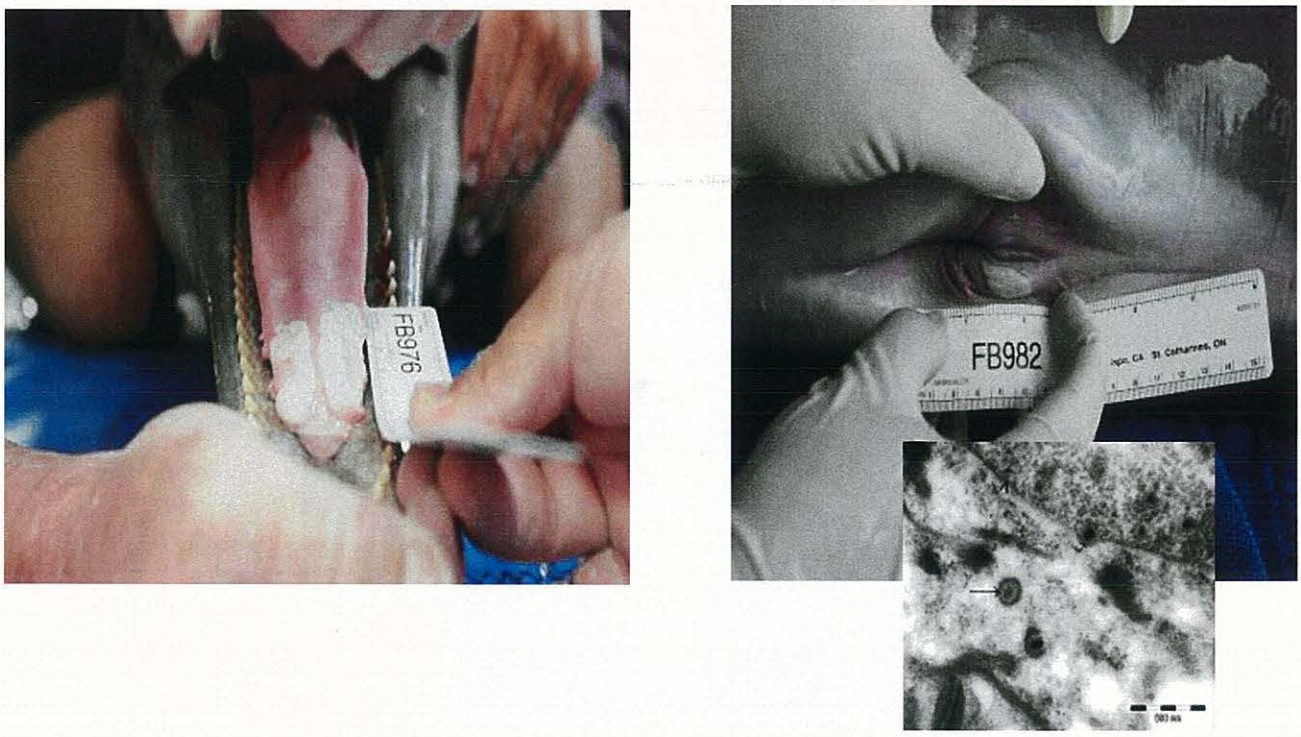

Figure 4 - Orogenital papillomas with herpes-like virus (inset, ultrastructural features with arrows indicating virions).

Clinicopathologic Findings from Atlantic Bottlenose Dolphins (*Tursiops truncatus)* Inhabiting the Indian River Lagoon, Florida

Juli D. Goldstein[1], E. Reese[1], R. A. Varela[1], R. H. Defran[1], S. D. McCulloch[2], J. S. Reif[2], P. A. Fair[3], and G. D. Bossart[1]

[1]Harbor Branch Oceanographic Institution, Ft. Pierce, FL, [2]Department of Environmental and Radiological Health Sciences, Colorado State University, Ft. Collins, CO; [3]National Oceanic and Atmospheric Administration, NOS Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC

A joint five-year comprehensive research project was initiated in 2003 between Harbor Branch Oceanographic Institution in Ft. Pierce, Florida, and the National Ocean Service Center for Coastal Environmental Health and Biomolecular Research at Charleston, South Carolina (NMFS Permit No. 998-1678-01). The goal of this project is to study the health of Atlantic bottlenose dolphin (*Tursiops truncatus)* populations inhabiting the Indian River Lagoon (IRL), Fl and the coastal waters of Charleston, SC. This presentation examined hematologic, serum biochemical, and cytologic variables in IRL dolphins in order to establish normal baseline values, which will be useful in subsequent health evaluations of these animals.

Sixty-three samples were analyzed for serum biochemistry and 60 samples for hematology. Sixty-two gastric, fecal and blowhole specimens were also examined. The ages of dolphins examined ranged from 3.5 years – 26 years, and only apparently-healthy dolphins were included in this study. Clinical health was based on veterinary physical exam evaluations. Animals exhibiting lesions such as lobomycosis or papillomas were automatically excluded.

One-way ANOVAs were preformed on all hematological and biochemical values comparing males vs. females, pregnant vs. non-pregnant females, and juveniles vs. adults. There were no significant differences between males and females or between pregnant females and non-pregnant female hematologic values. Juvenile animals (< 6 years) had a higher mean MCH than adults (≥6 years), and adults had a higher relative percentage of segmented neutrophils. Differences in serum biochemistry were also found: females had a greater mean value than males for sodium, BUN, BUN/creatinine ratios, CPK, cholesterol, triglycerides, and iron. Non-pregnant females had a higher mean TIBC and albumin from serum protein electrophoresis than pregnant females. Juveniles had higher mean values than adults for BUN, BUN/creatinine ratios, CPK, triglycerides, iron, PSAT, and lipase.

No significant pathologic findings were present in the fecal and blowhole cytologies for both years. However, 24% (7/29) of the dolphins examined in 2003 had evidence of gastric inflammation, and 13.8% (4/29) of those animals exhibited severe gastric inflammation. In 2004 only 4.2% (1/24) of the population exhibited mild or moderate gastric inflammation, no severe inflammation was present.

The Atlantic bottlenose dolphin represents an apex predator, yet is one of the least studied wildlife groups occurring within and along the IRL. No recent comprehensive health assessment of IRL bottlenose dolphins has been conducted for the IRL system, and it has been over 26 years since the last health study of IRL dolphins was completed.[1] These findings and analyses represent the most complete hematologic, serum biochemistry and cytologic analyses reported to date for the IRL dolphin population and will provide a useful baseline for future studies on these animals, as well as other free-ranging and captive populations.

Table 1. Hematology baseline values for clinically healthy IRL bottlenose dolphins

Name	Units	Min	Max	Mean	Stdev	N
WBC	10^3/µl	5.8	19.5	10.31	2.55	60
RBC	10^6/µl	2.8	4.8	3.59	0.28	60
Hb	g/dl	11.3	18.2	14.45	1.13	60
PCV	%	35	46	40	2.4	28
Hct	%	32	50	40.22	3	60
MCV	fl	96	126	112.27	6.56	60
MCH	pg	33	45	40.48	2.59	60
MCHC	g/dl	33	38	36	0.86	60
RDW	%	11.1	16.1	12.48	1	60
Basos	10^3/µl	0	0.3	0.04	0.08	60
Segs_Rel	%	25.35	68.37	44.74	9.9	60
Segs_Abs	10^3/µl	1.8	12.7	4.61	1.62	60
Bands_Rel	%	0	2.41	0.04	0.31	60
Bands_Abs	10^3/µl	0	0.2	0	0.03	60
Lymphs_Rel	%	2.04	47.33	19.04	7.85	60
Lymphs_Abs	10^3/µl	0.2	6.2	1.94	0.93	60
Monos_Rel	%	0	10.69	3.34	2.21	60
Monos_Abs	10^3/µl	0	1.6	0.36	0.3	60
Eosinophils_Rel	%	13.68	367.52	37.92	44.27	60
Eosinophils_Abs	10^3/µl	1.3	43	4	5.28	60
NRBC		0	1	0.5	0.71	2
Platelet	10^3/µl	73	281	166.35	41.44	60
MPV	fl	8.6	20.6	12.15	2.22	60
TP_Ref		6.6	9.7	7.6	0.52	60
Schalm_FIB		50	400	136.89	86.07	61

Table 2. Serum Analyte baseline values for clinically healthy IRL bottlenose dolphins

Name	Units	Min	Max	Mean	Stdev	N
Glucose	mg/dl	66	131	94.84	13.07	63
Sodium	mEq/l	151	164	154.95	2.4	63
Potassium	mEq/l	3.4	4.8	3.96	0.32	63
Chloride	mEq/l	106	119	113.48	2.96	63
BiCarb	mEq/l	9	28	20.87	4.11	63
Anion_Gap		14	40	24.63	5.62	63
Nak	mEq/l	33	44	39.93	3.18	28
BUN	mg/dl	46	87	66.43	9.47	63
Creat	mg/dl	0.6	1.8	1.09	0.27	63
BUN_Creat	mg/dl	25.56	110	64.89	18.95	63
T_Protein	g/dl	6.4	10	7.44	0.6	63
Albumin	g/dl	3.2	5.2	4.48	0.33	63
Globulin	g/dl	2	6.8	2.96	0.68	63
A_G	g/dl	0.47	2.48	1.58	0.34	63
T_Bilirubin	mg/dl	0	0.2	0.09	0.04	63
D_Bilirubin	mg/dl	0	0.1	0.02	0.04	63
I_Bilirubin	mg/dl	0	0.1	0.07	0.05	63
Calcium	mg/dl	8.3	10.5	9.33	0.47	63
Phosporus	mg/dl	3.6	7.1	5.17	0.7	63
Magnesium	mg/dl	1.2	1.9	1.44	0.15	63
Uric_Acid		0.2	2.3	0.97	0.51	28
AP	U/l	43	899	270.19	147.53	63
ALT	U/l	19	122	44.29	22.51	28
AST	U/l	142	733	254.22	91.65	63
SDH	U/l	1	51	10.47	11.09	60
LDH	U/l	378	706	488.96	82.54	28
CPK	U/l	82	291	151.6	41.1	63
Amylase	U/l	1	3	1.11	0.42	28
Lipase	U/l	1	27	9.75	4.95	28
GGT	U/l	17	39	27.33	3.89	63
Cholesterol	mg/dl	88	183	140.32	24.63	28
Triglyceride	mg/dl	41	182	83.73	32.66	63
Iron	µg/dl	32	206	99.22	35.94	63
TIBC	µg/dl	174	579	247.7	62.4	63
PSAT	%	15	73	40.62	12.7	63
Albumin_2	g/dl	3.05	4.64	3.73	0.33	63
T_Alpha	g/dl	0.68	1.81	1.2	0.24	63
Alpha_1	g/dl	0.11	0.84	0.35	0.2	63
Alpha_2	g/dl	0.37	1.17	0.85	0.18	63
T_Beta	g/dl	0.31	0.74	0.45	0.1	63
Beta_1	g/dl	0.12	0.57	0.28	0.12	46
Beta_2	g/dl	0.15	0.39	0.22	0.05	35
Gamma	g/dl	0.83	5.18	2.05	0.61	63
T_Glob	g/dl	2.67	6.92	3.7	0.65	63
Immune_T_Protein	g/dl	6.4	10	7.44	0.6	63

Immune_A_G	g/dl	0.44	1.74	1.04	0.21	63
Lipemia		0	34	14.65	8.05	63
Hemolysis		0	40	13.56	9.91	63
Icterus		0	0	0	0	63

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Evaluation of a Portable Clinical Analyzer for Assessment of Critical Care Blood Gases and Electrolytes in Atlantic Bottlenose Dolphins

René A. Varela[1], L. Schwacke[2], P. A. Fair[3]; G. D. Bossart[1]

[1]Division of Marine Mammal Research and Conservation, Harbor Branch Oceanographic Institution, Ft. Pierce, FL; [2]NOAA, Charleston, SC; [3]NOAA/NOS/CCEHBR, Charleston, SC

Atlantic bottlenose dolphins (*Tursiops truncatus*) are the most commonly stranded marine mammal on the southeast coast of the United States. It is also the most common marine mammal housed within oceanaria worldwide. Given both of these facts, the necessity to produce diagnostic tools for improving critical care and general health assessment of these animals takes on an added importance. In stranded bottlenose dolphins, a tool is necessary for rapidly assessing acid/base balance and electrolyte status, primarily to determine their long-term viability, and as with captive animals, to determine an appropriate treatment regime and response to current treatment. The i-Stat Critical Care Blood Gas Analyzer (Heska Corp., Fort Collins, CO) provides this potential functionality. In fact, this diagnostic tool has proven useful for determining metabolic health in other species. It has been shown to be precise, accurate, portable, rapid, and very field capable. With this in mind, this study had several objectives. Initially, our intent was to standardize i-STAT and its protocols for use in bottlenose dolphins. Once this was done, it was important to establish a normal range for wild dolphins and compare those values with those of a standard veterinary diagnostic laboratory. Finally, we intended to identify changes in the iStat values associated with sample and animal processing times associated with the Dolphin HERA Project study. For this study, 154 wild Atlantic bottlenose dolphins (*Tursiops truncatus*) (Charleston, SC and Indian River Lagoon, FL) and 15 Atlantic bottlenose dolphins in human care (Dolphin Discovery, Mexico) were utilized. Standard capture techniques as indicated in the HERA protocols and NMFS Permit # 998- 1678- 01 were utilized for the wild dolphins. Blood samples were taken from the periarteriolar venous rete (PAVR) of the flukes utilizing a 19ga, ¾" butterfly catheter and evacuated with a 1 ml vented blood gas syringe with lyophilized lithium heparin. In the wild dolphins, blood samples were taken upon initial capture and after they were processed on the animal processing boat. Those animals in human care were sampled once in a voluntary, behaviorally conditioned session. Samples were processed as soon as possible by an iStat Critical Care Blood Gas Analyzer (Heska Corp., Fort Collins, CO), utilizing an EC8+ Cartridge. For the pre and post samples, the time period between the initial set of the net and the beginning of the blood draw was recorded and is referred to as the time-to-bleed (TTB) for that sample. The time between the completion of the blood draw and the run of the blood in the i-Stat was also recorded and is referred to as the time-to-run (TTR) for each sample.

Effect of lag time in bleed and sample run on i-Stat results. A Generalized Linear Model was employed to investigate the effect of the continuous covariates, TTB and TTR, on each i-Stat parameter. In short, none of the parameters showed substantial deviation from model assumptions. Following the GLM analysis, adjusted values were calculated for

each parameter. For mean glucose and C02 values, samples were divided into three groups: time-to-run less than 10 minutes, time-to-run between 10 and 20 minutes, and time-to-run greater than 20 minutes, with the same treatment performed for time to bleed. It was found that the effect of TTR (i.e., lag in run time) and TTB (i.e., pre vs. post) work in opposite directions, TTR is negatively correlated and TTB is positively correlated.

Comparison of i-Stat and laboratory measurements. Pearson's correlation coefficient was calculated to determine deviation from a best-fit line, which would indicate the precision of the measurements. Additionally, a Coefficient of Accuracy, Ca, was calculated to determine the deviation of the best fit line from the ideal 45° line, which would indicate the accuracy of the measurements. Finally, the Concordance Correlation statistic was determined in order to provide a single statistic indicative of both accuracy and precision (Lin and Torbeck 1998). There was no good agreement on any of the parameters with the exception of BUN. Laboratory glucose values compared to i-Stat glucose values for a given individual showed a degree of scatter indicating low precision, but a relatively symmetric distribution around 45° line indicated good accuracy (i.e. no bias). Laboratory BUN value versus i-Stat BUN value for a given individual showed minimal scatter indicating good precision, but points distributed primarily above the 45° line indicated that i-Stat values tend to be slightly higher than laboratory values and lacked accuracy. Laboratory sodium, potassium, and chloride values versus i-Stat sodium, potassium, and chloride values for a given individual showed a lack of correlation indicating poor agreement, and i-Stat values tended to be lower as compared to laboratory values.

In summary, the i-Stat critical care blood gas analyzer system was successfully used for field sampling in dolphins. Also, a normal range for several iStat analytes were established in wild dolphins (Table 1), but more animals would need to be sampled to produce a dependable range for captive animals as well. Additionally, poor correlation existed between i-Stat and laboratory values, which preclude a lateral comparison of these values. However, there does appear to be good precision within the i-Stat, allowing for its independent use. Finally, significant differences were seen with increasing TTR and TTB, which can result in large degrees of sampling-induced error. It will be critical in future studies to limit the time to run a sample to less than 20 min. Likewise, reducing the time to the initial bleed during the HERA project is likely to result in fewer aberrations in the post bleeding indices.

Table 1: Wild *Tursiops truncatus* serum chemistry, hematology, and blood i-STAT values

	SERUM	BLOOD		iSTAT		
	(n=154)	Pre (n=144)	Post (n=114)	Pre (n=142)	Post (n=112)	Captive (n=15)
Glucose (mg/dL)	94.06 +/- 13.55			98.95 +/-14.93	126.13 +/- 22.01	103.20 +/- 15.40
Blood Urea Nitrogen (mg/dL)	62.76 +/- 9.20			66.39 +/-10.96	66.99 +/-11.19	60.20 +/- 10.79
Sodium (mmol/L)	155.37 +/- 3.44			151.56 +/- 3.91	151.70 +/- 4.17	154 +/- 2.27
Potassium (mmol/L)	3.89 +/- 0.37			3.84 +/- 0.53	3.61 +/- 0.31	3.88 +/- 0.27
Chloride (mmol/L)	113.37 +/- 3.30			117.80 +/- 3.34	117.46 +/- 3.40	118.67 +/- 2.06
TCO2 (mmol/L)	N/A			28.63 +/- 4.51	31.93 +/- 4.21	33.80 +/- 2.18
Anion Gap (mmol/L)	24.66 +/- 5.38			10.65 +/- 6.67	6.94 +/- 5.49	7.47 +/- 3.58
Hematocrit (%PCV)	N/A	39.86 +/- 3.02	39.43 +/- 2.34	46.01 +/- 9.71	42.35 +/- 7.10	37.47 +/- 4.60
Hemoglobin (g/dL)	N/A	14.13 +/- 1.09	14.08 +/- 0.88	15.63 +/- 3.31	14.47 +/- 2.38	12.87 +/- 1.55
pH	N/A			7.32 +/- 0.07	7.34 +/- 0.05	7.34 +/- 0.06
PCO2 (mmHg)	N/A			52.39 +/- 7.02	56.32 +/- 7.10	59.28 +/- 7.03
HCO3 (mmol/L)	21.35 +/- 4.00			27.08 +/- 4.36	30.50 +/- 3.10	32.07 +/- 2.09
Base Excess (mmol/L)	N/A			5.26 +/- 0.86	4.76 +/- 3.45	6.13 +/- 2.75

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Blubber Findings from Atlantic Bottlenose Dolphins (*Tursiops truncatus)* Inhabiting the Indian River Lagoon, Florida

Goldstein J. D., R. A. Varela and G. D. Bossart

Harbor Branch Oceanographic Institution, Ft. Pierce, FL

As a part of a five-year comprehensive HERA study, we initiated an investigation analyzing blubber thickness and morphology utilizing ultrasonography. The primary goal of this study was to develop a non-invasive method of health evaluation, utilizing ultrasonography to evaluate differential blubber thickness. In addition, we seek to determine the differential blubber thickness at various anatomical locations, as well as assess differences between and within geographically distinct populations. Finally we plan to compare our findings to previous blubber studies based on blubber samples from necropsies as opposed to ultrasonography. It is important to note that this abstract represents preliminary data, and no advanced statistical analyses have been conducted at this time.

The images for the study were obtained utilizing a Sonosite 180 ultrasound machine with a 5-2 MHz curvilinear probe. An Aquasonic gel pad (standoff) served as an interface between the probe and the skin. Due to the challenges of a placing a curved probe on a flat surface, the standoff also aided in providing a uniform image. Finally, a towel/shaded area or virtual reality glasses were used to overcome the challenges of viewing the images in the daylight. Seven locations were selected for imaging (Figure 1). Previous studies indicated that the blubber areas cranial to the anus were considered to be the most "active". In Koopman et al., (2002) which examined starved harbor porpoises, very little of the overall blubber loss came from the tailstock; more than 92% of the cumulative loss occurred cranial to the anus (Koopman et al. 2002). Sites 1-6 were measured in both 2003 and 2004, and site 7, which is the biopsy site, was measured only in 2004. Once the images were obtained, they were analyzed in a controlled environment with a laptop computer and specialized software. A full thickness measurement was obtained for site 1 (postnuchal fat pad). Internal, external, and total thickness measurements were obtained for sites 2-7.

Thus far, descriptive statistics for clinically healthy animals have been conducted and preliminary analyses completed. No adjustments were made based on age, length, weight, girth, or gender as of yet. (Figure 2) Based on this preliminary information, several observations were evident. Overall, internal blubber layers were thicker than external layers for Indian River Lagoon (IRL) and Charleston (CHS) animals. Charleston dolphins had higher total blubber thickness than IRL dolphins. Indian River Lagoon postnuchal fat pads appeared to be thicker than CHS dolphins. Finally, sites 3 and 5 had similar thickness as did sites 4 and 6.

Further analyses will include standardization for size, comparison of healthy vs. non-healthy animals, comparisons based on gender, age, and pregnancy status, comparison of geographic locations, as well as comparisons to the published literature. In addition,

studies are also being conducted to evaluate the significance of the postnuchal fat pad which is thought to be metabolically different than the other blubber locations.

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Figure 1. Blubber ultrasound site locations

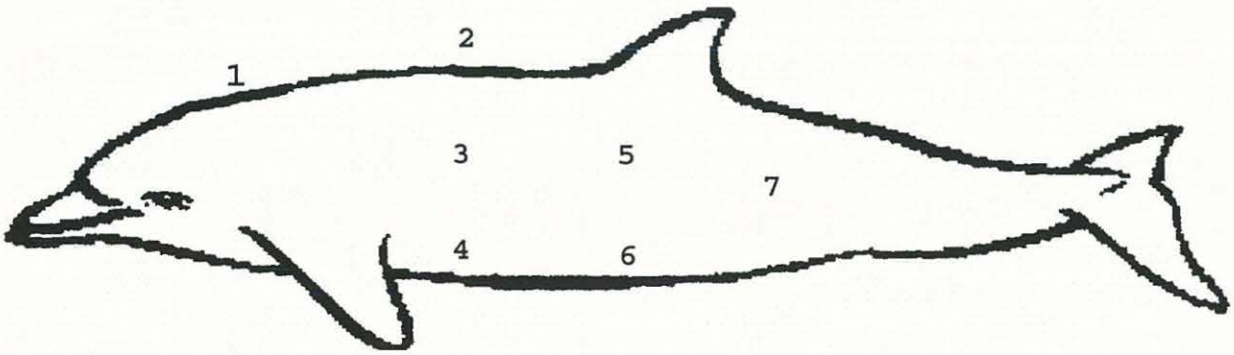
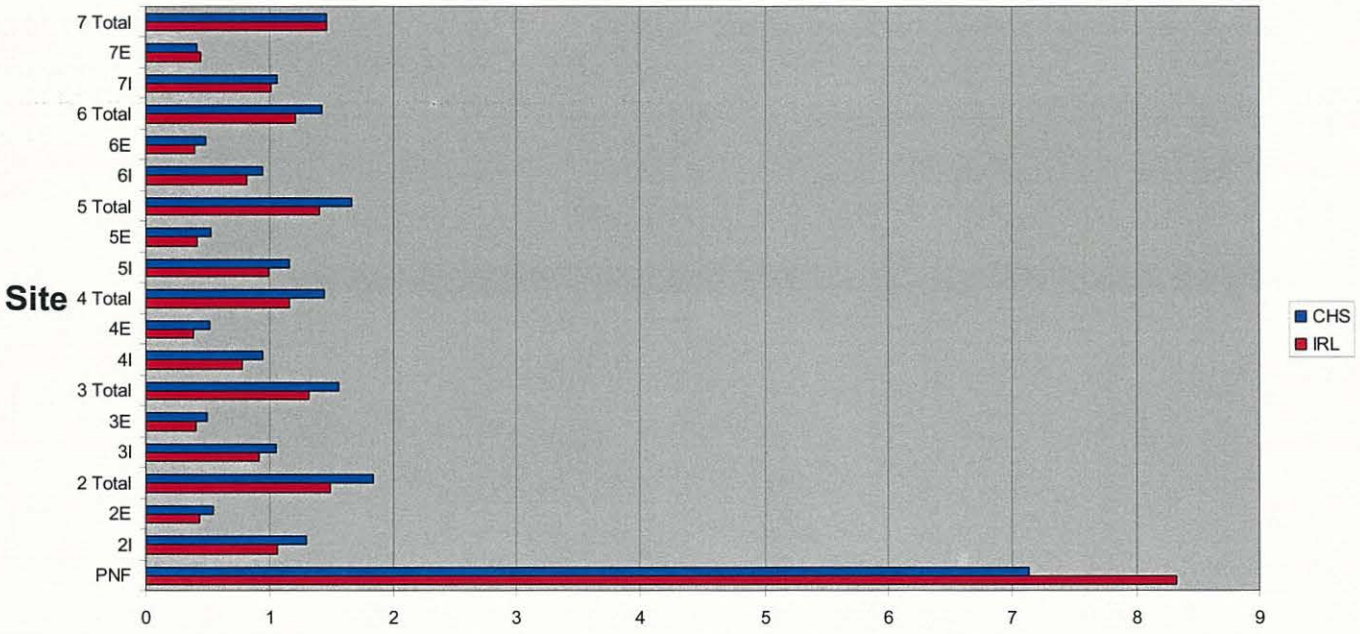


Table 1. Comparison of CHS and IRL blubber measurements by site location. Key: I=Internal Layer, E= External Layer, PNF=Post Nuchal Fat Pad.



	PNF	2I	2E	2 Total	3I	3E	3 Total	4I	4E	4 Total	5I	5E	5 Total	6I	6E	6 Total	7I	7E	7 Total
CHS	7.13	1.29	0.54	1.84	1.05	0.49	1.55	0.94	0.51	1.44	1.15	0.52	1.66	0.94	0.48	1.42	1.06	0.41	1.46
IRL	8.33	1.06	0.43	1.49	0.91	0.4	1.31	0.77	0.38	1.15	0.99	0.41	1.4	0.81	0.39	1.2	1.01	0.44	1.46

Centimeters

Isolation of a Novel Papillomavirus (Pv) from a Genital Lesion of Dolphin Fb892: *Tursiops truncatus* Papillomavirus Type 2.

Manuela Rehtanz[1,2], S-J Ghim[1,2], A. B. Jenson[1,2], G. D. Bossart[2]

[1]James G Brown Cancer Center, University of Louisville, KY
[2]Harbor Branch Oceanographic Institution, Ft. Pierce, FL

A novel papillomavirus, Tursiops truncatus Papillomavirus type 2, TtPV-2, isolated from a genital lesion of dolphin FB892 was identified by using the isothermal multiply primed rolling-circle-amplification technique. The complete nucleotide sequence was determined by sequencing transposon integration sites of the cloned viral DNA. A phylogenetic analysis revealed that TtPV-2 belongs to the close-to-root sea mammal genus together with TmPV-1, PsPV-1 and TtPV-1 with the highest L1 nucleotide sequence similarity to TtPV-1 (70%). The L1 nucleotide sequence encoding the major component of the viral capsid was identified and cloned. To create VLPs as a vaccine which can be applied to captive animals, insect cells were transfected with a baculoviral vector containing TtPV-2 L1, the coding sequence for the L1 protein of the PV isolated from *Tursiops truncatus* in the Charleston Harbor.

Antibodies against dolphin Ig were generated in mice, isolated and purified to serve as a control in future seroepidemiological studies to screen captive and free-ranging dolphins for active infections with TtPV-2. Therefore, antibodies against VLPs will be induced in mice.

Specimens probably containing Herpes- and Poxviruses, respectively, will be used to characterize those viruses, possibly to create vaccines.

Acknowledgements

Dr. Rehtanz is a Postdoctoral fellow of Harbor Branch Oceanographic Institution, Fort Pierce, FL and works at the Brown Cancer Center, University of Louisville, KY.

Development and Standardization of a Suite of Assays to Assess Immunotoxicity in the Bottlenose Dolphin

Margie M. Peden-Adams[1,2,3] and Tracy Romano[3,2]

[1]Department of Pediatrics, [2]Marine Biomedicine and Environmental Science Center, Medical University of South Carolina, Charleston, SC. [3]Mystic Aquarium and Institute for Exploration, Mystic, CT.

Increases in the incidence of mass mortality events (MMEs) involving bottlenose dolphins (BND) has led to concern over their health. Many of these mortality events are thought to be caused by infectious microbes such as morbillivirus and/or biotoxins such as brevetoxin. It is unclear if these agents were the primary cause of the MMEs or if compromised immunity, related to contaminant exposures, causes increased susceptibility to infectious agents. Studies with terrestrial and marine mammals have shown that organochlorine contaminants suppress immune function. In the 1980's, the National Toxicology Program proposed a two-tiered immunotoxicological testing scheme for rodents. Luster et al., (1988) and Weeks et al., (1992) proposed a three tiered testing scheme for assessing immunotoxicity in wildlife (reviewed in Keller et al. 2005). Based on these testing schemes a suite of assays that do not require *in vivo* procedures and can be performed non-lethally was developed for assessment of immune function in marine mammals. The assays chosen encompass measurements that can be conducted from blood samples to measure function of innate, cellular, and humoral immunity (Table 1).

Currently, various labs assess immune function in bottlenose dolphins; however, differences in assay methods and collection and storage techniques make comparing data from different studies difficult. Standardization and validation of functional immune assays are, therefore, required to be able to compare immune function data from different dolphin populations collected by various investigators. Thus this study began to standardize and validate immune assays proposed in Table 1. Assay validation requires that the assays be:

1. Performed in several labs yielding the same results
2. Reproducible within a lab and between labs
3. Specific for the type of immune function being assessed
4. Able to measure both normal and abnormal function
5. Able to measure altered function caused by exposure to know immunotoxicants
6. Undergo peer review

To begin to standardize and validate the assays for bottlenose dolphin, T- and B-cell proliferation using radioactive thymidine were optimized and standardized on paired blood samples by two labs. Incubation times, peripheral blood leukocyte collection methods, mitogen concentrations and media components were assessed and a Standard Operating Procedure (SOP) was finalized. Proliferation measurements using BrDU are currently be optimized for BND. Experiments will then be undertaken to compare the results of these two methods on paired samples. Finally, paired experiments in two labs

will be undertaken to assess *in vitro* exposure to known immunotoxicants to determine the repeatability of results between labs.

Table 1. Proposed *Comprehensive Screening Set* of immune assays for bottlenose dolphin that can be performed on blood samples from non-lethal collections.

Test	Immune Component	Comments/Status
MØ Phagocytosis/ Neutrophil phagocytosis	Innate	Available
MØ/ Neutrophil Respiratory Burst (nitric oxide, hydrogen peroxide, superoxide Production)	Innate	Available (Flow Method-hydrogen peroxide)/ Available (spectrofluorometer methods-superoxide- NBT reduction)/ Develop nitric oxide assay
Lysozyme Activity	Innate	Available
NK cell Activity	Innate	Available
Immunophenotyping (CD4, CD8, CD19, CD21, CD2, MHCII, CD16)	CMI	Need reagents (anti-CD8, anti-CD16)
Mixed Lymphocyte Response (MLR) (in vitro)	CMI	Needs to be developed[a]
CTL activity (in vitro)	CMI	Needs to be developed[a]
Lymphocyte Proliferation	CMI/HI	Available/ method validation ^{3}H vs. BrDU required
Total IgM/IgG	HI	Available
Pathogen ELISAs	HI	Available
PFC Response (M-D in vitro)	HI	Needs to be developed[a]
Cytokine Panel	All	Needs to be Validated
Stress Hormones	General	Available
WBC Differential	General	Available
Hematocrit	General	Available

[a]Needs to be developed in this species but has been done in mice and humans.
CMI= Cell Mediated Immunity, HI= Humoral Immunity

During the HERA live captures in 2003 two phagocytosis methods were assessed: *Listeria* uptake (Staining and counting via microscope) and a flow cytometric assay assessing *E. coli* phagocytosis. These assays when assessed in relation to sampling locations yielded the same results. During the 2004 HERA live capture, respiratory burst was assessed by two methods (superoxide flow cytometry method and NBT reduction measuring hydrogen peroxide). Although these assays did not correlate with each other, when assessed statically in relation to the study locations they yielded the same results. The lack of correlation between the measurements of these two different free radicals is not necessarily unexpected as the production rates of these upon cell stimulation are not clear and may not be 1:1. Regardless, the data showed that either method could be used to assess respiratory burst and that the same conclusion as to the differences between locations would be made. As contaminant data from these captured animals becomes available, these different methods will be correlated against specific contaminant class to determine if they yield similar relationships.

NK cell activity was optimized for two different methods (Cr51 release and flow cytometric analysis). These studies tested two target cell lines (K562 and Yac-1). Based on studies in beluga whales (DeGuise et al., 1997), it was expected that K562 cell line would be the better target for the assay. Studies with both methods, however, revealed that for bottlenose dolphins Yac-1 cells are better targets. These two assays will be compared between labs following *in vitro* exposure to known immunotoxiciants.

Studies assessing sample collection and storage were also undertaken to determine the similarity of results between fresh vs. frozen cells. Cell viability was assessed in several different types of freezing media. Origen medium from Fisher yielded slightly higher cell viability (although not statistically) than 90% FBS/10% DMSO. For freezing experiments, lymphocyte proliferation (measured with 3H-thymidine) and NK cell function (CR51 release) were assessed on fresh (24hr old) cells. Peripheral blood leukocytes (PBLs) collected and assessed for proliferation and NK cell activity were then frozen at 2e7 cells/mL in Origen medium for one month. Frozen cells did not function in the Cr51 NK cell assay. Preliminary experiments suggest that PBLs frozen in 90% FBS/10% DMSO do function in the Cr51 release assay. Further studies need to critically assess this function in fresh cells as compared to cells frozen in 90%FBS/10%DMSO.

Lymphocyte proliferation in cells frozen in Origen medium were assessed at three concentrations of Con A (T-cell mitogen) and LPS (B-cell mitogen). Frozen results have not been correlated to fresh results to date but will be. However, at optimum concentrations of each mitogen (ConA 2.5 μg/mL and LPS at 120 μg/mL), there were no significant differences between the mean of the samples assessed fresh and the mean of the same sample assessed frozen. At sub- and super- optimum concentrations the means were different from the fresh samples but this was only statistically significant for the sub-optimal concentration of LPS (60 μg/mL). Further studies are required to assess a frozen time course to determine how long PBLs can be frozen and still yield similar results in the proliferation assay.

Ongoing studies are assessing the feasibility of an *in vitro* antibody production assay (plaque forming cell response). Validation of lysozyme activity between two labs will occur in summer 2005 and NK cell activity will be compared in samples using flow cytometry and Cr51 release using samples from the HERA 2005 captures. Final validation of the immune assays will lead to finalized SOPs. Optimization, standardization, validation, and development of formalized SOPs for immune measurements will allow better assessment of immune status and comparison of immune status between bottlenose dolphin live capture studies in the future.

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Lymphocyte Proliferation, NK-cell Activity, and Lysozyme Activity in Free-Ranging Bottlenose Dolphins Captured as Part of the Health and Risk Assessment Project: Preliminary Data Summary 2003 and 2004

Margie M. Peden-Adams[1,2,3], J. EuDaly[1,2], L. Heesemann[2], M. Lee[1,2], and T. Romano[3,2]

[1]Department of Pediatrics, Medical University of South Carolina, Charleston, SC, [2]Marine Biomedicine and Environmental Science Center, Medical University of South Carolina, Charleston, SC. [3]Mystic Aquarium and Institute for Exploration, Mystic, CT.

Immune function is important to the health and ability of marine mammals to survive. Therefore, as part of the Bottlenose Dolphin Health and Risk Assessment (HERA) Project conducted by the National Ocean Service and Harbor Branch Oceanographic Institution, lymphocyte proliferation, natural killer (NK) cell activity, and plasma lysozyme activity was assessed from blood samples collected during live capture-release events conducted in Indian River Lagoon, FL and Charleston, SC during the summers of 2003 and 2004. Blood samples were collected and shipped cool, overnight for analysis. Differences between locations were noted both years and the trends between locations differed between years based on differences in numbers of animals/gender.

B-cell proliferation (LPS-induced) was measured by stimulating cells and assessed ^{3}H-thymidine incorporation. Preliminary data, with both years combined suggests there was no significant difference in response between locations, although 2003 data indicated that animals in IRL had lower B-cell function than those in CHS this was not seen in 2004 data and did not show up when data was combined for both years. Males typically exhibited lower B-cell proliferation than did females and this was significantly different within locations, but not between locations. With both years combined, females captured in the south part of IRL had lower B-cell proliferation than did females in the northern part of IRL, but this was not statistically significant.

T-cell proliferation (ConA-induced) was not significantly different in 2003 data between locations but was in 2004. In 2004 data, females from the IRL had lower T-cell proliferation than did females from CHS (for both pre and post samples). When the data was combined for both years, males had significantly lower T-cell proliferation than did females within locations. The only significant difference between CHS and IRL, when both years were combined, was in female post samples. T-cell proliferation in post samples from IRL were lower than post samples from CHS. Caution should be taken with this difference, however, as data has not been adjusted for differences that may have occurred in elapsed time between pre and post sampling for individual animals. With both years combined, males captured in the south part of IRL had significantly lower T-cell proliferation than did males in the northern part of IRL.

NK-cell activity was measured by a standard Cr^{51} release assay. Preliminary data from 2003 suggests there were no differences in NK-cell activity within or between locations. In 2004, females from IRL had significantly lower NK-cell activity than females from

CHS. With both years combined, females from IRL had lower NK-cell activity than females from CHS, but this was not statistically significant. There was no difference between North and South IRL.

Plasma lysozyme activity was significantly higher in animals captured in IRL than in CHS in the summer of 2003. This paralleled significantly increased monocyte phagocytosis in IRL as compared to CHS in 2003. In 2004, lysozyme did not differ between locations (nor did phagocytosis); however, with both years combined, males in IRL had significantly higher lysozyme activity than did males in CHS. There was no difference between North and South IRL.

A preliminary assessment of relationships between hormones and immune function revealed that significant correlations were observed with thyroid hormones and estradiol and that the relationships were what would be expected from published effects in rodents. Associations with perfluorinated contaminant (PFAs) measurements were also conducted (Table 1). Stronger correlations were observed in some cases when data was assessed by location and/or by gender/location (data not shown). Additionally, select PFAs not summarized in Table 1 also showed strong correlations with immune parameters (Figure 1).

Table 1. Brief summary of Spearman rank correlations (Rs) between B-cell proliferation (lps120 and lps60) and plasma lysozyme activity in relation to select perfluorinated compounds (PFOS and PFOA) and the summed total of all PFAs measured.

		All Animals		All Females		All Males	
Compound	Health Parameter	n	Rs	n	Rs	n	Rs
PFOS	lps120	84	***0.2692***	26	***0.3908***	58	0.199
	lps60	84	***0.347***	26	0.345	58	***0.3293***
	lysozyme	89	***-0.3404***	29	-0.1941	60	***-0.3995***
PFOA	lps120	84	***0.2662***	26	***0.4069***	58	0.2039
	lps60	84	***0.3432***	26	0.3525	58	***0.3456***
	lysozyme	89	***-0.3647***	29	-0.3146	60	***-0.4052***
ΣPFA	lps120	84	***0.2722***	26	***0.4202***	58	0.1998
	lps60	84	***0.3519***	26	0.3709	58	***0.3373***
	lysozyme	89	***-0.3846***	29	-0.22	60	***-0.4326***

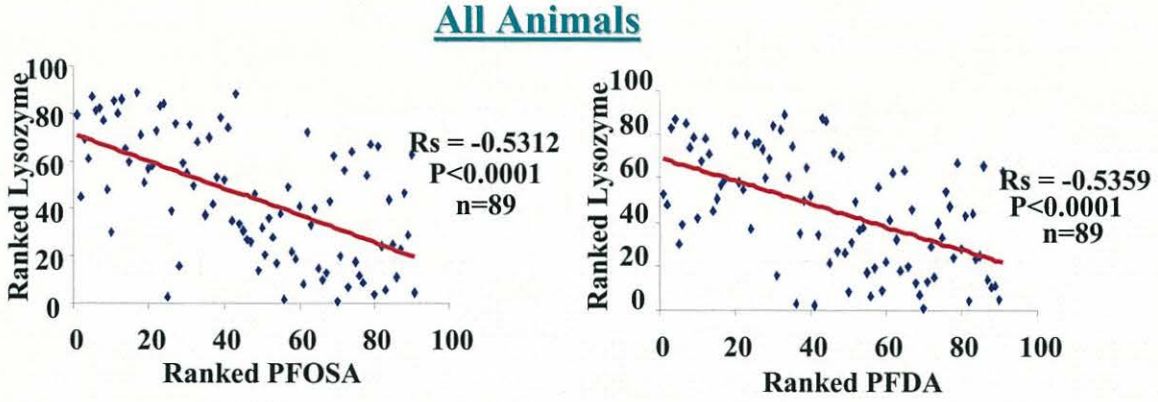

Figure 1. Spearman ranked correlations (Rs) for some specific parameters not summarized in Table 1.

In conclusion, preliminary data suggests differences in immunity within and between capture locations. The differences vary with capture year; therefore, alterations in environmental and health parameters between years should be assessed. As expected immunity exhibited relationships with plasma hormone levels. These relationships need to be further explored in relation to pre and post sampling and time elapsed between these samplings. Finally, data suggest for the fist time that immunity in wildlife may be modulated by exposure to PFAs. This needs to be followed with *in vitro* exposures and rodent dosing experiments and will be further clarified as the 2004 PFA data becomes available.

Assessment of Immune Function in Wild Bottlenose Dolphins (*Tursiops truncatus*) Live-Captured in the Indian River Lagoon, Fl and Charleston, SC

Tracy Romano[1], A. Linton[1,2], M. J. Keogh[3], D. Beal[1], M. Peden-Adams[4], G. D. Bossart[5], and P. A. Fair[6]

[1] Department of Research and Veterinary Services, Mystic Aquarium and Institute for Exploration, Mystic, CT; [2]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO; [3]Department of Veterinary Anatomy & Public Health, Texas A&M University, College Station; [4]Marine Biomedicine and Environmental Science Center, Medical University of South Carolina, Charleston, SC; [5]Harbor Branch Oceanographic Institute, Ft. Pierce, FL; [6]NOAA/NOS/CCEHBR Charleston, SC

The Bottlenose Dolphin Health and Risk Assessment (HERA) Project is a major collaborative effort involving a number of individuals and institutions. The study was designed to collect and analyze various health parameters from bottlenose dolphin populations in the Indian River Lagoon (IRL), FL and Charleston Harbor (CHS), SC. Resident populations in these two regions are exposed to various contaminants and environmental stressors that may potentially lead to immunosuppression and increased risk of illness and disease. Therefore, immune function was evaluated as part of an overall health assessment. The primary purpose of this study was to specifically assess immune function in free-ranging bottlenose dolphins live-captured in the Indian River Lagoon, FL, and Charleston, SC as part of the HERA Project.

After capture, blood samples were obtained both initially upon restraint (pre sample), and immediately before release into the wild (post sample). Flow cytometry was used to quantify peripheral blood lymphocyte subsets and to assess neutrophil and monocyte phagocytosis and respiratory burst. Cetacean-specific antibodies or antibodies shown to cross-react with dolphin counterparts from other species were used to quantify lymphocyte subsets. Absolute numbers and percentages were quantified for dolphin T and B lymphocytes, T helper and MHC class II positive cells. Two fluorophores were used to simultaneously measure phagocytosis and respiratory burst in dolphin neutrophils and monocytes. Propidium iodide-labeled *Staphylococcus aureus* (ATCC 14923) (wavelength of 585nm) was used to measure phagocytized bacteria, and 2', 7'-dichlorofluorescein diacetate (DCFDA), a non-fluorescent cell permeable dye which upon oxidation becomes fluorescent (wavelength of 525nm) was used to measure respiratory burst activity. Percent phagocytosis and mean fluorescence intensity (MFI) for respiratory burst activity were measured in whole bottlenose dolphin blood. Data from 2003 and 2004 for each location was combined and analyzed within each individual population with respect to sex (male/female) and location (north/south region of the IRL), and then analyzed between IRL and CHS populations. Immune data has been preliminarily analyzed with other data sets such as stress and reproductive hormones, and contaminants such as perfluorinated compounds. Preliminary statistical analyses were carried out at $P<0.05$.

In the IRL, males had significantly higher T/T helper cell ratios than females, while males in CHS had significantly higher neutrophil phagocytosis than females in CHS. Those animals sampled in the North of the IRL showed significantly higher B cells than those dolphins in the South IRL, along with higher T cell and MHC II percentages than those in the South. In parallel with the above findings, those animals in the South showed greater T/B cell ratios than those dolphins in the North IRL. Moreover, the absolute numbers of lymphocyte subsets in males of the North IRL were significantly higher than males in the South IRL. The T/B and T/T helper ratios of females in the South IRL were higher than the females in the North. When comparing the IRL vs. CHS population, B cells in CHS females are significantly higher than females in the IRL and B cells and T helper cells are higher in CHS males than IRL males. The T/B ratios of IRL males and females are higher than CHS males and females. Neutrophil phagocytosis is higher in IRL females than CHS females. Positive correlations ($P<0.05$) were observed with T helper cells, T and B cell percentages, and T/B ratios with testosterone and estradiol; negative correlations were observed with ACTH and B cell percentages, aldosterone and T , MHC II percentages, dopamine and epinephrine and T helper, B cell percentages and MHC II+ cells. Perfluorinated compounds showed a positive correlation with T, B, T helper, and MHC II+ cells.

It was determined at the first HERA Project review meeting last February that in order for conclusions to be drawn from the immune data that we need a starting data set of "healthy" animals identified by veterinary observations and body condition (Tier I) and CBC/serum chemistries (Tier II). Furthermore, data should be analyzed both categorically and statistically in a consistent manner among data sets. The data will be reanalyzed once this information is available and conclusions drawn in order to provide a "complete" health assessment of the bottlenose dolphin populations in the IRL and CHS.

Acknowledgments

The authors would like to thank the Live Capture Field Team for collection of dolphin blood samples and Dr. Jeffrey Stott (UC Davis) for the cetacean specific antibodies (CD2, CD21, and CD19). This project was funded by NOS (Contract's #WC1330-02-RT0030; #WC133C04CN0012) and ONR (Grant #N00014-00-1-0041). Studies on wild dolphins were conducted under Scientific Research Permit #998-1678-00 from the National Marine Fisheries Service.

Serum Biomarkers of Immune Function in Bottlenose Dolphins Inhabiting the Indian River Lagoon, FL and Charleston, SC

Charles Rice, Ph.D. Clemson University, Clemson, SC

Key monoclonal antibody (mAb) reagents have been developed in our laboratory that allow for the detection and quantification of both total and specific IgG in bottlenose dolphin (Beck and Rice, 2003). Two mAb antibodies (BB10-2 and BB32-2) were generated against bottlenose dolphin IgG previously purified using protein-G columns. In addition, rabbit polyclonal antisera was generated against this same IgG preparation. Antibody (IgG) levels specific to a particular pathogen can be quantified by indirect ELISAs using either of the mAbs, while total IgG levels can be measured by capture ELISAs using both mAbs or by using one mAb to capture IgG and the polyclonal anti-sera for detection. Serum samples from individual bottlenose dolphins were collected during live captures at Indian River Lagoon, FL and Charleston Harbor in 2003 and 2004 by NOAA field specialists. Serum samples were then sent to Clemson University where upon the ELISAs were performed. No differences in total IgG were detected between the two field sites in both years. In 2003 no differences in bacteria-specific antibody levels between the two sites were detected. However, several differences in antibody levels against specific bacteria could be detected in 2004. Indian River Lagoon females had lower antibody levels against *Erysipelothrix rhusiopathiae* than did males sampled in the Charleston Harbor. Males sampled from the Indian River Lagoon had higher antibody levels specific for *Mycobacteria marinum*, but lower levels of antibody specific to *Vibrio vulnificus*.

We are currently in the process of validating in-hand mAbs and polyclonal antibodies generated against bottlenose IgM purified using Hi-Trap columns (Pharmacia). Unfortunately, this method of IgM purification also co-purifies a certain fraction of IgG, thus which of our mAbs are specific for IgM and whether or not the polyclonal is specific for IgM is not clear. An *in vitro* transcription-translation system (rabbit reticulocyte lysate) that expresses a cloned bottlenose dolphin IgM fragment (generous gift from Dr. Greg Warr) is currently being used to screen our antibodies. If neither of our antibodies are specific for the clones IgM fragment then we will express this fragment as a recombinant protein to then generate more anti-IgM antibodies. Having IgM-specific reagents will allow us to quantify both pathogen-specific and total IgM in archived serums samples collected in 2003 and 2004.

Circulating acute phase proteins such as c-reactive protein (CRP) are indicative of early or persistent inflammation, thus should serve as a key biomarker of any ongoing inflammation. An in-hand mouse anti-sera generated against sharpnose shark (Karsten and Rice, 2004) recognizes a serum protein in bottlenose dolphins indicative of CRP (i.e., 25 kD denatured). Using standard indirect ELISAs, no differences between Indian River Lagoon and Charleston Harbor bottlenose dolphin CRP levels were detected, however CRP levels in select individuals were quite high. Lysoyzme protein levels were examined during 2004 using an in-hand pan-lysozyme-specific mAb (M24-2). No differences between sites or genders were detected. We did not, however, examine lysozyme activities in these serum samples.

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Characterization of Bottlenose Dolphin Cell Activation Using Monoclonal Antibodies

Sylvain De Guise, C. Mori, B. Morsey, and M. J. Levin

Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT

The immune system is central in health and one of the most susceptible systems to the effects of exogenous stressors. The complexity of the marine mammal immune system and its susceptibility to different types of stressors are increasingly studied and slowly unraveled. The present study consisted of using new methods to assess the expression on blood leucocytes of molecules known to be inducible upon cell activation, in order to possibly assess the level of activation in dolphins from different locations. The expression of the beta-2 integrin CD11/CD18 (using a cetacean-specific monoclonal antibody) as well as that of the pattern recognition receptors CD14, TLR2 and TLR4 (using cross-reacting antibodies to the human molecules) was assessed in different cell types using flow cytometry. CD11/CD18 was expressed on 7 +/- 5% (standard deviation), 11 +/- 6% and 1.5 +/- 2% of neutrophils, monocytes and lymphocytes, respectively, with ranges of 1-26%, 0-31% and 0-9%, respectively. CD14 was generally expressed on less than 1% of leucocytes of all types. TLR2 and TLR4 were both expressed on generally less than 1% of neutrophils and lymphocytes, and on 3 +/- 9% and 1 +/- 4% of monocytes, respectively. Expression of those molecules was not increased by a short-term in vitro stimulation. The expression of CD14 in monocytes and lymphocytes and that of CD11/CD18 in neutrophils were lower in Charleston than in the IRL when data from both years were combined (CD14) or using only the 2003 data (CD11/CD18). In contrast, the expression of TLR4 in neutrophils and lymphocytes was higher in Charleston than in the IRL in 2004. There were no differences between sexes in the expression of those molecules. Overall, the expression of CD14, TLR2 and TLR4 was detected for the first time in leucocytes from bottlenose dolphins, and the expression on the surface of leucocytes of molecules whose expression is induced upon activation may prove to be a useful tool in further health assessment of wild and captive populations of marine mammals.

Organochlorine Pesticides, Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers in Dolphins from the Indian River Lagoon, Fl and Charleston, SC

Patricia A. Fair and Gregory Mitchum

NOAA/NOS/CCEHBR, Charleston, SC

Persistent organic contaminants (POCs), including organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), are ubiquitous pollutants in coastal environments and are of significant concern due to their high toxicity and long environmental half-lives. Many POCs have the potential to induce toxicological impacts on both wildlife and humans (Jones and de Voogt 1999). Over the last several years, it has become clear that PBDEs and other flame retardant chemicals have joined PCBs, DDT and dioxin on the list of persistent, bioaccumlative chemicals. These PBDEs have been found in tissues of a wide range of animals, including marine mammals (Aguilar et al. 2002) and humans, with levels in breast milk of U.S. women highest in the world (Betts 2002, Schecter et al. 2004, She et al. 2004). Potential health risks of PBDEs for marine mammals include endocrine disruption in grey seals (Hall et al. 2003). On a global scale, evidence for declining concentrations of traditional POCs, such as PCBs and DDT, are occurring in some marine systems, while increasing levels of PBDEs and other emerging contaminants highlight the need to monitor marine mammals and the environment (de Wit 2002, Houde et al. 2005). These compounds were measured in blubber biopsy samples collected during the summer of 2003 from dolphins in the Indian River Lagoon (IRL), FL and Charleston (CHS), SC using GC/MS techniques. Full-depth biopsies were surgically removed from the left side of the animal at a site 5-10 cm caudal to the dorsal fin and 10 cm ventral to the dorsal ridge and placed immediately in a liquid nitrogen dewar.

POC analytes were measured in blubber samples from a total of 72 dolphins (38 CHS, 34 IRL). Measurements included **71 PCB congeners** (1, 2, 3, 5/8, 9, 12, 15, 18, 20, 26, 28/31, 29, 37, 44, 45,48, 49, 50, 52, 56/60, 61/74, 63, 66, 69, 70/76, 77, 82, 84, 87/115, 88/95, 89, 92, 99, 101/90, 105, 106/118/123, 107/108, 101/90, 110, 114, 119, 123, 126, 128/167, 130, 132/153/168, 141, 146, 149, 151, 154, 159, 169, 170/190, 172, 174, 177, 180, 183, 187, 188, 189, 193, 194, 195, 200, 201, 202, 206, 207, and 209), and **20 pesticides** (2,4'-DDD, 2,4'-DDD, 2,4'-DDE, 2,4'-DDT, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, Aldrin, Beta-HCH, Cis-chlordane, Cis-nonachlor, Dieldrin, Endosulfan I, Endosulfan II, Endosulfan Sulfate, Gamma-chlordane, Heptachlorepoxide, Hexachlorobenzene, Mirex, and Trans-nonachlor) and **6 PBDE congeners** (28, 47, 99, 100, 153, and 154).

Analysis of total PCBs were reported in Table 1 by the following age categories: adult males 15 yrs and older; adult females 7 yrs and older and juveniles were characterized as less than these ages; pregnant females were also grouped. Consistent with many other marine mammal studies, concentrations of PCBs were highest in male dolphins and lowest in females of reproductive age (Aguilar et al., 2002). The mean concentrations of PCBs and pesticides varied among age categories with fewer differences seen among the two sites. The total PCB data in Table 1 were based on our recently expanded analysis of

71 PCB congeners. These results, when compared to our previous published values on CHS and IRL animals, which assessed total PCB based on only 28 congeners (Hansen et al. 2004), found similar values which were approximately less than 50% of the total PCB concentrations measured using 71 congeners. Total DDT was greater for all CHS dolphins (22,570ng/g; n=38) compared to IRL dolphins (19,755ng/g; n=34) but age categories comparisons were similar to that for the PCB data.

Table 1. Total PCBs (ng/g lipid) in Blubber Samples 2003

Total PCBs	# of animals		Mean (± Std Dev)		Minimum		Maximum		Median	
Age category	CHS	IRL	CHS	IRL	CHS	IRL	CHS	IRL	CHS	IRL
Juvenile Male	10	14	64,634 (23,426)	62,078 (46,102)	28,575	22,763	100,219	192,649	59,006	50,506
Juvenile Females	3	3	43,687 (11,621)	36,686 (24,367)	30,628	9,282	52,892	55,916	47,541	44,859
Adult Males	16	10	117,378 (63,564)	113,867 (59,935)	59,796	41,710	255,010	227,291	88717	100,086
Adult Females	5	3	8,787 (2,745)	10,210 (9,347)	4,538	1,520	12,209	20,099	8,907	9,011
Pregnant Females	4	3	16,273 (12,631)	29,582 (13,102)	7,788	14,852	35,073	39,935	11,115	33,959

While the distribution pattern of blubber PBDEs were similar among dolphins at both sites, the CHS animals had higher total PBDEs (sum of 28, 47, 99, 100, 153, and 154) in their blubber tissue. Male dolphins in CHS had mean concentration of 2,994 ng/g lipid (±1,193 SD) compared to 1,275ng/g lipid (±855 SD) in IRL male dolphins. In all age categories adult females in both the IRL and CHS sites had the lowest PBDE concentrations (CHS mean concentration 363±127ng/g lipid; IRL mean concentration 693±800ng/g lipid). CHS juvenile dolphins, both females and males, also had 3-4X higher concentrations than IRL juvenile male and female dolphins with the juvenile male dolphins near the same levels as in adult males which are among the highest levels found in marine mammals. The distribution pattern of PBDE congeners were similar for both sites with 4 congeners comprising 98% of all PBDEs measured for CHS and 94% for IRL as follows: PBDE100>PBDE154>PBDE99>PBDE47. PBDE 100 was the largest component for both the IRL and CHS dolphins (51% CHS; 42% PBDEs) followed by PBDE 154 (CHS 24%; IRL 23%), then PBDE 154 (24%CHS; 23%) and PBDE 99 (13%CHS; 15%IRL).

Preliminary descriptive statistics conducted on 2003 POC blubber concentrations in IRL and CHS dolphins are presented. The high body burden of complex mixtures of POCs, including PCBs, DDT, DDE and PBDEs, carried by these populations of dolphins and the known toxicities of many of these compounds may pose a significant health risk. The POC 2004 blubber data for IRL and CHS dolphins will be available spring 2005 and further analysis will include comparison to 2003 data and examining the relationships between POC blubber concentrations with POC plasma concentrations, thyroid level and biomarkers of immune functions as well as other health parameters.

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Emerging Contaminants (Perfluoroalkyl Compounds and Hydroxylated-PCBs) in Bottlenose Dolphins from Charleston, SC and the Indian River Lagoon, FL

Magali Houde[1,2], P. Fair[3], G. B. Bossart[4], K. Solomon[1], D. Muir[1,2]

[1]University of Guelph, Ontario, Canada; [2]National Water Research Institute, Environment Ontario, Canada; National Oceanic and Atmospheric Administration, NOS Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC; [4]Division of Marine Mammal Research and Conservation, Harbor Branch Oceanographic Institution, Ft. Pierce, FL

As part of the HERA dolphin project, two main groups of emerging contaminants, perfluoroalkyl compounds and hydroxylated polychlorinated biphenyls (OH-PCBs), were assessed for the first time in free-ranging bottlenose dolphins. Perfluoroalkyl compounds (PFAs) have been used for decades in a variety of industrial and commercial products such as stain repellents, paints, polishers and fire-fighting foams. Perfluorinated carboxylic and sulfonic acids are persistent PFA degradation products known to affect development, peroxisome proliferation and cell-to-cell communication in rodents. OH-PCBs are metabolic PCB degradation products that are known to have thyroidogenic effects. Our objectives were to assess the PFA, PCB and OH-PCB concentrations in dolphin's plasma as well as look at the potential PFA bioaccumulation through the dolphin's food web by analyzing water, sediment and fish samples. A PFA body distribution was also assessed by analyzing tissues of a female dolphin from Charleston. Results show that high PFA levels, including high concentrations of perfluorooctane sulfonate (PFOS), were detected in plasma of animals from both populations. PFA concentrations detected in Charleston dolphins were higher that any report published to this date. PFAs were detected in all tissues analyzed with the highest levels found in liver, lung and thyroid. PFAs were also detected in sediment and fish. Prey/predator ratio suggests possible bioaccumulation and biomagnification of PFA in the dolphin's food chain. An extraction method is presently being tested for water sample extraction. Preliminary results for PCB showed that plasma levels were higher at Charleston compared to Indian River Lagoon (IRL). Numerous OH-PCBs were also detected in dolphin plasma from both locations with higher levels detected in animals from IRL compared to Charleston. PCB pattern of contamination were similar between populations but varied for OH-PCBs. Relationships between PFA plasma concentrations and biomarkers of immune functions, as well as PCB/OH-PCB levels and hepatic enzyme activities, respectively, will be investigated.

Changes in Gene Expression in the Skin-Blubber Biopsy as Measures of Chemical Exposure and Effect in the Bottlenose Dolphin (*Tursiops truncatus*)

Montie, E. W. [1,2], S. R. Garvin[1], P. A. Fair[3], G. D. Bossart[4], A. A. Hohn[5], G. B. Mitchum[3], W. E. McFee[3], M. J. Moore[1], and M. E. Hahn[1].

[1]Woods Hole Oceanographic Institution, Woods Hole, MA; [2]Massachusetts Institute of Technology, Cambridge, MA; [3]NOAA, National Ocean Service, Charleston, SC; [4]Harbor Branch Oceanographic Institution, Ft. Pierce, FL; [5]NOAA, National Marine Fisheries Service, Beaufort, NC

Background and Objectives. In support of the Bottlenose Dolphin Health and Risk Assessment (HERA) Project, our research goals were to assess changes in gene expression (in the skin-blubber biopsy) as measures of chemical exposure and effect. Cytochrome P4501A1 induction is a valuable biomarker of exposure to planar halogenated aromatic hydrocarbons (PHAHs) and has been used extensively in fish, birds, and marine mammals. Its advantages include the extensive literature demonstrating its relationship to PHAH exposure, the relatively robust methods that exist for its detection, and the fact that it can be measured in skin-blubber biopsy samples. Thus, our primary objective was to measure cytochrome P450 1A1 (CYP1A1) expression in bottlenose dolphin skin-blubber biopsy samples from i) Charleston, SC; ii) Titusville, FL (IRL-N); (iii) and Stuart, FL (IRL-S) sites. The initial CYP1A1 analysis of stained sections revealed that blubber morphology and CYP1A1 expression were highly variable with blubber depth. Consequently, our research goals expanded to provide a histological assessment of blubber stratification in these live-captured dolphins and to quantitatively test the hypothesis that CYP1A1 expression varies with depth in dolphin blubber.

Despite the value of CYP1A1 as a measure of chemical exposure, its value as a biomarker of adverse effect is debated, because its mechanistic link to PHAH toxicity is uncertain. There is a need for new biomarkers of PHAH effects, especially ones that are more directly linked to toxicity. Thus, an additional objective was to explore the feasibility of using suppression subtraction hybridization (SSH) to identify novel biomarkers (genes) for measuring chemical exposure in skin-blubber biopsies.

There is also evidence that certain PHAHs can interfere with the thyroid hormone system in rats, humans, and seals and cause hypothyroxinemia (low thyroxine or T4). Thus, in addition to the use of CYP1A1 and research to develop novel biomarkers of PHAH toxicity, we began to explore possible biomarkers of thyroid hormone status, in particular Type II 5'-deiodinase (or D2). D2 activates the prohormone T4 to form the active hormone T3. Observations have led to the theory that D2 produces T3 for local cellular demands, independent of circulating T3. D2 expression also increases with hypothyroidism. Thus, another objective was to explore the feasibility of measuring D2 in skin-blubber biopsies as a biomarker of thyroid hormone homeostasis.

Results – Blubber Morphology and CYP1A1 Expression. We used established histological and immunohistochemical methods to understand the structure of blubber and to assess the expression of CYP1A1 in skin-blubber biopsies of bottlenose dolphins (*Tursiops truncatus*) captured in the waters of Cape May, NJ (n=4), Charleston, SC (n=38), and Indian River Lagoon, FL (n=36) during the summer of 2003. Histological analysis of blubber revealed stratification of the blubber into three layers: a superficial layer (very high density of structural fibers and few adipocytes), a middle layer (more and larger adipocytes, and fewer structural fibers), and a deep layer (increased structural fiber densities and smaller adipocytes). Histological analysis also revealed individual and site-specific variability in structural fiber densities, adipocyte numbers, and adipocyte cell sizes among dolphins sampled at the three sites. New Jersey dolphins had more and larger adipocytes and lower densities of structural fibers than CHS and IRL dolphins. We plan to investigate other sources of variability in blubber morphology including sex, development, pregnancy, lactation, nutritional status, and disease effects.

CYP1A1 expression was strongest and most frequent in capillary endothelial cells within the blubber, similar to findings in other studies of cetacean CYP1A1. CYP1A1 expression differed among the blubber layers: deep blubber layer > middle blubber layer > superficial blubber layer ($p < 0.001$). CYP1A1 expression was greater in Charleston versus Indian River Lagoon bottlenose dolphins ($p = 0.011$). Males had higher CYP1A1 expression than females in the deep blubber layer ($p < 0.001$). Chemical analysis of biopsies and plasma are underway in the labs of Greg Mitchum and Derek Muir. When these data become available, we will determine the relationship between CYP1A1 expression and concentrations of non-*ortho* and mono-*ortho* PCBs in plasma and blubber of individual dolphins. In addition, we will assess the relationship between CYP1A1 expression and plasma levels of hydroxylated metabolites of PCBs. We also plan to determine if CYP1A1 expression in the middle and deep blubber layers is higher in thin dolphins compared to robust individuals.

SSH Experiments. To begin to identify novel PHAH-regulated genes, we exposed dolphin skin/blubber biopsies to PCB-126, isolated RNA, and measured changes in gene expression. Initially, we measured the expression of CYP1A1, to verify that the *in vitro* exposure had been effective at activating the AHR and inducing a known AHR-regulated gene. To do this, CYP1A1 (370 bp) and actin (1200 bp) cDNA fragments were amplified by RT-PCR (from FB814), cloned, and sequenced. The bottlenose dolphin CYP1A1 amino acid sequence showed 99% identity to striped dolphin (*Stenella coeruleoalba*) CYP1A1, 89% identity to pig (*Sus crofa*) CYP1A1, 87% identity to the mouse (*Mus musculus*) CYP1A1, and 86% identity to human (*Homo sapiens*) CYP1A1. The slice treated with PCB-126 showed elevated CYP1A1 expression compared to the DMSO-treated slice, while actin expression did not differ with treatment. These results indicate that the *in vitro* biopsy dosing technique was effective in inducing AHR-dependent gene expression.

As part of the HERA 2004 project, we plan to follow-up these initial results. We have designed primers for conducting real-time, quantitative RT-PCR analysis of CYP1A1 and actin expression. Using these primers, we will measure the expression of CYP1A1 and

actin in all DMSO- and PCB-126-treated slices. The biopsy samples demonstrating the greatest CYP1A1 induction in these assays will be used in SSH to identify novel PHAH-responsive genes.

Type II 5'-deiodinase (D2). To begin to determine the feasibility of measuring D2 as a biomarker of thyroid hormone homeostasis, we isolated total RNA from a skin-blubber biopsy (from FB814). A D2 (750 bp) cDNA fragment was amplified by RT-PCR (from FB814), cloned, and sequenced. The bottlenose dolphin D2 amino acid sequence showed 92% identity to pig (*Sus crofa*) D2, 89% identity to human (*Homo sapiens*) D2, 90% identity to the mouse (*Mus musculus*) D2, and 89% identity to rat (*Rattus norvegicus*) D2.

As part of the HERA 2004 project, we plan to follow-up these initial results. We have designed primers for conducting real-time, quantitative RT-PCR analysis of D2 and actin expression. Using these primers, we will measure the mRNA expression of D2 and actin in the 2003 skin-blubber biopsies. When the data become available, we will determine the relationship between D2 expression and concentrations of non-*ortho* and mono-*ortho* PCBs in plasma and blubber of individual dolphins. In addition, we will assess the relationship between D2 expression and plasma levels of hydroxylated metabolites of PCBs. We are also interested in the role that D2 plays in blubber dynamics. [Supported by NOAA and by an EPA STAR Graduate Fellowship].

Development of a Dolphin CDNA Microarray

Annalaura Mancia[1], M. Lundqvist[1], T. Romano[2], G. Bossart[3], P. Fair[4], and G. W. Warr[1]

[1]Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston SC; [2]Mystic Aquarium and Institute for Exploration, Mystic CT; [3]Harbor Branch Oceanographic Institution, Inc., Fort Pierce, FL.; [4]CCEHBR, National Ocean Service, Charleston SC

The Atlantic bottlenose dolphin (*Tursiops truncatus*) has been proposed as a sentinel species for the health of the marine environment. Dolphins, as top predators, are sensitive to the biointensification effects of marine toxins, pollutants and infectious disease agents. A number of dolphin pathogens have been described, including morbillivirus, caliciviruses and a significant number of human pathogens. The aim of this project is to generate molecular tools to assess the health of wild dolphins, thereby indicating the status of the local marine environment and providing information for marine resources management. Random Expressed Sequence Tag (EST) clones have been isolated and sequenced from dolphin Peripheral Blood Leukocyte (PBL) cDNA libraries. Genes known to be important in the innate and adaptive immune responses of terrestrial mammals and in responses to stress and contaminant exposure have been targeted for cloning using PCR-based techniques. We have cloned approximately 2200 unigenes from the dolphin cDNA libraries and 62 dolphin genes of known stress or immune function by targeted PCR (www.marinegenomics.org). These genes, together with other genes randomly selected without sequencing from the cDNA libraries, have been amplified and used to construct a cDNA microarray representing 3700 dolphin genes. The dolphin cDNA microarray will be used to analyze PBL RNA from captive dolphins of known health status with the aim to validate and optimize the cDNA microarray as a sensitive and informative tool. The dolphin cDNA microarray will be used to analyze PBL RNA from wild dolphins to determine the utility of "transcriptional signatures" in revealing dolphin health status.

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Establishment and Characterization of Epidermal Cell Cultures (Ds1) from the Skin of the Atlantic Bottlenose Dolphin (*Tursiops truncatus*)

Jin, Yu[1], M. S. Kindy[1,2,6], J. E. Baatz[2,3], M. Peden-Adams[2,3], T. J. Ellingham[4], D. J. Wolff[4], P. A. Fair[2,7], and S. Gattoni-Celli[2,5,6]

[1]Department of Neuroscience, Medical University of South Carolina, Charleston, SC; [2]Marine Biomedicine and Environmental Sciences Center, Charleston, SC; [3]Department of Pediatrics, Medical University of South Carolina, Charleston, SC; [4]Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC; [5]Department of Radiation Oncology, Medical University of South Carolina, Charleston, SC
[6]Ralph H. Johnson VA Medical Center, Charleston, SC

The Atlantic bottlenose dolphin (*Tursiops truncatus*), a marine mammal found off the Atlantic coast, has become the focus of considerable attention. Assessment of the impacts of environmental stressors on bottlenose dolphins (BND) has been difficult because of the protected status of these marine mammals. Because of the prevalence of skin pathologies in BND at the Indian River Lagoon (Florida), the studies presented herein focused on establishing epidermal cell cultures as a tool for future evaluation of environmental stressors on BND skin in vitro. Primary epidermal cell cultures (DS1) were established from skin samples obtained from Atlantic bottlenose dolphins. The *in vitro* epidermal cell cultures were subjected to karyotype analysis and further characterized using immunohistochemical methods demonstrating that DS1 cultures express cytokeratins. Further comparisons using 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE) demonstrated that the proteomic profile of BND skin tissue samples was similar to that of skin-derived DS1 cultures. Epidermal cell cultures derived from *Tursiops truncatus* will provide a unique tool for studying key features of the interaction occurring between dolphins and the environment in which they live at their most crucial interphase: the skin.

Prevalence of Zoonotic Protozoa Infecting Bottlenose Dolphins

Ron Fayer[1], M. Santin[1], J. P. Dubey[1], P. A. Fair[2], G. D. Bossart[3], C. Sreekumar[1], O. C. H. Kwok[1], and P. H. Thulliez[4]

[1]United States Department of Agriculture, Beltsville, MD, [2]National Oceanic and Atmospheric Administration/National Ocean Service, Charleston, SC, [3]Harbor Branch Oceanographic Institute, Fort Pierce, FL, [4]Institut de Puericulture, Paris, France

Although *Giardia* and *Cryptosporidium* have been detected in a small number of seals and sea lions, neither they nor any species of Microsporidia have been detected in dolphins. Fecal specimens obtained in 2004 from 32 bottlenose dolphins in the Indian River Lagoon, FL and from 29 dolphins from the Charleston, SC collection areas were cleaned of debris by sieving. A 2 µl suspension from each specimen was spotted onto a glass microscope slide, stained with fluorescein-labeled antibody to *Giardia* and *Cryptosporidium*, and examined by fluorescence microscopy. DNA was extracted from the remainder of each specimen and polymerase chain reaction (PCR) was applied using generic primers for the 18S ribosomal RNA genes of *Giardia*, *Cryptosporidium*, and Microsporidia. Neither microscopy nor PCR detected any of these parasites except for 3 dolphins from which DNA for fish Microsporidia were detected.

Toxoplasma gondii has not been isolated from dolphin tissues. In the present study, sera from 146 (60 from 2003, and 86 from 2004) dolphins from the IRL and Charleston sites were tested for antibodies to *T. gondii.* Sera from 2003 were tested by the direct agglutination test (DAT), modified agglutination test (MAT), indirect fluorescent antibody test (IFAT), the Sabin-Feldman dye test (DT), and an indirect hemagglutination test (IHAT). For sera from 2003, 60 dolphins were seropositive with MAT titers of 1:20 in 3, 1:40 in 19, 1:80 in 29, 1:160 in 2, 1:1,280 in 3, 1:2,560 in 2, and 1:5,120 or higher in 2 and these results were confirmed by DAT results. Dye test titers for these dolphins were < 1:10 in 53, 1:800 in 3, 1:1,600 in 2, and 1:3,200 in 2. IHAT titers were < 1:64 in 52, 1:128 in 1, 1:512 in 2, and 1:2,048 in 5. The IFAT titers were < 1:20 in 3, 1:20 in 47, 1:80 in 9. For sera from 2004, MAT antibodies were found in 86 of 86 dolphins with titers of 1:25 in 29, 1:50 in 23, 1:100 in 27, 1:200 in 3, 1:1,600 in 1, and 1:3,200 in 3; these sera were not tested by other tests. In summary, MAT antibodies were found in 100% of the 146 dolphin sera tested.

Detection of *Erysipelothrix rhusiopathiae* Antibodies in *Tursiops truncatus*

Rhonda A. Patterson

University of Southern Mississippi, Hattiesburg, MS

Erysipelothrix rhusiopathiae is a Gram positive bacilli which is pathogenic to a variety of mammals, birds and some aquatic animals. The consumption of contaminated fish is one possible cause of *E. rhusiopathiae* infection in captive marine mammals, and since acute erysipelas in a cetacean can lead to death before any symptoms are seen, erysipelas can be considered one of the most problematic diseases seen at aquatic parks housing captive cetaceans. A diagnostic assay which could be applied routinely to monitor antibody titers against this pathogen would be useful in monitoring ongoing immune status. The specificity and amplification of results using an enzyme linked immunosorbent assay (ELISA), as well as less complex protocols compared to equally sensitive assays (radioimmunoassays) are proof of the need for ELISA assays as the routine assay to detect *E. rhusiopathiae* antibody titers in captive or free ranging cetaceans. The assay developed by this laboratory uses a combination of capture antigens extracted from the surface of an isolate of *E. rhusiopathiae,* provide by the U.S. Navy Marine Mammal Program from a *Tursiops gilli* that succumbed to a septicemic case of erysipelas, to coat the wells. It also uses antisera specifically developed for *Tursiops truncatus* to detect their antibodies. Serum samples (collected in the summer of 2004) from twenty-two free ranging Atlantic bottlenose dolphins (*Tursiops truncatus*) in the Charleston, South Carolina area were assayed. Statistical analysis of the results was performed. Of the twenty-two samples assayed, one sample was found to have no titer (or a titer of <1/100, the lowest dilution tested); one sample had a titer of 1/100; five samples had titers of 1/1000; ten samples had titers of 1/10,000; four samples had titers of 1/100,000; and one sample had a titer of 1/1,000,000. These results were not unexpected and compare favorably with other free ranging dolphin samples that have been assayed as well as results seen in captive animals housed in open ocean pens with high human traffic and/or closed facilities with sand filtration systems. Further erysipelas analysis will include samples for 2004 IRL dolphins in addition to banked 2003 serum samples from animals at both sites.

Assessment of Microflora in Dolphins from the Indian River Lagoon, FL and Charleston, SC

John Pisani[1] and P. A. Fair [2]

[1]Micrim Labs, Fort Lauderdale, FL; [2]National Oceanic and Atmospheric Administration, National Ocean Service, Center for Coastal Environmental Health & Biomolecular Research, Charleston, SC

We report here the results of a study on the bacterial and fungal microorganisms recovered from dolphins of the Indian River Lagoon, Florida (IRL) and the Charleston Harbor System, Charleston SC. (CHS). Each dolphin's microbiota was assessed by culture of the blowhole, gastric contents and feces. All cultures were evaluated by the use of six different bacterial media (tryptic soy agar w/ 5% sheep blood, MacConkey, Hektoen enteric, thiosulfate citrate bile sucrose agar, Saboroud dextrose agar with chloramphenicol and thioglycollate nutrient broth) and for fungal assessment; fungal media used were (Saboroud, Saboroud's with Chloramphenicol, Malt extract, and Mycobiotic agar) cultured at room and elevated temperatures. Blowhole cultures from 76 dolphins evaluated in 2003 yielded a total of 22 different bacteria with a predominance of *Aeromonas hydrophila* (19%) followed by *Plesiomonas shigelloides* (17%), *Vibrio alginolyticus* (11%), *Pseudomonas fluorescens* (9%) and *Pseudomonas aeruginosa* (7%). The greatest incidences of mycotic isolation from blowhole cultures were in the Genus Candida, with several true fungi being represented. During 2004, blowhole cultures evaluated from 50 dolphins yielded a total of 24 different bacteria with a predominance of *Aeromonas hydrophila* (21%), *Pseudomonas fluorescens* (20%), *E. coli* (7%), *Plesiomonas shigellides* (7%), *Shewanella putrefaciens* (7%) and *Vibrio alginolyticus* (6%). Gastric cultures had a greater incidence of yeast isolations as well as 13 different bacterial isolates, with the greatest representations being *Bacillus sp.* (11% of isolates) and *Clostridium perfringens* (10% of isolates). Finally, 18 different bacteria were isolated from fecal cultures, with the greatest incidence being *E. coli* (32% of isolates) and 26% of isolates being *Plesiomonas shigelloides*. Over a two-year period, bacterial isolations from both sites (IRL and CHS) had negligible differences as well as no major difference in representations of genders. Microbial data will be subjected to statistical analysis and compared with other health parameters such as immune status and disease.

Antibiotic Resistant *Escherichia coli* Found in Wild Dolphin Populations

John A. Bemiss[1], B. R. Lyon[1], T. W. Greig[1], G. D. Bossart[2] and P. A. Fair[1]

[1]National Oceanic and Atmospheric Administration, National Ocean Service, Charleston, SC; [2]Division of Marine Mammal Research and Conservation, Harbor Branch Oceanographic Institution, Ft. Pierce, FL

It has been hypothesized that the wide spread use of antibiotics in humans, pets, and agriculture may be causing a carryover of antibiotics and antibiotic resistant bacteria into the natural environment. This carryover has been supported by documentation of antibiotic resistant bacteria in a variety of wild caught species. To investigate these finding further, fecal samples collected during a health assessment study of bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon (IRL), Florida and the Charleston Harbor area (CH), South Carolina in 2003 were tested for antibiotic resistant *Escherichia coli*. The IRL is a shallow water ecosystem characterized by residential and undeveloped areas with limited water exchange; while the CH harbor estuary is characterized by residential, urban and light industrial with a high rate of water exchange due to the confluence of the Cooper, Ashley and Wando Rivers. *E. coli* were cultured from fecal samples or rectal swabs and evaluated for resistance to 25 antibiotics using a modified Behring Microscan RUO panel. Of the 15 animals captured in the IRL, three (20%) exhibited antibiotic resistance to one or more of the antibiotics tested. Of the 23 animals captured in CHS 16 (70%) exhibited resistance to one or more of the antibiotics tested. Data from this study on antibiotic resistant *E. coli* in wild caught dolphins will be included in a larger assessment of health and environmental parameters to further our understanding on the transfer and potential impacts of antibiotic resistance in coastal dolphins.

The Dolphin HERA Project: What Can Fatty Acid Profiles Tell Us About Dolphin Health?

Holbrook, M.A. and G. T. Seaborn

NOAA/National Ocean Service, Marine Forensics Laboratory, Charleston, SC

Marine mammals use blubber for insulation, buoyancy control, maintaining body shape, hydrodynamic streamlining, and energy storage (Koopman 2001). Blubber tissue is composed primarily of fibrous proteins and adipocytes, which are specialized cells that store fat as triacylglycerols. Blubber is extremely important metabolically, as most marine mammals have very few internal adipose depots (Pond 1998). Lipid profiles of blubber may vary among different species, different populations within a single species, and age groups within populations. The prevailing assumption is that observed differences are largely dependent on individual differences in diet due to preference and/or availability, and may also be affected by variation in age, reproductive status, health status, temperature, and other metabolic and environmental factors (Bowen 1999). In the bottlenose dolphin (*Tursiops truncatus*), the fatty acid composition of blubber triacylglycerols changes seasonally and has been shown to change differentially among stratified layers in blubber depth. It has been suggested that the inner blubber layer is more metabolically active, reflecting seasonal changes, while the outer blubber is more representative of historical feeding habits and may be more useful in population studies (Recks 2004). Fatty acid profiles in inner and outer blubber biopsies from bottlenose dolphins captured in the HERA project may thus reflect both short- and long-term differences within and among populations and will provide baseline profiles to aid in assessing individual, population, and ecosystem health.

Blubber biopsies from bottlenose dolphins captured in Charleston, SC (CHS) and Indian River Lagoon, FL (IRL) during the summer season in 2003 and 2004 were divided into inner and outer blubber by gross morphology. Mean blubber depth of 2003 and 2004 samples were compared by location, indicating no difference between the two years in CHS samples, but a significant difference in IRL animals sampled in 2003 and 2004 ($p < 0.0001$). This is likely due to sample collection, rather than a physiological change in blubber depth in the animals. The need for standardized techniques in field sample collection was recognized by both laboratory and field scientists. No significant difference was observed between blubber depths measured in the field by ultrasound and in the laboratory at the time of analysis in the 2004 samples from both CHS and IRL, suggesting that sampling technique improved between 2003 and 2004.

Blubber lipids were extracted in hexane, transesterified to form fatty acid methyl esters (FAME), and analyzed by gas chromatography with flame ionization detection for quantitation and mass spectrometric detection used in conjunction with the analysis of reference standards for peak identification. Review and verification of chromatographic data are in progress. Preliminary results show 144 FAMEs identified in CHS and IRL 2004 outer blubber samples. Eliminating FAMEs with mean weight percent values less than 0.5% resulted in a list of 28 FAMEs that accounted for 88.2% of total weight percent

on average (range 83.4-90.8%). Multivariate statistical methods for data analysis will be used to examine fatty acid profile data in conjunction with other indicators of health. Correlations with age, adipocyte cell counts and areas from histological analysis, and post-nuchal fat pad depth measured in the field will be explored.

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An Exploration Into the Feeding Ecology of Bottlenose Dolphins and Their Potential Prey Using Fatty Acids and Stable Isotopes

Worthy, G. A. J., A. M. Samuel, and T. A. M. Worthy

Physiological Ecology and Bioenergetics Lab, Department of Biology, University of Central Florida, Orlando, FL

The Indian River Lagoon (IRL) system is a complex of three estuarine lagoons (Mosquito Lagoon, Banana River Lagoon, and Indian River Lagoon) that extends approximately 253 km (155 mi) along Florida's east coast from latitude 29° 05'N to 26° 58'N. This system is a relatively closed system with only three inlets to the ocean, and is home to a resident population of bottlenose dolphins (*Tursiops truncatus*) and many resident/limited migration fish species. Both dolphins and fish have been extensively studied in terms of their preferred prey species and behavior, making the IRL a unique system for examining feeding ecology using stable isotopes and fatty acid signatures. Preferred prey species for these dolphins have previously been identified through stomach content analyses, and represent a wide variety of feeding strategies, from benthic detritivores such as mullet, to omnivores and carnivores such as pinfish. The purpose of this on-going study is to examine the inter-relationships between fatty acid and isotopic signatures of bottlenose dolphins and their presumed prey in the IRL, with the ultimate goal of interpreting bottlenose dolphin feeding habits.

Fish were collected bimonthly in the IRL and were immediately frozen and stored at -20°C until analyzed. Blubber samples were collected from live dolphins using either biopsy darts on free-swimming animals or as part of a live-capture operation. Blubber samples were also opportunistically collected from fresh dead-stranded dolphins (condition code 2 or early 3) in the IRL. Prey species were individually measured and weighed, then ground to homogeneous consistency before being sub-sampled for further analyses. Lipids were extracted from each fish sub-sample using a solution of 2:1 chloroform/methanol. Fatty acid methyl esters were prepared from the extract using acid-catalyzed methylation. Esters were purified in hexane, then analyzed using gas-liquid chromatography. Resultant chromatograms were compared to known standard mixtures and secondary external reference standards to determine fatty acid composition. Sub-samples of each fish sample were hand ground to powder after lipid extraction, and aliquots analyzed for stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) using a Delta Finnigan Mat isotope ratio mass spectrometer. Isotope ratios were determined as:

$$\delta X = [(R_{sample}/R_{standard})-1] \times 1000$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ (‰).Statistical tests included both univariate and multivariate techniques.

Preliminary results indicated that fatty acid composition of blubber differed significantly between summer and winter seasons. Within each season, samples taken from live animals could not be distinguished from dead-stranded animals in terms of their fatty acid composition. Differences were noted, however, between samples from live animals that were full depth and samples that were shallow depth. As well, gender differences were

evident with male and female animals having different fatty acid composition in their inner blubber layers. Using fatty acids to distinguish prey items was more difficult with some species (e.g., mullet) having distinct signatures, but others (e.g., pigfish) had fatty acid signatures similar to other species. Strong seasonal differences in fatty acid composition were evident within each prey species.

Stable isotope signatures were used to further evaluate differences between prey items and dolphins. Prey items showed differences in $\delta^{15}N$, separating out by trophic level (detritivore vs. piscivore). Some prey items such as pinfish showed drastic changes in $\delta^{15}N$ concentrations, indicating a trophic shift for these fish during the year. There was also a difference in $\delta^{15}N$ concentrations between dolphins sampled in years 1995-1998 and those sampled in years 2000-2003. By examining prey size between earlier fish collections (1991-1992) and more recent collections (2000-2003), a shift is seen in the growth curve for many different dolphin prey species. This change in fish size corresponding to a change in $\delta^{15}N$ concentration in dolphin skin could indicate changes in prey size and therefore, feeding habits, over the last decade in the IRL.

Acknowledgements:
We thank Christy Alves, Nicole Browning, Michelle DiPiazza and Nathalie Greenwald for their invaluable assistance in processing samples; Dr. Richard Paperno for fish collections; Megan Stolen and Wendy Noke for stranded dolphin sampling; and Drs. Pat Fair and Greg Bossart, along with the rest of the HERA team for assistance with dolphin biopsy sampling.

DNA Strand Breaks in Blood of Bottlenose Dolphin from the East Coast of the United States

Richard F. Lee and Karrie Brinkley, Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA 31411

The interaction of chemicals with cellular DNA can result in a wide assortment of DNA lesions, including a large number of base modifications, strand breaks and cross linkages. Exposure to physical agents, such as sunlight and radioactivity, can result in DNA damage. Single strand breaks are the most prevalent type of DNA damage in animals exposed to genotoxicants. If these different lesions are not repaired there can be altered gene expression leading to a number of biological consequences, including effects on reproduction and development. Several methods have been used to measure DNA strand damage, including micronucleus and sister chromatid exchange assays. A more recent assay, which seems to be more sensitive and reproducible than these assays, is the comet assay, where DNA strand breaks are determined by measuring the migration of DNA from immobilized nuclear DNA. The DNA after electrophoresis is stained with a fluorescent DNA-specific dyes. The migration of DNA away from the nucleus is quantified in terms of percentage of DNA in the tail or tail moment using image analysis software.

For our studies the comet assay was carried out on dolphin blood. Since marine mammals have nonnucleated erythrocytes, DNA strand breaks in lymphocytes are quantified. The comet assay on dolphin blood kept at 3°C showed no changes when sampled at 1,2,3 and 4 days of storage. Dolphin blood in cold packs was sent to us by overnight mail and the comet assay was carried out within 24 hours of receiving blood sample. To date, the blood has been processed for over 200 individual dolphins and the comet assay results are being compared with a variety of parameters collected from the dolphins, including location of station, chemical analysis of blubber for a number of pesticides and other anthropogenic compounds, immune system status and time after blood collection. A strong correlation was found between T-cell proliferation and DNA tail moments. Thus, increased number of DNA strand breaks correlated with a decreased ability of the T-cells to proliferate, i.e. undergo mitosis, which related to the ability of the blood to respond to infection. Dolphins with compromised immune system, such as those with viral infection, would be expected to show high levels of DNA strand breaks. There seem to be some evidence that dolphins when first collected show increased DNA strand breaks but several hours after collection most of these dolphins show low levels of DNA strand breaks. The large number of blood samples processed suggest that our data set will be one of the largest comet assay studies ever conducted on a species other than humans.

Uric Acid Levels: Wild and Semi-Domestic Dolphin Populations

Forrest Townsend[1], W. Van Bonn[2], S. Wong[2], P. A. Fair[3], G. D. Bossart[4], C. Wabner[5], E. Jensen[2], and T. Smith[2]

[1]BayVet Hospital, Fort Walton, FL; [2]U.S. Navy Marine Mammal Program, San Diego, CA; [3]National Oceanic and Atmospheric Administration, National Ocean Service, Charleston, SC; [4]Division of Marine Mammal Research and Conservation, Harbor Branch Oceanographic Institution, Fort Pierce, FL; [5]Mission Pharmacal Labs, San Antonio, TX

Overview. Initial analyses have been conducted on urine samples collected from two wild dolphin populations and one semi-domestic population. Aliquots of urine were collected into acidified and non-acidified glass tubes and submitted to Mission Pharmacal Reference Laboratory (San Antonio, Texas) for determination of multiple physiochemical parameter values anticipated to be clinically useful in the evaluation of renal health.

Statistical Methods. Test results were received from Mission Pharmacal Reference Laboratory and analyzed using SAS Release 8.2. Results among wild and Semi-domestic populations were compared using a general linear model. Statistical significance was defined as a p-value $< .01$.

Results. The study population included 24 urine samples from 11 semi-domestic dolphins and 49 samples from 49 wild dolphins. Wild dolphins had significantly higher levels of urine uric acid and uric acid-to-creatinine ratios than semi-domestic dolphins (p-values $<.0001$ and .002, respectively). While animal health variables were not included in this initial study, it is interesting to note that two Semi-domestic dolphins receiving hydration therapy had the lowest uric acid-to-creatinine ratios compared to the rest of the study population.

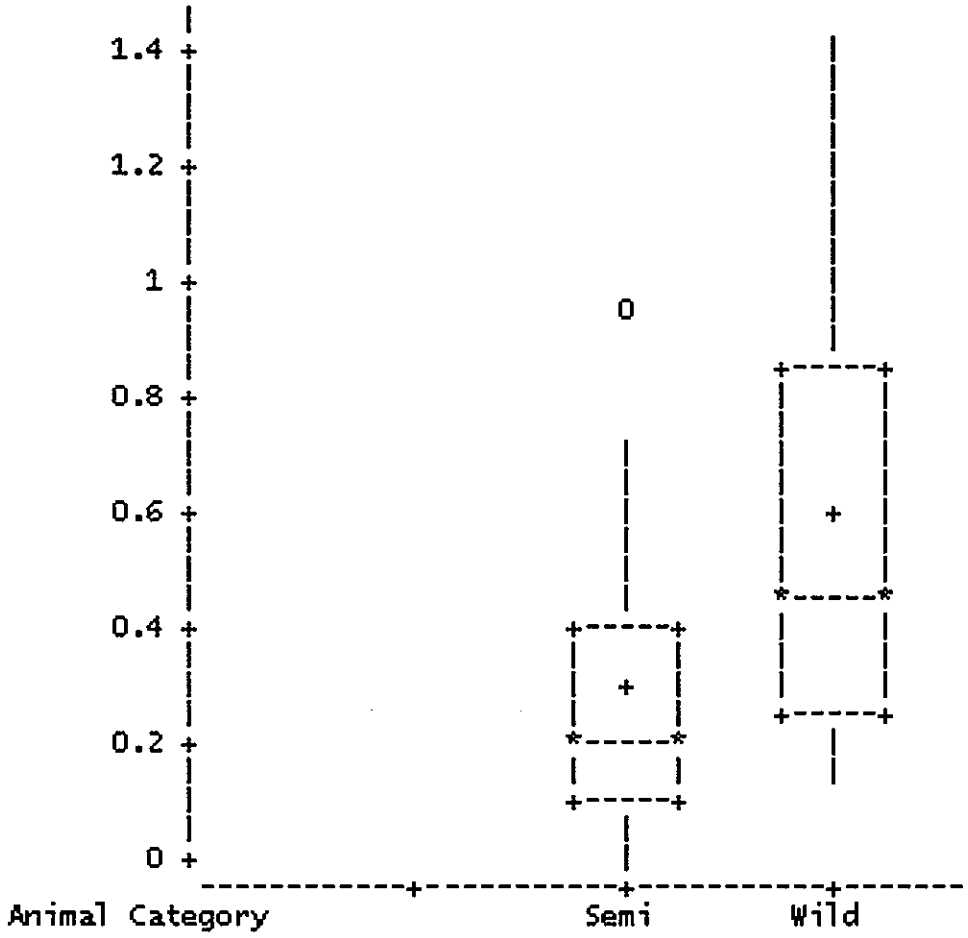
Semi-domestic dolphins were more likely to have higher urine sodium than wild dolphins (p-value .002). There were no significant differences among wild and semi-domestic dolphins when comparing urine pH, ammonia, or potassium. Semi-domestic dolphins did not have detectable levels of citrate in the urine.

Conclusions. There were significant differences in urine uric acid concentration when comparing wild and semi-domesticated dolphins. This study does not address potential confounders, including fasting status, gender, age, medical treatments, and animal health status. Preliminary results indicate, however, that urine uric acid may be a useful tool for assessing renal function in dolphins. Future investigations incorporating demographic data, complete blood counts, and serum chemistries are needed to help determine the importance of urine uric acid in assessing renal health.

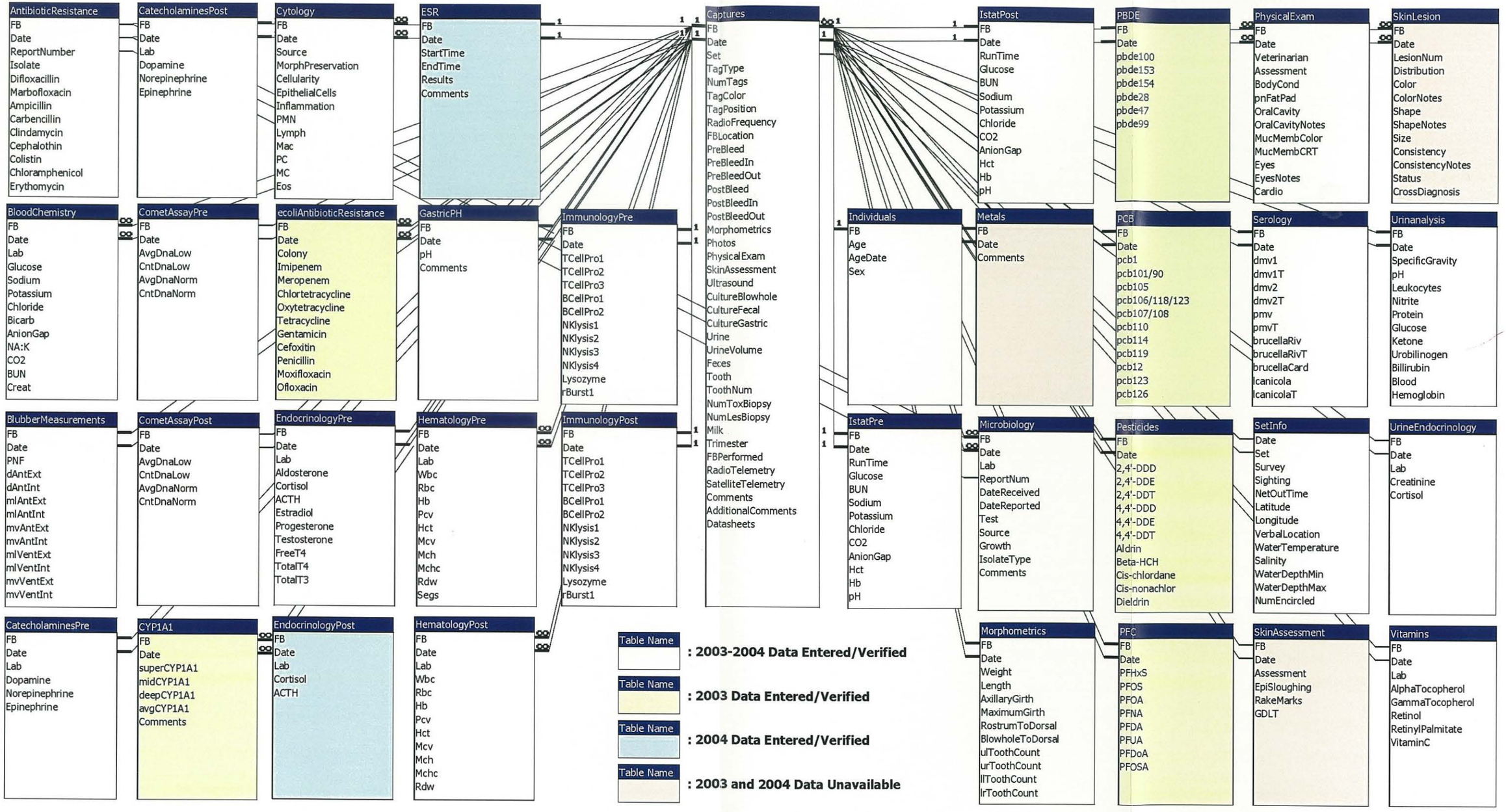
Graph 1. Box Plots of Urine UA: Creatinine in Wild v. Semi-domestic Dolphin Populations

The UNIVARIATE Procedure
Variable: UACreat (UACreat)

Schematic Plots



Appendix A. HERA database table relationships and data entry status.



AntibioticResistance

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Report Number	Text	Report number (assigned by lab).
Isolate	Text	Isolate tested.
Difloxacillin	Byte	Code representing sensitivity/susceptibility of isolate to difloxacillin (see luABResistance for code descriptions).
Marbofloxacin	Byte	Code representing sensitivity/susceptibility of isolate to marbofloxacin (see luABResistance for code descriptions).
Ampicillin	Byte	Code representing sensitivity/susceptibility of isolate to ampicillin (see luABResistance for code descriptions).
Carbencillin	Byte	Code representing sensitivity/susceptibility of isolate to carbencillin (see luABResistance for code descriptions).
Clindamycin	Byte	Code representing sensitivity/susceptibility of isolate to clindamycin (see luABResistance for code descriptions).
Cephalothin	Byte	Code representing sensitivity/susceptibility of isolate to cephalothin (see luABResistance for code descriptions).
Colistin	Byte	Code representing sensitivity/susceptibility of isolate to colistin (see luABResistance for code descriptions).
Chloramphenicol	Byte	Code representing sensitivity/susceptibility of isolate to chloramphenicol (see luABResistance for code descriptions).
Erythomycin	Byte	Code representing sensitivity/susceptibility of isolate to erythomycin (see luABResistance for code descriptions).
Furadantin	Byte	Code representing sensitivity/susceptibility of isolate to furadantin (see luABResistance for code descriptions).
Ceftazidime	Byte	Code representing sensitivity/susceptibility of isolate to ceftazidime (see luABResistance for code descriptions).
Gentamicin	Byte	Code representing sensitivity/susceptibility of isolate to gentamicin (see luABResistance for code descriptions).
Amikacin	Byte	Code representing sensitivity/susceptibility of isolate to amikacin (see luABResistance for code descriptions).
Oxacillin	Byte	Code representing sensitivity/susceptibility of isolate to oxacillin (see luABResistance for code descriptions).
Cefotaxime	Byte	Code representing sensitivity/susceptibility of isolate to cefotaxime (see luABResistance for code descriptions).
Penicillin	Byte	Code representing sensitivity/susceptibility of isolate to penicillin (see luABResistance for code descriptions).
Septra/bactrim	Byte	Code representing sensitivity/susceptibility of isolate to septra/bactrim (see luABResistance for code descriptions).
Tobramycin	Byte	Code representing sensitivity/susceptibility of isolate to tobramycin (see luABResistance for code descriptions).
Tetracyclines	Byte	Code representing sensitivity/susceptibility of isolate to tetracyclines (see luABResistance for code descriptions).
Metronidazole	Byte	Code representing sensitivity/susceptibility of isolate to metronidazole (see luABResistance for code descriptions).
Augmentin	Byte	Code representing sensitivity/susceptibility of isolate to augmentin (see luABResistance for code descriptions).
Ciprofloxacin	Byte	Code representing sensitivity/susceptibility of isolate to ciprofloxacin (see luABResistance for code descriptions).
Enrofloxacin	Byte	Code representing sensitivity/susceptibility of isolate to enrofloxacin (see luABResistance for code descriptions).
Pipercillin	Byte	Code representing sensitivity/susceptibility of isolate to pipercillin (see luABResistance for code descriptions).

Appendix B. HERA data dictionary (continued).

BloodChemistry

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Lab	Byte	Code representing lab conducting analyses (see luLab for code descriptions).
Glucose	Single	Glucose detected in serum from pre-processing blood sample (mg/dL).
Sodium	Single	Sodium detected in serum from pre-processing blood sample (mEq/L for Cornell & mmol/L for Miami).
Potassium	Single	Potassium detected in serum from pre-processing blood sample (mEq/L for Cornell & mmol/L for Miami).
Chloride	Single	Chloride detected in serum from pre-processing blood sample (mEq/L for Cornell & mmol/L for Miami).
Bicarb	Single	Bicarbonate detected in serum from pre-processing blood sample (mEq/L for Cornell & mmol/L for Miami).
AnionGap	Single	Anion gap calculated in serum from pre-processing blood sample (mEq/L for Cornell & mmol/L for Miami).
NA:K	Integer	Sodium to potassium in serum from pre-processing blood sample.
CO2	Single	CO2 detected in serum from pre-processing blood sample (mmol/L).
BUN	Single	Blood urea nitrogen detected in serum from pre-processing blood sample (mg/dL).
Creat	Single	Creatinine detected in serum from pre-processing blood sample (mg/dL).
BUN/Creat	Single	Ratio of blood urea nitrogen to creatinine in serum from pre-processing blood sample.
TProtein	Single	Total protein detected in serum from pre-processing blood sample (g/dL).
Albumin	Single	Albumin detected in serum from pre-processing blood sample (g/dL).
Globulin	Single	Globulin detected in serum from pre-processing blood sample (g/dL).
A/G	Single	Ratio of albumin to globulin in serum from pre-processing blood sample.
DBilirubin	Single	Direct bilirubin detected in serum from pre-processing blood sample (mg/dL).
IBilirubin	Single	Indirect bilirubin detected in serum from pre-processing blood sample (mg/dL).
TBilirubin	Single	DBilirubin + IBilirubin (mg/dL).
Calcium	Single	Calcium detected in serum from pre-processing blood sample (mg/dL).
Phosporus	Single	Phosphorus detected in serum from pre-processing blood sample (mg/dL).
Magnesium	Single	Magnesium detected in serum from pre-processing blood sample (mEq/L for Cornell & mmol/L for Miami).
UricAcid	Single	Uric acid detected in serum from pre-processing blood sample (mg/dL; det limit = 0.2).
AP	Single	Alkaline phosphatase detected in serum from pre-processing blood sample (U/L).
ALT	Single	Alanine aminotransferase detected in serum from pre-processing blood sample (U/L).
AST	Single	Aspartate aminotransferase detected in serum from pre-processing blood sample (U/L).
SDH	Single	Succinate dehydrogenase detected in serum from pre-processing blood sample (U/L; values reported as "< 2 U/L" were recorded as 1 U/L).
LDH	Single	Lactic dehydrogenase detected in serum from pre-processing blood sample (U/L).
CPK	Single	Creatine phosphokinase detected in serum from pre-processing blood sample (U/L).
Amylase	Single	Amylase detected in serum from pre-processing blood sample (U/L; Cornell values reported as "< 3 U/L" recorded as 1.5 U/L and Miami values reported as "< 30 U/L" recorded as 15 U/L).
Lipase	Single	Lipase detected in serum from pre-processing blood sample (U/L).
GGT	Single	Gamma-glutamyl transferase detected in serum from pre-processing blood sample (U/L).
Cholesterol	Single	Cholesterol detected in serum from pre-processing blood sample (mg/dL).
Triglyceride	Single	Triglyceride detected in serum from pre-processing blood sample (mg/dL).
Iron	Single	Iron detected in serum from pre-processing blood sample (ug/dL).
TIBC	Single	TIBC detected in serum from pre-processing blood sample (mg/dL).
%SAT	Single	Percent saturation of O2 in serum from pre-processing blood sample (%).
Albumin2	Single	Albumin2 detected in serum from pre-processing blood sample (g/dL).
Alpha1	Single	Alpha1 detected in serum from pre-processing blood sample (g/dL).
Alpha2	Single	Alpha2 detected in serum from pre-processing blood sample (g/dL).
TAlpha	Single	Alpha1 + Alpha2 (g/dL).
Beta1	Single	Beta1 detected in serum from pre-processing blood sample (g/dL).
Beta2	Single	Beta2 detected in serum from pre-processing blood sample (g/dL).
TBeta	Single	Total beta detected in serum from pre-processing blood sample (g/dL).
Gamma	Single	Gamma detected in serum from pre-processing blood sample (g/dL).
TGlob	Single	TAlpha + TBeta + Gamma (g/dL).
ImmuneTProtein	Single	Albumin2 + TGlob (g/dL).
ImmuneA/G	Single	Albumin2/TGlob (g/dL).
Lipemia	Single	Index representing degree of lipemia for blood sample used to derive blood chemistry profile.
Hemolysis	Single	Index representing degree of hemolysis for blood sample used to derive blood chemistry profile.

BloodChemistry (continued)

Field Name	Type	Description
Icterus	Single	Index representing degree of icterus for blood sample used to derive blood chemistry profile.
Comments	Text	Comments.

BlubberMeasurements

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
PNF	Single	Dorsal post-nuchal fat pad blubber measurement 10 cm behind blowhole (cm).
dAntExt	Single	Dorsal external blubber measurement 10 cm anterior to dorsal fin (cm).
dAntInt	Single	Dorsal internal blubber measurement 10 cm anterior to dorsal fin (cm).
mlAntExt	Single	Mid-lateral external blubber measurement 10 cm anterior to dorsal fin (cm).
mlAntInt	Single	Mid-lateral external blubber measurement 10 cm anterior to dorsal fin (cm).
mvAntExt	Single	Mid-ventral external blubber measurement 10 cm anterior to dorsal fin (cm).
mvAntInt	Single	Mid-ventral internal blubber measurement 10 cm anterior to dorsal fin (cm).
mlVentExt	Single	Mid-lateral external blubber measurement 10 cm ventral to dorsal fin (cm).
mlVentInt	Single	Mid-lateral internal blubber measurement 10 cm ventral to dorsal fin (cm).
mvVentExt	Single	Mid-ventral external blubber measurement 10 cm ventral to dorsal fin (cm).
mvVentInt	Single	Mid-ventral internal blubber measurement 10 cm ventral to dorsal fin (cm).
biopsyExt	Single	External blubber measurement at biopsy site (cm).
biopsyInt	Single	Internal blubber measurement at biopsy site (cm).
Comments	Text	Comments.

Captures

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Set	Long Integer	Set number on capture date.
TagType	Text	Type of tag (roto, roto/radio, satellite, none, etc.).
NumTags	Integer	Number of tags placed on animal.
TagColor	Text	Color of tags.
TagPosition	Text	Position on dorsal fin that tag was placed.
RadioFrequency	Text	Frequency of radio tag.
FBLocation	Text	Location of freezebrand (rt dorsal, lt dorsal, both dorsal, none, both dorsal/body, etc.).
PreBleed	Boolean	Indicates if pre-process bleed was performed.
PreBleedIn	Date	Needle in time for pre bleed.
PreBleedOut	Date	Needle out time for pre bleed.
PostBleed	Boolean	Indicates if post-process bleed was performed.
PostBleedIn	Date	Needle in time for post bleed.
PostBleedOut	Date	Needle out time for post bleed.
Morphometrics	Boolean	Indicates if morphometrics were recorded.
Photos	Boolean	Indicates if photos were taken.
PhysicalExam	Boolean	Indicates if a physical exam was performed.
SkinAssessment	Boolean	Indicates if a skin assessment was performed.
Ultrasound	Boolean	Indicates if ultrasound exam was performed.
CultureBlowhole	Boolean	Indicates if blowhole culture was obtained.
CultureFecal	Boolean	Indicates if fecal culture was obtained.
CultureGastric	Boolean	Indicates if gastric culture was obtained.
Urine	Boolean	Indicates if urine sample was obtained.
UrineVolume	Single	Volume of urine collected (ml).
Feces	Boolean	Indicates if a feces sample was obtained.
Tooth	Boolean	Indicates if a tooth was extracted.
ToothNum	Integer	Number corresponding to extracted tooth.

Captures (continued)

Field Name	Type	Description
NumToxBiopsy	Integer	Indicates number of blubber biopsies taken.
NumLesBiopsy	Integer	Indicates number of biopsies performed on lesions.
Milk	Boolean	Indicates if milk sample was obtained.
Trimester	Byte	Indicates trimester of pregnancy.
FBPerformed	Boolean	Indicates if animal was freezebranded.
RadioTelemetry	Boolean	Indicates if radio telemetry was attached.
SatelliteTelemetry	Boolean	Indicates if satellite telemetry was attached.
Comments	Text	Comments.
Additional Comments	Text	Additional comments.
Datasheets	Hyperlink/Memo	Link to datasheet.

CatecholaminesPost

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Lab	Byte	Code representing lab conducting analyses (see luLab for code descriptions).
Dopamine	Single	Dopamine detected in plasma from post-processing blood sample (pg/mL; values reported as "< 20 pg/mL" were recorded as 10 pg/mL).
Norepinephrine	Single	Norepinephrine detected in plasma from post-processing blood sample (pg/mL).
Epinephrine	Single	Epinephrine detected in plasma from post-processing blood sample (pg/mL).

CatecholaminesPre

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Lab	Byte	Code representing lab conducting analyses (see luLab for code descriptions).
Dopamine	Single	Dopamine detected in plasma from pre-processing blood sample (pg/mL; values reported as "< 20 pg/mL" were recorded as 10 pg/mL).
Norepinephrine	Single	Norepinephrine detected in plasma from pre-processing blood sample (pg/mL).
Epinephrine	Single	Epinephrine detected in plasma from pre-processing blood sample (pg/mL).

CometAssayPost

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
AvgDnaLow	Single	Average % DNA detected in tail region using a low pH (approximately 10) treatment on post-processing blood sample (%).
CntDnaLow	Integer	Number of cells used to copmute AvgDnaLow.
AvgDnaNorm	Single	Average % DNA detected in tail region using a normal pH (approximately 14) treatment on post-processing blood sample (%).
CntDnaNorm	Integer	Number of cells used to copmute AvgDnaNorm.

CometAssayPre

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
AvgDnaLow	Single	Average % DNA detected in tail region using a low pH (approximately 10) treatment on pre-processing blood sample (%).

CometAssayPre (continued)

Field Name	Type	Description
CntDnaLow	Integer	Number of cells used to copmute AvgDnaLow.
AvgDnaNorm	Single	Average % DNA detected in tail region using a normal pH (approximately 14) treatment on pre-processing blood sample (%).
CntDnaNorm	Integer	Number of cells used to copmute AvgDnaNorm.

CYP1A1

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
superCYP1A1	Single	Superficial blubber layer immunohistochemical (IHC) staining score for cytochrome P4501A1.
midCYP1A1	Single	Middle blubber layer immunohistochemical (IHC) staining score for cytochrome P4501A1.
deepCYP1A1	Single	Deep blubber layer immunohistochemical (IHC) staining score for cytochrome P4501A1.
avgCYP1A1	Single	Average immunohistochemical (IHC) staining score for cytochrome P4501A1.
Comments	Text	Comments.

Cytology

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Source	Byte	Code representing cytology source (see luSource for code descriptions).
MorphPreservation	Byte	Code representing assessment of morphologic preservation (see luCytology for code descriptions).
Cellularity	Byte	Code representing assessment of cellularity (see luCytology for code descriptions).
EpithelialCells	Byte	Code representing assessment of epithelial cells (see luCytology for code descriptions).
Inflammation	Byte	Code representing assessment of inflammmation (see luCytology for code descriptions).
PMN	Byte	Code representing assessment of PMN (see luCytology for code descriptions).
Lymph	Byte	Code representing assessment of lymph (see luCytology for code descriptions).
Mac	Byte	Code representing assessment of MAC (see luCytology for code descriptions).
PC	Byte	Code representing assessment of PC (see luCytology for code descriptions).
MC	Byte	Code representing assessment of MC (see luCytology for code descriptions).
Eos	Byte	Code representing assessment of Eos (see luCytology for code descriptions).
Bacteria	Byte	Code representing assessment of bacteria (see luCytology for code descriptions).
Fungus	Byte	Code representing assessment of fungus (see luCytology for code descriptions).
Nasitrema	Byte	Code representing assessment of nasitremia (see luCytology for code descriptions).
NonCellularDebris	Byte	Code representing assessment of non-cellular debris (see luCytology for code descriptions).
pH	Single	Fluid pH (gastric cytology only).
Comments	Text	Comments.

ecoliAntibioticResistance

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Colony	Byte	Ecoli colony from which antibiotic resistance was tested.
Imipenem	Byte	Code representing imipenem concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR1 for code descriptions).
Meropenem	Byte	Code representing meropenem concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR1 for code descriptions).
Chlortetracycline	Byte	Code representing chlortetracycline concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR3 for code descriptions).
Oxytetracycline	Byte	Code representing oxytetracycline concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR3 for code descriptions).

Appendix B. HERA data dictionary (continued).

ecoliAntibioticResistance (continued)

Field Name	Type	Description
Tetracycline	Byte	Code representing tetracycline concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR3 for code descriptions).
Gentamicin	Byte	Code representing gentamicin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR1 for code descriptions).
Cefoxitin	Byte	Code representing cefoxitin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR6 for code descriptions).
Penicillin	Byte	Code representing penicillin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR4 for code descriptions).
Moxifloxacin	Byte	Code representing moxifloxacin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR2 for code descriptions).
Ofloxacin	Byte	Code representing ofloxacin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR2 for code descriptions).
Chloramphenicol	Byte	Code representing chloramphenicol concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR6 for code descriptions).
NalidixicAcid	Byte	Code representing nalidixic Acid concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR3 for code descriptions).
Amikacin	Byte	Code representing amikacin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR4 for code descriptions).
Nitrofurantoin	Byte	Code representing nitrofurantoin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR7 for code descriptions).
Ceftriaxone	Byte	Code representing ceftriaxone concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR4 for code descriptions).
Amoxicillin	Byte	Code representing amoxicillin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR3 for code descriptions).
Ciprofloxacin	Byte	Code representing ciprofloxacin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR5for code descriptions).
Sulfathiazole	Byte	Code representing sulfathiazole concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR9 for code descriptions).
Cephalothin	Byte	Code representing cephalothin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR3 for code descriptions).
Ampicillin	Byte	Code representing ampicillin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR3 for code descriptions).
Trimethoprim	Byte	Code representing trimethoprim concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR8 for code descriptions).
TrimSulf	Byte	Code representing trimethoprim/sulfamethoxazole concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR10 for code descriptions).
Azithromycin	Byte	Code representing azithromycin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR5 for code descriptions).
Apramycin	Byte	Code representing apramycin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR6 for code descriptions).
Erythromycin	Byte	Code representing erythromycin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR3 for code descriptions).
Streptomycin	Byte	Code representing streptomycin concentration (ug/ml) at which ecoli growth was inhibited (see luEcoliABR7 for code descriptions).

EndocrinologyPost

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Lab	Byte	Code representing lab conducting analyses (see luLab for code descriptions).
Cortisol	Single	Cortisol detected in plasma/serum from post-processing blood sample (ug/dL).
ACTH	Single	ACTH detected in plasma/serum from post-processing blood sample (pg/mL; values reported as "> 1250 pg/mL" were recorded as 1250 pg/mL).

EndocrinologyPre

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Lab	Byte	Code representing lab conducting analyses (see luLab for code descriptions).
Aldosterone	Single	Aldosterone detected in plasma/serum from pre-processing blood sample (pg/mL).
Cortisol	Single	Cortisol detected in plasma/serum from pre-processing blood sample (ug/dL).
ACTH	Single	ACTH detected in plasma from pre-processing blood sample (pg/mL; values reported as "> 1250 pg/mL" were recorded as 1250 pg/mL).
Estradiol	Single	Estradiol detected in serum from pre-processing blood sample (pg/mL).
Progesterone	Single	Progesterone detected in serum from pre-processing blood sample (ng/mL; values reported as "< 0.05 ng/mL" were recorded as 0.025 ng/mL).
Testosterone	Single	Testosterone detected in serum from pre-processing blood sample (ng/mL; values reported as "< 0.05 ng/mL" were recorded as 0.025 ng/mL).
FreeT4	Single	Free-T4 detected in plasma/serum from pre-processing blood sample (ng/dL).
TotalT4	Single	Total T4 detected in plasma/serum from pre-processing blood sample (ug/dL).
TotalT3	Single	Total T3 detected in plasma/serum from pre-processing blood sample (ng/mL).

ESR

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
StartTime	Date	Start time for erythrocyte sedimentation rate test.
EndTime	Date	End time for erythrocyte sedimentation rate test.
Results	Integer	Erythrocyte sedimentation rate test results.
Comments	Text	Comments.

GastricPH

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
pH	Single	Gastric fluid pH.
Comments	Text	Comments.

HematologyPost

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Lab	Byte	Code representing lab conducting analyses (see luLab for code descriptions).
Wbc	Single	White blood cell count in post-processing blood sample (10 X 3).
Rbc	Single	Red blood cell count in post-processing blood sample (10 X 6).
Hb	Single	Hemoglobin in post-processing blood sample (GM%).
Pcv	Integer	Packed cell volume in post-processing blood sample (%).
Hct	Single	Hematocrit in post-processing blood sample (%).
Mcv	Single	Mean corpuscular volume in post-processing blood sample (U3).
Mch	Single	Mean corpuscular hemoglobin in post-processing blood sample (UUG).
Mchc	Single	Mean corpuscular hemoglobin concentration in post-processing blood sample (%).
Rdw	Single	Relative (or red cell) distributive width in post-processing blood sample (%).
Segs	Single	Segmented neutrophils in post-processing blood sample (% for U of Miami, count (10 X 3) for Cornell).
Bands	Single	Band neutrophil in post-processing blood sample (% for U of Miami, count (10 X 3) for Cornell).
Lymphs	Single	Lymphocytes in post-processing blood sample (% for U of Miami, count (10 X 3) for Cornell).
Monos	Single	Monocytes in post-processing blood sample (% for U of Miami, count (10 X 3) for Cornell).
Eosinos	Single	Eosinophils in post-processing blood sample (% for U of Miami, count (10 X 3) for Cornell).

HematologyPost (continued)

Field Name	Type	Description
Basos	Single	Basophils in post-processing blood sample (%).
SegsRel	Single	Segmented neutrophil in post-processing blood sample (%).
SegsAbs	Double	Segmented neutrophil count in post-processing blood sample (10 X 3).
BandsRel	Single	Band neutrophil in post-processing blood sample (%).
BandsAbs	Double	Band neutrophil count in post-processing blood sample (10 X 3).
LymphsRel	Single	Lymphocytes in post-processing blood sample (%).
LymphsAbs	Double	Lymphocyte count in post-processing blood sample (10 X 3).
MonosRel	Single	Monocytes in post-processing blood sample (%).
MonosAbs	Double	Monocyte count in post-processing blood sample (10 X 3).
EosinophilsRel	Single	Eosinophils in post-processing blood sample (%).
EosinophilsAbs	Double	Eosinophil count in post-processing blood sample (10 X 3).
Nrbc	Single	Nucleated red blood cells in post-processing blood sample (/100 WBC).
Platelet	Single	Platelets in post-processing blood sample (thou/uL).
Mpv	Single	Mean platelet volume in post-processing blood sample (fL).
tpRef	Single	Total protein (measured by refractometer) in post-processing blood sample (g/dL).
RbcMorph	Text	Red blood cell morphology of post-processing blood sample.
PltAppearance	Byte	Code representing platelet appearance for post-processing blood sample (see luPltAppearance for code descriptions).
WbcMorph	Text	White blood cell morphology of post-processing blood sample.
Parasites	Byte	Code representing parasite presence in post-processing blood sample (see luParasites for code descriptions).
PlsAppearance	Byte	Code representing plasma appearance for post-processing blood sample (see luPlsAppearance for code descriptions).
SchalmFIB	Single	Schalm FIB of post-processing blood sample (mg/dL; values reported as "< 100 mg/dL" were recorded as 50 mg/dL).

HematologyPre

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Lab	Byte	Code representing lab conducting analyses (see luLab for code descriptions).
Wbc	Single	White blood cell count in post-processing blood sample (10 X 3).
Rbc	Single	Red blood cell count in post-processing blood sample (10 X 6).
Hb	Single	Hemoglobin in post-processing blood sample (GM%).
Pcv	Integer	Packed cell volume in post-processing blood sample (%).
Hct	Single	Hematocrit in post-processing blood sample (%).
Mcv	Single	Mean corpuscular volume in post-processing blood sample (U3).
Mch	Single	Mean corpuscular hemoglobin in post-processing blood sample (UUG).
Mchc	Single	Mean corpuscular hemoglobin concentration in post-processing blood sample (%).
Rdw	Single	Relative (or red cell) distributive width in post-processing blood sample (%).
Segs	Single	Segmented neutrophils in post-processing blood sample (% for U of Miami, count (10 X 3) for Cornell).
Bands	Single	Band neutrophil in post-processing blood sample (% for U of Miami, count (10 X 3) for Cornell).
Lymphs	Single	Lymphocytes in post-processing blood sample (% for U of Miami, count (10 X 3) for Cornell).
Monos	Single	Monocytes in post-processing blood sample (% for U of Miami, count (10 X 3) for Cornell).
Eosinos	Single	Eosinophils in post-processing blood sample (% for U of Miami, count (10 X 3) for Cornell).
Basos	Single	Basophils in post-processing blood sample (%).
SegsRel	Single	Segmented neutrophil in post-processing blood sample (%).
SegsAbs	Double	Segmented neutrophil count in post-processing blood sample (10 X 3).
BandsRel	Single	Band neutrophil in post-processing blood sample (%).
BandsAbs	Double	Band neutrophil count in post-processing blood sample (10 X 3).
LymphsRel	Single	Lymphocytes in post-processing blood sample (%).
LymphsAbs	Double	Lymphocyte count in post-processing blood sample (10 X 3).

HematologyPre (continued)

Field Name	Type	Description
MonosRel	Single	Monocytes in post-processing blood sample (%).
MonosAbs	Double	Monocyte count in post-processing blood sample (10 X 3).
EosinophilsRel	Single	Eosinophils in post-processing blood sample (%).
EosinophilsAbs	Double	Eosinophil count in post-processing blood sample (10 X 3).
Nrbc	Single	Nucleated red blood cells in post-processing blood sample (/100 WBC).
Platelet	Single	Platelets in post-processing blood sample (thou/uL).
Mpv	Single	Mean platelet volume in post-processing blood sample (fL).
tpRef	Single	Total protein (measured by refractometer) in post-processing blood sample (g/dL).
RbcMorph	Text	Red blood cell morphology of post-processing blood sample.
PltAppearance	Byte	Code representing platelet appearance for post-processing blood sample (see luPltAppearance for code descriptions).
WbcMorph	Text	White blood cell morphology of post-processing blood sample.
Parasites	Byte	Code representing parasite presence in post-processing blood sample (see luParasites for code descriptions).
PlsAppearance	Byte	Code representing plasma appearance for post-processing blood sample (see luPlsAppearance for code descriptions).
SchalmFIB	Single	Schalm FIB of post-processing blood sample (mg/dL; values reported as "< 100 mg/dL" were recorded as 50 mg/dL).

ImmunologyPost

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
TCellPro1	Double	Ratio of stimulated (suboptimum concentration of concanavalin A; 1.25 ug/mL culture) to unstimulated T-cell proliferation from post-processing blood sample.
TCellPro2	Double	Ratio of stimulated (optimum concentration of concanavalin A; 2.5 ug/mL culture) to unstimulated T-cell proliferation from post-processing blood sample.
TCellPro3	Double	Ratio of stimulated (superoptimum concentration of concanavalin A; 5 ug/mL culture) to unstimulated T-cell proliferatio from post-processing blood sample.
BCellPro1	Double	Ratio of stimulated (suboptimum concentration of lipopolysacchardie; 60 ug/mL culture) to unstimulated B-cell proliferation from post-processing blood sample.
BCellPro2	Double	Ratio of stimulated (optimum concentration of lipopolysacchardie; 120 ug/mL culture) to unstimulated B-cell proliferation from post-processing blood sample.
NKlysis1	Double	Natural KillerCell lysis expressed as concentration difference of effector to target cells in dilution of 1 effector:100 leukocyte cells from post-processing blood sample.
NKlysis2	Double	Natural KillerCell lysis expressed as concentration difference of effector to target cells in dilution of 1 effector:50 leukocyte cells from post-processing blood sample.
NKlysis3	Double	Natural KillerCell lysis expressed as concentration difference of effector to target cells in dilution of 1 effector:25 leukocyte cells from post-processing blood sample.
NKlysis4	Double	Natural KillerCell lysis expressed as concentration difference of effector to target cells in dilution of 1 effector:12.5 leukocyte cells from post-processing blood sample.
Lysozyme	Double	Concentration of lysozyme (read at 5 min) in serum from post-processing blood sample (ug/uL).
rBurst1	Single	Measurement of superoxide production in serum from post-processing blood sample using calcium ionophore as a stimulant (SI).
rBurst2	Single	Measurement of superoxide production in serum from post-processing blood sample using phorboldibutyrate as a stimulant (SI).
Comments1	Text	Comments pertaining to TCell, BCell, NKlysis, Lysozome,and rBurst analyses.
MHCII+	Double	Number of cells positive for MHCII+ surface proteins in serum from post-processing blood sample.
CD2	Double	Number of T-cells positive for CD2 surface proteins in serum from post-processing blood sample.
CD21	Double	Number of B-cells positive for CD21 surface proteins in serum from post-processing blood sample.
CD19	Double	Number of B-cells positive for CD19 surface proteins in serum from post-processing blood sample.
CD4	Double	Number of T-cells positive for CD4 surface proteins in serum from post-processing blood sample.
Granulocytes	Double	Percent of phagocytosis by all phagocytic cell types in serum from post-processing blood sample.
Monocytes	Double	Percent of phagocytosis by macrophages in serum from post-processing blood sample.
Comments3	Text	Comments pertaining to MHCII+, CD, and phagocytosis analyses.

ImmunologyPre

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
TCellPro1	Double	Ratio of stimulated (suboptimum concentration of concanavalin A; 1.25 ug/mL culture) to unstimulated T-cell proliferation from pre-processing blood sample.
TCellPro2	Double	Ratio of stimulated (optimum concentration of concanavalin A; 2.5 ug/mL culture) to unstimulated T-cell proliferation from pre-processing blood sample.
TCellPro3	Double	Ratio of stimulated (superoptimum concentration of concanavalin A; 5 ug/mL culture) to unstimulated T-cell proliferatio from pre-processing blood sample.
BCellPro1	Double	Ratio of stimulated (suboptimum concentration of lipopolysacchardie; 60 ug/mL culture) to unstimulated B-cell proliferation from pre-processing blood sample.
BCellPro2	Double	Ratio of stimulated (optimum concentration of lipopolysacchardie; 120 ug/mL culture) to unstimulated B-cell proliferation from pre-processing blood sample.
NKlysis1	Double	Natural KillerCell lysis expressed as concentration difference of effector to target cells in dilution of 1 effector:100 leukocyte cells from pre-processing blood sample.
NKlysis2	Double	Natural KillerCell lysis expressed as concentration difference of effector to target cells in dilution of 1 effector:50 leukocyte cells from pre-processing blood sample.
NKlysis3	Double	Natural KillerCell lysis expressed as concentration difference of effector to target cells in dilution of 1 effector:25 leukocyte cells from pre-processing blood sample.
NKlysis4	Double	Natural KillerCell lysis expressed as concentration difference of effector to target cells in dilution of 1 effector:12.5 leukocyte cells from pre-processing blood sample.
Lysozyme	Double	Concentration of lysozyme (read at 5 min) in serum from pre-processing blood sample (ug/uL).
rBurst1	Single	Measurement of superoxide production in serum from pre-processing blood sample using calcium ionophore as a stimulant (SI).
rBurst2	Single	Measurement of superoxide production in serum from pre-processing blood sample using phorboldibutyrate as a stimulant (SI).
Comments1	Text	Comments pertaining to TCell, BCell, NKlysis, Lysozome,and rBurst analyses.
IgG1	Double	Concentration of IgG1 antibodies in serum from pre-processing blood sample (mg/mL).
CRP	Double	Concentration of C-reactive protein globulins in serum from pre-processing blood sample (ug/mL).
antiMmar	Double	Relative antibody activity per 100 ul of Mycobacteria marinum in serum from pre-processing blood sample (U/uL).
antiErhu	Double	Relative antibody activity per 100 ul of Erysipelothrix rhusiopathiae in serum from pre-processing blood sample (U/uL).
antiVchol	Double	Relative antibody activity per 100 ul of Vibrio cholerae in serum from pre-processing blood sample (U/uL).
antiEcoli	Double	Relative antibody activity per 100 ul of Ecoli OH-157 in serum from pre-processing blood sample (U/uL).
antiVpar	Double	Relative antibody activity per 100 ul of Vibrio parahemolyticus in serum from pre-processing blood sample (U/uL).
antiVcar	Double	Relative antibody activity per 100 ul of Vibrio carchariae in serum from pre-processing blood sample (U/uL).
antiVvul	Double	Relative antibody activity per 100 ul of Vibrio vulnificus in serum from pre-processing blood sample (U/uL).
Comments2	Text	Comments pertaining to IgG1, CRP, and antibody analyses.
MHCII+	Double	Number of cells positive for MHCII+ surface proteins in serum from pre-processing blood sample.
CD2	Double	Number of T-cells positive for CD2 surface proteins in serum from pre-processing blood sample.
CD21	Double	Number of B-cells positive for CD21 surface proteins in serum from pre-processing blood sample.
CD19	Double	Number of B-cells positive for CD19 surface proteins in serum from pre-processing blood sample.
CD4	Double	Number of T-cells positive for CD4 surface proteins in serum from pre-processing blood sample.
Granulocytes	Double	Percent of phagocytosis by all phagocytic cell types in serum from pre-processing blood sample.
Monocytes	Double	Percent of phagocytosis by macrophages in serum from pre-processing blood sample.
Comments3	Text	Comments pertaining to MHCII+, CD, and phagocytosis analyses.

Individuals

Field Name	Type	Description
FB	Text	Freezebrand number.
Age	Single	Age.
AgeDate	Date	Date on which age was determined.
Sex	Byte	Code representing sex of dolphin (see luSex for code descriptions).

ppendix B. HERA data dictionary (continued).

IstatPost

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
RunTime	Date	ISTAT run time.
Glucose	Single	Glucose detected in post-processing blood sample (G/dL).
BUN	Single	Blood urea nitrogen detected in post-processing blood sample (mG/dL).
Sodium	Single	Sodium detected in post-processing blood sample (mmol/L).
Potassium	Single	Potassium detected in post-processing blood sample (mmol/L).
Chloride	Single	Chloride detected in post-processing blood sample (mmol/L).
CO2	Single	CO2 detected in post-processing blood sample (mmol/L).
AnionGap	Single	Anion gap ratio of post-processing blood sample (mmol/L).
Hct	Single	Hematocrit of post-processing blood sample (%PCV).
Hb	Single	Hemoglobin in post-processing blood sample (g/dL).
pH	Single	pH of blood sample taken following exam.
PCO2	Single	Partial pressure,CO2 of post-processing blood sample (mmHg).
HCO3	Single	HCO3 (bicarbonate) detected in post-processing blood sample (mmol/L).
BEecf	Single	BEecf (standard bicarbonate) detected in post-processing blood sample (mmol/L).

IstatPre

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
RunTime	Date	ISTAT run time.
Glucose	Single	Glucose detected in pre-processing blood sample (G/dL).
BUN	Single	Blood urea nitrogen detected in pre-processing blood sample (mG/dL).
Sodium	Single	Sodium detected in pre-processing blood sample (mmol/L).
Potassium	Single	Potassium detected in pre-processing blood sample (mmol/L).
Chloride	Single	Chloride detected in pre-processing blood sample (mmol/L).
CO2	Single	CO2 detected in pre-processing blood sample (mmol/L).
AnionGap	Single	Anion gap ratio of pre-processing blood sample (mmol/L).
Hct	Single	Hematocrit of pre-processing blood sample (%PCV).
Hb	Single	Hemoglobin in pre-processing blood sample (g/dL).
pH	Single	pH of blood sample taken following exam.
PCO2	Single	Partial pressure,CO2 of pre-processing blood sample (mmHg).
HCO3	Single	HCO3 (bicarbonate) detected in pre-processing blood sample (mmol/L).
BEecf	Single	BEecf (standard bicarbonate) detected in pre-processing blood sample (mmol/L).

Lipid

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Lipid	Single	Average lipid content of PBDE, PCB, and Pesticide samples (%).

mdlPBDE

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
pbde100	Single	Minimum detection limit for pbde100 analysis (ng/g wet weight).
pbde153	Single	Minimum detection limit for pbde153 analysis (ng/g wet weight).
pbde154	Single	Minimum detection limit for pbde154 analysis (ng/g wet weight).
pbde28	Single	Minimum detection limit for pbde28 analysis (ng/g wet weight).

mdlPBDE (continued)

Field Name	Type	Description
pbde47	Single	Minimum detection limit for pbde47 analysis (ng/g wet weight).
pbde99	Single	Minimum detection limit for pbde99 analysis (ng/g wet weight).

mdlPCB

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
pcb1	Single	Minimum detection limit for pcb1 analysis (ng/g wet weight).
pcb101/90	Single	Minimum detection limit for pcb101/90 analysis (ng/g wet weight).
pcb105	Single	Minimum detection limit for pcb105 analysis (ng/g wet weight).
pcb106/118/123	Single	Minimum detection limit for pcb106/118/123 analysis (ng/g wet weight).
pcb107/108	Single	Minimum detection limit for pcb107/108 analysis (ng/g wet weight).
pcb110	Single	Minimum detection limit for pcb110 analysis (ng/g wet weight).
pcb114	Single	Minimum detection limit for pcb114 analysis (ng/g wet weight).
pcb119	Single	Minimum detection limit for pcb119 analysis (ng/g wet weight).
pcb12	Single	Minimum detection limit for pcb12 analysis (ng/g wet weight).
pcb123	Single	Minimum detection limit for pcb123 analysis (ng/g wet weight).
pcb126	Single	Minimum detection limit for pcb126 analysis (ng/g wet weight).
pcb128/167	Single	Minimum detection limit for pcb128/167 analysis (ng/g wet weight).
pcb130	Single	Minimum detection limit for pcb130 analysis (ng/g wet weight).
pcb132/153/168	Single	Minimum detection limit for pcb132/153/168 analysis (ng/g wet weight).
pcb141	Single	Minimum detection limit for pcb141 analysis (ng/g wet weight).
pcb146	Single	Minimum detection limit for pcb146 analysis (ng/g wet weight).
pcb149	Single	Minimum detection limit for pcb149 analysis (ng/g wet weight).
pcb15	Single	Minimum detection limit for pcb15 analysis (ng/g wet weight).
pcb151	Single	Minimum detection limit for pcb151 analysis (ng/g wet weight).
pcb154	Single	Minimum detection limit for pcb154 analysis (ng/g wet weight).
pcb156	Single	Minimum detection limit for pcb156 analysis (ng/g wet weight).
pcb157	Single	Minimum detection limit for pcb157 analysis (ng/g wet weight).
pcb159	Single	Minimum detection limit for pcb159 analysis (ng/g wet weight).
pcb169	Single	Minimum detection limit for pcb169 analysis (ng/g wet weight).
pcb170/190	Single	Minimum detection limit for pcb170/190 analysis (ng/g wet weight).
pcb172	Single	Minimum detection limit for pcb172 analysis (ng/g wet weight).
pcb174	Single	Minimum detection limit for pcb174 analysis (ng/g wet weight).
pcb177	Single	Minimum detection limit for pcb177 analysis (ng/g wet weight).
pcb18	Single	Minimum detection limit for pcb18 analysis (ng/g wet weight).
pcb180	Single	Minimum detection limit for pcb180 analysis (ng/g wet weight).
pcb183	Single	Minimum detection limit for pcb183 analysis (ng/g wet weight).
pcb187	Single	Minimum detection limit for pcb187 analysis (ng/g wet weight).
pcb188	Single	Minimum detection limit for pcb188 analysis (ng/g wet weight).
pcb189	Single	Minimum detection limit for pcb189 analysis (ng/g wet weight).
pcb193	Single	Minimum detection limit for pcb193 analysis (ng/g wet weight).
pcb194	Single	Minimum detection limit for pcb194 analysis (ng/g wet weight).
pcb195	Single	Minimum detection limit for pcb195 analysis (ng/g wet weight).
pcb2	Single	Minimum detection limit for pcb2 analysis (ng/g wet weight).
pcb20	Single	Minimum detection limit for pcb20 analysis (ng/g wet weight).
pcb200	Single	Minimum detection limit for pcb200 analysis (ng/g wet weight).

ppendix B. HERA data dictionary (continued).

mdlPCB (continued)

Field Name	Type	Description
pcb201	Single	Minimum detection limit for pcb201 analysis (ng/g wet weight).
pcb202	Single	Minimum detection limit for pcb202 analysis (ng/g wet weight).
pcb206	Single	Minimum detection limit for pcb206 analysis (ng/g wet weight).
pcb207	Single	Minimum detection limit for pcb207 analysis (ng/g wet weight).
pcb209	Single	Minimum detection limit for pcb209 analysis (ng/g wet weight).
pcb26	Single	Minimum detection limit for pcb26 analysis (ng/g wet weight).
pcb28/31	Single	Minimum detection limit for pcb28/31 analysis (ng/g wet weight).
pcb29	Single	Minimum detection limit for pcb29 analysis (ng/g wet weight).
pcb3	Single	Minimum detection limit for pcb3 analysis (ng/g wet weight).
pcb37	Single	Minimum detection limit for pcb37 analysis (ng/g wet weight).
pcb44	Single	Minimum detection limit for pcb44 analysis (ng/g wet weight).
pcb45	Single	Minimum detection limit for pcb45 analysis (ng/g wet weight).
pcb48	Single	Minimum detection limit for pcb48 analysis (ng/g wet weight).
pcb49	Single	Minimum detection limit for pcb49 analysis (ng/g wet weight).
pcb5/8	Single	Minimum detection limit for pcb5/8 analysis (ng/g wet weight).
pcb50	Single	Minimum detection limit for pcb50 analysis (ng/g wet weight).
pcb52	Single	Minimum detection limit for pcb52 analysis (ng/g wet weight).
pcb56/60	Single	Minimum detection limit for pcb56/60 analysis (ng/g wet weight).
pcb61/74	Single	Minimum detection limit for pcb61/74 analysis (ng/g wet weight).
pcb63	Single	Minimum detection limit for pcb63 analysis (ng/g wet weight).
pcb66	Single	Minimum detection limit for pcb66 analysis (ng/g wet weight).
pcb69	Single	Minimum detection limit for pcb69 analysis (ng/g wet weight).
pcb70/76	Single	Minimum detection limit for pcb70/76 analysis (ng/g wet weight).
pcb77	Single	Minimum detection limit for pcb77 analysis (ng/g wet weight).
pcb82	Single	Minimum detection limit for pcb82 analysis (ng/g wet weight).
pcb84	Single	Minimum detection limit for pcb84 analysis (ng/g wet weight).
pcb87/115	Single	Minimum detection limit for pcb87/115 analysis (ng/g wet weight).
pcb88/95	Single	Minimum detection limit for pcb88/95 analysis (ng/g wet weight).
pcb89	Single	Minimum detection limit for pcb89 analysis (ng/g wet weight).
pcb9	Single	Minimum detection limit for pcb9 analysis (ng/g wet weight).
pcb92	Single	Minimum detection limit for pcb92 analysis (ng/g wet weight).
pcb99	Single	Minimum detection limit for pcb99 analysis (ng/g wet weight).

mdlPesticides

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
2,4'-DDD	Single	Minimum detection limit for 2,4'-DDD analysis (ng/g wet weight).
2,4'-DDE	Single	Minimum detection limit for 2,4'-DDE analysis (ng/g wet weight).
2,4'-DDT	Single	Minimum detection limit for 2,4'-DDT analysis (ng/g wet weight).
4,4'-DDD	Single	Minimum detection limit for 4,4'-DDD analysis (ng/g wet weight).
4,4'-DDE	Single	Minimum detection limit for 4,4'-DDE analysis (ng/g wet weight).
4,4'-DDT	Single	Minimum detection limit for 4,4'-DDT analysis (ng/g wet weight).
Aldrin	Single	Minimum detection limit for Aldrin analysis (ng/g wet weight).
Beta-HCH	Single	Minimum detection limit for Beta-HCH analysis (ng/g wet weight).
Cis-chlordane	Single	Minimum detection limit for Cis-chlordane analysis (ng/g wet weight).
Cis-nonachlor	Single	Minimum detection limit for Cis-nonachlor analysis (ng/g wet weight).
Dieldrin	Single	Minimum detection limit for Dieldrin analysis (ng/g wet weight).
EndosulfanI	Single	Minimum detection limit for EndosulfanI analysis (ng/g wet weight).
EndosulfanII	Single	Minimum detection limit for EndosulfanII analysis (ng/g wet weight).
EndosulfanSulfate	Single	Minimum detection limit forEndosulfan Sulfate analysis (ng/g wet weight).
Gamma-chlordane	Single	Minimum detection limit for Gamma-chlordane analysis (ng/g wet weight).
Heptachlorepoxide	Single	Minimum detection limit for Heptachlorepoxide analysis (ng/g wet weight).
Hexachlorobenzene	Single	Minimum detection limit for Hexachlorobenzene analysis (ng/g wet weight).

mdlPesticides (continued)

Field Name	Type	Description
Mirex	Single	Minimum detection limit for Mirex analysis (ng/g wet weight).
Trans-nonachlor	Single	Minimum detection limit for Trans-nonachlor analysis (ng/g wet weight).

Microbiology

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Lab	Byte	Code representing lab conducting analyses (see luLab for code descriptions).
ReportNum	Text	Report number assigned by lab.
DateReceived	Date	Date when culture was received.
DateReported	Date	Date when results were reported.
Test	Byte	Code representing type of test conducted (see luTest for code descriptions).
Source	Byte	Code representing source of culture (see luSource for code descriptions).
Growth	Byte	Code representing culture growth (see luGrowth for code descriptions).
IsolateType	Byte	Code representing type of isolate (see luIsolateType for code descriptions).
Comments	Text	Comments.

Morphometrics

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Weight	Single	Weight (lb).
Length	Single	Straight line measurement, parallel to the body, from the tip of the rostrum to the fluke notch (cm).
AxillaryGirth	Single	Circumferential measurement of the torso immediately caudal to the pectoral fins (cm).
MaximumGirth	Single	Circumferential measurement of the torso immediately cranial to the origin of the dorsal fin (cm).
RostrumToDorsal	Single	Straight line measurement, parallel to the body, from the tip of the rostrum to the distal tip of the dorsal fin (cm).
BlowholeToDorsal	Single	Straight line measurement, parallel to the body, from the center of the blowhole to the distal tip of the dorsal fin (cm).
ulToothCount	Byte	Upper left tooth count.
urToothCount	Byte	Upper right tooth count.
llToothCount	Byte	Lower left tooth count.
lrToothCount	Byte	Lower right tooth count.
FlukeWidth	Single	Straight line measurement between fluke tips (cm).
rTesticle	Single	Length of right testicle (cm).
lTesticle	Single	Length of left testicle (cm).
Comments	Text	Comments.

PBDE

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
pbde100	Single	Non-normalized result of pbde100 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pbde153	Single	Non-normalized result of pbde153 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pbde154	Single	Non-normalized result of pbde154 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pbde28	Single	Non-normalized result of pbde28 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pbde47	Single	Non-normalized result of pbde47 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pbde99	Single	Non-normalized result of pbde99 analysis (ng/g wet weight; values < MDL reported as MDL/2).

ppendix B. HERA data dictionary (continued).

PCB

ield Name	Type	Description
B	Text	Freezebrand.
)ate	Date	Capture date.
ıcb1	Single	Non-normalized result of pcb1 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb101/90	Single	Non-normalized result of pcb101/90 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb105	Single	Non-normalized result of pcb105 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb106/118/123	Single	Non-normalized result of pcb106/118/123 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb107/108	Single	Non-normalized result of pcb107/108 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb110	Single	Non-normalized result of pcb110 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb114	Single	Non-normalized result of pcb114 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb119	Single	Non-normalized result of pcb119 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb12	Single	Non-normalized result of pcb12 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb123	Single	Non-normalized result of pcb123 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb126	Single	Non-normalized result of pcb126 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb128/167	Single	Non-normalized result of pcb128/167 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb130	Single	Non-normalized result of pcb130 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb132/153/168	Single	Non-normalized result of pcb132/153/168 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb141	Single	Non-normalized result of pcb141 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb146	Single	Non-normalized result of pcb146 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb149	Single	Non-normalized result of pcb149 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb15	Single	Non-normalized result of pcb15 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb151	Single	Non-normalized result of pcb151 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb154	Single	Non-normalized result of pcb154 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb156	Single	Non-normalized result of pcb156 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb157	Single	Non-normalized result of pcb157 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb159	Single	Non-normalized result of pcb159 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb169	Single	Non-normalized result of pcb169 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb170/190	Single	Non-normalized result of pcb170/190 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb172	Single	Non-normalized result of pcb172 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb174	Single	Non-normalized result of pcb174 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb177	Single	Non-normalized result of pcb177 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb18	Single	Non-normalized result of pcb18 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb180	Single	Non-normalized result of pcb180 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb183	Single	Non-normalized result of pcb183 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb187	Single	Non-normalized result of pcb187 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb188	Single	Non-normalized result of pcb188 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb189	Single	Non-normalized result of pcb189 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb193	Single	Non-normalized result of pcb193 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb194	Single	Non-normalized result of pcb194 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb195	Single	Non-normalized result of pcb195 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb2	Single	Non-normalized result of pcb2 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb20	Single	Non-normalized result of pcb20 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb200	Single	Non-normalized result of pcb200 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb201	Single	Non-normalized result of pcb201 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb202	Single	Non-normalized result of pcb202 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb206	Single	Non-normalized result of pcb206 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb207	Single	Non-normalized result of pcb207 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb209	Single	Non-normalized result of pcb209 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb26	Single	Non-normalized result of pcb26 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb28/31	Single	Non-normalized result of pcb28/31 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb29	Single	Non-normalized result of pcb29 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb3	Single	Non-normalized result of pcb3 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb37	Single	Non-normalized result of pcb37 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb44	Single	Non-normalized result of pcb44 analysis (ng/g wet weight; values < MDL reported as MDL/2).
ıcb45	Single	Non-normalized result of pcb45 analysis (ng/g wet weight; values < MDL reported as MDL/2).

.ppendix B. HERA data dictionary (continued).

PCB (continued)

Field Name	Type	Description
pcb48	Single	Non-normalized result of pcb48 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb49	Single	Non-normalized result of pcb49 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb5/8	Single	Non-normalized result of pcb5/8 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb50	Single	Non-normalized result of pcb50 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb52	Single	Non-normalized result of pcb52 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb56/60	Single	Non-normalized result of pcb56/60 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb61/74	Single	Non-normalized result of pcb61/74 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb63	Single	Non-normalized result of pcb63 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb66	Single	Non-normalized result of pcb66 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb69	Single	Non-normalized result of pcb69 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb70/76	Single	Non-normalized result of pcb70/76 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb77	Single	Non-normalized result of pcb77 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb82	Single	Non-normalized result of pcb82 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb84	Single	Non-normalized result of pcb84 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb87/115	Single	Non-normalized result of pcb87/115 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb88/95	Single	Non-normalized result of pcb88/95 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb89	Single	Non-normalized result of pcb89 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb9	Single	Non-normalized result of pcb9 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb92	Single	Non-normalized result of pcb92 analysis (ng/g wet weight; values < MDL reported as MDL/2).
pcb99	Single	Non-normalized result of pcb99 analysis (ng/g wet weight; values < MDL reported as MDL/2).

Pesticides

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
2,4'-DDD	Single	Non-normalized result of 2,4'-DDD analysis (ng/g wet weight; values < MDL reported as MDL/2).
2,4'-DDE	Single	Non-normalized result of 2,4'-DDE analysis (ng/g wet weight; values < MDL reported as MDL/2).
2,4'-DDT	Single	Non-normalized result of 2,4'-DDT analysis (ng/g wet weight; values < MDL reported as MDL/2).
4,4'-DDD	Single	Non-normalized result of 4,4'-DDD analysis (ng/g wet weight; values < MDL reported as MDL/2).
4,4'-DDE	Single	Non-normalized result of 4,4'-DDE analysis (ng/g wet weight; values < MDL reported as MDL/2).
4,4'-DDT	Single	Non-normalized result of 4,4'-DDT analysis (ng/g wet weight; values < MDL reported as MDL/2).
Aldrin	Single	Non-normalized result of Aldrin analysis (ng/g wet weight; values < MDL reported as MDL/2).
Beta-HCH	Single	Non-normalized result of Beta-HCH analysis (ng/g wet weight; values < MDL reported as MDL/2).
Cis-chlordane	Single	Non-normalized result of Cis-chlordane analysis (ng/g wet weight; values < MDL reported as MDL/2).
Cis-nonachlor	Single	Non-normalized result of Cis-nonachlor analysis (ng/g wet weight; values < MDL reported as MDL/2).
Dieldrin	Single	Non-normalized result of Dieldrin analysis (ng/g wet weight; values < MDL reported as MDL/2).
EndosulfanI	Single	Non-normalized result of EndosulfanI analysis (ng/g wet weight; values < MDL reported as MDL/2).
EndosulfanII	Single	Non-normalized result of EndosulfanII analysis (ng/g wet weight; values < MDL reported as MDL/2).
EndosulfanSulfate	Single	Non-normalized result of EndosulfanSulfate analysis (ng/g wet weight; values < MDL reported as MDL/2).
Gamma-chlordane	Single	Non-normalized result of Gamma-chlordane analysis (ng/g wet weight; values < MDL reported as MDL/2).
Heptachlorepoxide	Single	Non-normalized result of Heptachlorepoxide analysis (ng/g wet weight; values < MDL reported as MDL/2).
Hexachlorobenzene	Single	Non-normalized result of Hexachlorobenzene analysis (ng/g wet weight; values < MDL reported as MDL/2).
Mirex	Single	Non-normalized result of Mirex analysis (ng/g wet weight; values < MDL reported as MDL/2).
Trans-nonachlor	Single	Non-normalized result of Trans-nonachlor analysis (ng/g wet weight; values < MDL reported as MDL/2).

PFC

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
PFHxS	Single	Wet weight of perfluorohexane sulfonate (ng/g; 6 C molecule).
PFOS	Single	Wet weight of perfluorooctane sulfonate (ng/g; 8 C molecule).
PFOA	Single	Wet weight of perfluorooctanoate (ng/g; 8 C molecule).

ppendix B. HERA data dictionary (continued).

PFC (continued)

ield Name	Type	Description
FNA	Single	Wet weight of perfluorononanoic acid (ng/g; 9 C molecule).
FDA	Single	Wet weight of perfluorodecanoic acid (ng/g; 10 C molecule).
FUA	Single	Wet weight of perfluoroundecanoate (ng/g; 11 C molecule).
FDoA	Single	Wet weight of perfluorododecanoate (ng/g; 12 C molecule; values below detection limit of 1 ng/g were recorded as 0.5)
FOSA	Single	Wet weight of heptadecafluorooctane sulfonamide (ng/g; can be degradated in acids).

PhysicalExam

ield Name	Type	Description
B	Text	Freezebrand.
ate	Date	Capture date.
eterinarian	Byte	Code representing attending vet for physical exam (see luVet for code descriptions).
ssessment	Text	Subjective assessment of attitude on processing boat (e.g. ocular response, arching, breath holding, jaw tone, vocalizations, etc.).
odyCond	Byte	Code representing body condition index (see luBodyCondition for code descriptions).
nFatPad	Byte	Code representing post-nuchal fat pad index (see luPostNuchal for code descriptions).
ralCavity	Byte	Code representing normalcy of oral cavity (see luNormal for code descriptions).
ralCavityNotes	Text	Notes pertaining to abnormal oral cavity.
ucMembColor	Text	Color of mucus membrane.
ucMembCRT	Text	Color return time of mucus membrane (seconds).
yes	Byte	Code representing normalcy of eyes (see luNormal for code descriptions).
yesNotes	Text	Notes pertaining to condition of eyes.
ardio	Byte	Code representing normalcy of cardiovascular activity (see luNormal for code descriptions).
ardioRatePre	Integer	Heart rate (/min) pre-respiration.
ardioRatePost	Integer	Heart rate (/min) post-respiration.
ardioRhythm	Byte	Code representing rhythm of cardiovascular activity (see luCardioRhythm for code descriptions).
ardioNotes	Text	Notes describing abnormality of cardiovascular activity.
esp	Byte	Code representing normalcy of respiratory activity (see luNormal for code descriptions).
espAbnormality	Byte	Code representing respiratory abnormality (see luRespAbnormality for code descriptions).
lowOdor	Byte	Code representing presence of blow odor (see luYesNo for code descriptions).
ucus	Byte	Code representing presence/severity of mucus (see luMucus for code descriptions).
espNotes	Text	Notes describing abnormality of respiratory system/activity.
ITract	Byte	Code representing normalcy of gastrointestinal tract (see luNormal for code descriptions).
utSounds	Byte	Code representing presence/absence of gut sounds (see luYesNo for code descriptions).
astricFluid	Byte	Code representing normalcy of gastric fluid (see luNormal for code descriptions).
eces	Byte	Code representing normalcy of feces (see luNormal for code descriptions).
ecesNotes	Text	Notes describing abnormality of feces (i.e. texture, color, odor, etc.).
enitalSlit	Byte	Code representing normalcy of genital slits (see luNormal for code descriptions).
aginaOrPenis	Byte	Code representing normalcy of vagina or penis (see luNormal for code descriptions).
ightMammary	Byte	Code representing normalcy of right mammary (see luNormal for code descriptions).
eftMammary	Byte	Code representing normalcy of left mammary (see luNormal for code descriptions).
epNotes	Text	Notes describing abnormality of reproductive system/organs.

Serology

ield Name	Type	Description
B	Text	Freezebrand.
ate	Date	Capture date.
mv1	Byte	Code representing result of dolphin morbillivirus test conducted by Oklahoma lab (see luSerologyResult for code descriptions).
mv1T	Byte	Code representing titer dilution pertaining to dolphin morbillivirus test conducted by Oklahoma lab (see luTiterMorbOK for code descriptions).

Serology (continued)

Field Name	Type	Description
dmv2	Byte	Code representing result of dolphin morbillivirus test conducted by Miami lab (see luSerologyResult for code descriptions).
dmv2T	Byte	Code representing titer dilution pertaining to result of dolphin morbillivirus test conducted by Miami lab (see luTiterMorbMiami for code descriptions).
pmv	Byte	Code representing result of phocine morbillivirus test conducted by Oklahoma lab (see luSerologyResult for code descriptions).
pmvT	Byte	Code representing titer dilution pertaining to result of phocine morbillivirus test conducted by Oklahoma lab (see luTiterMorbOK for code descriptions).
brucellaRiv	Byte	Code representing result of brucella abortus RIV test conducted by NVSL (see luSerologyResult for code descriptions).
brucellaRivT	Byte	Code representing titer dilution pertaining to brucella abortus RIV test result (see luTiterBrucNVSL for code descriptions).
brucellaCard	Byte	Code representing result of brucella abortus card test conducted by NVSL (see luSerologyResult for code descriptions).
lcanicola	Byte	Code representing result of leptospira canicola test (see luSerologyResult for code descriptions).
lcanicolaT	Byte	Code representing titer dilution pertaining to leptospira canicola test result (see luTiterLeptoNVSL for code descriptions).
lgrippo	Byte	Code representing result of leptospira grippo test (see luSerologyResult for code descriptions).
lgrippoT	Long Integer	Code representing titer dilution pertaining to leptospira grippo test result (see luTiterLeptoNVSL for code descriptions).
lhardjo	Byte	Code representing result of leptospira hardjo test (see luSerologyResult for code descriptions).
lhardjoT	Long Integer	Code representing titer dilution pertaining to leptospira hardjo test result (see luTiterLeptoNVSL for code descriptions).
lictero	Byte	Code representing result of leptospira ictero test (see luSerologyResult for code descriptions).
licteroT	Long Integer	Code representing titer dilution pertaining to leptospira ictero test result (see luTiterLeptoNVSL for code descriptions).
lpomona	Byte	Code representing result of leptospira pomona test (see luSerologyResult for code descriptions).
lpomonaT	Long Integer	Code representing titer dilution pertaining to leptospira pomona test result (see luTiterLeptoNVSL for code descriptions).
lbratislava	Byte	Code representing result of leptospira bratislava test (see luSerologyResult for code descriptions).
lbratislavaT	Long Integer	Code representing titer dilution pertaining to leptospira bratislava test result (see luTiterLeptoNVSL for code descriptions).
vee	Byte	Code representing result of venezuelan equine encephalomyelitis test (see luSerologyResult for code descriptions).
veeT	Byte	Code representing titer dilution pertaining to venezuelan equine encephalomyelitis test result (see luTiterEENVSL for code descriptions).
eee	Byte	Code representing result of eastern equine encephalomyelitis test (see luSerologyResult for code descriptions).
eeeT	Byte	Code representing titer dilution pertaining to eastern equine encephalomyelitis test result (see luTiterEENVSL for code descriptions).
wee	Byte	Code representing result of western equine encephalomyelitis test (see luSerologyResult for code descriptions).
weeT	Byte	Code representing titer dilution pertaining to western equine encephalomyelitis test result (see luTiterEENVSL for code descriptions).
wnile	Byte	Code representing result of west nile virus test (see luSerologyResult for code descriptions).
wnileT	Byte	Code representing titer dilution pertaining to west nile test result (see luTiterEENVSL for code descriptions).
toxo1	Byte	Code representing result of toxoplasma test conducted by USDA lab (see luSerologyResult for code descriptions).
toxo1T	Byte	Code representing highest titer dilution pertaining to result of toxoplasma test conducted by USDA lab (see luTiterToxoUSDA for code descriptions).
toxo2	Byte	Code representing result of toxoplasma test conducted by Miami lab (see luSerologyResult for code descriptions).
toxo2T	Byte	Code representing titer dilution pertaining to result of toxoplasma test conducted by Miami lab (see luTiterToxoMiami for code descriptions).
crypto1	Byte	Code representing result of cryptosporidium PCR test conducted by USDA lab (see luSerologyResult for code descriptions).

Serology (continued)

ield Name	Type	Description
'ypto2	Byte	Code representing result of cryptosporidium test conducted by Miami lab (see luSerologyResult for code descriptions).
'ypto2T	Integer	Code representing titer dilution pertaining to cryptosporidium test conducted by Miami lab (see luTiterCryptoMiami for code descriptions).
iicro	Byte	Code representing result of microsporium PCR test conducted by USDA lab (see luSerologyResult for code descriptions).
lasto	Byte	Code representing result of blasto RID test conducted by Miami lab (see luSerologyResult for code descriptions).
occi	Byte	Code representing result of coccidiodes RID test conducted by Miami lab (see luSerologyResult for code descriptions).
sperRID	Byte	Code representing result of aspergillus radial immunodiffusion test conducted by Miami lab (see luSerologyResult for code descriptions).
sperAg	Single	Aspergillus antigen reported as index of sample reactivity over a negative control (>1.4 considered positive in other species).
hlam	Byte	Code representing result of chlamydia test conducted by Miami lab (see luSerologyResult for code descriptions).
hlamT	Byte	Code representing titer dilution pertaining to chlamydia result (see luTiterChlamMiami for code descriptions).
:omments	Text	Comments.

SetInfo

:ield Name	Type	Description
)ate	Date	Set date.
;et	Long Integer	Set number.
;urvey	Long Integer	Survey number.
;ighting	Byte	Sighting number.
Jet0utTime	Date	Time net was set.
.atitude	Double	Latitude of set location.
.ongitude	Double	Longitude of set location.
/erbalLocation	Text	Verbal description of set location.
Vater Temperature	Single	Surface water temperature (C).
;alinity	Single	Surface water salinity (ppt).
Vater DepthMin	Single	Minimum water depth around net compass (ft).
Vater DepthMax	Single	Maximum water depth around net compass (ft).
Jum Encircled	Long Integer	Total number of dolphins encircled.
Jum Escapes	Long Integer	Total number of dolphins that escaped.
Jum SetFree	Long Integer	Total number of dolphins intentionally set free and not processed.
Jum Calves	Long Integer	Total number of calves encircled.
:omments	Text	Comments.

SkinAssessment

:ield Name	Type	Description
:B	Text	Freezebrand.
)ate	Date	Capture date.
Assessment	Byte	Code representing general assessment of skin (see luSkinAssessment for code descriptions).
:piSloughing	Byte	Code representing characterization of epidermal sloughing (see luEpidermalSloughing for code descriptions).
Rake Marks	Byte	Code representing the prevalence of rake marks (see luRakeMarks for code descriptions).
GDLT	Byte	Code representing the prevalence of grossly distinct lesion types (see luGDLT for code descriptions).

SkinLesion

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
LesionNum	Byte	Lesion description number.
Distribution	Byte	Code representing lesion distribution.
Color	Byte	Code representing lesion color.
ColorNotes	Text	Textual description of colors other than provided options.
Shape	Byte	Code representing lesion shape.
ShapeNotes	Text	Textual description of shape other than provided options.
Size	Byte	Code representing lesion size.
Consistency	Byte	Code representing lesion consistency.
ConsistencyNotes	Text	Textual description of consistency other than provided options.
Status	Byte	Code representing lesion status.
CrossDiagnosis	Byte	Code representing cross diagnosis.
cdTraumatic	Byte	Code representing traumatic cross diagnosis.
cdInfectious	Byte	Code representing infectious cross diagnosis.
cdOther	Byte	Code representing other cross diagnosis.
BiopsyTaken	Byte	Code representing type of biopsy taken.
HistDiagnosis	Byte	Code representing histopathologic diagnosis.
HistNotes	Text	Textual description of histopathologic diagnosis other than provided options.

Urinanalysis

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
SpecificGravity	Byte	Code representing specific gravity detected in urine (see luSpecificGravity for code descriptions).
pH	Byte	Code representing pH detected in urine (see lupHurine for code descriptions).
Leukocytes	Byte	Code representing amount of leukocytes detected in urine (see luLeukocytes for code descriptions).
Nitrite	Byte	Code representing amount of nitrite detected in urine (see luNitrite for code descriptions).
Protein	Byte	Code representing amount of protein (mg/dL) detected in urine (see luProtein for code descriptions).
Glucose	Byte	Code representing amount of glucose (mg/dL & g/dL%) detected in urine (see luGlucose for code descriptions).
Ketone	Byte	Code representing amount of ketone (mg/dL) detected in urine (see luKetone for code descriptions).
Urobilinogen	Byte	Code representing amount of urobilinogen (mg/dL) detected in urine (see luUrobilinogen for code descriptions).
Billirubin	Byte	Code representing amount of billirubin detected in urine (see luBillirubin for code descriptions).
Blood	Byte	Code representing amount of blood (Ery/uL in 2004) detected in urine (see luBlood for code descriptions).
Hemoglobin	Byte	Code representing amount of hemoglobin (Ery/uL) detected in urine (see luHemoglobin for code descriptions).
Notes	Text	Notes.

UrineEndocrinology

Field Name	Type	Description
FB	Text	Freezebrand.
Date	Date	Capture date.
Lab	Byte	Code representing lab conducting analyses (see luLab for code descriptions).
Creatinine	Single	Creatinine in urine sample (mg/dL).
Cortisol	Single	Cortisol to creatinine ratio in urine sample (ug cortisol/g creat; values reported as "< 1.2 ug/g" were recorded as 0.6 ug/g).

ppendix B. HERA data dictionary (continued).

Vitamins

ield Name	Type	Description
FB	Text	Freezebrand.
)ate	Date	Capture date.
.ab	Byte	Code representing lab conducting analyses (see luLab for code descriptions).
\lphaTocopherol	Single	Alpha tocopherol detected in plasma from pre-processing blood sample (mg/L).
Gammatocopherol	Single	Gamma tocopherol detected in plasma from pre-processing blood sample (mg/L; values reported as "none detected" were recorded as 0 mg/L).
Retinol	Single	Retinol detected in plasma from pre-processing blood sample (mg/L; values reported as "< 0.06 mg/L" were recorded as 0.03 mg/L).
RetinylPalmitate	Single	Retinyl palmitate detected in plasma from pre-processing blood sample (mg/L).
/itaminC	Single	Vitamin C detected in plasma from pre-processing blood sample (mg/L).

Appendix C. HERA database lookup table code descriptions.

LookUp Table	Code	Description
luABResistance		
	0	Not Sensitive
	1	Sensitive
	2	Intermediate Susceptibility
	3	Moderately Sensitive
luAlive		
	0	Yes
	1	No
	2	Presumed Dead
luBillirubin		
	0	neg
	1	+ small
	2	++ moderate
	3	+++ large
luBlood		
	0	neg/neg
	1	trace/NA
	2	+ small/about 5-10
	3	++ moderate/about 50
	4	+++ large/about 250
luBodyCondition		
	1	Emaciated
	2	Underweight
	3	Ideal
	4	Overweight
	5	Obese
luCardioRhythm		
	0	Regular sinus arrhythmia
	1	Abnormal arrhythmia

LookUp Table	Code	Description
luCytology		
	0	None
	1	Mild/Few
	2	Moderate/Moderate Numbers
	3	Severe/Extensive Numbers
luDetection		
	0	Negative
	1	Positive
	2	Borderline
luEcoliABR1		
	0	≤2
	1	4
	2	8
	3	16
	4	>16
luEcoliABR10		
	0	≤2/28
	1	4/76
	2	>4/76
luEcoliABR2		
	0	≤1
	1	2
	2	4
	3	8
	4	>8
luEcoliABR3		
	0	≤4
	1	8
	2	16
	3	32
	4	>32

ppendix C. HERA database lookup table code descriptions (continued).

LookUp Table	Code	Description
uEcoliABR4		
	0	≤8
	1	16
	2	32
	3	64
	4	>64
uEcoliABR5		
	0	≤1
	1	2
	2	4
	3	>4
uEcoliABR6		
	0	≤8
	1	16
	2	32
	3	>32
uEcoliABR7		
	0	≤16
	1	32
	2	64
	3	128
	4	>128
uEcoliABR8		
	0	≤2
	1	4
	2	8
	3	>8
uEcoliABR9		
	0	≤250
	1	500
	2	>500
uEpidermalSloughing		
	0	None
	1	Generalized
	2	Focal
uGDLT		
	0	0
	1	≤2
	2	3-5
	3	>5

LookUp Table	Code	Description
luGlucose		
	0	neg
	1	50
	2	100 (1/10)
	3	250 (1/4)
	4	500 (1/2)
	5	1000 (1)
	6	2000 or more (2+)
luGrowth		
	0	None
	1	Light
	2	Moderate
	3	Heavy
luHemoglobin		
	0	negative
	1	about 10
	2	about 50
	3	about 250
luIsolateType		
	0	Bacterium
	1	Fungi
luKetone		
	0	neg
	1	5 trace
	2	15 small
	3	40 moderate
	4	80 large
	5	160 large
luLab		
	0	ARUP
	1	Cornell
	2	Miami
	3	Micrim
	4	NVSL
	5	USDA
	6	None

Appendix C. HERA database lookup table code descriptions (continued).

LookUp Table	Code	Description
luLeukocytes		
	0	neg
	1	trace
	2	+ small
	3	++ moderate
	4	+++ large
luMucus		
	0	None
	1	Mild
	2	Moderate
	3	Severe
luNitrite		
	0	neg
	1	low positive
	2	high positive
luNormal		
	0	WNL
	1	Abnormal
luParasites		
	0	None seen
lupHurine		
	0	5
	1	5.5
	2	6
	3	6.5
	4	7
	5	7.5
	6	8
	7	8.5
luPlsAppearance		
	0	Normal
	1	Slight hemolysis
	2	Moderate hemolysis
	3	Marked hemolysis
luPltAppearance		
	0	Adequate
	1	Increase
	2	Adequate/clumps

LookUp Table	Code	Description
luPostNuchal		
	1	Concave
	2	Spongy
	3	Firm
	4	Convex
luProtein		
	0	neg
	1	trace
	2	+ 30
	3	++ 100
	4	+++ 300
	5	+++ 500
	6	++++ 2000 or more
luRakeMarks		
	0	0
	1	≤2
	2	3-5
	3	>5
luRespAbnormality		
	0	Rales
	1	Wheezes
	2	Forced blows
	3	Sputtering
luSerologyResult		
	0	Negative
	1	Borderline/Incomplete
	2	Weak Positive
	3	Positive
	4	Nonspecific/Contaminated
	5	Not Tested
	6	No Sample/Quantity Not Sufficient
luSex		
	0	Female
	1	Male
	2	Probable female
	3	Probable male
luSkinAssessment		
	0	Normal
	1	Abnormal
	2	Not Examined

ppendix C. HERA database lookup table code descriptions (continued).

LookUp Table	Code	Description
uSource		
	0	Blowhole
	1	Fecal
	2	Gastric
uSpecificGravity		
	0	1
	1	1.005
	2	1.01
	3	1.015
	4	1.02
	5	1.025
	6	1.03
luTest		
	0	C/F
	1	C/S
luTiterBrucNVSL		
	0	1:25
	1	1:50
	2	1:100
	3	1:200
luTiterChlamMiami		
	0	1:5
	1	1:25
	2	1:50
	3	1:100
luTiterCryptoMiami		
	0	1:4
	1	1:8
luTiterEENVSL		
	0	1:10
	1	1:20
	2	1:40
luTiterLeptoNVSL		
	0	1:100
	1	1:200
	2	1:400
	3	1:800

LookUp Table	Code	Description
luTiterMorbMiami		
	0	1:10
	1	1:50
luTiterMorbOK		
	0	1:4
	1	1:6
	2	1:8
	3	1:16
	4	1:32
	5	1:64
	6	1:128
	7	1:256
luTiterToxoMiami		
	0	1:64
	1	1:128
	2	1:256
	3	1:512
	4	1:1024
	5	1:2048
luTiterToxoUSDA		
	0	1:20
	1	1:40
	2	1:80
	3	1:160
luUrobilinigen		
	0	Normal
	1	0.2
	2	1
	3	2
	4	4
	5	8
	6	12
luVet		
	0	Townsend
	1	Bossart
	2	Varela
luYesNo		
	0	Yes
	1	No