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The effect of temperature on hydrocarbon profiles and the microbial community composition in North Saskatchewan River water during mesoscale tank tests of diluted bitumen spills☆

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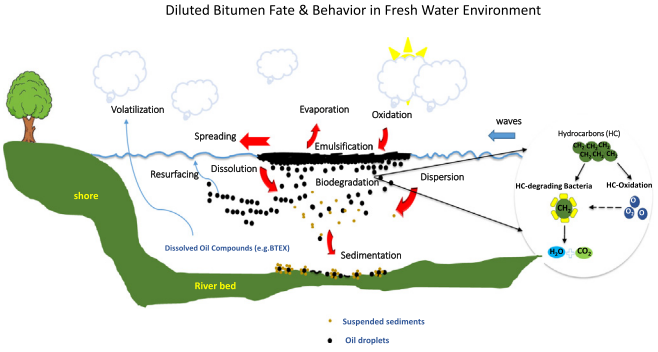
HIGHLIGHTS

- Diluted bitumen spill tests conducted for 35 days in fresh water including sediment.
- At 15 °C water, 78 wt% (at 2 °C water, 82 wt%) of oil mass recovered as floating oil.
- <1.0 % of the DB was recovered from the bottom sediment, regardless of the temperature.
- Microbial population changes in water reflected changes in PAH concentrations.
- Alpha- & Beta-Proteobacteria were the main DB-degraders in river water.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite many studies of diluted bitumen (DB) behavior during spills in saltwater, limited information is available on DB behavior in fresh water. This study examined the collective weathering processes on changes of fresh DB spilled in the North Saskatchewan River water and sediment mixture in a mesoscale spill tank under average air/water temperatures of 14 °C/15 °C and 6 °C/2 °C. Temporal changes of the hydrocarbon and microbial community compositions in the water column were assessed during the two 35-day tests under intermittent wave action. The contents of total organic carbon (TOC), benzene/toluene/ethylbenzene/xylenes (BTEX) and polycyclic aromatic hydrocarbons (PAHs) in water decreased with time during both tests. The final contents remained at higher values in warm water (15 °C) than in cold water (2 °C) after the collective weathering processes. A quick response of the main phyla, Proteobacteria and Actinobacteria, was observed, where the members of Proteobacteria enriched during both DB spills. In contrast, the members of Actinobacteria reduced with time. The microbial shifts coincided with the changes of PAHs in the waters at both temperatures. A comparison of the physical properties and chemical compositions of fresh and weathered DBs at both temperatures showed that the oil had undergone weathering that increased oil density and viscosity due to losing the light oil fraction with boiling points < 204 °C and emulsifying with water. This corresponded to losses of 19.0 wt% and 17.2 wt% of the fresh DB at 15 °C and 2 °C tests, respectively. For organic compounds in the DB with boiling points > 204 °C, there were small losses of saturates and 2- & 3-ring PAH aromatics (more during the 15 °C test than the 2 °C test), and negligible losses in the subfractions of resins and asphaltenes by the ends of the tests. <1.0 wt% of the DB was recovered from the bottom sediment, regardless of the temperature.

☆ His Majesty the King in Right of Canada as Represented by the Minister of Natural Resources, 2022.

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1. Introduction

Oil sands production from Western Canada has grown rapidly over the past two decades where Alberta's raw bitumen production in 2018 exceeded 3 million barrels per day (Alberta Energy Regulator, 2019). To meet the transportation specifications of pipelines to access desired markets, the density and viscosity of bitumen are reduced by diluting it with light oils (e.g., 30 vol% of naphtha-based condensate) (Alberta Energy Regulator, 2019). Consequently, this diluted bitumen (DB) is a major commodity transported in Canadian pipelines (Alberta Energy Regulator, 2019). This has raised public concern over the potential for pipeline spills and their impacts on aquatic environments (Lee et al., 2015).

Current literature on DB spills in aquatic environments identified knowledge gaps about DB fate, behavior, and biological effects when spilled into aquatic environments, particularly in fresh waters (Environment Canada et al., 2013; Witt O'Brien et al., 2013; Lee et al., 2015; NASEM, 2016; Fisheries and Oceans Canada, 2018). One report highlights that the fate and behavior of DB products are within the existing range for conventional petroleum products, with an acknowledgment that DB's viscosity and density change more rapidly (NASEM, 2016). Existing response measures are generally effective on DB and may require a faster change over to equipment designed for higher viscosity oils as weathering progresses (NASEM, 2016). Additional research specific to fresh water under cold conditions was recommended to better understand how these conditions affect the natural weathering processes and fate and behavior of DB in the aqueous environment (Fisheries and Oceans Canada, 2018).

Several in-depth research studies have been conducted using lab-scale (Hounjet et al., 2018; Hua et al., 2018; Xin et al., 2022), mesoscale (King et al., 2019; Ortmann et al., 2020; Monaghan et al., 2022) and field trials (Stoyanovich et al., 2019a; Stoyanovich et al., 2019b; Johannessen et al., 2020; Rodriguez-Gil et al., 2021; Stoyanovich et al., 2021; Stoyanovich et al., 2022) to characterize and understand the fate and behavior of DB in both salt and fresh water. Lab-scale jar mixing experiments investigated the distribution of DB at various water pH values (e.g., 5.0–10.0), salinities (e.g., 0.05–5.0 mS/cm), with or without sediment, and under high end-to-end mixing energy conditions (Hounjet et al., 2018; Hua et al., 2018; Monaghan et al., 2021; Xin et al., 2022). One study showed that oil-particulate aggregates were formed at a high sediment concentration (10,000 mg/L) condition (Hua et al., 2018). Another study conducted by King et al. (2019) examined the influence of salinity on DB density and viscosity at a water temperature of 6 °C in mesocosms to enhance the understanding of natural attenuation processes including evaporation and photochemical oxidation (King et al., 2019). This study showed that salinity had an insignificant effect on the viscosity and density of weathered oils, while the evaporation and photochemical oxidation processes considerably changed the oil's viscosity and density (King et al., 2019). Johannessen et al. (2020) modelled the fate of DB spilled in the coastal waters of British Columbia (Canada), including near river mouths. Their study predicted that highly weathered DB could submerge in the low-density surface waters during the summer and fall seasons, particularly after heavy rainfall, but will likely re-emerge at the surface as the outflowing layer of fresh water gets mixed with denser seawater (Johannessen et al., 2020). The experimental work by Ortmann et al. (2020), which examined the seasonal effects of DB weathering in marine conditions, reported that no DB sank over 14 days (Ortmann et al., 2020). Recently, a series of controlled field-scale DB spills into limnocorrals were conducted in the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) oligotrophic research lake to better understand the potential fate and behavior of DB spills in low-energy natural fresh water environments under a water temperature range of 13.5–22.7 °C (Stoyanovich et al., 2019a; Stoyanovich et al., 2019b; Yang et al., 2020; Rodriguez-Gil et al., 2021; Stoyanovich et al., 2021; Stoyanovich et al., 2022). The study showed that a large portion of the surface slick sank in the water on day 8 of the experimental period under field conditions.

Similar to spills of conventional oils, natural weathering processes change the physicochemical properties of spilled DB over time, which influences the fate, persistence, and toxicity of the oil in freshwater ecosystems. It is known that spilled oils affect the microbial properties of the oil-contaminated water (Feris et al., 2004; Mason et al., 2014; Ortmann et al., 2019) due to changes in concentrations of organic compounds available to microorganisms (Hazen et al., 2010; Mason et al., 2014), limitations of nutrients such as nitrogen (Mason et al., 2014) and phosphorus (Ortmann et al., 2019), as well as changes in the physicochemical properties of the water such as dissolved oxygen and pH (Hazen et al., 2010; Varjani, 2017). Studies of the potential effects of DB spills on microbial responses in coastal environments (Deshpande et al., 2018; Ortmann et al., 2019; Schreiber et al., 2019b) suggest that DB-degrading microbes immediately respond to DB spills (Ortmann et al., 2019) and change with the season, time, and distance from the oil-contamination sites (Schreiber et al., 2019b). Similarly, hydrocarbons in water can be transformed by the collective natural weathering processes, including the bioactivities of native microbes present in the water and/or sediment. For example, polycyclic aromatic hydrocarbons (PAHs) can be transformed into oxidized PAHs (Heshka et al., 2022).

The understanding of the fate and behavior of oil spills has continued to advance in recent years. The existing knowledge on oil impacts on aquatic ecosystems stems from the combined knowledge generated from previous ocean/coastal oil spill events and/or laboratory studies (Witt O'Brien et al., 2013; Ortmann et al., 2019; Schreiber et al., 2019b; Ortmann et al., 2020; Ortmann et al., 2021; Rodriguez-Gil et al., 2021; Stoyanovich et al., 2021; Stoyanovich et al., 2022; Monaghan et al., 2022; Zhong et al., 2022). However, knowledge gaps remain. One of the high-priority research areas remaining is to determine how DB weathering processes in inland rivers change under different Canadian seasonal conditions (Lee et al., 2015). This is of particular interest as oil weathering determines its subsequent transport and fate (Lee et al., 2015). Inland waters with their varieties of habitats support a wide range of aquatic species. Spills of any types of oils can pose high risks to these habitats and species (Owens et al., 1993) due to the limited dispersion and dilution capabilities, oil remobilization, and migration to downstream shorelines and the river bottom (Lee et al., 2015). To advance the existing knowledge on DB weathering in inland rivers, two DB spill tests were performed in an open, mesoscale spill tank under controlled conditions at air/water temperatures of 14 °C/15 °C and 6 °C/2 °C. The design of the spill tank allowed evaluation of the effects of evaporation, dispersion, dissolution, sedimentation, and emulsification processes on DB properties. North Saskatchewan River (NSR) water and sediment were used during the spill tests. The main objective of the study was to examine how temperature affects DB fate in the water and sediment over the 35-day test period. The temporal response of native microbial communities to the DB-derived hydrocarbons in the water was also assessed. The results include quantitative determinations of the DB distributions between the water surface and shoreline, the water, and the sediment after the 35-day periods.

2. Materials and methods

2.1. Crude oil, chemicals, water, and sediment sources

Cold Lake Winter Blend, a type of DB, was collected from a pipeline in barrels in the Edmonton area, (AB, Canada). North Saskatchewan River (NSR) water was collected from the Edmonton municipal water facility after the cyclone centrifugation process, before potable water treatment processing, on September 25, 2018 for the 15 °C water test and on January 21, 2019 for the 2 °C water test. The river sediment was collected from a floodplain of the NSR (Lara-Jacobo et al., 2021; Monaghan et al., 2022). The river sediment was composed of four major mineral components, 20 ± 6 wt% clay minerals, 20 ± 2 wt% carbonates, 8 ± 2 wt% feldspars, and 52 ± 2 wt% quartz. Nearly 100 % of the sediment consisted of particles having a diameter range of <250 μm, with over half of the sample consisting of particles having a diameter range of <45 μm (referred to as "fines") (Lara-Jacobo et al., 2021; Monaghan et al., 2022). Details of the sediment's preparation and characterization are available in previous

papers (Lara-Jacobo et al., 2021; Monaghan et al., 2022). All chemicals and solvents including dichloromethane (DCM) were purchased from Sigma Aldrich as "HPLC grade" and were used as received.

2.2. Controlled mesoscale diluted bitumen tank tests

The oil spill operating conditions have been reported previously (Lara-Jacobo et al., 2021; Heshka et al., 2022; Monaghan et al., 2022). Briefly, before the tests, the 3 m × 1 m × 1.5 m (L × W × H) sized mesoscale indoor tank (Fig. S1-a) was filled with 1200 L of NSR water to a height of 0.7 m. Approximately 2.4 kg of NSR sediment was added into the water and mixed via waves in the tank for 5 min to achieve a suspended sediment content of 2000 ppm (Fig. S1-b). This step was followed by the addition of approximately 10 L of fresh DB on the water surface through a pouring device to achieve an oil slick thickness of 5 mm. The high oil-water ratio of 1:120 was chosen to ensure that the water or sediment samples collected from the tanks would contain detectable quantities of organic compounds (Heshka et al., 2022; Monaghan et al., 2022). The tank conditions were selected to represent a low-energy lacustrine environment with occasional wind- and/or current-induced wave action; losses due to evaporation of the volatiles were allowed. The temperatures of the two tests were maintained at average air/water temperatures of 14 °C/15 °C and 6 °C/2 °C representing Alberta's fall and spring seasons, respectively. The evaporation of water during the tests was compensated for by the addition of Type IV deionized water to maintain a constant water height of 0.7 m without introducing extra dissolved ions. The oil spill tests were conducted under indirect daylight conditions since the tank is located within a building. The only exception was during sampling when one LED light (above the tank) was turned on (average 1.0 h per day). Consequently, photo-oxidation processes were expected to be minimized. During the 35-day tests, the wave-generating paddle was set to generate a 60 cm wavelength and a wave amplitude of 8 cm with a wave period of 0.5 s. The paddle was kept ON continuously for the first two days to provide surface mixing and then turned OFF for the next two days to mimic no-wind condition. This ON-OFF wave cycling pattern was repeated during both tests. The only exception in the ON-OFF cycling pattern was during weekends when the paddle status was kept the same as the status of Friday until the following working day. In terms of the suspended sediment content, we had conducted a 130-hour control tank test (without adding oil) to quantify the suspended sediment content in the water grabbed using a 1-L van Dorn water sampler from a similar height as the water sampling port under two conditions (with wave mixing and without mixing). Data showed that suspended sediment content dropped quickly during the first day (within 30 h) to below 0.05 g/kg (50 ppm) in the water and maintained this low level until the end of the test (Fig. S2).

2.3. Water and oil sampling

During the 35 day tests, water samples were collected at regular intervals from the middle of the depth of water through the spill tank sampling port, which was a stainless-steel tube with a diameter of 6.35 mm (1/4 in.) located at a height of 30.5 cm (Fig. S1-a). A series of water samples were taken at time points of T3 (three hours after the spill of DB on water), T120 (day 6), T144 (day 7), T312 (day 14), T336 (day 15), T504 (day 22), T648 (day 28), T672 (day 29), and T816 (day 35, the end of oil spill test). Analyses of the water samples included water solution parameters (pH, electrical conductivity, alkalinity), major and trace elements, hydrocarbon content, and microbiological analyses. Observations of the floating oil were recorded from both the over-head and window-side of the tank using an intrinsically safe camera (Smart-EX® 01). At the end of the tests, the weathered oil samples from the water surface, shoreline, and bottom sediment in the tank were recovered for mass balance and additional analyses. Over 95 % of the added sediment was collected from the bottom of the tank. It was found that some oil had adhered to the sides of the stainless-steel tank and glass windows. The oil collected from the sides and window was included in the "shoreline oil" mass balance calculation.

2.4. Water and sediment analysis

Water samples were not filtered prior to the following hydrocarbon analyses. The determination of benzene, toluene, ethylbenzene, and xylenes (BTEX) concentrations was performed by a commercial laboratory, using headspace GC–MS, following the USEPA methods 5021/8015 and 8260 (USEPA, 2003; USEPA, 2006; USEPA, 2014a). QA/QC protocols were followed for these analyses as described in the cited standard procedures. Polycyclic aromatic hydrocarbons (PAHs) were measured by a commercial laboratory using gas chromatography with mass spectrometry detection (GC–MS) following the USEPA methods 3510C and 8270 (USEPA, 1996; USEPA, 2014b). The TOC content of water samples was determined as per ASTM D7573-09 (ASTM, 2009) using a Shimadzu TOC V CPH instrument. The total organic carbon content of the sediment was determined by an in-house DCM extraction method described previously and was found to have a TOC content of 0.785 wt% (Lara-Jacobo et al., 2021; Mehler et al., 2022).

Water samples were filtered through 0.22 μm Nylon filters (Signa-Aldrich) before being subjected to water solution parameters (pH, electrical conductivity, alkalinity), major and trace elements. Electrical conductivity was measured with a handheld Exstix conductivity total dissolved solids (TDS) meter (Extech Instruments, Nashua, NH). Solution pH was measured with a pH electrode (Mantech, Guelph, ON). Major anions were measured by ion chromatography following the USEPA Method 300 (USEPA, 1993). Major cations were determined by inductively coupled plasma optical emission spectrometry (Radial Varian Vista-Pro, now Agilent Technologies, Santa Clara, CA) where the matrix for the aqueous introduction was a 5 % nitric acid solution. TDS was calculated as the sum of measured ions, except for sulfur (S) and phosphorus (P). Ion balance was calculated from the sum of total measured cations divided by the sum of the total measured anions and adopted as a quality control check.

2.5. Water sampling for microbial analysis

The microbial communities in NSR water, sediment-containing river water, and DB-contaminated water samples were analyzed by the 16S rRNA gene sequencing technique. A separate set of samples were collected at the sampling times for the microbiological study. Identical water samples (n = 3) at each sampling time point were initially filtered (Millipore, pore size, 0.22 μm) and sent to the Energy, Mining and Environment Research Centre (National Research Council Canada, Montreal, Canada) for the 16S rRNA gene amplicon sequencing analysis. A modified protocol by Ausubel (2002) was used to extract the genomic DNA. The description of the DNA extraction procedure is provided in the Supplementary Materials. The polymerase chain reaction (PCR) was performed using the primer set 515F-Y (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGYCAAT TYMTTTRAGTTT′-3′) targeting the V4-V5 variable regions of the 16S rRNA gene of both archaea and bacteria (Caporaso et al., 2011). PCR amplification for Illumina MiSeq amplicon sequencing was performed using the HotStarTaq Master Start polymerase (Qiagen, Toronto, ON, Canada). Reactions (25 μL) contained nuclease-free H_2O, template DNA, and 20 μM of forward and reverse primers with MiSeq forward overhang adapters. The thermal cycling conditions were as follows: initial denaturation at 95 °C/3 min followed by 8 cycles at 95 °C/30 s, 55 °C/30 s, and 72 °C/30 s and a final extension at 72 °C/5 min. The quality of PCR products was evaluated by gel electrophoresis (1.5 % Agarose or 1 % E-gel 96 SybrSafe agarose gel) and the PCR products were purified using the AMPure XP beads. The PCR products were then indexed and cleaned. Finally, the indexed PCR products were pooled before sequencing with the Illumina MiSeq platform and the 500-cycle MiSeq reagent kit v2. The sequencing data were quality checked and paired-end reads were assembled followed by the clustering of sequences (97 %) into Operational Taxonomic Units (OTUs) using the rRNA short amplicon analysis pipeline (Tremblay et al., 2015; Yergeau et al., 2015). The Ribosomal Database Project (RDP) classifier (v2.5) was used to assign the resulting OTUs to a taxonomic lineage (Wang et al., 2007). Finally, the taxonomic classifications and downstream analyses were

performed using QIIME v1.9.1 (Caporaso et al., 2010), in-house Perl, and R scripts, respectively. The 16S rRNA gene sequencing data were grouped as "abundant or major" and "rare or minor". The "abundant or major," taxonomic groups (e.g., phylum, class, genus) had a relative abundance (RA) of >3 %, while the "rare or minor," taxonomic group had a relative abundance of <3 % in each sample or sampling day.

2.6. Oil characterization

After collecting the required water samples for analyses at the end of the tests (T817 or Day 35), weathered DB from the water surface (referred to as floating oil) and oil adhering to the shoreline, side walls, and windows (referred to as shoreline oil) were recovered and weighed. No parts of the oil mat were observed to be submerged or sunken to the bottom of the tank at either temperature. However, oil was associated with the collected sediment and its content was included in the mass balance calculations. The density and viscosity of fresh oils were measured at 20 °C, 25 °C, and 30 °C using an Anton-Paar Stabinger viscometer as per the ASTM D7042 (ASTM, 2016b). The density of weathered oil was determined as per the ASTM D70 (ASTM, 2021) or D4052 (ASTM, 2018b). Dynamic viscosity of weathered oil was measured using a Brookfield viscometer as per the ASTM D5018 (ASTM, 2018c). Densities at 2 °C and 15 °C were calculated from the linear relationship between the measured densities and temperatures (K). Kinematic viscosities at 2 °C and 15 °C were estimated from the logarithmic relationship between the experimentally determined kinematic viscosities and the inverse of the absolute temperature (K^{-1}). The boiling point distributions of the fresh and weathered DB were measured using high temperature simulated distillation (HTSD) as per ASTM D7169 (ASTM, 2018a) using an Agilent gas chromatograph with Analytical Control software. Both fresh DB and floating oils were distilled to a cut point of 204 °C to separate the light oil fraction (initial boiling point [IBP] to 204 °C, referred to as IBP–204 °C) from the heavier fraction of the oil (boiling point [BP] higher than 204 °C, referred to as BP > 204 °C) as per ASTM D1160 (ASTM, 2018a). Detailed hydrocarbon analysis (DHA) of the light ends (IBP–204 °C) was performed by gas chromatography as per ASTM D6729 (ASTM, 2020). For the weathered floating oil, water content was measured by freezing the water in its IBP–204 °C fraction and then pouring the oil present off the ice, recording its weight (ASTM, 2018a). Elemental analysis was performed to determine the contents of carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) using an Elemental Analyzer as per ASTM D5291 (ASTM, 2016a). Oxygen (O) content was determined with an Elemental Oxygen analyzer using an in-house modified ASTM method whereby the sample was pyrolyzed under a stream of He and evolved CO_2 was quantified via gas chromatography using a thermal conductivity detector. Reported elemental data were normalized with respect to total CHNSO content. Separations of the fresh and weathered DB samples into saturates, aromatics, resins, and asphaltenes (SARA) subfractions were performed according to an in-house modification of ASTM D2007 (ASTM, 2019) and ASTM D2549 (ASTM, 2017). The PAHs in oil samples were measured by a commercial laboratory using gas chromatography with mass spectrometry detection (GC–MS) following USEPA methods 3570 and 8270 (USEPA, 2014b).

3. Results and discussion

In this study, the physical and chemical changes of fresh diluted bitumen (DB) during a spill into freshwater environments were assessed. Two 35-day controlled tests were conducted with the spill of fresh DB onto sediment-containing NSR water under average air/water temperatures of 14 °C/15 °C and 6 °C/2 °C, respectively. Fig. S3 shows the oil appearance on the water surface throughout the tests. The oil physical behaviors were a result of collective natural weathering processes including oil spreading and mixing, evaporation, dispersion, dissolution, emulsification, and sedimentation. Photo-oxidation was not assessed but it was expected to have an insignificant effect due to the tank setup mentioned in Section 2.2. The weathering processes mentioned above occur at different rates and with different onset times, resulting in progressive changes in oil composition and physical behavior, as well as in water composition after the spill (Lee et al., 2015). The results of this study are presented in three sections: (1) DB oil spill fate and behavior, (2) Changes in water composition during the DB spills, and (3) Bacterial community response during the DB spills.

3.1. DB oil spill fate and behavior

To characterize the weathered DB in the fresh water, the oil was recovered from the water surface and shoreline at the end of the spill tests. The oil contents of the water and bottom sediment were also determined. The determination of the overall mass balance for the oil in the system is described in the following section.

3.1.1. Oil mass balance analysis

To understand the behavior of DB in fresh water, it is important to determine where the oil goes during the 35-day weathering period. For this purpose, the remaining oils were recovered at the end of the tests from the spill tanks. Overall, the floating oil mat lost mass over time due to the evaporation of light compounds to the air as well as some oil adhering to the water's edge ("shoreline") of the tank. A portion of the fresh oil was also dispersed into the water phase, where it could possibly dissolve, be biodegraded, or interact with sediment. As described previously, the tank system does not have water flowing through it. Consequently, the only possible route for the oil to leave the tank was through evaporation into the air. It is estimated that <100 g of oil from the 9.4 kg (~10 L) of fresh DB added to the water at the start of the tests was lost due to incomplete tank cleanup at the end of the tests.

Masses of floating and shoreline oil were corrected for water trapped in the oil. Table S1 summarizes the water contents of the fresh and weathered oil, as well as the oil recovered from the tank shorelines at 15 °C and 2 °C. Nearly 77.9 wt% and 81.5 wt% of spilled oil mass were recovered as floating oil at the end of the 15 °C and 2 °C tests, respectively. Additionally, ~1.8 wt% and 0.4 wt% at 15 °C and 2 °C, respectively, of the oil was recovered from the tank shorelines. The spilled DB also underwent a slight emulsification process over the 35 days Overall, the recovered floating oil contained 32–39 wt% water and the shoreline oils contained a lower water content of 28 wt% at both temperatures.

Analyses showed that the lost organic compounds were mainly the light compounds in DB (referred to as the initial boiling point to 204 °C distillation fraction [IBP–204 °C fraction]), which were almost all removed from the floating oil in the 15 °C test, while 2.0 wt% of the IBP–204 °C fraction was retained within the floating oil during the 2 °C test (Fig. S4). This corresponded to losses of 19.0 wt% and 17.2 wt% of the fresh DB at 15 °C and 2 °C, respectively over the 35-day spill test periods.

Fig. 1 shows the contents of the IBP–204 °C fractions isolated from the fresh DB and weathered floating oil (2 °C test), each reported normalized on a whole oil basis. The results show that the IBP–204 °C fraction of the fresh DB consisted mainly of C5 to C8 hydrocarbons (>2.0 wt%, Fig. 1a), or classes of *n*-paraffins, isoparaffins, naphthenes and aromatics (>2.0 wt%, Fig. 1b). The olefin content of fresh DB was low. The 2.0 wt% of aromatic compounds in the fraction cover a range of C6 to C12 molecules, as shown in Fig. 1c. After 35 days of weathering during the 2 °C test, only some larger hydrocarbons in this boiling range remained (e.g., approximately 1.0 wt% each of C9 and C10 compounds) (Fig. 1d). The saturates contents had been reduced to similar contents as the olefins and aromatics, <1.0 wt% (Fig. 1e). Most of the BTEX compounds (C6 to C8 aromatics) were removed by the end of the 2 °C test; however, residual C9–10 aromatic compounds remained (Fig. 1f). Interestingly, most of the IBP–204 °C fraction compounds remaining at the end of the 2 °C test were removed during the 15 °C test. The warmer air and water temperature (14 °C/15 °C) were likely the cause of greater loss of these compounds from the oil mat, either due to evaporation or dissolution processes (Lee et al., 2015).

The total organic carbon content of the water column during the 35 days of the tests was estimated from the differences in TOC values

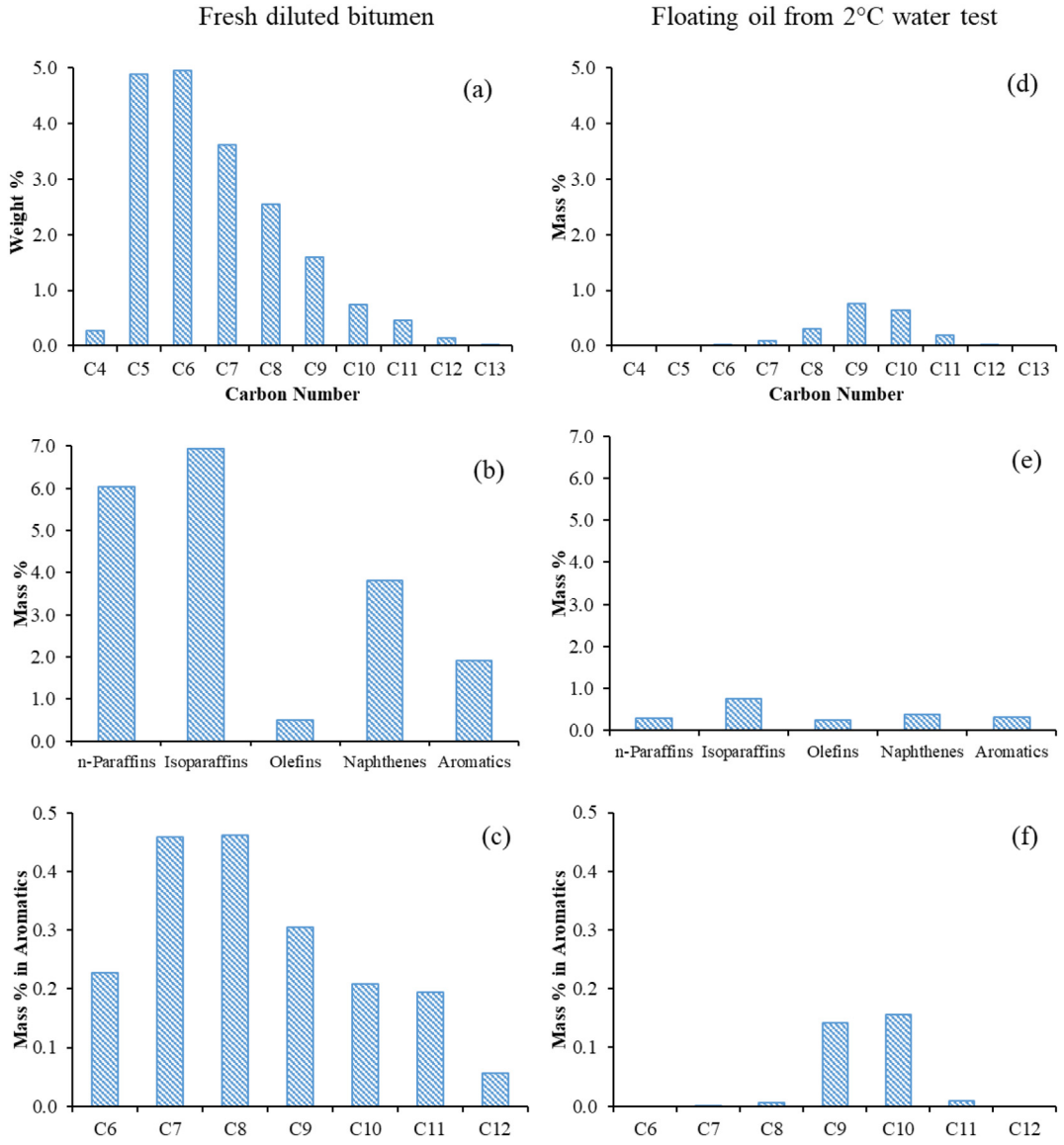

Fig. 1. Comparison of the contents of the IBP–204 °C fractions in fresh diluted bitumen (DB) (a,b,c) and floating oil collected from the 2 °C water test (e,d,f). Hydrocarbon contents (wt%) are reported by carbon number (a&d); hydrocarbon classes (b&e), and aromatics by carbon number (c&f). note: insufficient IBP–204 °C fraction could be recovered from floating oil from the 15 °C water test for analysis.

measured before and after the DB spills. The TOC in water accounted for only 0.1 wt% of the total spilled DB. Details of the TOC content change with time will be discussed in Section 3.2.

The bottom sediment was recovered after the removal of the oil and water from the tank at the end of the tests. Subsamples of the bottom sediment at the end of spill tests were extracted with dichloromethane (DCM) to determine the degree of oil sedimentation. It was found that 30 ± 2 mg oil per gram of dry sediment was associated with bottom sediment at the end of both tests. The HTSD results of the oils extracted from the bottom sediments (Fig. S5) showed that the extracted oils consisted of compounds having close to the full range of DB boiling point distributions, where compounds boiling below 100 °C were still present. This suggests that the oil sedimentation processes happened during the early stages of the spills when the dispersed oil in the water phase came in contact with the suspended sediment allowing the oil and sediment to sink together. Oil sedimentation accounted for approximately 1.0 wt% of the total spilled DB. Fig. S6 illustrates the oil mass distributions at the end (day 35) of the 2 °C and 15 °C spill tests.

A comparison of these results to those for a previously conducted test with conventional crude (CC, Mixed Sweet Blend) under similar conditions (e.g., the water temperature of 15 °C) showed that the loss of the IBP-204 °C fraction of DB at this temperature was considerably less than that of the 28-day CC oil spill test (19 wt% versus 42 wt%) (Lara-Jacobo et al., 2021). Similarly, the extent of DB sedimentation in this study was significantly less than that found for the CC spill test (30 ± 2 mg oil per gram of dry sediment for DB compared to 138 ± 57 mg oil per gram of dry sediment for CC) (Lara-Jacobo et al., 2021). Of note, both the CC and DB spills were carried out at the same water temperature, the same wave mixing energy, and the same sediment type and loading (2000 ppm) in a 1200 L freshwater environment. The only differences were the type of spilled oil (DB vs. CC oil) and the experimental period (e.g., 35 days vs. 28 days). The lower DB sedimentation could be explained by its lower tendency to disperse into the water due to its higher viscosity (532 cSt at 15 °C, below) compared to CC (5.9 cSt at 15 °C) (Lara-Jacobo et al., 2021) at the same water temperature, salinity, wave mixing pattern, and energy dissipation rate (Lee et al., 2015; Hospital et al., 2016).

3.1.2. DB property and composition changes

The fresh DB used in this study had a density of 0.9292 g/mL and a viscosity of 532 cSt at 15 °C (Table 1). Visual observations immediately after

Table 1
Density and viscosity of fresh diluted bitumen (DB) and floating oils in both tests.

Property		Fresh DB	Floating oils	
			15 °C water test	2 °C water test
		Unit: g/mL		
Density	At 2 °C[a]	0.9370	1.0028	1.0001
	At 15 °C[a]	0.9292	0.9963	0.9943
	At 20 °C	0.9290	0.9940	0.9922
	At 25 °C	0.9264	0.9910	0.9890
	At 30 °C	0.9231	0.9890	0.9874
	R^2	0.9953	0.9868	0.9643
Property		Fresh DB	Floating oils	
			15 °C water test	2 °C water test
		Unit: cSt		
Viscosity	At 2 °C[a]	1.39×10^3	1.07×10^6	7.12×10^5
	At 15 °C[a]	532	1.97×10^5	1.31×10^5
	At 20 °C	378	1.07×10^5	7.08×10^4
	At 25 °C	268	5.94×10^4	3.93×10^4
	At 30 °C	196	3.36×10^4	2.21×10^4
	R^2	0.9997	1.0	1.0

[a] Calculated from the measured data at 20 °C, 25 °C, and 30 °C as explained in Section 2.6; the density and viscosity measurement have a relative standard deviation of ≤5 %.

the oil was spilled onto the river water revealed that oil droplets were quickly dispersed into the water under the wave action provided by the paddle. However, the majority of DB resurfaced and accumulated in the shore area within 3 h of the onset of the tests (Fig. S3). Over the course of the 35 days, when the wave cycle was off, the floating oil fully covered the water surface (e.g., on day 14) during the 2 °C test, while weathered oil only partially covered the water surface (e.g., on day 16) during the 15 °C test. The colour of the floating oils and shoreline oils did not change and remained the same as the original DB colour (e.g., black).

As mentioned above, oil densities at 2 °C and 15 °C were calculated from the linear relationship between the measured densities at the corresponding temperatures. The oil density values at the end of the 15 °C test (e.g., T816 or day 35) had increased but were still under 1.0 g/mL. For the 2 °C test, the floating oil density had increased to a value 1.0 g/mL. However, despite their high densities the oil mats were still floating on the water surface at the end of both the 2 °C and 15 °C spill tests (Fig. S3). The measurement of oil viscosity before and after the tests showed that oil viscosity also increased from 532 cSt to 1.97×10^5 cSt in the 15 °C test, and from 1390 cSt to 7.12×10^5 cSt in the 2 °C test (Table 1) due to the loss of the volatile compounds from the oil (Fig. S4).

The SARA and elemental analyses were performed on the fresh DB and weathered floating oils to determine the DB changes during the tests. As summarized in Table 2, the SARA data for the BP > 204 °C fraction of the fresh DB and weathered floating oils showed negligible content change at the 2 °C test. Relatively a small portion of saturates and aromatics was lost at 15 °C during the 35-day weathering period, resulting in a slight proportionally increase of resins and asphaltenes. A comparison of the elemental contents of the BP > 204 °C fraction of the fresh DB to those of the weathered floating oils showed no major differences (Table 2). Fresh bitumen, as produced, has low contents of volatile compounds (Yang et al., 2011). The volatile compounds in fresh DB are mainly derived from the light oil diluent added to the bitumen to meet pipeline specifications for transportation outside of Alberta. Nevertheless, these light compounds with boiling points of <204 °C were removed over the course of the 35-days during the 15 °C test; a few percent of the light fraction remained in the weathered oil at the end of the 2 °C test. For the heavier oil fractions (BP > 204 °C), no change in composition at the "weight %-scale" was found at 2 °C, and only a slight reduction in the proportion of saturates and aromatics were detected at 15 °C.

Table 2
Saturates, aromatics, resins, and asphaltenes (SARA) and elemental (CHNSO) contents of the BP > 204 °C fractions of the fresh diluted bitumen (DB) and weathered floating oils (fraction [wt%]).

	Substance	Unit	BP > 204 °C fraction		
			Fresh DB	Floating oil	
				15 °C water test	2 °C water test
SARA[a]	Saturates	wt%	24.7	22.2	24.9
	Aromatics		40.6	39.1	40.4
	Resins		16.6	19.1	16.5
	Asphaltenes		18.1	19.6	18.2
Elemental[a]	Carbon	wt%	84.2	83.8	83.8
	Hydrogen		10.2	11.0	11.1
	Nitrogen		0.5	0.5	0.4
	Sulfur		4.3	4.3	4.1
	Oxygen		0.8	0.5	0.5

[a] Values represent the average of at least three replicate analyses having a relative standard deviation of ≤5 %. Values for elemental content of oxygen (O) have a relative standard deviation of ≤15 %. BP = boiling point.

3.2. Changes in water composition during the DB spills

Water chemistry analyses including conductivity, total dissolved solids (TDS), pH, and ion balances for the river water used during the spill tests were measured before and after the tests. Table S2 summarizes the results. The average values of the water properties are largely unaffected by the addition of DB. This is consistent with the water chemistry results obtained for spill tests of a conventional crude oil in fresh water (Lara-Jacobo et al., 2021; Heshka et al., 2022).

Crude oils with different origins vary in composition. This leads to differences in water hydrocarbon profiles after spilling into the water. Measurement of the TOC contents of the water shows the contents of both the natural background organic carbon present in the river water as well as the organic compounds whose origin is from the oil. Among all the different organic compounds originating from the oil that can dissolve in water, the contents of aromatic compounds, and their oxidized by-products, tend to correlate with toxicity (Lara-Jacobo et al., 2021; Heshka et al., 2022). Assessing their fate in water helps to understand their potential effects on aquatic communities (Lee et al., 2015). Therefore, the contents of BTEX and PAH compounds, selected as representative aromatics that originated from the IBP-204 °C and BP > 204 °C fractions of the oil, respectively, were also quantified for this study.

Before the addition of oil, the TOC content of the sediment-containing river water was 2.5 mg/L. With oil addition, the TOC content reached a maximum value of 11.2 mg/L approximately 30 min after the spill of DB on the water during the 15 °C test (Fig. S7). In contrast, a maximum TOC value of 7.5 mg/L was detected in the water two hours after the DB spill during the 2 °C test (Fig. S7). The delayed and lower maximum TOC content during the 2 °C test suggests the slower dispersion and dissolution of the fresh oil at 2 °C. This is likely due to the higher viscosity of the oil at 2 °C than at 15 °C (e.g., 1390 vs 532 cSt, in Table 1). The TOC data indicates that spilled DB had contributed 8.7 mg/L and 5.0 mg/L organic carbon to the water during these maximum values at 15 °C and 2 °C, respectively. Also, the TOC contents of the water during both tests decreased rapidly to approximately 6.0 mg/L (3.5 mg/L from DB) within the first day of the tests and remained relatively constant for the remainder of the spill tests. The BTEX contents of water (Fig. S8) show that over 50 % of the maximum TOC values are attributable to BTEX compounds due to their relatively high solubility in water. The rapid decrease of BTEX and TOC contents during the first 24 h is likely related to their high vapor pressures (Lee et al., 2015). As with the TOC, a slightly higher BTEX content was detected during the first few hours of the 15 °C test as compared to the 2 °C test, due to the temperature effect. Within 24 h, the total BTEX content of water diminished to close to 0.5 mg/L in both tests.

The PAH compounds are hydrophobic and do not readily dissolve in water or volatilize to the atmosphere (except naphthalene) (LaGrega et al., 2010b). The total PAH contents of the water with time during the 15 °C and 2 °C tests are shown either by parent & alkylated PAHs (Fig. 2 a-1 & b-1) or by different ring numbers (Fig. 2 a-2 & b-2). Three hours after the DB spill (T3), the total PAH content of water reached the maximum values of 66 μg/L and 164 μg/L, respectively, in the 2 °C and 15 °C tests (Table S3). The lower value in 2 °C water is likely due to both the higher viscosity of the oil and the lower dispersion and solubility of PAH compounds in the colder water (Miller et al., 1998). Noteworthy is that the contribution of total PAH compounds to the TOC content of water is one-tenth (1/10) of the contribution from the BTEX (e.g., >2.0 mg/L in both tests at T3 in Fig. S8-c). Table S3 summarizes the individual PAH compounds' values at T3 in the water. Approximately 16 parent and 36 alkylated PAH compounds, covering 2- to 6-rings in size were included in these analyses. Selected PAH contents of the water were compared to their maximum aqueous solubility, as reported by LaGrega et al. (2010a). It is seen that the PAH contents in the water are well below their solubility limits. For example, while the reported aqueous solubility of naphthalene is 19,000 μg/L at 10 °C, the naphthalene content of water in both tests was only 3.5–3.7 μg/L. Another example is for anthracene, a 3-ring PAH compound. The reported aqueous solubility for anthracene is 45 μg/L at 25 °C. However, the content of anthracene in the water was found to be 0.33–0.56 μg/L. These examples suggest that there was no solubility limitation for PAHs from the floating oil mats (Table S4) which continued to dissolve into the water. However, the total parent and alkylated PAH content decreased, instead of increasing, over time to relatively constant values after 504 h (21 days) during the 2 °C test and after 72 h (3 days) during the 15 °C water test (Fig. 2). There are two potential reasons for this trend. The first would be that the emulsification and high viscosity of the floating oils limited the continuous dissolution of PAH compounds into the water. A second reason would be the removal of PAH compounds from the water by microbial biodegradation.

Our analysis showed that over 90 % of the total PAHs in the water were associated with the alkylated PAHs in both the 2 °C and 15 °C tests (Fig. 2 a-1 & b-1), which was consistent with the abundance of alkylated PAHs in fresh DB (e.g., 4.4 g/kg) and small parent PAHs (e.g., 0.3 g/kg) (Table S4). The analysis of the fresh DB showed that the fresh DB contained more 2- to 3-ring PAH compounds (e.g., 4.1 g/kg in total) than the 4- to 6-ring PAHs (e.g., 0.6 g/kg in total). It is known that the 4–6 ring PAHs are generally less water-soluble than the 2–3 ring PAHs. Thus, it is not surprising to see that around 60 % of the total PAHs detected in the water were 2- and 3-ring PAHs at most sampling points (Fig. 2 b-1 & b-2). The most abundant 2- and 3-ring PAHs were C2, C3, and C4 naphthalenes, C1, C2, and C3 dibenzothiophenes, and C1, C2, C3, and C4 phenanthrenes/anthracenes in both the 2 °C and 15 °C tests, which was consistent with the PAH distribution in fresh DB (Table S4). The next most significant compounds were the 4-ring PAH compounds. Examples include C2, C3, and C4 fluoranthenes/pyrenes, C2 benz(*a*)anthracenes/chrysenes, and C3 benzanthracenes/chrysenes. The 5–6 ring PAHs such as C1 and C2 benzofluoranthenes/benzopyrenes and benzo (*g,h, i*) perylene were negligible in water. The observed trends are consistent with the decreased water solubility as the ring size of PAHs increased (Lee et al., 2015).

3.3. Bacterial community response during the DB spills

The bacterial community composition of the NSR water, sediment-containing NSR water, and DB-contaminated water were determined to assess the short-term (35 days) response of the native bacterial community to the DB spill. A comparison of the post-spill bacterial taxa with the bacterial

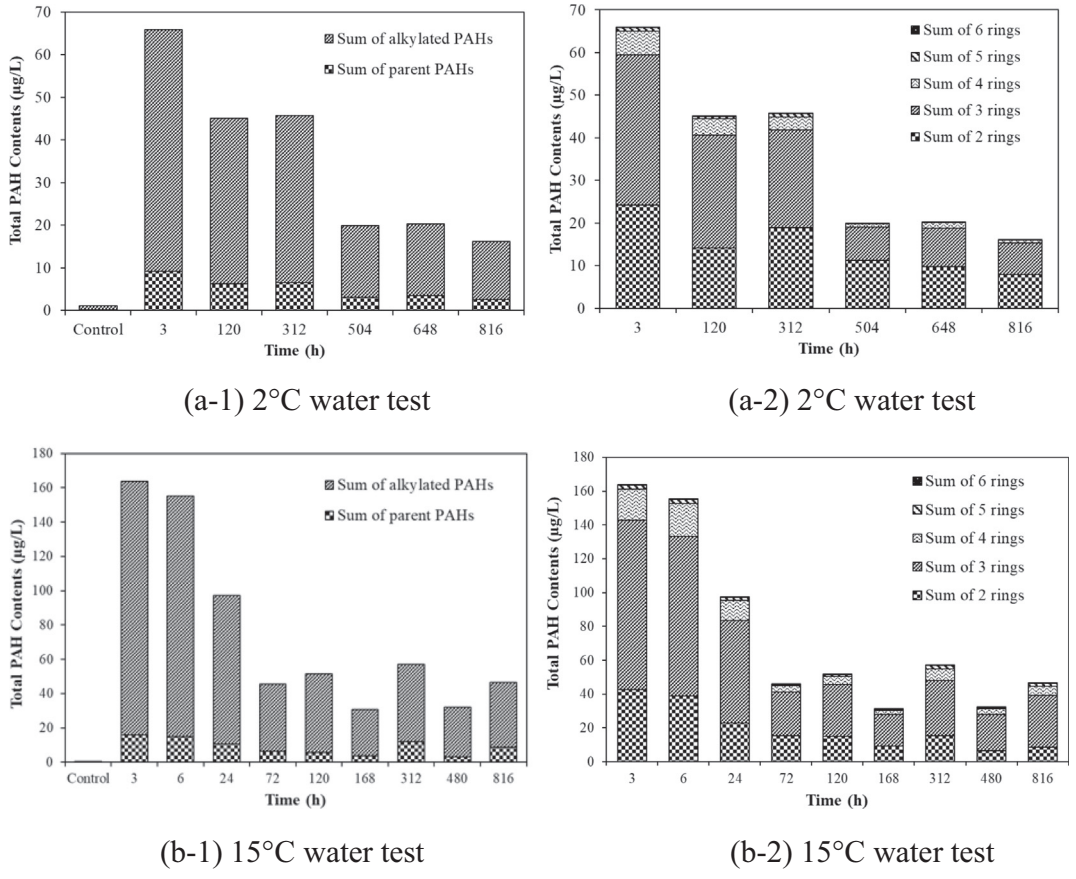

Fig. 2. Total polycyclic aromatic hydrocarbon (PAH) contents of the water over 816 h (35 days) of oil spill tests. (a-1) the sum of parent and alkylated PAH compounds in the 2 °C water test; (a-2) the sum of PAHs with 2–6 rings in the 2 °C water test; (b-1) the sum of parent and alkylated PAH compounds in the 15 °C water test; (b-2) the sum of PAHs with 2–6 rings in the 15 °C water test.

taxa in the river water or sediment-containing river water showed the presence of similar dominant (e.g., taxa with a relative abundance of >3 %) bacterial taxa pre- and post-spill at temperatures of 2 °C and 15 °C (Fig. S9). However, the relative abundances (RA) of the taxa were different in the pre-spill samples (e.g., NSR water or sediment-containing water) than in the post-spill samples (e.g., T3 to T840). For example, at the phylum level Proteobacteria, Actinobacteria, and Bacteroidetes were dominant across all samples at both temperatures (Fig. S9 & Table S5). These phyla accounted for 97.2 % of the total taxa in the pre-spill samples. The members of Proteobacteria accounted for 50 % and 65 %, respectively, at 2 °C and 15 °C and remained dominant post-spill. In contrast, the members of Actinobacteria (RA of 25.1 %), Planctomycetes (RA of 4.6 %), and Verrucomicrobia (RA of 5.4 %) decreased post-spill at both temperatures. Bacteroidetes with a RA of 22.1 % were enriched at 2 °C to an average of 29.3 % (range from 12.5 % to 37.5 % from T3 to T840), while the abundance of this phylum decreased to an average of 17.2 % (range from 7.5 % to 43.75 % from T3 to T840) at 15 °C, even though several members of these phyla are known to utilize hydrocarbons as the sole carbon source under aerobic or anaerobic conditions (Gran-Scheuch et al., 2017; Kostka et al., 2011).

As observed for the phylum level discussed above, noticeable changes were observed among the dominant classes of the bacterial communities at the class level (Fig. 3) following the DB spill in response to the bioavailability of hydrocarbons in the water and/or water temperature. Consequently, the bacterial communities at the end of the spill tests became less similar to their starting communities. Alpha-, beta-, & gamma-Proteobacteria, Flavobacteriia, Cytophagia, and Verrucomicrobiae were enriched during the spill test (T3 to T840) from an average RA of 8.4 % to 12.3 % and 13.2 %, at 2 °C and 15 °C, respectively. While the abundances of other dominant classes including Actinobacteria decreased from 35.5 % (2 °C) and 20.0 % (15 °C) at T3 to 12.5 % and 3.8 % at the end of spill tests at 2 °C and 15 °C, respectively. The maximum enrichment was observed among the members of Betaproteobacteria (RA of 22.4 %) at both temperatures (Fig. 3).

In general, three distinct microbial patterns were observed during the DB spill, in which the relative abundances of some dominant bacterial communities either (i) steadily increased or (ii) decreased, or (iii) fluctuated (Fig. S9). Examples of the first pattern were the steady increase of the alpha- and beta-Proteobacteria classes throughout the experimental period at both temperatures (Fig. 3). These classes contained representatives of the Alphaproteobacterial Sphingomonas, Sphingobium, Sphingopyxis, and Novosphingobium that contain known hydrocarbon-degrading bacteria (Deshpande et al., 2018). Moreover, the genera *Polynucleobacter* and *Methylotenera* (Deng et al., 2019; Fujii et al., 2012; Kostka et al., 2011; Waigi et al., 2015) were also dominant in the spill tank. Enrichment of hydrocarbon-degrading bacteria was also reported in a study conducted by Deshpande et al. (2018) that revealed a strong relationship between the biodegradation of DB-derived aliphatic and aromatic compounds with the enrichment of the members of Proteobacteria and to a lesser extent the members of Sphingobacteriia (Deshpande et al., 2018). Studies showed that the members of these classes are known to degrade a range of organic compounds including dissolved organic matter (Hahn, 2003; Li et al., 2019; Watanabe et al., 2009), phenanthrene (Deshpande et al., 2018; Gran-Scheuch et al., 2017; Pinyakong et al., 2000), anthracene (Waigi et al., 2015), and other PAH compounds (Cederwall et al., 2020; Schreiber et al., 2019b). Their high abundances in the spill tank suggest that the members of these classes either developed the necessary enzymes to degrade DB-derived hydrocarbons (e.g., through historical exposure to petroleum hydrocarbons during oil and gas development in the region) (Gran-Scheuch et al., 2017; Waigi et al., 2015) or they used specific metabolic pathways to enhance hydrocarbon bioavailability (e.g., through biosurfactant production) (Belcher et al., 2012; Waigi et al., 2015). Examples of the second pattern were the steady decrease of the taxa (e.g., hgcl clade, Sporichthyaceae, and Candidatus Planktophila) belonging to the class

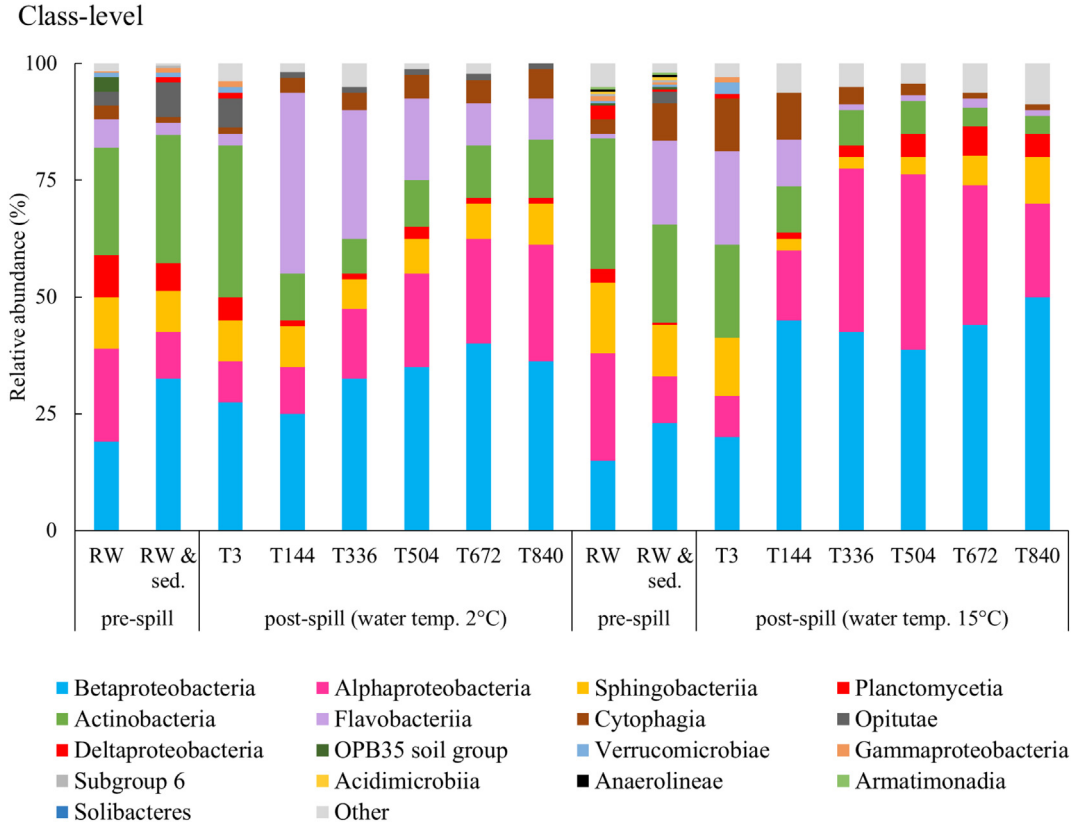

Fig. 3. Relative abundance of bacterial classes pre- & post-spill at 2 °C and 15 °C. "Other" represents the classes with relative abundances of <3 % of the total bacterial taxa in at least one sampling time. RW represents the microbial taxa in the NSR water before the DB spill; RW & sed. represents the microbial taxa in the sediment-containing NSR water before the DB spill. Values represent the average of three replicate samples having a relative standard deviation of ≤5 %.

Actinobacteria at 15 °C and their decrease from T3 to T504 at 2 °C (Figs. S9 & 3). Despite the high abundance (32.5 % and 20 %, respectively at 2 °C and 15 °C) of Actinobacteria at T3 and their known ability to degrade a broad range of hydrocarbons (Deshpande et al., 2018; Kostka et al., 2011) including diesel (Saborimanesh and Mulligan, 2015), light crude oil (Saborimanesh and Mulligan, 2015), and n-alkanes, phenol, anthracene, pyrene (Margesin et al., 2013), the low abundance of Actinobacteria after the DB spill suggests that members of this class (e.g., candidatus planktophila, sporichthyaceae, hgcl clade) either were affected by the elevated levels of oil-derived organic compounds or were dependent on the metabolites produced by the primary DB-degrading bacteria (e.g., Proteobacteria). This may explain their slow recovery after T336 until the end of the spill test at 2 °C and their survival until the end of the spill test at 15 °C (Fig. 3). Examples of the third pattern were observed among members of Flavobacteriia (e.g., genus *Flavobacterium*). Although this class contributed a significant portion of the bacterial community composition at T3 in the 15 °C test, they quickly decreased until the end of the test (Fig. 3). However, a slightly different behavior was observed at 2 °C. At 2 °C, Flavobacteriia increased from a RA of 2.5 % at T3 to a maximum RA of 38.8 % at T144 and then gradually decreased until the end of the DB spill. These different behaviors at 2 °C and 15 °C suggest that these bacteria were able to degrade light hydrocarbons (e.g., BTEX or small saturates or volatile- & semi-volatile PAH compounds) (Kostka et al., 2011; Saborimanesh and Mulligan, 2015) that were removed primarily due to evaporation during the early days of the DB spill tests (Fig. S7) (Fujii et al., 2012). However, the significant enrichment of Flavobacteriia at 2 °C under similar experimental conditions suggests the influence of their sensitivity to temperature where members of this psychrophilic class grow well at temperatures below 10 °C (Zhu et al., 2003).

At the genus level, different dominant genera were present at T3 and only *Albidiferax* was shared among the dominant genera present at the start of both tests (Fig. S10). Although *Albidiferax* remained among the most dominant genera until the end of the tests at the 2 °C and 15 °C, new genera emerged at the end of the 2 °C and 15 °C tests. The taxa hgcl clade and Sporichthyaceae (at the family- level) were replaced by *Methylotenera* and *Flavobacterium* at 2 °C, while *Flavobacterium* and *Pseudarcicella* were replaced by *Polynucleobacter* and *Sediminibacterium* at the end of 15 °C. Taxa related to *Albidiferax* showed the ability to grow heterotrophically on some organics (e.g., acetate) using iron as an electron acceptor in subglacial environments (Achberger et al., 2016). Despite the emergence of some new species at the end of both oil spill tests, the Shannon diversity index showed that the bacterial community diversity only slightly increased post-spill at 2 °C ($H = 1.8 \pm 0.3$) and 15 °C ($H = 1.8 \pm 0.1$) (Fig. S11).

A closer look at the bacterial community composition changes with water temperature showed that the bacterial communities were primarily affected in two ways. Firstly, lower rates of change were observed at 2 °C than 15 °C. Secondly, some of the dominant taxa (e.g., Betaproteobacteria) had a shorter lag phase or acclimation time at 15 °C than at 2 °C. This could be due to the effect of temperature on the availability of organic compounds in the water (Benbouzid et al., 2012). Overall, the effect of temperature was different in the dominant classes. For example, Alphaproteobacteria with a RA of 8.75 % in the river water only enriched to 25 % by the end of the 2 °C test (T840), while the members of this class enriched to a maximum of 37.5 % by the end of the 15 °C test (T540). Betaproteobacteria also showed similar behavior, in which a lower abundance (RA of 36.25 %) was observed at 2 °C in comparison to 50 % at 15 °C (Table S5). In contrast, lower growth was observed among the members of the class Flavobacteriia at 15 °C than at 2 °C (Fig. 3). The over-enrichment of the genus *Flavobacterium* at 2 °C suggests its sensitivity to water temperature as it enriched at 2 °C despite its low abundance at T3, while it disappeared at 15 °C. As mentioned above, this is consistent with the psychrophilic nature of several members of this class (Chaudhary et al., 2019; Gomes et al., 2022; Zhu et al., 2003). The same behavior was observed with the members of the genus *Methylotenera* (belonging to the Nitrosomonadales order) that significantly enriched from below 1 % to 9 % at 2 °C, while it remained absent at 15 °C (Fig. S10). *Methylotenera* resides in different habitats and can metabolize several carbon sources (e.g., methylamine, machine oil, and aromatic oil compounds) (Afshin et al., 2015; Ramachandran et al., 2021; Wang et al., 2019). On the other hand, it seems that *Polynucleobacter* enriched better at 15 °C (Fig. S10). These results are in agreement with a previous study that reported the presence of similar microbial communities with different relative abundances at temperatures of 5 °C and 25 °C as fresh water microbial consortium was exposed to Cold Lake Blend and Western Canadian select DB as the only carbon source (Deshpande et al., 2018).

Studies have shown that the natural attenuation of spilled oil depends on several factors including oil properties, time, the distance from the spill source, and environmental variables (Schreiber et al., 2019a; Yang et al., 2020). As the changes in the concentration and composition of oils impact the microbial community composition (Cederwall et al., 2020) a comparison of the changes in the relative abundance of bacterial communities and the organic carbon content (measured as PAH contents) of water were measured to assess the effects of organic compound availability on microbial community composition. Given the evaporative loss of light molecular weight compounds during the early hours of the DB spills in this study, the total PAH compounds at T3 and not T0 were considered as the starting PAH content of the water for estimating the removal rate of PAHs by microbial degradation in water. It should be noted that the contribution of the evaporation process to the removal of semi-volatile PAH compounds was also considered. A correlation was observed between the relative abundance of specific taxa (e.g., Alpha- and Beta-Proteobacteria, Sphingobacteriia) and the concentrations of organic compounds (e.g., PAHs) in the water. As can be seen from Fig. 4, the shift in the bacterial community composition was coincidental with the availability of PAHs in water. For example, the abundance of several PAH-degrading bacteria (e.g., *Albidiferax*) increased in the water until the end of the 2 °C and 15 °C tests, while the total PAHs, particularly, 2–4 ring PAHs in

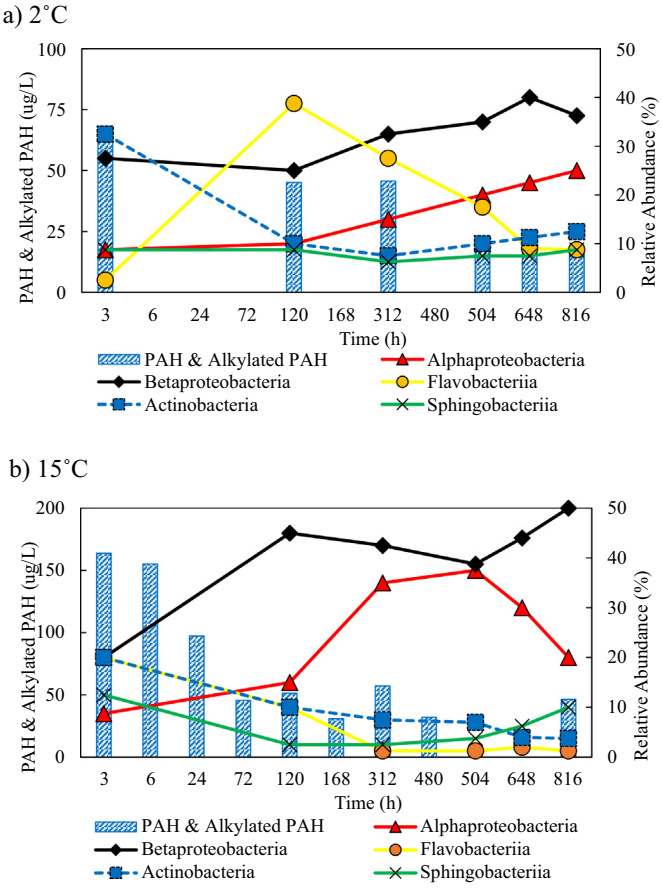

Fig. 4. Changes in PAH and alkylated PAH and dominant microbial classes in water in the (a) 2 °C and (b) 15 °C tests.

water, steady decreased in water. This highlighted the important effect of organic availability on shaping the microbial community composition following the DB spill. However, the disappearance of several members of Flavobacteriia (e.g., genus *Flavobacterium*) during the spill tests, despite the availability of PAH compounds and BTEX (Hendrickx et al., 2006; Pruden et al., 2003; Sedran et al., 2002; Shokrollahzadeh et al., 2008) suggests that the members of this class might only contribute to the removal of smaller PAH compounds during the early days of the DB spill tests. Overall, the biodegradation processes resulted in growth of the bacterial communities discussed by utilization of organic compounds from the water, as indicated by the reduction in PAH contents over the 35 days of the spill tests (e.g., 73–75 % of 2–3 ring PAHs and 52–87 % of 4–5-ring PAHs).

4. Conclusions

During this study, the contribution of collective weathering processes and temperature effects on the fate and behavior of diluted bitumen (DB) in a fresh water environment were assessed. Two 35-day mesoscale tank tests were conducted using sediment mixed with North Saskatchewan River (NSR) water at two different temperatures. Through the detailed characterization of DB-contaminated water sampled from the tank during the experimental period, changes in the hydrocarbon profile in the water and the response of bacterial communities to hydrocarbon availability in water at the two temperatures (2 °C and 15 °C) were analyzed.

Results suggested that the water temperatures had minor effects on the behavior of the oil during the 35-day spill test, including its distribution between floating oil, water, sediment and air. The majority (circa 80–82 %) of spilled DB was recovered from the water surface and shoreline as water-in-oil emulsions. <1.0 % of the spilled DB was recovered from the bottom sediment in the tank. A slightly higher portion of the oil was lost due to evaporation and/or biodegradation processes in the 15 °C test (19.0 %) as compared to the 2 °C test (17.2 %). Since the tank is a closed vessel without water flow in and out, most of the oil lost was by evaporation of the volatile fraction containing organic compounds that boil below 204 °C, over the first 24 h after the spill. The saturates-aromatics-resins-asphaltenes (SARA) measurements, and elemental analyses of BP > 204 °C fractions, confirmed that negligible composition changes were found in the weathered floating oil at 2 °C, while a relatively small portion of saturates and aromatics with boiling points higher than 204 °C were lost from the 15 °C test during the experimental period (35 days).

The organic contents of the water with time supported the conclusion that early losses of light oil compounds were primarily due to evaporation and volatilization of compounds from the floating oil and water. These processes resulted in rapid decreases in the total organic carbon (TOC) and benzene-toluene-ethylbenzene-xylenes (BTEX) contents of the water at both temperatures. The rapid decrease of PAH concentrations in the first 3 h of the spill tests was likely also due to the resurfacing of dispersed oil droplets, and the sedimentation processes, as observed by the tank observation during the early test period. Thereafter, the changes in the semi-volatile and non-volatile PAHs in the water appeared to be a function of time and microbial biodegradation. The biodegradation processes by the identified bacterial communities (e.g., Betaproteobacteria) contributed to the removal of organic compounds from the water over 35 days (e.g., 73–75 % of 2–3 ring PAHs and 52–87 % of 4–5-ring PAHs). Relatively similar starting bacterial communities were observed at the start of both tests. However, different taxa were enriched at the end of 2 °C and 15 °C due to the effect of temperature and organic compound availability. Overall, the cold-water temperature (2 °C) slowed down the oil biodegradation. This could be due to the lower DB dispersion of organic compounds into the water at 2 °C and/or slower bacterial activity. However, a short-term lag phase (e.g., <7 days) in the dominant bacterial communities at 2 °C suggests the pre-exposure of the communities to petroleum-source contaminants in the NSR water and their ability to quickly respond to DB spills.

This study is among the very few studies that have looked at the fate, behavior, and biological effects of a DB spill in sediment-containing fresh water at two temperatures. Given the continuing transportation of bitumen-derived products by pipelines and rail, information from this work will contribute to the knowledge-base for the behavior of DB if spilled in fresh water. These results support spill responders during development of response and remediation plans for potential DB spills.

CRediT authorship contribution statement

Qin Xin: Conceptualization, investigation, methodology, oil and water data analyses, writing original draft, review & editing. **Nayereh Saborimanesh**: Biological data analysis, methodology, writing microbes-related sections, review & editing. **Heather D. Dettman**: Review & editing, technical input. **Charles W Greer**: Providing biological data, review & editing. **Hena Farooqi**: Investigation, review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160161.

References

Achberger, A.M., Christner, B.C., Michaud, A.B., Priscu, J.C., Skidmore, M.L., Vick-Majors, T.J., Adkins, W., Anandakrishnan, S., Barbante, C., Barcheck, G., 2016. Microbial community structure of subglacial lake Whillans, West Antarctica. Frontiers in Microbiology 7, 1457.

Afshin, Y., Delherbe, N., Kalyuzhnaya, M.G., 2015. Methylotenera. Bergey's Manual of Systematics of Archaea and Bacteria, pp. 1–11.

Alberta Energy Regulator, 2019. Alberta Energy Outlook, Executive Summary, ST98.

ASTM, 2009. D7573-09: Standard Test Method for Total Carbon and Organic Carbon in Water by High Temperature Catalytic Combustion and Infrared Detection. ASTM International, West Conshohocken, PA, USA.

ASTM, 2016. D5291: Standard Test Methods for Instrumental Determination of Carbon, Hydrogen, and Nitrogen in Petroleum Products and Lubricants. ASTM International, West Conshohocken, PA, USA.

ASTM, 2016. D7042: Standard Test Method for Dynamic Viscosity and Density of Liquids by Stabinger Viscometer (and the Calculation of Kinematic Viscosity). ASTM International, West Conshohocken, PA, USA.

ASTM, 2017. D2549-02: Standard Test Method for Separation of Representative Aromatics and Nonaromatics Fractions of High-boiling Oils by Elution Chromatography. ASTM International, West Conshohocken, PA, USA.

ASTM, 2018. D1160: Standardtest Method for Distillation of Petroleum Products at Reduced Pressure. ASTM International, West Conshohocken, PA, USA.

ASTM, 2018. D4052: Revision 18A: Standard Test Method for Density, Relative Density, and API Gravity of Liquids by Digital Density Meter. ASTM International, West Conshohocken, PA, USA.

ASTM, 2018. D5018: Standard Test Method for Shear Viscosity of Coal-tar and Petroleum Pitches. ASTM International, West Conshohocken, PA, USA.

ASTM, 2019. D2007: Standard Test Method for Characteristic Groups in Rubber Extender and Processing Oils and Other Petroleum-derived Oils by the Clay-Gel Absorption Chromatographic Method. ASTM International, West Conshohocken, PA, USA.

ASTM, 2020. D6729: Standard test method for determination of individual components in spark ignition engine fuels by 100 metre capillary high resolution gas chromatography. ASTM International, West Conshohocken, PA, USA.

ASTM, 2021. D70/D70M-21: Standard Test Method for Specific Gravity and Density of Semi-solid Asphalt Binder (Pycnometer Method). ASTM International, West Conshohocken, PA, USA.

Ausubel, F.M., 2002. Short Protocols in Molecular Biology: A Compendium of Methods From Current Protocols in Molecular Biology. Wiley.

Belcher, R.W., Huynh, K.V., Hoang, T.V., Crowley, D.E., 2012. Isolation of biosurfactant-producing bacteria from the Rancho La Brea tar pits. World J. Microbiol. Biotechnol. 28, 3261–3267.

Benbouzid, H., Le Floch, S., Stephan, L., Olier, R., Privat, M., 2012. Combined effects of salinity and temperature on the solubility of organic compounds. J. Chem. Thermodyn. 48, 54–64.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108, 4516–4522.

Cederwall, J., Black, T.A., Blais, J.M., Hanson, M.L., Hollebone, B.P., Palace, V.P., Rodríguez-Gil, J.L., Greer, C.W., Maynard, C., Ortmann, A.C., 2020. Life under an oil slick: response of a freshwater food web to simulated spills of diluted bitumen in field mesocosms. Can. J. Fish. Aquat. Sci. 77, 779–788.

Chaudhary, D.K., Kim, D.-U., Kim, D., Kim, J., 2019. Flavobacterium petrolei sp. nov., a novel psychrophilic, diesel-degrading bacterium isolated from oil-contaminated Arctic soil. Sci. Rep. 9, 1–9.

Deng, Y., Gui, Q., Dumont, M., Han, C., Deng, H., Yun, J., Zhong, W., 2019. Methylococcaceae are the dominant active aerobic methanotrophs in a chinese tidal marsh. Environ. Sci. Pollut. Res. 26, 636–646.

Deshpande, R.S., Sundaravadivelu, D., Techtmann, S., Conmy, R.N., Santo Domingo, J.W., Campo, P., 2018. Microbial degradation of cold Lake blend and Western Canadian select dilbits by freshwater enrichments. J. Hazard. Mater. 352, 111–120.

Environment Canada, Fisheries and Oceans Canada, Natural Resources Canada, 2013. Behaviour, fate and transport of two diluted bitumen products from the Canadian oil sands. Environment Canada, Ottawa, ON, p. 85.

Feris, K., Hristova, K., Gebreyesus, B., Mackay, D., Scow, K.M., 2004. A shallow BTEX and MTBE contaminated aquifer supports a diverse microbial community. Microb. Ecol. 48, 589–600.

Fisheries and Oceans Canada, 2018. Status Report on the Knowledge of the Fate and Behaviour of Diluted Bitumen in the Aquatic Ecosystems. Center for Science Advice, National Capital Region, Fisheries and Oceans Canada.

Fujii, M., Kojima, H., Iwata, T., Urabe, J., Fukui, M., 2012. Dissolved organic carbon as major environmental factor affecting bacterioplankton communities in mountain lakes of eastern Japan. Microb. Ecol. 63, 496–508.

Gomes, A., Christensen, J.H., Gründger, F., Kjeldsen, K.U., Rysgaard, S., Vergeynst, L., 2022. Biodegradation of water-accommodated aromatic oil compounds in Arctic seawater at 0°C. Chemosphere 286, 131751.

Gran-Scheuch, A., Fuentes, E., Bravo, D.M., Jiménez, J.C., Pérez-Donoso, J.M., 2017. Isolation and characterization of phenanthrene degrading bacteria from diesel fuel-contaminated Antarctic soils. Front. Microbiol. 8, 1634.

Hahn, M.W., 2003. Isolation of strains belonging to the cosmopolitan polynucleobacter necessarius cluster from freshwater habitats located in three climatic zones. Appl. Environ. Microbiol. 69, 5248–5254.

Hazen, T.C., Dubinsky, E.A., DeSantis, T.Z., Andersen, G.L., Piceno, Y.M., Singh, N., Jansson, J.K., Probst, A., Borglin, S.E., Fortney, J.L., 2010. Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 330, 204–208.

Hendrickx, B., Junca, H., Vosahlova, J., Lindner, A., Rüegg, I., Bucheli-Witschel, M., Faber, F., Egli, T., Mau, M., Schlömann, M., 2006. Alternative primer sets for PCR detection of genotypes involved in bacterial aerobic BTEX degradation: distribution of the genes in BTEX degrading isolates and in subsurface soils of a BTEX contaminated industrial site. J. Microbiol. Methods 64, 250–265.

Heshka, N.E., Peru, K.M., Xin, Q., Dettman, H.D., Headley, J.V., 2022. High resolution orbitrap mass spectrometry analysis of oxidized hydrocarbons found in freshwater following a simulated spill of crude oil. Chemosphere 292, 133415.

Hospital, A., Stronach, J.A., Matthieu, J., 2016. A review of oil mineral aggregates formation mechanisms for the Salish Sea and the lower Fraser River. Proceedings of the Thirty-ninth AMOP Technical Seminar, Environment and Climate Change Canada, Ottawa, ON. 2016, pp. 434–454.

Hounjet, L.J., Stoyanov, S.R., Chao, D., 2018. Distributions of diluted bitumen and conventional crude oil in a range of water types. Chemosphere 211, 1212–1218.

Hua, Y., Mirnaghi, F.S., Yang, Z., Hollebone, B.P., Brown, C.E., 2018. Effect of evaporative weathering and oil-sediment interactions on the fate and behavior of diluted bitumen in marine environments. Part 1. Spill-related properties, oil buoyancy, and oil-particulate aggregates characterization. Chemosphere 191, 1038–1047.

Johannessen, S.C., Greer, C.W., Hannah, C.G., King, T.L., Lee, K., Pawlowicz, R., Wright, C.A., 2020. Fate of diluted bitumen spilled in the coastal waters of British Columbia, Canada. Mar. Pollut. Bull. 150, 110691.

King, T.L., Robinson, B., Toole, P., Clyburne, J.A.C., 2019. Access Western Blend (Dilbit) weathered on cold fresh and marine salt waters. Forty-second AMOP Technical Seminar. Forty-second AMOP Technical Seminar, Ottawa, ON, Canada, pp. 847–858.

Kostka, J.E., Prakash, O., Overholt, W.A., Green, S.J., Freyer, G., Canion, A., Delgardio, J., Norton, N., Hazen, T.C., Huettel, M., 2011. Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico Beach sands impacted by the Deepwater Horizon Oil spill. Appl. Environ. Microbiol. 77, 7962–7974.

LaGrega, M.D., Buckingham, P.L., Evans, J.C., 2010a. Hazardous Waste Management. Waveland Press.

LaGrega, M.D., Buckingham, P.L., Evans, J.C., 2010b. Hazardous Waste Management. Second edition. Waveland Press.

Lara-Jacobo, L.R., Gauthier, C., Xin, Q., Dupont, F., Couture, P., Triffault-Bouchet, G., Dettman, H.D., Langlois, V.S., 2021. Fate and fathead minnow embryotoxicity of weathering crude oil in a pilot-scale spill tank. Environ. Toxicol. Chem. 40, 127–138.

Lee, K., Boufadel, M., Chen, B., Foght, J., Hodson, P., Swanson, S., Venosa, A., 2015. The Behaviour and Environmental Impacts of Crude Oil Released Into Aqueous Environments. The Royal Society of Canada, p. 489.

Li, X., You, C., Qu, L., Zhou, B., Tang, X., Xiao, H., 2019. Bacterial communities fluctuate in abundance and diversity under simulated oil-contaminated seawater conditions. J. Oceanol. Limnol. 37, 615–627.

Margesin, R., Moertelmaier, C., Mair, J., 2013. Low-temperature biodegradation of petroleum hydrocarbons (n-alkanes, phenol, anthracene, pyrene) by four actinobacterial strains. Int. Biodeterior. Biodegradation 84, 185–191.

Mason, O.U., Scott, N.M., Gonzalez, A., Robbins-Pianka, A., Bælum, J., Kimbrel, J., Bouskill, N.J., Prestat, E., Borglin, S., Joyner, D.C., 2014. Metagenomics reveals sediment microbial community response to Deepwater Horizon oil spill. ISME J. 8, 1464.

Mehler, W.T., Xin, Q., Heather, D.D., Greg, G., 2022. Sediment toxicity evaluations of diluted bitumen and conventional crude oil spills via meso-scale spill tanks. Environ. Toxicol. Chem. 00, 1–11.

Miller, D.J., Hawthorne, S.B., Gizir, A.M., Clifford, A.A., 1998. Solubility of polycyclic aromatic hydrocarbons in subcritical water from 298 K to 498 K. Journal of Chemical and Engineering Data 43, 1043–1047.

Monaghan, J., Richards, L.C., Vandergrift, G.W., Hounjet, L.J., Stoyanov, S.R., Gill, C.G., Krogh, E.T., 2021. Direct mass spectrometric analysis of naphthenic acids and polycyclic aromatic hydrocarbons in waters impacted by diluted bitumen and conventional crude oil. Sci. Total Environ. 765, 144206.

Monaghan, J., Xin, Q., Aplin, R., Jaeger, A., Heshka, N.E., Hounjet, L.J., Gill, C.G., Krogh, E.T., 2022. Aqueous naphthenic acids and polycyclic aromatic hydrocarbons in a meso-scale spill tank affected by diluted bitumen analyzed directly by membrane introduction mass spectrometry. J. Hazard. Mater. 440, 129798.

NASEM, 2016. Spills of Diluted Bitumen From Pipelines: A Comparative Study of Environmental Fate, Effects, and Response. National Academies Press.

Ortmann, A.C., Cobanli, S.E., Wohlgeschaffen, G., Thamer, P., McIntyre, C., Mason, J., King, T.L., 2019. Inorganic nutrients have a significant, but minimal, impact on a coastal microbial community's response to fresh diluted bitumen. Mar. Pollut. Bull. 139, 381–389.

Ortmann, A.C., Cobanli, S.E., Wohlgeschaffen, G., MacDonald, J., Gladwell, A., Davis, A., Robinson, B., Mason, J., King, T.L., 2020. Measuring the fate of different diluted bitumen products in coastal surface waters. Mar. Pollut. Bull. 153, 111003.

Ortmann, A.C., Cobanli, S.E., King, T.L., Greer, C.W., Wohlgeschaffen, G., Robinson, B., 2021. Integrating physical, chemical and biological data to understand fate, behaviour and effects of diluted bitumen in coastal waters. International Oil Spill Conference, p. 1141488.

Owens, E., Taylor, E., Marty, R., Little, D., 1993. An inland oil spill response manual to minimize adverse environmental impacts. International Oil Spill Conference. American Petroleum Institute, pp. 105–109.

Pinyakong, O., Habe, H., Supaka, N., Pinpanichkarn, P., Juntongjin, K., Yoshida, T., Furihata, K., Nojiri, H., Yamane, H., Omori, T., 2000. Identification of novel metabolites in the degradation of phenanthrene by Sphingomonas sp. strain P2. FEMS Microbiology Letters 191, 115–121.

Pruden, A., Sedran, M., Suidan, M., Venosa, A., 2003. Biodegradation of MTBE and BTEX in an aerobic fluidized bed reactor. Water Sci. Technol. 47, 123–128.

Ramachandran, A., McLatchie, S., Walsh, D.A., 2021. A novel freshwater to marine evolutionary transition revealed within methylophilaceae bacteria from the Arctic Ocean. MBio 12, e01306–e01321.

Rodriguez-Gil, J.L., Stoyanovich, S., Hanson, M.L., Hollebone, B., Orihel, D.M., Palace, V., Faragher, R., Mirnaghi, F.S., Shah, K., Yang, Z., 2021. Simulating diluted bitumen spills in boreal lake limnocorrals-part 1: experimental design and responses of hydrocarbons, metals, and water quality parameters. Sci. Total Environ. 790, 148537.

Saborimanesh, N., Mulligan, C.N., 2015. Effect of sophorolipid biosurfactant on oil biodegradation by the natural oil-degrading bacteria on the weathered biodiesel, diesel and light crude oil. J. Bioremediat. Biodegrad. 6, 1–8.

Schreiber, L., Fortin, N., Tremblay, J., Elias, M., Mason, J., Sanschagrin, S., Cobanli, S., King, T., Lee, K., Greer, C.W., 2019. Evaluation of the natural attenuation potential of diluted bitumen by microbial communities in the Douglas Channel and Hecate Strait, British Columbia. Applied and Environmental Microbiology 85 (10), AEM.00086-19.

Schreiber, L., Fortin, N., Tremblay, J., Wasserscheid, J., Elias, M., Mason, J., Sanschagrin, S., Cobanli, S., King, T., Lee, K., 2019. Potential for microbially mediated natural attenuation of diluted bitumen on the coast of British Columbia (Canada). Applied and Environmental Microbiology 85, e00086-19.

Sedran, M.A., Pruden, A., Wilson, G.J., Suidan, M.T., Venosa, A.D., 2002. Effect of BTEX on degradation of MTBE and TBA by mixed bacterial consortium. J. Environ. Eng. 128, 830–835.

Shokrollahzadeh, S., Azizmohseni, F., Golmohammad, F., Shokouhi, H., Khademhaghighat, F., 2008. Biodegradation potential and bacterial diversity of a petrochemical wastewater treatment plant in Iran. Bioresour. Technol. 99, 6127–6133.

Stoyanovich, S., Rodriguez-Gil, J.L., Hanson, M., Hollebone, B.P., Orihel, D., Palace, V., Faragher, R.J., Mirnaghi, F.S., Blais, J., 2019. The physical fate and behaviour of diluted bitumen spilled into freshwater limnocorrals. Forty-second AMOP Technical Seminar. ECCC, Ottawa, ON, Canada, pp. 295–300.

Stoyanovich, S., Rodríguez-Gil, J., Hanson, M., Hollebone, B., Orihel, D., Palace, V., Faragher, R., Mirnaghi, F., Shah, K., Yang, Z., 2021. Simulating diluted bitumen spills in boreal lake limnocorrals-part 2: factors affecting the physical characteristics and submergence of diluted bitumen. Sci. Total Environ. 790, 148580.

Stoyanovich, S., Yang, Z., Hanson, M., Hollebone, B., Orihel, D., Palace, V., Rodriguez-Gil, J., Mirnaghi, F., Shah, K., Blais, J., 2022. Fate of polycyclic aromatic compounds from diluted bitumen spilled into freshwater limnocorrals. Sci. Total Environ. 819, 151993.

Stoyanovich, S.S., Yang, Z., Hanson, M., Hollebone, B.P., Orihel, D.M., Palace, V., Rodriguez-Gil, J.L., Faragher, R., Mirnaghi, F.S., Shah, K., 2019b. Simulating a spill of diluted

bitumen: environmental weathering and submergence in a model freshwater system. Environ. Toxicol. Chem. 38, 2621–2628.
Tremblay, J., Singh, K., Fern, A., Kirton, E.S., He, S., Woyke, T., Lee, J., Chen, F., Dangl, J.L., Tringe, S.G., 2015. Primer and platform effects on 16S rRNA tag sequencing. Front. Microbiol. 6, 771.
USEPA, 1993. EPA Method 300.0: Determination of Inorganic Ions by Ion Chromatography. United States Environmental Protection Agency, Washington, DC.
USEPA, 1996. EPA Method 3510C (SW-846): Separatory Funnel Liquid-Liquid Extraction, 3510C. United States Environmental Protection Agency, Washington, DC.
USEPA, 2003. EPA Method 8015D (SW-846): Nonhalogenated organics using GC/FID, 8015D. United States Environmental Protection Agency, Washington, DC.
USEPA, 2006. EPA Method 8260D (SW-846): Volatile Organic Compounds by Gas Chromatography-Mass Spectrometry (GC/MS). United States Environmental Protection Agency, Washington, DC 8260D.
USEPA, 2014. EPA Method 5021A: Volatile Organic Compounds (VOCs) in various sample matrices using equilibrium headspace analysis, 5021A. United States Environmental Protection Agency, Washington, DC.
USEPA, 2014b. EPA Method 8270E (SW-846): Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC-MS). United States Environmental Protection Agency, Washington, DC 8270E.
Varjani, S.J., 2017. Microbial degradation of petroleum hydrocarbons. Bioresour. Technol. 223, 277–286.
Waigi, M.G., Kang, F., Goikavi, C., Ling, W., Gao, Y., 2015. Phenanthrene biodegradation by sphingomonads and its application in the contaminated soils and sediments: a review. Int. Biodeterior. Biodegradation 104, 333–349.
Wang, M., Deng, B., Fu, X., Sun, H., Xu, Z., 2019. Characterizations of microbial diversity and machine oil degrading microbes in machine oil contaminated soil. Environ. Pollut. 255, 113190.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.
Watanabe, K., Komatsu, N., Ishii, Y., Negishi, M., 2009. Effective isolation of bacterioplankton genus polynucleobacter from freshwater environments grown on photochemically degraded dissolved organic matter. FEMS Microbiol. Ecol. 67, 57–68.
Witt O'Brien, Polaris Applied Sciences, Western Canada Marine Response Corporation, 2013. A Study of Fate and Behaviour of Diluted Bitumen Oils on Marine Waters: Dilbit Experiments, Gainford, AB, p. 67.
Xin, Q., Hounjet, L.J., Hartwell, A., 2022. Spill behaviours of pipeline-transportable processed bitumen products in fresh water. Fuel 309, 122040.
Yang, C., Wang, Z., Yang, Z., Hollebone, B., Brown, C.E., Landriault, M., Fieldhouse, B., 2011. Chemical fingerprints of Alberta oil sands and related petroleum products. Environ. Forensic 12, 173–188.
Yang, Z., Shah, K., Laforest, S., Hollebone, B.P., Situ, J., Crevier, C., Lambert, P., Brown, C.E., Yang, C., 2020. Occurrence and weathering of petroleum hydrocarbons deposited on the shoreline of the North Saskatchewan River from the 2016 husky oil spill. Environ. Pollut. 258, 113769.
Yergeau, E., Bell, T.H., Champagne, J., Maynard, C., Tardif, S., Tremblay, J., Greer, C.W., 2015. Transplanting soil microbiomes leads to lasting effects on willow growth, but not on the rhizosphere microbiome. Front. Microbiol. 6, 1436.
Zhong, X., Li, P., Lin, X., Zhao, Z., He, Q.S., Niu, H., Yang, J., 2022. Diluted bitumen: physicochemical properties, weathering processes, emergency response, and recovery. Front. Environ. Sci. 10, 910365.
Zhu, F., Wang, S., Zhou, P., 2003. Flavobacterium xinjiangense sp. nov. and Flavobacterium omnivorum sp. nov., novel psychrophiles from the China No. 1 glacier. Int. J. Syst. Evol. Microbiol. 53, 853–857.