

Presence of emerging pollutants in surrounding waters of
Bamfield, British Columbia, Canada



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Non-Technical Summary:

Pollutants have long been a problem in our world's oceans, directly affecting organisms within and indirectly affecting human health and resources. Research was conducted in Barkley Sound, British Columbia, to determine if pollutants are present in the waters of British Columbia, specifically around fish pen locations. To do this, seawater samples were taken at 22 stations in six different water channels and analyzed for a suite of emerging pollutants. Each sample was filtered and extracted, eventually concentrating a 4L water sample down to a 4uL sample. A combination of filtering and GC-mass spectrometry, which determines the composition of the sample provides the various compounds present in each of the 22 samples. Within the samples pesticides, hormones, musks, and antibiotics were found. Thymol, a pesticide, was the most common amongst all stations along with all four of the hormone compounds found near fish pen stations. Overall more natural emerging pollutants were found than man-made pollutants within Barkley sound, concluding that Barkley Sound is a reasonably clean water area.

Acknowledgments:

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Abstract:

The ocean has long been a home to general pollutant compounds, but recently emerging pollutants have become more difficult to study. Throughout various transects of Bamfield, British Columbia, research was conducted to determine if the waters of Bamfield, BC are home to pollutant compounds, specifically near Atlantic Salmon fish pens. To do this, seawater samples were taken at 22 stations during March 23-27 within various bays, channels, and inlets and analyzed for 38 specific pollutant compounds. Each sample was ran through a GC-MS and detected for compounds, resulting in 32 out of the 38 compounds present within samples. Thymol was present within all 22 stations with an average concentration of 1.12 ng/L, and surface waters have a higher average concentration than depth. All four hormone compounds were also found near fish pen stations with the highest concentration in San Mateo Bay #1 with 24.851 ng/L of 17 α -ethynylestradiol present. The pollutant concentrations were compared to Puget Sound concentrations where it was found that the Barkley Sound had higher frequencies for 8 out of 14 emerging pollutants analyzed, but overall Puget Sound had higher pollutant levels for all emerging pollutants at all stations.

The various channels, inlets, and bays of Bamfield, British Columbia are an area of little research, especially relating to pollution in surrounding waters and environmental impacts of drugs used in fish farming (Bjorklund et al. 1990). Although marine fish farming ranks among one of the fastest growing industries in the world, emerging pollutants still have unknown environmental effects (Holmer et al. 2003). Emerging pollutants are defined as chemicals or toxins people believe are a problem, but are not governmentally regulated yet. Currently emerging pollutant levels in the Puget Sound range from 0 to 992.4 ng/L. The focus of this study will be to look at the abundance of general emerging pollutants and compounds, specifically around fish pens, and state whether the waters throughout Bamfield, BC are home to general pollutant compounds. A comparison of locations within Bamfield and Puget Sound will also be done for any difference in pollutant abundances, specifically of xenobiotics (completely man-made). Some of the general environmental pollutants found in water today are metals, pesticides, and organics (Scott and Sloman, 2004), with three categories of input, sewage, nutrients, and synthetic-organic compounds, being a priority to analyze (McIntyre, 1992). Pollution in the world's oceans is becoming a major problem with toxic pollutants in ecosystems having enormous impacts on plants and animals, especially within the benthic environments (Marine Pollution, 2003). Decomposition of particulate waste matter from marine fish farming is increasing the organic loading of benthic environments causing water column hypoxia and higher rates of nutrient cycling (Holmer et al. 2003). Hypoxic sediment was particularly seen in Effingham Inlet, British Columbia even where fish pens were not present, although many shellfish farms were. Hypoxic sediment is not the only means of pollutant transportation as pollutants are reaching coastal waters from rivers, direct discharges, and spills but rarely making it to the open ocean. Two of the

main transports for pollutants reaching the open ocean are atmospheric and shipping (McIntyre, 1992).

The goal of this study is to determine whether the waters of British Columbia are home to emerging pollutant compounds by combining water samples and mass spectrometry to see if the presence of compounds heightens around Atlantic salmon pen locations. Specific pollutant concentrations will also be compared to previously analyzed Puget Sound waters during the month of March. The pollutant compounds thymol, isothymol, acetylsalicylic acid, ibuprofen, diazinon, musk xylene, musk moskene, musk ketone, chlordane, bisphenol a, 17a-ethynylestradiol, mestranol, B-estradiol, and progesterone, will be broken up into four groups, antibiotics, hormones, musks, and pesticides.

Methods:

Sampling: Samples were collected for pollutant compound analysis at 11 stations aboard the Barkley Star from March 23-27. The cruise track extended into Effingham Inlet (EF-1, 2, 3), Trevor Channel (TC), Alberni Inlet (AI), Imperial Eagle Inlet (IE), San Mateo Bay (SMB), and Sarita Bay



Figure 1: Barkley Sound sampling stations. 10 stations marked throughout Effingham Inlet (EF 1,2,3), Imperial Eagle Inlet (IE), Trevor Channel (TC), San Mateo Bay (1&3), and Sarita Bay (SB2).

(figure 1). The sampling scheme consisted of CTD casts (10-200m) where samples were

collected from 2-3 Niskins on all casts. From each Niskin taken at depth seawater was drawn into 4L plastic containers and stored until analysis. Surface samples at 5 m were also taken at all stations using a hand lowered Niskin.

Filtering: Water samples were initially individually pre-filtered at Bamfield Marine Sciences Center using a vacuum and 47mm glass-fiber filter to extract all particulate matter. Samples were then returned to their original container and labeled with the exact amount filtered, which was 4L. Samples were then acidified with approximately 3300 uL of 12M (37%) HCL allowing the sample to reach a pH of 2. Samples were filtered again using 6cc/200mg cartridges, which were pre-treated and ran using (Keil and Neibauer, 2009) protocol. Once each sample was completely filtered the cartridges were removed from the manifold and stored in the fridge for further analysis at the University of Washington labs.

Cartridge Elution: Elution's were done on all 22 samples according to the (Keil and Neibauer, 2009) protocol. This allowed the samples to be filtered again using the vacuum manifold and the addition of ethyl acetate. After filtration the samples were moved to the TurboVap, which emits pressure individually to each sample, causing it to slowly evaporate. Samples were checked periodically and taken out of the TurboVap when only 0.5mL of liquid was left. The vials were then weighed for the exact 0.5mL weight. All sample weights were recorded and vials were returned to the freezer.

Analysis: Samples were analyzed using a gas chromatography - mass spectrometer (GC-MS), which combines both methods in order to identify specific substances within a sample by identifying trace elements that may have thought to be completely disintegrated. The analysis method of GC – MS was used to predict concentration values by filtering and eluding 4L seawater samples taken from various locations throughout the

Barkley Sound. By using the GC-MS method we were able to identify any trace amounts of specific pollutant compounds present in the Barkley Sound samples. Once samples were ran through the GC – MS, peak areas were analyzed. In order to get accurate concentration values of pollutant compounds, any peak area with a q-value lower than 70 was changed to zero. Of the 39 compounds being identified by the GC-MS, 14 were specifically analyzed for patterns and trends. Once the sample is filtered down to a GC vial, 50 uL of sample and 50 uL of BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) are combined and analyzed. Once the concentration of the compound is found by injecting standards of known concentrations (i.e. 0.03mM, 0.05mM, and 0.07mM), steps are traced backwards to get compound concentrations from the initial 4L sample.

Mapping: Data from the 14 compounds was plotted against each other at all stations. Of particular interest was spatial distribution of the compounds present in San Mateo Bay and Sarita Bay and the difference between compounds at surface and depth.

Results:

GC-Mass Spectrometry helped to determine the predicted pollutant compounds for each station. By using samples taken from both surface and depth, concentrations were calculated within the GC vial, and concentrations from the original samples were calculated. Four antibiotic, four hormone, two musk's, and four pesticide pollutant compounds were found within the waters of Bamfield, BC. Of the 14 compounds being looked for 13 were found, with Musk Xylene not being found at any stations. Each hormone compound was found at fish pen stations, i.e. San Mateo Bay #1, #3, and Sarita Bay #2 (figure 2).

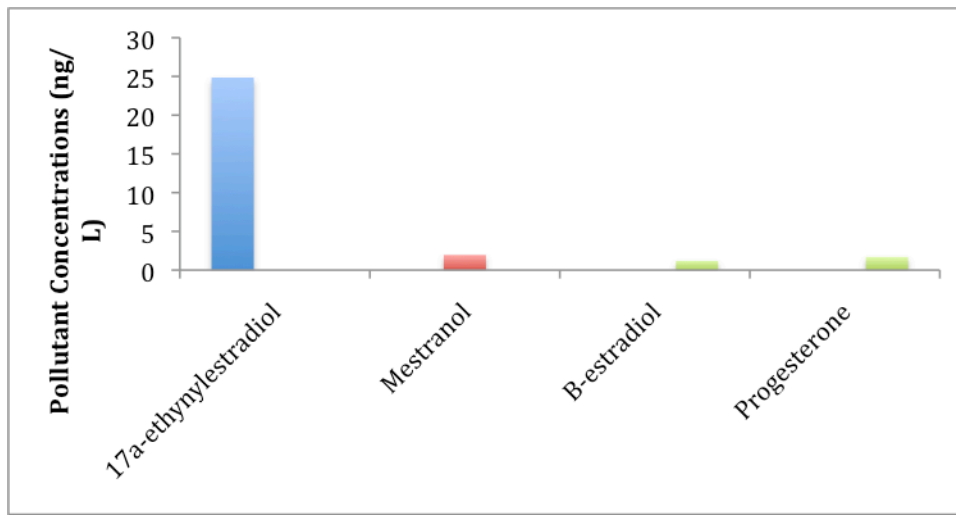


Figure 2: Average pollutant concentrations from all fish pen stations showing 17a-ethynylestradiol with the highest average level of the 4 emerging pollutant hormones.

Compound concentrations were B-Estradiol (San Mateo Bay #3, 1.191 ng/L at 5m), Mestranol (Sarita Bay #2, 1.499 ng/L at 5m and 2.409 ng/L at 140m), Progesterone (Sarita Bay #2, 1.704 ng/L at 140m), and 17a-ethynylestradiol (San Mateo Bay #1, 24.851 ng/L at 5m). Thymol was found at every station ranging from concentrations of 0.18 ng/L to 2.574 ng/L. Thymol concentrations were on average higher at the surface, 1.3 ng/L, than at depth, 0.87 ng/L (figure 3).

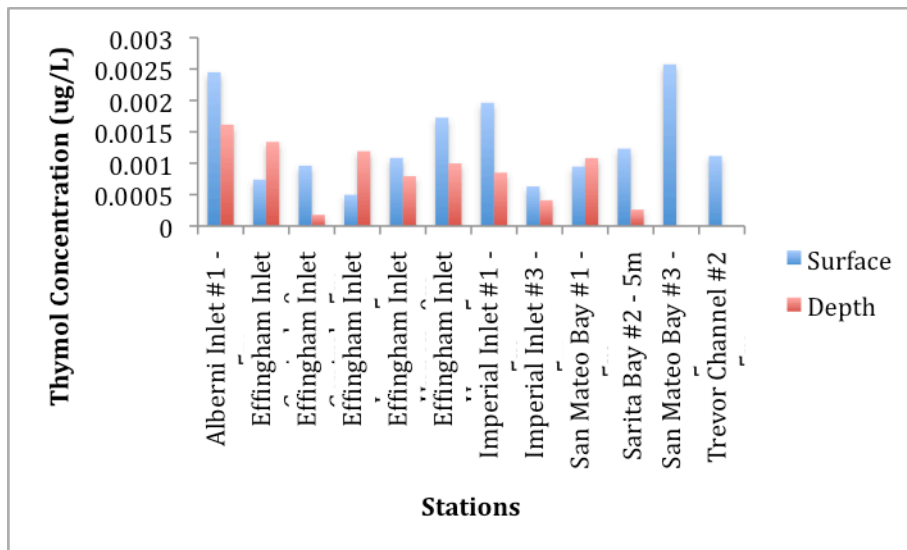


Figure 3: Thymol concentrations within Barkley Sound looking at Surface versus Depth. Surface values were higher for at all sampling spots except for 3 locations.

Bisphenol A, an antibiotic found in plastics was found at 14 of the 22 stations with concentration values ranging from 0.19 ng/L to 10.98 ng/L.

Emerging pollutant frequencies in Barkley Sound are seen in figure 4 as frequency percentages for all 14 analyzed

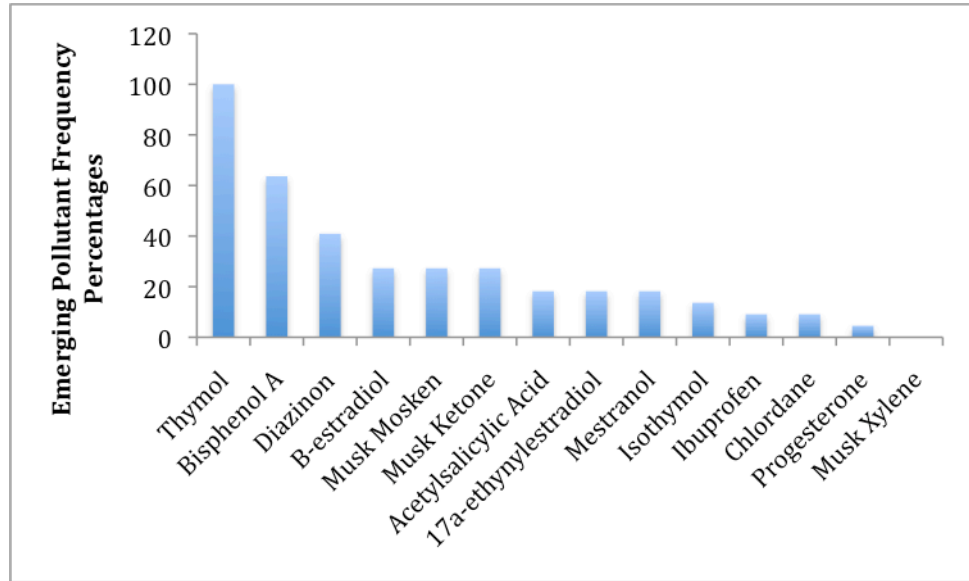


Figure 4: Emerging pollutant frequency percentages for all 14 analyzed emerging pollutants within Barkley Sound. Thymol has the highest percentage (100%) with Bisphenol A at a close 63%. Musky Xylene is the lowest with 0%.

pollutants. When compared to the Puget Sound, Barkley Sound had higher frequency percentages for 8 of the 14 emerging pollutants (figure 5), but overall, Puget Sound had higher pollutant levels for all 14 pollutants.

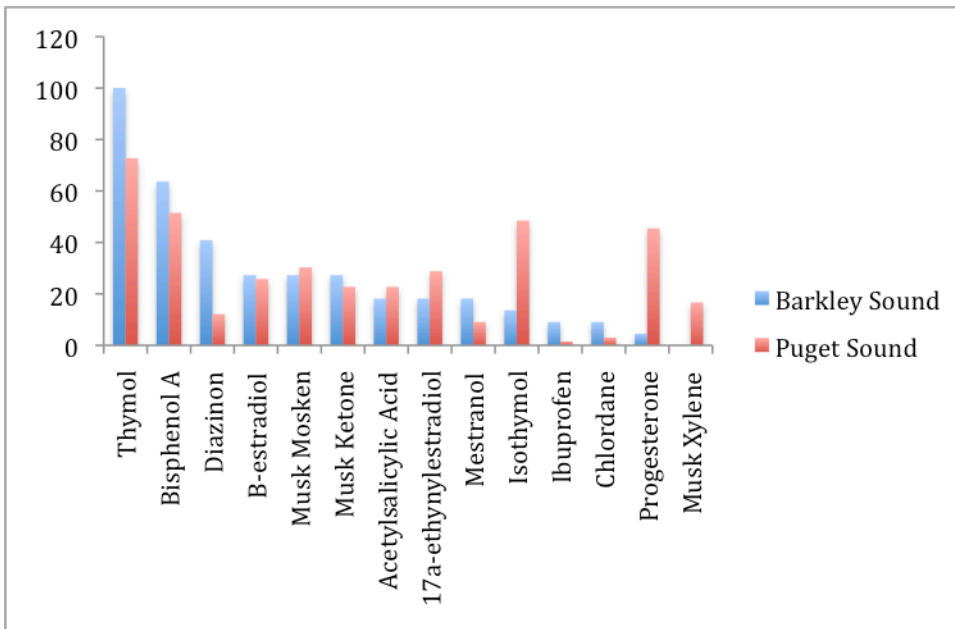


Figure 5: Comparison of frequency percentages for Barkley Sound vs. Puget Sound. All 14 emerging pollutants are compared with 8 out of the 14 having higher frequency percentages in Barkley Sound.

Barkley Sound had higher average frequencies of natural emerging pollutants (21.6%) versus xenobiotic (man-made) pollutants (19.6%) (Figure 6).

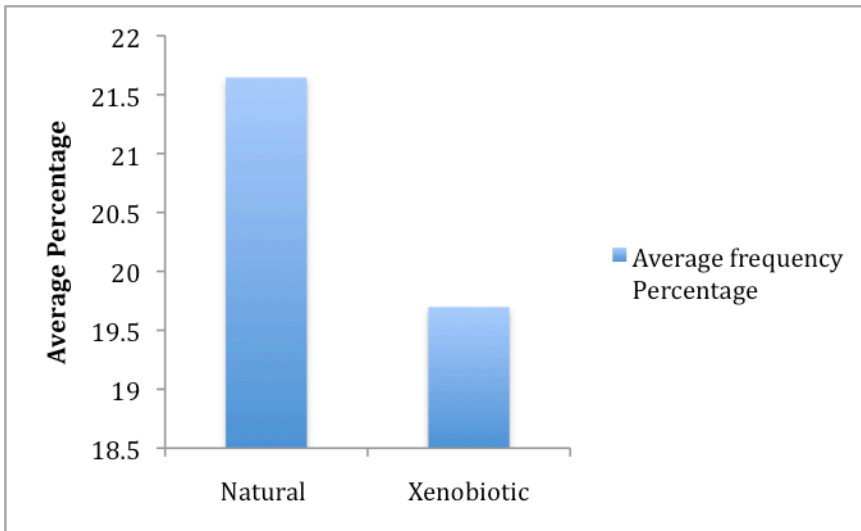


Figure 6: Average frequency percentages for natural vs. xenobiotic pollutants within Barkley sound. There were 3% more natural pollutants than xenobiotic.

Of the three musk’s being analyzed (ketone, xylene, moskene), all but musk xylene were found in both Barkley Sound and Puget Sound. Puget Sound had a higher frequency percentage of Musk Moskene than Barkley Sound, 30.3% versus 27.3%, but a lower frequency percentage of Musk Ketone (figure 7).

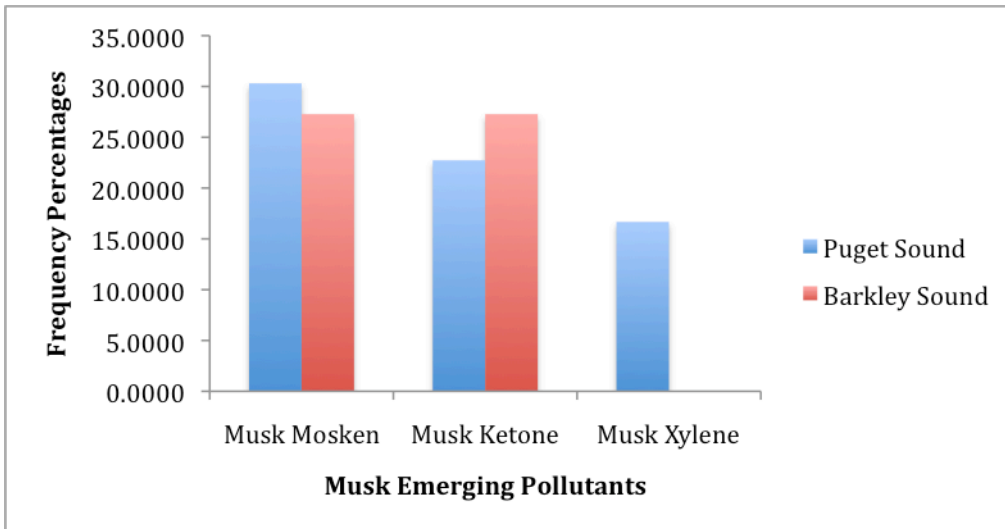


Figure 7: Frequency percentages for Musk Moskene, Ketone, and Xylene taken in both Barkley Sound and Puget Sound. Musk Xylene was not present within Barkley Sound. Puget Sound had a higher frequency percentage of Musk Moskene, but not Musk Ketone.

Discussion:

Once peak areas were converted to concentration (mM) within the GC vials, $M1V1 = M2V2$, conversions were made again to get the sample into mmol of compound. During this step we must make the assumption that everything came off of the cartridge during the elution process, thus stating that our mmol of compound is equivalent to the amount of the compound on the cartridge. By simple conversion the compound concentration is then converted to ng/L, and the concentration of compounds from our original sample are found. After final concentrations are recorded, standards must be accounted for and subtracted from compound numbers if present.

The low pollutant concentrations discovered show that overall the Barkley sound water was very clean. Although 32 of the 38 compounds were present in water samples, all had very low concentrations. Ten other compounds were only present in three stations or less, resulting in 22 compounds with significant presence. Thymol, a natural antiseptic pesticide was the only pollutant compound found in all 22 stations with greater concentrations at the surface since liquid thymol is mainly found floating amongst the surface (Gildemeister, 1913). Thymol had the highest frequency percentage for both Puget Sound and Barkley Sound waters, as well as Bisphenol a at a close second. Bisphenol a is anticipated to be seen in high levels because of its presence in the coatings of food and beverage cans, as well as it's ability to absorb into sediments (Staples et al. 1998).

At the three fish pen locations, San Mateo Bay #1, San Mateo Bay #3, and Sarita Bay #2, all four hormone compounds were found in the waters of at least one of the stations. As well as hormone compounds, 15 other pollutant compounds were present within fish pen stations, which were predicted due to the fact that fish pens are

responsible for an estimated 5,000 tons of organic pollutants (Raswson et al. 2007). One particular pollutant, 17 α -ethynylestradiol, was only found in the San Mateo Bay #1 at 5m with a concentration of 24.851 ng/L, one of the higher concentration values. 17 α -ethynylestradiol is a xenobiotic version of estrogen, which makes it particularly interesting to be found at the surface in a main fish pen location. 17 α -ethynelestradiol is also found in birth control pills, which may be a factor in its presence at the fish pen location due to women living at these sites. The presence of Progesterone, a natural female steroidal hormone, at only Sarita Bay near the fish pens, may also be due to female presence. This type of human impact is particularly interesting because of the remote location of Barkley Sound.

Natural pollutants analyzed within Barkley Sound were Thymol, Isothymol, Musk Moskene, B-estradiol, Progesterone, and Coprostanol. Xenobiotic pollutants measured were Acetyl Acid, Ibuprofen, Chlordane, Bisphenol a, 17 α -ethynylestradiol, and Mestranol. From these emerging pollutants measured within Barkley Sound, natural pollutants had a higher average frequency percentage than xenobiotic, 21.6% versus 19.7%. Although they were very close in percentage there were 1.9% more natural pollutants found which is good for British Columbia waters. Natural pollutants are likely to be seen at higher concentrations because of human and animal impacts. Human and animal impacts also play a large role in the high percentage of xenobiotic pollutants found within Barkley Sound waters. This is due to thymol as a natural antiseptic, mestranol and 17 α -ethynelestradiol in birth control pills, and isothymol as a food preservative.

Along with thymol, isothymol, mestranol, and 17 α - ethynelestradiol being present due to human impact, diazinon and chlordane are also frequently seen in Puget Sound

waters near urban areas, often times at levels above chronic guidelines (Ebbert et al. 2000). Within Barkley Sound, diazinon had a 41% frequency; having an average pollutant concentration of 0.25 ng/L whereas diazinon levels in Puget Sound were 1.2 ng/L. Although indoor use of diazinon has been recently outlawed, it is still used as a pesticide for fields, lawns, and soils. Chlordane, another xenobiotic pesticide, had a 9.1% frequency with a concentration of 0.23ng/L within Barkley Sound. Chlordane is expected to have higher concentrations within Puget Sound, with a pollutant level of .8ng/L, due to the higher population of humans and agricultural landscape.

Compared to Puget Sound (Keil et al. 2009), compound concentrations in Barkley Sound were significantly lower. The presence of thymol in the Puget Sound was 23 ng/L whereas the average concentration of thymol throughout all 22 stations in Barkley sound was 1.12 ng/L. Based on surface and depth; thymol had higher concentrations at the surface for 9 of the 12 stations. This could be due to its presence in natural antiseptics, allowing it to accumulate at the surface from agricultural run-off. In general, all pollutant compound concentrations found within the Puget Sound by Rick et al. 2009 were on average 22.3 ng/L higher than Barkley Sound.

Conclusions:

- 32 of the 38 pollutant compounds were found
- Only 10 compounds were present in 3 stations or less
- 22 compounds had significant presence
- Antibiotics, hormones, musk, and pesticides were found at all 22 stations
- Progesterone, a steroidal hormone was only found in Sarita Bay near fish pen locations
- Barkley Sound is an overall very clean water area with although a lot of pollutants present, found in very small and rather insignificant amounts
- Thymol (found at all stations) has a greater average concentration at the surface than at depth (1.3 ng/L-surface vs. 0.87ng/L-depth)
- Natural pollutants have a higher average frequency in Barkley Sound than xenobiotic pollutants

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