NOS Analytical Capabilities for Harmful Algal Blooms and Molecular Diagnostics for Understanding Exposure and Effects of Coastal Stressors on Marine Mammals

Frances M. Van Dolah

Marine Biotoxins Program and Marine Genomics Core Laboratory



Marine Biotoxins Program

- Multidisciplinary program established in 1992. Conducts research to reduce the impact of harmful algal blooms on human health and the coastal environment.
- Analytical Response Team established as a national resource to provide analytical support for investigating HAB related events.
 - over 200 investigations supported
 - >60% marine mammal related





 toxin analyses conducted by the Analytical Response Team have contributed significantly to the determination of algal toxins as major causes of UMEs

TOOLS & TECHNOLOGY

to improve capacity to address future HAB threats



High throughput laboratory toxin methods

- Receptor-based assays
- ELISA, Radioimmunoassays
- Directed cytotoxicity assays



Toxin field methods

- Blood collection cards
- Lateral flow test kits



In situ toxin sensor development
simultaneous monitoring of HAB species and toxins



Toxin analytical methods •Tandem LC-mass spectrometry

EVENT INITIATED RESEARCH

Investigation of biotoxins in stranded *Kogia* in North Carolina found 68% positive for domoic acid

- Retrospective analysis (1997-2008) of domoic acid exposure in pygmy/dwarf sperm whales (*Kogia spp*.) and 11 other cetacean species stranding in the southeast U.S.
 - Domoic acid detected exclusively in *Kogia spp*.
- OHHI project to identify the presence and toxicity of *Pseudo-nitzschia spp.* in south Atlantic Bight (Collaborators: SC DNR, College of Charleston)
 - Identified and confirmed toxicity of *Pseudo-nitzschia* spp. in both inshore and offshore waters
 - *Pseudo-nitzschia* spp. added to routine phytoplankton monitoring in SC



TOXIN BIOMONITORING

LC-MS identification of ciguatoxin in stranded NWHI Hawaiian monk seals



Hawaiian Monk Seal (Monachus schauinslandi)

Status: Critically Endangered with declining populations in Northwest Hawaiian Islands

P-CTX1B



 Blood biomonitoring identified high incidence of ciguatoxin exposure in apparently healthy monk seals in the western atolls of Northwest Hawaiian Islands (Collaborator: NMFS Hawaiian Monk Seal Recovery Program)



EVENT INITIATED TOOL DEVELOPMENT

Domoic acid induced mortalities and reproductive failure in California sea lions

- Project: to develop a molecular diagnostic tool to identify domoic acid exposed animals in the population (Collaborator: Marine Mammal Center)
 - modified canine microarray
 - blood samples : 33 domoic acid toxicosis, 24 leptospirosis 4 healthy
 - identified 110 genes that completely separated two disease states



Agglomerative clustering correctly classifies animals using 110 genes



Domoic acid

Leptospira

PCA shows complete discrimination of two disease states and healthy controls using SVM selected genes



Blood Transcriptomics: further development as a tool for health and exposure assessment in dolphins

 Blood transcriptomics is used clinically to identify disease state, monitor therapeutic responses, and define disease prognosis in humans



Baseline information needed for application in marine mammals:

- between-subject variation
- temporal changes within subjects

 Longitudinal study of intra- and inter-individual variation of the blood transcriptome in captive dolphins using RNA-seq (Collaborator: Dolphin Quest)



- 4 individuals fasted blood samples collected ~monthly over one year for gene expression
 - hematology and blood chemistry measurements
 - health status notes
- RNA sequencing in progress

Skin Transcriptomics: tool development for monitoring health and exposure in free ranging cetaceans

- Need for health indicators that can be collected from free ranging animals
- Blubber biopsies used for monitoring contaminant levels; molecular biomarkers show correlation with contaminants
- Since genes function in networks of coordinated pathways, the measurement of global gene expression has the potential to more precisely define the health and physiological status of cetaceans from remote biopsies.





Skin Transcriptome of Bottlenose Dolphins: season is a major driver of gene expression

- 94 dolphins from 3 bays in the northern Gulf of Mexico, in three seasons (spring, summer, winter) over two years
- bottlenose dolphin microarray



- 32.6% of all variance in gene expression profiles was associated with season
- Differentially expressed gene sets: cell proliferation, cell migration, purine metabolism, glucose transport
- seasonal differences reflect decreased metabolic rate and rate of regeneration of skin in winter



- Persistent organic pesticides (ΣPOPs) were significantly lower in blubber in winter than warmer seasons (Balmer et al, in press)
- Gene expression profiles did not correlate with blubber contaminant levels within a season



Current Investigations into Skin Transcriptomics for Monitoring Health and Exposure in Cetaceans

- RNA-seq analysis of *Tursiops* skin biopsies from N. GOM and two locations in the southeast U.S. (Charleston Harbor, ACE Basin)
 - Further characterize transcriptome variability due to location and season
 - Better document skin xenobiotic pathway responses to contaminants
 - Build database of transcriptomes for comparative analysis in future exposure events
- RNA-seq mapping of skin xenobiotic pathways using a bottlenose dolphin epidermal cell line (Collaboration: Medical Univ. of SC)
- Initiate skin and blood transcriptome analyses in Beluga Whales from Bristol Bay, Alaska (Collaboration: NIST)







Thank you!



NATIONAL CENTERS FOR COASTAL OCEAN SCIENCE NATIONAL OCEAN SERVICE

