Assessing Monitored Natural Recovery (MNR) for remediating crude oil spills in freshwater environments: The Freshwater Oil Spill Remediation Study (FOReSt) at the Experimental Lakes Area, Canada

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ABSTRACT (#667537)

Monitored natural recovery (MNR) was assessed as a non-invasive method for limiting residual oil exposure in the aquatic environment following contained spills of Cold Lake Blend diluted bitumen (CLB) and conventional heavy crude (CHV) at the IISD-Experimental Lakes Area in Canada. Oils were applied and left in place for 72h to simulate potential spill cleanup response times. After physical removal of free surface oil, biological response and recovery (microbes, zooplankton communities, emergent insects, and benthic invertebrate) was assessed over 80d and exposure of polycyclic aromatic compounds (PACs) and their alkylated forms (aPACS) in water and sediment were characterized. Embryonic development of fathead minnow eggs exposed to water from each of the enclosures was used to determine potential impacts on fish early life stage development. There were significantly different concentrations of PACs in the enclosures treated with diluted bitumen and CHV immediately after application and attenuation differed between the two products throughout the study period. Water contained primarily 3 ring PACs and aPACs. Microbial taxa with known oil degrading capacity increased in water relative to total community abundance. Emergent insect abundance was significantly lower in both oil treated enclosures relative to reference enclosures, but fish development was not significantly impacted by oil treatments. Monitored natural recovery could be successfully applied to oil spill affected freshwater shorelines, but additional data are required to determine long term recovery trajectories.

INTRODUCTION

Environmental impacts of oil spills are concerning to the public, oil industry and regulatory community and there is uncertainty about methods to employ when cleaning oil spills in some freshwater environments. The Canadian Energy Regulator (CER) tasked industry with augmenting knowledge regarding the fate of spilled oil by identifying environmental effects from accidents and contingency planning for spills as key considerations in their review of proposed projects (NEB 2016). In response, the Canadian Association of Petroleum Producers (CAPP) and the Canadian Energy Pipeline Association (CEPA) commissioned an expert panel review by the Royal Society of Canada (RSC) regarding current knowledge of the behaviour and toxicity of crude oil in the environment (Lee et al. 2015). The RSC report identified several priority research areas specific to freshwater, including the need to develop and improve methods for remediation of affected habitats and to assess the efficacy of conventional and new oil spill remediation options. The RSC report also noted that studies conducted on spills of opportunity are not adequate to address these research needs because rigorous pre-spill baseline information is typically lacking at affected sites: instead, controlled field studies are needed to understand ecosystem level impacts of spilled oil (Lee et al. 2015). Canada's Department of Fisheries and

Oceans (Dupuis and Ucan-Marin 2015) and the US National Academy of Sciences (2016) also identified this as high priority research.

In response to the identified research needs, the International Institute for Sustainable Development's Experimental Lakes Area (IISD-ELA) initiated a large collaborative project, the Freshwater Oil Spill Remediation Study (FOReSt). The FOReSt project was developed to evaluate and compare methods for cleaning oil spills from affected freshwater shorelines. Using contained and controlled spills in a pristine lake environment, minimally invasive options for removing oil deposited on freshwater shorelines, and minimizing ecological effects, are being examined. The intent is to model oil that has spread in the aquatic environment and that has become secondarily deposited onto the shoreline (i.e. the so-called "bathtub ring" effect). Shorelines and littoral areas are major contributors to the productivity of freshwater systems and are sensitive to perturbation (Devlin et al. 2016). Therefore, non-invasive methods are needed to eliminate disturbance to sensitive riparian areas that can occur with typical cleanup operations. Unlike marine systems, oil spill cleanup options such as dispersants and other chemical methods are not currently registered for use in freshwater systems in Canada. Moreover, aggressive removal of oil from shoreline areas could impede, rather than accelerate, recovery of the affected area (Lee et al. 2015). As a result, there is a pressing need to conduct studies to determine potential natural recovery rates of freshwater systems affected by oil spills (Whelan et al. 2014).

In the spring of 2018, two enclosures that isolated sections (2.5 X 15m) of shoreline were deployed in Lake 260 at the IISD-ELA field station in Northwestern Ontario, Canada. Model spills of either CLB or CHV were applied to separate enclosures and 2 untreated enclosures served as reference systems. After an initial removal of floating oil, Monitored Natural Recovery (MNR) was implemented and concentrations of polycyclic aromatic compounds (PACs) were determined in the water and sediment of the enclosures over a period of 80d. Potential effects on multiple trophic levels of the aquatic food web were also examined.

MATERIALS AND METHODS

Study site: Lake 260 (surface area = 32.8 ha, vol. = 1,975,971 m^3, max. depth = 15.7m) is a typical boreal lake at the IISD-ELA field station. It was selected based on existing baseline information, ease of access, and isolation from development and publicly accessed waterways. Water and sediment and samples were collected early in the 2018 field season prior to the model oil spill applications for analysis of baseline metrics. Beginning in April, when water temperatures allowed work to begin (>8°C), two enclosures (15 X 5m) (Curry Industries, Winnipeg), divided in half longitudinally, were deployed in a mixed sand-organic shoreline area of the Lake 260 shoreline (Fig. 1).

The enclosures were sealed to the aquatic and terrestrial sediment/soil using a double row of sandbags. Wave energy loggers were placed in each enclosure and in the nearby lake environment. A 2wk acclimation period allowed characterization of baseline water chemistry, bacterial communities and ecological trajectories of the zooplankton and emergent insects.

Oil additions and cleanup: CLB and CHV were provided by an independent third party, sourced from CAPP's member companies' supplies and met pipeline specifications. Before application 7kg of both products was left exposed to air and sunlight for 36 hrs in separate stainless steel evaporation pans containing 25cm depth of freshwater at the IISD-ELA field station. Weathered oil was collected into glass jars using stainless steel utensils. Details regarding the physical and chemical characterization of the pre-weathered and weather oils are provided in SI Appendix 1 (SI materials at https://github.com/IISD-ELA/FOReSt_2018_water_quality_IOSC_Manuscript).

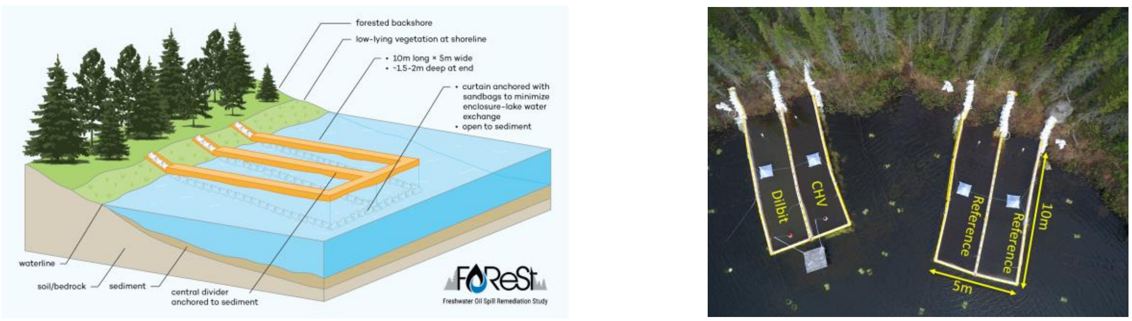

Figure 1: Schematic cross-sectional view of a shoreline enclosure (left) and overhead drone image of the enclosures (right) deployed in Lake 260 at the IISD-Experimental Lakes Area.

The oil applied to each enclosure (~1.25L) targeted an oil thickness of 0.1 cm for the 2.5m linear shoreline and an estimated vertical band (i.e. wave height) of 15cm. Two enclosures were unoiled as reference systems. No cleanup was initiated for 72h to simulate conservative response times (CEPA 2105). After 72h, oil on the water surface was removed using pre-weighed polypropylene sorbent pads (Spill Ninja, MEP Brothers, Winnipeg Canada). This method is effective for up to 8d for heavy oil spills with recoveries of 70-95% (PAS 2013). After the initial removal of floating oil, Monitored natural recovery (MNR) was implemented (Magar et al. 2009) as the sole remediation method for 80d.

Oil and water chemistry: Surface water samples (1L) were collected for PAC and aPAC analysis from each enclosure at 10 timepoints during the study: 3d before oil addition and 1,2,3,5,9,13, 18, 53, and 80d after application. Surface grab samples of lake sediments were collected prior to oil addition and 1 and 2 months after the oiling. Concentrations of PACs and aPACs were determined in extracted water and sediment samples using a validated GC-MS procedure (Idowu et al. 2017). Basic water chemistry parameters (pH, °C, O_2, Cond.) were determined using a YSI probe (YSI Inc., Yellow Springs, OH) and nutrients (NH_3, NO_3,NO_2, total dissolved nitrogen, total dissolved phosphorous, suspended P, dissolved inorganic carbon, dissolved organic carbon, suspended C/N) and suspended Fe, chlorophyll *a*), major anions and cations, alkalinity, soluble reactive silica and turbidity were measured biweekly in water samples (SI Appendix 2).

Microbial community: Microbial community: DNA was extracted from 1L water obtained prior to oil additions and 1 month after the additions for microbial community analysis. Taxonomic 16S and 18S rRNA gene amplifications were performed on all the gDNA samples for prokaryotes and eukaryotes, respectively. Alpha and beta diversity analyses were applied to study and compare the structure of microbial communities and analyze significant differences through time for each of the enclosures. Detailed methods are available in SI Appendix 3.

Biological effects: Zooplankton densities and community composition were determined in the littoral zone of each enclosure using standard techniques (Cadmus et al. 2016). Enclosures can affect community structure so differences were also compared in reference enclosures and the lake environment. Total abundance and richness, as well as temporal trends in emerging insects, were determined using floating emergence traps (surface area of 0.5625 m^2) adapted from Cadmus et al (2016). Traps were positioned ~2.5 m from shore (~1 m depth), prior to the addition of treatments and sampled weekly for 12 weeks. Kick net samples were obtained in fall using standard Environmental Effects Monitoring protocols (Environment Canada 2012). Benthic stages were enumerated and identified to genus or species according to the Canadian

Biomonitoring Network protocols, while adults (from emergence traps) were identified to family using standard keys (Marshall 2017).

Fish: Potential impacts of residual oil was assessed 14d after primary oil removal by exposing embryo-larval fathead minnows to water from the enclosures and a reference location in the lake using an adapted Environment Canada 7d test (Environment Canada 2011). Briefly, fathead minnow eggs (<24h post fertilization) were exposed to undiluted water for 7 days. Static renewal exposures of 40 eggs per replicate (X3) were conducted in beakers containing 400 ml of water that was replaced 80% every other day with fresh water from the appropriate location. This protocol typically uses larvae with inflated swimbladders, but because oil exposure may impact swimbladder inflation (Madison et al. 2015), 1d post hatch embryos were used in the protocol. Mortality was assessed daily. At 7d embryos were photographed to determine length and assess deformities using a dissecting microscope.

Vegetation: The Alberta Riparian Habitat Management Society (ARHMS) standardized approach (Ambrose et al. 2009) was applied to quantify the health and functionality of riparian vegetation for each enclosure. Nine (9) key attributes of the ARHMS assessment were scored. WorldView-3 satellite imagery with 30-cm resolution was acquired on Aug.9, 2018 covering experimental Lake 260 and three control lakes (Lakes 373, 375 and 442). Imagery was comprised of four electromagnetic channels; Red, Green, Blue and Near Infrared (NIR) allowing for the Normalized Difference Vegetation Index (NDVI) to be calculated. NDVI is a measure of vegetative vigor or plant health using the Red and NIR channels of satellite imagery with values ranging from +1.0 (healthy vegetation) to 0.0 (unhealthy vegetation) to -1.0 (lack of vegetation presence). 2018 imagery was compared to 2017 20-cm resolution aerial photo imagery NDVI values from the North West Ontario Orthophotography Project (NWOOP) collected May 7 to 13, 2017.

RESULTS AND DISCUSSION

Study Site: When utilizing enclosures to model ecosystem processes it is prudent to consider the potential influences imposed by isolating sections of the water column and sediments. For example, attenuated wave action and water circulation can reduce dissolved oxygen concentrations and other important parameters that influence biological productivity (eg. pH, nutrients and suspended solids (Liber 1994). Enclosures can also affect trophic cascade processes including grazing of phytoplankton by zooplankton, and fish consumption of periphyton and zooplankton. Two reference enclosures were included in this study's design to acknowledge these confounding influences and to allow direct comparisons among enclosures treated with oil and untreated enclosures. Modest wave heights, typical of small boreal shield lakes, were further attenuated inside the enclosures relative to the lake shoreline (SI Appendix 4). Rates of bacterially mediated degradation rely on water circulation to deliver oil and nutrient and oxygen cofactors. Lower wave energy in the enclosures from this study likely limited water circulation and potential bacterial rates of degradation relative to unenclosed shoreline environments in the lake (Lee et al. 2015).

Basic water chemistry parameters were different inside the enclosed shoreline environments relative to unenclosed reference locations in the lake. For example, temperatures (range 17-24°C) and oxygen saturation (range 54-90%) were similar among all four enclosures throughout the study period, but were generally less than values obtained in the lake environment (SI Appendix

2). Lower pH values were recorded inside all enclosures relative to the lake, and pH in the oil treated enclosures was consistently lower than the reference enclosures. Lower pH has also been observed in smaller freshwater enclosures treated with crude oil likely resulting from the presence of napthenic acids and/or organic sulfur in the oil (Cederwall et al. 2020).

Water nutrient chemistry was performed throughout the exposure period to infer potential effects on primary production and/or respiration in the oil treated and reference enclosures. Oil addition had no obvious effects on nutrients (C,N and P) or their ratios in the well-mixed littoral enclosures environments (SI Appendix 2). However, lower alkalinity and dissolved inorganic carbon (DIC) and calcium concentrations, coupled with peaks of chlorphyll a (Chla) in mid summer (July 10 = 24d after oil addition), suggest primary productivity may have been enhanced in the oil treated enclosures relative to the reference systems (Khan et al. 2020). While elevated DO and pH would also be expected with enhanced primary production, the addition of oil may have concurrently increased respiration of organic carbon by microbes, algae and fungi from the source oil, resulting in depletion of oxygen (Lee et al. 2015). Finally, the oil treated enclosures had significantly lower concentrations of soluble reactive silica relative to unoiled enclosures. Silica can be depleted from surface waters to support diatoms. While the growth of many diatoms is inhibited by crude oils, production of some smaller diatoms taxa can actually be enhanced with exposure to oil (Ozhan et al. 2014). Additional analysis of the effects of crude oil spills on phytoplankton and secondary effects on water chemistry are warranted in littoral freshwater habitats.

Oil additions and cleanup: Seventy two (72) hours after oils were applied, floating oil was removed from the water surface using sorbent pads. The objective was not to remove as much oil as possible but, instead, to facilitate study of the remaining unrecovered product. Each pad was pre-weighed to the nearest 0.1g prior to oil recovery. After recovery, pads were drained overnight to remove any trapped water before being re-weighed. The total mass of recovered oil was determined for each enclosure: i.e. 24.9% from the CHV treated enclosure and 18.5% from the enclosure receiving dilbit. In addition to the primary recovery, we also quantified the mass of oil that adhered to the enclosure walls. This analysis, performed 100d after oiling, used scaled digital images of the entire curtains to determine oiled areas and masses of oiled sections to calculate total mass of oil on the curtains. We included this in the total recovered mass of oil because floating product that adhered to the curtains would have been available for absorbent recovery in the aquatic environment in the absence of the curtain. Including secondary recovery, 31.6% of CHV and 30.5% of the dilbit was recovered. The total recoveries (= primary + secondary) are comparable to reported recovery efficiencies for industrial cleanup of heavy oil spills reported from 1993 to 2012 (Petz 2013).

Table 1: Masses of oil applied to shoreline enclosures and recovery, after 72 hrs with absorptive media (primary recovery), as well as secondary recovery 100d after oil application.

Product	Total Applied	Primary Recovery	Oil on enclosure	Total Recovery
Diluted Bitumen	1563g	289.2g	187.5g	476.7g (30.5%)
Conventional Heavy Crude	1460g	363.5g	98.8g	462.3g (31.6%)

Oil and water chemistry: Oil remaining in the enclosures after primary recovery, including the oil adhering to enclosure curtains, was available for degradation via Monitored Natural Recovery (MNR), a method that relies on microbial degradation and quantification of the recovery trajectory using chemical analysis (Magar et al. 2009). For this project, recovery was framed in the context of potential exposure of aquatic organisms to PACs.

Our objective was to assess the potential for chronic toxicity over time in the aquatic environment after model spills. Acute toxicity is a lesser concern because both oils were weathered prior to application, allowing soluble low molecular weight compounds capable of inducing narcosis to evaporate rapidly within the first 24 hours of weathering, and prior to application (Lee et al. 2015). Concentrations of total PACs and alkylated PACs (aPACs), which are the most bioaccumulative and chronically toxic compounds in crude oils (Madison et al. 2015), were elevated above reference levels in water from the enclosures treated with both types of oil (Fig. 2). Highest PAC concentrations observed were in the CHV enclosure 1d after oil application (= 500ng/L), but were still well below the range reported to cause chronic toxicity to freshwater organisms (~2-80 µg/L) (Lee et al. 2015). Three ringed aPACs, primarily C1-C3 phenanthrenes and dibenzothiophenes, contributed most to the elevated water concentrations (Fig. 2), similar to previous results from a model diluted bitumen spills in freshwater mesocosms (Stoyanovich et al. 2020). However, unlike the previous study we also observed elevated concentrations of 4-ringed PACs in the water column of both the dilbit and CHV treated enclosures. Except for low and comparable profiles of 2-ringed PACs, the enclosures treated with diluted bitumen consistently had greater concentrations of 3-,4- and 4^+-ringed PACs at most sample times throughout the 80d exposures. Concentrations of 2-ringed PACs remained elevated in the CHV treated enclosure, and 2 and 4-ringed PACs were elevated in both oil treated enclosures at the 80d sampling period. Concentrations of 3 and 4-ringed PACs were near reference levels in enclosures treated with both types of oil by the final sampling time (Fig.2). Microbial degradation of 3-ringed compounds, including phenanthrene, has been demonstrated over relatively short timeframes (e.g. weeks) in freshwater (Rehman et al. 2018). We are currently performing additional studies using replicated enclosures deployed for extended treatment times (>500d) to explore the potential of microbial degradation of crude oils in freshwater shorelines.

Alkylated PAC concentrations were elevated in sediments of both oil treated enclosures 2 months after the oil was introduced, and this effect was most striking in the CHV treated enclosure (Table 2). As noted below in the *Microbial Community* section, water and sediment contain very different communities with potentially different degradative capacities and trajectories of recovery.

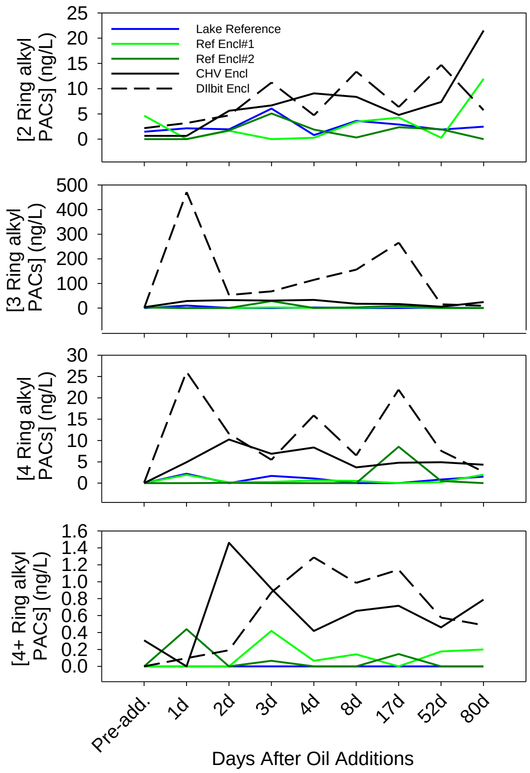

Figure 2: Concentrations of 2,3,4 and 4+ ring alkylated polycyclic aromatic compounds (PACs) in water of reference enclosures (light and dark green lines) enclosures treated with model spills of diluted bitumen (solid black line) and conventional heavy crude oil (dashed black line) and in Lake 260 outside the enclosures (blue line).

Microbial community: Prokaryotic (16S rRNA gene) and eukaryotic (18S rRNA gene) microbial communities were characterized in water from the enclosures and the lake before oil treatment and at 1-month post-oiling. Principle Component Analysis (PCA) revealed that lake water from outside the enclosures exhibited a slight community shift over time but that

Table 2: Concentrations of 2,3,4 ring alkylated polycyclic aromatic compounds (PACs) in sediment of enclosures and at reference locations in the lake. Data are presented as ng/g dry sediment.

	Lake Ref	Ref Encl#1	Ref Encl#2	CHV Encl	Dilbit Encl
2-Ringed alkylated PACs					
Pre Oil	14.5	6.3	13.2	16.1	22.5
1 month post oil	19.7	16.7	6.3	298.0	33.1
2 months post oil	16.5	4.1	3.8	686.4	57.4
3-Ringed alkylated PACs					
Pre Oil	61.1	58.4	93.0	42.2	75.6
1 month post oil	45.6	30.3	16.4	847.6	147.3
2 months post oil	14.3	85.9	40.5	3459.7	519.9
4-ringed alkylated PACs					
Pre Oil	17.7	2.8	5.3	5.0	19.5
1 month post oil	13.2	10.3	3.2	186.4	25.2
2 months post oil	4.6	11.1	1.5	1458.5	30.7

waters from the reference enclosures were similar and had no apparent differences arising from the crude oil and dilbit treatments. However, a community shift was evident in water when the top 100 Operational Taxonomic Units (OTUs) were compared among treatments 1-month post-oiling. Prior to oil application there were 50 OTU's common to all sample locations and 19 unique OTU's in the lake water, while the CHV and dilbit enclosures had just three and one unique OTU's, respectively (Figure3, upper left). One month after oil was applied the common OTU's among water collected from all locations remained similar at 49, but the number of unique OTU's in water from the CHV and dilbit treated enclosures increased to 18 and 11, respectively (Figure 3, upper right). Similar results were obtained for the eukaryotic bacterial community analysis; of the 100 most abundant OTU's, 47 of them were shared among all sample locations (controls, crude oil, dilbit and reference lake) prior to oil treatments. There were three common taxa among the two oiled treatments (two from the order *Chromulinales* and one from the order *Chrysosphaerales*). One month after oil treatment there were still 47 OTU's shared among all the treatments but unique OTU's in the oil treated enclosures increased to four and were now different (orders *Pseudellipsoidion-Eustigmatales, Bivalvulida, Oligohymenophorea;Stokesia Mougeotia*, and *Mougeotia*). These shifts are coincident with altered water chemistry within each of the oil treated enclosures but it is difficult to conclude whether the presence of oil in either enclosure was directly responsible for inducing changes in the resident bacterial communities.

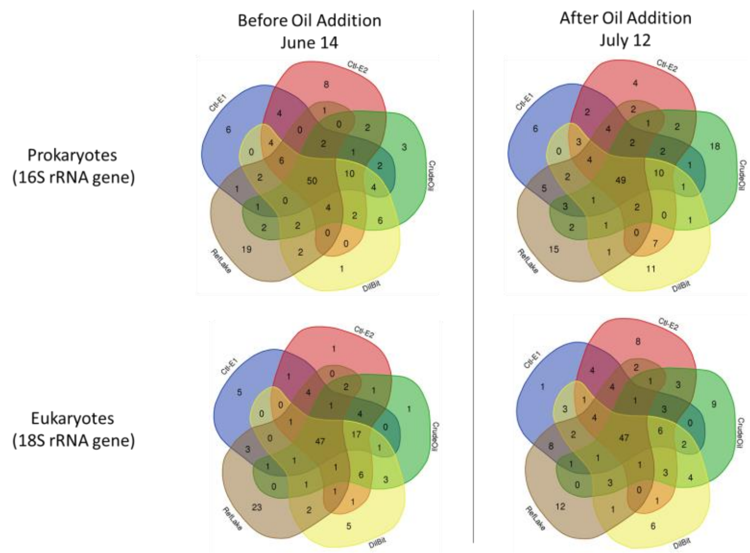

Figure 3: Venn diagram illustrating the number of Operationally Defined Taxonomic Units (OTUs) for prokaryotic bacterial taxa, based on 16S rRNA genetic analysis (top row), and eukaryotic bacterial taxa, based on 18S rRNA genetic analysis (bottom row), in water samples prior to treatment with oil on June 14 (left column) and approximately one month after oil treatments were applied on July 12 (right column).

Other Biological Effects: Residual oil had a significant impact on the insect community. A total of 52 samples were collected from emergence traps in the four enclosures over the 80-day study; 15 families were identified. The kick-net samples taken at the end of the exposure had 28 taxa identified. Using both collection approaches, organisms in the taxonomic group *Diptera*, specifically the family *Chironomidae,* were the most abundant. Total abundance of emerging insects and benthic invertebrates were lower by >50% in the dilbit and CHV treatments compared to the reference enclosures (Figs. 4 and 5). Family richness among emergence samples were 20% lower in dilbit and 40% lower in CHV enclosures, respectively (Fig. 4). Species richness of benthic invertebrates was lower by 23% and 46% in dilbit and CHV treatments, respectively (Fig. 4 inset).

Application rates of crude oil at 10 L/m^2 and 0.24 L/m^2 resulted in declines in certain species of Chironomidae and Trichoptera in previous field studies (Mozley and Butler 1978) while some species of Chironomidae are considered to be very tolerant to the presence of oil (Lacerda et al. 2014). However, application rates in the current study were only approximately 0.075L/m^3 prior to primary cleanup. Toxicity testing of post-Kalamazoo spill in sediment samples using *Chironomus dilutus* and *Hyalella azteca* caused significant mortality, a result of dilbit sinking to the sediments and causing acute toxicity (NAS 2106). It is unclear if the response observed in this study is related to direct toxicity or perhaps other mechanisms (e.g., oil sheen from residual oil impairing emergence and egg laying) which can impact surface dwelling insects (Black et al. 2020).

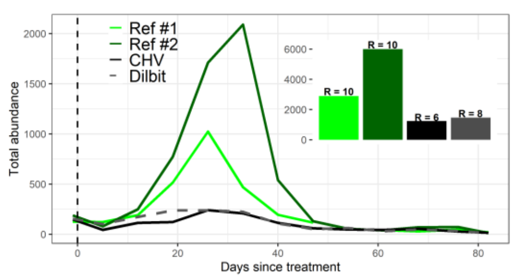

Figure 4: Emergence of organisms per trap in days post oil treatment applications and total abundance per treatment. Total family richness (R) is represented above each treatment column.

Relatively few field studies have considered the potential for spilled crude oil to affect the development of early life stages of freshwater fish. Early life stages of sockeye salmon (parr) exposed to environmentally relevant concentrations of the water-soluble fraction of diluted bitumen had compromised cardiac function that was reflected in reduced swimming performance, but only at exposures >66.7 µg PACs/L (Alderman et al. 2017). Increased rates of cardiac deformities have also been demonstrated in early life stages of fathead minnows, the same species used for the current study, exposed to WAFs of two Canadian diluted bitumens products, with EC_{50} for this effect ranging from ~0.6 to 1.6 µg PACs/L (Alsaadi et al. 2018). Mortality appeared to be slightly higher among fatheads exposed to CHV, and deformities were greater among dilbit exposed fish than in fathead eggs/embryos raised in water from the reference enclosures (Table 2). However, mortality in the range of 15% has been reported in previous studies using the same test methods (Colavecchia et al. 2004).

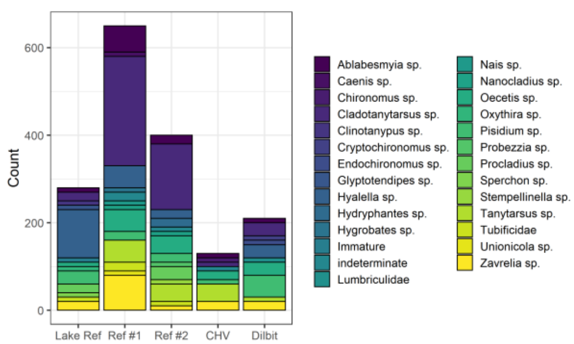

Figure 5: Total abundance of benthic invertebrates in each enclosure at the end of the study (80d post oil application) via kick net sampling. Species richness (R) is represented above each treatment column.

and guidance for applying the protocol allows up to 20% cumulative mortality among reference fish (Environment Canada 2011). Deformities of ~5% among reference fish have been reported for highly replicated test (Vignet et al. 2019). Additional studies are required to examine the potential for low PAC-exposures arising from residual oil in freshwater shorelines to affect development of early life stages of fathead minnows.

Riparian NDVI values were greatly influenced by collection dates and seasonality. Riparian areas had significantly lower NDVI values in early May 2017 (mid-spring) verses early August 2018 (summer). Vegetation health scores were high with 33 of 36 riparian sites scoring greater than 85%. Lack of invasive species, physical shoreline alteration and human activity combined with good tree and shrub establishment resulted in generally healthy riparian systems within the project lake area. There were also no indications that oil applied to the shorelines affected vegetation health. However, the riparian area shoreline adjacent to the experimental spills scored significantly lower at 66.7%. This area scored poorly on RHA endpoints pertaining to human

Table 2: Summary of endpoints for 7d exposures of fathead minnow embryo-larvae to water sourced from the four shoreline enclosures in Lake 260. Data for mortality and deformities are expressed as means from the triplicate exposures, while length, measured in survivors to the end of the 7d exposure, is given as Σ±SEM.

Location	% Mortality	Length (mm)	% Deformities
Ref Encl. #1	7.2	5.67 ± 0.02	5.2
Ref. Encl. #2	15.9	5.61 ± 0.03	12.1
Dilbit Encl.	6.1	5.59 ± 0.03	20.8
CHV Encl.	20.4	5.63 ± 0.03	11.4

activity, related to foot traffic within the riparian zone from researchers accessing the site to obtain water, sediment and biological samples and not resulting from oil contamination or direct toxicity to vegetation (Figure 7). Ongoing recovery will be monitored using interseason imagery to compare the model spills sites to reference sites on Lake 260.

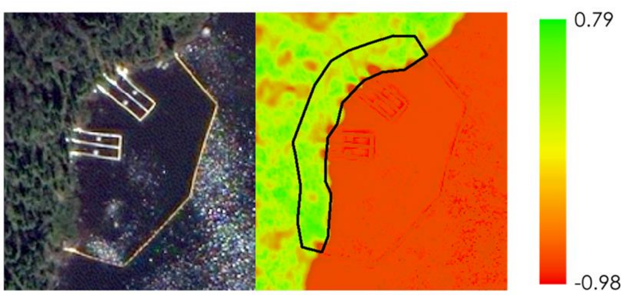

Figure 7. Experimental site in 2018 WorldView-3 RGB pseudo true colour satellite imagery (left); Oil treated enclosure sites in NDVI values ranging from +0.79 (healthy) to -0.98 (lack of vegetation).

CONCLUSIONS

We used enclosures to isolate sections of freshwater lake shoreline to study the impacts of model spills of conventional heavy crude and dilute bitumen. The enclosures attenuated the already low energy in shoreline areas and may have limited microbial degradation rates. Water chemistry analysis indicated few effects of oil on nutrients but several parameters suggested both enhanced phytoplankton production and respiration in the presence of oil that warrants further investigation. Following non-invasive recovery of spilled CHV (31.6%) and dilbit (30.5%) from the water surface using absorbent pads, concentrations of total and alkylated PACs were elevated above reference levels in both CHV and dilbit treated enclosures. The dilbit enclosure had greater concentrations of 3-,4- and 4+-ringed PACs at most sample times. By 80d post spill, concentrations of 3 and 4-ringed PACs were near reference levels in both oil treated enclosures. A bacterial community shift, based on OTUs, was evident in water 1-month post-oiling but it is difficult to attribute this to the presence of oil. There were no significant effects of oil on fish development or riparian vegetation health indices, but negative impacts were evident in enclosures treated with both oils in terms lower total abundance and family and species richness. Studies using replicated enclosures and extended treatment times are warranted to fully examine more fully the potential for microbial degradation of crude oils in freshwaters, and mitigation of ecological effects over time.

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