# Microbial Comparison of Anoxic Saanich Inlet and Oxic Hood Canal using 16S Community Analysis

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**Abstract**

Microbes play a critical role in the marine environment. They cycle nutrients, fix carbon, and can severely alter the water chemistry. Around the world, a place that they are especially important is in marine oxygen minimum zones (OMZs) where some are able to use alternative electron receptors other than oxygen and make up a majority of the organisms in these regions. This study compares the microbial community and water chemistry of Saanich Inlet and Hood Canal. Saanich Inlet is a seasonally anoxic fjord located on the southern tip of Vancouver Island and Hood Canal is a fjord located in the Puget Sound. Bacterial counts were taken throughout the water column at each location and compared to the water chemistry. 16S genome analysis was done to determine the microbial community composition. Bacterial concentrations and the water profile of the two locations showed that Hood Canal was fairly consistent throughout the water column, however Saanich Inlet had multiple regimes and showed a large change in chemistry at 100m depth where the oxygen approached 0 mg/L. This difference was corroborated by the genomic results that showed microbial community differences that could be explained by the oxygen deficiency and the metabolic characteristics of the dominant groups in each location. We found that Saanich Inlet was dominated by the sulfur oxidizing clade of chemolithoautotrophic bacteria SUP05 whereas Hood Canal was dominated by the Heterotrophic clade of bacteria SAR11. These results show how as OMZs grow, newly anoxic regions may show a shift in their microbial communities.

## Introduction

Oxygen is important to a majority of organisms on the planet as an electron acceptor in cellular respiration. There are several places on earth where oxygen limited and organisms are forced to use alternative terminal electron acceptors. Oxygen deficiency is an especially prevalent phenomenon in the marine environment. There are several places across the world's oceans known for being anoxic or hypoxic and these areas are expected to grow with time (Deutsch et al., 2011). Within these environments most organisms struggle to live as they rely on oxygen, but there are organisms that have adapted to these extreme conditions and some organisms that are associated specifically with anoxia and hypoxia (Glaubitz et al., 2013). These organisms and their novel pathways are able to shape the water chemistry in many ways including using oxygen, producing nitrite, fixing carbon (Ulloa et al., 2012).

The largest oxygen depleted zones are located in the Subarctic, the Eastern Tropical Pacific and the Indian Ocean (Helly and Levin, 2004) however many coastal areas and fjords are also known to be seasonally or permanently oxygen deficient (Smethie, 1987). Fjords in particular have sills that cause low water renewal that allows for all of the oxygen to be consumed (Herlinveaux, 1962). Fjords behave differently based on bathymetry, river input, water inflow and based on seasons (Pawlowicz, 2017) which makes them an excellent place to study how biology changes when a marine environment loses its oxygen or as anoxic zones grow.

Bacterial communities are crucial everywhere in the oceans for the recycling of nutrients and fluxes of energy through dissolved organic matter (Azam and Malfatti, 2007). Anoxic regions of water are especially controlled by anaerobic microbial processes (Ulloa et al., 2012). Looking at the microbiology of these areas can give insight into what is happening in these areas

and how it may differ from areas that contain standard amounts of oxygen. Nutrient concentrations can give us insight into what processes are being carried out by anaerobic bacteria and the water chemistry can even allude to what types of organisms are found in these specific areas (Glaubitz et al., 2013).

In this study I sought to characterize the potential microbial differences in Saanich Inlet, a seasonally anoxic fjord (Herlinveaux, 1962) located on the southern tip of Vancouver Island, and Hood Canal, a seasonally low oxygen fjord prone to occasional fish die offs (Newton et al., 2007) located in the Puget Sound. I evaluated the bacterial community composition of the deep waters of both fjords to compare what organisms are located in the areas of decreased oxygen. Water column microbial counts were also analyzed at the same stations and compared to nutrient concentrations to see how microbes may be affected by or be affecting the water chemistry of the water.

## Methods

### Sample Collection:

Water was collected aboard the RV Thomas G Thompson from two stations using niskin bottles attached to a CTD. One station in Saanich Inlet and the other station in Hood Canal. Water samples cell counts were collected throughout the water column in both locations and samples were taken at three depths for DNA analysis. The three depths of collection were the Chlorophyll maximum, the oxycline and the oxygen minimum.

**Cell counts:**

Cells in 450 µL of water was fixed with 0.2µm filtered 1% formalin then frozen at -80˚Cuntil further processing at the University of Washington. 12 samples were taken in Saanich Inlet and 6 samples were taken in Hood Canal due to the difference in depth. In the laboratory, samples were thawed and 100 µL of each sample was transferred to a 96 then stained with SYBR Green 1 (Invitrogen, Eugene, OR, USA) at a dilution of 1:2000 for 60 minutes. Cells were enumerated using an EasyCyte Plus Flow Cytometer (Guava Technologies, Hayward, CA, USA) at a flow rate of 0.59µL and cells were detected by quantifying the green fluorescence with a 488 nm laser and a 525/30 nm photomultiplier.

**DNA extraction:**

From each depth designated for DNA analysis, 2L of water were taken (1L if chlorophyll was high enough to potentially clog filters) and pumped through a series of 47 mm filters (3 µm Polycarbonate and 0.2 µm isopore). Once water samples were run through the filters, the filters were put into cryovials and frozen at -80˚C on the ship for further processing once back at the University of Washington. Two samples were then prepped for DNA extraction and purification (Saanich Inlet station 140 m; Hood Canal station 62 m).

Each filter was transferred to a microcentrifuge tube and mixed with 560µL TE (pH 8.0) and bead beat on maximum speed for 30 seconds. Tubes were then stored at -80˚C for 30 min and transferred to a 95˚C heat block for 10 min and bead beat once more for 30 seconds. 30µL 10% SDS, 3µL Lysozyme (20mg/mL), and 3µL proteinase K (20mg/mL) was added to the tubes and mixed. Samples were incubated in 55˚C water bath for 2 hours. 100µL NaCl (5M) and 80

µL CTAB-NaCl (10%CTAB in 0.7% NaCl) were added and resulting sample was incubated in 55˚C water bath for 10 min.

An equal volume of chloroform was added to the DNA solution and mixed vigorously then centrifuged at 8000 x g for 3 min. The supernatant was transferred to a new tube and an equal volume of phenol, chloroform isoamyl (Sigma-Aldrich P2069 pH range 7.8-8.2) is added to the resulting DNA solution. The mixture was shaken vigorously and centrifuged at 8000 x g for 3 minutes. The resulting supernatant was added to a new tube and mixed with an equal volume of Chloroform. Mix vigorously and centrifuge the solution at 8000 g for 3 minutes. Supernatant was transferred to a new tube and pre chilled 100% ethanol (2.5x the volume of solution) was added and resulting solution stored at -20˚C overnight.

Tubes were centrifuged at max speed (20817 x g) for 45 minute and liquid was removed. I then washed the centrifuge pellet with 50-70-100% EtOH adding 1 mL of -20˚C EtOH and centrifuging the tube then removing the ethanol each time. Following the 100% EtOH wash I let the pellet air dry in empty fume hood until completely dry. Pellet was resuspended in 90µL TE (pH 8) and measured to assess DNA concentration and purity. DNA was further cleaned and purified using a Zymo DNA clean and concentrator kit following catalog instructions.

**16S analysis:**

DNA samples were sent to MR DNA (Molecular Research LP) in Shallowater, TX for 16S processing and analysis. The primers used to analyze the DNA were the modified V4 primers illCUs515F (GTGYCAGCMGCCGCGGTAA) and new806RB (GGACTACNVGGGTWTCTAAT) (Apprill et al., 2015). The DNA was processed using their

standard procedures for MiSeq processing (http://www.mrdnalab.com/16s-ribosomal-sequencing.html). OUT's were taxonomically classified based on the BLASTn and compared to the RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu).

## Results

### Bacterial counts:

Surface oxygen concentration in Saanich Inlet started higher than Hood Canal at 10.23 mg/L and 8.67 mg/L respectively. Both stations decreased oxygen over the first 20 m of the water column where Hood Canal then leveled off and was stable throughout the water column at approximately 4 mg/L. Saanich Inlet continued to decrease in oxygen concentration (fig 1). Saanich Inlet oxygen decreased to 1.66 mg/L at 100 m depth and maintained levels below 0.5 mg/L throughout the rest of the water column at nearly undetectable levels. Similar to the oxygen profiles both stations saw an initial decrease in bacterial counts with depth from the surface. In Saanich Inlet the bacteria count drastically increased from 100 m to the bottom. This coincides with the oxygen concentration reaching near depletion as well as the $NO_3$ concentration being almost completely depleted and the $NH_4$ concentration starting to increase from 0 µM (figure 2). This is a stark difference from the profile of Hood Canal $NO_3$ concentrations increase and remain above 25µM below 20m depth and the $NH_4$ concentration does not increase at all. The highest concentration of bacterial cells was more than three times higher than the highest value in Hood Canal with $3.09x10^6$ cells $mL^{-1}$ and $9.82x10^5$ cells $mL^{-1}$. In Saanich Inlet Sulfur was detected with smell from the deep depth bottles but was not quantified.

**16S**

At both stations proteobacteria dominated the community composition. However Saanich Inlet was dominated by a subdivision of proteobacteria called gammaproteobacteria and Hood Canal was dominated by alphaproteobacteria (figure 3). Saanich Inlet was comprised of 46.3% gammaproteobacteria and the next closest class was alphaproteobacteria with 12.2% of the total community. Hood Canal was more diverse with alphaproteobacteria making up 37.0% of the community composition with gammaproteobacteria at 20.5% and thaumarcheota at 18.2%.

When looking at the species level, Hood Canal and Saanich Inlet show more differences (figure 4). The only species that has any overlap between the two locations is the pelagibacter sp. It was the most common species found in Hood Canal with 18460 matches but is the third most common species seen in Saanich inlet with 10289 matches. Where Hood Canal had more diversity at the class level, it has much less diversity than Saanich Inlet at the species level. Hood Canal had two species that were seen at an abundance higher than 4000 matches (pelagibacter sp. and thioglobus sp.) whereas Saanich Inlet had 5 species (ruthia magnifica, arenicella xantha, pelagibacter sp., scalindua marina, and cytophaga spp.). Both locations had a most abundant species that peaked at approximately 32000 matches.

## Discussion

The oxygen content seen in this study saw a slightly different profile in Saanich Inlet than previous studies at approximately the same time of year. In January, previous studies saw oxygen content reaching minimum levels at or below 150m (Zaikova et al., 2009; Herlinveaux, 1962) which was 50m below what was seen in this study. In a similar study, Zaikova et al. found that

the cell abundance in Saanich Inlet between the months of April to November had a similar profile but the increase in abundance was not as drastic as the increase seen in this study. My data shows bacterial count was much higher in the anoxic water in Saanich Inlet. This relationship has implications of causation based on how important microbial organisms are to large scale water chemistry and especially oxygen consumption (Azam and Malfatti, 2007). Microbes are a large source of respiration and oxygen use which is likely a large factor in the depletion of oxygen when paired with low water circulation. The oxygen content and microbial distribution of Hood Canal matched with previous studies of the same area (Urakawa et al., 2010).

When looking at the water chemistry, Saanich Inlet appears to have multiple regimes as you move down in the water column whereas Hood Canal is relatively stable throughout. In both locations, the bacterial counts appear to be an inverse of the $NO_3$ concentration. The sharp decline in the $NO_3$ concentration seen in Saanich Inlet is most likely indicative of a shift from aerobic to anaerobic respiration in the water column (Devol, 1978). This is congruent with the fact that there is not a similar decrease in the $NO_3$ concentration in Hood Canal where the oxygen does not decrease in the same way and aerobic respiration is likely happening throughout the water column. Another notable metric of the water column is the $NH_4$ concentration. In Hood Canal there was a low concentration at the surface that went to zero and remained there for the entirety of the water column whereas in Saanich Inlet the $NH_4$ was at zero but started to increase with depth at slightly above 100m. 100m depth marks fairly drastic change in the water chemistry as this is the depth at which the water becomes anaerobic and the $NO_3$ concentration declines and the $NH_4$ concentration begins to increase. This all coincides with the drastic

increase in the microbial concentration. It is likely that all of the water chemistry changes are based on the microbial structuring of the Saanich Inlet ecosystem.

Anoxic waters in Saanich Inlet are often dominated by sulfur oxidizing bacteria from the SUP05 clade of gammaproteobacteria (Glaubitz et al., 2013). This is congruent with the Sulphur that was detected at the deep depths at the station. This is also similar to the findings of a previous study that found that at 125m depth the microbial community was comprised of greater than 50% SUP05 related sequences and 215m was comprised of greater than 75% SUP05 related sequences (Zaikova et al., 2009). This is contrasted by Hood Canal's microbial community which is dominated by alphaproteobacteria, a group mainly made up of the SAR11 clade (Herlemann et al., 2014). The difference in the $NO_3$ levels in the two stations can be explained by the dominate clade of bacteria and the way in which they respire. SAR11 are aerobic heterotrophs extremely common in oxic waters (Sun et al., 2011) and the SUP05 clade is a facultatively anaerobic chemolithoautotrophs that are common in anoxic waters (Shaw et al., 2017). Nitrogen reduction is common in anoxic waters (Dalsgaard et al., 2012) and explains the nitrate concentrations of Saanich Inlet. Nitrate is eventually used as a terminal electron acceptor in the place of oxygen so as the oxygen is used up, the facultatively anaerobic chemolithoautotrophs will consume the nitrate.

The Sulphur oxidation of SUP05 does not explain the $NH_4$ concentration that was seen in Saanich Inlet however. A possible source is the presence of organisms that use Dissimilatory nitrate reduction to ammonia (DNRA). Studies have found evidence that organisms found in the SUP05 clade may be carrying out DNRA (Shaw et al., 2017; Hawley et al., 2014). Another potential source is the deltaproteobacteria, many of which are known to carry out DNRA (van

den Berg et al., 2015). Deltaproteobacteria were more abundant in Saanich Inlet than in Hood Canal by nearly a factor of 3.

This study provides an excellent view into the extent that bacteria and microbes are able to shape and influence the environment in which they live. The direct comparison between two fjords of similar location had vastly different water chemistries explained by the oxygen concentrations and the microbial regimes that dominate the location. Further studies that perform 16S on the entire water column of both sites would help provide more insight into the differences between oxic and anoxic waters and how future anoxic locations may look as anoxia becomes a more common water feature.

## References

Apprill, Amy, Sean McNally, Rachel Parsons, and Laura Weber. "Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton." (2015).

Azam, Farooq, and Francesca Malfatti. "Microbial structuring of marine ecosystems." *Nature Reviews Microbiology* 5, no. 10 (2007): 782.

Dalsgaard, Tage, Bo Thamdrup, Laura Farías, and Niels Peter Revsbech. "Anammox and denitrification in the oxygen minimum zone of the eastern South Pacific." *Limnology and Oceanography* 57, no. 5 (2012): 1331-1346.

Deutsch, Curtis, Holger Brix, Taka Ito, Hartmut Frenzel, and LuAnne Thompson. "Climate-forced variability of ocean hypoxia." *science* 333, no. 6040 (2011): 336-339.

Devol, Allan H. "Bacterial oxygen uptake kinetics as related to biological processes in oxygen deficient zones of the oceans." *Deep Sea Research* 25, no. 2 (1978): 137-146.

Glaubitz, Sabine, Katrin Kießlich, Christian Meeske, Matthias Labrenz, and Klaus Jürgens. "SUP05 dominates the gammaproteobacterial sulfur oxidizer assemblages in pelagic redoxclines

of the central Baltic and Black Seas." *Applied and environmental microbiology* 79, no. 8 (2013): 2767-2776.

Hawley, Alyse K., Heather M. Brewer, Angela D. Norbeck, Ljiljana Paša-Tolić, and Steven J. Hallam. "Metaproteomics reveals differential modes of metabolic coupling among ubiquitous oxygen minimum zone microbes." *Proceedings of the National Academy of Sciences* 111, no. 31 (2014): 11395-11400.

Helly, John J., and Lisa A. Levin. "Global distribution of naturally occurring marine hypoxia on continental margins." *Deep Sea Research Part I: Oceanographic Research Papers*51, no. 9 (2004): 1159-1168.

Herlemann, Daniel PR, Jana Woelk, Matthias Labrenz, and Klaus Jürgens. "Diversity and abundance of “Pelagibacterales”(SAR11) in the Baltic Sea salinity gradient." *Systematic and applied microbiology* 37, no. 8 (2014): 601-604.

Herlinveaux, R. H. "Oceanography of Saanich Inlet in Vancouver Island, British Columbia." *Journal of the Fisheries Board of Canada* 19, no. 1 (1962): 1-37.

Pawlowicz, R. "Seasonal Cycles, Hypoxia, and Renewal in a Coastal Fjord (Barkley Sound, British Columbia)." *Atmosphere-Ocean* 55, no. 4-5 (2017): 264-283.

Shah, Vega, Bonnie X. Chang, and Robert M. Morris. "Cultivation of a chemoautotroph from the SUP05 clade of marine bacteria that produces nitrite and consumes ammonium." *The ISME journal* 11, no. 1 (2017): 263.

Smethie Jr, William M. "Nutrient regeneration and denitrification in low oxygen fjords." *Deep Sea Research Part A. Oceanographic Research Papers* 34, no. 5-6 (1987): 983-1006.

Sun, Jing, Laura Steindler, J. Cameron Thrash, Kimberly H. Halsey, Daniel P. Smith, Amy E. Carter, Zachary C. Landry, and Stephen J. Giovannoni. "One carbon metabolism in SAR11 pelagic marine bacteria." *PLoS One* 6, no. 8 (2011): e23973.

Ulloa, Osvaldo, Donald E. Canfield, Edward F. DeLong, Ricardo M. Letelier, and Frank J. Stewart. "Microbial oceanography of anoxic oxygen minimum zones." *Proceedings of the National Academy of Sciences* 109, no. 40 (2012): 15996-16003.

Urakawa, Hidetoshi, Willm Martens-Habbena, and David A. Stahl. "High abundance of ammonia-oxidizing Archaea in coastal waters, determined using a modified DNA extraction method." *Applied and environmental microbiology* 76, no. 7 (2010): 2129-2135.

Van Den Berg, Eveline M., Udo Van Dongen, Ben Abbas, and Mark Cm Van Loosdrecht. "Enrichment of DNRA bacteria in a continuous culture." *The ISME journal* 9, no. 10 (2015): 2153.

Walsh, David A., Elena Zaikova, Charles G. Howes, Young C. Song, Jody J. Wright, Susannah G. Tringe, Philippe D. Tortell, and Steven J. Hallam. "Metagenome of a versatile chemolithoautotroph from expanding oceanic dead zones." *Science* 326, no. 5952 (2009): 578-582.

Zaikova, Elena, David A. Walsh, Claire P. Stilwell, William W. Mohn, Philippe D. Tortell, and Steven J. Hallam. "Microbial community dynamics in a seasonally anoxic fjord: Saanich Inlet, British Columbia." *Environmental microbiology* 12, no. 1 (2010): 172-191.

Figure 1.

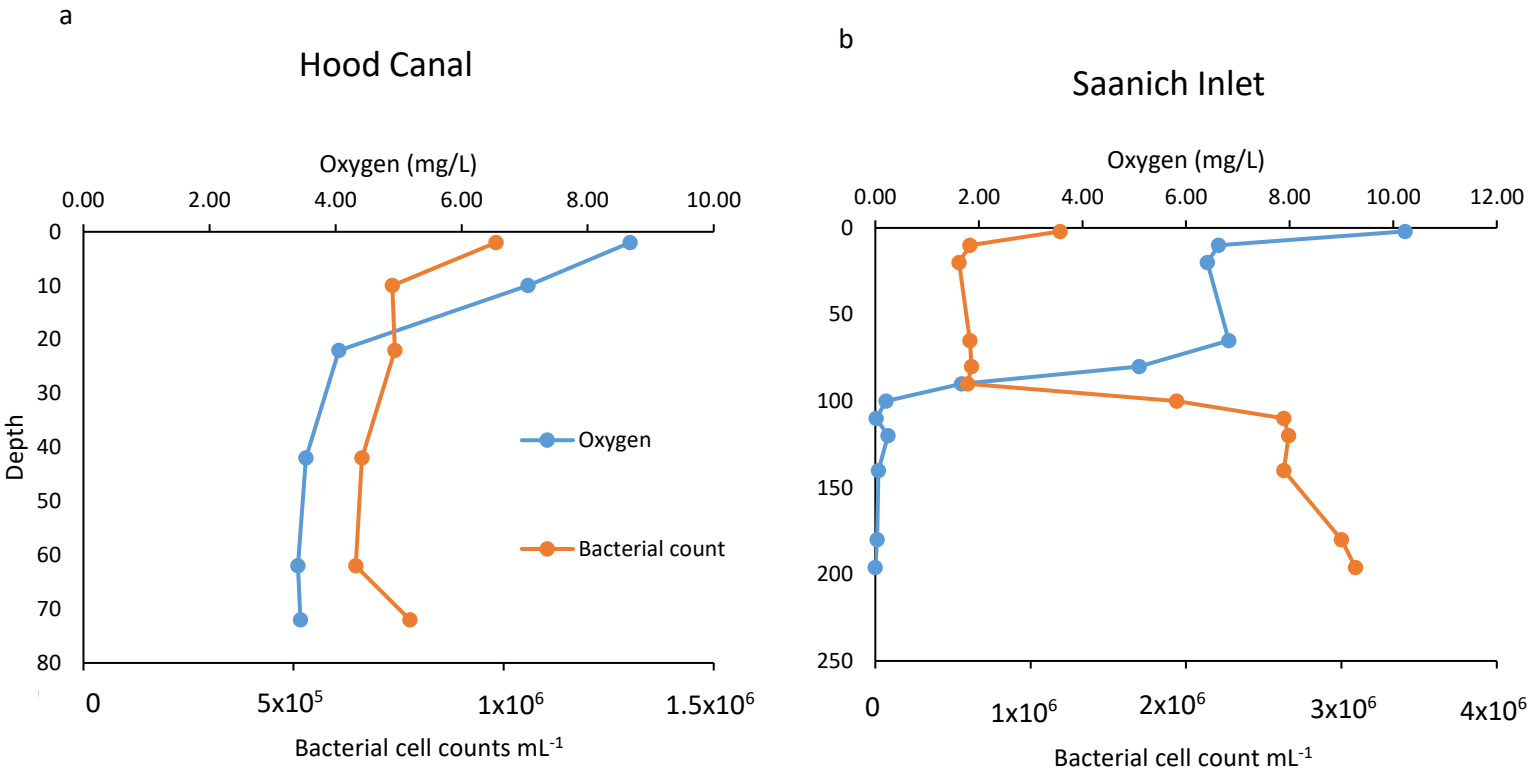


Figure 1 shows the water column from January 2018 of both Hood Canal and Saanich Inlet. Bacterial counts are compared to the oxygen level in order to assess the potential relatedness between oxygen level and bacterial concentration.

Figure 2.

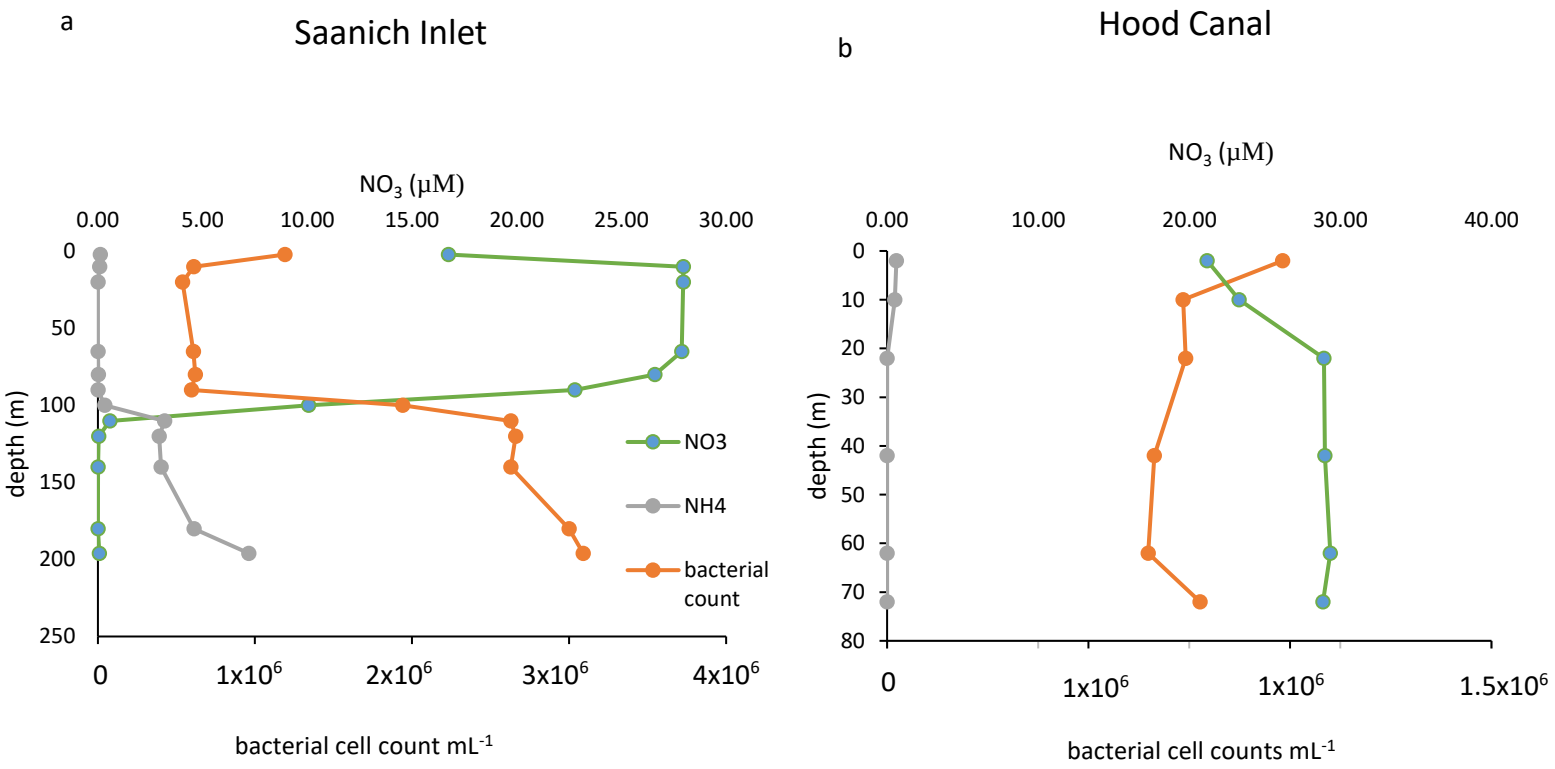


Figure 2. Comparison between the water profiles from January 2018 of Saanich Inlet (a.) and Hood Canal (b) when comparing the bacterial counts the $NO_3$ and the $NH_4$. The profiles looks at how the water chemistry may affect the bacterial concentration or vice versa.

Figure 3.

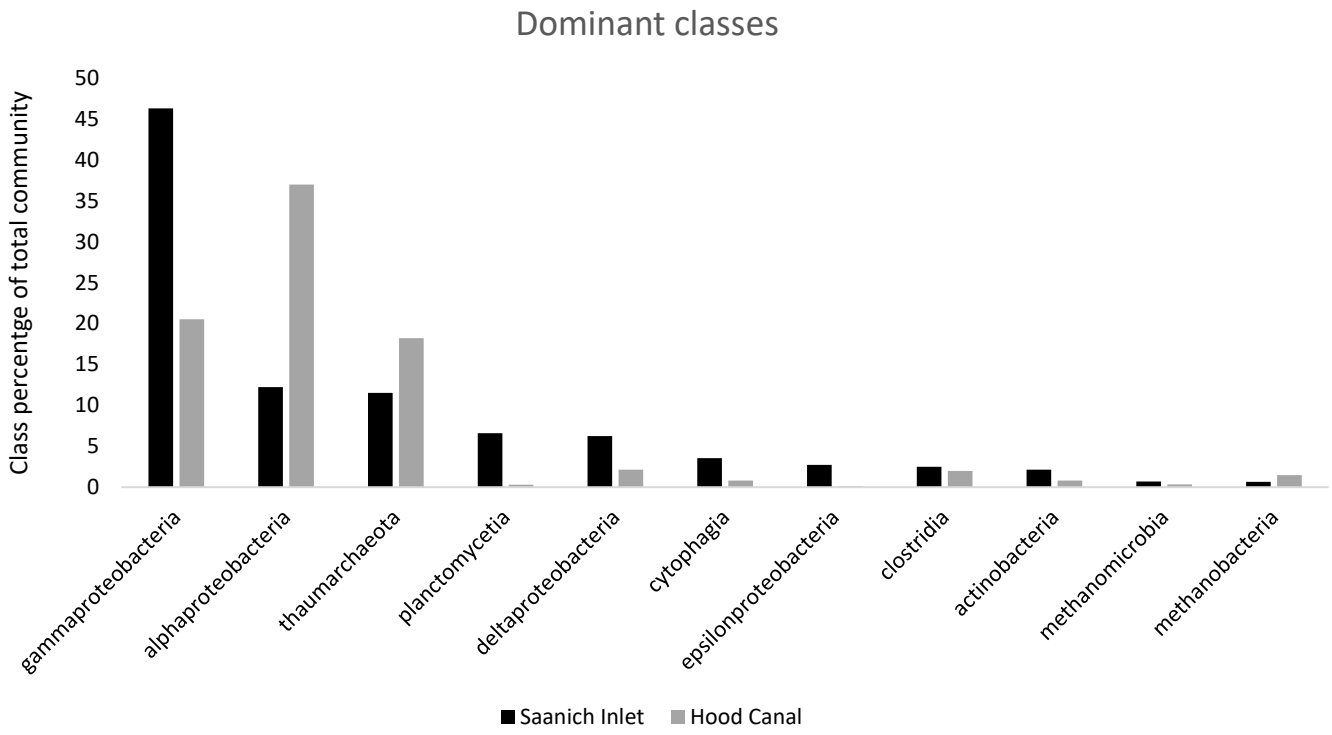


Figure 3 shows the proportion of the entire community in Saanich Inlet and Hood Canal in January 2018. Percentages were determined by 16S DNA analysis in order to characterize the microbial community in each location and identify the dominant groups.

Figure 4.

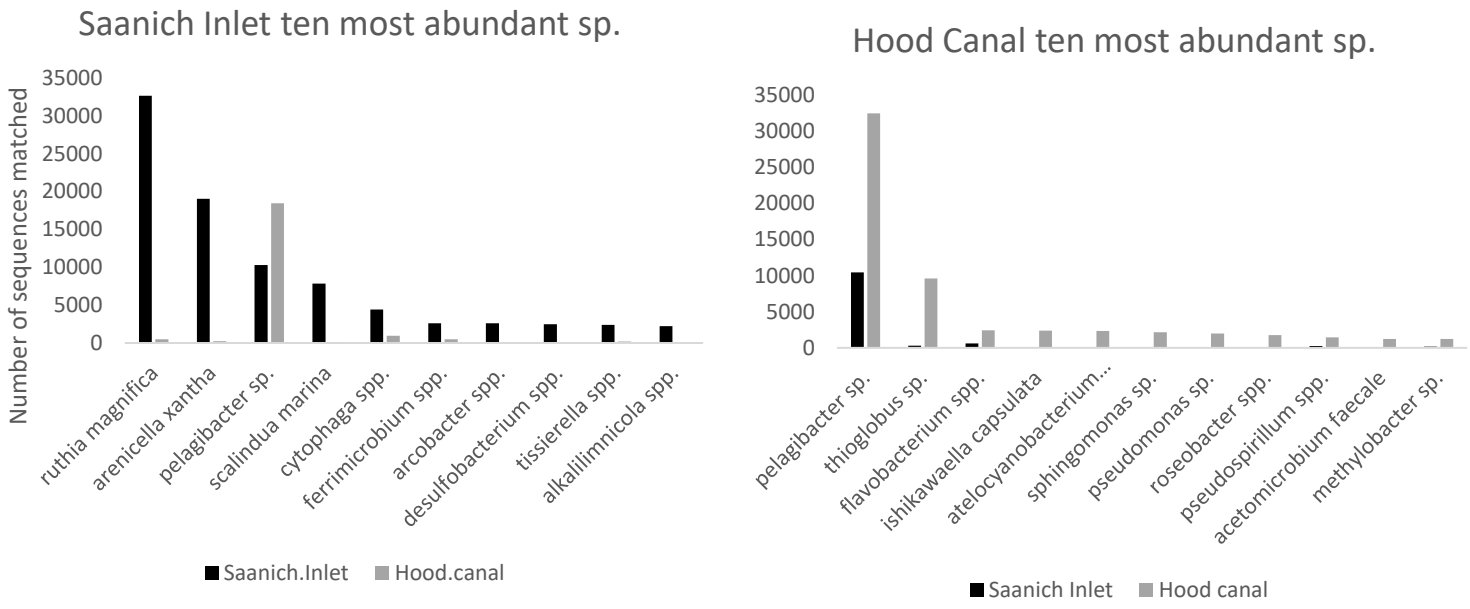


Figure 4 shows a comparison of the ten most abundant species found in Saanich Inlet and Hood Canal. 16S genomic analysis was performed on each location to determine the species that dominate the location giving insight into how the areas differ in bacterial composition.