The marine methane cycle in the Canadian Arctic Archipelago during summer.

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Abstract

In the Arctic Ocean region, methane (CH₄) concentrations are higher than the global average, with particularly high concentrations of dissolved CH₄ observed along many subarctic and Arctic continental shelf margins. Despite this, the Arctic Ocean emits only minimal methane fluxes to the atmosphere across the air-sea interface, suggesting that water column oxidation of methane may be an important process.

In this study, we paired thermohaline, chemical, and biological data collected during the Northwest Passage Project transit through the Canadian Arctic Archipelago (CAA) waters in the summer of 2019 with in-situ and in-vitro methane data. Our findings suggested that the most elevated in-situ concentration of dissolved methane was present in the near-surface waters of the Pacific, particularly in meltwater regions. The highest methane concentrations were observed within shallow waters, averaging at 5.8±2.5 nM within the upper 30m depth. Furthermore, the methane distribution showed a distinct pattern from east to west, with higher concentrations and oxidation rate potential in the western region. In our study, we observed generally low methane oxidation rate constants, averaging at 0.006±0.002 d⁻¹. However, surface waters from Wellington Channel and Croker Bay exhibited relatively higher methane oxidation rates, averaging at 0.01±0.0004 d⁻¹. These regions were distinguished by a significant proportion of meltwater, including both meteoric water and sea ice meltwater, mixed with water of Pacific origin. We identified microbial taxa of Pacific-origin likely associated with methane oxidation, including Oleispira (γ-proteobacteria) and Aurantivirga (Flavobacteria), in the Pacific and meteoric waters. In contrast, deeper layers (>200m depth) showed lower methane concentrations (av. 3.1±1.1 nM) and lower methane oxidation rate constants (av. 0.005±0.001 d⁻¹). Within the sea ice, dissolved methane concentrations were found to be higher than the concentrations at equilibrium with atmospheric capacity, with an average of [CH₄]=9.2±5 nM. The sea ice temperature data (Table S2) indicated the presence of ice permeability, which likely facilitated the release of dissolved methane that was either trapped or produced since the previous freezing period. Notably, methane concentrations were 25% higher in waters collected in the western CAA in comparison to the ice-free waters (eq. S1).

The overall picture suggested supersaturation of in-situ methane in shallow waters, coupled with faster oxidation rates in meltwater and Pacific dominant layers, suggesting rapid seasonal cycling of methane and prevention of the methane migration into the atmosphere.

1. Introduction

Methane (CH₄) is a significant climate-relevant gas with a global warming potential 28 times greater than carbon dioxide (CO₂) over a 100-year timescale (Pachauri et al., 2014). It is the second most
significant greenhouse gas after carbon dioxide, contributing to approximately 23% of climate change in the twentieth century. Anthropogenic emissions from agricultural practices, fossil fuel production, and waste disposal account for most of the methane released into the atmosphere, while minor sources such as wildfire, biomass burning, permafrost, termites, dams, and the ocean, contribute to the remaining 20% (Saunois et al., 2020). Methane concentrations in the Arctic region are 8-10% higher than the global average, with a dry air mole fraction of 1890 ppb (Saunois et al., 2020; Oh et al., 2020). Recent studies have measured elevated concentrations of dissolved CH$_4$ in the subarctic and Arctic shelf areas, with limited gas exchange at the air-sea interface (e.g., Fisher et al., 2011; Shakhova et al., 2014; Steinle et al., 2015; Ferré et al., 2020).

In-situ measurements of methane concentration in the water column can reveal information about past processes that have affected its distribution. Methane is relatively stable in the water column, persisting for a significant amount of time after it has been produced, transported, or dissolved (Yamamoto et al., 1976). Therefore, in-situ measurements can provide a historical record of methane in the water column. In contrast, in-vitro methane experiments, such as methane oxidation rate assays, provide information on the potential microbial processes that could occur. These experiments measure the rate at which methane is consumed by microorganisms present in the water sample. This information helps to understand the potential for methane to be removed from the water column through microbial processes and provides insight into the cycling of methane. Hence, understanding the role of marine microbial metabolism in methane production and oxidation is crucial for assessing the amount of methane vented into the atmosphere. Environmental drivers control the methanotrophy (microbial aerobic oxidation of methane) by supplying suitable substrates for metabolic processes (Singh et al., 2010). Observations of microbial community structure can be used to identify ecosystem states, estimate biological activity rates, and better understand the mechanisms underlying changes in biogeochemical processes. Previous studies have investigated the methane flux in various regions of the Arctic Ocean, including the Northwest Passage (NWP), and assessed the methane budget (Kitidis et al., 2010; Damm et al., 2010; Sultan et al., 2020; Manning et al., 2020, 2022).

In the NWP, methane undersaturation was attributed to meltwater, while methane oversaturation was detected underneath multi-year sea ice (Kitidis et al., 2010). Multi-year observations have suggested that riverine freshwater is not a major source of CH$_4$ in the Canadian Arctic Archipelago (CAA), at least during summer and early fall (Manning et al., 2022). Furthermore, it was determined that the rate of CH$_4$ exchange between the ocean and atmosphere was low, indicating that this region has a minor role in regulating global atmospheric concentrations of methane across the North American Arctic Ocean (Manning et al., 2020, 2022). Therefore, the primary sources of CH$_4$ in the CAA remain poorly identified.

This study aims to further elucidate the methane cycle in the NWP, quantify seawater methane oxidation as a sink, and detect its main association with environmental features and biogenic control in the water column. These analyses are supported by thermohaline, chemical, and biological characteristics of the water masses in relation to the methane cycle. The uniqueness of this study lies in the analysis of paired datasets of dissolved methane and potential oxidation rates with associated marine microbial communities and physical-chemical properties of the water masses in the CAA, such as temperature, salinity, chlorophyll-α fluorescence, turbidity, and nutrients.

### 1.2 Study area

The study area was centered around Parry Channel, between 71 – 77 °N, and 100 – 79 °W (Fig. 1). From East to West, named parts of the Channel are, Lancaster Sound, Barrow Strait, and Melville Sound. This Channel connects Baffin Bay to the east with the Beaufort Sea to the west.
Figure 1: The left panel displays the bathymetric chart of the Arctic Ocean, with the study area delineated by a white rectangle. The right panel shows the study area during the Northwest Passage cruise in July-August 2019, with sampling stations represented by yellow dots and sea ice coring sites by red dots.

The Arctic Ocean (AO) features a vast estuarine system that exerts thermohaline (buoyancy) forcing on the inputs and disposition of freshwater components (Stigebrandt, 1984; Carmack et al., 2016). The Canadian Arctic Archipelago (CAA) serves as a watershed discharge in the AO, as it receives advection from the relatively fresh upper layers of the AO, ice melt, local river discharge, and net precipitation (Ingram and Larouche, 1987). One of three main routes connecting the AO to the Labrador Sea and the North Atlantic is the Northwest Passage (McLaughlin et al., 2007). A limiting sill is present in the Lancaster Sound further east in Barrow Strait, where the depth is approximately 125 m. Continuing eastward, water depths increase gradually to approximately 500m in Lancaster Sound, then sharply to over 2000 m in the center of Baffin Bay (McLaughlin et al., 2007). The flow through the CAA, from the Pacific to the Arctic to the Atlantic Oceans, results from the higher sea level of the Pacific Ocean (McLaughlin et al., 2007). This sea level difference arises because Pacific waters (PW) are fresher, and assuming a level of no-motion among the three ocean basins, the Arctic is thought to be 0.15 m higher than the Atlantic (Stigebrandt, 1984). During the eastward transit through Parry Channel, the PW and Atlantic Water (AW) undergo mixing. The shallow sill at Barrow Strait restricts the eastward flow across Lancaster Sound, constraining the deep layer of AW (McLaughlin et al., 2007). The riverine runoff supplied by Cunningham River, Garnier River, and Mecham River has a significant impact on the hydrodynamics and biogeochemistry of Lancaster Sound (Brown et al., 2020). The Special Report on the Ocean and Cryosphere in a Changing Climate published by the IPCC in 2021 showed that the runoff into the AO increased by 3.3 ± 1.6% and 2.0 ± 1.8% for Eurasian and North American rivers, respectively (1976–2017). Tidal energy also affects the hydrodynamics within Parry Channel, entering the CAA primarily from the Atlantic Ocean and being mainly semi-diurnal. As a result, waters transiting the Northwest Passage are significantly modified by tidally-driven mixing, and tidal currents are particularly strong, reaching 50–150 cm s⁻¹ in the vicinity of Barrow Strait (Prinsenberg and Bennetr, 1989).
The water column structure is characterized by AW in the deep layers, with Pacific-origin waters overlaid, and seasonal mixed water at the top (McLaughlin et al., 2007). In the summer, the seasonal mixed layer (Polar Mixed Layer, PML) contains freshwater from river outflow and sea-ice melt, characterized by low salinities, warm temperatures, low nutrient concentrations, and high dissolved oxygen saturations. This water constitutes the uppermost layer, and its depth varies according to the meltwater supply (approximately 50 m deep in Parry Channel). Below this layer is the Pacific-origin summer water, which is characterized by relatively warm temperatures and higher salinities, with higher nutrient concentrations and decreasing oxygen saturations. Atlantic-origin water in deep layers shows maximum nutrient concentrations (McLaughlin et al., 2007). The western part of the CAA is characterized by more consistent sea-ice coverage compared to the eastern side (Agnew and Howell, 2003). According to data released by the Canadian Ice Service, the thickness of first-year sea ice in the CAA can vary between 2.5 m and 2.0 m in the northern and southern sections, respectively, with multi-year ice reaching a thickness of 3–5 m (Canadian Ice Service, 2002). From the time of freeze-up in January to the break-up in late July, the ice is usually immobilized as landfast ice, and is frequently accompanied by polynyas because of strong winds (Dunbar, 1969).

2. Materials and methods

The list of the sampling locations and their acronyms used in this paper is the following (Fig. 1): Westernmost station (WS), Wellington Channel (WC), Peel Sound (PS), Prince Regent Sound (PRS), West of Navy Board Inlet (WNBI), Crocker Bay (CB), Jones Sound (JS), Pond Inlet (PI).

2.1 Sampling procedures

Seawater and sea ice samples were collected in the vicinity of Parry Channel during the Northwest Passage Project cruise held between 17 July – 4 August 2019 onboard the Swedish icebreaker RVIB Oden. The stations include both single points and transects (see Fig. 1), covering the area with longitude 78°14.94’ W - 99°16.63’ W, seawater was collected using a SeaBird 32 Water Carousel CTD rosette (24 x 12L), set with a SeaBird SBE 911+ CTD with dissolved oxygen and WETLabs Ecopuck sensors. The CTD sensor was owned and calibrated by the Swedish Polar Research Secretariat (SPRS). CTD casts and rosette bottle data are hosted at Swedish National Data Service (https://snd.gu.se/en/catalogue/study/2021-119#dataset), whereas the whole dataset and the processed CTD data are stored in Arcticdata.io (https://doi.org/10.18739/A2BN9X45M).

The sea ice concentration data was provided by the University of Bremen data archive, with 1-km space resolution (seaice.uni-bremen.de, Ludwig et al., 2019 and 2020). Sea ice charts from the Canada Ice Center were also used (www.canada.ca/en/environment-climate-change/services/ice-forecasts-observations/publications/interpreting-charts).

Methane – A total of 132 seawater samples (28x2 experimental and 76 discrete) (Table S1), and 5 sea ice cores (Table S2), were collected and analyzed to determine methane concentration and isotopic ratio. The sea ice cores were sampled using a Kovacs ice corer drill, while seawater was sampled through the CTD rosette as previously described. Sea ice was examined for in-situ dissolved methane, while in seawater, we also measured the microbial oxidation rates. Methane concentrations and isotopic ratios were analyzed in both in-situ and in-vitro samples using the method described by Uhlig and Loose (2017). Together, in-situ measurements and in-vitro experiments provide complementary information on methane in the water column. In-situ measurements reveal the past processes that have influenced methane concentrations, while in-vitro experiments provide insight into the potential for methane metabolism and
removal from the water column. All the samples were collected using multi-layer foil gas sampling bags (Restek, Bellfonte, PA – USA) and processed for further analysis following the details provided in paragraphs 2.2 and 2.3.

Microbial community – A total of 18 seawater samples were obtained from the in-vitro methane incubations at the end of the experiments to identify the most abundant microbial taxa. These samples were then juxtaposed with in-situ samples for comparative analysis. To collect the samples, a cylindrical, 0.22 µm Sterivex membrane filter (Millipore Sigma, Billerica, MA) was attached to the valve of each bag. DNA was extracted from the filters by drawing water from the Sterivex filter. The filters were then stored at -80 °C until they were transported to the University of Rhode Island (URI), Graduate School of Oceanography (GSO) for DNA extraction using the DNeasy PowerWater Sterivex Kit (Qiagen, Germantown, MD), following the manufacturer’s protocol (See paragraph 2.4 for further details).

Nutrients – A total of 239 seawater samples were collected to measure nutrients (doi: 10.18739/A2BN9X45M). Each sample had a volume of approximately 45 mL and was filtered through a 0.22-micron Milllex-GP Sterile Syringe Filter with PES Membrane (Thermo Fisher Scientific). The filtered samples were transferred to a Corning Falcon 50 mL Conical Centrifuge Tube (Fisher Scientific) and stored at -20°C onboard RVIB Oden for post-cruise analysis. Although the nutrient results are not discussed in this context, they will be utilized in the analysis to determine the Arctic Nitrate-Phosphate tracer in the water mass analysis, as described in Jones et al. (1998) and Tomczak (1981).

δ¹⁸O-Salinity - A total of 125 water samples were collected from 52 CTD casts following the CLIVAR/GO-SHIP protocol to investigate δ¹⁸O-Salinity relationship. To collect samples for water stable isotopes analyses, 30-mL Nalgene bottles were filled to the brim, tightly closed, sealed with parafilm, and stored in labeled sample bags. Two samples were taken per depth. All water samples were transported to the Atmosphere, Climate, and Ecosystems lab at University of Illinois at Chicago (UIC) for processing. The δ¹⁸O-Salinity dataset, which comprises more than 200 new and paired δ¹⁸O-Salinity measurements in the CAA, is publicly available through Pangaea at https://doi.pangaea.de/10.1594/PANGAEA.937543. As for the nutrients data, we utilized the δ¹⁸O samples to evaluate water mass fractions solving a simple 4-endmember mass balance analysis, as described in Tomczak (1981) and developed by Östlund and Hut (1984). For more information, refer to paragraph 2.5.

2.2 Methane concentration and isotope ratio measurements for in-situ samples

Samples processing – Seawater and sea ice for methane concentration and isotopic analysis was collected through vacuum multi-layer foil gas sampling bags equipped with a polypropylene combo valve and septum (capacity 3L, # 22951, Restek, Bellfonte, Pennsylvania, U.S.A.). For seawater, experiments were performed with approximately 2.8 L. The sea ice cores were put in 3L bags and melted at room temperature for the analysis. In all the bags a headspace of approximately 50 mL was created using hydrocarbon-free air, and the samples were stored in water at room temperature (approximately 10°C). Bags were analyzed for [CH₄] and isotopic signature (δ¹³CH₄) directly after preparation, following the method of Uhlig and Loose (2017). For assessing the methane saturation of the seawater and sea ice, we calculated the methane seawater and sea ice at equilibrium with the normal atmosphere, derived by the Bunsen solubility coefficients (Gas-Solubility Codes in R, https://github.com/URIGSO/Gas-248 Solubility-Codes), using in-situ potential temperature and practical salinity. The saturation anomaly was calculated as [(CH₄)_{in-situ} / (CH₄)_{equilibrium} - 1] *100, considering the solubility of the atmospheric methane at in-situ marine potential temperature and practical salinity.
Calibration - Standard gases 1001 (2500ppmv, $\delta^{13}$CH$_4$ = -23.9 ‰), 1002 (250ppmv, $\delta^{13}$CH$_4$ = -38.3 ‰), 1003 (2500ppmv, $\delta^{13}$CH$_4$ = -66.5 ‰) (Isometrics, Victoria, British Columbia, Canada) were used to generate linear calibrations for mixing ratios and isotope ratios in order to correct for any transient deviations in the G2201-i analyzer, while the standard 1003 was used almost simultaneously during the measurements to calibrate the measurements via daily drift (Uhlig and Loose, 2017). The 1003 standard revealed distinct instrumental drift while onboard the vessel Oden, as compared to the measurements on land; hence, we evaluated the dataset in two periods, from July 19th to August 4th (when the data were measured at sea), and from August 5th to August 14th (when the data were measured in the National Science Foundation facilities at Thule airbase, Greenland). The measurements of methane concentration were not calibrated, because the standard deviation between replicate dissolved methane water samples was significantly smaller than the standard deviation in gas standards that were introduced to the Picarro analyzer, likely due to a manual artefact during syringe sampling of the gas standards. A complete calibration of $\delta^{13}$CH$_4$ isotopic ratio was carried, because the isotope ratio was not affected in the same way as concentration during syringe sample injection. For the isotope ratio, we calculated the slope and intercept of the standard 1003 overtime and used the slope and intercept from the days of full calibrations (July 19 and July 29) into the equation for the data correction.

\[
\text{data.corrected} = \text{slope}. \text{day}. \text{cal} \times (\text{data} - \text{std}. \text{1003.overtime}) + (-66.5)
\]

Where, -66.5 ‰ was the expected isotopic signature of the standard 1003.

Quality control – We ran multiple measurements for each sample, in order to assess the uncertainty between the replicates and we only used data showing a standard deviation < 5% within the duplicated measurements.

2.3 Methane concentration and isotope ratio measurements for in-vitro experiments

We followed the methods of Uhlig and Loose (2017) with modifications.

Samples processing – Seawater for gas and isotope analysis was collected using vacuum multi-layer foil gas sampling bags fitted with a polypropylene combo valve and septum (#22950 Restek, Bellfonte, Pennsylvania, U.S.A.) with a capacity of 1 L. We used approximately 0.8-0.9 L seawater for each experiment. The headspace was created with approximately 100 mL of hydrocarbon-free air, after which a known volume (1 to 2.5 mL, as shown in Table S4) of gas standard 1003 with a known concentration and isotopic composition (as described earlier) was injected into the samples. The samples were left for several days before beginning the measurements. The incubation time ranged from 5 to 24 days (as indicated in Table S3), which was driven by logistical constraints. As the stable isotope and mass balance method is less sensitive to changes in methane, a longer incubation time was required compared to the radioisotope method. Samples collected earlier in the cruise had a longer incubation time, while those collected towards the end had a shorter time. Post-cruise, we extended the incubation times by continuing the analysis at a laboratory in Thule Air Base. However, the extension period could not exceed 10 days. While the smallest oxidation rates were not resolvable given the incubation time, we determined the lower limit using the uncertainty, as reported in Uhlig and Loose (2017). The samples were stored in a cold room at approximately 1°C throughout the experiment. For data calibration, we followed the same procedure used for the in-situ samples.

Estimating the oxidation rates from mass balance and isotopic fractionation – Microbial methane oxidation was quantified through incubation experiments using the method described in Uhlig and Loose (2017). In each incubation, a known volume of methane standard 1003 was added to monitor changes in mole and isotope ratio. In contrast to Uhlig and Loose (2017), we used an upper bound fractionation factor
\( \alpha_{ox} \) of 1.025 to calculate the oxidation rate constant for isotopic ratio, as it was consistent with the values obtained from the oxidation rate constants of the mass balance and isotope ratio (Fig. 2). The microbial oxidation rates were determined from the first-order rate constant \( k_{ox} \) and the concentration of dissolved methane in the water \([\text{CH}_4]_w\) during the incubation experiments, following the equation \( r_{ox} = k_{ox} \cdot [\text{CH}_4]_w \) (Uhlig and Loose, 2017). Figure 2 shows the trend line, which exhibited a Spearman’s correlation of 0.52.

Figure 2: Comparison between first order oxidation rate constants (\( k_{ox} \)) determined from \( \text{CH}_4 \) concentration (mass balance) and isotope ratios. The red line displayed the linear model between the values (\( R^2: 0.2564 \) p-value: 7.985e-12), whereas the red dashed line represented the 1:1 regression line.

Finally, to obtain an average \( k_{ox} \) value, we calculated the mean between \( k_{ox,\text{mass.balance}} \) and \( k_{ox,\text{isotope.ratio}} \). To assess the success of the experiments and measure the microbial activity, a killed control was prepared for each sample by adding 0.1M NaOH and injecting it into the sample after the final measurement. The \([\text{CH}_4]\) and \( \delta^{13}\text{C-CH}_4 \) were measured three times within a one-week time frame.

Quality control – We applied a stepwise criterion to evaluate and eliminate incubations of uncertain outcome. Duplicate incubation samples were collected for every in-vitro determination of methane oxidation rate. This resulted in a 2 by 2 matrix, with two independent measurements (mass balance and isotopic fractionation) for each in-vitro bag, to ensure a robust determination of methane oxidation. The following criteria were used to select the data for analysis:

1) A significance test using the Student’s T distribution was performed to ensure that the slope or rate of change in \([\text{CH}_4]\) or \( \delta^{13}\text{CH}_4 \) vs. time (oxidation rate) was unique from zero, within error. A confidence interval of 0.95 was chosen, and the number of independent measurements for each incubation was less than 15 (N<15).

2) The sign of \( k_{ox} \) from determinations from isotope ratio and molar mass determination for each incubation bag were compared. The bags showing agreement between the isotope ratio and the molar mass oxidation constants (\( k_{ox,\text{mass.balance}} \) and \( k_{ox,\text{isotope.ratio}} \) showing the same sign) were selected for further analysis.

3) In some cases, incubation bags agreed on the sign of \( k_{ox} \), but either mass balance or isotope ratio failed the significance test. For example, if \( k_{ox,\text{mass.balance}} \) accepted the null hypothesis and \( k_{ox,\text{isotope.ratio}} \)
rejected it for the same sample, this estimate was flagged. If the replicate incubation bag agreed in
sign of $k_{\text{ox}}$ with mass balance and isotope ratio passed the significance test, then the $k_{\text{ox}}$ estimates
were included. This amounts to requiring at least 3 agreements within the 2 x 2 matrix criteria (2
for mass balance and 1 for isotope ratio, or vice versa) were selected. Overall, 37 incubation
estimates from $N_{\text{tot}}=56$ was eliminated by this criterion.

Finally, if replicated bags did not pass the significance test, these were counted as zero detectable
methane oxidation, suggesting this water sample did not come from water hosting active methane
oxidizing microbes. These were counted as zeros, rather than being eliminated ($N = 2$).

The analyses were performed using R version 4.1.2 in RStudio Version 1.2.5033. Plots were prepared using
the base and ggplot2 packages.

2.4 DNA extraction and sequencing

In this study, we analyzed the diversity of methane-oxidizing communities in seawater using 16S
rRNA gene sequencing. The analysis was performed on both in-situ water samples and methane-incubated
samples collected at the final time point of the experiment. The methods used for this analysis were in
accordance with the protocols described in Uhlig et al. (2018) and Kerrigan and D’Hondt (2022).

DNA extraction – DNA extraction was performed using the PowerWater® DNA extraction kit
(Qiagen, Germantown, MD) and the DNeasy PowerWater Sterivex Kit (Millipore® cat. no. SVGPL10RC).
The latter involved a novel filter membrane treatment, where lysis buffer was added to Sterivex units and
mixed, followed by additional mechanical lysis in a 5 ml bead beating tube. After protein and inhibitor
removal, genomic DNA was captured on an MB Spin Column under vacuum, washed, and eluted from the
MB Spin Column filter membrane in a 50–100 µl volume. The extracted DNA was of high quality and
suitable for downstream applications, including PCR and qPCR.

PCR amplicon construction and sequencing - To construct PCR amplicons, the V4-V5
hypervariable region of the 16S rRNA gene was amplified using forward and reverse primers from Parada
et al., 2016. The procedure described in Kerrigan and D’Hondt (2022) was followed. A 20-µl PCR reaction
was performed for each sample using 0.4 µl Platinum SuperFi II DNA Polymerase (Invitrogen, Carlsbad,
CA), 4 µl SuperFi II Buffer (5x), 0.2 mM dNTPs, 0.5 µM of each primer, and 0.1 µl Bovine Serum Albumin
(Thermo Scientific, Carlsbad, CA). Each water sample was amplified in a single PCR reaction with 5 µl of
DNA template. To account for possible PCR reagent contamination, three samples were amplified, each
one containing only laboratory water and no extract. Additionally, a sterile Sterivex filter was used for each
DNeasy PowerWater Sterivex kit to account for possible kit contamination. The thermal cycler program
for all reactions began with an initial denaturation temperature of 98°C for 30 seconds, followed by 35
cycles of 98°C for 10 seconds, 60°C for 10 seconds, and 72°C for 15 seconds, followed by a final extension
of 72°C for 5 minutes. The samples were cleaned using the Agencourt AMPure PCR Purification Kit
(Beckman Coulter Life Sciences, Indianapolis, IN). The cleaned samples were sent to the University of
Rhode Island Genomics and Sequencing Center and sequenced on an Illumina MiSeq platform using the
Illumina MiSeq V3 chemistry at 2 x 300 cycles (NCBI BioProject PRJNA718862).

2.5 Water mass analysis

The water samples analyzed in this study were classified as a mixture of known water types defined
by their source water masses (SWM), whose physical and chemical characteristics have been well
documented (e.g., Tomczak, 1981). The SWM considered in this study were the Atlantic Water (AW),
Pacific Water (PW), Meteoric Water (MW), and Sea Ice Meltwater (SIM). To assess the contributions of
these SWM to the mixed water samples, we employed a simple 4-endmember mass balance analysis (Östlund and Hut, 1984) (see Fig. S1). This method assumes that the mixing processes between the waters are linear, that the water tracer properties are conservative, i.e., they are not subject to any chemical or physical alteration during mixing. The 4-endmember mass balance method was used to estimate the fractional contribution of each end member (f_i) to the mixed water samples at each measured point. The f_i values indicate the amount of each source water mass present in the given sample as described by Pardo et al. (2012) and Newton et al. (2013).

\[
\begin{align*}
 f_{AW} + f_{PW} + f_{MW} + f_{SIM} &= 1 \\
 f_{AW}(SA) + f_{PW}(SA) + f_{MW}(SA) + f_{SIM}(SA) &= SA_{obs} \\
 f_{AW}(ANP) + f_{PW}(ANP) + f_{MW}(ANP) + f_{SIM}(ANP) &= ANP_{obs} \\
 f_{AW}(\delta^{18}O) + f_{PW}(\delta^{18}O) + f_{MW}(\delta^{18}O) + f_{SIM}(\delta^{18}O) &= \delta^{18}O_{obs}
\end{align*}
\]

To constrain the mixing calculation, we used conservative tracers’ absolute salinity (SA), \(\delta^{18}O\), and an N:P-based tracer (ANP) (Jones et al., 1998). The \(\delta^{18}O\) was measured using a Picarro l2130-I CRDS water isotope analyzer (University of Illinois at Chicago) with a wire mesh inserted in the vaporizer inlet to trap salt from the seawater (doi: 10.1594/PANGEA.937538). The ANP ratio is a quasi-conservative water-mass tracers, which allowed us to identify the contributions of different water masses in the mixing layers. Specifically, we employed a four-component linear endmember mixing model using nutrients. Past studies have used nutrients in their Redfield ratios to distinguish between Pacific and Atlantic-derived waters (Ekwurzel et al., 2001; Whitmore et al., 2020). We calculated the Arctic Nitrate-Phosphate tracer (ANP) following the method described by Newton et al. (2013).

Arctic Nitrate-Phosphate tracer – ANP was determined using Euclidean geometry by calculating the distance between the sample and the trendlines for the Pacific and Atlantic, as shown in equations 3 and 4 (Jones et al., 1998; Whitmore et al., 2020 – Fig. 3).

\[
\begin{align*}
 NO_3^{-}_{AW} &= (17.499 \times [PO_4]^{3-}) - 3.072 \\
 NO_3^{-}_{PW} &= (12.368 \times [PO_4]^{3-}) - 10.549 \\
 ANP_{AW} &= \frac{abs(5.6 - 17.499 + 0.2 + 3.072)}{\sqrt{1^2 + (7.499^2)}} \\
 ANP_{PW} &= \frac{abs(5.6 - 12.368 + 0.2 + 10.549)}{\sqrt{1^2 + (12.368^2)}}
\end{align*}
\]
Figure 3: Nitrate-Phosphate relationships, utilizing the Jones 1998 model and incorporating data (represented by red dots). The orange line represents [PO$_4^{3-}$] / NO$_3^{-}$AW for Atlantic waters, while the blue line is [PO$_4^{3-}$] / NO$_3^{-}$PW for Pacific waters.

ANP is a modified version of the N* tracer that considers the specific N:P ratios of the Arctic water column and is adjusted to the dynamic range of the pelagic Arctic Ocean (Newton et al., 2013). Unlike Redfield ratios, ANP can be influenced by processes other than photosynthesis and respiration. Bacterial nitrification and denitrification, which mainly occur in anoxic regions of the continental shelf benthos, can cause deviations from Redfield ratios (Newton et al., 2013). Therefore, ANP is well-suited for our dataset. We determined the concentrations of phosphate, and nitrate+nitrite using a Quik Chem Series 8500 Lachat analyzer (Serial Number 061100000379 – Hach, Loveland, Colorado, USA) with a heater configuration of 500 W Max. Reagents and standards were prepared using Quik Chem Protocols: Nitrate + Nitrite 31-107-04-1 A, Phosphate 31-115-01-1 H. To ensure the quality of the nutrient data, we first excluded data showing a standard deviation greater than 5% between replicated measurements. We then compared our dataset to literature values (e.g., Torres-Valdés et al., 2013; Bhatia et al., 2021) and data storage (e.g., DiTullio and Lee, 2019) to further verify the accuracy of our measurements. A flag value of 1 was assigned to the nutrient values that showed agreement with the reference data, indicating reliable data. We also attempted to identify outliers using N:P:Si ratios to achieve Redfieldian consistency, but this approach was not suitable for our dataset due to the significant impact of denitrification in shaping nutrient ratios in Pacific waters and the influence of freshwater inputs on nutrient utilization (e.g., Sterner and Elser, 2002). Other outlier identification procedures were also not effective for our dataset. The methods and sources for selecting endmembers were carefully chosen to ensure the accuracy and reliability of the calculated fractions. The study’s DOI for further reference is 10.18739/A2BN9X45M.

**End Member determination** - The selection of endmembers was based on the data from the study and previous literature (Table 1). The salinity endmembers included data from the study for Atlantic Water (AW) and Pacific Water (PW) and values from literature for Meteoric Water (MW) and Sea Ice Meltwater (SIM) (Ekwurz et al., 2001; Newton et al., 2013; Whitmore et al., 2020). For ANP, the values for AW, PW, and MW were adopted from Newton et al. (2013), while the upper 50m values of SIM were used. For δ$^{18}$O,
end member values from the study for AW and PW were utilized, along with a value of -20 ‰ for MW from Whitmore et al. (2020) and surface values of +2.6 ‰ for SIM from Newton et al. (2013).

Table 1: MP endmembers. AW, PW, MW, SIM are the source water masses (SWMs); SA is the absolute S (g/kg), ANP is the Arctic nitrate-phosphate tracer, MB is the mass balance; δ¹⁸O was measured in ‰. For SIM, the ANP and δ¹⁸O tracers were calculated as averaged surface values per each CTD station; for δ¹⁸O tracer, we also added 2.6 ‰ as in Newton et al., (2013).

<table>
<thead>
<tr>
<th></th>
<th>AW</th>
<th>PW</th>
<th>MW</th>
<th>SIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA (g/kg)</td>
<td>34.50</td>
<td>32.50</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>ANP</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>Surface values</td>
</tr>
<tr>
<td>δ¹⁸O (‰)</td>
<td>0</td>
<td>-2.50</td>
<td>-20</td>
<td>Surface values + 2.6</td>
</tr>
<tr>
<td>MB</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

All the tracers were standardized as follows:

\[
\frac{data - mean(data)}{SD(data)}
\]

as they were measured in different measurement units (Table 1). Standardization gives each tracer a similar dynamic range and equivalent weighting in the least squares calculation. The mass balance constraint was assigned a higher weight to ensure all water mass fractions would sum to 1. To avoid interpreting samples that do not conform to the linear mixing model, we adopted the criteria that the mass fractions cannot deviate by more than 5% from the constraint of 1 (Tomzak, 1981; Tomczak and Large, 1989). The error for every endmember was calculated as follows:

\[
a = f_i \times endmember(obs)
\]

\[
b = \text{sum}(a)
\]

\[
error = \left(\frac{endmember(obs) - b}{endmember(obs)}\right) \times 100
\]

Where \( f_i \) is the water mass fraction for every SWM.

Any samples not conforming to this criterion was excluded from the water mass analysis (see Figure S2).

3. Results
3.1 Dissolved in-situ methane concentration and isotopic ratio

Overall, the range of methane concentrations values varied between 1.35 and 16.38 nM across the entire dataset, whereas the isotopic ratio varied between -64.2 and -17 ‰, with lowest isotope ratios in the surface ocean. The average of the [CH$_4$] was 4.17 ± 2.5 nM, and the values below the average were recorded within deeper layers (av. depth = 190 m). The supersaturation of methane in seawater relative to atmospheric methane concentrations (average 3.42±0.05 nM) in the CAA varied spatially due to variations in temperature and salinity. In the surface waters (down to ~40 m depth), the saturation of CH$_4$ was mostly above equilibrium, with a maximum of methane saturation anomaly of ~ 360%, while in deeper layers, the methane saturation anomaly was mostly negative (Fig. 4). The negative correlation (-0.56 of Spearman’s rank correlation coefficient, Fig. S8) between CH$_4$ and salinity (Fig. S3) corroborated that freshwater was associated with higher [CH$_4$]. According to Figure 4, the methane enrichment and depletion across a longitudinal scale did not show a clear trend.

![Figure 4: Methane saturation anomaly (%) across the longitudinal scale. The grey dots represent methane depletion (sat. anomaly <0%).](https://doi.org/10.5194/bg-2023-157)

The entire data set showed an inverse correlation (-0.50 of Spearman’s rank correlation coefficient, Fig. S8) between methane concentration and isotope ratio (Figure 5). At the low range of concentrations, the variability in δ$^{13}$CH$_4$ becomes quite large, suggesting the potential for a variety of inputs. In Figure 5, we showed the methane oxidation curve calculated using a Rayleigh distillation model with initial concentration and isotope ratio corresponding to an approximate hypothetical initial condition of δ$^{13}$CH$_4$ = -63 and [CH$_4$] = 15 nM, as well as a kinetic isotope fractionation factor of 1.025 (Kendall and Caldwell, 1998; Fenwick et al., 2017). Rayleigh distillation model assumes that isotopic fractionation during methane degradation occurs because of the preferential removal of the lighter isotope, $^{12}$C, over the heavier isotope, $^{13}$C. As the degradation proceeds, the remaining methane becomes progressively enriched in the heavier isotope, resulting in a depletion of the lighter isotope.
Figure 5: Methane oxidation curve calculated using a Rayleigh distillation model, with an initial concentration of 15 nM, a δ¹³CH₄ value of -63 ‰, and an isotopic fractionation factor of 1.025. The red line describes the Rayleigh curve. The curve shows the relationship between the remaining methane concentration and the corresponding isotopic composition of the remaining methane. The slope of the Rayleigh curve represents the isotopic fractionation factor, which is a measure of the degree of isotopic fractionation that occurs during the degradation of methane.

Figures 6 a and b show the discrete methane profiles of isotope ratio (a) and concentration coupled with the methane concentration at the atmospheric equilibrium (b) in Croker Bay. All the in-situ methane profiles can be found in Figure S4. Croker Bay represented the site with the highest supply of meltwater (mainly meteoric origin, MW) and recorded the highest methane concentration (Fig. 6b, ~17nM) coupled to the lowest isotopic signature (Fig. 6a, ~-64.3 ‰). In Croker Bay, the subsurface [CH₄] was close to atmospheric equilibrium, while concentrations were higher in the upper 25 m (Fig. 6b). West of Navy Board Inlet was the only site showing deep water methane enrichment (see Fig. S4), with an average of 3.4±0.03 nM in waters below 100m.
Figure 6: Methane data from samples collected in Croker Bay. a) methane isotope ratio profile; b) in-situ methane concentration and methane concentration at the equilibrium relative to the atmosphere capacity (methane equilibrium).

In summary, the in-situ methane concentration and isotopic ratio showed overall methane excess in the upper 200 m of the water column (Figs 6 and S4), suggesting methane production in shallow waters and methane uptake in deeper waters during the past.

3.2 In-vitro methane oxidation potential

The microbial oxidation rates ($r_{ox}$) with rate constants ($k_{ox}$) were calculated as in Uhlig and Loose (2017), and the averaged values, per site and depth, are shown in Table 2.

Table 2: Methane oxidation rate constant ($k_{ox}$) averaged per site and depth ($k_{ox,av}$ (d$^{-1}$)), and the associated microbial methane oxidation rates ($r_{ox}$ (nM/d)). In the table we also show the location (Site) and the depth of the sample collected; moreover, we show the standard deviation between the averaged $k_{ox}$ ($k_{ox,SD}$).

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth</th>
<th>$k_{ox,av}$ (d$^{-1}$)</th>
<th>$k_{ox,SD}$</th>
<th>$r_{ox}$ (nM/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JS</td>
<td>609</td>
<td>0.005</td>
<td>0.005</td>
<td>0.997</td>
</tr>
<tr>
<td>JS</td>
<td>7</td>
<td>0.003</td>
<td>0.001</td>
<td>0.458</td>
</tr>
<tr>
<td>WNBI</td>
<td>70</td>
<td>0.007</td>
<td>0.005</td>
<td>4.040</td>
</tr>
<tr>
<td>WNBI</td>
<td>15</td>
<td>0.005</td>
<td>0.001</td>
<td>2.000</td>
</tr>
<tr>
<td>WNBI</td>
<td>450</td>
<td>0.006</td>
<td>0.001</td>
<td>2.700</td>
</tr>
<tr>
<td>WNBI</td>
<td>70</td>
<td>0.004</td>
<td>0.002</td>
<td>1.030</td>
</tr>
<tr>
<td>WNBI</td>
<td>743</td>
<td>0.003</td>
<td>0.002</td>
<td>1.530</td>
</tr>
<tr>
<td>WNBI</td>
<td>401</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>WC</td>
<td>10</td>
<td>0.009</td>
<td>0.002</td>
<td>2.600</td>
</tr>
<tr>
<td>PRS</td>
<td>25</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>CB</td>
<td>241</td>
<td>0.008</td>
<td>0.008</td>
<td>0.760</td>
</tr>
</tbody>
</table>
In total, 83% of in-vitro samples exhibited significant methane oxidation, while the remaining 17% revealed negligible or insignificant metabolic activity with respect to methane.

In Figure 7, we showed the vertical distribution of the methane oxidation rate constant ($k_{\text{ox}}$). The vertical trend of the methane oxidation rates showed widespread oxidation potential for methane (positive $k_{\text{ox}}$), with highest values within WC and CB shallow waters, reaching up to 0.01 d$^{-1}$. During the in-vitro experiments, we recorded flat trend of methane concentrations and isotope ratio over time in two samples (shown by grey dots in Fig.7), suggesting no methane metabolism. We did not take these values into account for the microbial methane oxidation rate assessment, to not bias the results.

The methane oxidation rate constant results ($k_{\text{ox}}$) indicate a spatial trend across the study area, with the potential for the highest oxidation rates observed towards the western Channel. This trend is visually represented in Figure 7 through a color-coded longitudinal scale, where the darker blue dots matched with higher $k_{\text{ox}}$ values.

In summary, our findings reveal both spatial and vertical gradients within the water column, with the highest $k_{\text{ox}}$ values recorded towards the west, descending to a depth of 200m, followed by a gradual decrease towards the east (as represented by the light blue dots in Figure 7). Overall, the waters of the CAA exhibited a potential oxidative environment, in the summer of 2019, where in-situ methane excess could have served as food source for methane oxidizers. The oxidation detection limit was passed between 5 and 18 incubation days after incubation began, revealing the range of rates we observed.

3.3 Microbial community composition

The 16S rRNA gene analysis of the in-situ water column community revealed phylogenetic diversity. The in-situ microbial community structure was dominated by Flavobacteriaceae, including *Polaribacter sp.*
incubation period, most of the 16S rRNA gene sequences clustered into operational taxonomic units (OTUs) affiliated with Alphaproteobacteria, Gammaproteobacteria, and Flavobacteriaceae (Bacteroidetes). The dominant taxa observed in samples displaying methane oxidation were *Oleispira* (γ-proteobacteria), *Planctomarina* (α-proteobacteria), and *Aurantivirga* (flavobacteria, Song et al., 2015). While Alphaproteobacteria and Gammaproteobacteria include known methane-oxidizing bacteria, *Oleispira* and *Planctomarina* are not yet classified as such. However, these taxa were also found in Arctic methane incubations by Uhlig et al. (2018) and Gründger et al. (2021), suggesting their association with methane oxidation. Flavobacteria are typically secondary consumers of methane, oil, or cellular decay products (e.g., Radajewski et al., 2002; Jensen et al., 2008; Redmond and Valentin, 2011). While methane oxidizers have limited ability to consume multi-carbon substrates (Hanson & Hanson, 1996), many oil degraders cannot consume methane (Rojo, 2009). Flavobacteria are often associated with the degradation of high molecular weight dissolved organic carbon compounds (Cottrell and Kirchman, 2000), which may coincidentally be associated with methane uptake. In summary, our dataset highlights the occurrence of Chloroplast genomes, *Oleispira*, *Planctomarina*, and *Aurantivirga* in samples showing potential methane oxidation, consistent with the findings of Uhlig et al. (2018) and Gründger et al. (2021).

### 3.4 Dissolved methane and isotope ratio in sea ice

Methane dissolved in sea ice was in the range of 3.4 and 21.2 nM (Fig. S5), showing concentrations higher than in seawater ([CH₄] max in CB = 16.4 nM). On average, the sea ice recorded [CH₄] of 9.2 ± 5 nM, while seawater showed an average of 4±2 nM. The methane maximum was recorded within Westernmost Station sea ice core (21 nM), however, all the samples exhibited methane oversaturation with respect to the atmospheric concentration (av. 4.7±0.01 nM) above the bottom. Core 1 at the surface and Core 2 at the bottom showed methane depletion (3.4 and 4.1 nM, respectively) (Fig. 8). Both these cores were characterized by thicker multi-year ice (Canadian Ice Service and Table S2) and were collected in the vicinity of Westernmost Station.

![Figure 8: Methane concentrations and isotope ratios along the vertical profiles within sea ice. The red dots indicated the in-situ methane concentrations along the sea ice core, with the methane concentrations at the](https://doi.org/10.5194/bg-2023-157)
equilibrium relative to the atmosphere capacity (blue dots). The green dots showed the isotope ratio ($\delta^{13}\text{CH}_4$) of cores 1 and 2. The headers displayed the core numbers as exhibited in Fig. 1.

Cores 3, 4, and 5 were collected respectively in Peel Sound, close to Cunningham River, and in Prince Regent Sound (see Fig. 1), and they were characterized by first-year ice (Canadian Ice Service). There was no trend of methane concentrations from first-year to multi-year ice, hence from west to east (Fig. S6). The $\delta^{13}$C isotopic signature in sea ice cores was between -51.4 and -35.4 ‰, showing less variability in comparison to the in-situ water samples data.

3.5 [CH$_4$] and $k_{\text{ox}}$ in relation to water masses.

Dissolved methane was inversely correlated to AW (-0.4 Spearman’s rank correlation coefficient) and positively correlated with PW (0.3 Spearman’s rank correlation coefficient) (Fig. 9a and b), while the data showed positive correlation with meteoric and sea ice meltwaters (0.44 and 0.37, respectively) (Fig. 9c and d). Similarly, methane oxidation was weakly anticorrelated with AW (-0.17 Spearman’s rank correlation coefficient) (Fig. 9e), and positively correlated with PW (0.17 Spearman’s rank correlation coefficient) (Fig. 9f). The meltwaters exhibited a positive correlation with $k_{\text{ox}}$, as depicted in Figure 9g and h, with meteoric waters showing the highest Spearman’s rank correlation coefficient of 0.76 (Fig. 9g). Spearman’s matrices were separately computed for the in-situ and in-vitro data due to the limited availability of the latter, resulting in fewer data points in Figure 9e, f, g, and h.
Figure 9: The correlation between methane concentrations measured in situ and the methane oxidation rate constant ($k_{ox}$) was examined in relation to water mass fractions. Panels a, b, c, and d illustrate the methane concentrations measured in situ in relation to Atlantic and Pacific waters (a and b) as well as meltwater masses (c and d). Panels e, f, g, and h depict the methane oxidation rate constant in relation to Atlantic and Pacific waters (e and f) and the meltwaters (g and h). It should be noted that the results for the meltwaters may potentially increase, as they do not include the meteoric supply from Devon Ice Cap.

Summarizing, the analysis revealed the presence of two distinct environments with regards to methane activity. One environment exhibited active methane metabolism, while the other displayed non-metabolic behavior towards methane. This differentiation led to a distinct distribution of methane oxidation rates across the study area, as depicted in Figure 10. In the Atlantic water regime, dissolved methane was depleted with concentrations below the equilibrium saturation range, where we also recorded weaker methane microbial metabolism. The occurrence of no detectable methane oxidation in the deep layers of West Navy Board Inlet and in shallow Prince Regent Sound waters was not associated to unique thermohaline characteristics of the two sites, and unfortunately, the community structure was not analyzed in those sites.
Figure 10: Parry Channel segment with color-coded $k_{oa}$ averages by transect and depth, Excluding non-methane metabolism data (selected $k_{oa}>0$). Arrows Indicate Atlantic Water (AW) and Pacific Water (PW) intrusion into the Channel. White dots represent data points, with location details displayed in text boxes above. The x-axis measures distances (in km) from Wellington Channel (longitude = 93.11°W), 200 km away, to the Easternmost Point (longitude = 78.26°W), spanning 1400 km. Refer to the map in the lower left corner for further details.

4. Discussion

In the waters of the Canadian Arctic Archipelago, dissolved methane showed the strongest variations vertically in the water column, with higher concentrations detected in shallow waters. This distribution pattern suggests an influence of the water column structure on methane concentrations. The water column structure in the CAA was characterized by a seasonal meltwater mixed with Pacific Water (PW), overlying layers of PW and Atlantic Water (AW). Pacific Water was found to comprise more than 50% of the water above 200 m (Fig. S1), which correlated with areas of highest methane oxidation. The vertical distribution of methane concentrations was evident through various indicators. In addition to the positive correlation with PW and negative correlation with AW (Section 3.5), methane concentrations and absolute salinity (SA) were strongly correlated (Figure S3). Additionally, the Pearson correlation coefficient between them was -0.56, further supporting this relationship. Similarly, the potential methane oxidation rate exhibited a vertical profile, with higher values found in shallow waters. This pattern was also supported by a negative correlation with SA ($R=-0.47$). Conversely, lower oxidation rates were observed in samples collected from deeper layers, indicating that the AW-origin layers did not support potential methane microbial metabolism. The hydrographic context of these findings provides valuable insights into the main drivers of methane metabolism during the summer in this specific Arctic region. The relatively weak oxidation rates in near-bottom waters and indeed throughout the subsurface distribution of AW (see Fig. 10), did not suggest subsurface inputs of methane such as cold seeps, nor any strong role for AW in the methane cycle within the CAA. Previous research conducted near methane sources, such as Arctic shelf gas seeps, has shown that seasonal variations of water masses can affect methane oxidation rates, resulting in system-wide changes in the efficiency of water column methane oxidizers (Steinle et al., 2015, 2017; Gründger et al., 2021).
Instead, the distribution of $k_{ox}$ and $[\text{CH}_4]$ suggest a connection between methane cycling (both production and consumption) in near-surface waters. Potential explanations include the possibility of methane production within phosphate-limited PW (Repeta et al., 2016), methane production associated with breakdown of primary products such as DMSP (Damm et al., 2015 and 2008), or methane production associated with terrestrial freshwater in rivers (Manning et al., 2020 and 2022) or from glaciers (Pain et al., 2021). We explore each of these possible explanations in the sections that follows.

### 4.1 Possible sources of dissolved methane

Overall, the dissolved methane in-situ suggested a water column that is strongly affected by methane oxidation to first order (see Fig. 5), but the large scatter of isotopic ratios, especially at low methane concentrations also suggests multiple sources in an open system as the area of study. The highest dissolved methane concentrations were recorded in shallow meltwaters mixed with PW, concurrently with the lowest nutrient supply and high chlorophyll-a fluorescence data (e.g., Westernmost Station and Prince Regent Sound). More recently, several methanogenesis metabolism pathways have been identified that may produce $\text{CH}_4$ in situ in the surface ocean mixed layer, providing a more direct conduit to the atmosphere (Karl et al., 2008; Lenhart et al., 2016; Schmale et al., 2018).

The highest dissolved methane concentrations (>5 nM) were observed in the western CAA (with longitudes greater than or equal to 90 degrees west), except for Crocker Bay (av. $[\text{CH}_4]=12.9$ nM). Crocker Bay was not representative of the eastern Channel either, due to its influence from the Devon Ice Cap drainage. The highest values were recorded in: Barrow East ($[\text{CH}_4]_{av}=10.53$ nM), Peel Sound ($[\text{CH}_4]_{av}=8.5$ nM), Westernmost Station ($[\text{CH}_4]_{av}=7.7$ nM), and Prince Regent Sound ($[\text{CH}_4]_{av}=6.4$ nM). Barrow East and Peel Sound waters were characterized by high freshwater supply (Fig. S1) provided by Cunningham and Garnier Rivers (McLaughlin et al., 2014) and watershed drainage of marine-terminating rivers from the southern Canadian Arctic Archipelago, respectively (Brown et al., 2020). All sites were distinguished by substantial meltwater input (with meteoric water and sea ice meltwater exceeding 10%, Fig. S1) and anticipated detrital organic matter supply, which supports the hypothesis that methanogenesis occurred in the past and was influenced by particle supply within the water masses. Notably, methanogenesis in terrestrial fluvial systems and beneath terrestrial glaciers could explain the high marine methane concentrations observed at these sites (Valentine et al., 1994; Bange et al., 2010). Furthermore, the isotopic signature of methane in shallow Crocker Bay waters reached values lower than -64‰ (very depleted in $\delta^{13}$C), suggesting subglacial methanogenesis from the Devon Ice Cap runoff (Pain et al., 2021).

The only site showing methane excess in deep waters (at 690 m depth) was West Navy Board Inlet, and we recorded the methane maxima concurrently with relatively high turbidity (0.005 NTU). This outcome likely suggested methane release from sediments (Damm et al., 2005; Graves et al., 2015; Silyukova et al., 2020).

The positive correlation between $[\text{CH}_4]$ and SIM (Fig. 9) suggested that the sea ice melt cycle may somehow play a role in seasonal methane production, and indeed elevated methane in ice cores could suggest sea ice melt is a methane source, or the same mechanism that produces methane in sea ice is also active in the water column. The Western CAA contained the greatest sea ice cover (see the sea ice concentration in Fig. 11), mostly as multi-year ice (Canadian Ice Service). However, due to the warm season, the sea ice also occurred in the form of permeable sea ice and sea ice meltwater. Notably, the satellite-derived sea ice cover (Spreen et al., 2008) and the water mass estimates of sea ice meltwater tended to coincide with greatest contributions in the West, with >10% occupying the water volume.

As described earlier, the sea ice cores showed higher dissolved methane than seawater, and the western waters were methane enriched. This outcome suggested that the methane excess in the sea ice was...
due to past freezing processes and that the increase of ice permeability influenced the methane enrichment within the underneath seawater. Comparing our results with the ones from previous studies in the same location in July 2005 (Kitidis et al., 2010), sea ice similarly influenced the methane oxidation rates. Our outcome, confirmed by previous studies conducted in Utqiagvik (Loose et al., 2011) and the Central Arctic (Damm et al., 2015; Damm et al., 2018), suggested that the residence time of methane gas in sea ice could allow it to accumulate during the freezing period and be released during the melt period, supporting the metabolic consumption in Sea Ice Meltwater. Verdugo et al. (2021) and Damm et al. (2015) described how increased ice permeability at the ice bottom triggers methane release, causing low methane concentrations at the bottom of the ice and methane enrichment in the water underneath. This process was well described by the methane concentration in Core 2 (collected close to the Westernmost station). Here, the sea ice enriched in methane could have released the gas and the relict detritus, favoring the conditions for microbial methane production, explaining the methane excess in the waters underneath. The same condition, but backwards, was recorded in Core 1, where we recorded sea ice oversaturated in methane, with the top centimeters showing methane deficit. The methane initially entrapped in the sea ice was probably released into the atmosphere when the sea ice surface became permeable (Verdugo et al., 2021). The in-situ dissolved methane was distributed across the surface CAA waters mainly within meltwaters (see Fig. S1 and S4), without showing any clear relationship with sea ice concentration (Fig. 11), however logistical constraints prevented the expedition from transiting very far through ice-covered regions, so this relationship was difficult to establish.

In conclusion, we can state that the marine methane in the CAA was defined by two different environments during the summer of 2019, partly influenced by the sea ice melting. In the past, other studies showed the correlation between the sea ice and the greenhouse gases in the Arctic Ocean during the summer (Damm et al., 2018, Verdugo et al., 2021), however, no data were yet referred to our study area.
To summarize, it is likely that dissolved methane in the CAA waters during the summer is primarily driven by microbial metabolism, facilitated by the primary production and organic debris supplied by the melts waters (both meteoric and sea ice), with additional inputs from terrestrial or subglacial runoff. However, the specific metabolic pathway for any aerobic methanogenesis remains unknown and was not within the capabilities of this study to determine.

### 4.2 Possible sources of methane oxidation potential

Although we were unable to obtain incubation results from Westernmost Station, Barrow East, and Peel Sound, we were able to observe the potential for microbial methane oxidation in Croker Bay, Prince Regent Sound, and West Navy Board Inlet. Our incubation experiments at Croker Bay and West Navy Board Inlet showed the potential for microbial methane oxidation in both surface and deeper layers (CB average \( k_{\text{ox}} = 0.0085 \, \text{d}^{-1} \), WNBI average \( k_{\text{ox}} = 0.0024 \, \text{d}^{-1} \)). This finding suggests that the excess methane dissolved in the water column could be oxidized before reaching the atmosphere. Additionally, our observations along the West Navy Board Inlet transect showed no detectable oxidation at 15m and 400m depth, which, in conjunction with the low \( k_{\text{ox}} \) rates, suggested weak methane metabolism in waters above the bottom layer. In Prince Regent Sound, we only analyzed one depth for methane oxidation rates (25 m) and found no detectable oxidation potential. The results of genomic community composition analysis revealed the occurrence of *Oleispira*, *Planctomarina*, and *Aurantivirga* in the “time final” samples, where methane oxidation was detected. This finding suggested a potential linkage between these microorganisms and methane uptake. In a previous study by Uhlig et al. (2018), *Oleispira* was found to be more abundant in long-incubation samples than in situ samples, which were mainly dominated by \( \alpha \)- and \( \gamma \)-proteobacteria.

In our study, after incubating the samples with methane standard, we observed a shift towards higher fractions of \( \alpha \)-proteobacteria, with *Planctomarina* being the dominant genus. The occurrence of *Oleispira*, likely belonging to *Oleispira lenta* (Wang et al., 2012), and *Aurantivirga* taxa, highlighted the potential influence of Pacific water in methane oxidation, as these taxa have been predominantly found in North Pacific waters (Wang et al., 2012; Song et al., 2015). Notably, *Aurantivirga* occurred only in deeper layers, indicating its association with *Aurantivirga profunda*, a species isolated from deep seawater (Song et al., 2015).

The present study showed an average value of the oxidation rate constant (\( k_{\text{ox}} \)) of 0.006±0.002 \( \text{d}^{-1} \) (excluding non-methane metabolism), which fell within the range of values reported in previous Arctic research (e.g., Mau et al., 2017; Uhlig and Loose, 2017). The microbial oxidation rates (\( r_{\text{ox}} \)) reported in this study also fell within the same range of values showing a good agreement with global methane data, as depicted in Figure S7. In comparison, the only available data on methane oxidation in the Canadian Arctic Archipelago waters was from 2005 (Kitidis et al., 2010) and reported significantly higher \( k_{\text{ox}} \) values compared to other measurements in the Arctic, including those from seeps. The average \( k_{\text{ox}} \) reported by Kitidis et al. (2010) was 0.09 \( \text{d}^{-1} \). It is noteworthy that the oxidation rate measurements reported by Kitidis et al. relied solely on methane mass balance, which can result in high oxidation rate anomalies if methane gas leaks from the incubation chamber.

Our results indicated only a weak correlation between in-situ dissolved methane concentration and methane oxidation potential (\( k_{\text{ox}} \)) (Spearman’s rank correlation coefficient = 0.15, Fig. S9). However, methane oxidation can be expected in waters with high methane concentrations that are oversaturated with respect to atmospheric levels. Previous studies conducted in the Arctic and in cold seeps located on continental slopes worldwide (Mau et al., 2013; Boeiu & Wenzhöfer, 2013) have shown a positive correlation between elevated methane oxidation activity and high dissolved CH\(_4\) concentrations in marine
environments. These studies suggested that methane oxidizers may rapidly increase their oxidation rates in response to the abundance of their food source. Our findings were not inconsistent with this thesis, as we observed a relationship between methane oxidation and CH$_4$ concentrations in the CAA waters, however the correlation was weak. While we acknowledge that the number of coupled in-situ and experimental samples in our study was limited, the spatial coverage of our data is currently the most comprehensive record of methane metabolism in these waters. Overall, methane oxidation rates showed direct correlation with freshwater masses (Fig. 9), suggesting the influence of the CAA meltwater runoff on the methane oxidizers.

In summary, we found weak or absent methane microbial oxidation in AW, while it was stronger in meltwaters and PW. These results tend to support the role of surface processes in the CAA during the summer, including input of meltwater, biology, and processes associated to the particulate organic matter. While the dominant taxa associated with our methane oxidation process were not known methane oxidizers, they have recently been connected to methane metabolism, highlighting the need for further investigation.

5. Conclusions

Collectively, the CAA waters showed methane oversaturation in the upper water column with respect to atmospheric equilibrium saturation. The methane oversaturation was mainly associated with the meltwaters, turbidity, and biology (in nutrient depleted environments), likely following one of the paths explained by Repeta et al. (2016) and Sosa et al. (2020). In total, 83% of in-vitro samples were exhibited significant methane oxidation, while the remaining 17% revealed negligible or insignificant metabolic activity with respect to methane. The fastest oxidation rates were recorded within surface freshwater – both sea ice melt and meteoric waters – as well as within Pacific-origin waters. The community structure likely responsible for the methane oxidation was characterized by three main groups that have been recently associated with the methane metabolism (Aurantivirga, Oleispira and Planktomarina); Aurantivirga and Oleispira were both isolated from PWs. This outcome suggested that the PW predominance of the CAA waters could have defined hotspots for methane oxidations. The sea ice melting also influenced the methane distribution within the Sound, strengthening the hypothesis that the sea ice acted as a barrier for the gas exchange during the former winter, releasing dissolved methane during the summer. The overall picture suggested supersaturation of in-situ methane in shallow waters, coupled with faster oxidation rates in meltwater and Pacific dominant layers, suggesting prevention of the methane migration into the atmosphere towards the western CAA.

Summarizing, we can define the CAA waters into two different environments according to the methane metabolism: a) methane-metabolic active and b) non-methane metabolic active. The west side of the study area, including Croker Bay, showed great methane metabolism, where we had both in-situ dissolved methane excess, and faster methane oxidation rates potential. The eastern side, including Jones Sound and Pond Inlet data, characterized by Atlantic Water regime, exhibited lower methane concentrations and oxidation rate constant. Due to the Atlantification of the Arctic Ocean (Polyakov et al., 2017, 2020), we would expect higher intrusion of AW in the CAA at the expense of PW, with consequent sea ice melting. This would exacerbate the stratification of the dissolved methane, enhancing isolated methane-associated community.

Data availability
The produced database of this study has been archived in Arctic Data Center and can be assessed using the following link: https://doi.org/10.18739/A2BN9X45M. The sequence analysis and taxonomical classification is stored at National Center for Biotechnology Information (NCBI BioProject PRJNA718862).

Author contribution
B.L. designed the research. B.L. and A.D. implemented the study. A.D., C.G-E., Z.K., J.S., F.C., N.V., H.R., T.E., S.U., B.L., were involved in the sampling activities. A.D., B.L., N.V., H.R., T.E., S.U., performed the analysis. A.D., B.L., A.L.K., M.G-M., F.C., C.G-E. processed the data. A.D. and B.L. wrote the manuscript, with input from all authors.

Competing interests
The authors declare that they have no conflict of interest.

Acknowledgements
We would extend our gratitude to the entire crew of the RVIB Oden, and the Swedish Polar Research Secretariat team for the logistic effort. We greatly appreciated the involvement of all the undergraduates and scientists participating in the expedition. We thank our Arctic guide Sarah Scriver for assistance. We acknowledge the Inuit community for their collaboration, and the Marine Science Research Facility at GSO – URI (https://web.uri.edu/marinefacility/) for carrying out the nutrients analysis. We deeply thank NSF facilities at Thule airbase, Polarfield, Greenland for the help provided in Thule, Greenland. The study was supported by the National Science Foundation Awards #1748318 and # 1821900, with additional support from the Heising Simons Foundation. We gratefully acknowledge the NSF Program Officer for the Northwest Passage Project, Valentine Kass, and the lead PI of the project, Gail Scowcroft (Associate Director, Inner Space Center, University of Rhode Island).
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