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

Patrick J Kearns Correspond., 1, Jennifer L Bowen 1, Michael F Tlusty 2, 3

1 Biology Department, University of Massachusetts Boston, Boston, Massachusetts, USA
2 John H. Prescott Marine Lab, Anderson Cabot Center for Ocean Life, New England Aquarium, Boston, MA, USA
3 School for the Environment, University of Massachusetts Boston, Boston, MA, USA

Corresponding Author: Patrick J Kearns
Email address: lele123@gmail.com

Public aquarium exhibits offer numerous educational opportunities for visitors while touch tank exhibits offer guests the ability to directly interact with marine life. However, despite the popularity of these exhibits, the effect of human interactions on the host-associated microbiome or the habitat microbiome remains unclear. Microbial communities, both host-associated and habitat associated can have great implications for host health and habitat function. To better understand the link between human interactions and 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 its 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%), suggesting a potentially important role of these taxa in ray health. Further, there was no difference between dorsal and ventral surface of the ray in terms of microbial composition or diversity, and a very low presence of common human-associated microbial taxa (<1.5%). Our results suggest that human contact has a minimal effect on the skin and habitat microbiome of the cow-nose ray and that the ray skin harbors a distinct and lower diversity microbial community than its environment.
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Patrick J. Kearns¹, Jennifer L. Bowen¹†, and Michael F. Tlusty²,³

1. Biology Department, University of Massachusetts Boston, Boston, MA 02125 USA.
3. School for the Environment, University of Massachusetts Boston, Boston, MA 02125.

† Current address: Department of Marine and Environmental Science, Northeastern University, 430 Nahant Road, Nahant, MA 01908, USA.

Correspondence and request for materials should be addressed to MFT (mtlusty@neaq.org).

Abstract

Public aquarium exhibits offer numerous educational opportunities for visitors while touch tank exhibits offer guests the ability to directly interact with marine life. However, despite the popularity of these exhibits, the effect of human interactions on the host-associated microbiome or the habitat microbiome remains unclear. Microbial communities, both host-associated and habitat associated can have great implications for host health and habitat function. To better understand the link between human interactions and 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 its 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%), suggesting a potentially important role of these taxa in ray health. Further, there was no difference between dorsal and ventral surface of the ray in terms of microbial composition or diversity, and a very low presence of common human-associated microbial taxa (<1.5%). Our results suggest that human contact has a minimal effect on the skin and habitat microbiome of the cow-nose ray and that the ray skin harbors a distinct and lower diversity microbial community than its environment.
Introduction

In an effort to deepen the connection between visitors and the animals on exhibit, many zoos and aquariums have touch-tanks. These are exhibits where visitors can have direct contact with any number of animals. Many of the touch-tank exhibits in aquariums are themed around elasmobranchs, and are popular because they allow visitors to enter the exhibit and encounter animals they otherwise would likely never touch. These exhibits are important to promote science education within public aquariums because they allow visitors to practice the application of scientific reasoning (Kisiel et al. 2012), as well as engage in lengthier ecology discussions when guided by a trained interpreter (Kopczak et al. 2015).

Despite the popularity of touch tanks, there is a paucity of research about animal health in these exhibits. The few studies focusing on elasmobranchs in and associated with touch tanks examined shark growth rates (Payne and Rufo 2012), plasma biochemistry values (Persky et al. 2012), goiter onset (Morris et al. 2012), stingray abscess development (Clarke III et al. 2013), behavior (Casamitjana 2004), as well as the human stress response and emotional responses to contact with the animals (Sahrmann et al. 2015). Casamitjana (2004) suggested that touch tank exhibits may be maladaptive to elasmobranchs and other studies reported that abnormalities are occasionally observed (Persky et al. 2012; Morris et al. 2012). Other studies have shown that animals can breed on exhibit, suggesting that basic biological needs are being met (Payne and Rufo 2012). In any exhibit where there is contact between animals and visitors, concern for both animal and visitor health and safety is paramount.

One way that visitors may affect aquatic animals in touch tanks is through the addition of novel bacteria that may take up residence on the animal. All animals have a thin biofilm of microbes on their epithelial layer. The microbial communities (microbiome) on the host may provide protection against pathogens and disease and therefore promote animal health and well-being (Nayak 2010; Cho and Blaser 2012; Russell et al. 2014; Llewellyn et al. 2015). The assumption within animal husbandry is that maintenance of a healthy biofilm (e.g. slime coat maintenance in fish) is a key aspect of proper care of the exhibit animals (Schmidt et al. 2015). Thus, elasmobranch touch-tanks offer a potential challenge to animal husbandry where visitor contact may reduce the functional biofilm of the exhibit animals and potentially introduce novel bacteria that could be harmful to animals on exhibit.

To examine if the skin microbiome of exhibit animals is affected by contact with visitors, we characterized the microbial communities on the dorsal and ventral surface of cow-nose rays \((\text{Rhinoptera bonasus}; \text{hereafter, rays})\) being held in a touch-tank exhibit at the New England Aquarium. We hypothesized that if human contact had an effect on the ray skin microbiome, the dorsal surface of the rays would be altered and potentially contain a larger proportion of human-associated bacteria compared to the ventral surface, since only the dorsal surface of the rays is in contact with visitors.
direct contact with human hands. Additionally, we hypothesized that the ray skin microbiome would be conserved among different individuals and would be distinct from the microbial communities found in the exhibit environment. We expected that the rays would harbor a reduced subset of the microbial taxa found in their environment. Our results demonstrate that cow-nose rays have distinct skin microbial communities that are consistent across several animals, and that there is no evidence that the skin microbiome of cow-nose rays in the New England Aquarium are significantly altered by human contact.

Methods

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 along 40% of the periphery. The Aquarium has nearly 1.3 million visitors per year, and an estimated 50% visit the touch tank. We sampled ray skin microbiomes in the morning, prior to opening the exhibit to visitors, thus the rays had been untouched since the previous evening (14hr untouched). Dorsal and ventral surfaces of cow-nose rays were swabbed for their microbiome during a routine spine clipping procedure that was overseen by veterinary staff at the aquarium in September 2013. Rays (n= 5) were removed from the water, gently swabbed with cotton-tipped applicators on both surfaces (independent 5 cm swaths), clipped, and returned to the water. To document the microbiome of the ray environment, two 1-liter water samples were collected, one at the inflow pipe and one immediately in front of the outflow. Each liter of water was filtered on site through a 0.22 µM Sterivex™ filter. We also collected gravel from the tank using a sterile 15-mL centrifuge tube as well as a swab of biofilm growing on the tank wall. All samples were placed into cryovials (swabs and sediment) or whirlpack bags (water filters) and stored on dry ice until they were returned to the lab and stored at -80°C until nucleic acid extraction.

DNA extraction and sequencing

DNA was extracted from ray skin swabs, sediment, and biofilm samples using the MoBio PowerSoil® DNA total Isolation Kit (Carlsbad, CA USA) following manufacturer’s instructions. Water samples were extracted using the MoBio PowerWater® Total DNA Isolation Kit following the manufacturer’s instructions. DNA extractions were verified on a 1.5% agarose gel stained with ethidium bromide. To assess bacterial community composition, genomic DNA was amplified by polymerase chain reaction in triplicate using universal bacterial primers 515F and 806R (Caporaso et al. 2011) following conditions outlined by Caporaso et al. (2011) using Illumina adaptors and 12-bp GoLay barcodes on the reverse primers. Proper product formation was verified with electrophoresis and purified with the Qiagen PCR purification kit (Qiagen, Valencia, CA, USA). Samples were quantified fluorometrically with a Qubit (ThermoFisher, Waltham, MA, USA) and pooled in equal molar amounts for sequencing on the Illumina MiSeq with paired-end, 151-bp sequencing using V2 chemistry (Caporaso et al. 2012).
Sequence processing and analysis

A total of 495,524 reads (average length 253bp +/- 0.12bp) were first quality filtered following a previously published protocol (Boulich et al. 2013, Kearns et al. In Press) in QIIME (version 1.9; Caporaso et al. 2010a) following joining of paired end reads with fastq-join (Aronesty et al 2011). Reads were then screened for chimeras using USEARCH (Edgar et al. 2011) with both de novo and reference based methods and chimeric reads were discarded. After quality filtering a total of 491,746 sequences remained. Operational taxonomic units (OTUs) were picked with USEARCH (Edgar et al. 2011) at 97% sequence identity and we discarded OTUs appearing only once across the dataset. Taxonomy was assigned with UCLUST (Edgar 2011) using the GreenGenes database (version 13.5). A representative sequence was chosen, aligned with PyNast (Caporaso et al. 2010b), and a phylogenetic tree was constructed using FastTree (Price et al. 2010).

We calculated beta diversity using weighted UniFrac (Louzopone and Knight 2005) on an OTU table normalized to the lowest sequencing depth (16,500 sequences). Weighted UniFrac is similar to other beta diversity metrics in that it calculates pair-wise similarity, however UniFrac incorporates phylogenetic information of the microbial taxa, providing a more information rich estimation of community similarity. Results of the beta diversity analyses were visualized with a principal coordinates analysis (PCoA). To assess significance of sample groups we used a non-parametric multivariate analysis of variance (MANOVA) with 10,000 permutations (adonis, Anderson 2002). Alpha diversity metrics, including Shannon Diversity Index and phylogenetic diversity, were calculated on a rarefied OTU table (steps of 100, 10,000 restarts at each step) and significant differences in alpha diversity were assessed using a one-way ANOVA in R (R Core Team 2012). Shannon Diversity provides an estimate of total species richness and evenness but does not include any assessment of the relatedness of microbial taxa. Phylogenetic diversity, however, includes an estimation of phylogenetic relatedness by calculating the total branch length between microbial taxa.

Core microbiome and significantly different OTUs

To focus on specific OTUs important to the ray skin, we defined a core microbiome of OTUs that were present in all ray samples. The results of the core microbiome analysis were visualized with a venn diagram constructed in R. To assess significant differences in OTU frequencies among sample types we used a non-parametric ANOVA (Kruskal-Wallis test) in QIIME, defining significance with a Bonferroni corrected p-value <0.05.

To further assess the effect of human interaction with the ray skin microbiome we compared the taxonomic composition of the ray skin 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. We defined a taxonomic match to the human skin microbiome with a similarity threshold of 95%, such that if the OTUs present
in our study were 95% similar to the OTUs from the human skin they were considered a match.
We tested significant differences with a one-way ANOVA in R.

Results

Diversity and community composition

Following clustering at 97% sequence identity, a total of 14,535 OTUs were recovered from all sample types. Alpha diversity metrics including the Shannon Diversity Index and phylogenetic diversity were greater for the environmental samples than for the ray skin microbiome samples (Table 1; ANOVA, \( p < 0.01, F = 4.35 \)). Shannon values in the tank environment ranged from 6.4-9.3 with higher diversity found in the biofilm, sediment, and outlet water than in the water entering the tank. Although Shannon diversity was high in the biofilm, the Phylogenetic Diversity of the tank biofilm was low suggesting that it was likely constructed of closely related taxa. By contrast, Shannon values for the rays’ dorsal and ventral surfaces ranged between 3.42 and 5.43 and phylogenetic diversity ranged from 24.20 to 63.57 (Table 1). There was no significant difference in diversity observed between the dorsal and ventral surfaces of the rays, indicating a minimal effect of human contact on the diversity of bacteria on the ray skin microbiome.

Similarly, the ray skin microbial community structure did not demonstrate any observable influence of human contact. A principal coordinates analysis based on weighted UniFrac similarity indicated that the cow-nose ray skin microbiome was distinct from its environment (Fig. 1; adonis, \( F = 14.21, p < 0.001 \)). There was, however, no significant difference between the dorsal and ventral surfaces of the ray body (\( p > 0.5, F = 2.29 \)).

Taxonomic Composition

Ray skin was typically dominated by the Betaproteobacterial order Burkholderiales (~55%) and had a consistent presence of the orders Flavobacteriales (~19%) and Pseudomonadales (~12%; Fig. 2). However, the ventral sample from ray 1 (Figure 2) had a considerable presence of Gammaproteobacteria from the order Vibrionales (62.3%), replacing the consistent presence of the three dominant orders. Additionally, the most abundant orders in the ray skin microbiome (Burkholderiales, Flavobacteriales, and Pseuomonadales), were significantly less abundant in the surrounding environment (Kruskal-Wallis test; \( p < 0.01, F = 23.2 \)) suggesting these taxa may play an important role in the ray skin microbiome.

Communities from the ray tank environment were considerably different from those found on the ray directly. The input water was dominated by the Gammaproteobacterial order Vibrionales, but this order was not found abundant in the sediment, biofilm, or in the outlet water, suggesting that the conditions in the tank do not appear to be conducive to the growth of this order. While the order Vibrionales order was found in considerable abundance on the dorsal surface of ray 1, the inlet water was dominated by a member of the genus *Vibrio*, while the ventral surface of ray
I was dominated by the genus *Salinivibrio*. The outlet water, sediment and biofilms all had large proportion of the Alphaproteobacterial order Rhodobacterales, and the outlet water and biofilms had abundant taxa associated with the Gammaproteobacterial order Thiotrichales, whereas the sediment had abundant Alteromonadales instead. The biofilm also displayed a large portion of taxa associated with the Oceanospirillales (Fig. 2) that were in relatively low abundance in the rest of the samples.

**Core Microbiome**

To further assess the similarity between the dorsal and ventral surfaces of the ray we calculated the core microbiome (Fig. 3, Tables 2 and 3). The core microbiome of the ray skin consisted of 48 OTUs. Many OTUs (22 of 48) were shared between the ventral and dorsal ray skin while the remaining OTUs were unique to either surface. The abundance of the shared OTUs did not vary significantly between the dorsal and ventral surface of the rays (Kruskal-Wallis test, \( p > 0.3 \)). Moreover, they accounted for, on average, 89% of the sequences from both surfaces of the rays.

The OTUs that were unique to either surface of the rays, while significantly different between the two surfaces (Kruskal-Wallis test; \( p < 0.01 \)) were in low abundance, with no OTU accounting for more than 0.5% of the sequences from a given sample (Fig. 4).

**Human influences on community composition**

To assess whether there was evidence of human skin-associated bacteria on the microbiome of the rays and their environment, we compared our data to previously published data on the human skin microbiome (Grice et al. 2009; Oh et al. 2014; Table 4). Our results revealed a small percentage of taxa in the sediment (0.96%), water (0.43%), tank biofilm (0.43%), and ray skin (1.18%) that were commonly associated with human skin (Table 4). Further, there was no difference between the dorsal and ventral surfaces in the number of human-associated microbial taxa. This result suggested that human interactions do no significantly introduce human-associated bacteria to the ray skin and the touch tank habitat. Three OTUs, OTUs 4, 9, and 33 were identified as common components of the human skin microbiome and were also a part of the core skin microbiome of the cow-nose rays. Collectively, however, they accounted for only 735 sequences out of the >125,000 sequences that were identified as a part of the ray skin core microbiome.

**Discussion**

In recent years, host-associated microbial communities have received extensive attention due to their important role in immune defense and host wellness (Cho and Blaser 2012). The microbiome is a little studied characteristic of public aquarium exhibits, but has great implications as an indicator of animal health. Human interactions may introduce foreign and potentially pathogenic bacteria to animal skin and understanding the effects of human contact on aquarium animal microbiomes can provide valuable information about the health of the animals on exhibit. The goals of our study were to identify the skin associated microbiome of the cow-
nose ray and determine the effect of human interactions on the microbiome of the ray and its
habitat at the New England Aquarium.

The microbial communities associated with cow-nose rays displayed distinct communities and
lower diversity than water, sediment, and biofilm communities collected from within their tank
(Table 1, Figure 2). Our results suggest habitat filtering of skin microbial communities due to
lower niche space typically available on hosts (Rawls et al. 2006; Ogilvie et al. 2012; Frazenburg
et al. 2013). The filtering of taxa due to similar environmental conditions has been show to select
for constricted lineages of microbial taxa in host-associated and environmental microbial
communities (Webb et al. 2002; Cornwell et al. 2006; Horner-Devine and Bohannan 2006; Kraft
et al. 2007; Larsen et al. 2015; Schmidt et al. 2015). The presence of several
Gammaproteobacterial orders (Vibrionales, Thiotrichales, Alteromonadales, and
Oceanospirillales) in the tank and their absence or low abundance on the ray skin suggests
selection against these microbial taxa by the ray. Further, the consistent presence of the orders
Burkholderiales, Flavobacteriales, and Pseudomonadales (Fig. 2) and their very low abundance in
the environment suggests these taxa may be beneficial to maintaining the health of the ray skin
and are likely adapted to living on ray skin. All three of these orders are predominantly
heterotrophic bacteria, while members of the Pseudomonadales order have been consistently
shown to produce a myriad of extracellular compounds that have antibacterial and antifungal
properties (Holmström and Kjelleberg 1999). Our results suggest these three orders are likely
important to protection and maintenance of ray health.

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 seen on other groups of fish. For example, the skin microbiome of the rainbow trout
(Oncorhynchus mykiss) contained approximately ~15% of taxa from the Burkholderiales order
and ~40% from the Flavobacteriales order (Lowrey et al. 2015) in comparison to our ~55% and
~19% respectively, suggesting this group of taxa may be important to other fish groups, both
freshwater and marine. However, the third dominant order in our study, Pseudomonadales, was
not in meaningful abundance on the Black Molly (Poecilia sphenops), the rainbow trout (Lowrey
et al. 2015), the Gulf Killifish (Fundulus grandis; Larsen et al. 2015), or other bony fish/sharks
(Givens et al. 2015). Further, members of the Vibrio order appear to be abundant on the skin of
many fish species (Givens et al. 2015; Larsen et al. 2015; Lowrey et al. 2015), however their
abundance outside of the bottom of ray 1 (Fig. 2) was low in the rays we studied (<3%). This
may suggest these taxa are only beneficial to some fish and not others. Relative to other studies
on fish microbiomes, our study was conducted in the controlled setting of the New England
Aquarium. While the touch tank receives water from the Atlantic Ocean, there may be filtering
of microbial taxa potentially altering the ray microbiome. Future work on this group of fish in
their natural environment could further solidify the true composition of their skin microbiome.

Investigations into host-associated microbiomes have demonstrated that alterations to diet,
disease, or other environmental perturbations can have profound effects on microbial community
composition and diversity (Ley et al. 2005; Schmidt et al. 2015; Larsen et al. 2015). The rays in
our study are housed in an Elasmobranch touch-tank in the New England Aquarium where they
receive extensive contact by visitors on a daily basis. We demonstrated that there was no
significant difference in diversity or community composition between the dorsal and ventral skin
microbiome of the cow-nose ray (Table 1, Figure 2). Additionally, comparison to the human
microbiome revealed a very low percentage (<3%) of taxa that are commonly associated with
human skin, of which only 3 were found in the core microbiome (OTUs 4, 9, 33; Table 4).
Human skin associated bacteria were also a low percentage of sediment, biofilm, and water
communities (0.96, 0.43, and ~0.43% respectively). Taken together, our results indicate that
human contact with the dorsal surface of the ray on a daily basis has minimal effect on the ray
microbiome or its habitat, suggesting resistance to invasion by human associated bacteria.

Similar studies demonstrated environmental perturbations such as changes in salinity, disease
state, and temperature can have dramatic effects on microbial communities associated with fish
(Altinok and Grizzle 2001; Larsen et al. 2014; Schmidt et al. 2015). The apparent lack of a
significant response in the cow-nose ray skin microbiome to human contact may be because
human contact is a weak perturbation and one that the rays normally experience when interacting
with their environment. Conversely, our ray samples were collected prior to the exhibit opening
and the ray’s had not been touched for 14 hours prior to sampling and we may see a stronger
response closely following a human-ray interaction. However, this lack of difference first thing
in the morning indicates that any potential human-induced shift in ray skin microbiome is
ameliorated after a brief period of no contact and indicates the robustness of these communities
as a part of host defenses.

In conclusion, our results demonstrate a distinct difference in community composition and
diversity of cow-nose ray skin relative to their environment. Ray skin was dominated by three
main orders (Burkholderiales, Flavobacteriales, and Pseudomonadales) and the composition of the
ray skin microbiome was not affected by visitor contact at the aquarium. It will be important to
understand how the microbiome of animals compares to that of the exhibit environment, how the
microbiome can be altered through routine exhibit procedures (filtration, as animals shift onto
touch-tank exhibits), 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.

Acknowledgments

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and the UMass Boston High Performance Computing facility for access and assistance in using
their cluster. This work was funded by the University of Massachusetts Boston Civic
Engagement Scholars Initiative. Sequences from their dataset can be found in Qiita under study number 10572.

References


**Figures and Tables**
Table 1 - Alpha diversity metrics, including Shannon Diversity Index and phylogenetic diversity, for the samples in this study. Number in parentheses are the standard deviation of both dorsal (top) and ventral (bottom) ray samples.

<table>
<thead>
<tr>
<th></th>
<th>Shannon</th>
<th>Phylogenetic diversity</th>
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<tbody>
<tr>
<td>Sediment</td>
<td>9.28</td>
<td>193.91</td>
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<tr>
<td>Inlet</td>
<td>6.40</td>
<td>20.41</td>
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<td>Outlet</td>
<td>8.66</td>
<td>191.94</td>
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<tr>
<td>Biofilm</td>
<td>8.74</td>
<td>55.84</td>
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<tr>
<td>Ray Top</td>
<td>5.16 (1.39)</td>
<td>49.24 (12.47)</td>
</tr>
<tr>
<td>Ray Bottom</td>
<td>4.13 (0.5)</td>
<td>33.69 (11.25)</td>
</tr>
<tr>
<td>Ray1 Top</td>
<td>4.20</td>
<td>29.81</td>
</tr>
<tr>
<td>Ray1 Bottom</td>
<td>3.42</td>
<td>31.13</td>
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<tr>
<td>Ray3 Top</td>
<td>5.01</td>
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<td>Ray12 Bottom</td>
<td>4.85</td>
<td>55.80</td>
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Table 2- Taxonomic composition of OTUs that comprise the core OTUs in figure 3B. OTUs are significantly different between dorsal and ventral surfaces of the rays (Kruskal-Wallis test, p<0.01).

<table>
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<tr>
<th>OTU</th>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus/Species</th>
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<td>Dechloromonas</td>
</tr>
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<tr>
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<td>Dechloromonas</td>
</tr>
<tr>
<td>OTU.21</td>
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<td>Pseudomonadales</td>
<td>Pseudomonadaceae</td>
<td></td>
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<tr>
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<td>Rubrobacteria</td>
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<td>Rhodocyclaceae</td>
<td>Zoogloea</td>
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<td>Acinetobacter</td>
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</table>
Table 3: Taxonomic composition of OTUs that comprise the core OTUs in figure 3B. OTUs are not significantly different between dorsal and ventral surfaces of the rays (Kruskal-Wallis test, p>0.1).

<table>
<thead>
<tr>
<th>OTU</th>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus/Species</th>
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</thead>
<tbody>
<tr>
<td>OTU.27</td>
<td>Proteobacteria</td>
<td>Gammaproteobacteria</td>
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<td>Moraxellaceae</td>
<td>Enhydrobacter</td>
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<td>Novosphingobium</td>
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<td>Psychrobacter</td>
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<td>Rhodocyclaceae</td>
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<td>Gammaproteobacteria</td>
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<td>Pseudomonadaceae</td>
<td>Pseudomonas</td>
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<td>Bacillales</td>
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</tr>
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<td>Gammaproteobacteria</td>
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<td>Enterobacteriaceae</td>
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<td>Xanthomonadales</td>
<td>Xanthomonadaceae</td>
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<td>OTU.43</td>
<td>Proteobacteria</td>
<td>Gammaproteobacteria</td>
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<td>Vibrionaceae</td>
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<td>Flavobacteriales</td>
<td>Weeksellaceae</td>
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<td>Moraxellaceae</td>
<td>Acinetobacter</td>
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<td>Burkholderiales</td>
<td>Comamonadaceae</td>
<td>Limnohabitans</td>
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</table>
Table 4: Percentage of sequences found in samples previously found in human skin microbiome (Grice et al. 2009; Oh et al. 2014). For ray samples, numbers in parentheses are standard error of the mean.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% human associated</th>
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<tbody>
<tr>
<td>Sediment</td>
<td>0.96</td>
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<tr>
<td>Biofilm</td>
<td>0.43</td>
</tr>
<tr>
<td>Inlet water</td>
<td>0.41</td>
</tr>
<tr>
<td>Outlet water</td>
<td>0.45</td>
</tr>
<tr>
<td>Ray top</td>
<td>1.06 (+/- 1.41)</td>
</tr>
<tr>
<td>Ray bottom</td>
<td>1.29 (+/- 0.90)</td>
</tr>
</tbody>
</table>
Figure 1. Principal coordinates analysis of Weighted Unifrac 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 place in the ‘other’ category.

Figure 3. Venn diagram of the core skin microbiome of the cow-nose rays (A). Core microbiome is defined as bacterial taxa found in all samples within a category. The relative abundance of OTUs present in the core microbiome of the ray skin. OTUs core to all ray samples (B) and OTUs core to only one surface of the ray (C). Taxa in (C) are present on either all the dorsal or all the ventral surfaces, but are not present in all of the opposite surfaces. All taxa in (B) are not significantly different between the dorsal and ventral surfaces, while the taxa in (C) are significantly different as determined by a Kruskal-Wallis test.