

# **The Atlantic Bottlenose Dolphin Health and Environmental Risk Assessment Project from the Southeastern USA: 2003-2018**

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# **Health and Environmental Risk Assessment (HERA) Project for Atlantic bottlenose dolphins..a sentinel species model**

- A multidisciplinary and multi-institutional study of individual and population health
- 360 dolphins captured, given comprehensive health examinations and released in Indian River Lagoon (IRL), FL and Charleston (CHS) , SC, 2003-2015
- 95 peer-reviewed publications from 2003-2018
- Recent HERA review publications:

Bossart GD, Fair PA, Schaefer AM, Reif JS. Health and Environmental Risk Assessment Project for bottlenose dolphins *Tursiops truncatus* from the southeastern USA. I. Infectious diseases. *Diseases of Aquatic Organisms*. 125: 141-153, 2017.

Reif JS, Schaefer AM, Bossart GD, Fair PA. Health and Environmental Risk Assessment Project for bottlenose dolphins *Tursiops truncatus* from the southeastern USA. II. Environmental Aspects. *Diseases of Aquatic Organisms*. 125:155-166, 2017.

# The Indian River Lagoon, Florida ....



Examples of Skin Disorders in the Indian River Lagoon Dolphin Population





# Health and Environmental Risk Assessment Project for Atlantic bottlenose dolphins from the Indian River Lagoon, FL...capture, exam, release, re-exam



# Cetacean morbillivirus (CMV) infection in the Indian River Lagoon (IRL), FL...



- **Positive fluctuating CMV titers with seroconversion** in 10% IRL dolphins in the **absence of an epizootic. New infection occurring ...**
- **No pathologic evidence of disease but evidence of immunologic dysfunction** (impairment of cell-mediated adaptive immunity, similar to the immunologic pattern reported with acute morbillivirus infection in other species)
- Data suggest that recurring morbillivirus transmission and **subclinical infections** occurring in the absence of widespread mortality in IRL dolphins with clinicoimmunopathologic disturbances that may impact dolphin and **ecosystem health**

Bossart et al. Clinicoimmunopathologic findings in Atlantic bottlenose dolphins (*Tursiops truncatus*) with positive morbillivirus titers. Diseases of Aquatic Animals 97: 103–112, 2011.

Bossart et al. Morbillivirus infection in free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus*) from the southeastern United States: Seroepidemiologic and pathologic evidence of subclinical infection. Veterinary Microbiology 143: 160-166, 2010.

# Other seroepidemiologic findings...

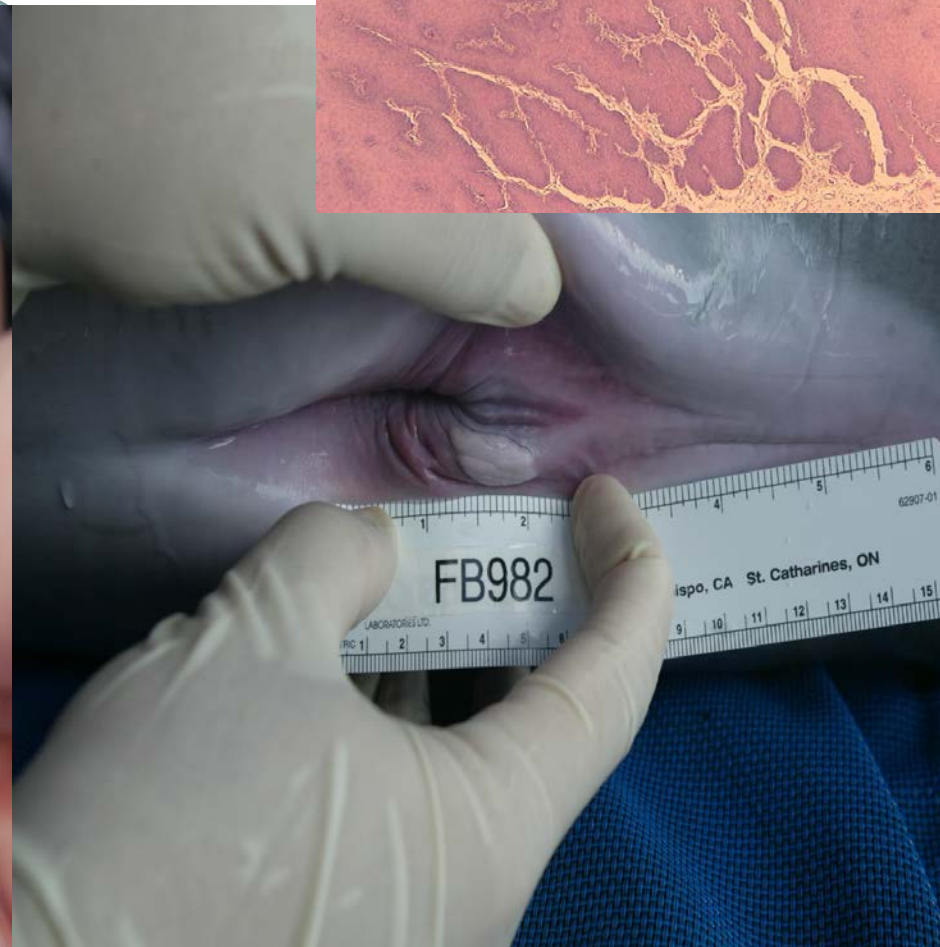
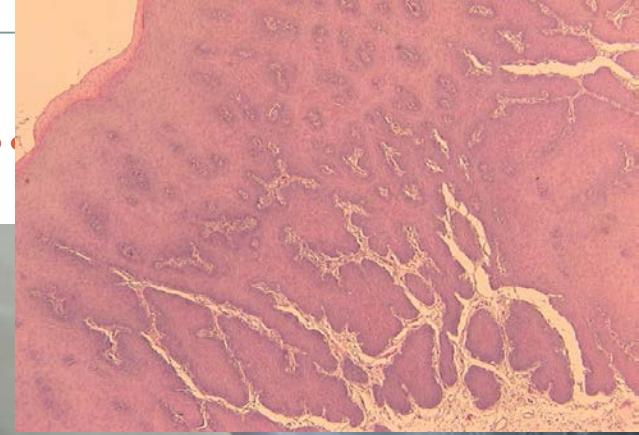


- Dolphin sera from IRL (n=122) and CHS (n = 82) were analyzed for antibodies to several pathogens
- Antibodies to **Eastern and Venezuelan encephalitis viruses and to West Nile virus** were detected only in IRL dolphins
- Positive titers to *Toxoplasma gondii* were identified in dolphins from both locations
- Serological evidence of exposure to Eastern equine encephalitis virus and West Nile virus represents **the first reports of these zoonotic pathogens in cetacean populations**

Schaefer et al. Serological evidence of exposure to selected pathogens in free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon, Florida and Charleston, South Carolina. *Aquatic Mammals* 35: 163-170, 2009.



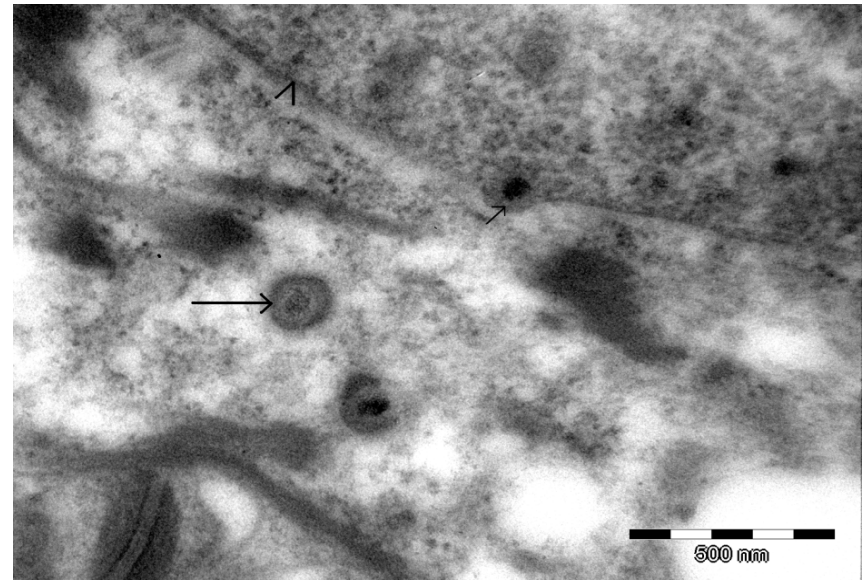
# Dolphin orogenital papillomas...



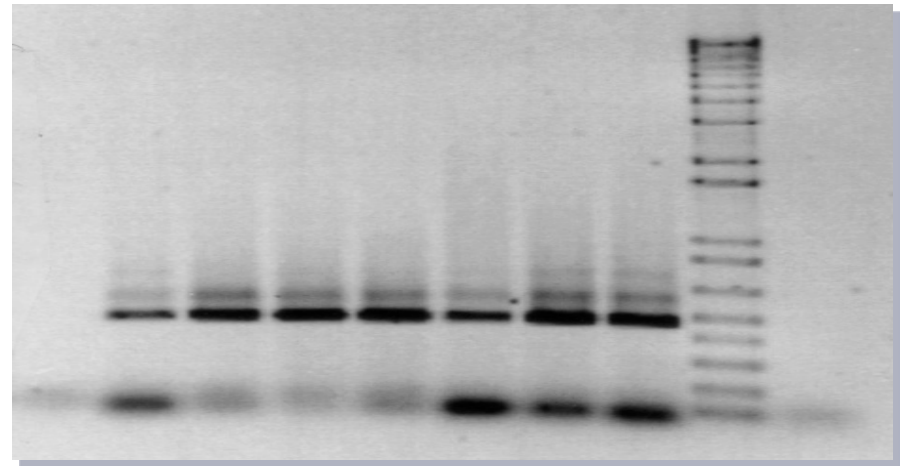
Bossart, GD et al. Hematological, biochemical and immunological findings in Atlantic bottlenose dolphins (*Tursiops truncatus*) with orogenital papillomas. *Aquatic Mammals* 34(2): 166-177, 2008.

Bossart GD, et al. Orogenital neoplasia in Atlantic bottlenose dolphins (*Tursiops truncatus*). *Aquatic Mammals* 31(4): 473-480, 2005.

# Herpesvirus-associated genital papillomas ...



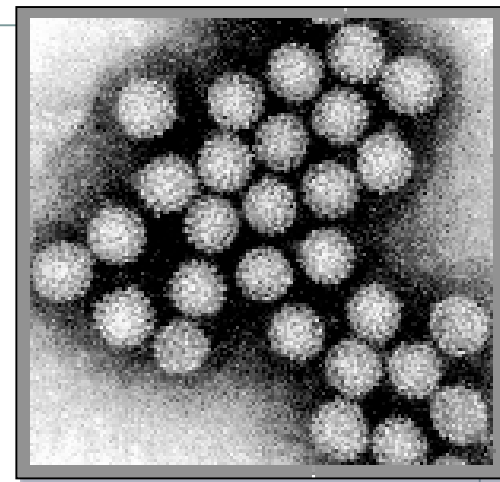
Transmission electron photomicrograph of a genital papilloma with virions suggestive of herpesvirus



PCR from a genital papilloma demonstrating a herpesvirus DNA polymerase gene part



# Viral isolation and sequencing...dolphin (TtPVs)



1) 2006: Genital lesions, isolated viral DNA , cloned and sequenced a new PV in 2006 named **TtPV-2**

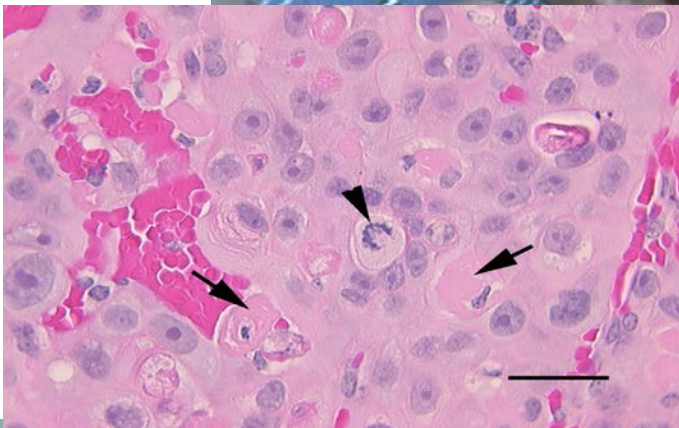
2) 2006-2013: The number of Tt papillomavirus types discovered grew to 7

3) 2014-2018: Next generation sequencing approaches and PCR from HERA dolphin samples identified 2 more novel PVs: **TtPV-8 and TtPV-9** (HERA sponsored University of Florida research; multiple investigators and publications)

Rehtanz et al. Isolation and characterization of the first American bottlenose dolphin papillomavirus: *Tursiops truncatus* papillomavirus type 2. *Journal of General Virology* 87: 3559-3565, 2006.

Rodrigues T, Subramaniam K, Cortes-Hinojosa G, Wellehan J, Ng T, Delwart E, McCulloch S, Goldstein J, Schaefer A, Fair P, Reif J, Bossart G, Waltzek T. Characterization of Potentially Oncogenic Viruses in Bottlenose Dolphin (*Tursiops truncatus*) Tumor Issues. 6<sup>th</sup> Florida Marine Mammal Health Conference. Orlando, FL. March 28-30, 2018.

# Malignant transformation...



# Summary: HERA dolphin orogenital papillomas....



- First reported oral papillomas and genital papillomas in free-ranging bottlenose dolphins from Atlantic coastal waters
- Tumors represent one or more progressive emerging diseases
- Evidence suggests that tumors are infectious (herpesvirus and PVs) most likely having an orogenital route of transmission (a STD)

Bossart et al. Hematological, biochemical and immunological findings in Atlantic bottlenose dolphins (*Tursiops truncatus*) with orogenital papillomas. *Aquatic Mammals* 34(2): 166-177, 2008.

Rehtanz et al. Papillomavirus antibody prevalence in free-ranging and captive bottlenose dolphins (*Tursiops truncatus*). *Journal of Wildlife Disease* 46: 136-145, 2010.

Rehtanz et al. Papillomaviruses and herpesviruses. Who is who in genital tumor development of free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus*). *Veterinary Microbiology* 160:297-304, 2012.



# Chlamydiosis...



- >80% seroprevalence of ***Cp. psittaci*** in IRL/CHS dolphins
- ***Cp. psittaci*** is an **avian and zoonotic pathogen**
- Serologic data indicate reservoir source are marine birds
- In dolphins, occurs in absence of clinical disease, but subclinical infection produces **immunologic perturbations** that may impact overall health
- **Potential impact on both environmental and public health**



Bossart et al. Clinicoimmunopathologic findings in Atlantic bottlenose dolphins (*Tursiops truncatus* with positive *Chlamydiaceae* antibody titers. Diseases of Aquatic Organisms 108: 71-81, 2014.

# Lobomycosis...

- Historically, cutaneous fungal disease thought to be caused by *Lacazia loboi*
- Disease reported **only** in **dolphins and humans**



Map from: Paniz-Modolfi et al.,  
Lobomycosis: an emerging  
disease in humans and  
delphinidae. *Mycoses*, 55:298-  
309, 2012.



## New HERA Data

- Cause is now determined to be due to a novel uncultivated strain of *Paracoccidioides brasiliensis*
- Disease now termed paracoccidioidomycosis ceti
- Disease associated with significant impairment in adaptive immunity (depression of both B and T cell responses)



Vilela R, et al Cutaneous granulomas in dolphins caused by novel uncultivated *Paracoccidioides brasiliensis*. *Emerging Infectious Diseases* 22(12): 2097-2103 , 2016.

Reif JS, Mazzoil M, McCulloch SD, Varela RA, Goldstein JD, Fair PA, Bossart GD. Lobomycosis in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon, Florida. *J. Am. Vet. Med. Assoc.* 228: 104-108, 2006.

Reif JS, Peden-Adams MM, Romano TA, Rice CD, Fair PA, Bossart GD. Immune dysfunction in Atlantic bottlenose dolphins (*Tursiops truncatus*) with lobomycosis. *Medical Mycology* 4: 1–11, 2008.



# Antibiotic resistance...



- **High prevalence of antibiotic resistant bacteria** ( *E. coli* and other opportunistic bacteria) in fecal, gastric and blowhole HERA dolphin samples including MRSA
- Organisms demonstrated resistance primarily to erythromycin, ampicillin, cephalothin
- Dolphins as sentinels- **potential public health risk from pathogenic antibiotic resistant microorganisms via environmental exposure** (CDC considers antibiotic resistance bacteria as one of the most critical emerging issues for human health; 2 million people are infected with antibiotic-resistant infections each year; 23,000 die )

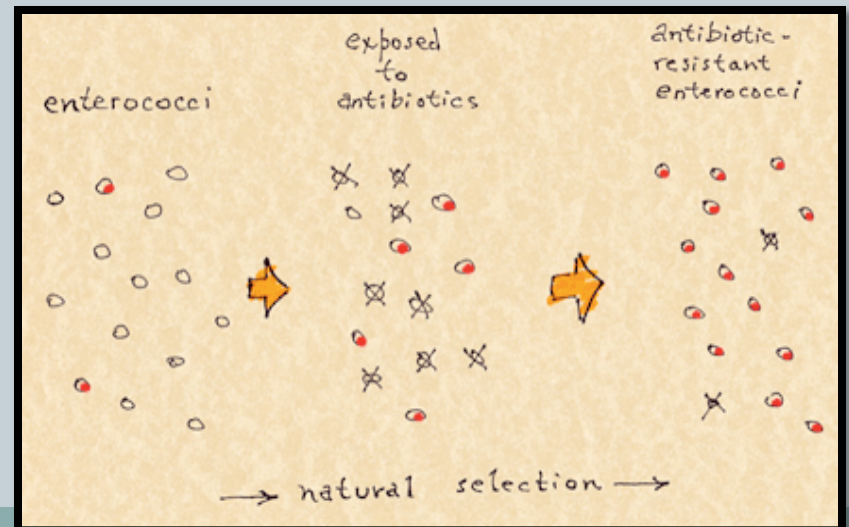
Schaefer AM, Bossart GD, Mazzoil M, Fair PA, Reif JS. Risk factors for colonization of *E. coli* in Atlantic bottlenose dolphins (*Tursiops truncatus*) in the Indian River Lagoon, Florida. Journal of Environmental and Public Health. doi:10.1155/2011/597073, 2011.

# Antibiotic resistance in the environment...



- Where are the resistant bacteria coming?
  - ✦ Direct transfer of organisms
  - ✦ Selection in the environment
- What drives selective pressure and gene exchange?
  - ✦ Unused antibiotics
  - ✦ Mercury and other metals
  - ✦ Salinity flux

Schaefer AM, Goldstein JD, Reif JS, Fair PA and Bossart GD. Antibiotic-resistant organisms cultured from Atlantic bottlenose dolphins (*Tursiops truncatus*) inhabiting estuarine waters of Charleston, SC and Indian River Lagoon, FL. Ecohealth. DOI: 10.1007/s10393-009-0221-5, 2009.



# Anthropogenic toxins...



1) **Higher** concentrations of persistent emerging contaminants such as polybrominated diphenyl ethers -in CHS dolphins - (**PBDEs**-flame retardants; impair development of nervous system and have hormone disrupting effects, particularly on estrogen and thyroid hormones)

2) Levels of PBDEs and perfluorooctanesulfonates (**PFOS**; stain repellents, etc; immunosuppressive effects and low birth weights ) represent some of the **highest** measured-in CHS dolphins-

Fair PA, Houde M, Hulseley TC, Bossart GD, Adams J, Balthis L, Muir DC. Assessment of perfluorinated compounds (PFCs) in plasma of bottlenose dolphins from two southeast US estuarine areas: Relationship with age, sex and geographic locations. *Mar Pollut Bull* 64: 66-74, 2012.

Fair P, Adams J, Mitchum G, Hulseley T, Reif J, Houde M, Muir D, Wirth E, Wetzel D, Zolman E, McFee W, and Bossart G. Contaminant blubber burdens in Atlantic bottlenose dolphins (*Tursiops truncatus*) from two southeastern US estuarine areas: Concentrations and patterns of PCBs, pesticides, PBDEs, PFCs, and PAHs. *Science of the Total Environment* 408: 1577-1597, 2010.

Fair PA, Mitchum G, Hulseley TC, Adams J, Zolman E, McFee W, Wirth E, and Bossart GD. Polybrominated diphenyl ethers (PBDEs) in blubber of free-ranging bottlenose dolphins (*Tursiops truncatus*) from two southeast Atlantic estuarine areas. *Arch Environ Contam Toxicol* 53: 483-494, 2007.

Houde M, Pacepavicius G, Darling C, Fair P, Alaei M, Bossart GD, Solomon K, Letcher R, Bergman A, Marsh G, and Muir D. Polybrominated diphenyl ethers and their hydroxylated analogs in plasma of bottlenose dolphins (*Tursiops truncatus*) from the United States east coast. *Environmental Toxicology and Chemistry* 28: 2061-2068, 2009.



# Mercury...



- **Total Hg-IRL dolphins –4-5 X higher blood and skin Hg than Charleston, SC dolphins**
- **Methyl Hg- IRL dolphins -skin- higher than beluga whales with suspected Hg-associated neoplasia (1.17 ug/g)**
- THg and MeHg levels in skin of IRL dolphins **exceeds** EPA standards by **21X (skin) for THg and 11X for MeHg**
- **Public health implications : fetus and child toxicity (neurodevelopment impairment); adult toxicity (muscle weakness, tremors, impaired speech, vision and hearing, motor skills and coordination impairment)**

Schaefer AM, Stavros HW, Bossart GD, Fair PA, Reif JS. Effects of mercury on hepatic, renal, endocrine and hematological parameters in Atlantic bottlenose dolphins (*Tursiops truncatus*) along the Eastern coast of Florida and South Carolina. Archives of Environmental Contamination. doi: 10.1007/s00244-011-9651-5, 2011.

Stavros HW, Bossart GD, Hulsey TC, Fair PA. Trace element concentrations in skin of free-ranging bottlenose dolphins (*Tursiops truncatus*) from the southeast Atlantic coast. Science of the Total Environment 388: 300-315, 2007.

Stavros HW, Bossart GD, Hulsey TC, Fair PA. Trace element concentrations in blood of free-ranging bottlenose dolphins (*Tursiops truncatus*): Influence of age, sex and location. Marine Pollution Bulletin 56: 348-379, 2008.

# Public health implications...IRL recreational fishermen



Study	Location	Population	Mean Hg
<b>Schaefer et al. 2012.</b>	<b>Indian River Lagoon, FL USA</b>	<b>Recreational anglers</b>	<b>1.5 µg/g</b>
Kosatsky T, et al. 2000.	Montreal, Canada	Consume St. Lawrence River sport fish	0.82 µg/g
Knobeloch L, et al. 2007.	Wisconsin, USA	Recreational anglers	0.86 µg/g
Lincoln RA, et al. 2011.	Gulf Coast, Louisiana	Recreational anglers	1.1 µg/g

Schaefer AM, Jenson E, Tremain D, Bossart GD, Reif JS. An Integrated Approach to the Study of Mercury in the Indian River Lagoon: Dolphins, Fish, and Humans. Annual Meeting of the International Society for Environmental Epidemiology, August 2012.

# Human/dolphin mercury study-Indian River Lagoon, FL...



## Key Findings:

- Mean recreational fisherman mercury concentration was above the recommended EPA level of **1 ppm** (highest acceptable exposure that will not cause long-term health effects)
- Mercury concentration was significantly associated with total seafood consumption (consuming seafood once a day or more were **3.71** times more likely to have a **high mercury concentration** [ $> 1\text{ppm}$ ] compared to those who consumed seafood once a week or less)
- Males were **twice** as likely to have a mercury concentration at or above 1 ppm compared to females
- First study to examine potential **shared environmental exposure** to mercury between human and dolphin populations in the same geographical region of Florida demonstrating an animal sentinel identifying a public health hazard
- **2017-2018** Study expansion to include analysis of pregnant women living in IRL coastal regions to evaluate question of possible fetal mercury toxicity...

Reif JS, Schaefer AM, Bossart GD. Atlantic bottlenose dolphins (*Tursiops truncatus*) as a sentinel for exposure to mercury in humans: closing the loop. *Veterinary Sciences* 2: 407-422, 2015.

## Summary:



1. The HERA dolphin populations are affected by complex infectious and neoplastic diseases often associated immunologic disturbances and environmental exposure to anthropogenic contaminants.
2. High concentrations of persistent organic pollutants including legacy contaminants as well as ‘emerging’ contaminants were detected in dolphins from CHS, with lower concentrations in the IRL.
3. Conversely, the concentrations of mercury in the blood and skin of IRL dolphins were among the highest reported worldwide and approximately 5 times as high as those found in CHS dolphins.



## Summary:



4. A high prevalence of resistance to antibiotics was detected in bacteria isolated from fecal, blowhole, and/or gastric samples at both sites, including methicillin resistant *Staphylococcus aureus* (MRSA) at CHS.
5. This is the first long-term study documenting the various types, progression, seroprevalence and pathologic interrelationships of infectious diseases and environmental exposures in dolphins from the southeastern USA.
6. Collectively, these studies illustrate the importance of long-term surveillance of estuarine populations of bottlenose dolphins and demonstrate that the bottlenose dolphin is a valuable sentinel animal that may reflect environmental health concerns and parallel emerging public health issues.



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