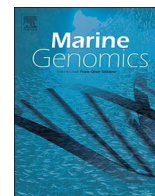




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Method paper

Skin Transcriptomes of common bottlenose dolphins (*Tursiops truncatus*) from the northern Gulf of Mexico and southeastern U.S. Atlantic coasts



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ABSTRACT

Common bottlenose dolphins serve as sentinels for the health of their coastal environments as they are susceptible to health impacts from anthropogenic inputs through both direct exposure and food web magnification. Remote biopsy samples have been widely used to reveal contaminant burdens in free-ranging bottlenose dolphins, but do not address the health consequences of this exposure. To gain insight into whether remote biopsies can also identify health impacts associated with contaminant burdens, we employed RNA sequencing (RNA-seq) to interrogate the transcriptomes of remote skin biopsies from 116 bottlenose dolphins from the northern Gulf of Mexico and southeastern U.S. Atlantic coasts. Gene expression was analyzed using principal component analysis, differential expression testing, and gene co-expression networks, and the results correlated to season, location, and contaminant burden. Season had a significant impact, with over 60% of genes differentially expressed between spring/summer and winter months. Geographic location exhibited lesser effects on the transcriptome, with 23.5% of genes differentially expressed between the northern Gulf of Mexico and the southeastern U.S. Atlantic locations. Despite a large overlap between the seasonal and geographical gene sets, the pathways altered in the observed gene expression profiles were somewhat distinct. Co-regulated gene modules and differential expression analysis both identified epidermal development and cellular architecture pathways to be expressed at lower levels in animals from the northern Gulf of Mexico. Although contaminant burdens measured were not significantly different between regions, some correlation with contaminant loads in individuals was observed among co-expressed gene modules, but these did not include classical detoxification pathways. Instead, this study identified other, possibly downstream pathways, including those involved in cellular architecture, immune response, and oxidative stress, that may prove to be contaminant responsive markers in bottlenose dolphin skin.

1. Introduction

Common bottlenose dolphins (*Tursiops truncatus*) are year-round inhabitants and top predators in waters along the southeastern U.S. Atlantic (SE) and Gulf of Mexico (GoM) coasts (Waring et al., 2015)

and, as such, are exposed to anthropogenic chemicals, particularly those that biomagnify through the food web (Aguilar et al., 1999; Wells et al., 2004). Persistent organic pollutants (POPs), such as polychlorinated biphenyl (PCB), legacy pesticides, etc. are particularly an issue because they magnify and bioaccumulate in the blubber. Remote

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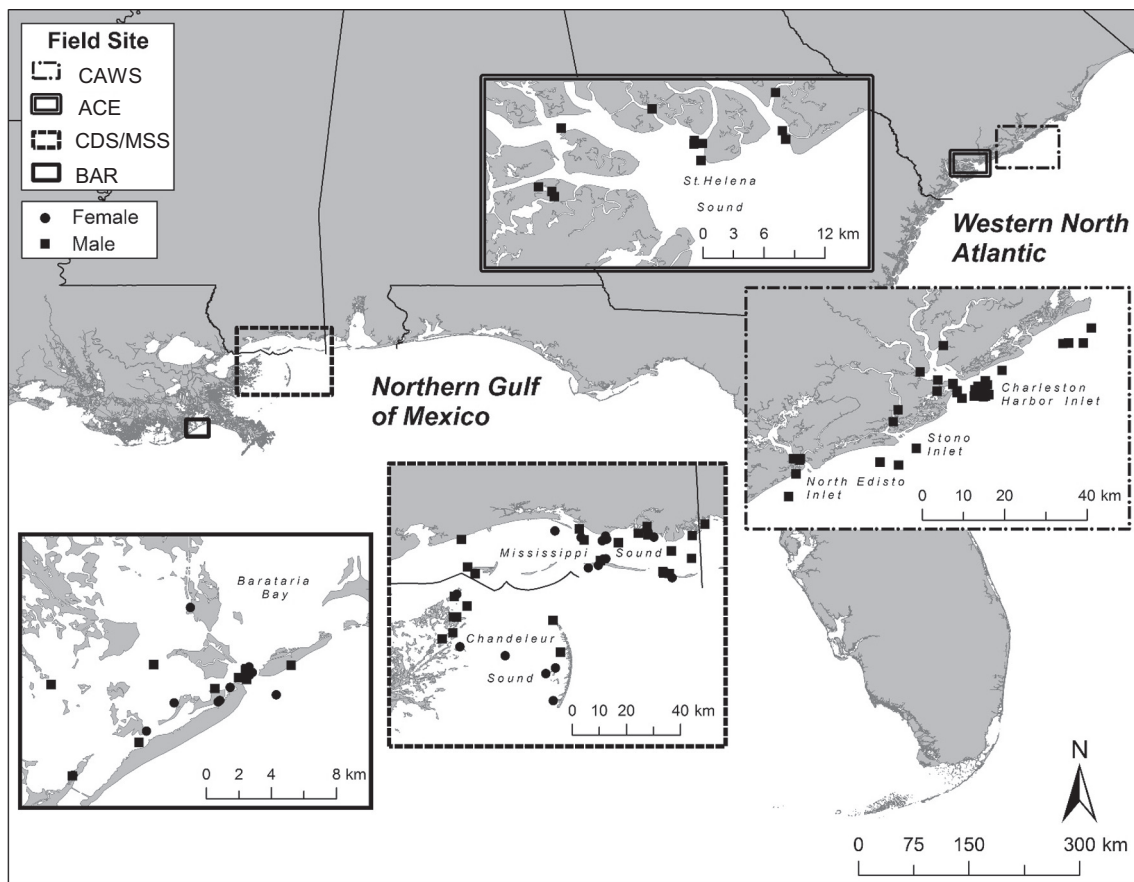


Fig. 1. Location of biopsy samples collected near Charleston, SC: the Cooper, Ashley, Wando, and Stono River Basin (CAWS) and further south in the Ashepoo, Combahee, and Edisto River Basin (ACE) in waters along the southeastern U.S. Atlantic (SE) and in the northern Gulf of Mexico: Barataria Bay (BAR), Chandeleur Sound (CDS), and Mississippi Sound (MSS).

biopsies of skin and blubber have been used extensively to obtain information on contaminant exposure in bottlenose dolphins (Kucklick et al., 2011; Balmer et al., 2015).

Because bottlenose dolphins have a lipid-rich blubber layer, this tissue compartment serves as a primary storage site for lipophilic contaminants. In males, the concentration of blubber contaminants is generally reflective of their environment (Kucklick et al., 2011), however reproductively mature females transfer much of their stored contaminant loads to their offspring through lactation, and thus the concentration and composition of blubber POPs in females may change substantially in relation to reproductive state. For this reason, POP concentrations are typically monitored in males (Yordy et al., 2010). In addition to blubber, skin is often sampled to investigate exposure of bottlenose dolphins to inorganic pollutants, such as mercury (Bryan et al., 2007; Woshner et al., 2008).

Organic or inorganic contaminant concentration in cetacean blubber and skin can help us understand levels of exposure, but these measures do not provide direct insight into the health effects of such exposures. Health assessment studies, which involve temporary capture and restraint to conduct physical and/or ultrasound examination and sampling of blood for diagnostics, have explored health endpoints in bottlenose dolphins in relation to some chemical exposures. For example, hypothyroidism, anemia, and immunosuppression were associated with high, site-specific polychlorinated biphenyl (PCB) contamination in dolphins resident to an embayment adjacent to a Superfund site in Georgia (Wells et al., 2004). Similarly, resident dolphins in Barataria Bay, Louisiana, which was heavily oiled following the explosion of the *Deepwater Horizon* (DWH) drilling platform in the Gulf of Mexico, were found to have a high prevalence of lung disease and adrenal insufficiency, both consistent with exposure to petroleum

hydrocarbons (Reif et al., 2006). However, such studies are expensive and labor-intensive to conduct, and are generally limited to dolphin stocks in shallow estuarine waters where the dolphins can be safely captured and temporarily restrained for processing. Thus, health assessment methods that can be conducted remotely (e.g. visual assessment), or via remote sampling of skin or blubber, are highly desirable.

Efforts have been made to interrogate remotely collected samples of blubber and skin for the expression of genes or proteins that demonstrate the activation of detoxification pathways or changes in expression of receptors, which may relate to altered endocrine or other adverse health effect pathways. For example, the expression of the xenobiotic detoxification enzyme cytochrome P450 1A1 (Cyp1A1) correlates with organic contaminant exposure in bottlenose dolphins (Wilson et al., 2007; Montie et al., 2008) and sperm whales (*Physeter macrocephalus*) (Godard-Coddling et al., 2011) as assessed by immunohistochemistry. Similarly, qPCR measurements of transcript abundance of selected nuclear receptors involved in xenobiotic detoxification and immune function correlate with persistent organic contaminant levels in harbor seals (*Phoca vitulina*) (Tabuchi et al., 2006) and killer whales (*Orcinus orca*) (Buckman et al., 2011). Therefore, the transcript abundance of this suite of genes has been proposed as a suitable suite of biomarkers for adverse health effects from some contaminant classes in cetaceans.

To broaden the scope of the genes and pathways interrogated in dolphin skin, we recently investigated global gene expression in skin from 94 common bottlenose dolphin remote biopsy samples using a dolphin-specific microarray (Van Dolah et al., 2015). This study using samples from dolphins in the northern Gulf of Mexico (NGoM) identified for the first time a significant seasonal influence on skin gene expression, with one-third of the genes queried being significantly

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