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BRIEF REPORT

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The skin microbiome of cow-nose rays (*Rhinoptera bonasus*) in an aquarium touch-tank exhibit

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Public aquaria offer numerous educational opportunities for visitors while touch-tank exhibits offer guests the ability to directly interact with marine life via physical contact. Despite the popularity of touch-tanks, there is a paucity of research about animal health in these exhibits and, in particular, there is little research on the microbial communities in these highly interactive exhibits. Microbial community structure can have implications for both host health and habitat function. To better understand the microbiome of a touch-tank we used high-throughput sequencing of the 16S rRNA gene to analyze the microbial community on the dorsal and ventral surfaces of cow-nose rays (*Rhinoptera bonasus*) as well as their environment in a frequently visited touch-tank exhibit at the New England Aquarium. Our analyses revealed a distinct microbial community associated with the skin of the ray that had lower diversity than the surrounding habitat. The ray skin was dominated by three orders: Burkholderiales (~55%), Flavobacteriales (~19%), and Pseudomonadales (~12%), taxonomic groups commonly associated with other fish species. Our results provide a survey of ray-associated bacterial communities in a touch-tank environment, thereby laying the foundation for future studies examining the role of potential challenges to ray microbiota and their associated health.

KEYWORDS
16S rRNA gene, cow-nose ray, elasmobranch, microbiome, touch-tank

1 | INTRODUCTION

In an effort to deepen the connection between visitors and the animals on exhibit, many zoos and aquariums have touch-tanks that promote science education (Kisiel, Rowe, Vartabedian, & Kopczak, 2012; Kopczak, Kisiel, & Rowe, 2015). The few studies focusing on human interaction with touch-tanks examined animal well-being and growth, or human stress and emotional response (Casamitjana, 2004; Clarke III et al., 2013; Morris et al., 2012; Payne, 2012, Persky et al., 2012; Sahrmann, Niedbalski, Bradshaw, Johnson, & Deem, 2015). Studies have reported occasional abnormalities among tank residents (Morris et al., 2012; Persky et al., 2012), however, animals can breed on exhibit, suggesting that basic biological needs are typically being met (Payne, 2012). In any exhibit where there is contact between animals and visitors, concern for both animal and visitor health and safety is paramount.

In recent years, host-associated microbial communities have received extensive attention due to their important role in immune defense and host wellness (Cho & Blaser, 2012). The microbiome is a little studied characteristic of public aquarium exhibits, but has great implications as an indicator of animal health. The maintenance of a healthy microbiome is a key aspect of animal care (Schmidt, Smith, Melvin, & Amaral-Zettler, 2015) and understanding the microbiome of animals on exhibit will allow aquarium staff to better evaluate animal health and well-being. In this study we characterized the skin microbiome of cow-nose rays (*Rhinoptera bonasus*; hereafter, rays) in a touch-tank exhibit at the New England Aquarium. We hypothesized that the ray microbiome would be conserved among rays but would be distinct from the tank microbiome and that there would only be a small percentage of common human-associated taxa present in the ray skin microbiome.

2 | METHODS

2.1 | Sample collection

The New England Aquarium touch-tank exhibit features a 25,000-gallon tank where visitors can interact with rays and other tank organisms. The Aquarium has nearly 1.3 million visitors per

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year, and an estimated 50% visit the touch-tank. In September 2013, we sampled ray skin microbiomes in the morning prior to allowing visitors access to the exhibit. As a part of routine veterinary care, rays were pulled from the water and the dorsal and ventral surfaces of five cow-nose rays were swabbed by aquarium veterinary staff to capture spatial variability of ray-associated bacteria. To document the microbiome of the touch-tank environment, two 1 L water samples were collected, one at the inflow pipe and one immediately in front of the outflow. Each liter of water was filtered through 0.22 μM Sterivex™ filters. We also collected gravel from the tank and the biofilm growing on the tank wall. All samples were stored on dry ice for transport to the lab and stored at −80°C until molecular analyses.

2.1.1 | DNA extraction and sequencing

DNA was extracted from swabs and sediment using the MoBio PowerSoil® Total DNA Isolation Kit (Carlsbad, CA) and water samples were extracted using the MoBio PowerWater® Total DNA Isolation Kit. DNA extractions were verified with gel electrophoresis and bacterial community composition was assessed by PCR in triplicate using universal bacterial primers (Caporaso et al., 2011) following conditions outlined by Caporaso et al. (2010, 2012). PCR product was purified with the Qiagen PCR Purification Kit (Valencia, CA). Paired-end sequencing of samples was performed on an Illumina MiSeq.

2.1.2 | Sequence processing and analysis

A total of 495,524 reads were first joined with fastq-join (Aronesty, 2011) and quality filtered following Bokulich et al. (2013) in QIIME (Caporaso et al., 2010). Operational taxonomic units (OTUs) were picked with the USEARCH pipeline (Edgar, Haas, Clemente, Quince, & Knight, 2011) at 97% sequence identity. We discarded OTUs appearing only once and taxonomy was assigned with UCLUST (Edgar et al., 2011) using the GreenGenes database (version 13.5). We calculated community similarity in QIIME using Bray-Curtis on an OTU table normalized to the lowest sequencing depth (16,500 sequences) and visualized these similarities with a principal coordinates analysis (PCoA). We tested for significant differences in microbial community composition using permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001), and determined which OTUs were significantly different among samples using a Kruskal–Wallis test in Qiime. To better understand taxa associated with the rays, we calculated the core microbiome and defined a taxon as core if it was present in 100% of ray samples. Although, a formal test for the presence of human-derived microbes on the ray skin would require a control group of rays that were not part of a touch-tank exhibit, we can, nonetheless, screen the sequences derived here to determine whether there is the presence of a large degree of sequences typically found in human skin microbiomes. If these human associated microbes were present, we would not be able to substantiate, based on this experimental design, that it was specifically due to human interaction with the animals, however, the absence of human associated microbes on the rays on exhibit would suggest that human contact is not transmitting human associated bacteria to the skin of the rays. Thus, we compared the taxonomic composition of the touch-tank to previously published work on the human skin microbiome (Grice et al., 2009; Oh et al., 2014). We generated taxonomic profiles from these datasets as described above and filtered our dataset using QIIME.

3 | RESULTS

3.1 | Community composition

A principal coordinates analysis based on weighted Bray–Curtis similarity indicated that the cow-nose ray skin microbiome was distinct from its environment (Figure 1; PERMANOVA, $F = 14.21$, $p < 0.001$). There was, however, no significant difference between the dorsal and ventral surfaces of the ray ($F = 2.29$, $p > 0.5$). Ray skin was dominated by the Betaproteobacterial order Burkholderiales (~55%) and had a consistent presence of Flavobacteriales (~19%) and Pseudomonadales (~12%; Figure 2). These three orders were significantly less abundant in the surrounding environment (Kruskal–Wallis test; $F = 23.2$, $p < 0.01$). The ventral sample from ray 1 had a considerable presence of Vibrionales from the genus *Salinivibrio* (62.3%), but this order was in low abundance on the remainder of the rays.

Microbial communities in the ray tank environment were considerably different from those found on ray skin. The input water was dominated by the Gammaproteobacterial genus *Vibrio* (order Vibrionales). The outlet water, sediment, and biofilm were dominated by the Alphaproteobacterial order Rhodobacterales, and the Gammaproteobacterial order Thiotrichales was also abundant in the outlet

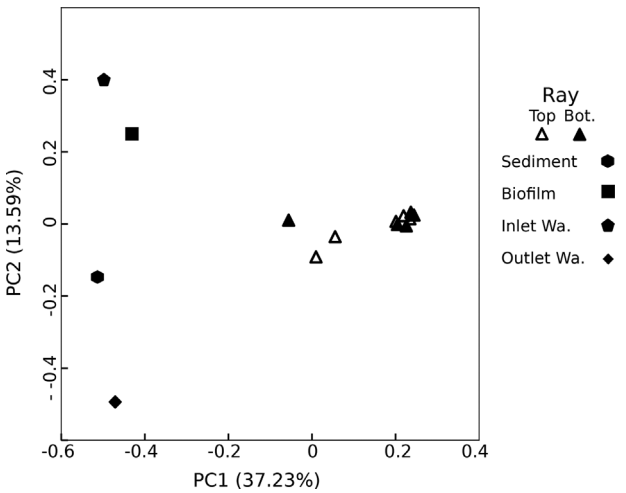

FIGURE 1 Principal coordinates analysis of Bray–Curtis similarity for the dorsal (open symbols) and ventral surfaces (closed symbols) of five rays from the New England Aquarium Touch–tank. Microbial communities of the cow-nose ray tank environment (inlet and outlet water, biofilm, and sediment, are also depicted (black symbols)

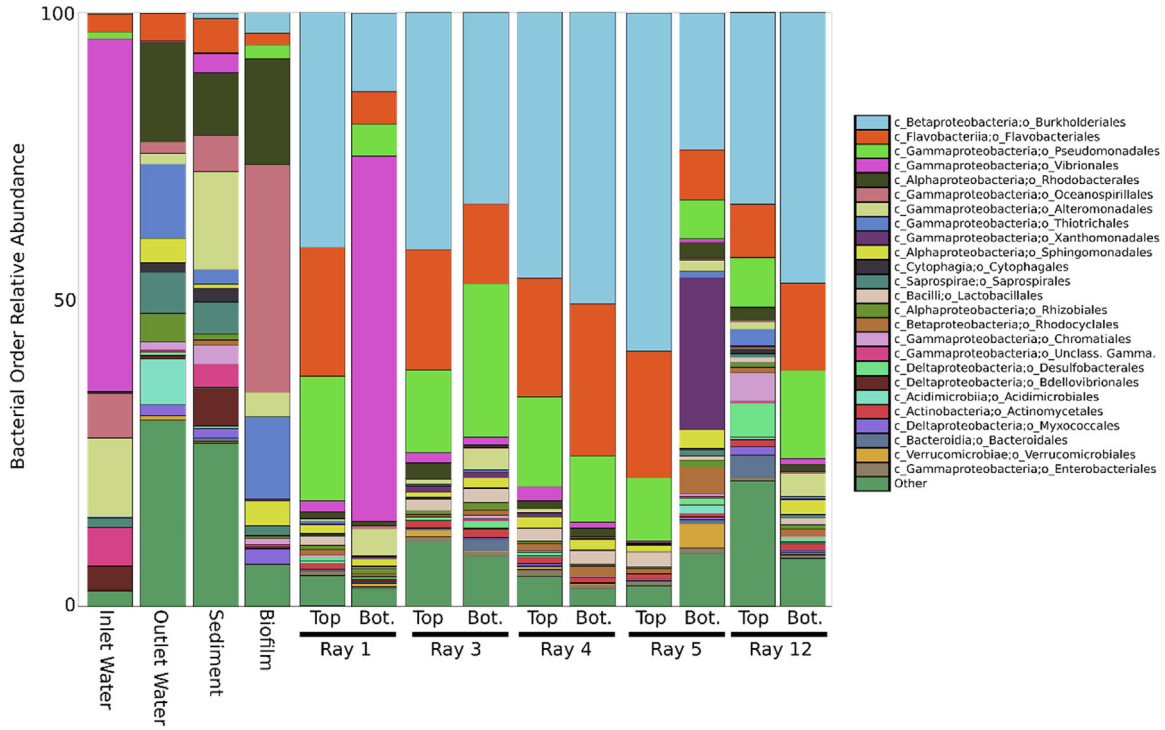

FIGURE 2 Stacked bar plot showing the top 25 most abundant bacterial orders accounting for 90% of all sequences. The remaining 10% of sequences are placed in the "other" category

water and biofilm, whereas the sediment had abundant Alteromonadales. The biofilm also displayed a large portion of taxa associated with the Oceanospirillales (Figure 2) that were in lower abundance in the rest of the samples. Analysis of the core microbiome (Table 1) revealed 22 core taxa present across all rays. In addition to more abundant orders highlighted above, several lower abundance groups including the orders Xanthomonadales, Flavobacterales, Enterobacterales, Sphingomonadales, and the class Bacilli were identified as being important to the captive ray skin microbiome. In addition, comparison of our data to previous studies on the human skin microbiome (Grice et al., 2009; Oh et al., 2014) revealed a very small percentage of taxa (<1.5%) commonly associated with human skin (Table 2) and while some taxa were closely related to pathogens (e.g., *Vibrio* sp.) no human pathogens were detected. This result suggests that human interactions do no significantly introduce human-associated bacteria to the ray skin and the touch tank habitat.

4 | DISCUSSION

The microbial communities associated with cow-nose rays displayed distinct structure compared to their tank (Figure 1) likely due to the lower niche space that is often found on hosts (Ogilvie, Overall, & Jones, 2012). The consistent presence of the orders Burkholderiales, Flavobacteriales, and Pseuomonadales (Figure 2) and their very low abundance in the environment suggests these taxa may be beneficial to maintaining the health of the ray skin. All three orders are predominantly heterotrophic bacteria, while members of the Pseudomondales order have been shown to produce numerous antimicrobial compounds (Holmström & Kjelleberg, 1999). Additionally, members of the order Burkholderiales have shown antibiotic activity (El-Banna & Winkelmann, 1998). Taken together, our results suggest rays may recruit taxa beneficial to host health as was recently shown with trout (Lowrey, Woodhams, Tacchi, & Salinas, 2015).

To date, there have been few investigations into ray or shark microbiomes and all studies have focused on gut microbiota (Givens, Ransom, Bano, & Hollibaugh, 2015). The microbial communities present on ray skin displayed a strong core of taxa dominated by Proteobacteria (Burkoldariales and Pseudomonadales) and Flavobacteria, taxonomic groups that have been documented on other groups of fish. For example, the skin microbiome of the rainbow trout (*Oncorhynchus mykiss*) was composed of ~15% of taxa from the order Burkholderiales and ~40% from the order Flavobacterales (Lowrey et al., 2015) compared to ~55% and ~19%, respectively on the New England Aquarium rays, suggesting these groups of taxa may be important to both freshwater and marine fish species. However, the third dominant order in our study, Pseudomonadales, was not in meaningful abundance on the Black Molly (*Poecilia sphenops*; Schmidt et al., 2015), the rainbow trout (Lowrey et al., 2015), the Gulf Killifish (*Fundulus grandis*; Larsen, Mohammed, & Arias, 2014), and other bony fish (Givens et al., 2015), but was present on the mosquito fish (*Gambusia affinis*; Leonard et al., 2014). Further, the phyum Firmicutes and Cyanobacteria, which were, on average <1% of the ray community, were consistently abundant (>15%) on

TABLE 1 Taxonomic composition of OTUs that comprise the core microbiome of ray skin

OTU	Phylum	Class	Order	Family	Genus/species
1	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Enhydrobacter*
2	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadadeae	*Novosphingobium*
3	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Psychrobacter*
4	Firmicutes	Bacilli	Lactobacillales		
5	Firmicutes	Bacilli	Lactobacillales		
6	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Methyloversatilis*
7	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Salinivibrio*
9	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	*Facklamia*
10	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	
11	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	
12	Firmicutes	Bacilli	Bacillales		
13	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Trabulsiella*
14	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	
15	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	
16	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	
17	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio shilonii*
18	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	
19	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Dechloromonas*
20	Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae	*Cloacibacterium*
21	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*
22	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Limnohabitans*

Taxa are considered core if they are present in 100% of the ray samples.

several fish species (Givens et al., 2015; Larsen et al., 2014; Lowrey et al., 2015). The low abundance of these phyla on rays may be a result of being held in captivity (Muegge et al., 2011) or it could simply be that these taxa do not confer a benefit to ray skin microbial communities. The effect of captivity on host-associated microbial communities remains unclear (Alfano et al., 2015; Muegge et al., 2011) and additional work on rays in their natural environment is needed to understand how their microbiome may differ when in captivity. We did not observe a signal of human derived bacteria in the microbiome of the rays on exhibit, suggesting that human to ray transfer of microbes in this exhibit at the time of sampling appears to be small. However, our experimental design does not allow us to detect whether human contact may have other negative effects on the ray microbiome, such as extirpation of important host-associated taxa.

TABLE 2 Percentage of sequences found in samples previously found in human skin microbiome [Grice et al., 2009; Oh et al., 2014]

Sample	% human associated
Sediment	0.96
Biofilm	0.43
Inlet water	0.41
Outlet water	0.45
Ray top	1.06 (±1.41)
Ray bottom	1.29 (±0.90)

For ray samples, numbers in parentheses are standard error of the mean.

5 | CONCLUSIONS

In conclusion, our results demonstrate a distinct difference in both community composition and diversity of ray skin microbial communities relative to their environment. Ray skin was dominated by three main orders (Burkholderiales, Flavobacteriales, and Pseuomonadales), orders that display antibacterial properties and are common to other fish skin microbiomes.We did not detect an appreciable number of bacterial typically associated with human skin in the touch-tank exhibit. It is important to document how the microbiome of the rays is altered through routine exhibit procedures and how it changes with veterinary procedures such as chemical treatment for parasites or disease. Ultimately, understanding the microbiome of exhibit animals may allow for a more sophisticated index of health and well-being.

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