Metagenomics analysis of coelomic fluid and gut microbiomes of the sea star, *Patiria miniata*

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Abstract

Sea star wasting disease (SSWD) is killing off large numbers of animals from multiple species of sea stars on the North American Pacific coast and appears to be different from earlier outbreaks. While a sea star-associated densovirus (SSaDV) has been identified as a likely infectious agent, much remains unknown regarding the roles other microbes may have in SSWD and about the composition of microbiomes from asymptomatic sea stars. We utilized next-generation sequencing to analyze the biodiversity of the coelomic fluid and pyloric caeca (gut) prokaryotic microbiomes of the sea star, *Patiria miniata*. Herein we report that the spirochaetaceae family of bacteria is a main constituent of the pyloric caeca (gut) microbiome in *P. miniata*. This novel finding provides insight into the bacterial composition of the gut microbiome of the sea star, *P. miniata*.

Introduction

Sea star wasting disease (SSWD) is killing off large numbers of animals from multiple species of sea stars on the North American Pacific coast and appears to be different from earlier outbreaks during the 70s, 80s, and 90s (Pacific rocky, 2015). The current SSWD was first observed in ochre stars (*Pisaster ochraceus*) in June 2013 in intertidal pool habitats along the coast of Washington State, and by the summer of 2014 the disease had spread from Mexico to Oregon (Pacific rocky, 2015). A sea star-associated densovirus (SSaDV) of the Parvoviridae family has been identified as a likely infectious agent involved in SSWD (Hewson *et al*., 2014) and metatranscriptome analysis of the echinoderm holobiont of asymptomatic and symptomatic sunflower stars indicates that bacteria from genera *Pseudomonas* and *Vibrio* are the more abundant in tissue samples take from the body walls of symptomatic sea stars (Gudenkauf and Hweson, 2015). However, much remains unknown regarding the role other microbes may have in SSWD and about the composition of microbiomes from asymptomatic sea stars.

Characterizing the microbiomes of sea stars is important in understanding sea star physiology and the pathophysiology associated with SSWD. For example, do changes in the sea star microbiome make the animal more susceptible to disease and microbial agents such as the SSaDV? One of the objectives of the URGE: Undergraduate Research Group on Echinoderms at LaGuardia Community College is to analyze the biodiversity of the prokaryotic microbiomes of the coelomic fluid (CF) and pyloric caeca (gut; GT) of the sea star, *Patiria miniata*. In 2014, the URGE performed metagenomics analysis of the CF and GT prokaryotic microbiomes from five to seven sea stars.

The objective of this research study is to validate these results obtained in 2014 and to identify commensal microbes in the CF and GT of the sea star, *P. miniata*.

Methods & Materials

Sample Collection

- Sea stars of the species *P. miniata* were purchased from South Coast Bio-Marine, LLC (San Pedro, CA) and housed in a custom 36-gallon Aqua Logic aquarium (San Diego, CA) with constant-flowing artificial seawater at 15-16°C. Animals were received in September 2016 and December 2016.

  Seawater samples (SW)
  - 5 from September and 4 from December

  ***P. miniata* samples of coelomic fluid (CF) and pyloric caeca (gut, GT)**
  - 2 from September and 7 from December

  Aquarium water samples (AqW)
  - 2 from the sump of the tank

Isolation of genomic DNA (gDNA)

- Genomic DNA was isolated using the Quick-gDNA MiniPrep kit from Zymo Research (Irvine, CA), according to the manufacturer's protocol.

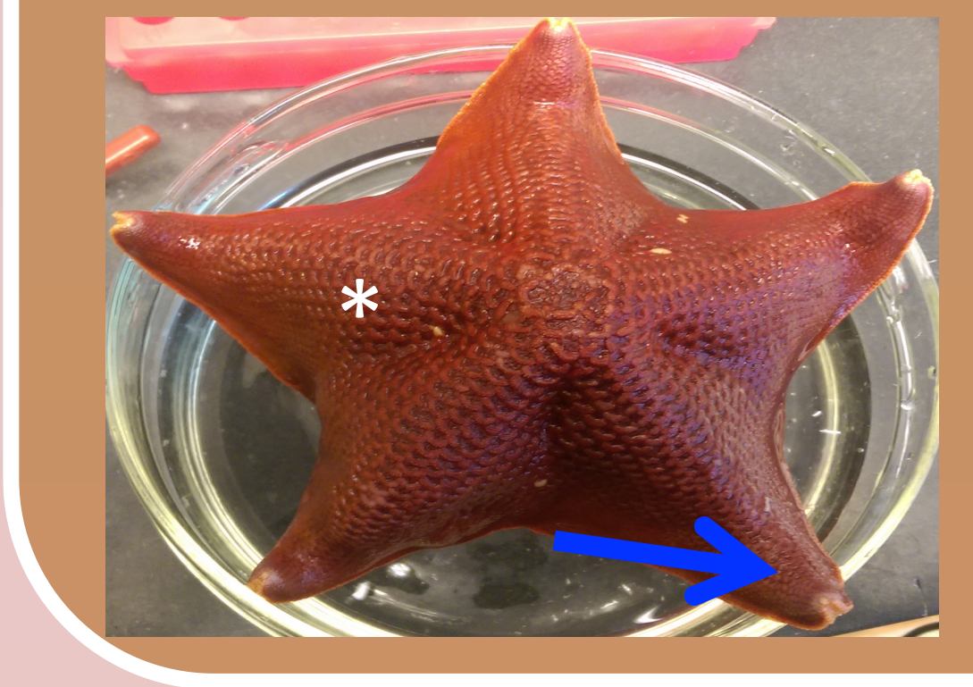
Determination of gDNA concentration

- The concentration of gDNA was determined was determined by reading the A_{260nm}.

16S rRNA diversity assay by Next-Generation DNA sequencing

- Genomic DNA samples were sent to Molecular Research Laboratory (MR DNA; www.mrdnalab.com; Shallowater, TX) for MiSeq Illumina next-generation paired-end sequencing of the 16S rRNA gene V4 variable region. The diversity assay produced 2x300bp paired end reads and the average number of reads per sample was 20,000.
- Sequence data were processed using MR DNA's proprietary analysis pipeline software.

Sump were the aquarium water samples were collected



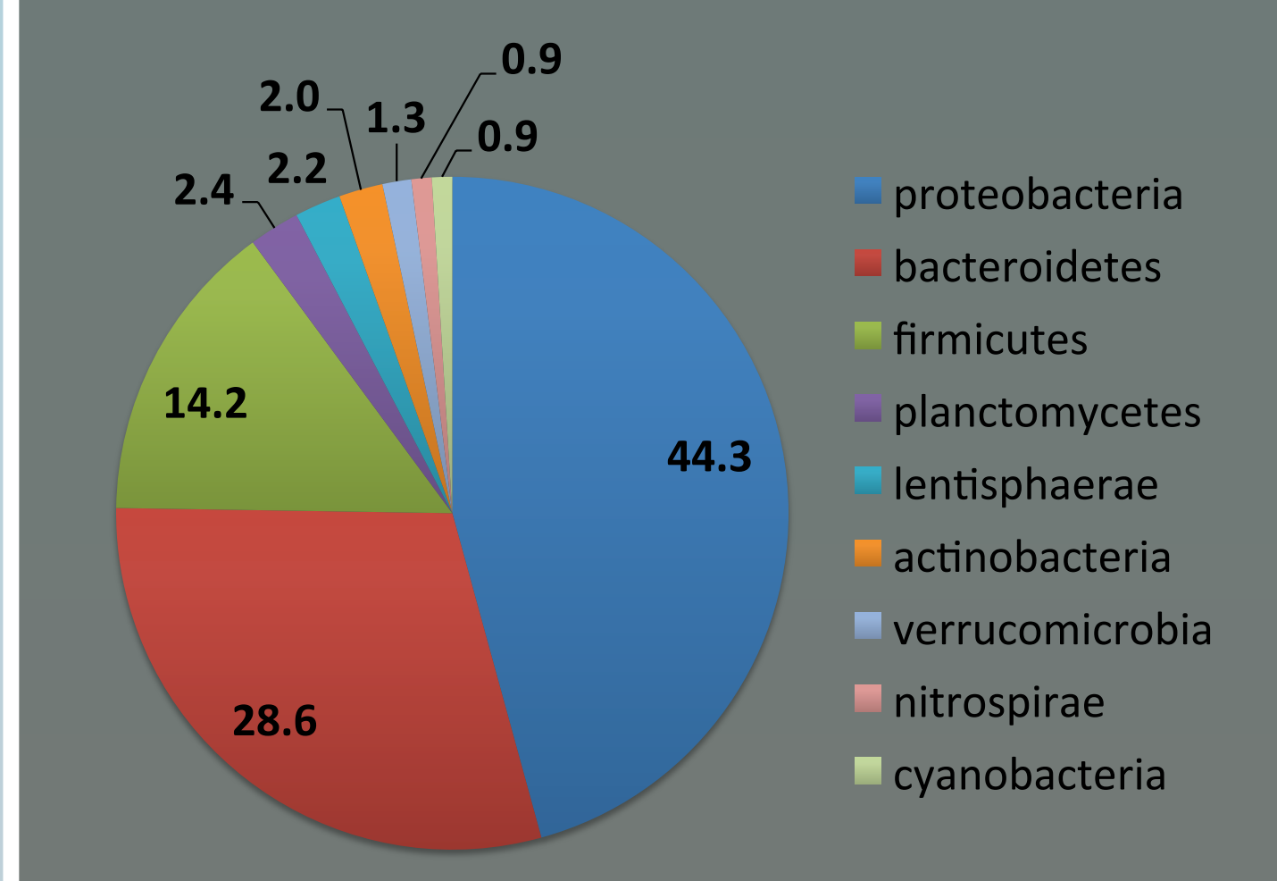
* area where bore a hole to collect pyloric caeca (gut; GT) samples

→ where inject 26 ½ gauge needle to collect coelomic fluid (CF) samples

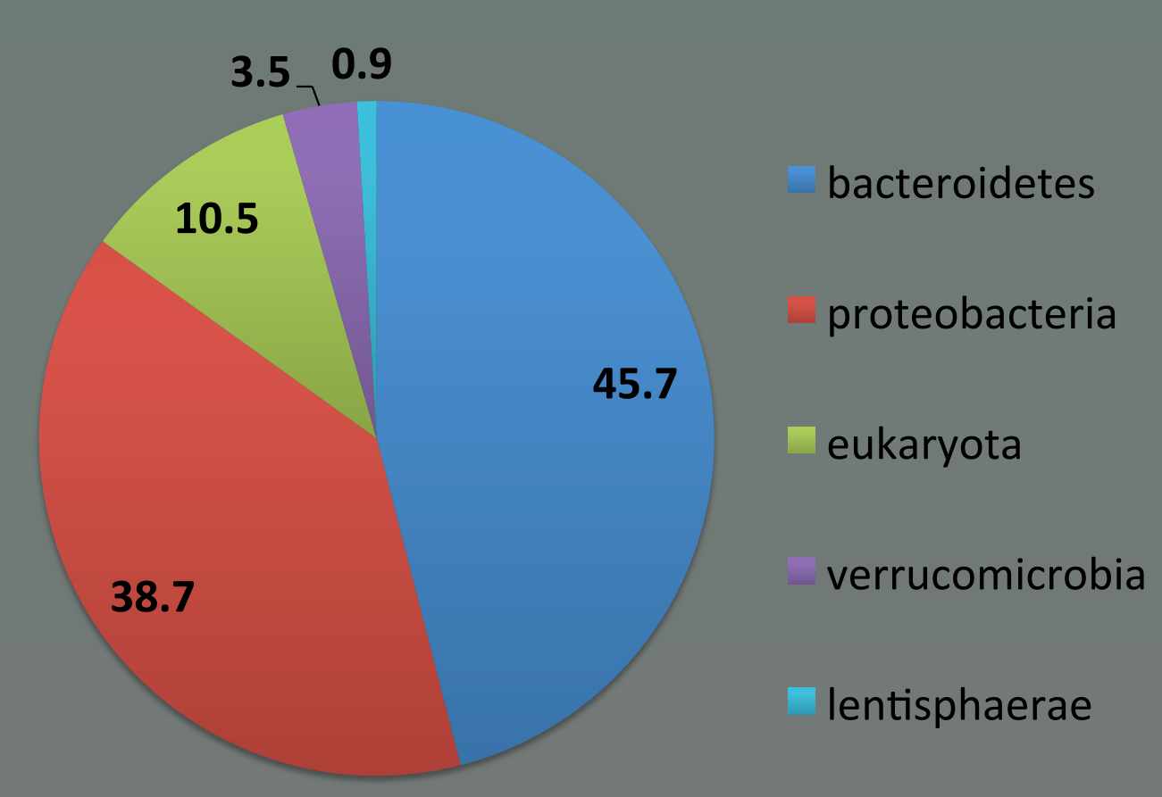
Results

Percentages of the most abundant phyla

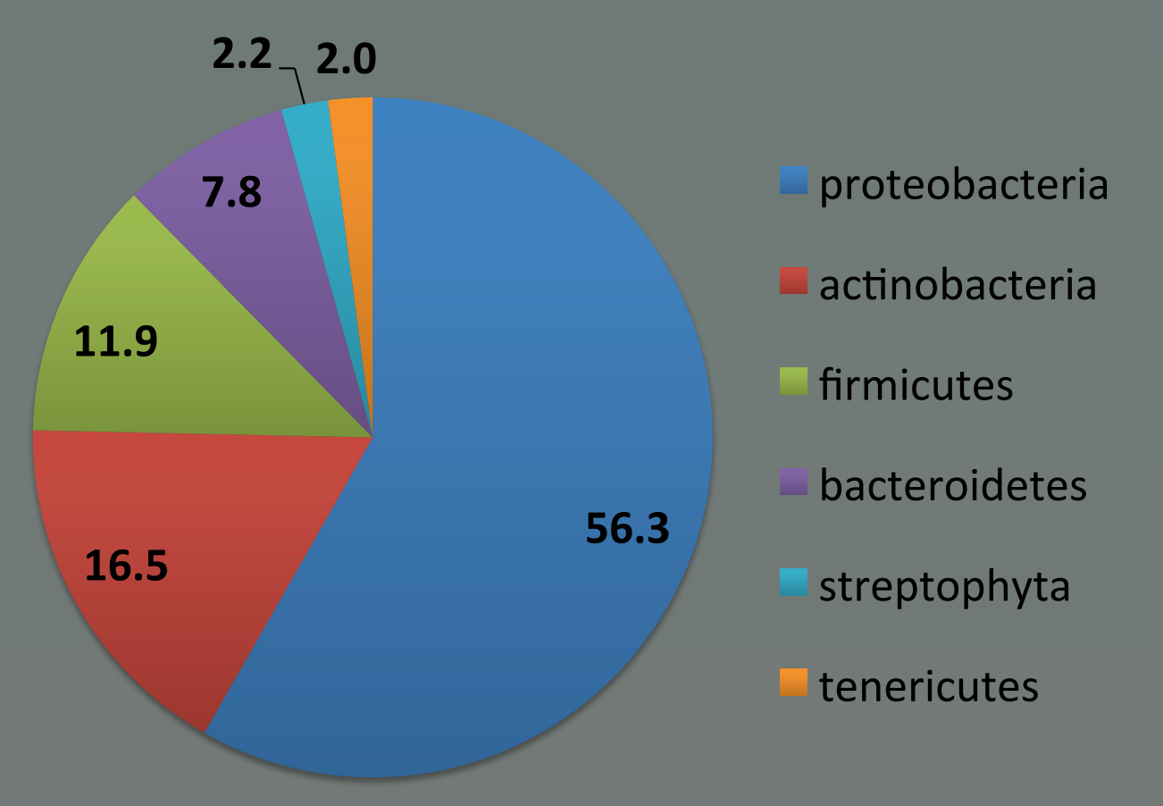
Aquarium water (AqW)



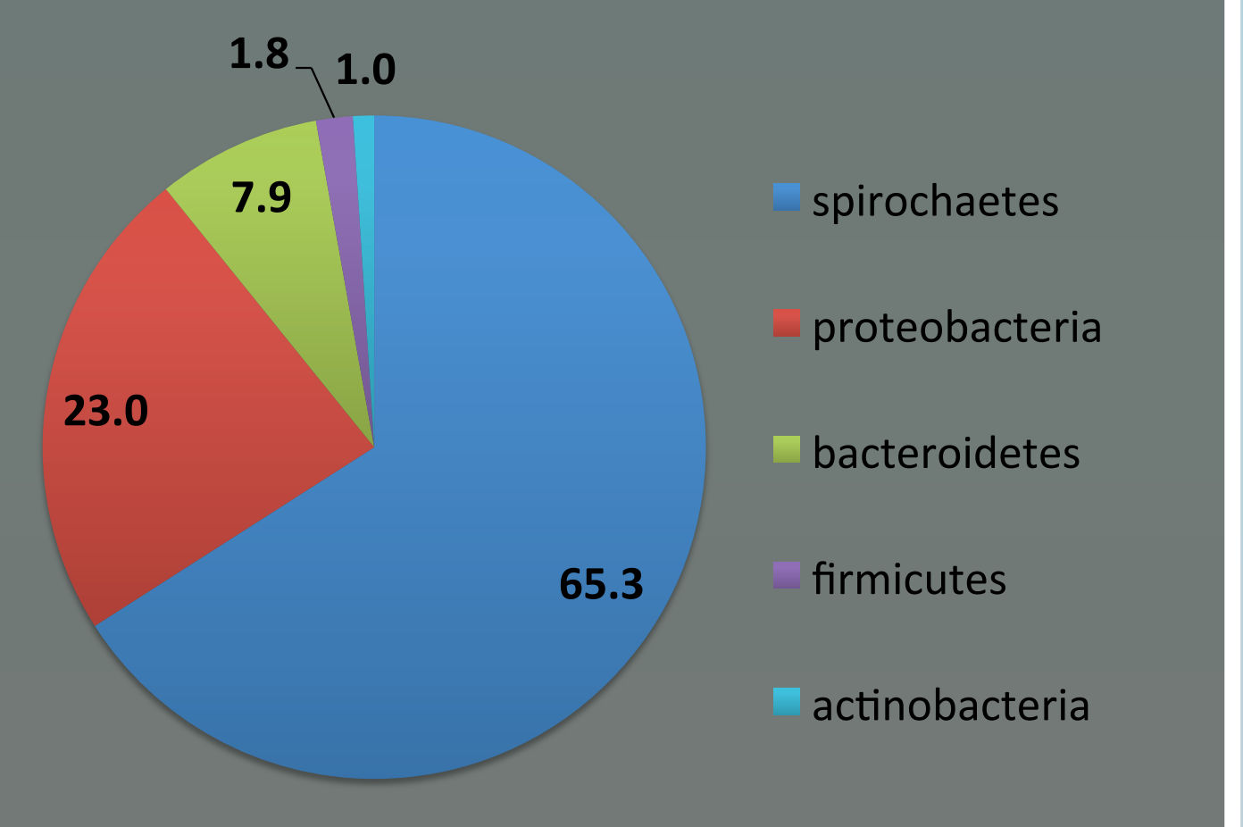
Seawater (SW)



Coelomic fluid (CF)

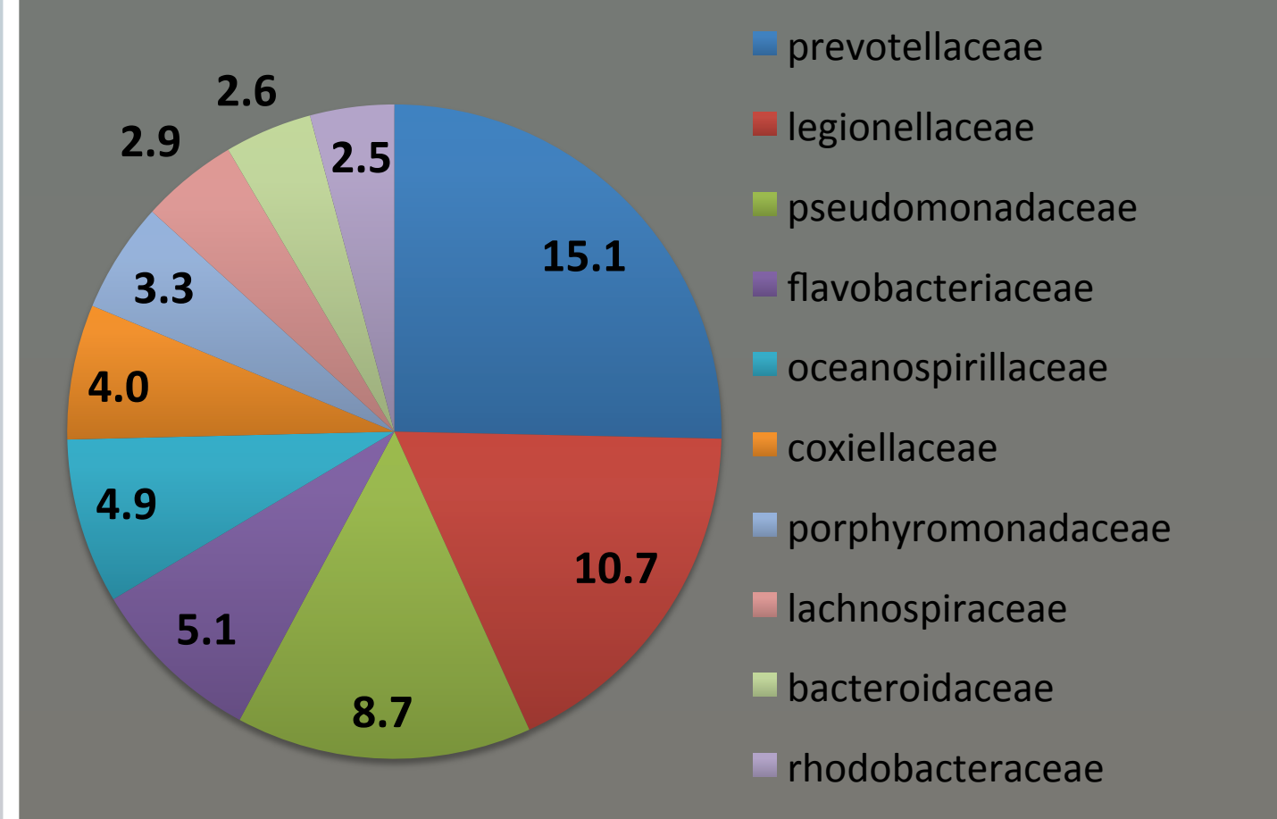


Pyloric caeca/gut (GT)

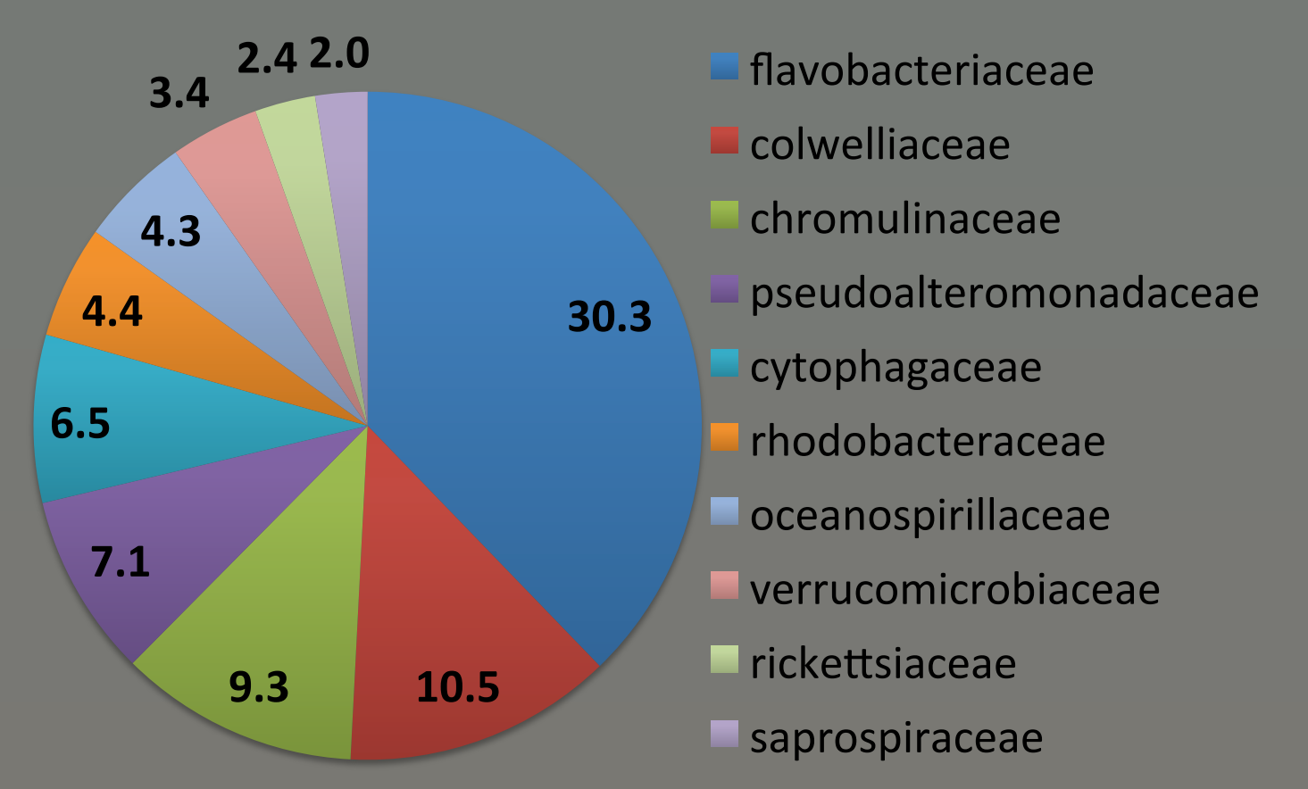


Percentages of the 10 most abundant families

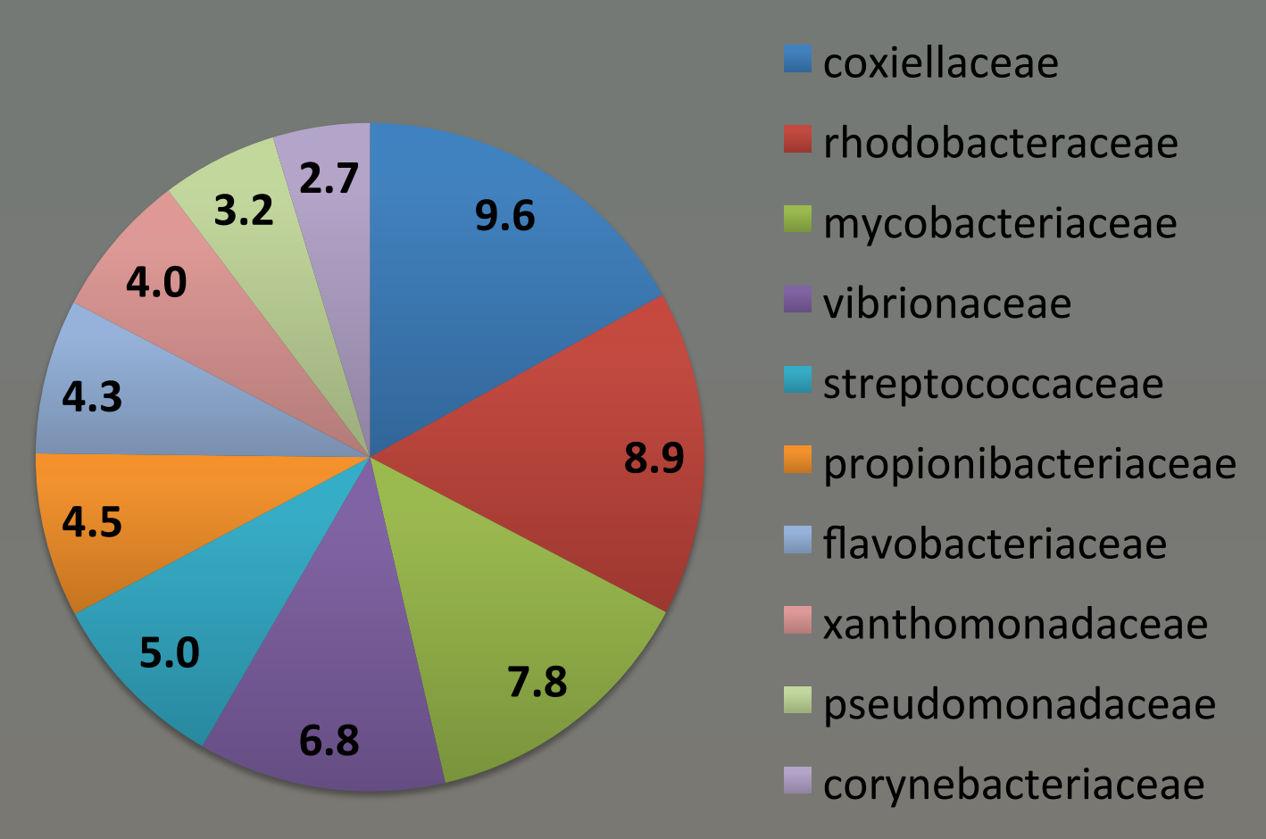
Aquarium water (AqW)



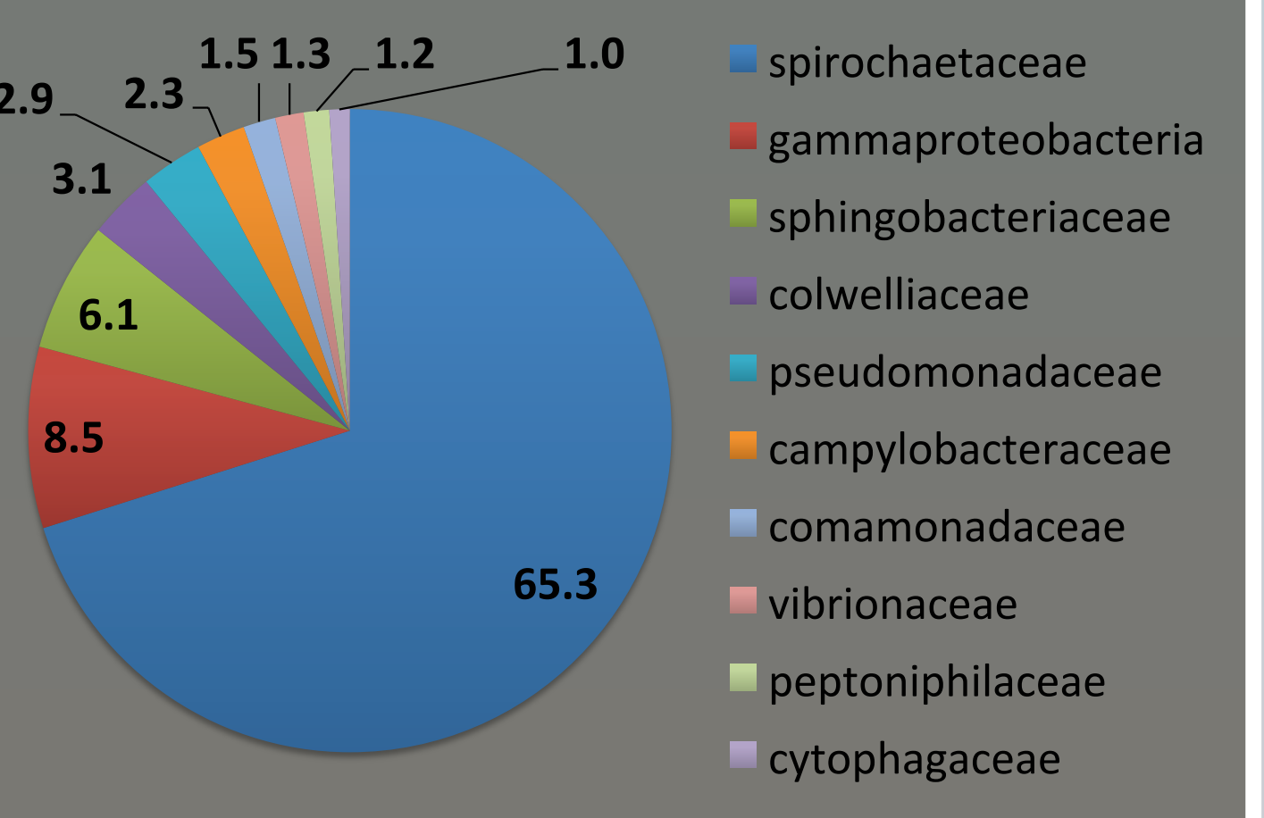
Seawater (SW)



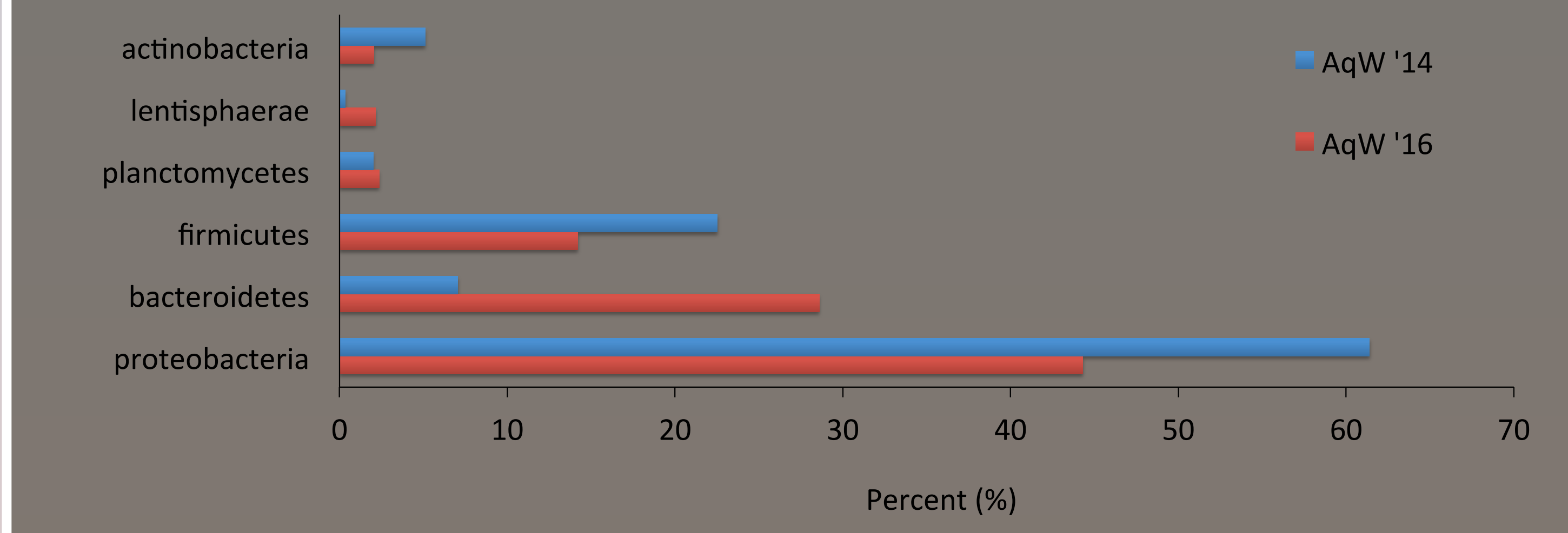
Coelomic fluid (CF)



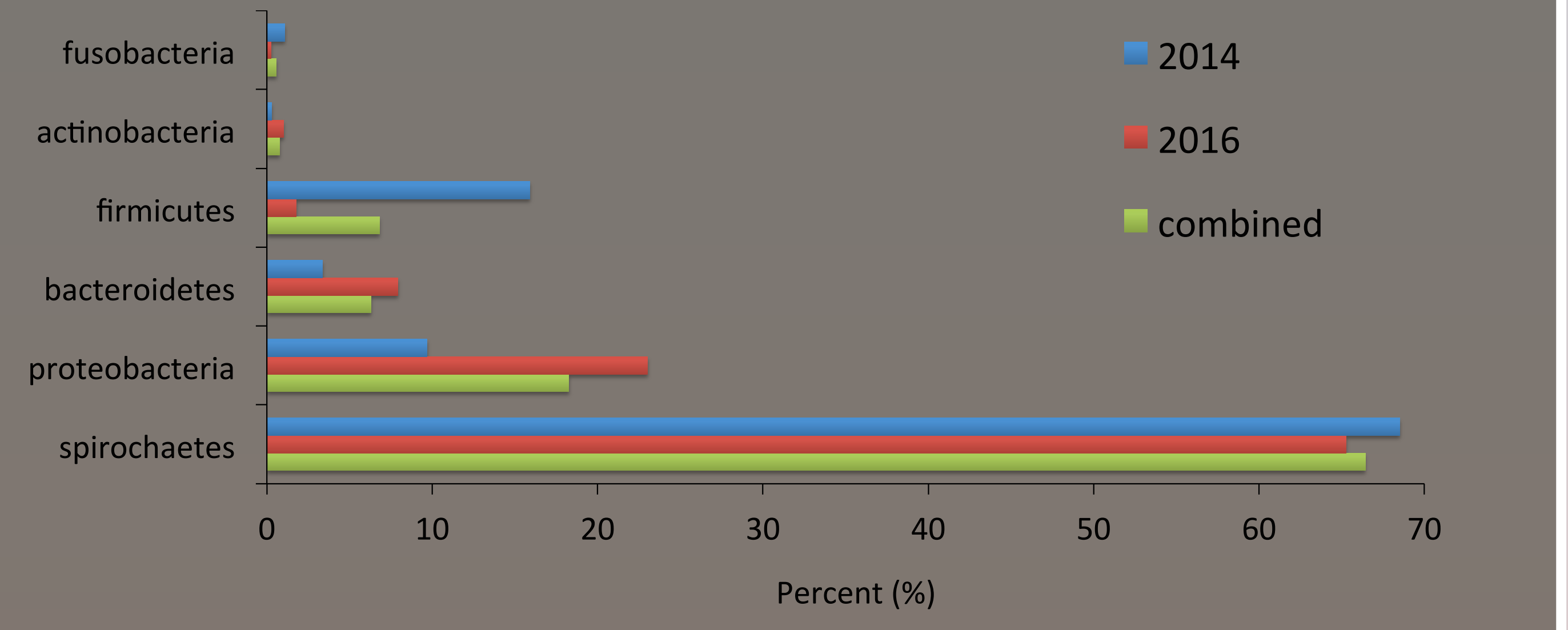
Pyloric caeca/gut (GT)



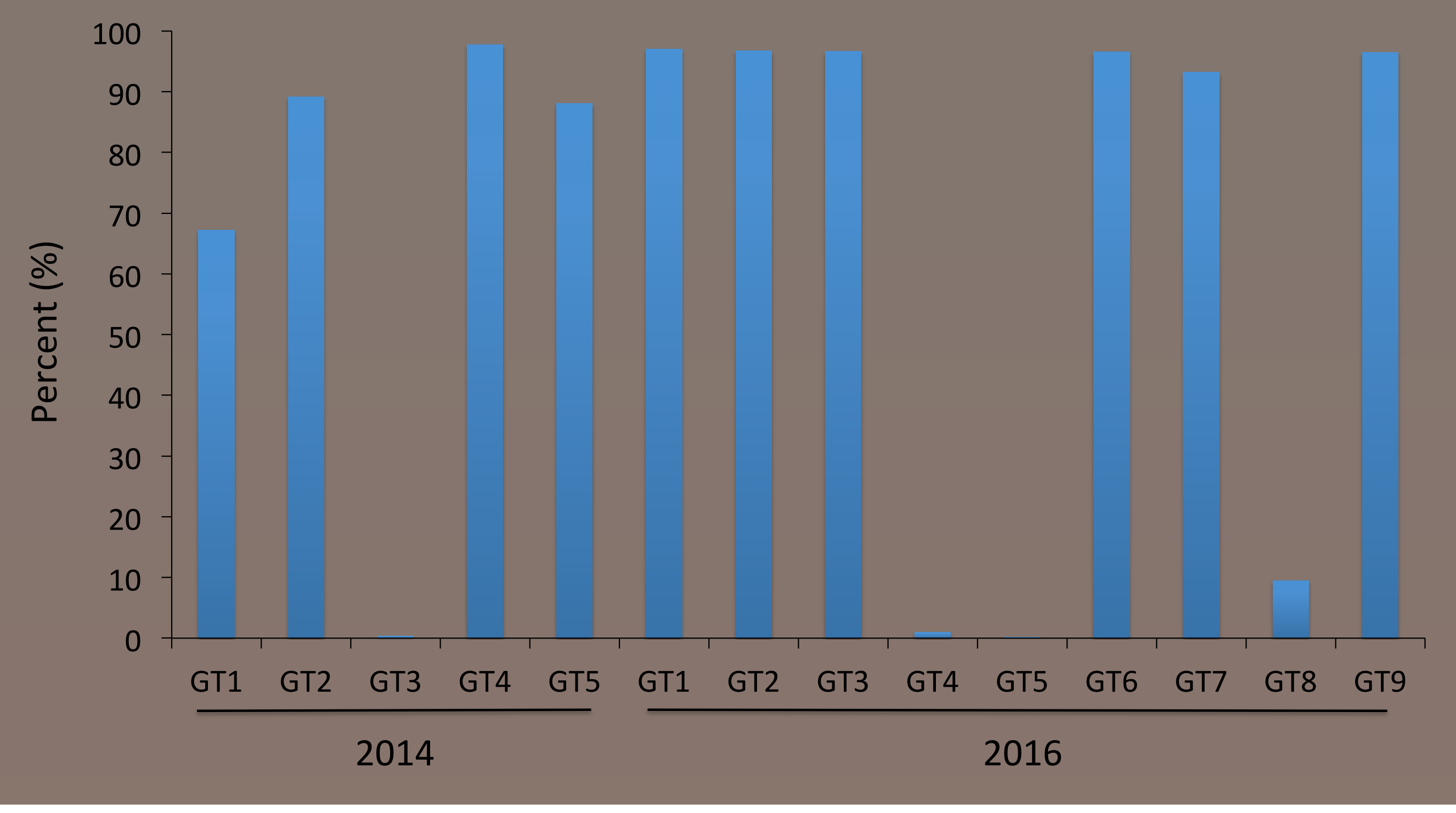
Comparison of phyla present in aquarium water from 2014 & 2016



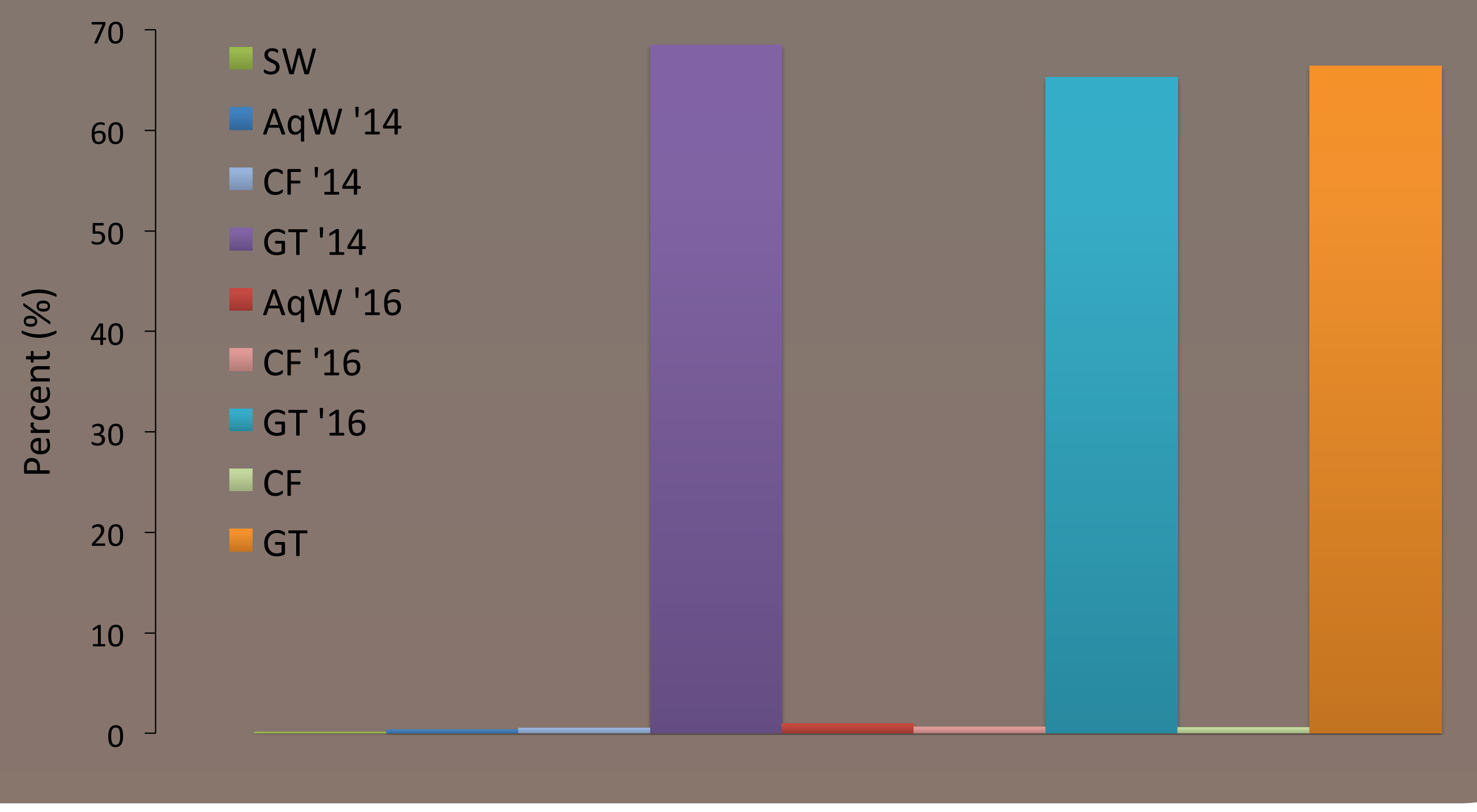
Comparison of phyla present in *P. miniata* gut from 2014 & 2016



Percentages of spirochaetaceae family present in gut of individual *P. miniata*



Percentage of spirochaetaceae family present in seawater, aquarium water, and *P. miniata* tissue from 2014 and 2016



Discussion

- The spriochaetaceae family of bacteria comprises a large percent of the gut microbiome but not the coelomic fluid microbiome of *P. miniata*. It is highly unlikely that this is a result of contamination from microbes present in seawater and aquarium water, since very low percentages of spirochaetaceae were present in the seawater and aquarium samples.
- When combined with the data collected from 2014, the average percentage of spirochaetaceae present in GT from *P. miniata* tested in 2014 and 2016 are similar, 68.5% and 65.3%, respectively.
- Of the 14 animals tested, a majority of them contain over 85% of spirocheataceae in their gut microbiome. However, 4 animals had less than 10% and 1 animal had about 67% of spirochaeteceae present in the gut microbiome.
- Members of the spirochaetaceae family are common in marine coastal environments and can grow at temperatures between 15-40ºC (Leschine *et al*., 2006). Spirochetes have been detected in tissue of the intertidal marine sponges (Alex and Antunes, 2015) and the gastrointestinal tract microbiome of the limpet, *Patella pellucida* (Dudek et al., 2014).
- **Spirochaetaceae family of bacteria appear to comprise a large percentage of the gut microbiome of the sea star, *P. miniata*.**
- Future experiments need to be conducted to identify the species of spirochetes present in the gut of *P. miniata* and their role in sea star physiology.

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