Nasal microbiome changes in the northern elephant seal as influenced by gender, migration and maternal transmission.

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Abstract:

Marine mammals occupy important niches in the oceans and perform ecosystem function by serving as sentinels of oceanographic conditions in the ocean. Marine mammal's health therefore can provide insight into ocean ecosystem health. Microbiome are now regarded as important factors that contribute to mammalian health, hence understanding the microbiome changes that occur in migrating mammals, such as that of elephant seals is crucial. Male and female elephant seals also exhibit differences not only in migration patterns but also in behavior, which provides an opportunity to understand the influence of gender and maternal transmission on microbiome composition. Here we investigated the microbiome assemblage from the upper respiratory tract of 41 wild northern elephant seals (*Mirounga angustirostris*) along the coast of California. Nasal swabs were collected from 14 adult and juvenile male seals, with three being sampled before and after migration to determine temporal changes in the bacterial community as the animals traverse the Pacific Ocean. Fourteen adult female seals were swabbed along with their respective pups, to determine if maternal transmission plays a role on seal pup's microbiome composition. Seventeen of these adult animals had biologging instruments deployed during their migration that provided high-resolution geographic location of individuals that allowed for the determination of regional oceanic influences on microbiome composition. Sequence data were generated using Illumina Miseq sequencing platform that targeted the V3 to V4 regions of the 16S rDNA gene. Our findings suggest that migration does not change the nasal microbiome composition but gender strongly influences the microbiome composition in elephant seals. Maternal transmission also dictates the composition of microbiome in seal pups but the presence of pathogenic bacteria in seal pup's nasal microbiome consortia indicates that environmental transmission may also play a role. To our knowledge, this is the first paper that characterized the changes in nasal microbiome composition in elephant seals as influenced by migration, gender and maternal transmission. Our data provides novel insights into microbial pathogenic bacteria transfer in mammals and microbiome development of free ranging animals

Introduction

On a global scale, marine mammals can serve as sentinels of oceanographic conditions and can provide insight into ocean ecosystem health (Moore, 2008). Furthermore, marine mammals fulfill the roles of apex predators and secondary consumers, displaying top-down control over the ocean's trophic food web (Estes *et al.* 2011*,* Wells *et al.* 2004). Many species of marine mammals have been impacted by human activities, including the Northern Elephant seal (*Mirounga angustirostris*), and was almost entirely wiped out by commercial sealing operations in the early 1900s. The population reached as low as 20 seals, as evident from strong founder effect seen in the limited genetic diversity of the current population approximated around 300,000 (Lowry *et al.* 2014). Due to the profound effects humans have had on the seals population, they have been the focus of many studies and are one of the most well-studied among pinnipeds (Robinson *et al.* 2012, Le Boeuf *et al*. 1989, Le Boeuf *et al.* 2000). Northern elephant seals have been tracked and recorded to travel thousands of kilometers in the North Pacific Ocean twice a year, before returning to their native colonies with high return rates (Robinson *et al.* 2012). During their migration, they reach the remote northeast Pacific to forage before returning to their colonies (Le Boeuf *et al.* 2000).

Recent sequencing technology has enabled the characterization of microbiome in virtually any organism or environment, including the open ocean (Shokrolla *et al*. 2012). Globally, marine mammal-associated microbiome studies on non-domestic animals have only recently been conducted, due to the difficulty of sampling. Of the studies conducted, they have focused on captive, stranded, or diseased animals, which are not fully representative of the population (Nelson *et al.* 2015). Gut microbiome of some marine mammals such as dolphins, sea lions, seals, and baleen whales (Bik *et al. 2016*, Smith *et al.* 2013, Glad *et al.* 2010, Sanders *et al*.

2013) as well the skin microbiome of humpback whales (Apprill *et al.* 2014) have been characterized.

At present, there are limited studies that investigate the microbiome of the mammalian upper respiratory tract (URP). In a recent study on the URT of bottlenose dolphins, it was concluded that the blowhole environment of the dolphins contained novel bacteria adapted to living in that environment relative to other mammal microbiomes (Johnson *et al.* 2009). Notably, the lung microbiome is expected to be distinct from the gut microbiome, solely because the two systems provide a different environment for bacteria. The gastrointestinal tract is a uniform temperature throughout its entire length, while the mucosa in the respiratory tract possess a gradient from ambient temperature to the core body temperature (Ingenito, 1987). The lungs of the northern elephant seal is a unique environment that is subjected to high pressures and punctuated gas exchange, as elephant seals spend 90% of the time under water (Le Boeuf *et al*. 1989). The airways of the elephant seal have been shown to have elevated levels of carbon monoxide as a result of unique endogenous metabolism (Tift *et al.* 2014). These traits provide a potentially extreme environment that could host a variety of unique bacteria, as no environment is too extreme to host microbial life (Johnson *et al.* 2009).

Female elephant seals serve as an excellent instrument to measure mesopelagic oceanographic environments. Generally, female elephant seals travel straight out towards the open ocean before turning north, largely avoiding the near shore environment along North America (Le Boeuf *et al.* 2000). Once away from the immediate near shore environment, direct human contact is considered to be unlikely (Goldstein *et al.* 2013). Male elephant seals, however, forage and move north along the coast, specializing on benthic feeding (Le Boeuf *et al.* 2000). Seals exposed to the terrestrial environment or nearshore likely have higher exposure to fecal

bacterial inputs from freshwater systems, contributing to diseases (Stoddard *et al.* 2008). These differing patterns in annual migrations allow them to occupy different ecological niches and could allow them to have varying microbiomes.

Northern elephant seals demonstrate extreme capacity for extended foraging dives under apneic conditions at depths of up to 2000m and temperatures down to nearly 2^{o}C (Robinson *et al.* 2012). Other large predator tracking data from loggerhead sea turtles and albacore tuna has indicated that they forage along a high chlorophyll zone called the Transition Zone Chlorophyll Front (Laurs *et al.* 1991, Polovina *et al.* 2000). But female elephant seal satellite data indicates that the seals travel along a gyre-gyre boundary between the sub-polar gyre and the sub-tropical gyre rather than the Transition Zone Chlorophyll Front (Robinson *et al.* 2012). Furthermore, the seals often dive deeper than the deep-sea scattering layer, believed to be in the hunt of small deep ocean lanternfish (Naito *et al.* 2013). At this depth, the seal's nasal surfactant is potentially exposed to mesopelagic bacteria. Mucus coated surfaces have been found to serve as a primary entry point of environmental bacteria to get stuck in mucus in mammalian cells (Barr *et al.* 2013).

Maternal transmission of microbiomes has been studied in the birth of humans comparing the risk of C-section births compared to vaginal birth. Studies have linked vaginally-transmitted microbiome as necessary for the baby's health, as C-section babies may have increased risk of celiac disease (Marild *et al.* 2012), asthma (Couzin-Frankel, 2010), type 1 diabetes (Algert et al. 2009), and obesity (Huh *et al.* 2012, Ajslev *et al.* 2011) in the newborn. Female elephant seals give birth to their young shortly after returning from migration and then nurse a single pup for 24-28 days before abruptly weaning (LeBoeuf *et al.* 1972). Mothers are in close physical contact with their pups, nudging them after birth to create a bond between them through scent

recognition. The pup mainly interacts with their mom (ca. 28 days) until they are large enough to venture away. So far, no studies have investigated if maternal transfer of microbiome exists in marine mammals.

Previous studies have not been able to well-characterize marine mammal microbiomes due to the difficulties associated with marine mammal sampling. In addition, maternal transmission is not well understood. Furthermore, linking geography to the microbiome of free-ranging marine mammal poses difficulty due to the lack of adequate animal tracking under most conditions. In our present study, we investigated various factors that can influence the nasal microbiome composition in elephant seals using advanced DNA sequencing technology, animal tracking, and non-invasive sampling to compare the microbiome composition between male and female elephant seals, the influences of migration on microbiome composition and maternal transmission on microbiome composition of newborn seals.

Methods:

Field Sampling:

Flocked sterile nasopharyngeal swabs (FLOQSwabs #502CS01, Copan flock technologies, Italy) were collected from multiple age classes of seals captured at Año Nuevo State Reserve in San Mateo County, California. Seals were chemically immobilized for sample collection and instrument attachment and recovery using established techniques (Le Boeuf *et al.* 1989, Le Boeuf *et al.* 2000). Animals were free-ranging wild seals, and had limited contact with humans. Swabs were inserted into either nostril of animals, and rotated along the mucosa cell lining of the nose. Swabs were then inserted into a sterile 1.5mL centrifuge tube and clipped using isopropyl-wipe sterilized micro-wire clippers. After collection, swabs were transported on ice prior to freezing temporarily at -20^{o}C and then stored at -80^{o}C until laboratory analysis.

Nasal swabs were collected from adult female Northern elephant seals at Año Nuevo (4-8 years of age, n= 14) and their respective pups (3-5 days, n= 13) during the 2015-2016 breeding season to recover satellite instruments (SPOT5, MK10-AF and MK9, Wildlife Computers, Redmond, WA or SMRU, St. Andrews, UK) after the animals returned from their annual migration. Pups were documented daily from birth until sampling to ensure pairing and age of pup.

Nasal swabs were collected from male Northern elephant seals (4-10 years of age, n= 13) at Año Nuevo State Reserve in San Mateo County, California, during their molt haul out in 2015. Animals were part of ongoing physiological studies or to be equipped with advanced satellite tracking instruments (SMRU, St. Andrews, UK) prior to their annual migration to Alaska and

back. Upon return to Año Nuevo State Reserve in the 2015-2016 breeding season, nasal swabs were again collected during satellite instrument recoveries at Año Nuevo, and San Simeon Pier, San Simeon, CA. (n= 3).

Nasal swabs were collected from a juvenile male Northern elephant seal (1 year of age, n= 1) prior to translocation from Año Nuevo to Hopkins Marine Station in Monterey, California. The seal was equipped with a satellite instrument (SPOT6, Wildlife Computers, Richmond, WA) and returned to Año Nuevo after swimming across Monterey Bay over the course of 36 hours, and was nasally-swabbed during instrument recovery. This animal was excluded from statistical comparisons due to different age, time, and location of experiment.

16S rDNA Extraction and Quantification

Nasal bacterial samples were processed in a type A2 biological safety cabinet sterilized with ethanol and UV. DNA was extracted from thawed swab samples by adding two pieces of 2mm sterile glass beads and 50μg 0.1mm sterile glass beads, mixed with 400 μL of ATL buffer (Qiagen, USA) and bead beat for two minutes using a MoBio 2-ml vortex adapter at maximum speed. A 25-μL proteinase K was added and incubated at 56^{o}C for 2 hours. DNA was isolated with a DNeasy Blood and Tissue DNA extraction Kit (Qiagen, USA). Isolated DNA was eluted from the DNeasy spin column using 50 or 100 μL of PCR-grade water (Ambion). Control isolations were extracted after every 6 samples, and were processed the same way as the samples but without the swab. Isolated DNA samples were stored at -20^{o}C.

To check for quality of DNA extract, 16S rDNA was amplified from all samples. Preparation of PCR reactions was carried out under a PCR hood station that was both UV-

sterilized and surface-sterilized with ethanol. A 25uL PCR reaction was prepared using GoTaq Green Mix (Promega, USA) Taq polymerase with universal bacterial primers pairs 515F and 806R on a Thermocycler (C1000 Touch, Bio-Rad). An aliquot (10uL) of each PCR product was ran on a 1% agarose gel stained with Gel Red (Biotium, USA). A 100bp small ladder (Fischer Scientific) was used as a marker. Agarose was ran for 35mins at 110V. Agarose gels were visualized on a UV transilluminator (Spectroline). Single banding at 300bp indicated presence of bacterial DNA from swabs.

A 2-μL aliquot of each isolated DNA sample was processed through a Quant-iT PicoGreen dsDNA Assay (Molecular Probes, Life Technologies) to determine dsDNA quantity and quality. Assays were carried out on black 96-well plates in sterile, dark cabinet according to kit protocols. Standards were done in triplicates using 0-200 ng/mL concentrations to generate standard curves. Assays were analyzed in duplicate on a SpectraMax M2e spectrophotometer (Molecular Devices). Sample concentrations were quantified based on 480 nm absorbance.

Illumina Sequencing

DNA aliquots were shipped on dry ice to the University of Illinois, Chicago DNA Services (DNAS) for sequencing of the 16S rDNA gene using primers 515F 5'-GTGCCAGCMGCCGCGGTAA -3' and 806R 5'-GGACTACHVGGGTWTCTAAT -3' on an Illumina MiSeq platform (Illumina) employing V2 chemistry (2x 256). Forward and reverse reads were merged using PEAR (Paired-End reAd mergeR) software (Zhang *et al*. 2014). Ambiguous nucleotides and adapter sequences were trimmed from the ends, and reads with internal ambiguous nucleotides discarded. Reads were trimmed to a quality threshold of $p = 0.01$. After trimming, reads that were less than 350 base pairs were discarded. Reads were screened for

chimeric sequences using the USEARCH algorithm with the GreenGenes 13.8 database as a reference (Edgar, 2010, Mcdonald *et al.* 2012).

Sequence Data Preparation

16S rDNA reads were analyzed using Quantitative Insights Into Molecular Ecology. (QIIME 1.8 pipeline (Caporaso *et al* 2010). Operational Taxonomic Units (OTUs) were clustered at 97% sequence similarity using UCLUST (Macdonald *et al.* 2012) and representative sequences from clustered OTUs were used for taxonomic identification. OTU representative sequences were aligned with PyNast (Caporaso *et al.* 2010) using Greengenes core alignment as a reference. Singleton sequences were removed prior to using the 16S Greengenes alignment lane to generate a phylogenetic tree using Fast Tree 1.4.2 OTUs were assigned taxonomy by RDP classifier 2.2 (Prince *et al.* 2010)

Core microbiome analysis and taxa determination

Samples were rarified at a minimum of 760 sequences and a maximum depth of 7600 sequences in 10 steps. The microbial diversity of all samples were grouped into categories (age, sex) and assessed using 2 alpha diversity indices (number of observed OTU's, phylogenetic diversity) at 7600 sequence depth. Microbiome analysis assignment of bacterial taxon was performed using the UCLUST (Edgar *et al.* 2010) taxonomy assigner in QIIME. To determine the differences of bacterial communities of different ages and sexes, beta diversity, and UPGMA tree clusters were jackknifed at an equal depth of 1000 sequences with 1000 iterations. Unweighted UniFrac distance matrices derived from 7600 sequence rarified beta diversities was used to test the significance of different microbial communities between age, sex, and migration using ANOSIM

and PERMANOVA. Animals were grouped according to ages adult (above 4 and 6 years old for males and females respectively) , subadult (3-5 year old males), pup (5-9 days old), juvenile (~1 year old).

Determination of animal tracks

All equipped animals contained a 0.5W ARGOS satellite transmitter (Wildlife Computers, Belleview, WA, USA: SPOT4, SPOT5, MK10-AF; or Sea Mammal Research Unit, St. Andrews, Scotland: SRDL-CTD) with a ~45 second repetition rate. ARGOS poor quality location classes (A and B), like previous investigations, were prevalent (Robinson *et al.* 2012). A state-space model was used to smooth the tracking data and obtain hourly position estimates using the CRAWL package in R (Johnson *et al.* 2008, R-Development, 2011), incorporating estimates of at-sea ARGOS error (Costa *et al.* 2010).

Ethics Statement:

This work was completed under National Marine Fisheries Service Marine Mammal permit # 19108. The animal use protocol for all procedures on free-ranging elephant seals was reviewed and approved by the University of California at Santa Cruz Institutional Animal Care and Use Committee and followed the guidelines established by the Canadian Council on Animal Care and the ethics committee of the Society of Marine Mammalogy.

Results:

Microbiome composition of elephant seals within age classes

The 16S rDNA gene was sequenced from 46 samples and 1 pooled extraction control. Of those, 4 samples failed to generate more than 100 reads and were excluded from further analysis. After denoising, samples generated 48,304 to 184,270 sequences per sample while the control generated 18,893 sequences. After singleton removal, and removal of control OTUs, 15,163 OTUs were represented. 271 OTUs were present in 50% of the elephant seals representing the elephant seal core microbiome from these samples. The most abundant bacteria genera found were *Helcococcus* (23.4%), *Porphyromonas* (8.1%), *Corynebacterium* (5.3%), *Psychrobacter* (4.9%) and some unknown taxa (9.1%).

In order to define the microbiome of elephant seals, we compared microbiome diversity of different age classes of animals to each other consisting of adult animals of both sexes, sub adult males, pups of both sexes, and a juvenile male. Pups in this study exhibited higher bacterial diversity than any other age class in this study. (Figs.1 and 2), while adult and subadult animals contained similar bacterial diversities.

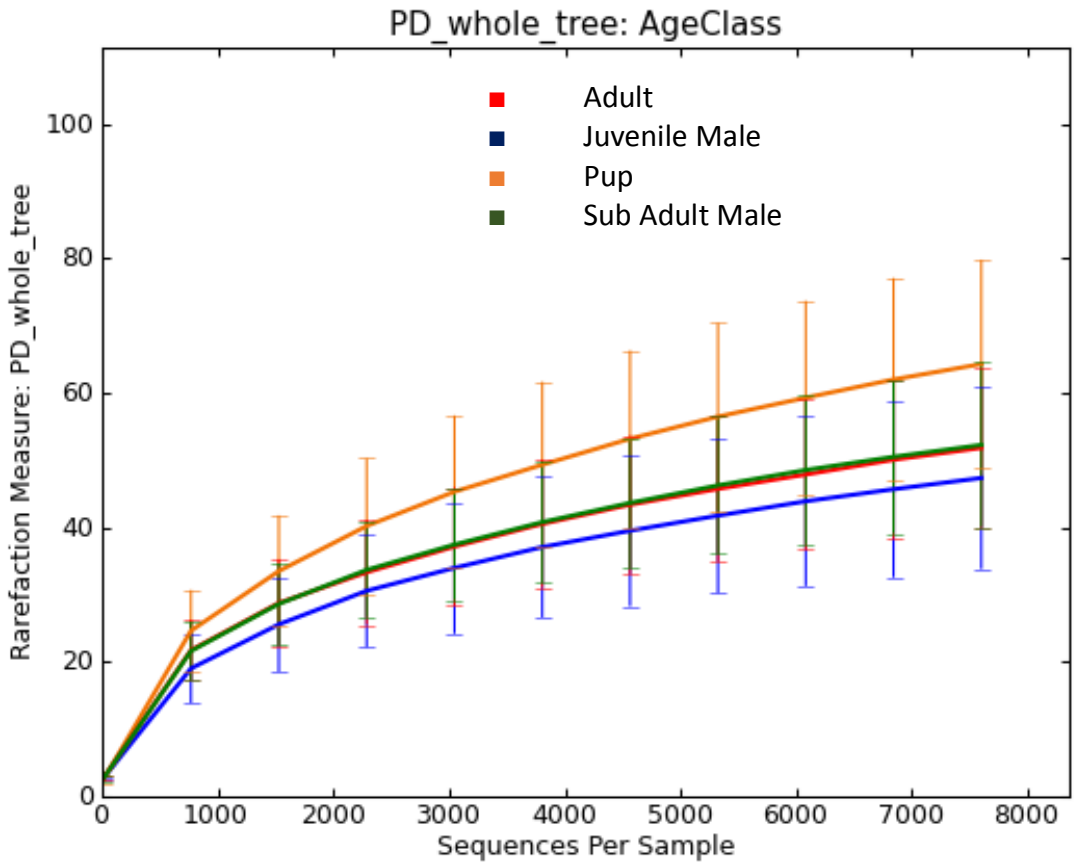

Figure 1. Rarefaction curve generating alpha diversity within sample. Alpha diversity of all OTUs. Error bars represent standard deviation.

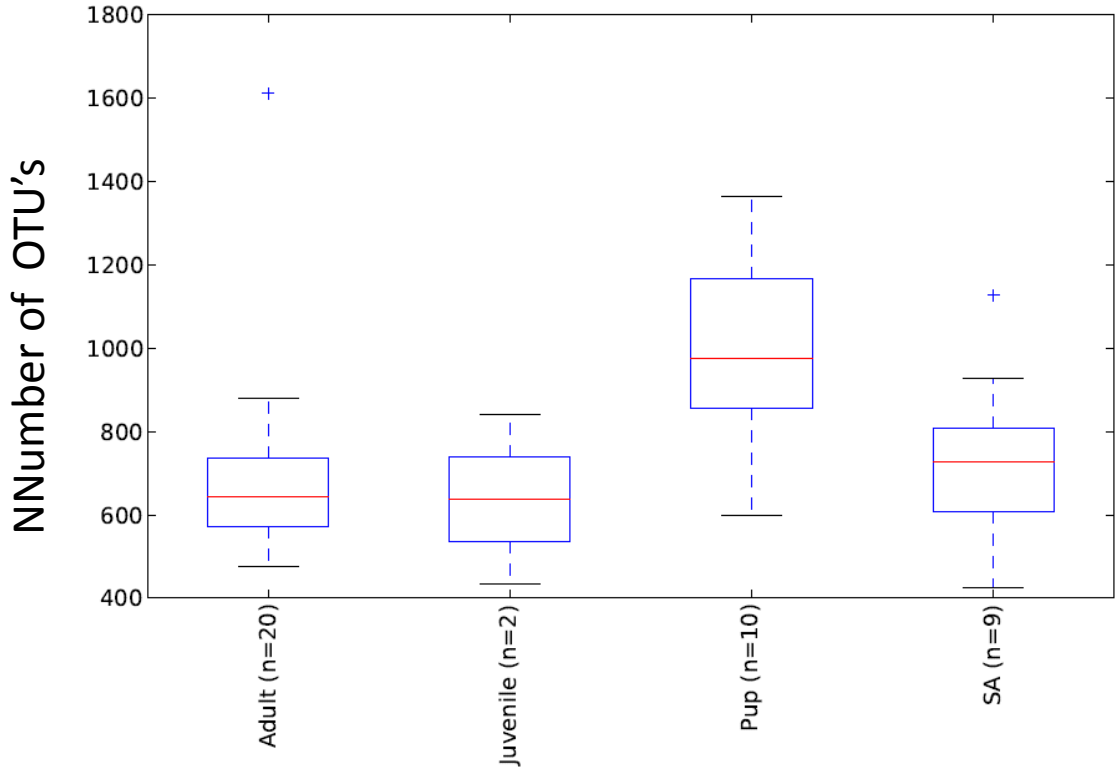

Figure 2. Differences in OTU diversity between age classes. Boxplots of observed number of OTU's at 7600 sequence. Red line represents median, edges of blue box are 25th and 75th percentiles, whiskers extend to extreme data points. Outliers are graphed as individual blue points. SA: Subadult

To investigate the effect of age, we sampled male seals that demonstrate dominance behavior and aggressive control over females by mass and nose size. Sub-adult male seals suffer high death rates due to inexperience in foraging, and are harassed easily by larger animals as they lack experience in fighting. We found that the microbiomes of male seals are similar regardless if they are sub-adult or adult (Fig. 3B).

Microbiome composition of elephant seals between genders

Male and female elephant seals have distinct social structure in a colony, and distinct migration routes. Here, we ask if elephant seals exhibit different microbiome composition based on gender and compared the microbiome composition between adult female and adult male animals. We

compared adults that have recently came back from migration to minimize temporal variation between samples. Overall, the elephant seal microbiome contained a small number of dominant genera, appearing to be different between adult males and females (Fig. 3A). The difference in adult microbiome composition between males and females was found to be statistically significant (Table 1).

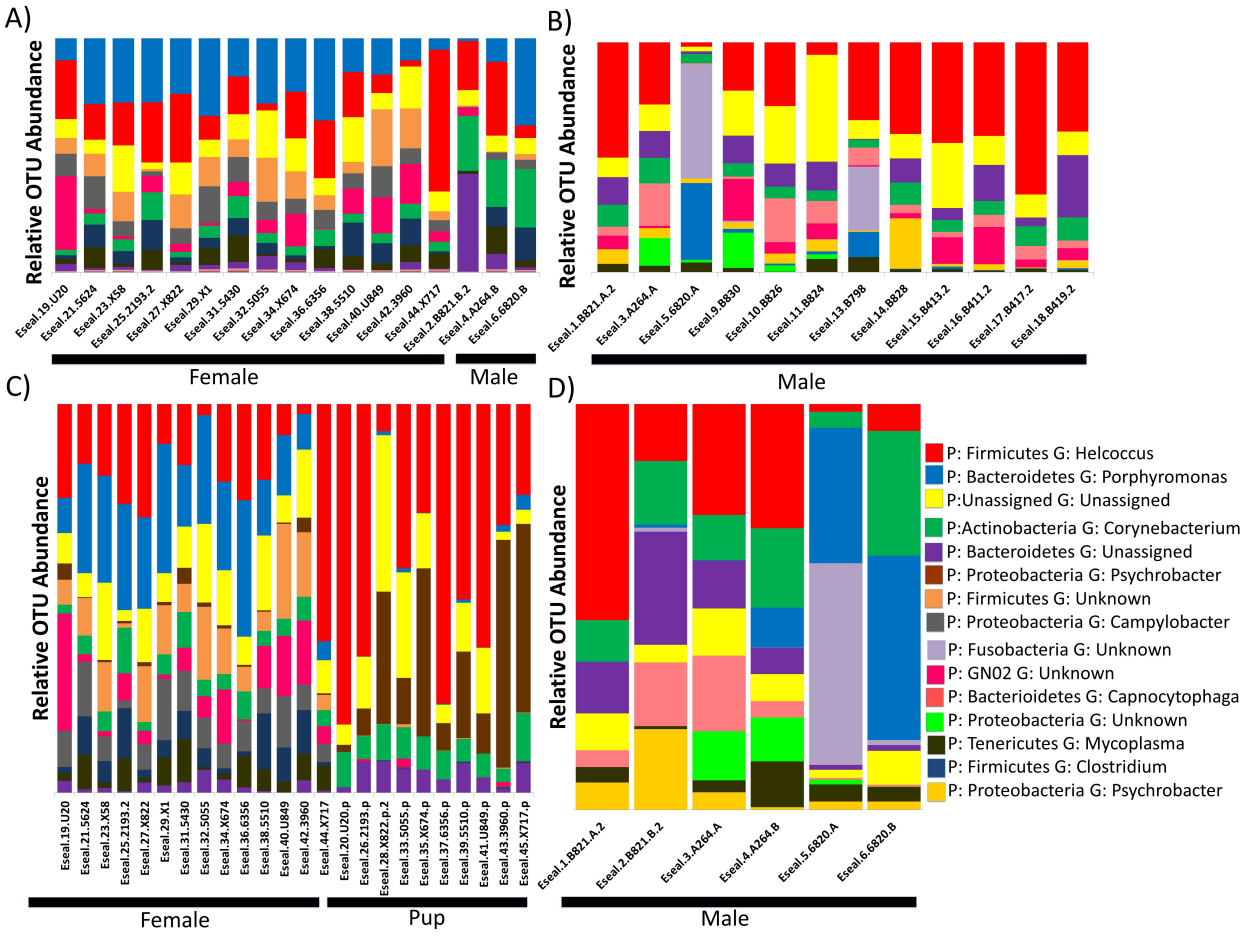

Figure 3. Bacterial genera representation of most dominant 16 OTUs. Cutoff was top 10% across all samples. A) Comparison between females and males during breeding season. B) Comparison between males after molting. C) Comparison of adult females and pups during breeding season. D) Comparison of before and after migration in adult males

Comparison	ANOSIM R	p-value	PERMANOVA pseudo-F	p-value
Male vs Female after migration	0.9666	0.01*	2.3911	0.003*
Male Before Vs Male after migration	0.0741	0.39	1.689	0.305
North Male vs North Female After Migration	0.1667	0.34	1.1537	0.291
Female after migration vs Pup	0.9104	0.01*	5.7738	0.001*

Table 1. Statistical summary of different age classes and migration patterns of elephant seals. Asterisks indicate statistical significance at $p<0.05$. Statistical comparisons were conducted using ANOSIM and PERMANOVA between categories. Comparisons between male (n=3) and female animals (n=12) after migration, comparison of the same individual male seals of before (n=3) and after migration (n=3), male (n=3) and female (n=2) seals with similar northern migrations, and comparison of female seals (n =12) and pups (n=10). ANOSIM and PERMANOVA was generated using similarity matrix comparison generated from 7600 rarified sequences.

We investigated the differences in microbiome by comparing the dominant genera between individuals. We observed that an unclassified bacteria genera from Firmicutes phylum was dominant only during the breeding season, mainly in the adult females, and not in males. We also observed a decrease in occurrence of an unknown genera in GN02 phylum in males compared to females (Fig. 3A).

Microbiome composition of elephant seals as influenced by migration

To determine if migration influences the nasal microbiome of elephant seals, we compared animal microbiomes of satellite tracked animals (Fig. 4A). We compared the same individual animals' microbiome before and after migration in adult males. Interestingly, a changed in relative abundance of the microbiome members was observed among adult male animals before and after migration (Fig. 3D) but the changes were not statistically significantly (Table 1).

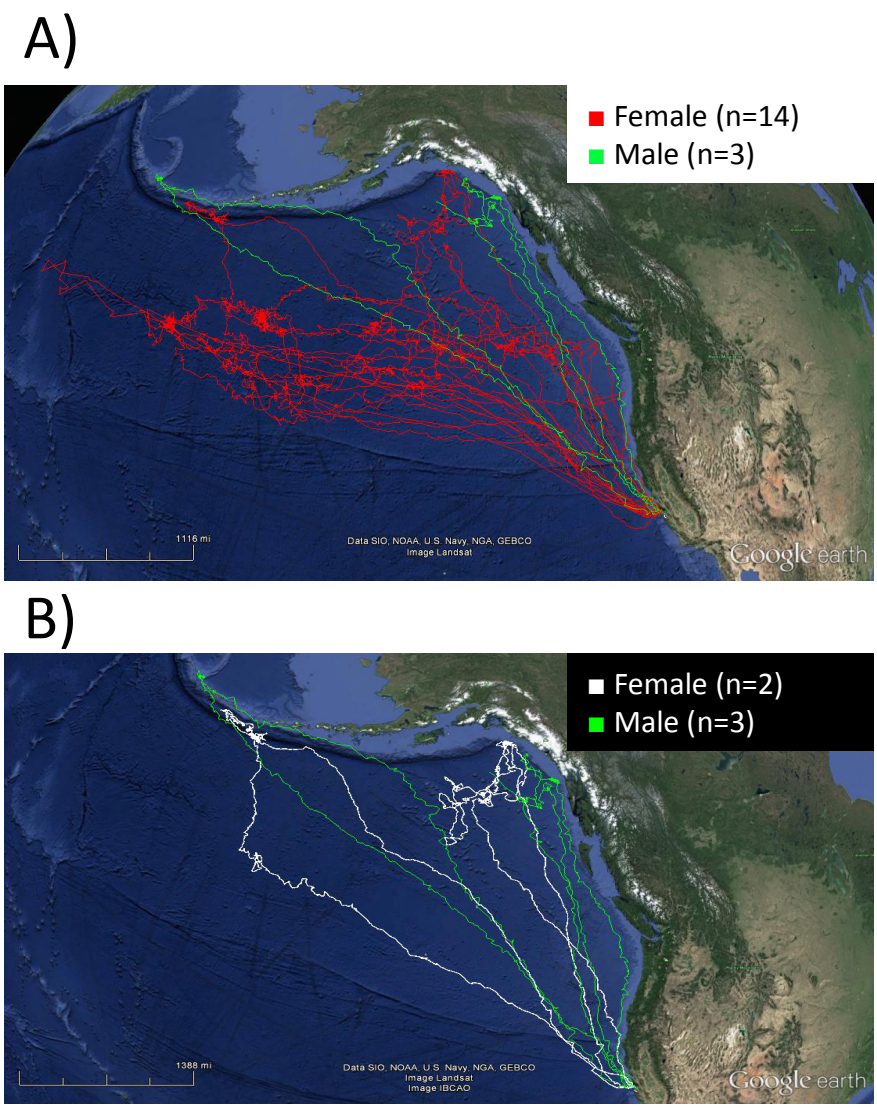

Figure 4. Argos satellite positions of equipped animals in this study. A) Animals in this study follow the trend that female seals and male seals forging in different environments of the north pacific. B) Female animals compared to males whose tracks overlap. These animals represent the northern migration animals used to determine statistical comparisons in bacterial composition between male and female. Tracks corrected using CRAWL package in R. Animals originate from Año Nuevo, CA.

To further investigate the consequence of migration on the elephant seal microbiome, we looked at the microbiome composition of males and females with the same migration route. Two females were found to have similar migration routes as the male seals (Fig. 4B). Interestingly, we found that their microbiome composition were not significantly different. (Table 1)

Maternal transmission of microbiome

To determine if there is maternal transmission of microbiome from adult female to pup, we compared the microbiome between a seal mother and its pup (Fig. 3C). We found that the microbiome of the mothers and their pups is significantly different (Table 1). Similarity of bacterial microbiome composition and structure between animals was shown as principal coordinates of the unweighted UniFrac distances (Fig. 5). We found that adult females and pups form distinct clusters. Adult female seals appear to have a consistent microbiome composition, and distinct from the pups or males. The first principal coordinate (PC1) explains 14% of the inter-sample variation and the second principal coordinate explains 7% of the inter-sample variation.

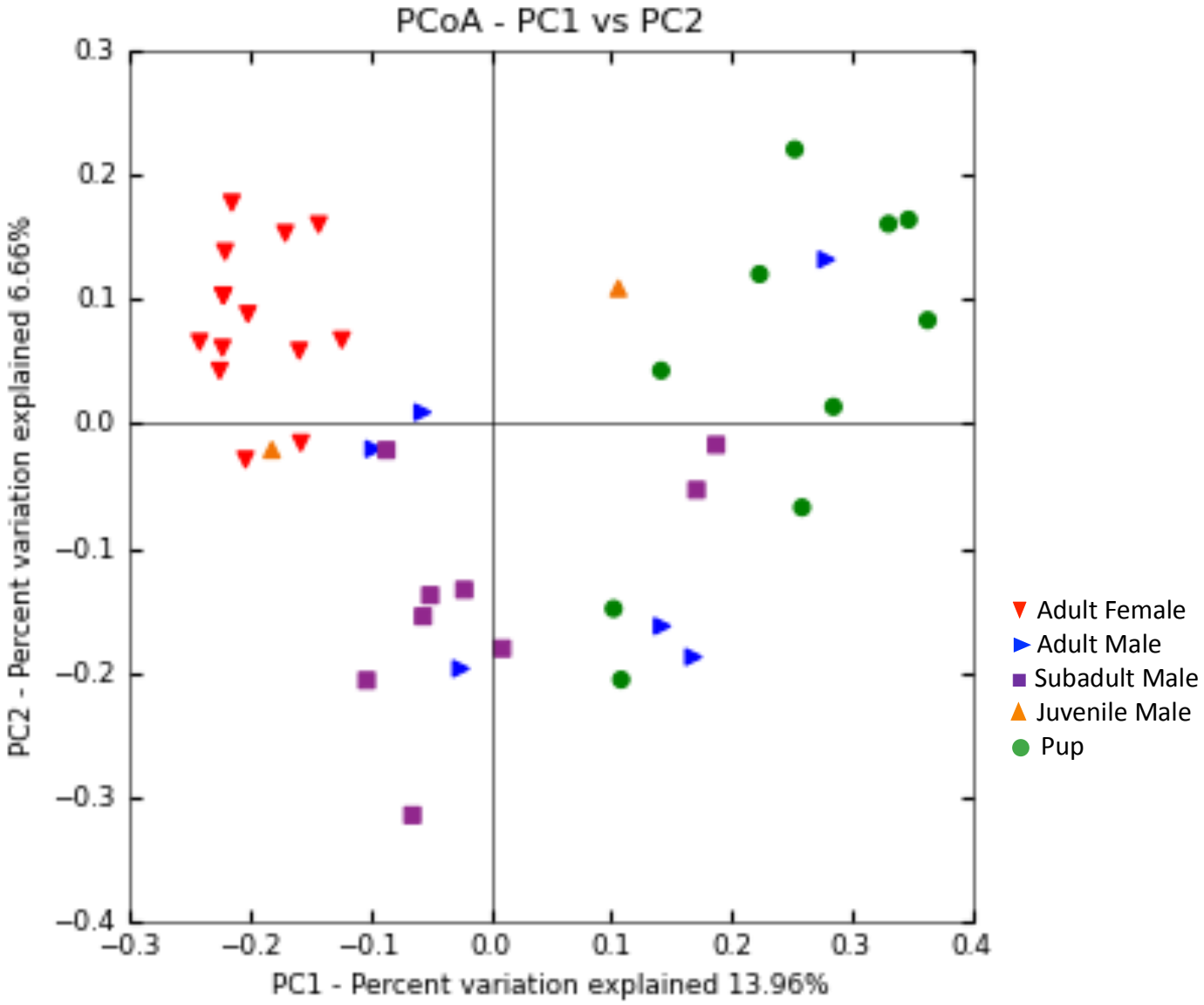

Figure 5. Beta diversity sample difference was generated from 7600 rarified sequences and used to construct a principal coordinate plot. Each axis represents maximum variation between samples, constructed from Unweighted UniFrac distances. All animals included in PCoA plot.

We investigated the dominant bacteria found in mothers and pups to see which bacterial genera were dominant and possibly transmitted. *Psychrobacter* were observed as minor

components in adult female animals, but in their respective pups there is an increase in relative abundance. The genera *Clostridium*, *Mycoplasma, Camplyobacter* and *Porphyromonas* were not the dominant bacteria in the pups but they were high in relative abundance among their mothers.

Discussion:

This is the first study that reports the microbiome of northern elephant seals, but more importantly the first to pair satellite tracked wild animals and investigate the influence of gender, migration and maternal transmission in elephant seals. Previous studies on living cetaceans found no relationships between gender or location on respiratory microbiomes (Bik *et al.* 2016, Lima *et al.* 2012), and to our knowledge no studies have linked adults to their newborn offspring for microbiome comparison in marine mammals. We found differences in OTU diversity between age classes which cannot be explained by the beach environment, as nearly every animal was on the same beach and mothers and pups were less than a meter apart, indicating an age-related effect. The microbiome is understudied in developing animals, and this provides support that young individuals do not necessarily exhibit the same microbiome as other individuals in the population. Furthermore, because the pups harbor more diverse bacteria, it is possible that these bacteria are able to opportunistically infect the pup, but not mature adult individuals. Additionally, if the pup microbiome is immature, it could be more susceptible to environmental bacteria from the beach they inhabit, as their nose is buried in the ground or just above it as they lay on it. Often during sampling, the pups had sand present in their nose, providing a means for environmental bacteria to enter.

We speculate that the observed variation in microbiome between gender seen in elephant seals could be caused by a number of factors. It is possible that the nose morphology brought about the difference, as male noses are larger and the nostril openings are always open, in comparison to females whose nose can open and close, as similar to canines. In previous studies investigating gender-linked microbiomes in humans, it has been found that gender influences diversity of OTUs (Dominianni *et al.* 2015). The cause of gender-linked microbiomes has been

elusive and has been implicated with differences in hormones, physiology, and behavior between human genders (Markle *et al.* 2013). Males and female elephant seals are also different in many aspects such as size, dominance behavior and migratory path at sea that may likely contribute to this variation in microbiome between gender.

We observed that the adult female microbiome has significantly different dominant genera than their pups. Over the ca. 5 days of age in the measured pups, adult female seals spend all of their time with their pups; they do not forage or leave the beach. During this time, mothers are nearby their pups, protecting them from other females and nursing them. Paired samples from mother and pup were taken within minutes of each other, limiting the effects of time on the microbiome sampling between the animals. The observed differences and distinct clustering within samples (Fig. 5) suggest that maturation of the microbiome is yet to occur pups. In humans, initial composition of babies changes into a permanently colonized adult microbiome over the course of years (Palmer *et al. 2007*). These early microbiome of the nursing pups may either be reservoirs of pathogenic bacteria or may play an important role in scent recognition.

Although we detected differences in microbiome composition between genders, we found that male and female seals that have taken somewhat similar migration route (both that went to the far North Pacific) had microbiome compositions that were not significantly different. This may hint that migration may play a role in changing the nasal microbiome composition of the seals. This comparison unfortunately does not have a large sample size (n =2 for females, n=3 for males). It is difficult to predict female seals that will go north, so deploying satellite instruments on a female demonstrating male migration behavior is rare, and in our study, appears in less than 10% of females. Interestingly, we did not detect any change in microbiome composition in males that went through the same migration route.

We detected a number of deep-water marine bacteria present in the majority of pups, despite the animals having never entered that water, including *Sphingopyxis alaskensis*, and *Psychrobacter pacificensis*. This indicates that marine bacteria transfer vertically from adult female to her pup, and that adult females can carry the bacteria to shore and give it to their pups. Transmission could have occurred through mother pup bonding as often seal pups rub their noses on the faces and bodies of their mother, providing a route for bacteria to enter their nose.

The genus *Mycoplasma* was only detectable in adult animals; this suggests that the zoonotic bacteria that cause "sealfinger" in humans enter the microbiome via the ocean, not inshore. In addition, the genera *Psychrobacter* displayed more dominance in pup microbiomes, than in adult females. *Psychrobacter* bacteria generally occur in saline environments, and are found in deep trenches of the Pacific ocean (Maruyama *et al.* 2010). In addition, several OTUs of the genus *Ornithobacterium*, a common infectious bird respiratory microbe, were found in all of the pups. It is possible that birds serve as a vector for this group of bacteria. The possibility of microbial transfer between birds and elephant seals has been brought up in the viral isolation of pandemic H1N1 in elephant seals (Goldstein *et al.* 2013). They concluded that elephant seals and marine mammals can be asymptomatic carriers of zoonotic diseases. Our study reinforces that potential pathogenic bacteria can be present in these animals but without the animals expressing clinical symptoms.

This study provides novel insights into the factors that can shape the microbiome of wild marine mammals, implicating that gender, age and possibly migration can dictate the structure of elephant seal microbiome. Future investigations such as the understanding the association of deep-sea *Pyschrobacter* with the elephant seal pups and to determine why and how it can thrive in a strictly land-based organism are an interesting venue for research. Moreover, future studies

may be able to illuminate novel transmission and disease vectors in elephant seals, which can contribute to understanding the health of these species and further improve ways on how to conserve this endangered marine organism.

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