

Unlocking the secrets of a breath: Characterizing the microbiome and immunome of the respiratory tract of the blue whale (*Balaenoptera musculus*)

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SUMMARY REPORT

The composition of the microbiome of the respiratory tract is determined by the balance of essential factors such as microbial migration and regulation of communities due to competition among microorganisms and elimination of microorganisms by the immune system. The microbiota of the respiratory tract plays an important role in preventing the colonization and establishment of respiratory pathogens, as well as in the maturation and maintenance of respiratory homeostasis. In this study we used next generation sequencing (NGS) and non-invasive drone-based blow collection to characterize the respiratory microbiome of blue whales. Our research aimed to establish baseline levels of the bacterial communities. More importantly, we propose using this information to infer potential pathogenic threats to blue whale respiratory health.

Our study was conducted in the Bahía de Loreto National Park located in the Gulf of California, Mexico. Focal follows of blue whales was carried out in 2016 and 2017, during the months of February to April. Blow samples were collected using a Phantom 3[®] quadcopter drone, which flew three to four meters above the whales. The genomic DNA was obtained with the QIAamp[®] DNA Mini Kit as per the manufacturer's instructions. V3-V4 hypervariable regions of the 16s rRNA gene were amplified using MiSeq 2- by 250pb (Illumina[®] technology) in the Institute of Genomic Medicine (Inmegen) in Mexico. Raw sequences were assembled and filtered according to quality ($Q > 25$) with Mothur (v.1.39.5). The amplicon sequence variants (ASV) were inferred with the R package dada2 (v.1.9.1). The phyloseq and microbiome packages in R were used to calculate the alpha and beta diversities, as well as the diversity indices of Shannon and Simpson.

Seventeen blow samples and three technical controls were sequenced (seawater, human sneeze and PCR blank). In total 379,813 sequences were identified, corresponding to 7,826 operational taxonomic units (OTUs). The rarefaction curves show that the sampling coverage was greater than 96% in all the samples, indicating that the sampling effort of the communities was good. Sample richness ranged from 192 to 1191, with an average of 563 OTUs. The variability in sample richness could be affected by the volume of sample collected during each drone flight, which unfortunately could not be accounted for. Blow samples contained bacterial classes that were not identified in any of the control samples such as *Acidimicrobia*, *Actinobacteria*, *Alphaproteobacteria*, *Campylobacteria*, *Erysipelotrichia*, *Leptospirae*, *Mollicutes*, and *Oxyphotobacteria*. Only one whale blow harbored *Mycoplasma* spp. Seventeen ASV were present in more than 50% of the samples and were considered as the core microbiome in the respiratory tract of blue whales in the Gulf of California.

This is the first study to characterize the bacterial microbiome of the blue whales' respiratory tract. We show that the blue whale population in the Gulf of California harbors similar bacterial composition among individuals, with a respiratory microbiome shared by more than 50% of all individuals sampled. Being able to determine the central microbiome of a particular anatomical region in a host is very important because the persistence of this bacteria in the majority of individuals suggests that these microorganisms can be beneficial for the species. In addition, our results of relative abundance are comparable to those for healthy domestic animals and humans, so we propose that the identified core respiratory microbiome of blue whales can be used as a reference for future long-term health studies.

These results have been shown in the 2018 International Association for Aquatic Animal Medicine Meeting and Conference with the title **What's in there? Discovering the Blue Whales' (*Balaenoptera musculus*) Respiratory Tract Microbiome**. Also, a manuscript (Core respiratory microbiome of the blue whale, *Balaenoptera musculus*, by Domínguez-Sánchez CA, Álvarez-Martínez RC, Gendron D and Acevedo-Whitehouse K) has been written and will shortly be submitted to the Journal of Environmental Microbiology. This manuscript gives credit to the SMM grant received.