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Detection of cyanotoxins (microcystins/nodularins) in livers from estuarine and coastal bottlenose dolphins (*Tursiops truncatus*) from Northeast Florida

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ABSTRACT

Microcystins/Nodularins (MCs/NODs) are potent hepatotoxic cyanotoxins produced by harmful algal blooms (HABs) that occur frequently in the upper basin of the St. Johns River (SJR), Jacksonville, FL, USA. Areas downstream of bloom locations provide critical habitat for an estuarine population of bottlenose dolphins (*Tursiops truncatus*). Since 2010, approximately 30 of these dolphins have stranded and died within this impaired watershed; the cause of death was inconclusive for a majority of these individuals. For the current study, environmental exposure to MCs/NODs was investigated as a potential cause of dolphin mortality. Stranded dolphins from 2013 to 2017 were categorized into estuarine (n = 17) and coastal (n = 10) populations. Because estuarine dolphins inhabit areas with frequent or recurring cyanoblooms, they were considered as a comparatively high-risk group for cyanotoxin exposure in relation to coastal animals. All available liver samples from estuarine dolphins were tested regardless of stranding date, and samples from coastal individuals that stranded outside of the known cyanotoxin bloom season were assessed as controls. The MMPB (2-methyl-3-methoxy-4-phenylbutiric acid) technique was used to determine total (bound and free) concentrations of MCs/NODS in liver tissues. Free MCs/NODs extractions were conducted and analyzed using ELISA and LC–MS/MS on MMPB-positive samples to compare test results. MMPB testing resulted in low-level total MCs/NODs detection in some specimens. The Adda ELISA produced high test values that were not supported by concurrent LC–MS/MS analyses, indicative of false positives. Our results indicate that both estuarine and coastal dolphins are exposed to MCs/NODs, with potential toxic and immune health impacts.

1. Introduction

1.1. Background

Harmful algal blooms (HABs) are large, toxic and/or nuisance algae blooms that can negatively impact aquatic habitat and threaten human and animal health. Due to substantial human health risks associated with cyanotoxin ingestion, respiration, or skin contact (WHO, 1998), detection of these toxins above recommended guidance levels has resulted in prolonged closure of public water supplies (McCarty et al., 2016), municipal or regional emergency declarations (Executive Order, 2016), and substantial economic losses (Anderson et al., 2000). Environmental exposure to cyanotoxins can pose both acute and chronic adverse health impacts. Symptoms of exposure in mammals include dermatitis, gastroenteritis, anorexia, impaired immune function, respiratory compromise, tumor production, and death, depending on the cyanotoxin type, concentration, route, and duration of exposure (Codd et al., 1995; Codd, 2000; Dow and Swoboda, 2000; Zhou et al., 2002; Shen et al., 2003). With increased anthropogenic watershed manipulation, nutrification, and urbanization altering flow and natural filtration of waterways worldwide, humans are becoming more susceptible to adverse health effects posed by more frequent and severe cyanobacterial blooms. Moreover, warmer temperatures associated with climate change are hypothesized to increase toxic HAB events (Paerl, 2008; Davis et al., 2009; Ye et al., 2011).

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An excellent example of an ecosystem experiencing recurrent HAB events is the large, north-flowing, brackish St. Johns River in the southeastern United States (SJR, Jacksonville, FL, USA). The SJR is severely impacted by anthropogenic pollution (e.g., noise, chemical and nutrient runoff, septic system failure, and heavy metal pollution), which can accentuate existing ecological stressors (e.g., seasonal freshwater discharge, harmful algal blooms, daily pH and salinity fluctuations). In this complex river system, northward flow plus strong tidal influences prevent natural flushing and cause periodic reverse directional flow (up to 161 miles upstream), allowing for prolonged retention of pollutants and biotoxins (Environmental Protection Board, 2017).

Water sampling in the lower SJR and surrounding freshwater tributaries has documented recurrent cyanobacterial blooms throughout the year since at least 2005 (Aubel et al., 2006). Many of the cyanobacteria species that predominate during blooms (e.g. Microcystis, Anabaena/Dolichospermum, Aphanizomenon/Chrysosporum, and Cylindrospermopsis) can produce potent toxins that can adversely affect public health, and cause widespread morbidity and mortality of aquatic organisms (Environmental Protection Board, 2017). Since 2005, the St. Johns Water Management District (SJWMD) has documented cyanotoxins in all areas of the river, from low salinity tributaries, to the brackish main channel (SJWMD, unpublished). Regardless of bloom presence, the most prevalent and persistent toxins in SJR water samples are microcystins/nodularins (MCs/NODS), whose concentrations have ranged from 0.15 to >2000 $\mu g\,L^{-1}$ (ppb) (SJWMD, unpublished). MCs and NODs are classified together as they are biochemically similar, potent and environmentally persistent hepatotoxins that share the unique Adda moiety (Rinehart and Harada, 1988). The U. S. Environmental Protection Agency (EPA) has drafted a nationally recommended ambient water quality criterion for recreational exposure to MCs/NODs at 4 $ng\,mL^{-1}$ (EPA, 2016). Under these guidelines, waterbodies exceeding this limit may require swimming and recreational advisories to protect human health (EPA, 2016).

In contrast, health risks for animals continuously or periodically inhabiting the river and feeding in cyanotoxin-impaired riverine systems are less well characterized, although their MC/NODs exposure may be substantial (D'Anglada, 2017). The SJWMD did not consistently collect toxin data or track toxin prevalence following dissipation of visible bloom events. The detection of MCs/NODs throughout the year suggests that the SJR ecosystem retains cyanotoxins long after visible blooms have dissipated (SJWMD, unpublished). As a result, marine mammals utilizing this habitat could be repeatedly or chronically exposed to MCs/NODs, including levels exceeding human recreational exposure limits.

Although the precise metrics of post-bloom toxin persistence are unknown, marine mammals utilizing this habitat can serve as sentinels for the SJR ecosystem, and for associated human health risks, due to their high trophic level and ecological and physiological similarities to humans (Bossart, 2011). The SJR provides critical habitat for >300 common bottlenose dolphins (*Tursiops truncatus*), approximately half of which inhabit the river year round (Gibson, unpublished). This population has suffered two unusual mortality events (UME) since 2010 (Environmental Protection Board, 2017). The cause of the 2010 UME was not determined, but followed a significant harmful algal bloom of Aphanizomenon flos-aquae, and a mass fish die-off as cited in the State of the River Report for the St. Johns River (Environmental Protection Board, 2017). Dolphins stranding during the 2010 UME exhibited mild to severe skin lesions, and a few individuals exhibited strange swimming patterns (TtNEFL1021, TtNEFL1024, TtNEFL2027) prior to stranding (FWC-FWRI, unpublished). Skin lesions in cetaceans can be associated with environmental and anthropogenic pollution and/or prolonged freshwater exposure (Wilson et al., 1999; Fury and Reif, 2012). Exposure to bloom byproducts may be associated with skin and respiratory disease, although specific byproducts remain poorly characterized. Exposure to water-borne cyanotoxins has been associated with development of a rash and other skin ailments in humans and terrestrial animals (Torokne et al., 2001; Stewart et al., 2006a,b). Pre-existing or concurrent skin disease in dolphins might further enhance their susceptibility to freshwater cyanotoxin exposure since the animals are in direct contact with the water. Since the 2010 UME, behavioral and stranding data suggest substantial declines in population health, characterized by widespread dermatitis, emaciation, and routinely utilizing and stranding in oligohaline (0.5-5 ppt) and low mesohaline (5–18 ppt) areas of the river (Gibson, unpublished; Borkowski, personal comm.; Environmental Protection Board, 2017). Although most of these stranded estuarine dolphins had no definitive cause of death, gross necropsy findings included dermatitis, congested and hemorrhagic-appearing livers, kidneys, and lungs, and pneumonia (FWC-FWRI, unpublished data). Although these lesions are relatively non-specific, all have been associated with cyanotoxin exposure in prior studies (Codd et al., 1995; Codd, 2000; Dow and Swoboda, 2000; Zhou et al., 2002; Shen et al., 2003) Because dolphin illness and strandings overlapped temporally and spatially with confirmed cyanobacterial blooms and cyanotoxin presence in the SJR ecosystem (SJRWMD, unpublished data), there is concern that estuarine dolphin health may be declining due to chronic or intermittent exposure to HAB events and associated cyanotoxins.

The high anthropogenic activity and additional environmental stressors that characterize the SJR could enhance the susceptibility of estuarine dolphins to the adverse effects of cyanotoxin exposure both during bloom and non-bloom periods, similar to findings from Grasman (2002), who documented enhanced wildlife disease impacts in pollution-impaired ecosystems. Exposure to pollution and environmental stressors has been associated with increased frequency and severity of UME events in eastern US estuarine dolphins, indicated by a higher proportion of infectious disease, physiological stress, and exposure to algal toxins (Hohn, 2002; Van Bressem et al., 2009). Marine mammals could also be exposed to biotoxins outside of bloom periods due to toxin persistence in sediment and biota, bioaccumulation, biomagnification, and disturbance of contaminated sediment during foraging activity (Welker and Steinberg, 2000; Miller et al., 2010; Papadimitriou et al., 2012; Corbel et al., 2014; Zastepa et al., 2017). Direct and recurrent exposure to biotoxins have been associated with 50% of declared marine mammal UMEs (NOAA, 2010). The SJR dolphins are classified as a genetically unique population, so continued deaths could result in significant loss of genetic diversity (Caldwell, 2001).

Microcystins (MCs) are a class of heptapeptides, consisting of over 150 structurally-related variants, while nodularins (NODs) are pentapeptides with over ten naturally occurring variants (Namikoshi et al., 1990; Stirling and Miles, 1999; Mazur-Marzec et al., 2006; Bortoli and Volmer, 2014; Niedermeyer, 2014). Both MCs/NODs are hepatotoxins that share the unique Adda moiety ([2*S*,3*S*,8*S*,9*S*,4*E*,6*E*,]-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) (Rinehart and Harada, 1988) and are jointly classified due to lack of specificity in some analyses. For instance, the Adda-specific enzyme-linked immunosorbent assay (ELISA) (Fischer et al., 2001) and the MMPB (2-methyl-3-methoxy-4-phenylbutiric acid) technique (Foss et Aubel, 2015) detect MCs/NODs indiscriminately. MCs/NODs are actively absorbed by liver hepatocytes through a specific energy-dependent transport process involving the rifampicin-sensitive hepatic bile acid carrier (Eriksson et al., 1990; Hooser et al., 1991; Runnegar et al., 1991; Fischer et al., 2010). This process allows MCs/NODs to concentrate in hepatocytes (Yu, 1995) as free, covalently bound, and conjugated