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REVIEW

Health and Environmental Risk Assessment Project for bottlenose dolphins *Tursiops truncatus* from the southeastern USA. II. Environmental aspects

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ABSTRACT: Bottlenose dolphins *Tursiops truncatus* are the most common apex predators found in coastal and estuarine ecosystems along the southeastern coast of the USA, where these animals are exposed to multiple chemical pollutants and microbial agents. In this review, we summarize the results of investigations of environmental exposures evaluated in 360 free-ranging dolphins between 2003 and 2015. Bottlenose dolphins inhabiting the Indian River Lagoon, Florida (IRL, n = 246), and coastal waters of Charleston, South Carolina (CHS, n = 114), were captured, given comprehensive health examinations, and released as part of a multidisciplinary and multi-institutional study of individual and population health. High concentrations of persistent organic pollutants including legacy contaminants (DDT and other pesticides, polychlorinated biphenyl compounds) as well as 'emerging' contaminants (polybrominated diphenyl ethers, perfluorinated compounds) were detected in dolphins from CHS, with lower concentrations in the IRL. 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. A high prevalence of resistance to antibiotics commonly used in humans and animals was detected in bacteria isolated from fecal, blowhole, and/or gastric samples at both sites, including methicillin-resistant *Staphylococcus aureus* (MRSA) at CHS. Collectively, these studies illustrate the importance of long-term surveillance of estuarine populations of bottlenose dolphins and reaffirm their important role as sentinels for marine ecosystems and public health.

KEY WORDS: Cetacean · Bottlenose dolphins · Persistent organic pollutants · Mercury · Antibiotic resistance

INTRODUCTION

As apex predators, bottlenose dolphins *Tursiops truncatus* serve as a sentinel species for monitoring the health of the environment (Reddy et al. 2001, Wells et al. 2004, Bossart, 2011). We initiated the bottlenose dolphin Health and Environmental Risk Assessment (HERA) Project in 2003 in order to evaluate individual and population health at 2 geographic locations in the southeastern USA. We applied classical and novel methods and diagnostic tools to detect and assess anthropogenic and environmental factors

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that affect dolphin health in estuarine ecosystems (see Bossart et al. 2017 [this issue]). This review summarizes the key findings from the environmental components of our investigations between 2003 and 2015.

The Indian River Lagoon, Florida (IRL), and Charleston, South Carolina (CHS), study sites were selected in part based on their divergent environmental characteristics and the fact that both sites have long-term, on-going photo-identification studies with evidence for site fidelity (Zolman 2002, Mazzoil et al. 2008, Speakman et al. 2010). Dolphins living in these locations are likely to be affected by anthropogenic activities and may serve as indicators of coastal pollution. Therefore, it is important to determine the sources and levels of exposure to chemical pollutants and microbial agents in order to understand the potential risks they pose and to mitigate sources of exposure. These resident cetacean populations are also useful resources for evaluating the relationship between exposure to biological and chemical agents and adverse health effects (Bossart 2011). In this paper, we describe environmental exposures to chemical and microbial agents in free-ranging common bottlenose dolphins captured and released during health assessment studies conducted between 2003 and 2015.

The IRL is a shallow-water ecosystem that comprises 40% of Florida's central east coast (US EPA 1996). From an environmental perspective, the long, narrow shape and hydrological features of the IRL, specifically its shallow depth and restricted tidal flushing, lead to the retention of pollutants which enter the estuary. Dense human development along the eastern coast of Florida and intense agricultural activity have resulted in increased freshwater inputs, changes in drainage patterns, and altered water quality (i.e. chemical contamination, high nutrient input, decreased salinity, decreased sea grass habitat, and eutrophication) (Sigua et al. 2000, Scott et al. 2002).

The CHS study site is an estuarine environment that is formed by the merging of the Cooper, Ashley, and Wando Rivers that flow into the Atlantic Ocean. The environmentally salient features of the site include its proximity to industrial, urban, and residential development. The CHS ecosystem receives substantial exposure to anthropogenic contaminants. High concentrations of trace metals, polychlorinated biphenyls (PCBs), and pesticides have been found in the sediments of CHS Harbor, as well as the Ashley and Cooper Rivers (Long et al. 1998). The CHS ecosystem is surrounded by rapidly expanding urban development and population growth, supports an active seaport, and contains a former US Navy base (Long et al. 1998). Two US Environmental Protection Agency (EPA) hazardous waste designated Superfund sites contaminated with polycyclic aromatic hydrocarbons, lead, chromium, copper, arsenic, zinc, and dioxin are located on the estuary (US EPA 2007a,b). Maps of the study sites are provided in the accompanying review (Bossart et al. 2017).

PERSISTENT ORGANIC POLLUTANTS

Legacy persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and dichlorodiphenylethanes (DDTs), as well as emerging chemicals such as polybrominated diphenyl ethers (PBDEs) and perfluoroalkyl acids (PFAAs) bioaccumulate in marine food webs, resulting in high concentrations in top trophic-level marine mammal species. Marine mammals from industrialized coastal regions with dense human populations have high concentrations of POPs in their tissues (O'Shea & Tanabe 2003, Houde et al. 2005). Concern over the potential toxicity of POPs for marine mammals coincided with a series of mortality events during the late 1980s and 1990s attributed to morbillivirus. Contaminants were suggested as a contributing factor (Aguilar & Borrell 1994). The toxicological implications of PCBs, PBDEs, and PFAAs are well established in laboratory animals (ATSDR 2000, Birnbaum & Staskal 2004, DeWitt 2015) but remain uncertain in marine mammals. An important component of the HERA Project was to determine the distribution and concentration of POPs in dolphins in order to evaluate the potential risks posed by exposure to these chemicals and to mitigate sources of exposure.

The concentrations of POPs were measured in blubber biopsy samples collected from 139 wild bottlenose dolphins from both sites between 2003 and 2005 using gas chromatography/mass spectrometry (Fair et al. 2010). The use of standardized collection and analytical methodology supported a robust comparative study assessing levels of POPs stratified by age and sex. The predominant class of POPs measured was PCBs, with the sum of all PCBs (ΣPCB) comprising 64 and 72% of the contaminant burden in adult males from CHS and IRL, respectively, followed by ΣDDT (20% CHS, 17% IRL), Σchlordane (7% for both sites), and ΣPBDEs (4% CHS, 2% IRL) (Fair et al. 2010). The Σpesticide, ΣDDT, and ΣPDBE concentrations in adult male dolphins from CHS were significantly higher than those in IRL males.

The ΣPCB concentration in adult males from CHS was also higher than that in IRL males, but the difference was not statistically significant (Table 1). Adult females transfer a large proportion of their body burden of POPs to their young during gestation and lactation; an age-dependent accumulation is observed in males (Ylitalo et al. 2001, Fair et al. 2010).

PCB compounds

PCBs are mixtures of up to 209 individual chlorinated compounds which were used as coolants and lubricants in a wide range of industrial applications. Although the concentrations of PCBs are declining worldwide, PCB levels in dolphins remain elevated along the eastern coast of the USA (Kucklick et al. 2011). Our findings showed that plasma concentrations of PCBs in dolphins sampled from CHS in 2003 and 2004 were approximately twice as high as those obtained from the IRL (Houde et al. 2006b). Exposures to PCBs (and DDT) are associated with suppressed immune responses in dolphins (Lahvis et al. 1995, Schwacke et al. 2012) and have been linked to an increased risk of morbillivirus infection in seals (Hall et al. 1992).

An individual-based model to predict PCB concentrations in the bottlenose dolphin population of CHS was developed with HERA data to gain a better understanding of the bioaccumulation and health risk of dietary PCBs across the population and their prey (Hickie et al. 2013). The modeled cumulative distribution of ΣPCB estimated the dietary concentration of PCBs that would be required to reach a condition where 95% of the population would have tissue levels below the health effect threshold. We found that 88% of the CHS and IRL population with all age classes included exceeded the 17 mg kg^{-1} PCB threshold established by Kannan et al. (2000) (Fair et al. 2010). Further, the mean total PCB value in adult male dolphins, which had the highest PCB contaminant loads, exceeded this threshold by more than 5-fold. This model used a novel approach to estimating the maximum acceptable dietary concentration for PCBs in dolphins and thus provided a useful tool for bottlenose dolphin conservation and site-specific risk evaluation.

Pesticides

A wide range of organochlorine pesticides has been routinely used in the USA, including the currently banned but environmentally persistent DDT. Compounds in the DDT group were the predominant pesticides detected in dolphin blubber, particularly the major DDT metabolite, 4,4′-DDE (Fair et al. 2010). Adult CHS males had significantly higher levels of DDT compounds, chlordane, mirex, endosulfansulfate, and hexachlorobenzene than their IRL counterparts.

Polybrominated compounds

PBDEs are a class of brominated flame-retardant chemicals used widely in consumer products. Substantial levels of PBDEs were measured in blubber samples from both dolphin populations collected during 2003–2005 with concentrations 4 times higher in CHS dolphins (Table 1) (Fair et al. 2010). However, little is known about PBDE toxicity and metabolism in cetaceans. Long-term monitoring data from photo-identification surveys found positive correlations between dolphin PBDE levels and developed land use in 2 adjacent watersheds in the CHS area (Adams et al. 2014).

Perfluorinated compounds

Exposure to PFAAs, fluorine-containing chemicals used widely in industrial and commercial applications for their stain-and water-resistant properties, is

Table 1. Summary of PCBs, pesticides, and polybrominated diphenyl ethers (PBDEs) detected in blubber (geomean, range) from adult male bottlenose dolphins *Tursiops truncatus* (Age = mean age) sampled in Charleston, South Carolina (CHS), and the Indian River Lagoon, Florida (IRL), during 2003–2005 (Fair et al. 2010). ΣPCBs measured 74 congeners, Σpesticides were analyzed for 23 compounds, and ΣPBDEs were analyzed for 13 congeners. **Significant difference between the 2 sites ($p < 0.01$)

Site	Dolphins		Contaminant (ng g^{-1} lipid)		
	n	Age (yr)	ΣPCBs	Σpesticides	ΣPBDEs
IRL	33	15.3	79800 (35000–227000)	27300 (9909–87891)	1490 (463–3790)
CHS	36	17.5	94000 (28600–255000)	43000 (19725–134862)**	5917 (1711–13200)**

pervasive in wildlife and humans (Kannan et al. 2004, Houde et al. 2011). PFAA concentrations in dolphin plasma were measured by high performance liquid chromatography tandem mass spectrometry (Fair et al. 2012a). Mean plasma concentrations in CHS dolphins were significantly higher than in IRL dolphins (Table 2) and similar to those reported for occupationally exposed humans (Fair et al. 2012a).

The identification of high PFAA levels in CHS dolphins led to an investigation of the dolphin food web and the environment (Houde et al. 2006a). Higher body burdens of specific PFAAs were found in CHS dolphins inhabiting areas with greater developed land use (Adams et al. 2008). PFAA sediment levels in CHS were higher than in any other urban US area, with over half of the sites exceeding the median global perfluorooctane sulfonate (PFOS) sediment concentration (White et al. 2015). The high levels of PFAAs found in CHS dolphins led to studies to measure these chemicals in humans residing in the region. A likely route of human exposure is through consumption of contaminated fish, thus we examined Gullah-ethnicity African Americans in the Charleston area where locally obtained seafood is a dietary staple. This community lives on coastal sea islands and is ideally situated for the study of environmental influences on disease due to its genetic and geographic homogeneity and high prevalence of systemic lupus erythematosus (Kamen et al. 2012). While temporal trends from 2003–2013 showed decreases in several PFAAs, substantial heterogeneity exists among individuals and age groups (Kamen et al. 2012, Gribble et al. 2015). The effects of exposure to these compounds and mechanisms of toxicity remain controversial. Recently, we also reported the detection in CHS dolphins of perfluoroalkyl phosphinic acids, one of the PFAA sub-groups used in a variety of industrial processes including pesticide formulation (DeSilva et al. 2016).

Table 2. Summary of perfluoroalkyl acids (PFAA) concentrations (ng g^{-1} wet weight) in plasma (geomean, range) from bottlenose dolphins *Tursiops truncatus* sampled in the Indian River Lagoon, Florida (IRL), and Charleston, South Carolina (CHS), 2003–2005 (Fair et al. 2012a). **Significant difference between the 2 sites (p < 0.01)

Dolphins	IRL		CHS	
	n	ΣPFAAs	n	ΣPFAAs
Adults	37	665 (112–4470)	32	1570 (574–3630)**
Juveniles	26	948 (113–3530)	26	2340 (596–8670)**

Our exposure studies documented concentrations of POPs in IRL and CHS dolphins above established thresholds for concern, thus warranting additional investigation. Some of the highest PFAAs and PBDEs found globally in marine mammals have been observed in bottlenose dolphins from CHS (Fair et al. 2010, 2012a, 2013). Detection of high concentrations of PFAAs in CHS dolphins led to the first investigation of associations between PFAA compounds and immune and clinical chemistry parameters in marine mammals. Age-adjusted linear regression models showed statistically significant associations between exposure to total PFAAs and/or individual analytes and markers of the immune, hematologic, hepatic, and renal systems (Fair et al. 2013). Identification of effects of exposure to chemical contaminants in free-ranging dolphins led to a number of laboratory studies in rodent models designed to corroborate results from field studies with dolphins, assess the sensitivities between the mouse model and dolphins, and evaluate risk (Wirth et al. 2014, 2015). The contaminant data from the HERA Project have been used to inform toxicological assessments using both *in vivo* and *ex vivo* approaches at environmentally relevant concentrations. For example, we examined the immunotoxic effects of PFAAs and PBDEs using a murine model and found that immunological function and gene expression are sensitive to exposure to these chemicals at concentrations found in CHS dolphins (Mollenhauer et al. 2009, 2011, Fair et al. 2011, 2012b). Compared to other toxicological endpoints, the immune system appears to be particularly sensitive to exposure to PFAAs (DeWitt 2015).

Triclosan

The first detection of triclosan, an anti-bacterial compound, in marine mammals was reported by Fair et al. (2009) in IRL and CHS dolphins at levels similar to those reported for humans. The continuous exposure of aquatic organisms to triclosan, coupled with its bioaccumulation potential, leads to detectable levels of triclosan throughout the aquatic food chain (Chalew & Halden 2009). Fair et al. (2009) added to the evidence on the persistence of these antimicrobials and contributed in part to a recent federal rule which removed these chemicals from over-the-counter antibacterial washes (US FDA 2016).

Although the contaminants measured in dolphins during HERA are described separately, they occur in tissues as complex mixtures. Little is known about the variation, interactions, cumulative effects, and risks of potentially toxic elements found in marine mammal tissues. Given the increasing environmental concentrations of emerging chemicals and the health effects associated with exposure, longitudinal monitoring of contaminants in resident dolphin populations is needed to assess temporal trends and aid in management and conservation (Fair et al. 2010).

MERCURY

Mercury (Hg) is a ubiquitous global contaminant released into the environment from multiple industrial processes with important ecosystem and public health implications (Driscoll et al. 2013). Principal among these in humans are neurotoxic effects on the fetus from pre-natal exposure characterized by cognitive and developmental impairment, deficits in memory, language skill, attention span, vision, and fine motor skills (National Research Council 2000, Oken et al. 2005). Mercury is transported through the atmosphere and re-deposited in rainfall. Much of southern Florida, including the estuarine ecosystems, is heavily impacted by mercury deposition. Bioaccumulation of mercury through the food chain is enhanced by high concentrations of sulfates and sulfate-reducing bacteria in sediments that promote methylation to the more toxic form of the element, methylmercury (Guentzel et al. 2001). Total mercury concentration (THg) was determined in blood and skin using combustion atomic absorption spectrometry according to EPA Method 7473 as described previously (Schaefer et al. 2015). IRL dolphins sampled between 2003 and 2005 had high concentrations of THg in blood and skin (Table 3). These concentrations significantly exceeded those in dolphins from CHS (Stavros et al. 2007, 2008) and are among the highest recorded worldwide (Reif et al. 2015). Similarly, the concentrations of THg in the liver of stranded IRL dolphins were approximately 10 times higher than those in dolphins stranded near CHS and were significantly correlated with skin concentrations (Stavros et al. 2011).

Table 3. Mean concentrations (± SD) of total mercury in blood and skin samples collected from Charleston, South Carolina (CHS), and Indian River Lagoon, Florida (IRL) bottlenose dolphins *Tursiops truncatus* (males and females, all ages included). **Significant difference between the 2 sites ($p < 0.01$). Adapted from Stavros et al. (2007, 2008); used by permission

Site	n	Mercury concentration Blood ($\mu g\ l^{-1}$)	Skin ($\mu g\ g^{-1}$)
IRL	75	658 ± 519.0	7.0 ± 5.9
CHS	74	147 ± 88.0**	1.7 ± 0.9**

In an analysis of 121 dolphins from both sites with complete health information, no statistically significant differences in the concentrations of THg in blood and skin were found between males and females. Age was not significantly associated with THg in either tissue (Schaefer et al. 2011b). Further analysis showed that the high THg levels in IRL dolphins impacted multiple organ systems. Decreases in total serum thyroxine and triiodothyronine with increasing concentrations of THg in blood and skin demonstrated an effect on the endocrine system. Further, concentrations of THg in blood and skin were positively associated with increases in serum adrenocorticotropic hormone. Increases in blood urea nitrogen, a marker of decreased renal function, and gamma-glutamyl transferase, a marker of liver disease, were significantly associated with increases in blood and skin THg. Changes in the hematopoietic system were also found, characterized by significant decreases in the absolute numbers of lymphocytes, eosinophils, and platelets and an increase in the absolute number of segmented neutrophils associated with increasing concentrations of THg in blood and skin (Schaefer et al. 2011b). The results were similar to those reported by Woshner et al. (2008) in a study of highly exposed dolphins from Sarasota Bay, Florida, and with pathologic changes in the liver in the same population (Rawson et al. 1993).

The immune system is also affected by exposure to high concentrations of Hg (Reif et al. 2009). A significant positive association between blood THg concentration and total globulins was found in an analysis of 142 dolphins from the IRL and CHS. Changes in the innate arm of the immune system occurred as shown by increases in monocyte phagocytic activity and plasma lysozyme concentration with increases in blood THG concentrations. The adaptive arm of the immune system was also affected. B lymphocyte markers (CD19+ [immature] and CD21+ [mature]) and class II major histocompatibility complex antigen-presenting cells decreased significantly with increasing concentrations of blood THg. In mitogen-induced lymphocyte proliferation assays, both B and T cell proliferation indices were reduced in a stepwise, dose-dependent manner with increasing blood THg. Dolphins with high concentrations of Hg in tissues may have an increased susceptibility to infectious diseases as shown by decreases in the concentrations of antibody to common marine organisms such as *Erysipelothrix rhusiopathiae* and *Mycobacterium* *marinum* and reduced lymphocyte proliferation (Reif et al. 2009). In summary, the evidence suggests that high concentrations of THg in the tissues of bottlenose dolphins produce detrimental effects on the endocrine, hepatic, immune, and hematopoietic systems.

Temporal trends and spatial distributions in THg concentrations in blood of IRL dolphins were also evaluated (Schaefer et al. 2015). THg concentrations in IRL dolphins decreased significantly between 2003 and 2012. Declines in the concentrations of Hg in US fish populations have also been reported in recent years (Chalmers et al. 2011). These national declines have been attributed to reduced exposures from point sources and emissions, rather than to a decline in global atmospheric deposition. However, Hg contamination patterns in the southeastern USA show increases, reflecting hydro-geological factors that favor the deposition of Hg from the atmosphere, rather than emissions from local point sources (Chalmers et al. 2011). There is very little industrial activity along the borders of the IRL. Therefore, the temporal changes in the concentrations of THg in dolphins reflect the fact that mercury deposition in southern Florida is due predominantly to transport and atmospheric deposition from global sources (Guentzel et al. 2001).

Significant spatial variation in THg concentrations was also observed with a decreasing gradient in IRL segments from north to south (Fig. 1). Dolphins were assigned to segments based on capture location (Mazzoil et al. 2008). The concentration in dolphins from the north Indian River was significantly higher than that in the 3 southernmost segments (Schaefer et al. 2015). The basis for this observation remains unclear.

The principal source of exposure to Hg in humans is the consumption of fish and shellfish (NRC 2000). Thus we reasoned that the high levels of THg we detected in IRL dolphins could indicate that the adjacent human population of approximately 2.5 million persons might be at risk of serious health consequences if they consume seafood from the IRL. Therefore, we conducted a study to determine exposure to Hg in persons living near the IRL by measuring the concentration of THg in hair and assessing patterns of seafood consumption (Schaefer et al. 2014). The sources of seafood, frequency of consumption, and species consumed were measured in 135 coastal residents. Their mean hair mercury concentration (1.53 µg g^{-1}) was higher than the mean concentrations found in recreational anglers and coastal residents in other studies conducted in the USA and Canada (Reif et al. 2015). Over half the participants had a hair Hg concentration which exceeded the exposure guideline established by the US EPA of 1.0 µg g^{-1} (Schaefer et al. 2014). Although the hair Hg concentrations were significantly lower in women (0.96 µg g^{-1}) than in men (2.02 µg g^{-1}), the concentrations were approximately 5 times higher than those from a nationally representative sample of US women of childbearing age (Reif et al. 2015). Hair mercury concentrations in all participants were significantly associated with the consumption of seafood and with the consumption of locally caught fish (Schaefer et al. 2014). Those persons who reported eating seafood 3 times a week or more and those who obtained over half of their fish and shellfish from recreational sources had the highest levels of hair THg and were significantly more likely to exceed the US EPA exposure threshold than those who ate only commercially obtained seafood (Schaefer et al. 2014).

In light of the public health hazard identified from our studies of IRL dolphins and the adjacent human population, we are currently conducting a study of

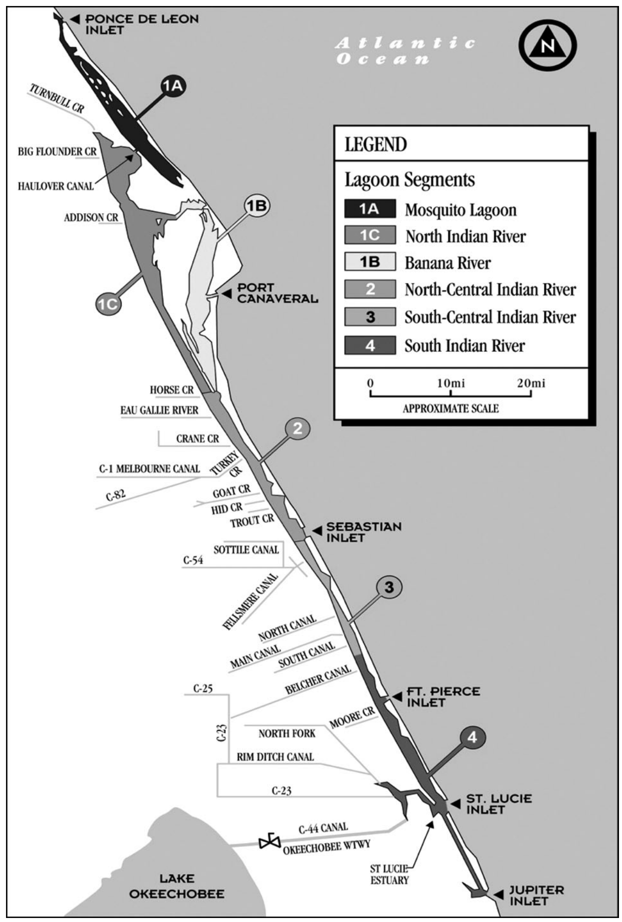

Fig. 1. Indian River Lagoon, Florida (USA), showing hydrogeological segments. Adapted from Mazzoil et al. (2008); used by permission

pregnant women in Florida. The goal is to determine the extent of their knowledge regarding the risks of exposure to mercury from seafood consumption and their dietary practices. The issue is timely since fish consumption is recommended widely due to high concentrations of omega-3 polyunsaturated fatty acids which have multiple health benefits, particularly reducing the risk of cardiovascular disease. This interrelationship between dolphin and human health is a classic example of the 'One Health' concept. Our studies of mercury exposure and health effects in HERA dolphins led to the investigation of exposure to mercury in the adjacent human population. By 'closing the loop' directly between an animal sentinel and human health, we have made a contribution to public health that is virtually unique (Reif et al. 2015).

MICROBIAL ISOLATIONS AND ANTIBIOTIC RESISTANCE

Several published investigations have described microbial isolations from fecal, blowhole, and gastric samples and antibiotic sensitivity patterns in dolphins (Buck et al. 2006, Greig et al. 2007, Venn-Watson et al. 2008, Schaefer et al. 2009, 2011a, Morris et al. 2011, Stewart et al. 2014). Microbial isolation, identification, and antibiotic sensitivity testing for samples collected from the blowhole, stomach, and anus during HERA were conducted in a single commercial laboratory using standard methods as described previously (Schaefer et al. 2009). In IRL and CHS dolphins, the most frequently cultured Gram-negative organisms between 2003 and 2005 were *Plesiomonas shigelloides*, *Aeromonas hydrophila*, *Escherichia coli*, and *Pseudomonas fluorescens* in descending order (Morris et al. 2011). Distribution patterns of *P. shigelloides* and *E. coli* were similar for CHS and IRL dolphins, with the highest frequency in fecal swabs. Among the Gram-positive bacteria cultured, *Clostridium perfringens*, *Bacillus* sp., and *Staphylococcus* (coagulase negative) were predominant. *Bacillus* sp. was isolated from blowhole and gastric samples, but not from fecal swabs. The most abundant fungal species isolated were *Candida glabrata* and *C. tropicalis*. In general, a higher prevalence of bacteria and fungi (4-fold increase) was found in the IRL compared to CHS dolphins (Morris et al. 2011).

Pathogenic strains of *E. coli* cause gastrointestinal disease in humans and animals. Human epidemics have been associated with recreational exposure to water (Keene et al. 1994) and with the ingestion of contaminated food or water. Fecal coliform counts are commonly used as an indicator of water quality and fecal pollution in domestic systems. We assessed risk factors for *E. coli* colonization in IRL dolphins in order to assess the potential for anthropogenic sources of microbial pollution (Schaefer et al. 2011a). The overall prevalence of colonization from 2003 to 2007 was 52%. A spatial gradient was found, with the highest colonization rates in the northern segments of the IRL (Fig. 1). From an environmental perspective, human population density and the number of sewage treatment plants bordering an IRL segment were not associated with dolphin colonization rates. However, at the county level, cumulative rainfall 48 h prior to capture and the number of septic systems were significantly associated with risk for *E. coli* colonization in the adjacent dolphin population (Schaefer et al. 2011a). Interestingly, the highest colonization rates were measured in 2004, a year during which multiple hurricanes impacted the Florida coast. These results suggested that increased runoff from terrestrial sources and overloaded septic systems during storm events may have led to introduction of fecal coliforms and bacterial loading in the estuarine ecosystem. The findings illustrate the potential for using dolphins as sentinels for climatic phenomena. Since *E. coli* and other coliform bacteria can act as either primary or opportunistic pathogens in humans, the findings also have important implications for public health (Schaefer et al. 2011a).

Bacterial resistance to antibiotics is one of the leading public health issues worldwide, with important individual and population level ramifications (WHO 2014). At least 2 million people become infected with antibiotic-resistant bacteria in the USA annually and at least 23 000 people die as a result of these infections (CDC 2016a). As resistance to antibiotics continues to increase, the probability of successfully treating infections caused by common pathogens decreases (Hawkey & Jones 2009). Therefore, it is essential to understand the role of the environment in the emergence of resistance among pathogens that can impact both wildlife and public health. Aquatic environments have the potential to play a significant role in the emergence of antibiotic resistance. They can serve as a reservoir of resistance due to the amount and diversity of microbial loading, particularly with clinically relevant bacteria. Further, discharges from terrestrial sources contain antibiotics that are not degraded during sewage treatment. Therefore, aquatic ecosystems accumulate human pathogens released into the environment and facilitate the exchange of resistance genes between bacterial species (Rizzo et al. 2013).

Antibiotic-resistant bacteria have been cultured from bottlenose dolphins from coastal and estuarine populations. Greig et al. (2007) used fecal samples collected from dolphins during HERA in 2003 to assess the level of antibiotic resistance to *E. coli*. Isolates were screened for resistance to 25 antibiotics. The prevalence of resistance was significantly higher at CHS than in the IRL. Evidence of resistance was detected in *E. coli* from dolphins at either site for 19 of the 25 antibiotics tested. Resistance to penicillin was most common, followed by cephalothin, ampicillin, and amoxicillin; all are commonly used in both humans and animals. Within-animal isolate and sampling locale variability were examined. Isolates from the CHS area exhibited a greater complexity of resistance patterns and within-individual diversity compared to those from the IRL (Greig et al. 2007).

Schaefer et al. (2009) analyzed data collected during HERA from 2003–2005 to evaluate the prevalence and distribution of antibiotic-resistant organisms cultured from blowhole, gastric fluid, and feces of IRL and CHS dolphins. In descending frequency, organisms demonstrated resistance to erythromycin, ampicillin, and cephalothin. These findings have implications for public health because many of these organisms are human pathogens and because bacteria displaying these patterns of resistance have colonized a marine species. The discharge of many antibiotics commonly used in human and veterinary medicine directly via septic tanks and effluents or indirectly through estuarine drainage systems (Sime 2005) is likely responsible for the shared patterns of resistance found in dolphins, humans, and domestic animals.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from blowhole and gastric samples from 3 CHS dolphins (Schaefer et al. 2009). Although an uncommon finding in marine mammals, MRSA infections were also diagnosed in 5 dolphins and 3 walruses in a marine park with apparent inter-species transmission (Faires et al. 2009). The incidence of community- (versus hospital-) acquired infections in humans has increased in recent years (Klevens et al. 2007). The Centers for Disease Control and Prevention estimates an annual incidence of 62 000 cases in the USA, of which 27% are community acquired (CDC 2016b). Unlike hospital-acquired strains of MRSA, community infections affect healthy people with none of the known risk factors for MRSA, thus posing a greater threat to public health (Naimi et al. 2001).

Recently, temporal trends in resistance were examined in bacterial isolates from HERA dolphins from 2003 to 2015 (Table 4; A. M. Schaefer et al. unpubl.). Between 2003 and 2015, analysis for antibiotic resistance was performed on a total of 897 IRL and 449 CHS isolates from 171 and 80 dolphins, respectively. Of those, 733 (81.7%) isolates from the IRL and 378 (84.2%) from CHS were suitable for screening. The most commonly cultured pathogens included *Acinetobacter baumannii*, *Aeromonas hydrophila*, *Clostridium perfringens*, *Escherichia coli*, and *Vibrio alginolyticus*. When compared to the 2003–2007 sampling period, isolates collected in the IRL during 2010–2015 were more likely to be resistant to ceftazidime, cefotaxime, and gentamicin. This finding is consistent with a recent report describing increases in resistance in bacteria isolated from pinnipeds in the

Table 4. Prevalence of antibiotic resistance in Indian River Lagoon, Florida (IRL) bacterial isolates collected from bottlenose dolphins *Tursiops truncatus*, for 2003–2007 and 2010–2015. **Bold text** highlights significant differences ($p < 0.05$) between the 2 sampling periods

Antibiotic	2003–2007		2010–2015		p
	No. resistant/ no. tested	Percent resistant	No. resistant/ no. tested	Percent resistant	
Amikacin	4/369	1.08	7/259	2.70	0.21
Ampicillin	307/385	79.74	196/266	73.68	0.07
Augmentin	214/392	54.59	138/263	52.47	0.59
Cefotaxime	**27/368**	**7.34**	**57/260**	**21.92**	**<0.01**
Ceftazidime	**14/370**	**3.78**	**26/260**	**10.00**	**0.02**
Cephalothin	246/392	62.76	160/265	60.38	0.54
Chloramphenicol	112/392	28.57	71/265	26.79	0.62
Ciprofloxacin	10/392	2.55	13/265	4.91	0.11
Enrofloxacin	12/391	3.07	13/265	4.91	0.23
Erythromycin	356/392	90.82	246/265	92.83	0.36
Furadantin	140/369	37.94	80/260	30.77	0.07
Gentamicin	**4/370**	**1.08**	**11/260**	**4.23**	**0.01**
Marbofloxacin	8/359	2.23	11/259	4.25	0.15
Piperacillin	184/371	49.60	120/260	46.15	0.39
Tetracycline	98/392	25.00	66/263	25.10	0.98

Northwest Atlantic (Wallace et al. 2013). Changes in the resistance patterns among isolates from wild bottlenose dolphins may reflect trends in the usage and disposal of antibiotics from terrestrial sources. It is not clear whether resistance is acquired in humans and transferred to the marine environment through discharges of wastes or whether the marine environment is the origin of resistance. Plausibly, the exchange of genes among bacteria may be occurring in the marine ecosystem (Rizzo et al. 2013, Vaz-Moreira et al. 2014). These data provide insight into the changing patterns of antibiotic resistance among bacteria in a marine environment and generate hypotheses regarding anthropogenic influences on resistance and risk to public health. They also provide evidence that dolphins serve as sentinels for microbial, as well as chemical, environmental, and public health hazards.

CONCLUSIONS

Bottlenose dolphins are the most common coastal cetacean species found in the southeastern USA. Their long life span, trophic position as an apex predator, and widespread geographical distribution in temperate coastal environments create the ideal conditions for a sentinel animal (Wells et al. 2004, Bossart 2011). Longitudinal studies such as HERA are vital for monitoring temporal trends in the distribution of emerging and legacy chemical contaminants, marine biotoxins, antibiotic-resistant bacteria, and other environmental hazards. Exposure data collected during the dolphin HERA Project identified the CHS harbor and surrounding estuaries as hotspots for emerging chemicals (PFAAs, PBDEs) and led to further studies of dolphin health effects, the environment, and local human populations. Similarly, the IRL was identified as an ecosystem with extremely high levels of Hg in resident dolphins with associated health effects. This finding led to our recent study of Hg levels in coastal human residents along the IRL, a seminal investigation which directly applied the findings from a sentinel species to identify a public health hazard in a contiguous human population. Multiple contemporary environmental threats face animals and humans in coastal ecosystems. Marine mammal sentinels may serve as indicators of risk from climate change (Fury & Reif 2012), harmful algal blooms (Bossart et al. 1998), emerging chemical contaminants (Fair et al. 2010), and oil spills (Schwacke et al. 2014). The sentinel species approach can also inform regulatory or management strategies that protect both ecosystem and human health. Additional studies of marine mammal sentinels should be conducted globally, as an important component of the 'One Health' initiative.

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