

# Effects of Long-term Chlorinated Water Exposure on the Nasal Microbiome

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## Abstract.

It is known from many studies that the human body carries trillions of microorganisms at many different body sites. The composition and diversity of the microbes can be analyzed through the diversity of microbial genetic information in a sample also known as the microbiome. The composition of the human microbiome at these different body sites is influenced by both environmental factors and host genetics. For this study we focused on an environmental factor, more specifically the effect of long term chlorine exposure on the composition of the microbiome in the human nares. To accomplish this task nasal swab samples were collected from competitive swimmers as well as semi-professional basketball players to compare the nasal microbiome of athletes the exposed to chlorine on a daily basis and athletes that are not. The swab samples were used to extract bacterial DNA, which was used to do 16S rRNA gene sequence analysis with Qiime 2 microbiome analysis software. The 16S rRNA gene is the most commonly used marker in microbiome research that allows for the composition of the microbial community to be determined in a sample. Preliminary results in this experiment show that there are no significant differences between the microbiome of athletes who are exposed to chlorine regularly and those athletes that are not. Further experimentation will be done though quantifying the amount of bacteria and fungi in each sample to determine whether prolonged chlorine exposure has any effect on the nasal microbiome.

## Introduction.

Environmental factors are known to play a role in the composition of the microbiome at many different body sites. Of these body sites, the nares are one that are constantly exposed to the external environment. There are many different chemical compounds that can access the nares as an individual goes through their daily tasks and prolonged exposure could affect the composition of the nasal microbiome, and potentially overall health. In this study we studied the affect of prolonged exposure to chlorinated water and what effect this would have on the composition of the nasal microbiome. We hypothesized that chronic exposure would alter microbiome diversity or composition.

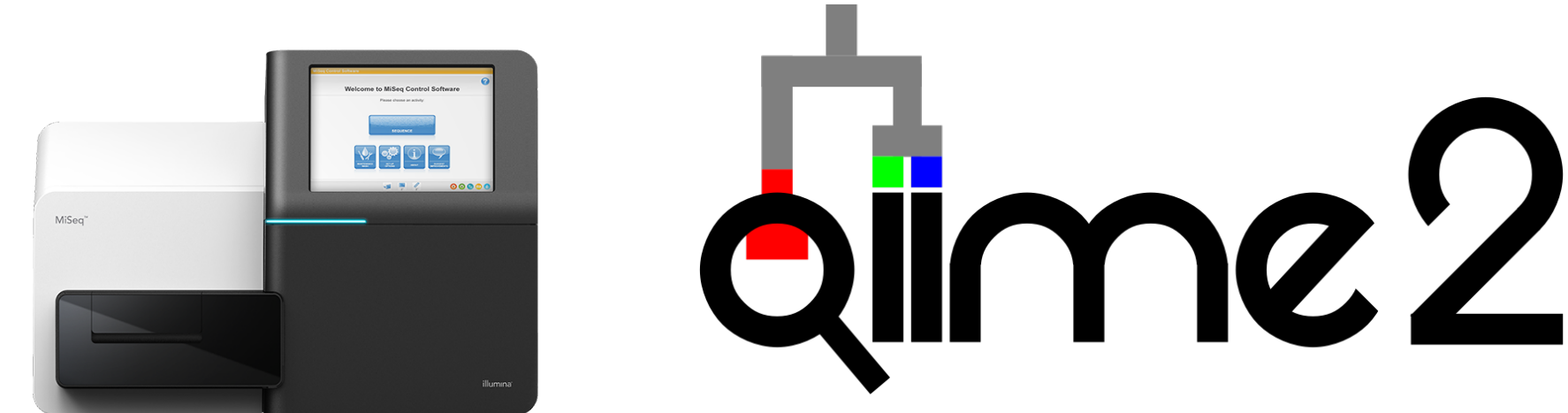
## Methods.

**Table 1:** Number of samples collected for each test group

Sampling	Number of Samples
Chlorine Exposed, Baseline	21
Chlorine Exposed, 2nd	29
Chlorine Non-exposed	12

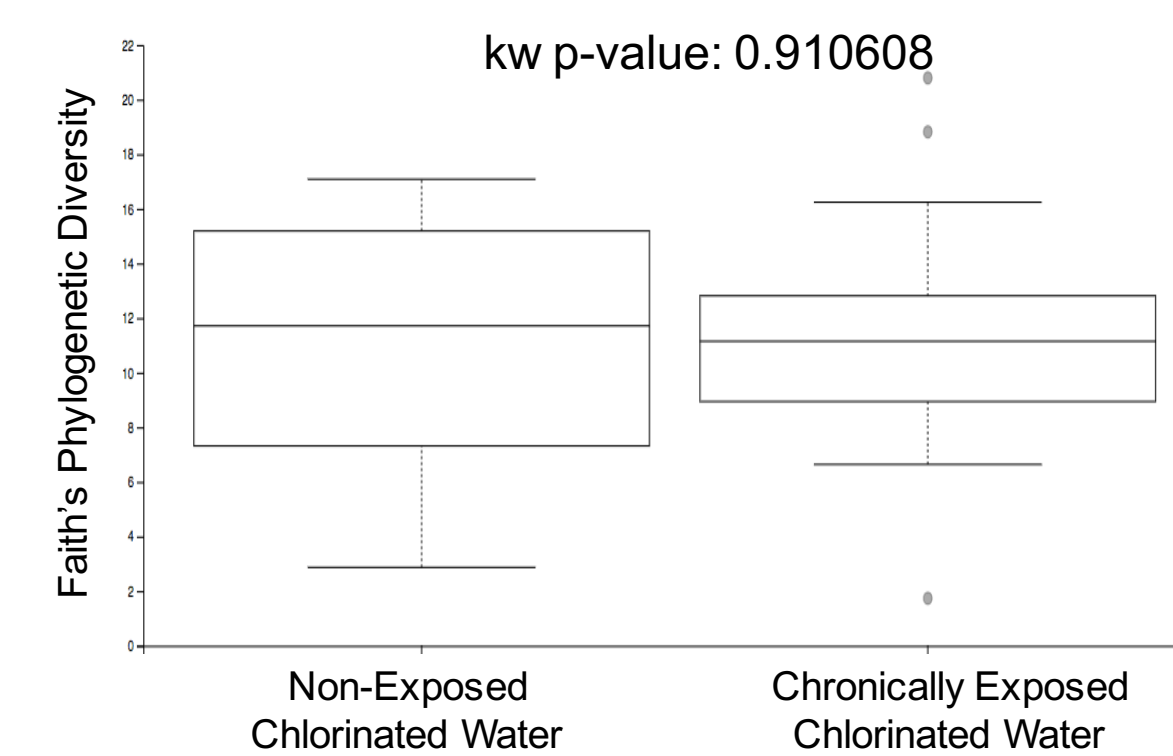
### 16s Sequencing on Illumina MiSeq platform.

Nasal swab samples were extracted using a Qiagen Powersoil kit per manufacturer instructions. PCR primers F515/806 were used to amplify the V4 region of the 16s rRNA gene and sequenced on the Illumina MiSeq. Sequences were quality filtered and processed using QIIME2 2017.12.

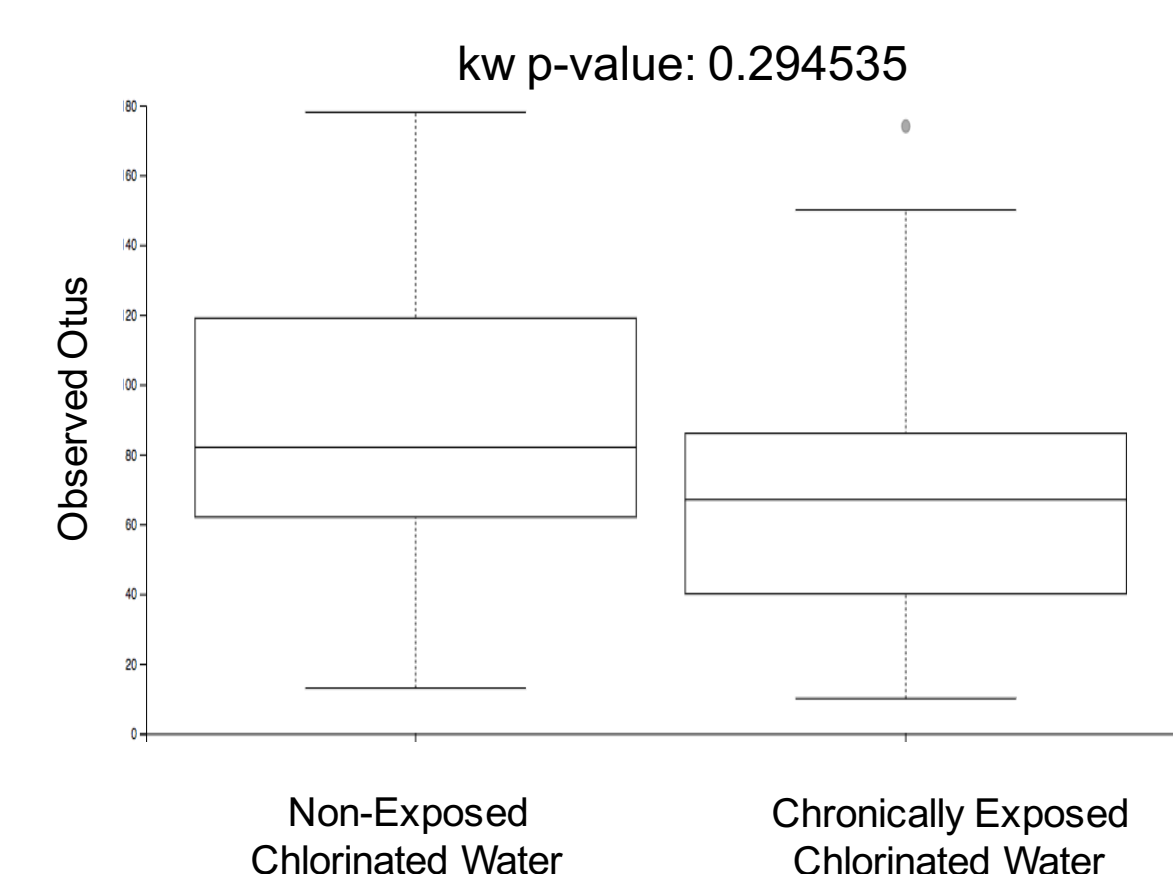


## α-Diversity

### Baseline Sampling: Participants who are Chronically Exposed to Chlorinated Water v. Non-Exposed Age- and Sex-matched participants

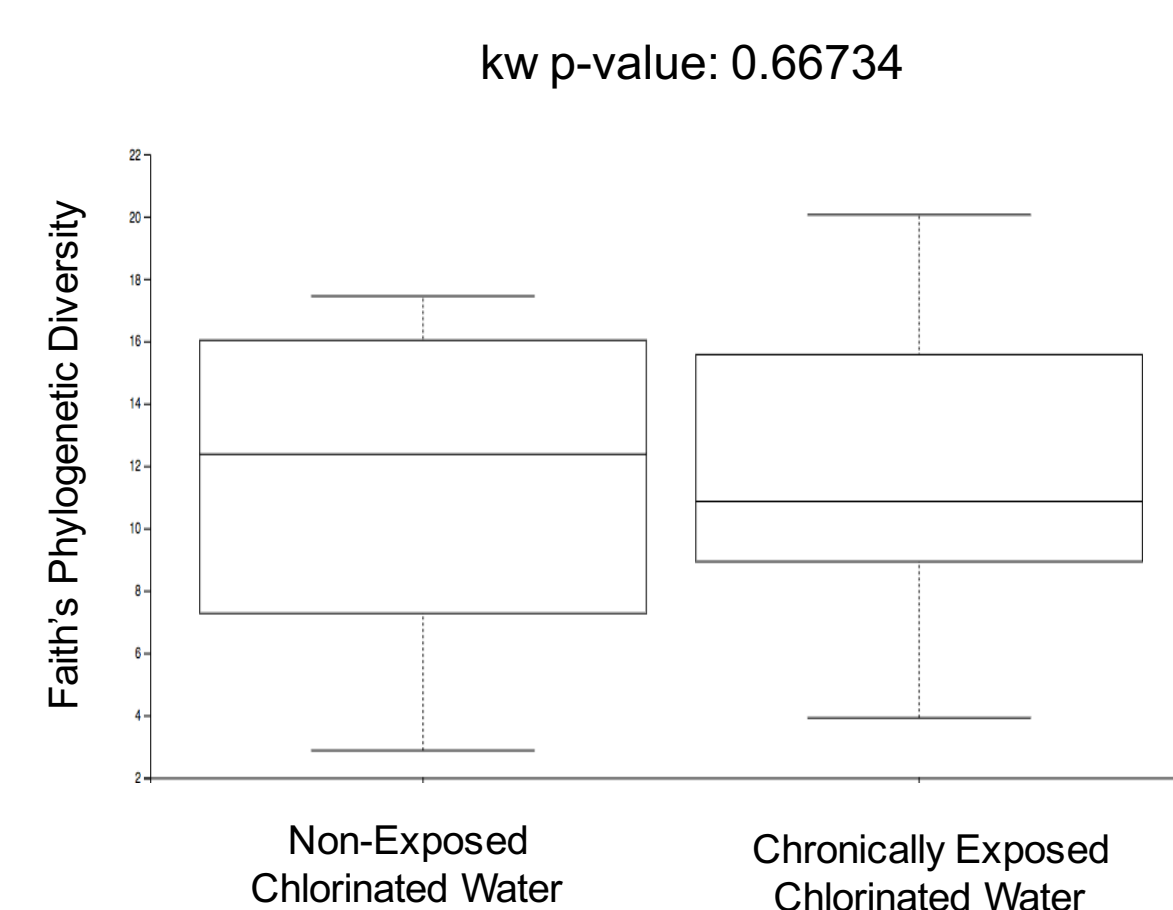


**Figure 1:** Faith's Phylogenetic Diversity of individual's nasal microbiota from participants who are exposed to chlorinated water at time point 1 and participants who are not exposed. No significant differences in phylogenetic diversity were noted [ $p > 0.05$ , Kruskal Wallis (kw) test for significance]

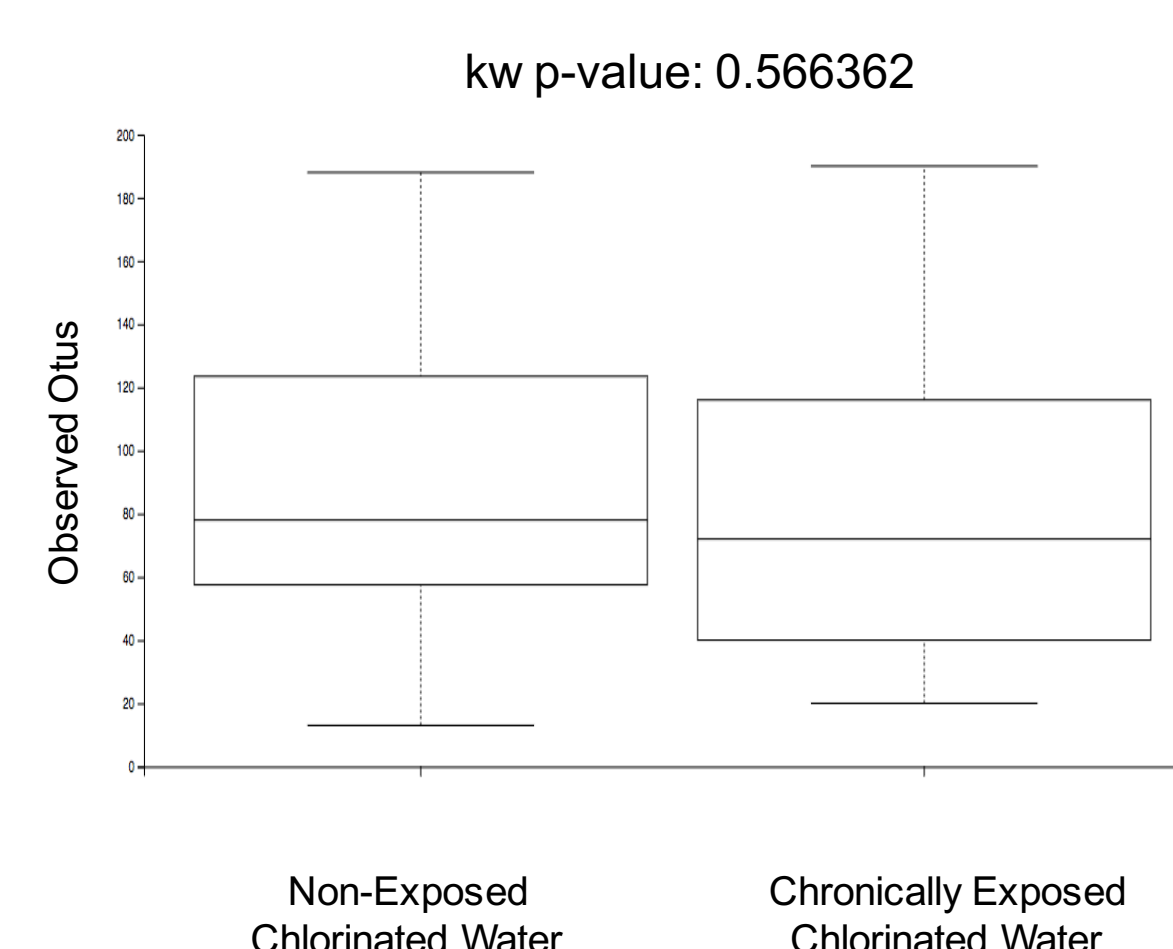


**Figure 2:** Richness of individual's nasal microbiota from participants exposed to chlorinated water at time point 1 and participants who are not exposed. No significant differences in phylogenetic diversity were noted [ $p > 0.05$ , Kruskal Wallis (kw) test for significance]

### 2<sup>nd</sup> Sampling: Participants who are Chronically Exposed to Chlorinated Water v. Non-Exposed Age- and Sex-matched participants



**Figure 3:** Faith's Phylogenetic Diversity of individual's nasal microbiota from participants who are exposed to chlorinated water at time point 2 and participants who are not exposed. No significant differences in phylogenetic diversity were noted [ $p > 0.05$ , Kruskal Wallis (kw) test for significance]

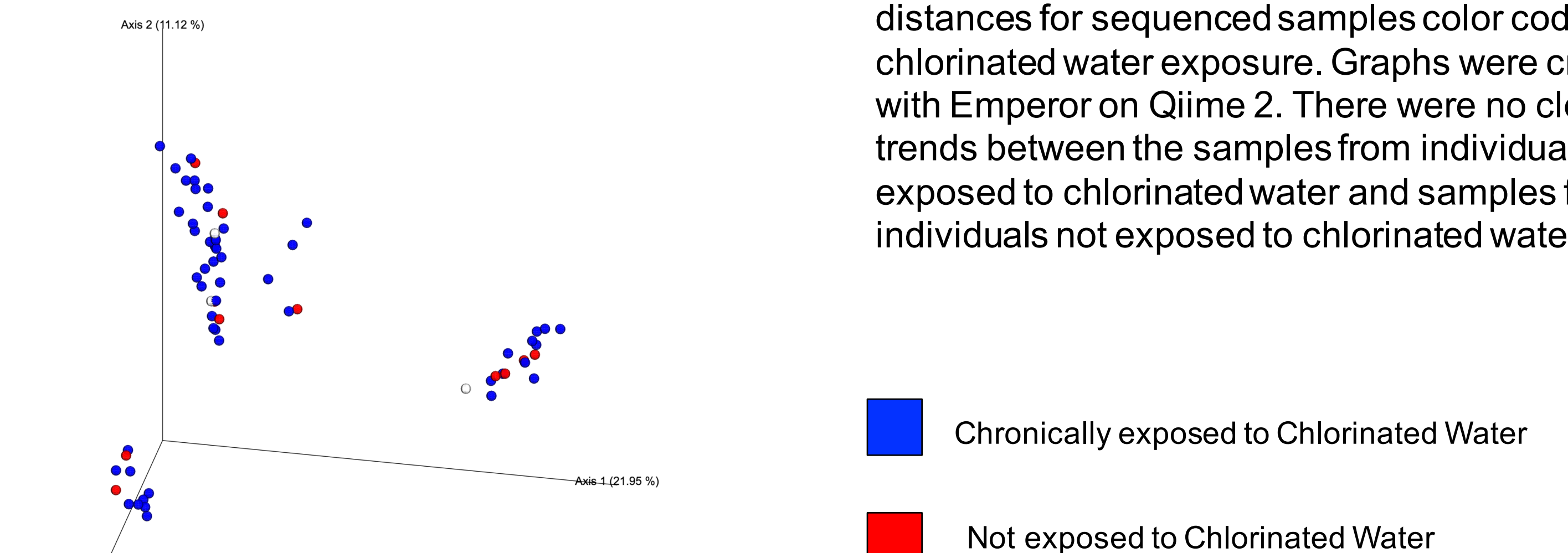


**Figure 4:** Richness of individual's nasal microbiota from participants exposed to chlorinated water at time point 2 and participants who are not exposed. No significant differences in phylogenetic diversity were noted [ $p > 0.05$ , Kruskal Wallis (kw) test for significance]

## Results.

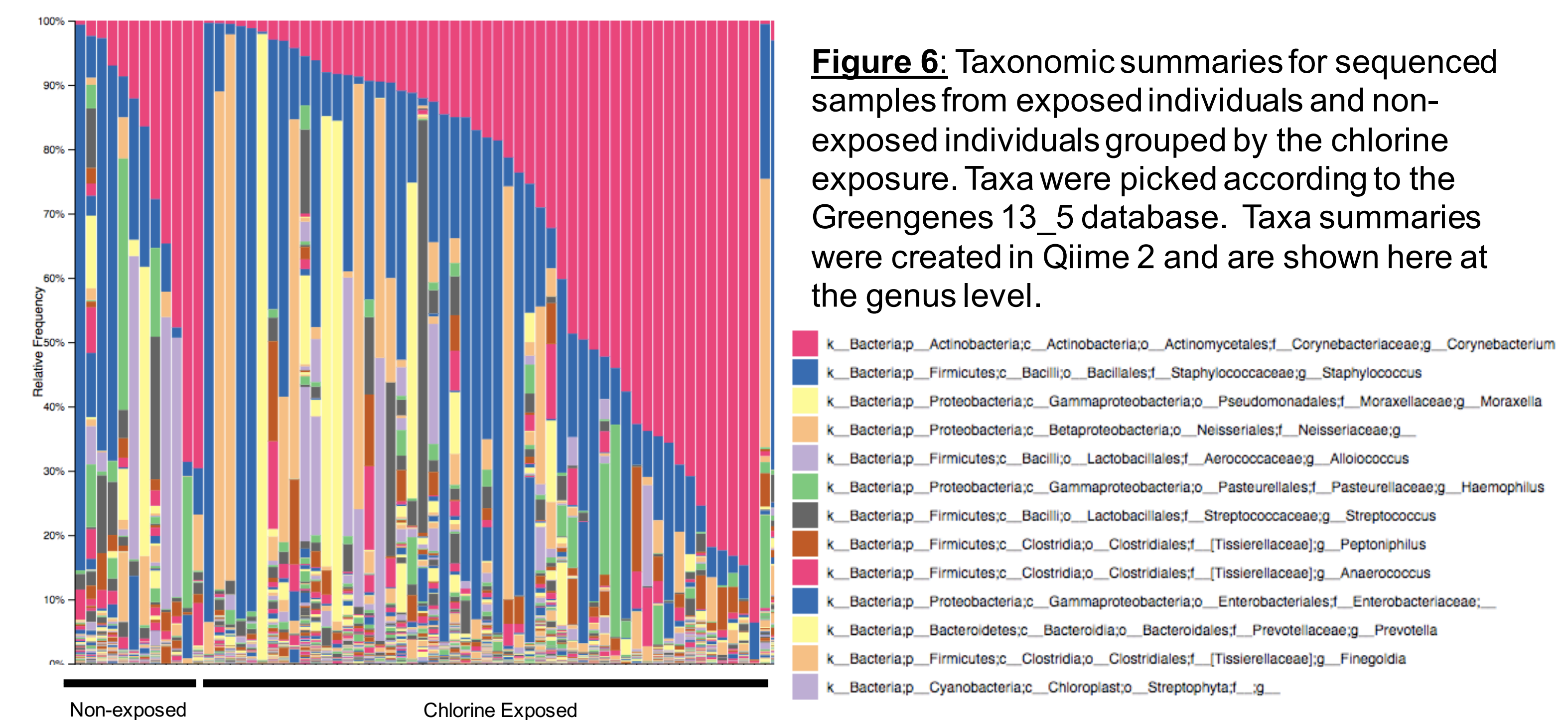
## β-Diversity

### PCoA of Unweighted Unifrac Distances for Samples Chronically Exposed to Chlorinated Water v. Non-Exposed Samples



**Figure 5:** PCoAs of unweighted Unifrac distances for sequenced samples color coded by chlorinated water exposure. Graphs were created with Emperor on Qiime 2. There were no clear trends between the samples from individuals exposed to chlorinated water and samples from individuals not exposed to chlorinated water.

### Taxonomic Summaries for Samples Chronically Exposed to Chlorinated Water v. Non-Exposed Samples



**Figure 6:** Taxonomic summaries for sequenced samples from exposed individuals and non-exposed individuals grouped by the chlorine exposure. Taxa were picked according to the Greengenes 13\_5 database. Taxa summaries were created in Qiime 2 and are shown here at the genus level.

## Conclusions.

- There are no statistically significant differences in the richness or phylogenetic diversity of the nasal microbiome of individuals exposed to chlorinated water (swimming pool) compared to those who are not chronically exposed.
- In both groups, *Corynebacterium*, *Staphylococcus*, *Neisseria* and *Moraxella* are the predominant members of the nasal microbiota.

## Acknowledgements.

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