The Effects of Simulated Spills of Oil and Secondary Cleanup Methods on Macroinvertebrates in a Freshwater Boreal Lake

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Abstract

There is significant uncertainty around the impacts of oil spills and subsequent cleanup methods, specifically chemical and biological responses, in freshwater ecosystems. To address these gaps, the multi-year Freshwater Oil Remediation Study (FOReSt) was conducted at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA). The FOReSt initiative consisted of controlled spills contained in littoral enclosures installed in a freshwater lake at the ELA. A 2018 pilot study examined the fate, behaviour, and effects of diluted bitumen (dilbit) and conventional heavy crude (CHV) followed by physical removal of residual oil. The second phase was a full-scale study in 2019 on the behaviour, fate, and effects of dilbit and two secondary cleanup methods, a shoreline washing agent (SWA, Corexit EC9580-A) and a biological cleanup method (enhanced monitored natural recovery or EMNR) on two different substrates (Rock Cobble and Peat Organic). In both studies, the macroinvertebrate communities were monitored pre- and post-experimental spills using insect emergence traps (at multiple points in time) and by standard kick net sampling at the end of the exposure periods, respectfully. In the 2018 pilot study, insect emergence was reduced relative to untreated control enclosures within both dilbit and CHV treatments over the duration of the field season (>50% reduction in total abundance relative to control enclosures). A similar trend was observed in the macrobenthos community, with a >50% reduction in abundance and lesser overall taxa richness relative to untreated control enclosures. In the 2019 study, both SWA and EMNR showed no statistically significant effects on the total abundance or taxa richness for emergent insects and benthic invertebrates in both shoreline types relative to control enclosures. There was a statistically significant decrease in total diversity (Inverse Simpson) for the EMNR for emergent insects in the Peat Organic shoreline relative to control enclosures. Results from the 2018 pilot study indicate that dilbit negatively impacts the macroinvertebrate community, and from the 2019 full scale study, EMNR and SWA additions to a dilbit release did not affect the macroinvertebrate community to the same degree as dilbit alone. Overall, the findings of this thesis can be utilized to better address spill response and cleanup methods for freshwater shorelines.

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Acronyms

BOREAL	Boreal Lake Oil Release Experiment by Additions to Limnocorrals
BTEX	Benzene, Toluene, Ethylene and Xylene
CHV	Conventional Heavy Crude
CLB	Cold Lake Blend
Dilbit	Diluted Bitumen
EMNR	Enhanced Monitored Natural Recovery
EPT	Ephemeroptera, Plecoptera, and Trichoptera
ETO	Ephemeroptera, Trichoptera, and Odonata
FOReSt	Freshwater Oil Spill Remediation Study
HMW	High Molecular Weight
IISD-ELA	International Institute of Sustainable Development-Experimental Lakes Area
LMW	Low Molecular Weight
LPL	Lowest Possible Level of taxa
NEBA	Net Environmental Benefit Analysis
OPA	Oil Particle Aggregate
PAC	Polyaromatic Compounds
PAH	Polycyclic Aromatic Hydrocarbons
SCAT	Shoreline Cleanup Assessment Technology
SWA	Shoreline Washing Agent
tPAC	Total Polyaromatic Compounds

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Chapter 1: Background on Oil Properties, Transport, Spills, Spill Response, and their Effects on Freshwater Macroinvertebrate Communities

1.1 Introduction

Oil production, refining, and transportation by truck, rail, or pipeline all contribute to accidental oil releases in Canada (TSB, 2017). Oil spills can be detrimental to ecosystems; however, it is unclear if the majority of effects are direct responses from the oil spills, indirect impacts from cleanup response methods, or a combination of both (Dew et al., 2016). There are multiple ways to clean up an oil release including natural, physical, chemical, and biological methods. For each, consideration of habitat type, product type, and any additional adverse effects the methods may have on the ecosystem must be considered. The Royal Society of Canada Report on the *Behaviour and Environmental Impacts of Crude Oil Released into Aqueous Environments* identified many gaps in our current understanding of both spills and response (Lee et al., 2015). They highlight the need for additional research to determine the effects of different crude oil types, cleanup methods, and the type of impacts to freshwater aquatic organisms (including invertebrates) and ecosystems (Lee et al., 2015). Based on the findings and recommendations of the Royal Society of Canada Report, a multi-year study was developed at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) called the **Freshwater Oil Spill Remediation Study (FOReSt)** project. The IISD-ELA is a remote freshwater research laboratory in northwestern Ontario, Canada that includes 58 boreal lakes designed for research purposes by the Province of Ontario and the Federal Government of Canada.

This thesis is one component of the FOReSt project and examines the effects of both crude oil and dilbit product on freshwater aquatic macroinvertebrates by examining community structure, total abundance, richness and diversity of emerging insects and sediment dwelling invertebrates (i.e. benthic macroinvertebrates). Additionally, it evaluates potential effects on aquatic invertebrates regarding the addition of shoreline treatment methods on two different shoreline types (Rock Cobble and Peat Organic) using community structure, abundance, richness and diversity endpoints. The core purpose of this thesis is to advance scientific knowledge regarding the impacts of conventional heavy crude oil and diluted bitumen (dilbit) spills and cleanup methods in controlled field studies.

1.2 Chemical and Physical Properties of Crude Oil and Dilbit

Bitumen is considered an unconventional oil because of its unique extraction and transport methods and it was not commonly extracted in Canada until 1967. (CAPP, 2022 and Conmy et al., 2017). In Canada, it is produced from natural oil sand deposits in the Western Canada Sedimentary Basin (WCSB) (Figure 1.1). Bitumen is extracted by two processes including direct mining using Steam Assisted Gravity Drainage (SAG-D) and in-place extraction or open pit mining (NRCAN, 2016; Environment Canada, 2013). Bitumen as a raw product is too viscous to be transported by pipeline (King et al., 2015) without heating or dilution. Bitumen can be diluted with a naptha based condensate, often by-products of natural gas; this product forms a diluted bitumen or "dilbit". Diluents are rich with low molecular weight (LMW) saturates and contain an appreciable portion of high molecular weight (HMW) compounds, altering the dilbit chemical compound from that of bitumen (Lee et al., 2015). Bitumen can also be diluted with a synthetic crude oil to form synthetic bitumen or "synbit" or a mix of diluent, synthetic oil and bitumen, which is commonly called "dilsynbit" (Crosby et al., 2013; Environment Canada, 2013; Zhou et al., 2015). The addition of diluent will decrease viscosity and density. Blends of dilbit typically have 70-80% bitumen and 20-30% diluents while synbits are typically a 50:50 mixture (Crosby et al., 2013; Lee et al., 2015).

Dilbit contains pentanes, heavier hydrocarbons, and more volatile organic carbons (VOCs) specifically benzene toluene ethylene and xylene (BTEX) than synbit with approximately 21% more BTEX (Dew et al., 2016). In addition, dilbit has 22% more light end hydrocarbons and more organic solvents from its greater bitumen content than synbit. There are over 61 different bitumen blends exported from Canada (CER, 2016). Due to the variety of different blends, it is difficult to predict fate and effect outcomes as the chemical and physical properties can vary significantly among products. For example, unresolved complex mixtures (UCM) in the blend indicate a complex mixture of hydrocarbons are present. This generally indicates both extensive biodegradation of oil, and that the existing hydrocarbon mixtures will be resistant to further biodegradation (Environment Canada, 2013). Further, the viscosity of different blends is temperature dependent and pipeline transport usage will vary seasonally (Lee et al., 2015). For example, Cold Lake Blend (CLB) is one of the most common blends of dilbit transported by pipeline in Canada. It is classified as a winter blend indicating that diluent makes up ~30% of its mass (King et al., 2015), which will influence its propensity to spread and sink when spilled into freshwater.

Crude oils, including conventional and unconventional types, are complex mixtures primarily consisting of hydrocarbons, some compounds containing heteroatoms, small amounts of inorganic sulphur, metals, and minerals (Lee et al., 2015). Crude oil constituents are divided into four chemical classes: saturates, aromatics, resins, and asphaltenes (Lee et al., 2015). Different petroleum products have different compositions, which alter their physical and chemical properties classifying them as light, medium, heavy, and bitumen crude oils (Conmy et al., 2017). Dilbit is most similar to conventional heavy crudes regarding its composition of BTEX, polycyclic aromatic hydrocarbons (PAHs), total acid number (TAN), sulphur, and metals compared to light or medium conventional crudes (Zhou et al., 2015). Bitumen contaminants of concern to ecosystems are primarily PAHs, naphthenic acids, and metals (Lee et al., 2015; see Section 1.6 and 1.7 for further details). It is important to understand the chemical composition of oil as it determines its fate and effects to the environment (see Sections 1.6 and 1.7).

1.2.1 Chemical Composition of Oil

Oil composition is characterized based on the solubility of the four main chemical classes of hydrocarbon components; saturates, aromatics, resins and asphaltenes (Aske et al., 2001). Saturates are often the major component of petroleum. They are comprised of hydrocarbon molecules having single carbon bonds and are considered readily biodegradable and the least toxic of the major petroleum fractions (Lee et al., 2015). Saturates are broken down into three subclasses: paraffins, isoparaffins, and naphthenes (Lee et al., 2015). Saturates make up the majority of chemical compounds in light and medium crude oils and a smaller amount in heavy crude and bitumen (Conmy et al., 2017).

In contrast to saturates, aromatics are either cyclic or planar unsaturated compounds having structures based on a single benzene ring or multiple fused rings (Lee et al., 2015). They have one or more alkyl groups and the most common in petroleum are benzene, toluene, ethylene, and xylene (BTEX). These alkyl monoaromatics that are the most water soluble compounds and are mobile in the water phase (Lee et al., 2015). Light and medium crude oils have a higher content and variation of BTEX than dilbit, heavy crude, and bitumen oils (Zhou et al., 2015). The majority of saturates are considered to be relatively non-toxic due to their high volatility and rapid biodegradation by weathering. However, naphthenes or cyclo-alkanes that are low in molecular weight can have acutely toxic effects to both vertebrate an invertebrate species as they can partition readily into biological membranes, similar to BTEX compounds (Lee et al., 2015; Conmy et al., 2017; Zhou et al., 2015).

Polycyclic aromatic hydrocarbons (PAHs) have two or more fused aromatic rings and are less volatile and more persistent than monoaromatics (Lee et al., 2015). Their persistence is associated with greater chronic impacts impairing survival, growth or reproduction of freshwater aquatic species (Conmy et al., 2017). The chronic toxicity of oil is primarily from alkyl PAHs having 3-5 rings (Lee et al., 2015). The content of PAHs in dilbit, specifically dibenzothiophenes and 3-5 ringed alkyl PAHs, have been compared with conventional crudes (Zhou et al., 2015; Lee et al., 2015). Alkylated PAHs and EPA priority PAH content is lower in dilbit, which should pose a reduced health hazard to humans and toxicity to aquatic life than light or medium crudes (Zhou et al., 2015).

Resins are not exclusively hydrocarbons as they include other elements in their chemical structures (Lee et al., 2015). Asphaltenes are the most structurally complex and highest molecular weight (HMW) components of petroleum. Both resins and asphaltenes are not that susceptible to biodegradation (Lee et al. ,2015) are non-volatile, and play significant roles in oil colloid and droplet formation (Environment Canada, 2013). These four fractions contribute to the viscosity, specific gravity, and biodegradation rates of oil (Lee et al., 2015).

Bitumen, with more high molecular weight components are thought to be less toxic because of it's low solubility and inability to readily cross biological membranes (Lee et al. ,2015). Bitumen also has higher concentrations of heteroatoms such as sulphur, nitrogen, and oxygen. It has been suggested that heteroatom-containing compounds are more water soluble (Zhou et al., 2015).

1.2.2 Bulk Properties of Oil

Bitumen and heavy crude oils are close to the density of water (Table 1). As temperature decreases, density increases for bitumen and heavy crude oils and is a major mechanism causing oil to sink (Environment Canada, 2013). American Petroleum Institute (API) gravity is the petroleum industry standard and is the inverse measure of density used to compare petroleum types (Environment Canada, 2013; Lee et al., 2015). Oils of API gravity >10% will float on freshwater at 15˚C. Weathering of oil affects density as low molecular weight (LMW) compounds evaporate first, leaving behind higher molecular weight (HMW) residuals, decreasing specific gravity (Lee et al., 2015). Oil may sink via three main pathways: mixing with heavier material such as sediment or mineral fines, wave action, or weathering of the product (Environment Canada 2013; Zhou et al., 2015). It has been determined that evaporation alone can cause sinking of oils, while other mechanisms such as temperature, uptake of solid matter, photooxidation, natural dispersion, and water uptake can also contribute (Environment Canada, 2013; Zhou et al., 2015). Weathering typically increases density between 7-8% from fresh oil to the most weathered state. Temperature decreases oil density by approximately 2% from 0-15˚C and density increases with evaporation are usually more moderate (Environment Canada, 2013). Conventional heavy crude (CHV) and Cold Lake Blend (CLB) dilbit vary in their chemical composition and their physical properties such as densities (Table 1). As the density of diluted bitumen may exceed that of water during weathering, submerged oil may pose challenges for spill response and recovery; this is because the majority of response methods are designed to clean up floating oil (Environment Canada, 2013).

Whether an oil product sinks or floats after a spill strongly affects ecosystem impacts. Hence, it is important to understand if and how fast the density of spilled dilbit will approach or exceed that of water during weathering (Zhou et al., 2015). Previous studies have demonstrated that CLB has poor natural dispersion effectiveness with little oil in the water column; ~6% in spring and summer months (King et al., 2015). Zhou et al., (2015) observed that in the absence of sediments, dilbit will often stay afloat for at least 10 days. Similarly, in the Boreal Lake Oil Release Experiment by Additions to Limnocorrals (BOREAL) project conducted at IISD-ELA, dilbit started to sink within the 8 days of introduction to limnocorrals (Stoyanovich et al., 2019). Both BOREAL and FOReSt observations were similar to observations from the Kalamazoo River dilbit release that occurred in Marshall Michigan in 2010 (Black 2019; Stoyanovich et al., 2019). The sinking of oil typically causes oil sediment aggregates to form, which may be aggravated by agitation from cleanup techniques (Zhou et al., 2015). Oil sediment aggregates have been observed up until two years after an oil release indicating that they are stable in freshwater environments. If oil aggregates are freed from the sediments they may re-float (Environment Canada, 2013).

Table 1.1: Physical properties of cold lake blend and conventional heavy crude (+/- SD) adapted from Environment Canada Oil Properties Database (2020), Environment Canada (2013), and Crude Quality Inc. (2020) based on 5 yr average prior to June 8 2018.

Oil Type	*Density kg/m³*	*API Gravity (˚)*	*wt% Sulphur*	*Light Ends (vol%)*	*BTEX (vol%)*
Cold Lake Blend (CLB)	0.9277 ± 0.005	22.6	3.78 ± 0.08	20.40 ± 1.5	1.06 ± 0.17
Conventional Heavy Crude (CHV)	0.9285 ± 0.0039	20.9	3.46 ± 0.29	17.59 ± 3.6	0.86 ± 0.17

Oil viscosity affects the rate of spreading and resistance to being dispersed into droplets. Surface and interfacial tensions are important at smaller scales governing the size of dispersed oil droplets and the final thickness of a spreading film (Environment Canada, 2013). Viscosity is determined by chemical composition. High proportions of light end low molecular weight alkanes and aromatics lower viscosity whereas asphaltenes and resins increase it (Lee et al. ,2015). In studies on dilbit viscosity, viscosity was found to increase by approximately a factor of 10 for each increasing stage of percent weathered oil (5% steps from 0%), ranging from 0-25% of oil weathered by mass (Environment Canada, 2013). Dilbit has a higher viscosity than light and medium conventional crude oils but is similar to heavy crude oils (Zhou et al., 2015). Dilbit has a higher concentration of resins and asphaltenes (HMW compounds), sulphur, metal and organic acid content making it more viscous and dense compared to other petroleum types (Conmy et al. 2017). Since dilbit is more viscous than conventional crude oils it has a greater chance of sticking to shoreline surfaces and coating wildlife and vegetation (Zhou et al., 2015). The volatility of the diluent is also significantly higher than that of the bitumen and it is expected that much of diluent will evaporate from diluted bitumen upon release (Zhou et al., 2015).

With weathering of dilbit, the proportion of saturates and aromatics decreases while the proportion of non-volatile resin and asphaltene groups increases (Environment Canada 2013). In a study by Environment Canada (2013), within 6 hrs at 15˚C, 11.7% of the total mass of CLB was evaporated and within 147 hrs, 20% of its total mass was lost. Concentrations of alkanes n-C9 to n-C14 fell slowly with increasing weathering (Environment Canada 2013). This indicates that a dilbit spill would potentially have the same initial impacts as that of a light or medium crude oil spill and becomes similar to heavier fuel oils as weathering occurs (Environment Canada 2013).

1.3 Oil Transport in Canada

Canada has one of the largest oil reserves in the world at an estimate 27.5 billion m^3 and with the majority found in the Alberta oil sands (Dupuis et al. 2015). Canada has the third largest global oil reserve, the Western Canada Sedimentary Basin (Figure 1.1); and is the fourth largest crude oil exporter in the world (Robidoux et al., 2018). Because of domestic and international oil demand, petroleum products are moved from production site to markets. There were approximately 4.21 million barrels per day (b/d) of crude oil products produced in Canada in 2017 (CER, 2018). In 2013, 74% of production was exported to the US and overseas (Alsaadi et al., 2017) through various means of transport. In total, the oil sands account for 63% of Canada’s oil production in 2019 (NRCan, 2020).

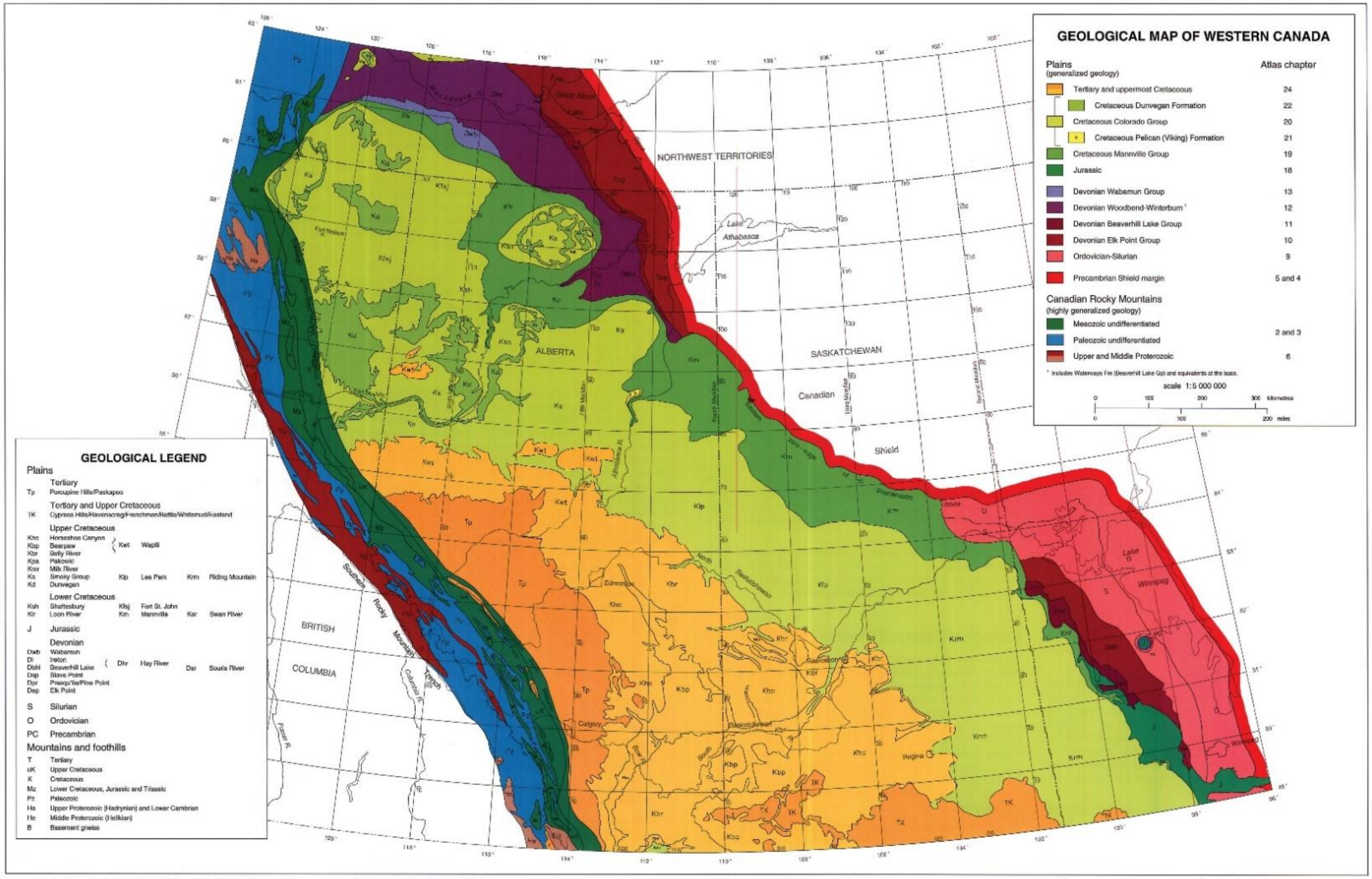

Figure 1.1 Geological map of the Western Canada Sedimentary Basin, showing the regionally and stratigraphically generalized distribution of Phanerozoic rocks in the Interior Plains (commonly mantled by Quaternary cover) and the schematic distribution of major Proterozoic and Phanerozoic tectonic wedges in the Canadian Rocky Mountains.

Figure 1.1: Geological map of the Western Canada Sedimentary basin (taken from Alberta Geological Survey, 2020).

The most efficient way to connect oil production and refining to markets is via pipeline due to the large volumes that need to be transported. Canada has 840,000 km of pipelines carrying crude oil to domestic refineries and for export to the US (Figure 1.2). The current pipeline capacity is 3.9 Mb/d (NRCan, 2019). Another common means of transport is by rail and accounts for 6.2% of the volume of rail freight in Canada (CER, 2018). In 2018, oil exports via rail increased to 327,229 b/d (CER, 2018), while the estimated rail loading capacity out of western Canada is approximately 1 million b/d (NRCan, 2019). Transport by truck is the final means of transporting oil inland; in 2011, 31 million cubic meters of oil was transported via truck (Lee et al., 2015).

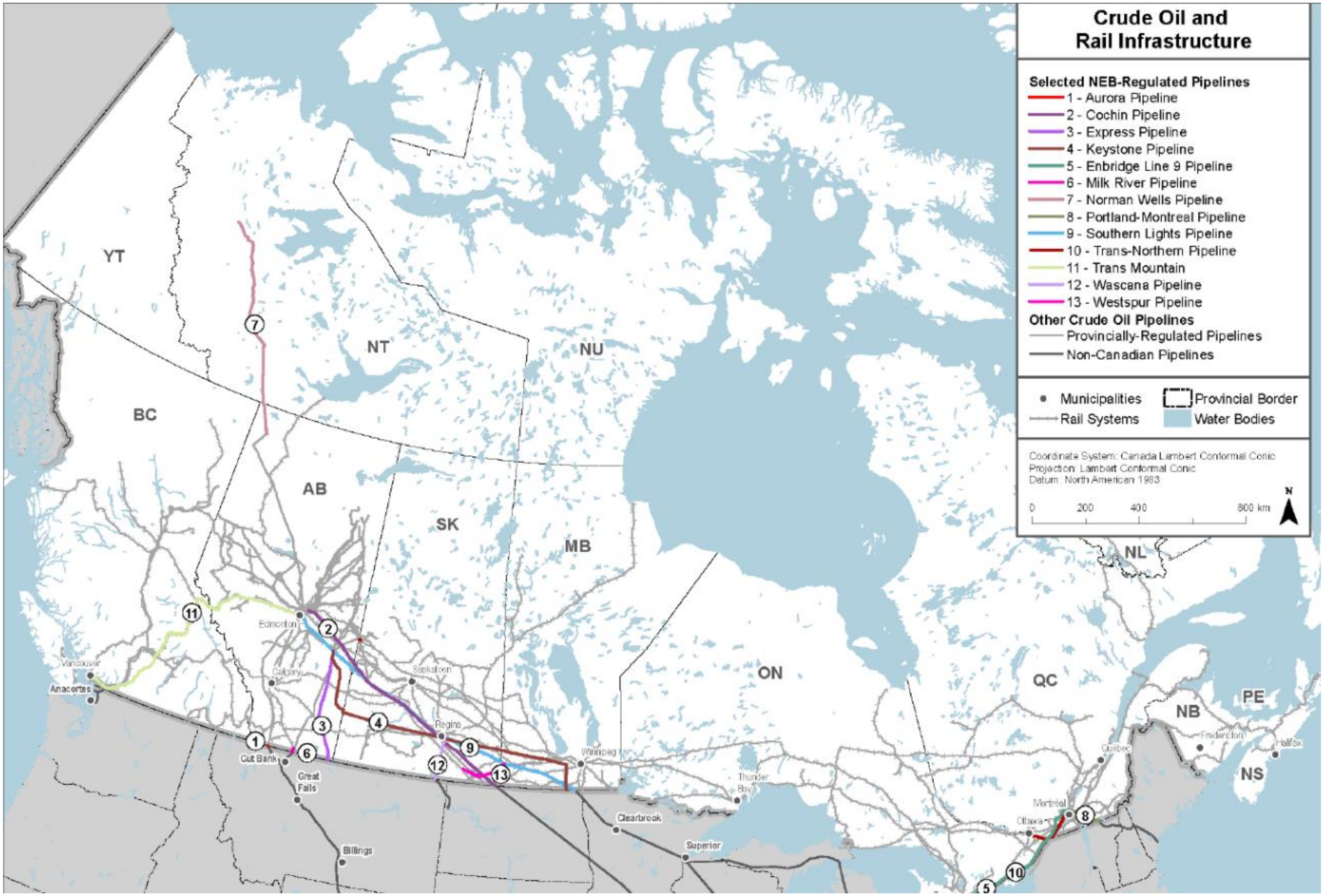

Figure 1.2: Crude oil and rail infrastructure in Canada (taken from NRCan, 2020).

When transporting oil across the country, pipelines, railways, and highways cross a multitude of different ecosystems and environmentally sensitive areas, including freshwater lakes, rivers and wetlands. Each method of transport involves risks that may lead to an accidental release (Table 1.2), with potential adverse effects to the receiving ecosystem. Oil spill impacts to the environment have the potential to last months to years depending on the location, product released, environmental effects and cleanup efforts implemented (Barron et al., 2020; IPIECA, 2014). Spilled oil may enter a water body either directly from an overland release or indirectly through groundwater transfer (Alsaadi et al., 2017).

Typically, a few thousand oil releases to freshwater occur in North America each year at the various stages of transport, extraction, consumption and natural seeps (Vandermeulen et al., 1994; Lee et al., 2015). For all Alberta Energy Regulated pipelines in Canada, there were 93 crude oil releases, three of which impacted water (two in stagnant water/muskeg, one in flowing water) from 2015-2019 (AER 2020). In North America, there have been four major dilbit spills entering waterbodies since 2007. In 2007, the Trans Mountain Pipeline had a punctured line in Burnaby BC releasing 224,000 L of oil. In July 2010, Enbridge pipeline release of 3,320,000 L of dilbit released into the Talmadge Creek and Kalamazoo River near Marshal Michigan, in 2013 Exxonmobil Pegasus spill released 800,000 L of oil in Mayflower Arkansas and in 2016 the Husky Energy Pipeline releasing 250,000L into the North Saskatchewan River (Conmy et al., 2017 and Government of Canada, 2018).

Table 1. 2: Number of accidents, volume shipped, and accident rate by mode of crude oil transport in Canada (Lee et al., 2015).

Year	*Mode*	*Number of Accidents*	*Volume Shipped (m^3)*	*Rate (per million m^3)*
2011	Pipeline	259	736,285,714	0.35
2012	Rail	1	3,908,266	0.26

1.4 Spill Responses

Prior to the Exxon Valdez oil release in Prince William Sound Alaska 1989, there was limited information available for constructing risk assessment models to predict ecological impacts for oil spills (Peterson et al., 2003). Spill impacts were often considered from a mechanical perspective on how to physically remove oil and not from an ecological one that would consider risks and impacts to the environment (Vandermuelen et al., 1994). The Exxon Valdez Spill demonstrated that delays in recovery where oil persisted for over a decade could lead to chronic impacts on the ecosystem. The high persistence of oil resulted in delayed population impacts that impacted health, growth and reproduction of affected populations and led to indirect trophic level interactions (Peterson et al., 2003).

In response to this discovery, Shoreline Cleanup and Assessment Techniques (SCAT) and surveys were developed by Owens Coastal Consultants and Environmental Mapping Ltd (Reid, 2014; Owens and Sergy, 2003). SCAT has since been adapted by the American Petroleum Institute (API) and Environment Canada and Climate Change (ECCC). While on-water recovery is often a good measure for bulk oil cleanup, the likelihood that some oil will reach a shoreline is very high. SCAT surveys help assess shorelines and determine the need for treatment by defining the location, extent, and characteristics of oiling. Additionally, they are used to develop shoreline treatment recommendations that minimize or eliminate additional harm to the environment and provide appropriate end points for cleanup following the principles of As Low As Reasonably Practicable (ALARP) (IPIECA, 2014).

SCAT surveys utilize the American Petroleum Institute Inland Oil Spills summary tables (Reid, 2014). These tables identify the different oil types and cross reference them to the relative environmental impact from response methods for spills in a variety of waterbodies and shoreline habitats to determine which cleanup method should cause the least impact for that type of environment (Reid, 2014). The current drawback to these tables is that they focus heavily on mechanical removal for medium and heavy oils. There is still insufficient information on the impacts of utilizing chemical and biological response methods (API, 1994; API, 2016) as well as impacts of diluted bitumen products.

In the event of an oil release there are three phases of spill response (Reid 2014). The first phase is the reactive phase, where reconnaissance is used to track product spread and determine which techniques should be used to contain and recover oil. The second phase is containment and recovery where a containment plan is executed to prevent additional movement of oil and collection at designated points. The third phase is the cleanup where field data are gathered to determine long-term effects and select the least impactful cleanup methods suitable for the shoreline type (API, 2016). This third phase is where the SCAT surveys are reviewed and a Net

Environmental Benefit Analysis (NEBA) is created. A NEBA is a structured approach to compare the environmental benefits of different response tools (IPIECA, 2015). This approach compiles and evaluates data, predicts outcomes, balances tradeoffs, and selects the best response option (IPIECA, 2015). Since every scenario is different because of varying ecological and socioeconomic sensitivities, locations, and product types, a NEBA must be completed for each spill. With the current information gap on potential impacts and benefits of biological and chemical cleanup methods, the NEBA may not recommend the response method that would be most appropriate for some scenarios.

1.5 Oil Spill Cleanup Methods

While an oil release itself can cause significant damage to a freshwater ecosystem, many spill response techniques can also result in unintended ecological damage. For example, cleanup efforts during the Exxon Valdez event caused additional impacts to the environment. When the beach at Eleanor Island was cleaned using high pressure and hot water washing, silty sediments were washed out into the water, along with many of the organisms that lived along the shoreline (NOAA, 2000). Many of these organisms were not able to recolonize until the beach sediments had re-stabilized. Overall, spill response methods are designed to minimize adverse impacts to the environment; however in some situations access to a spill location may be difficult, impacting what type of cleanup option can be used. Additionally, getting access to a site may cause adverse impacts (Vandermuelen et al., 1994). For instance, access to remote shorelines or larger waterbodies can be limited, resulting in brushing and extensive vegetation removal; or the use of heavy equipment can cause soil compaction, erosion, and sedimentation and siltation. This can lead to additional impaired habitat, reducing overall species abundance and productivity and it may allow for the introduction of invasive species, further impairing ecosystem integrity. Shorelines are particularly susceptible to large economic losses and environmental damages from oil releases. Rapid oil cleaning on shorelines is difficult and mechanical removal often causes further damage to the environment (Bizzel et al., 1999)

Currently, there are three different types of oil spill response methods employed both in Canada and internationally; they are Physical, Natural, and Biological & Chemical methods.

1.5.1. Physical Processes

Physical Processes involve physically removing oil from the environment in which it was spilled. Physical methods of oil cleanup are the most commonly used to date; therefore, more information regarding their impacts tare known (API, 2016). There are many different physical removal techniques. '**Containment and Recovery**' is the standard response to a release of petroleum on water and typically involves the use of booms, skimmers, barriers, and sorbents. This method can recover greater than 50% of spilled product on occasion but is dependent on water and weather conditions (Lee et al., 2015). Booms are used to control the spread and divert or deflect flow paths of oil and to prevent additional impacts to sensitive shorelines (EPA, 2013a). Skimmers are used to recover oil from the water's surface. Barriers are used to prevent oil from spreading and can be used for releases that occur on land and are flowing into a water body to prevent additional flow or can be used on water such as dams (API, 1994). '**Mechanical Removal'** is the process of manually removing residual product either by hand shoveling, vacuum, pumping, use of sorbents, scraping, bagging and removal of bulk product, trenching, or use of sorbents (Lee et al., 2015). '**Washing Recovery'**, also known as physical herding, is when flooding or pressure washing of surfaces is used to lift oil and direct it to a local collection point. This works best where banks or shoreline are easily accessible and where there are minimal flows or currents (Reid, 2014; API 2016). '***In situ* Treatment'** are methods that can be used on site such as burning (using fire resistant booms) or natural berms on shore (Lee et al., 2015). Oil needs to be 2-3mm thick to burn and while smoke can have an impact on wildlife it can be effective in ice or frozen conditions (API, 1994). Mixing of sediments (reworking) to lift up settled oil at the bottom of a water body can be used to float oil product to the surface, where it can be collected or allow for the increased natural removal process from the reworking. **Vegetation removal** is another *in situ* treatment that involves removing contaminated vegetation and debris to prevent additional wildlife impacts or long-term effects. A NEBA helps to determine if removing habitat will be more detrimental than keeping oil in place.

Drawbacks of physical removal are that it can often cause additional harm to the shoreline environment. Mechanical removal and washing recovery can damage and displace sensitive shoreline habitat, increasing adverse impacts beyond that of the oil itself (Lee et al., 2015). Booming and skimming are the most commonly used on-water response, although sheening problems often persist and will eventually reach shorelines. Additionally, booming and skimming only collect oil on the water's surface (IPIECA, 2015). Oil sorbent pads need to be replaced and removed often as they can sink or be trampled into sediments leaving excess waste at the release site (Vandermeulen et al., 1994).

1.5.2 Natural Processes

The second response method is ***Natural Process.*** This approach makes no attempt to remove any stranded oil. Oil is left in place to break down and degrade naturally via biological, chemical and physical processes (API, 2016). This response strategy is the preferred method for remote or inaccessible locations or in spill response situations where human cleanup actions are likely to cause more harm to the environment than the spill itself. The primary mechanisms for natural recovery of petroleum hydrocarbons are biodegradation from oil degrading microbes or evaporation and photooxidation, which rapidly removes toxic, light weight components of oil (Lee et al., 2015).

1.5.3 Biological and Chemical Processes

The third response method is ***Biological and Chemical Process***. These processes involve the addition of a substance (either biological of chemical) to an oil spill to enhance breakdown of the contaminants and prevent further environmental contamination. Biological treatments are similar to natural attenuation; however, there is a human intervention component to increase reduction of the oil (Lee et al., 2015), and is also referred to as enhanced natural attenuation. As the contaminant is being treated *in-situ* there is an increased risk of effects, and therefore natural attenuation as well as enhanced attenuation should be monitored to ensure provincial and federal remediation criteria are met.

Bioremediation exploits the ability of microorganisms to convert pollutants, such as petroleum hydrocarbons, into biomass, carbon dioxide, water, and innocuous oxygenated end products (Lee et al., 2015). While biological methods are less disruptive to the environment, remediation rates are considered slow and enhance the risk of contaminants migrating from the site of spill (Lee et al., 2015). Bioremediation has been used in marine spills such as the Exxon Valdez but infrequently in freshwater spill situations (Vandermuelen et al., 1994). A form of bioremediation is nutrient enrichment or bio-stimulation. Enrichment of nutrients such as phosphorus and nitrogen can speed rates of natural attenuation by increasing oil degradation by microbes. Nutrients including nitrogen, phosphate and iron are essential to biological processes, and crude oils are naturally deficient in these nutrients (Atlas et al., 2011). Adding nutrients or growth enhancing co-substrates can improve habitat quality and stimulate the growth of indigenous oil degraders, increasing degradation rates (Lee et al., 2001). Nutrient enrichment is best used after bulk oil cleanup is completed and shorelines are left lightly oiled. Nutrient enrichment is if often applied with water as a spray but can also be applied as a solid and oleophilic formulations. It is not recommended for shallow waterbodies where nutrient overloading can occur or toxicity of nutrients may pose a concern (API, 1994). Seeding with oil-degrading microbes is a form of bioaugmentation and is best used in areas with light oiling in locations where it is difficult to bring mechanical equipment. Seeding has only been studied in a few trials; a field test during the Exxon Valdez found no significant difference of biodegradation between treatment and controls (Venosa et al., 2003). Very little information is available on bioremediation effects in freshwater to date. The operational use of bioremediation is can take weeks to months to have an effect; this is often not a feasible option when immediate response and cleanup is required by regulating bodies (Zhu et al., 2001).

While not commonly used for oil spills, previous field studies in freshwater wetlands have shown that the addition of ammonium nitrate and triple super phosphate, a phosphorous fertilizer ($Ca(H_2PO_4)2 \cdot H_2O$), reduced residual oil toxicity to background levels within a one-week period, while sodium nitrate was less effective (Lee et al., 2001). Purandare et al. (1999) performed a freshwater wetland mesocosm experiment using a light crude and six different treatment types of nutrient combinations to determine degradation rates. Results showed addition of nitrate and phosphate enhanced degradation of alkanes and PAHs. Venosa et al. (2002) did not show any significant enhancement of degradation of alkanes or total PAHs in a field study using nutrient additions in freshwater wetlands. Many field tests have not been properly designed, well controlled or correctly analyzed, leading to skepticism and confusion among the user community (Zhu et al., 2001).

Chemical treatments with surfactants are used to either disperse oil allowing it to be more easily degraded or to prevent or reduce adhesion of oil onto sensitive shorelines allowing oil to be washed off and collected. Chemical agents have seldom been employed for inland freshwater oil releases in the US and Canada because of the potential for consequent contamination to the environment and to potable water sources (Vandermuelen et al., 1994). If the addition of chemicals to an oil spill is being considered, it must be determined that the added chemical to the oil is less toxic to the environment than the oil itself (Scott et al., 1984) in order to get regulatory approval.

Chemical dispersants reduce the oil-water interfacial tension (NRC, 2005), thereby decreasing the energy needed for the slick to break up into small droplets and mix into the water column. These droplets allow oil to move into the water column where microbes can enhance biodegradation rates (Bhattacharyya et al., 2003). Chemical dispersants have surfactants as their active ingredient (Wrenn and Venosa, 2008); they are amphipathic compounds that have hydrophobic and hydrophilic components within the same molecule (Hemmer et al., 2011). Dispersants reduce oil exposure to surface water organisms and are typically applied by spraying from aircraft and/or boats.

Surface washing agents (SWA) (also known as shoreline cleaners) contain surfactants with greater hydrophilic-lipophilic balance (HLB) than those in dispersants (Fingas, 2013). Surface washing agents are used to remove oil product from shorelines by letting the cleaner soak into the surface for a specified time after which it is rinsed off and recovered in adjacent water using mechanical response equipment, typically skimmers (Bhattacharyya et al., 2003). The use of cleaners can reduce adverse effects of shoreline contamination. These products are typically hydrocarbon solvents that alter the viscosity of oil surfactants and alter surface tension of oil by a mechanism referred to as detergency so oil will not stick to a substrate (Owens et al., 2010). The effectiveness of these techniques depends on the type of oil and their chemical and physical properties. Success also depends on the mixing of agents with the oil and the ability to contain and recover oil (Owens et al., 2010). Shoreline cleaners can either be applied as a pretreatment just prior to stranding of oil, or on shorelines with oil impacts.

With the latter option, cleaners are sprayed on the shoreline to lift and soften oil, typically followed by flushing to wash residues off (API, 1994).

Surface washing agents have not been used on a large scale and have been limited to laboratory and microcosm studies in both fresh and salt water. At this time Corexit EC9580-A (formally Corexit 9580) is an approved spill-treating agent as per the Canada Oil and Gas Operations Act for offshore (marine) waters and is on the US EPA National Contingency Plan (NCP) (Fiocco et al., 1991; Fingas, 2013; Government of Canada, 2016). Corexit 9580 is a formulation of biodegradable surfactants using a low toxicity and a highly refined hydrocarbon solvent (Fiocco et al., 1991). Of 44 test products in a laboratory study, Corexit 9580 was found to remove the greatest oil percentage (26%) and to have the lowest dispersion (7% at a one-minute settling time) (Fiocco et al., 1991). Corexit 9580 has been used in marine spill response and has shown significant effectiveness and low aquatic toxicity (Fiocco et al., 1991).

Although the primary intent of a washing agent is not to disperse oil, if it is used with high water energy SWA may in fact disperse oil (Fingas, 2013). Some studies identified an elevated hydrocarbon concentration after the treatment was applied (Fingas, 2013) as chemical cleaners and dispersants are often themselves hydrocarbon based. Dispersant-enhanced toxicity of oil may increase toxicity and research is required to define an effective minimum dispersant-to-oil ratio. To attain regulatory approval, chemical cleaners should allow recovery without dispersion (Fiocco et al., 1991). In addition, the toxicity of SWAs is highly variable and can range from highly toxic to non-toxic (Fingas, 2013).

1.6 Impacts of Oil Spills and Toxicity to Aquatic Ecosystems

Releases of both crude oil and its dilution blends into to the environment can be detrimental to aquatic ecosystems. In the initial release phase of an oil spill, the oil can be lethal to mammals, fish, birds and shoreline organisms through coating and smothering (EPA, 1999). Oil will submerge into the water column and collect on shorelines and as weathering occurs oil will become more adhesive and difficult to remove. Littoral zones can be very sensitive as oil tends to coat shoreline sediments and aquatic vegetation (EPA, 1999).

The physical aspects of an ecosystem such as temperature, size of the water body, season, currents, and basin morphology will influence oil toxicity (Lee et al., 2015). Weathering of oil plays a significant role in determining oil toxicity and its fate in the spill environment. Most commonly used toxicity tests and protocols do not take site specific information and weathering into account, which prevents unbiased comparisons of toxicity among oil types and test conditions that could impact ecological risk assessments (Adams et al., 2017) and cleanup recommendations.

The chemical aspects of oil also impact toxicity within ecosystems. The main constituents of concern in oil are BTEX and PAHs. PAHs can react with oxygen and with sunlight to create more water-soluble products than the parent compounds. These can be carcinogenic, affect embryo toxicity, mobility, and persistence of toxic photo-oxidized products in the water column (Lee et al., 2015). Once a dilbit product is released into the environment, weathering occurs and evaporation and degradation of the LMW hydrocarbons and the BTEX fraction will tend to evaporate quickly leaving the heavy ends behind, which have similar properties to that of parent crude oil (Robidoux et al., 2018; Stoyanovich et al., 2019). The HMW hydrocarbons left behind increase adhesion and viscosity causing the oil to stick to surfaces, interact more with particles, and increase the likelihood of sinking in the water column (Lee et al., 2015; Environment Canada, 2013). Photooxidation, emulsification, dissolution, and natural dispersion can cause oil constituents to dissolve more easily in water and become more bioavailable to organisms resulting in adverse toxic effects.

While oil concentrations decrease over time following a spill, chemical analyses often do not distinguish between dissolved and particulate hydrocarbons that potentially affect oil toxicity (Adams et al., 2017). The focus of toxicity testing should be on chronic exposures as PAHs are persistent in the environment. Toxicity test methods for PAHs are heavily focused on the list of 16 EPA priority (or parent) PAHs. Many other PAH representatives in the class of polycyclic aromatic compounds (PACs) (i.e. heterocyclic aromatic compounds or alkylated derivatives) have not have been studied sufficiently to understand their occurrence in an environment and their toxicity to a variety of different organisms (Anderson et al., 2015). Several highly toxic PAHs found in heavier oil products are not included in the 16 EPA priority PAHs, such as alkylated derivatives, specifically 3-5 ringed alkyl PAHs and heterocyclic aromatic compounds (Anderson et al., 2015; Let et al., 2015). Relying on this list in the analysis of petroleum contaminated sediments for a toxicity estimate underestimates the load of PAH contaminations (Anderson et al., 2015). Due to the variability of chemical components in crude oils and its dilution blends, there is no single standard test for conventional crudes and dilbit. Site specific tests are required on exposure to aquatic organisms to oil and toxicity due to the unique environmental conditions at each spill (Adams et al., 2017).

Information on elements of oil toxicity can be limited to a few laboratory test species that are not necessarily representative of the wide array of species that may be exposed in the event of a release. An example of this was demonstrated in a study by Black et al. (2021) identifying significant adverse oiling effects on pleuston species in freshwater that can play major roles in aquatic food webs, but that are not commonly used in toxicity tests. Toxicity test methods in laboratories are restrictive and may not be relevant to environmental conditions for actual oil spills. Only a few field studies on oil toxicity have been completed utilizing controlled spills (see Black et al., 2021a; Stoyanovich et al., 2019; Cederwall et al., 2020; Sergy & Blackwell, 1987; and Venosa et al., 1996). The remainder of research on oil spills comes from opportunistic research following accidental oil release to the environment (Dupuis et al., 2015). There are limited data on diluted bitumen toxicity in natural systems; therefore, it is inconclusive if this is a greater risk to an ecosystem compared to other crude oils.

To date, research on impacts of oil releases in aquatic ecosystems has largely focused on marine habitats where there is potential for offshore releases. The reason for this is in the past, more oil was shipped in tankers. Now, more oil is being moved over land, shifting the focus on inland releases. Currently, little is known about the impacts of inland releases to freshwater (Vinson et al., 2008, Steen et al., 1999). In particular, dilbit has been relative understudied as compared to conventional crudes and most studies have largely focused on fish (Barron et al., 2018). When interpreting the effects of dilbit or bitumen on an aquatic ecosystem it is important to consider trophic cascades and indirect effects (Fleeger et al., 2003) as some taxa that are not directly exposed to oil or affected by toxicity but may still be affected through food web interactions. A trophic cascade can occur when organisms from one trophic level are affected by a contaminant to a greater extent than other trophic levels in an ecosystem (Dew et al., 2016). There are two types of cascades depending on which trophic level is affected. A top down cascade is when top predators or grazers are affected by contaminants resulting in population level effects on prey species. A bottom up cascade is when the primary producers or consumers are affected causing trophic levels above to be affected (Dew et al., 2016). Investigations into lower trophic levels, such as macroinvertebrates, which support fish and birds, are important to reduce uncertainty when characterizing ecological risk. For example, in the BOREAL study, different tropic levels in freshwater limnocorrals were monitored and trophic interactions identified (Stoyanovich et al., 2019; Black 2019; Cederwall et al., 2020). While some of the information was inconsistent, crustacean zooplankton had large decreases that may have impacted fish and shifting predators may have allowed for juvenile copepods and rotifers to increase (Black, 2019).

1.7 Impacts on Aquatic Macroinvertebrates

Aquatic macroinvertebrates perform essential consumer functions in aquatic ecosystems, serve as food for many other species, and are generally important components of food webs and biodiversity in freshwater systems (Britton et al., 1988). Invertebrates integrate chemical and ecological disturbance in their habitat and have a wide range of species from secondary producers to top predators, making them effective biomonitors (Basset et al., 2004; Lacerda et al., 2014). Due to their ease of sampling and varying tolerance to pollution (Elliot 1978), macroinvertebrates have been widely used as indicators of pollution and other stressors including oil releases. In the event of an oil release, aquatic macroinvertebrates can be subject to both acute (e.g. mortality) and chronic toxicological effects such as impaired growth and reproduction. Changes in invertebrate population density, diversity, and abundance can be significantly altered by oil releases, and population changes can indirectly affect the survival of organisms within the food web (IOGP, 2015; Steen et al., 1999).

1.7.1 Effects from Dilbit

To date, little is known about freshwater oil spills and their impacts on the aquatic environment. Currently, the majority of data describing effects of dilbit on freshwater aquatic organisms were collected in relation to the Kalamazoo River release or laboratory studies (Dew et al., 2016). Toxicity testing of post-Kalamazoo spill sediment samples with the midge *Chironomus dilutus* and the amphipod *Hyalella azteca* found significant increase in mortality. *C. dilutus* was the more sensitive of the two species with reduced survival resulting from a combined effect of oil residues (presumably oil particle aggregates) and sediment characteristics; whereas reduced survival of *H. azteca* was caused by effects of Total Extractable Hydrocarbons (TEH) (Fitzpatrick et al., 2012; Fitzpatrick et al., 2015). The findings from the Fitzpatrick et al., (2012) study confirms that weathered dilbit sank to the bottom impacting sediments. The chemical analysis (PAHs and total extractable hydrocarbons) of the sediment in the Kalamazoo River indicated that there could be risks for acute and chronic toxicity on benthic fauna depending on how heavily oiled the sediment has become (Fitzpatrick et al., 2015). Changes in community structure were also found post-spill and were largely due to the removal of vegetative cover during cleanup efforts and increased light penetration. It is unclear whether the shift in community structure was because of photodegradation of bitumen or simply an abiotic effect on the habitat (Dew et al., 2016).

A component of the Boreal Lake Oil Release Experiment by Additions to Limnocorrals (BOREAL) served to improve the understanding of dilbit releases in freshwater environments with a focus on weathering. The study consisted of nine limnocorrals (10-m diameter, 2-m deep, 157 m^3) within a lake with seven different concentrations of dilbit varying from 1.5L-180L and a control. Black (2019) demonstrated that over the post spill period there was a strong negative relation between emergence rates of all insects within the community and overall volume of added oil. Changes in Chironomid emergence contributed the most to the observed changes followed by Ceratapogodinae and Leptoceridae. The proportion of insects that emerged pre spill accounted for 89% and 74% of the two highest treatments while it ranges from 11-59% in the lower treatments and pre-spill emergence accounted for 5.6% of total emergence in the control (Black, 2019). Surface dilbit started to sink within the first month (Stoyanovich et al., 2019), which was also reported in a pilot study (Stoyanovich et al., 2019), similar to observations at the Kalamazoo spill. The benthic communities showed no apparent change with increasing oil volume (Black et al., 2021a).

Sheen presence on the water has also been shown to impact emergence. Surface sheen could limit emergence for organisms that pass through the air water interface, as well as impair oviposition, which can limit fecundity and reproductive success of benthic communities (Black, 2019). Black (2019) found that presence of oil concentrations greater than 0.07 L/m^2 significantly reduced total emergence by nearly half over a 90 day period. Mozley and Butler (1978) found that *Agabus* larva and adults were trapped in floating crude oil. In laboratory experiments, hatching of *Chironomus, Procladius*, and Tanytarsini were not affected by oil exposure, but pupae were trapped by oil slicks thicker than 0.5 mm (Mozley and Butler 1978).

1.7.2 Effects of Crude Oil

Crude oil releases have been shown to affect the diversity and abundance of aquatic communities (Steen et al., 1999). Some taxa, such as Chironomids, have been found to be relatively tolerant to crude oils (Arimoro et al., 2008; Poulton et al., 1997), while others such as Trichoptera and Crustacea are less tolerant (Steen et al., 1999). Effects include impaired respiration in organisms with external gills, such as Plecoptera, Ephemeroptera, and Trichoptera and abnormalities in tracheal gills (Crunkilton et al., 1990). Studies have also shown Trichoptera, Plecoptera, and snails were significantly impacted by being physically trapped in oil on plant stems and surface films (Barsdate et al., 1980). Crunkilton et al. (1990) studied a freshwater stream after an oil release and found persistent community-level effects. For example, it took 64 days for certain Dipteran species to start proliferating and 266 days for midges (Chironomidae) and blackflies (Simulidae) to return to background levels. Overall, the benthic community's recovery took between 336-523 days (Crunkilton et al., 1990). In a similar study in a freshwater marsh site, survival was only seen by day 186 in tubifex worms (Battacharyya et al., 2002). A study by Mozely et al. (1978) assessed effects on aquatic invertebrates in two ponds dosed with 0.24 L/m^2 and 10 L/m^2 of Prudhoe Bay crude oil.

They determined that sample cores and emergence traps exposed to Prudhoe Bay crude oil had similar benthic invertebrate composition by subfamily in the first year but emergence deceased drastically in the following years, with the family Tanytarsini most reduced. In the benthic samples, larval development was arrested before metamorphosis and there was no indication of recovery for species in the genera *Nemoura*, *Asynarchus*, or *Tanystasini* seven years after the spill, although overall abundance and biomass remained high (Mozley et al., 1978). These studies demonstrated varying effects of residual oil at different trophic levels as shredder species and burrowing deposit feeders were least impacted while small particulate filter feeders or sediment gatherers were greatly impacted (Crunkilton et al., 1990; Mozley et al., 1978). Oil may decrease the number of predators allowing more oil tolerant species to become dominant within the community.

Previous laboratory studies have shown that 500 µg/g of residual crude oil in sediments was toxic to mayfly species *Hexagenia bilineata* (Poulton et al., 1992). Steen et al. (1990) determined that weathered oil was significantly less toxic than fresh oils. Acute toxicity for BTEX was not detected in *Chironomus attenuatus* and *Chironomus tentans.* Both species did however demonstrate acute impacts to PAHs, specifically for anthracene having an LC50 (48hr) of 15 mg/L for *C. attenuatus* and LC50 (48hr) 2.8 mg/L *for C. tentans*. In a study using Bonny Light crude, *Chironomus* had a mean cumulative mortality (total mortality between four observation periods 12-48h) of 65% for 10 ml/L, while mosquitos had mean cumulative mortality of 90%, indicating they are more sensitive than *Chironomus* species (Arimoro et al., 2008). Similar studies have indicated that impacts may be on the species level and looking at order or family may leave some gaps in determinations of taxonomic sensitivity. Previous studies have also demonstrated lab and field studies may yield different results. Mozely et al. (1978) compared oil impacts to benthic invertebrates in laboratory studies as well as freshwater ponds. Two species in the genera *Nemoura* and *Asynarchus* were eliminated in the treatment ponds, yet in the laboratory samples observed no mortality although concentrations were six times higher than what was applied to the ponds (Mozely et al., 1978). One genus, *Tanytarsus,* was nearly eliminated from the ponds, although the laboratory tests were not sensitive other than some minor effects on emergence (Barsdate et al., 1980). These differences are likely due to trophic level impacts in the ponds that cannot be identified in a laboratory study as well natural variation of species composition between ponds (Mozely et al., 1978). The Mozely study is a demonstration of the benefit of having both laboratory as well as field studies completed to understanding oil impacts to the benthic community.

1.7.3. Effects from Cleanup Methods

While oil itself can be both acutely and chronically toxic to aquatic macroinvertebrate communities, methods used to clean up oil spills may cause further impacts. Traditional cleanup methods are based on the physical recovery of oil slicks on water surfaces such as booming and skimming. These methods are ineffective for spilled oil once it becomes submerged and develops oil particle aggregates that are transferred to the benthic environment (Fitzpatrick et al., 2015). Previous spills such as the Exxon Valdez caused mass mortality of benthic invertebrates (Peterson et al., 2003). These impacts on oil shores occurred from a combination of chemical toxicity, smothering by oil, delays in recovery, and physical displacement from the habitat (Peterson et al., 2003; Fitzpatrick et al., 2015). Agitated dredging, raking, or pressure washing likely created significant habitat displacement and disturbed settled oil particle aggregates (OPAs) affecting benthic communities. The movement of the sediment and OPAs likely caused smothering of some benthic organisms and made some of the oil components, specifically PAHs more bioavailable contributing to the lack of recovery (Fitzpatrick et al., 2015).

Use of both chemical and biological treatments in response to oil releases can also have adverse effects on aquatic invertebrate communities. Previous studies have shown that the addition of ammonium nitrate and triple super phosphate reduced residual oil toxicity to background levels within a one-week period, while sodium nitrate was less effective (Lee et al., 2001). In the same study it was determined that *Hyalella azteca* (20-100% mortality) was more sensitive to the oil than *Daphnia magna* (0-20% mortality) likely because *H. azteca* were in contact with the sediment (Lee et al., 2001). The unoiled treatments with only ammonium nitrate caused negligible impacts to *Daphnia magna* and toxicity was lower for the *H. azteca* compared to the oiled treatments (Lee et al., 2001). The species dependent responses indicate that impact assessments should be based on a multispecies trophic level approach to improve ecological relevance (Lee et al., 2001).

In both freshwater and marine benthic communities, greater impacts have been noted on intertidal benthos than subtidal benthos (Mozley et al., 1978) as oil will collect and stick to the shoreline. The effectiveness of different cleanup efforts needs to be considered for different shoreline types and their potential impacts to the environment.

The addition of dispersants or cleaners can drive oil into the water column, adhering to sediment particles and collecting in the sediment.

Corexit 9580 has been used on oil spills and has a toxicity that is relatively low in marine systems, however little research is done on its effectiveness and toxicity for freshwater spills (Bhattacharyya et al., 2003). Marine tests of this product in the late 1980's and early 1990's demonstrated no significant toxic effects between reference oiled sites and oiled sites with an addition of cleaner, indicating the cleaner did not attribute to toxicity of the oil itself (Fiocco et al., 1991). Additionally, Corexit 9580 allowed shorelines to be cleaned faster and more effectively reducing cleanup times (177.7 m^2/hr compared to 40.7 m^2/hr with regular water washing) (Fiocco et al., 1991). Environment Canada tested Corexit 9580 and 20 other shoreline cleaner products with 96hr trout exposure tests and it proved to be the least toxic, having an LC50 over 5600 mg/L (Fiocco et al., 1991).

A laboratory study by Bhattacharyya et al. (2003) assessed the toxicity of weathered South Louisiana Crude oil (SLC), and the addition of a chemical dispersant (Corexit 9500) and a shoreline cleaner (Corexit 9580) on *Daphnia pulex*, the medaka *Oryzias latpis,* and *Chironomus tentans* in laboratory microcosms. Results indicated both dispersant and cleaner treatments without oil became less toxic over time and were nontoxic by day 186 of the study. The crude oil only treatment started showing survival at day 31 and slightly increased by day 186 for *C. tentans* while toxicity for *D. pulex* and *O. latpis* was drastically decreased by day 186 (Bhattacharyya et al., 2003). While the addition of dispersant to the crude oil was more toxic than the cleaner addition to the crude oil, both increased toxicity compared to the oil treatment alone for all three test species. The most significant effects of SLC and cleaner were to *Chironomus tentans* with 0% survival during the 186 day study (Bhattacharyya et al., 2003). Due to the significant effects on the Chironomids, a secondary study was completed, using sediment assays to examine effects on benthic invertebrates (*C. tentans, H. azteca,* and *Tubifex tubifex*). Similarly, significant effects occurred for all test species; amphipods were the most sensitive while tubificid were the most tolerant. The results indicated that toxicity of crude oil was increased with the addition of chemical treatments. It should be noted that both portions of the study were based on a worst-case oil spill scenario; when the concentration of the cleaner was reduced by half the toxicity was relatively minor in the chironomids assay (Bhattacharyya et al., 2003).

The effects from combined oil and SWAs like Corexit EC9580-A have the potential to increase hydrocarbons concentrations as many cleaners are formulated with hydrocarbon-based solvents (Black et al., 2021; Fiocco et al., 1991). Klerks et al. (2004) found that the water column dwelling species *Daphnia pulex* (48h exposure), had a stronger relationship between toxicity of hydrocarbons, than that of sediment dwelling chironomid larvae (96hr exposure). Additionally, the results demonstrated that sediment dwelling organisms were affected by different PAHs as the dissolved fraction of PAHs will affect the water column species while benthic species will ingest sediments and can accumulate hydrocarbons from both interstitial water and sediments (Klerks et al., 2004). This determination emphasizes the importance of looking at effects on different trophic levels, i.e. sediment dwelling and water column species. Of the 41 PAHs that were analyzed only three contributed to chironomids toxicity, which were C4 phenanthrenes, and the C2 and C3- dibenzothiophenes.

While the chemical components of Corexit 9580A can aid in toxicity, the physical impacts of the treatment addition can also impair certain insect species. Black et al. (2021) evaluated physical impacts associated with dilbit, Cold Lake Blend (CLB) and Corexit 9580A on freshwater pleuston species specifically, adult whirligig beetles (Family Gyrinidae) and water striders (*Metrobates sp.*). This study assessed the physical impairment (reduced movement, compared to control) and immobility of water striders and beetles. Oil treatments ranged from a nominal sheen thickness of 0.0026-0.2070 µm, treatments with Corexit EC9580-A had application rate of 0.006 L/m^2; these concentrations assume a worst-case scenario. In a real life scenario, containment response would be employed to reduce spread of sheen where possible, limiting the concentration of oil reaching the shoreline and reducing the concentration of Corexit EC9580-A. As well, Corexit EC9580-A should be applied only to the impacted shoreline, limiting exposure beyond impacted shore, the Black et al (2021) study applied Corexit EC9580-A on all surface water in the test chamber. During the pilot study using just dilbit (CLB), water striders were impaired in all treatments within 15 minutes and immobilized after 96 hr. Beetles were less sensitive, with some immobility occurring initially, but with recovery by the end of the study period that was extended an additional 96 hr due to high recovery. In a second study, only water striders were tested; oil treatments (at nominal thickness as low as 7.7x10^{-3} µm) demonstrated 75% immobility within 24 hours showing physical impairment of their movement and attempts to clean themselves after exposure. Concentration-response analysis determined an effective sheen thickness causing 50% immobility after 48 hr at 2.77+/-0.91x10^{-3} µm. As for treatments with oil and Corexit EC9580-A 100% immobility occurred within 5 minutes, with no change in the 48 hours that followed, within hours water striders were found in mats of clumped oil and Corexit EC9580-A just below the surface. Water striders can play significant roles in the food web (Cooper 1984; Marczak et al., 2007; Sushchik et al., 2016); due to their dependence on surface tension, they are extremely susceptible to acute impacts from both oil spills and shoreline treatments (Black et al., 2021). A benefit of conducting field studies are that a variety of species can be observed rather than common laboratory test species that may not be reflective of community impacts. This is a good indication that the addition of oil and Corexit EC9580-A cause both chemical and physical impacts to species and both need to be considered when looking at impacts when considering possible risk to aquatic organisms.

1.8 Freshwater Oil Spill Remediation Study (FOReSt)

Studies on the fate and effects of bitumen (or dilbit) and dispersants have been largely based in marine ecosystems. Relatively few controlled studies have investigated effects of dilbit spills and remediation efforts on freshwater ecosystems, as identified in the report on *The Behaviour and Environmental Impacts of Crude Oil Released into Aqueous Environments* (Lee et al., 2015). Additional research is required to understand impacts of oil spills on aquatic macroinvertebrates and effects to populations and community structure and whether they are driven by direct or indirect effects in aquatic food webs. More research is needed to understand physiology, life history and habitat characteristics of invertebrates and their susceptibility to oil and its toxicity (Lee et al., 2015).

A controlled field research study is needed to determine effects of oil spills on a variety of sites representing different ecosystems to enhance recommendations for spill cleanup methods and monitoring. Additional studies for cleanup methods are required to determine if alternate response techniques such as biological or chemical methods are effective and can reduce impacts associated with physical destruction from mechanical equipment. Currently, in Canada chemical cleaners can only be used as a last resort if all other cleanup methods were used with diligence and the use of a surfactant will provide a net environmental benefit (Owens et al., 2010).

Bridging these research gaps will provide the oil industry along with spill response agencies more accurate information on how to clean up an oil spill in a variety of different freshwater habitats. Knowledge of impacts to aquatic invertebrates and other aquatic communities will help determine what potential impacts need to be monitored and how long recovery can take. Lastly, having a better understanding of all cleanup methods and their impacts to the environment will help the SCAT teams ensure they are choosing a cleanup method that will not cause additional environmental damage to the impacted site (Reid, 2014; API, 2016).

The FOReSt project examined the ecological impacts of crude oil as well as diluted bitumen and the effectiveness of alternative shoreline cleanup procedures in the littoral zone of a boreal lake. FOReSt overcame some of the limitations of laboratory studies that examined smaller scales of biological organization and the responses of single taxa to toxicant exposure (Preston, 2002). The use of *in situ* enclosures where multiple taxa and trophic levels can react to a toxicant exposure while interacting with the producer and consumer communities provided data regarding indirect and direct toxicant effects (Fleeger et al., 2003; Preston, 2002). This project was designed to help provide new remediation techniques to improve the Shoreline Cleanup and Assessment Techniques (SCAT) process, benefiting response teams, industry and regulatory bodies. This study focused on residual oils migrating to the shoreline after being dispersed into the environment and had two separate field seasons (2018-2019). Each field season monitored changes in the chemical composition of residual oil, and effects on the microbial, plankton, benthic invertebrate, fish, amphibian, and shoreline vegetation communities. The specific field seasons were:

Year 1 2018: Pilot Study: Four enclosures were used to examine differences in aquatic exposures of both conventional heavy crude and diluted bitumen. Oil was weathered prior to addition, and effects were assessed after 72 hours and initial cleanup efforts had taken place. No secondary cleanup efforts were used. Results for aquatic macroinvertebrates are presented in Chapter Two of this thesis

Year 2 2019: Full Scale Study: Sixteen enclosures were used to examine the effectiveness of different secondary cleanup methods for removing residual oil on 2 different shorelines, Rock Cobble and Peat Organic. The cleanup methods used were a shoreline washing agent (SWA), Corexit EC9580-A and enhanced monitored natural recovery (EMNR). This overall study determined the fate of spilled oil in freshwater shorelines with different substrates, effectiveness of alternative cleanup methods with differing substrates and the fate and chemical/physical weathering of oil after remediation. Results for aquatic macroinvertebrates are presented in Chapter Three of this thesis.

1.9 Thesis Objectives and Hypotheses

Objective 1 (Pilot Study 2018): Characterize the response of macroinvertebrate communities (emergent insects and benthos) to experimental dilbit and heavy crude oil spills under field conditions at the IISD-Experimental Lakes Area (IISD-ELA).

Hypothesis 1: Benthic invertebrate and emergent insect communities are negatively impacted by oil spills.

Prediction 1: Benthic invertebrate and emergent insect abundance and richness will decrease with the addition of weathered crude oil and dilbit. Effects from both product types should be similar as weathered dilbit has similar chemical properties to heavy crude.

Objective 2 (Shoreline Cleanup Study 2019): Characterize the response of macroinvertebrate communities (emergent insects and benthos) following experimental dilbit spills and the application of two secondary treatment methods (the surface washing agent [SWA] Corexit EC9580-A and enhanced monitored natural recovery {EMNR]) at the IISD-ELA on two shoreline types (Peat Organic and Rock Cobble).

Hypothesis 2: Benthic invertebrate and emergent insect communities will be impacted by secondary treatment methods in both shoreline types as compared with enclosures treated with oil, but without secondary treatment.

Prediction 2: Benthic invertebrate and emergent insect abundance will decrease with the addition of weathered dilbit and secondary treatment SWA (Corexit EC9580-A) in both shoreline types and increase with the addition of ENMR treatment in both shoreline types.

Statistical hypothesis:

- H_{o1}: abundance of organisms in control enclosures will be equal to abundance in dilbit and SWA enclosures
- H_{a1}: abundance of organisms in control enclosures is < abundance in dilbit and SWA enclosures
- H_{o2}: abundance of organisms in control enclosures will be equal to abundance in dilbit and EMNR enclosures
- H_{a2}: abundance of organisms in control enclosures is > abundance in dilbit and EMNR enclosures

Chapter 2: Simulated spill and recovery of conventional heavy crude and diluted bitumen on a boreal lake shoreline and the effects on benthic invertebrate communities: Data from the pilot-scale study of The Freshwater Oil Spill Remediation Study (FOReSt)

2.1 Abstract

A pilot-scale study at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) was undertaken to examine the fate, behaviour, and effects of diluted bitumen (dilbit) and conventional heavy crude (CHV) oil in boreal lake shoreline environments. In 2018, controlled spills in littoral enclosures (n=1 per treatment, n=2 for controls) were performed (approximately 1.25L of weathered oil product per 20,000L water), followed by recovery after 72 hours using mechanical cleanup methods and natural attenuation of residual oil constituents. The aquatic insect and benthic invertebrate communities were monitored after the experimental spills using insect emergence traps deployed for 83 days and standard kick sampling at the end of the exposure period. In both the dilbit and conventional spill scenarios, insect emergence was significantly reduced over the duration of the study (>50% reduction in total abundance relative to control enclosures). A similar trend was observed in the macrobenthos community, with >50% reduction in abundance and lower overall taxonomic richness relative to control enclosures. Our results indicate that even following mechanical oil product recovery efforts, impacts on emergent insect and benthic invertebrate communities are possible. These data were used to inform the design of a full-scale study performed in 2019 to compare the efficacy of oil removal using nutrient additions and a shoreline cleaner relative to natural attenuation.

2.2 Introduction

Unconventional oils extracted via mining and *in situ* methods accounted for 63% of Canada's oil production in 2019 (NRCan, 2017). Unconventional crude oil (also known as bitumen) is highly viscous and needs to be heated or diluted to be transported effectively (Crosby et al., 2013). Bitumen is upgraded to diluted bitumen (referred to commonly as "dilbit") by adding gas condensates to decrease the viscosity and facilitate transport through pipelines. Dilbit blends are typically 70:30 oil to condensate but can vary during different seasons to ensure flow as temperatures fluctuate (Dupuis et al., 2015). Dilbit is diluted with gas condensates that contain a mixture of naphthenic acids, polycyclic aromatic hydrocarbons (PAHs), and metals (Robidoux et al., 2018). Approximately 4.2 million barrels per day of crude oil and its constituents, including dilbit, are transported throughout Canada either by truck, rail, or pipeline (CER, 2018). The most efficient way to move product to markets is via pipelines. This long-distance and continuous transport increases the risk of an oil release into a freshwater environment. This is because many pipeline routes cross waterbodies and watersheds and can therefore enter surface water either directly from an overland release or via groundwater transfer (Robidoux et al., 2018; Alsaadi et al., 2017).

Releases into the environment of both conventional heavy crude (CHV) oil and dilution blends can be detrimental to aquatic ecosystems. Once a dilbit product is released into the environment, the light end hydrocarbons and the BTEX fraction evaporate quickly leaving the heavy ends behind resulting in similar properties to that of the parent crude oil (Robidoux et al., 2018; Stoyanovich et al., 2019). Increases in oil density following evaporation may cause dilbit to sink and bind to suspended solids in the water column (Environment Canada, 2013; Dew et al., 2016), potentially increasing impacts to taxa inhabiting the water column and sediments. Additionally, greater physical impacts such as oil smothering and physical displacement from cleanup activities (Peterson et al., 2003; Fitzpatrick et al., 2015) have been seen on shorelines following spill events as this is where oiling is greatest (Mozley et al., 1978).

Research on impacts of oil releases to aquatic ecosystems has largely focused on marine habitats and significant gaps exist in our understanding of impacts to freshwater ecosystems (Vinson et al., 2008; Steen et al., 1999; Lee et al., 2015). As well, dilbit has been relatively understudied as compared to conventional crudes, regardless of ecosystem type (Lee et al., 2015). Few toxicological studies have been completed for dilbit, and these have largely focused on fish (Barron et al., 2018). Most studies are laboratory-based, which do not necessarily reflect the responses of an entire ecosystem or the complex changes oil can undergo in the field (Mozely et al., 1978; Barsdate et al., 1980). Laboratory studies are limited to observing effects of select test species and cannot determine trophic level effects or take into account environmental factors (weather, evaporation, etc.) that play a role in oil degradation. Studies completed following releases such as the Enbridge pipeline release into the Kalamazoo River typically lack background (pre-spill) data and effects at these locations will also have physical environmental impacts to consider (Lee et al., 2015).

Releases of crude oil have been shown to cause adverse effects on diversity and abundance of macroinvertebrate communities within freshwater environments (Steen et al., 1999). Community-level effects can persist for years – for example, Crunkilton et al. (1990) looked at a domestic crude oil spill in Asher Creek, Missouri, USA and found that it took over a year for the benthic community to recover. Varying effects were observed at different trophic levels; for example Chironomidae and shredder species were least impacted by residual oil (recovery started after Day 38) while small particulate filter feeders or sediment gatherers were greatly affected (recovery after Day 176-266) (Crunkilton et al., 1990). Predators initially decreased but then increased as the more oil tolerant species began to recover (Crunkilton et al., 1990; Lacerda et al., 2014). Some insect groups like chironomids are relatively tolerant while Ephemeroptera, Plecoptera, and Trichoptera (EPT) are more sensitive (Arimoro et al., 2008; Poulton et al., 1997; Steen et al., 1999). Surface sheen of oil may also affect emergence; chironomid pupae were caught in oil slicks greater than 0.5 mm (Mozely and Butler, 1978). The physical effects of oiling such as sheening and surface cover can obstruct emergence and oviposition and had greater effects than chemical toxicity as the Polycyclic Aromatic Compounds (PAC) values were below known toxic levels (Black et al., 2021).

Laboratory tests are not always reflective of natural ecosystems and results can vary considerably. Laboratory tests by Mozely et al. (1978) using 12- 21 day mortality tests for *Chironomus*, *Procladius*, *Tanytarsini*, *Asynarchus*, and *Nemoura* showed no mortality at oil concentrations six-fold greater than in pond experiments where the same species were eliminated. Further, laboratory tests cannot test all species, leaving gaps in determinations of species sensitivity for any particular ecosystem (Barsdate et al., 1980).

The aim of spill cleanup is to minimize long-term impacts of an oil release to the exposed environment (IPECA, 2015). While an oil release itself can cause significant damage to a freshwater ecosystem, the spill response techniques can also cause further unintended ecological damage (API, 2014; EPA, 1999). This can lead to additional habitat impairment, reducing overall species abundance and productivity, and may allow for the introduction of invasive species, further impairing ecosystem integrity (Reid, 2014; IPECA, 2014). Shoreline environments can suffer large economic losses (commercial use [e.g. fishing], recreational use, property value decline, etc.) and environmental damages from oil releases. Rapid oil cleaning of shorelines is difficult and mechanical removal often causes further damage to the environment (Bizzel et al., 1999). To determine which cleanup method will be least impactful to the environment the type of oil must be identified, along with consideration of the location of release. Different water bodies and shoreline types will require different cleanup methods (API, 2016). To date, there is little information on the effectiveness or impact of oil spills and different cleanup approaches for freshwater, particularly boreal lake ecosystems. Boreal regions contain more than 60% of the world's surface freshwater (Schindler and Lee, 2010).

Directed research is needed to determine how heavy crude oil and diluted bitumen may impair freshwater ecosystems and determine the most effective and clean up options. The objective of this study was to characterize the effects of both crude oil and dilbit spills in a boreal lake and their effects on abundance and diversity in the littoral aquatic macroinvertebrate community. This research is the pilot study for the larger scale Freshwater Oil Spill Remediation Study (FOReSt) at the IISD-ELA, which was undertaken in 2019 and discussed in Chapter 3. In summer 2018, an experimental spill in an open environment was executed. Two shoreline enclosures were used to model the impacts of weathered Conventional Heavy Crude (CHV) and Diluted Bitumen (dilbit) and two control enclosures. Following oil addition, mechanical cleanup methods were implemented and impacts to the macroinvertebrate community were observed over the 83 day study. Data from this study will help regulators develop guidance on invertebrate sampling and appropriate end points for spill response and cleanup efforts as well as inform the full-scale FOReSt study in 2019 (Chapter 3 of this thesis). We hypothesize that benthic invertebrate and emergent insect communities will be negatively impacted by oil spills. We predict that benthic invertebrate and emergent insect abundance and richness will decrease with the addition of weathered crude oil and dilbit. Effects from both product types should be similar as weathered dilbit has similar chemical properties to heavy crude.

2.3 Methods

2.3.1 Study Location

This 83-day study simulated a freshwater oil spill and mechanical cleanup using large shoreline enclosures to determine, in part, the impacts to the aquatic macroinvertebrate community. The study was conducted in Lake 260 (49°41'56.4"N, 93°46'01.2"W), an oligotrophic, low energy (minimal wave/current action) boreal lake at the International Institute of Sustainable Development-Experimental Lakes Area (IISD-ELA) facility in Ontario, Canada. Lake 260 has a surface area of 32.8 ha, volume of 1,975,971 m^3 and a maximum depth of 15.7 m. The lake is on non-porous metamorphic rock, therefore groundwater inputs are negligible and there was only one outflow to the north of the lake and one inflow from Lake 112 which is a small marsh-like lake (Perry, 2021). The sediments in the enclosures were characterized as silty-sandy with small pebbles scattered throughout with minimal organic material on/within the sediments (Black, 2019). An additional benefit of using this lake is it has historical data available on biota and water quality.

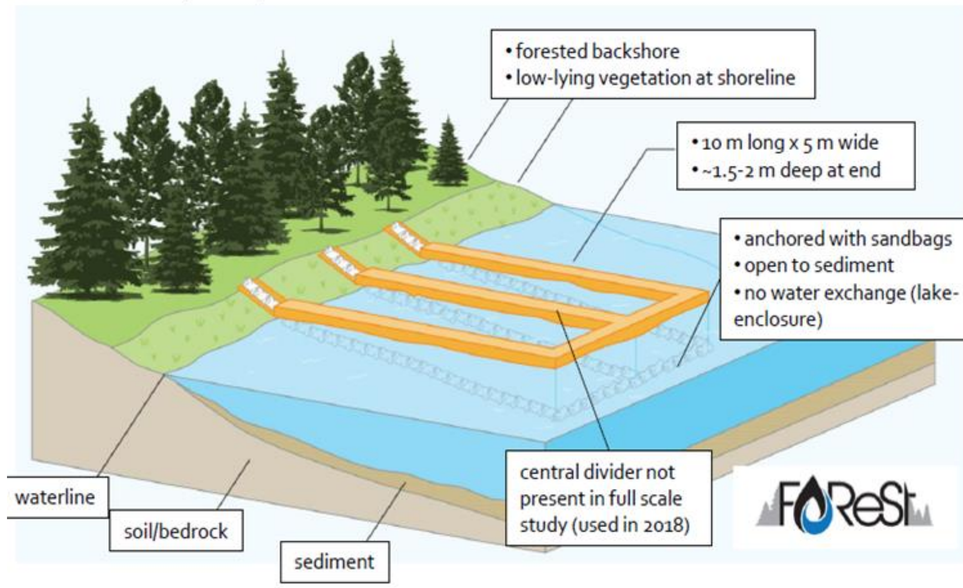

Figure 2.1: Schematic of a shoreline enclosure (taken from Palace et al., 2021).

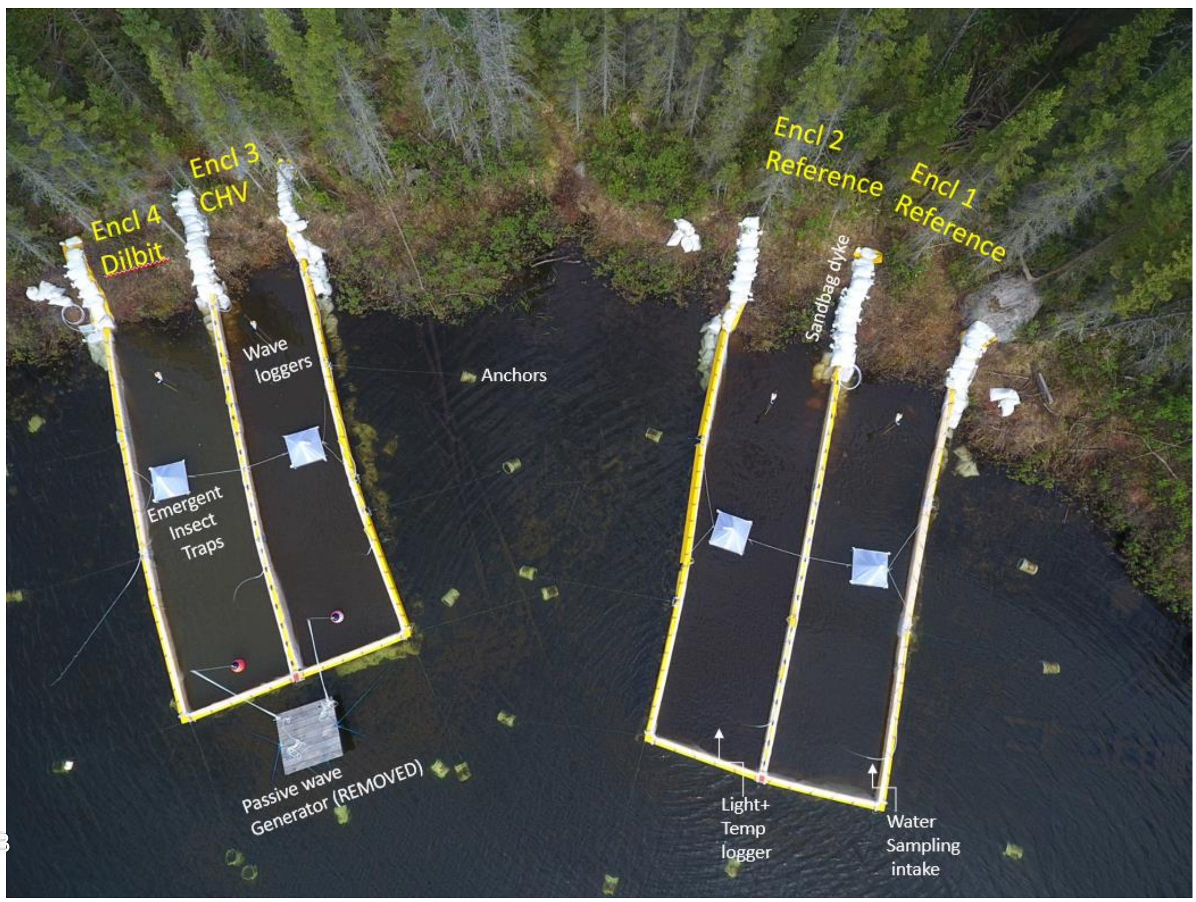

Figure 2.2: Drone image of the enclosures in Lake 260 at the IISD-ELA (photo taken from Palace et al., 2021).

2.3.2 Enclosure Design and Treatment Application

The shoreline enclosures were installed on the northeastern side of the lake. They were open to the atmosphere and sediments and contained natural and complex food webs reflective of a boreal lake ecosystem. Two shoreline enclosures were deployed, with a divider in each creating four separate enclosures (15 m x 2.5 m x 1.5 m) in the littoral zone of Lake 260, with the total volume of each enclosure being approximately 18.75 m^3 (Figure 2.1 and Appendix Figure B.1). The enclosure locations were not randomly selected for this study; since there was a divider, the controls were together and the treatments were together to minimize impacts of product leaking into controls. The enclosures were designed with a floating collar attached to polypropylene curtains (made by Curry Industries in Winnipeg, MB). The curtains were sealed with sandbags to prevent water, aquatic fauna and treatments from leaving or entering the enclosure. The floating collars extended onshore (5 m of the 15 m length) to act as a barrier for potential wave action (Perry, 2021) (Figure 2.2). The enclosures were anchored using buckets of cement attached at each bracket to keep the enclosure in place. Within the enclosures, wave loggers were added, along with light and temperature loggers and the water sampling intake for the water samples (Figure 2.2). Prior to oil addition, fish were removed from the enclosures using cage traps and nets to ensure each was similar regarding piscivorous predatory influence on consumer communities. A total of ten free-swimming (not caged within enclosure) test fish *(Pimephales promelas)* were added to each enclosure prior to the oil addition and were removed at the end of the study.

Of the four enclosures, one was treated with dilbit using a Cold Lake Blend (CLB), one with conventional heavy crude (CHV), and two were control enclosures (no oil added). The enclosures were installed and allowed to acclimate for two weeks. Prior to application, both oil products were naturally weathered by introducing 7 kg of CHV or dilbit over 25 cm depth of water sourced from Lake 260 in stainless steel evaporation pans and exposed to air and sunlight for 36 hours to simulate the residuals left over from a spill. From this, 1.25 L of weathered product was added to both treatment enclosures. The enclosures were dosed with the dilbit and CHV on June 22, 2018. The oil was applied to each enclosure within 50 cm of the shoreline with a target oil thickness of 0.1 cm on the vegetation (targeted 1 mm-thick coating for entire 2.5 m of linear shoreline). Wave and wind action was simulated by a leaf blower and moving the enclosure collar up and down (Perry, 2021). Mechanical cleanup occurred on June 25, 2018, 72 hours after dosing. The oil was recovered by flushing the oiled region of shoreline and vegetation with water from inside the enclosures using low pressure streams at 10cm intervals including the control enclosures (Perry, 2021). Oil products were recovered from the water surface using pre-weighted polypropylene sorbent pads (Spill Ninja Oil Only Pads, MEP Brothers, Winnipeg, MB) to mimic that of common recovery efforts in freshwater. Shoreline vegetation and enclosure curtains and collar were not scrubbed to remove oil. Rather, the oil deposited on the curtains was quantified using digital scaling images of the curtains to determine total mass (Palace et al., 2021).

2.3.3 General Water, Oil Chemistry, and Sediment Sampling

Surface water samples were collected from each enclosure at ten different time points: Day -3 (prior to oiling) then on Days 1, 2, 3, 5, 9, 13, 18, 53, and 80 after application (Palace et al., 2021). Water samples were collected using a Spectra Field Pro II professional grade peristaltic pump with food grade PVC tubing (3/16' x 5/16' 70 F). Water was pumped through tubing for 10-15 seconds, to clear any residual water prior to a triplicate rinse of each sample bottle with enclosure water (Perry, 2021). Water samples were collected from the midpoint of each enclosure at ~10-15cm depth (V. Palace personal communication). Dissolved inorganic carbon (DIC) and pH/conductivity samples were collected using a 125 mL HDPE ID2 Nalgene bottle while the remainder of water chemistry samples were collected using 500 mL HDPE ID2 Nalgene bottles. Total petroleum hydrocarbons (TPH), saturates, aromatics, including polycyclic aromatic hydrocarbons (PAH and alkylated PAHs) were collected in 1 L water samples and analyzed using gas chromatography mass spectrometry (GC/MS). Temperature, pH, dissolved oxygen, and conductivity, were determined at the same time points using a handheld YSI probe (YSI Inc., Yellow Springs OH) (Palace et al., 2021). Nutrients (NH_3, NO_3, NO_2, total dissolved nitrogen (TDN), total dissolved phosphorous TDP), suspended P, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and suspended C/N) and suspended Fe, chlorophyll *a*, major anions and cations, alkalinity, soluble reactive silica, and turbidity were determined biweekly over the duration of the study (Palace et al., 2021). Analyses were completed at the IISD-ELA chemistry lab under the direction of Dr. Sonya Havens-Higgins following the protocols of Stainton et al., (1977).

Sediment samples were collected and analyzed for concentrations of PACs and alkylated PACs following the procedure outlined in Idowu et al., (2018).

2.3.4 Macroinvertebrate Sampling

The emergent insect and macroinvertebrate community was assessed via: (a) floating emergence traps throughout the 83 day study; and (b) by kicknet sampling at the end of the study, respectively. Both methods evaluated abundance and taxonomic richness of emergent insects and benthic invertebrates. The two methods were also used to obtain a better understanding of the impacts on two life stages (larval and adult) and if oil impacts primarily result from the surface sheen or from oil decomposition in the water column and sediments.

2.3.4.1 Insect Emergence

Emergence traps are passive samplers that intercept aquatic insects as they emerge across the water-air interface and can provide continuous monitoring of emerging insects from fixed locations and are typically used to monitor timing and abundance of emergence (Malison et al., 2010). Floating emergence traps were used to minimize agitating residual oil or mixing of sediment, as water column or submerged traps would have been impacted by the oil layer, as well as potentially re-suspending oil following collections. To further reduce disturbance to the experimental treatment and enclosure sediment, samples from the emergence traps were collected from outside the enclosure by pulling the trap to the side using the attached rope (Cadmus et al., 2016).

Previous studies have shown success in studies using a pyramid style surface-floating trap (Davies et al., 1984; Malison et al., 2010; Tweedy et al., 2013; Cadmus et al., 2016), including enclosures at the IISD-ELA (Black, 2019). The floating portion or base of the emergence traps was constructed with a PCV pipe (Whiles et al., 2001; Cadmus et al., 2016). The top portion was made with a PVC frame and a fine mesh cover (250 µm). Many insect species tend to exhibit positive phototaxis behavior and studies have shown using opaque covers in emergence traps can reduce the number of insects up to 80% (Davies, 1984). The final design is as described by Black et al., (2021) and had an 80 cm^2 surface area. Each emergent trap was built with a 2” DR17 HDPE Frame, No-see-um polyester fabric, white – 1.5 m^2 sheet (non-opaque), 250-mL HDPE Nalgene x 2 with modified lids, PVC Schedule 40 Frame with T-joints and 90˚ joint, and 1-1/4” plastic clips (Figure 2.3). No additional floats or foam were used on the frame to prevent additional oil adhesion. There was a collection bottle, which was easily attached and detached from the trap, and propylene glycol was used as a preservative. Traps were secured to brackets by rope along the side of each enclosure (Figure 2.4) to aid in sample collection (Cadmus et al., 2016). Because traps floated on the surface and were anchored, they collected insects repeatedly at the same location in each enclosure over a depth of ~1 m as recommended by Malison et al. (2010) (Appendix Figure B.2 and B.3).

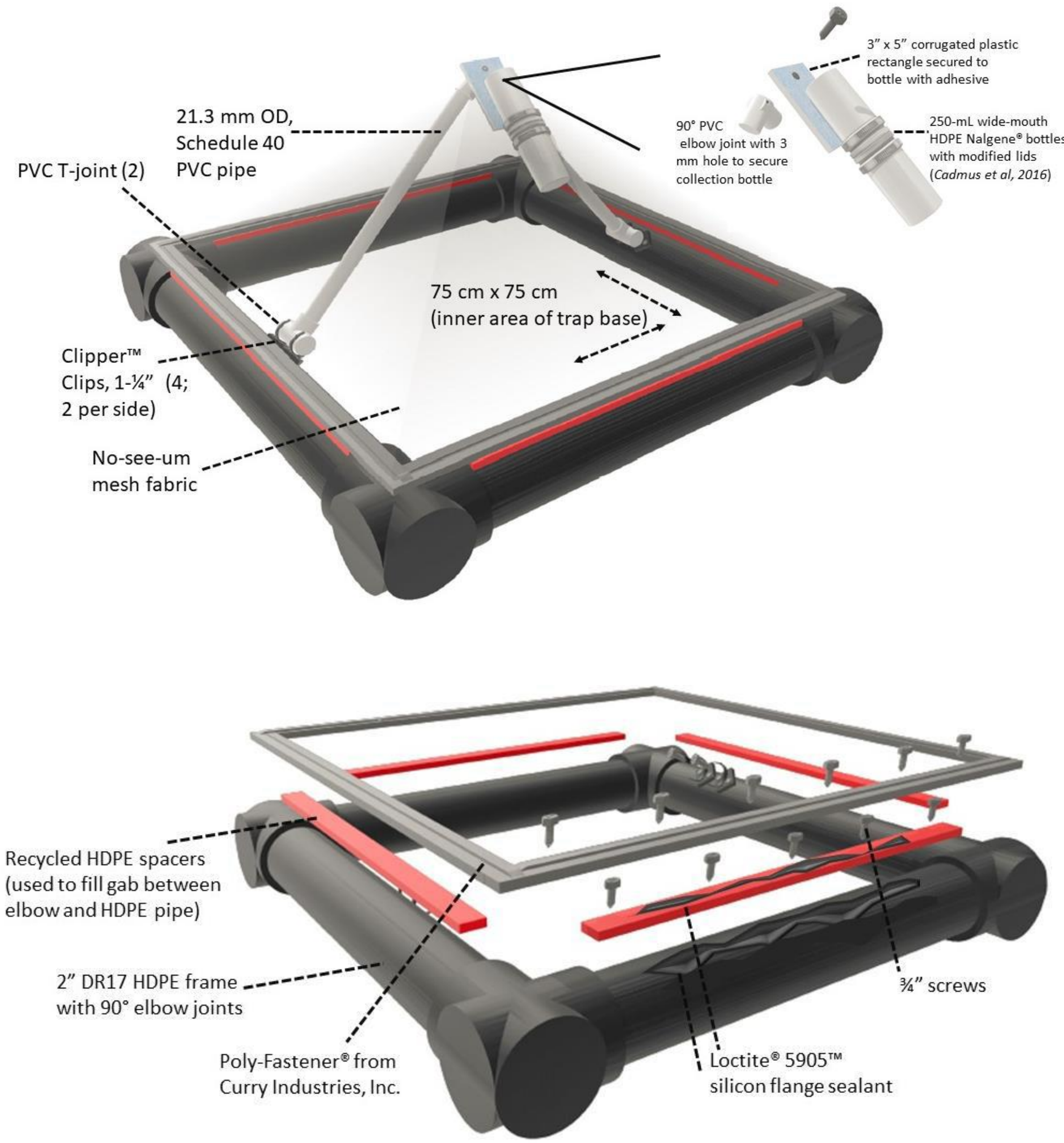

Figure 2.3: Diagrams of emergence trap used in passive sampling of the emerging insect communities within FOReSt enclosures (image taken from Black, 2020).

Lake 260 has at least six different insect orders where most species are from the Diptera and Trichoptera orders based on historical collections (Kidd et al., 2014 and Black, 2019). Collections of total abundance will likely be greater by placing the trap in the littoral zone of the lake, therefore traps should be placed where greatest emergence appear (Malison et al., 2010). Lake 260 is an oligotrophic lake and emergence data from these systems have demonstrated gradual declines in emergence with depth compared to that of eutrophic lakes where emergence tends to be greatest near the shoreline (Davies, 1984). In previous studies at ELA dipteran emergence at different depths was found to be highly variable, though samples from the same locations were nearly constant (Davies, 1980).

Each enclosure contained one trap deployed on June 3rd. 2018 (Day -19). Only one trap was added to each enclosure in efforts to limit agitation of oil. The traps were set out in the middle of the enclosure to avoid the sides and placed at an average depth of approximately 1 m to maintain consistency with emergent counts (Davies, 1980). Traps were inspected weekly for holes and to ensure sample bottle and preservative were intact, that predators were not present (i.e., spiders and dragonflies), and that there was no larval colonization (Davies 1984); none of which were identified during inspections. In western Ontario, where IISD-ELA and Lake 260 are located, the emergence of aquatic insects typically occurs from early May to mid-September (Pinkney et al., 2000; Rosenberg et al., 2001). Samples were taken pre- and post-treatment, at regular intervals, (Days -1, 5, 12, 19, 26, 33, 40, 47, 54, 61, 68, 76, and 82). Samples were analyzed with a dissection microscope and specimens were identified to Order and Family using standard keys (Marshall, 2017; Merritt et al., 1978).

All insects collected were IDed, only aquatic macroinvertebrates were IDed to family level by H. Kajpust.

Figure 2.4: Image of enclosure set up with emergence trap in place on June 3, 2018 (photo taken by Holly Kajpust).

2.3.4.2 Benthic Sampling

Kick net samples within each of the four enclosures and a lake reference station (i.e. outside of the enclosures) were taken September 4^{th}, 2018 (Day 74 of the study), following CABIN (Canadian Aquatic Biomonitoring Network) sampling protocols (Environment Canada, 2012). The kick-and-sweep path was defined before entering the enclosure. The benthic invertebrate sampling procedure involved a travelling kick sample collected using a standard CABIN kicknet (triangular aperture with each side measuring 36 cm in length, 400 µm mesh and collection cup). The samples were collected following a 'zig-zag' pattern across the enclosure, with start position at a depth of one meter. Each sample was collected by moving backward across the enclosure while working the net up and down through the water column and progressively moving shallower with each cross of the enclosure until the shoreline was reached. As per CABIN protocol (Environment Canada 2019), sampling effort was standardized by time, where each enclosure was sampled for two minutes. Benthos were dislodged from the substratum by gently tapping the bottom of the kicknet on the substrate, as well as gently swiping feet along the substrate (Environment Canada 2019). Material collected within the kicknet was immediately transferred into labeled, 1 L wide-mouth plastic jars and preserved with 10% buffered formalin.

In the laboratory, samples were washed through a 500-µm sieve and the retained material was sorted to remove benthic invertebrates for enumeration and identification. Subsampling was required due to the large quantity of debris collected. As per CABIN protocol (Environment Canada, 2014), each sample was split into 100 equal cells using a Marchant box (Marchant, 1989). To avoid confounding taxon richness calculations, the number of cells subsampled was standardized so that search effort remained consistent among samples, thus, 10% of each sample was sorted (10 of 100 cells). Organisms were identified to the lowest practical level (LPL) (genus or family) using the following taxonomic keys: Chironomidae (Anderson et al., 2013; Merritt et al., 2008); Oligochaeta (Brinkhurst 1986; Peckarski et al., 1990); all other insects (Merritt et al., 2008; Peckarski et al., 1990; Wiggins 2014), Mollusca (Clarke 1981; Peckarski et al., 1990), other Arthropoda (Peckarski et al., 1990; Pennak 1989). Chironomidae, Ceratopogonidae, and Oligochaeta were mounted on slides for identification using a compound microscope with 60-1500x magnification. A dissecting microscope with 10-80x magnification was used to identify all other organisms. The taxonomist who identified the samples was Dr. Michael White.

2.3.5 Statistical Analysis

For the benthic and emergence samples, total abundance, and total taxa richness were determined. All data were compiled in Microsoft Excel, and statistical analyses were conducted using R version 3.5.3 and R Studio version 1.1.456 with *vegan* version 2.5-5 and *ggplot2* for data visualization. Richness was calculated according to the richness function in Microsoft Excel. Traditional statistics were not applicable in this study as there was no replication.

2.4 Results

2.4.1 Water Chemistry and Oil Composition

After the initial cleanup using sorbent pads, total oil recovered (including material deposited on the curtains) was 31.6% (0.855 L) in the CHV enclosure and 30.5% (0.869

L) in the dilbit enclosure. Cleanup of the shoreline oil reduced concentrations in both enclosures from 0.05 L/m^2 to 0.03 L/m^2 (Palace et al., 2021).

The addition of oil had no observed effects on nutrients (C, N, and P) or their ratios in the enclosures (Palace et al., 2021). Lower alkalinity and DIC and calcium concentrations, coupled with peaks of chlorophyll *a* in mid-summer, suggest primary productivity may have been enhanced in the treatment enclosures (Palace et al., 2021). The temperature and oxygen saturation were similar in all enclosures, while pH was observed to be consistently lower in the treatment enclosures (~6.6) compared to the control enclosures (~6.8) (Palace et al., 2021).

Concentrations of Total Polycyclic Aromatic Compounds (PAC) were elevated above control levels in both crude and dilbit enclosures (Palace et al., 2021). The highest concentration of PACs observed in this study was measured at 0.5 µg/L in the CHV enclosure, while for dilbit it was ~0.03 µg/L., one day after the initial exposure. The 3-ringed PACs (C1-C3 phenanthrenes and dibenzothiophenes) contributed most to elevated water concentrations, at ~0.47 µg/L CHV and ~0.028 µg/L dilbit one day after treatment (Palace et al., 2021). The C4 ring PACs were also found to have elevated concentrations in both treated enclosures (~0.026 µg/L CHV and ~0.005 µg/L dilbit one day after treatment) (Palace et al., 2021). The CHV treated enclosures had higher concentrations of 3-, 4-, and 4^{+}-ringed PACs than the dilbit enclosure at most times during the exposure period. Concentrations of 2-ringed PACs remained elevated In CHV enclosure and 2- and 4-ringed PACs were elevated in both enclosures (Palace et al., 2021). At the end of the 80-day sampling period, the 3- and 4-ringed PACs in the treatment enclosures were reduced to concentrations similar to the controls (Palace et al., 2021). A light sheen was observed during initial oiling and prior to cleanup efforts, afterwards there was minimal sheening around the shoreline and around the emergence traps. Alkylated PAC concentrations in the sediment were increased in both treatment enclosures relative to the control enclosures and continued to increase throughout the study as the oil sunk. This increase was noticeably greater for CHV than dilbit (Table 2.1).

Table 2.1: Concentrations of Polycyclic Aromatic Compounds (PACs) in sediment of enclosures, data from Palace et al. (2021).

Enclosures	*Control 1*	*Control 2*	*CHV*	*Dilbit*
2-ringed alkylated PACs (ng/g)				
Pre-oil	6.3	13.2	16.1	22.5
1 month post-oil	16.7	6.3	298.0	33.1
2 months post-oil	4.1	3.8	686.4	57.4
3-ringed alkylated PACs (ng/g)				
Pre-oil	58.4	93.0	42.2	75.6
1 month post-oil	30.3	16.4	847.6	147.3
2 months post-oil	85.9	40.5	3459.7	519.9
4-ringed alkylated PACs (ng/g)				
Pre-oil	2.8	5.3	5.0	19.5
1 month post-oil	10.3	3.2	186.4	25.2
2 months post-oil	11.1	1.5	1458.5	30.7

2.4.2 Insect Emergence Response

A total of 52 emergent insect samples were collected over the 83-day (13 time points) study from the four enclosures and a total of 15 families were identified. The taxonomic group *Diptera*, specifically the Family *Chironomidae,* were the most abundant (~93% for control enclosures, ~94% for treatments) followed by *Ceratopogonidae* (~5% for control enclosures and ~3.5% in treatments). Very few Trichoptera (~0.75% for control enclosures, ~1% for treatments) were present from the families Leptoceridae and Hydroptilidae. Few Ephemeroptera and Odonata in the control enclosures (0.07%) and one Hymenoptera in the dilbit enclosure were also observed.

Total abundance of all emerging insects was supressed by 68% in the dilbit and 73% in the crude oil treatments compared to the control enclosures (Figure 2.5). Family richness evaluated from emergence were 20% lower in dilbit and 40% lower in CHV relative to the control enclosures (Figure 2.6). Due to the dominance of *Chironomidae,* no further analysis was conducted as the remaining families were too few to distinguish treatment-related family specific impacts

2.4.3 Benthic Macroinvertebrate Response

Twenty-eight taxa were identified in kick-net samples collected at the end of the study period. Organisms in the taxonomic group *Diptera*, specifically the family *Chironomidae,* were the most abundant (~70% of control enclosures, ~43% of dilbit, and ~77% of CHV). Total abundance of benthic invertebrates was supressed by 60% in the dilbit and 80% crude oil treatments compared to the average of the control enclosures (Figure 2.7). The taxa that were most impacted from both dilbit and CHV were from the *Chironomidae* family, *Cladotanytarsus sp.,* that averaged ~38% of total abundance in the control enclosures and decrease to 15% in CHV and 10% in dilbit treatments. The taxa *Tanytarsus sp*. had a slight decrease in the dilbit (~5% total abundance) compared to the control enclosures (average ~10% total abundance). The taxa *Hyalella sp.* was present in all enclosures except the CHV enclosure. The only taxa that increased compared to the control enclosures was *Pisidium sp*. In the dilbit enclosure *Pisidium sp*. accounted for ~24% of the total abundance compared to ~4% in the control enclosures. Similar to the emergent community, the remaining taxa were too low to distinguish treatment related effects. Taxa richness of benthic invertebrates was reduced relative to control enclosures by 23% and 46% in dilbit and CHV treatments, respectively (Figure 2.7).

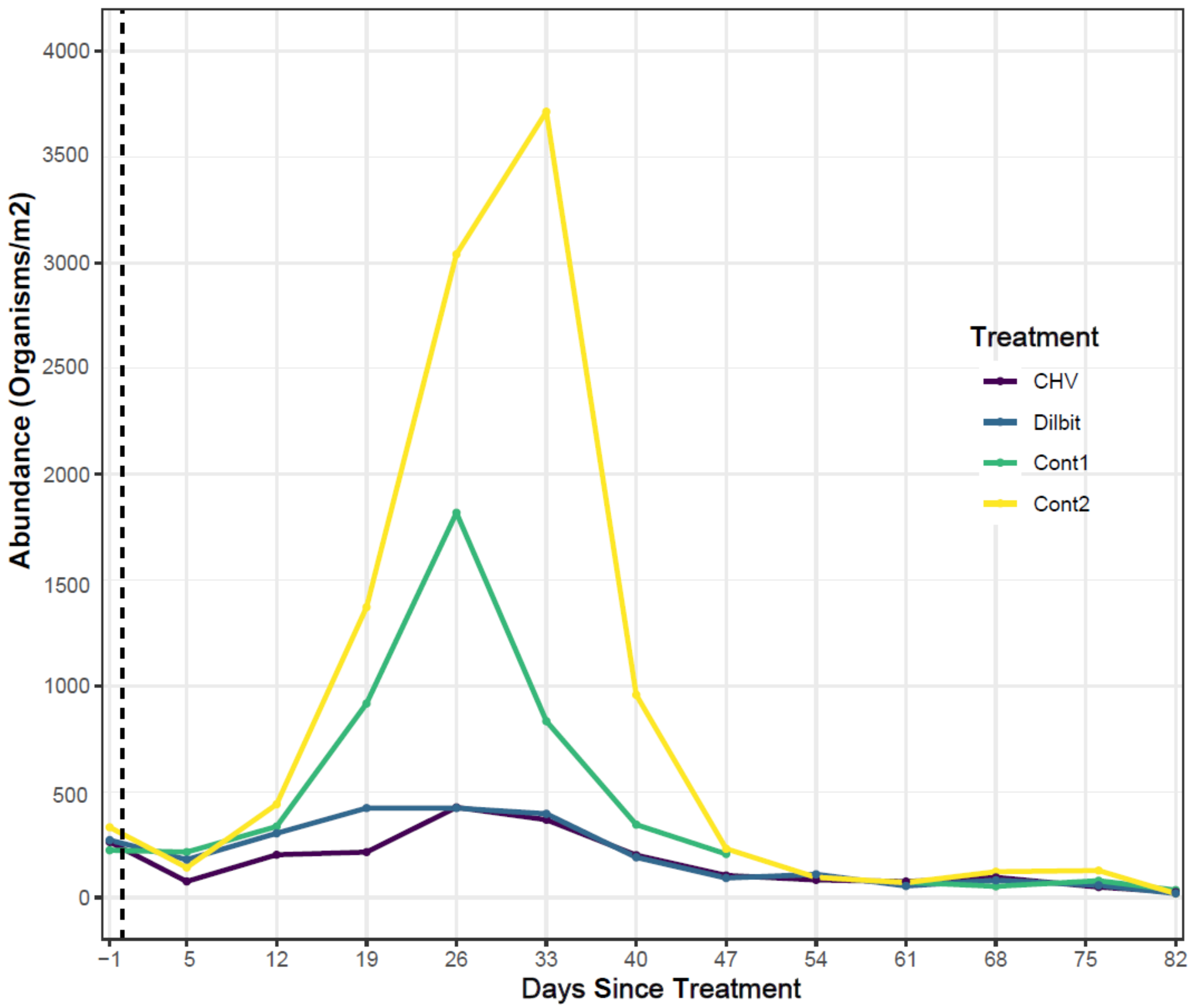

Figure 2.5: Emergence of all insects (organisms/m^2) (one trap per enclosure) in enclosures treated with dilbit or heavy crude (CHV) relative to the control enclosures over the course of the 83-d study (June 21 to Sept 12, 2018). Dashed line indicates the day of oil introduction (Day 0).

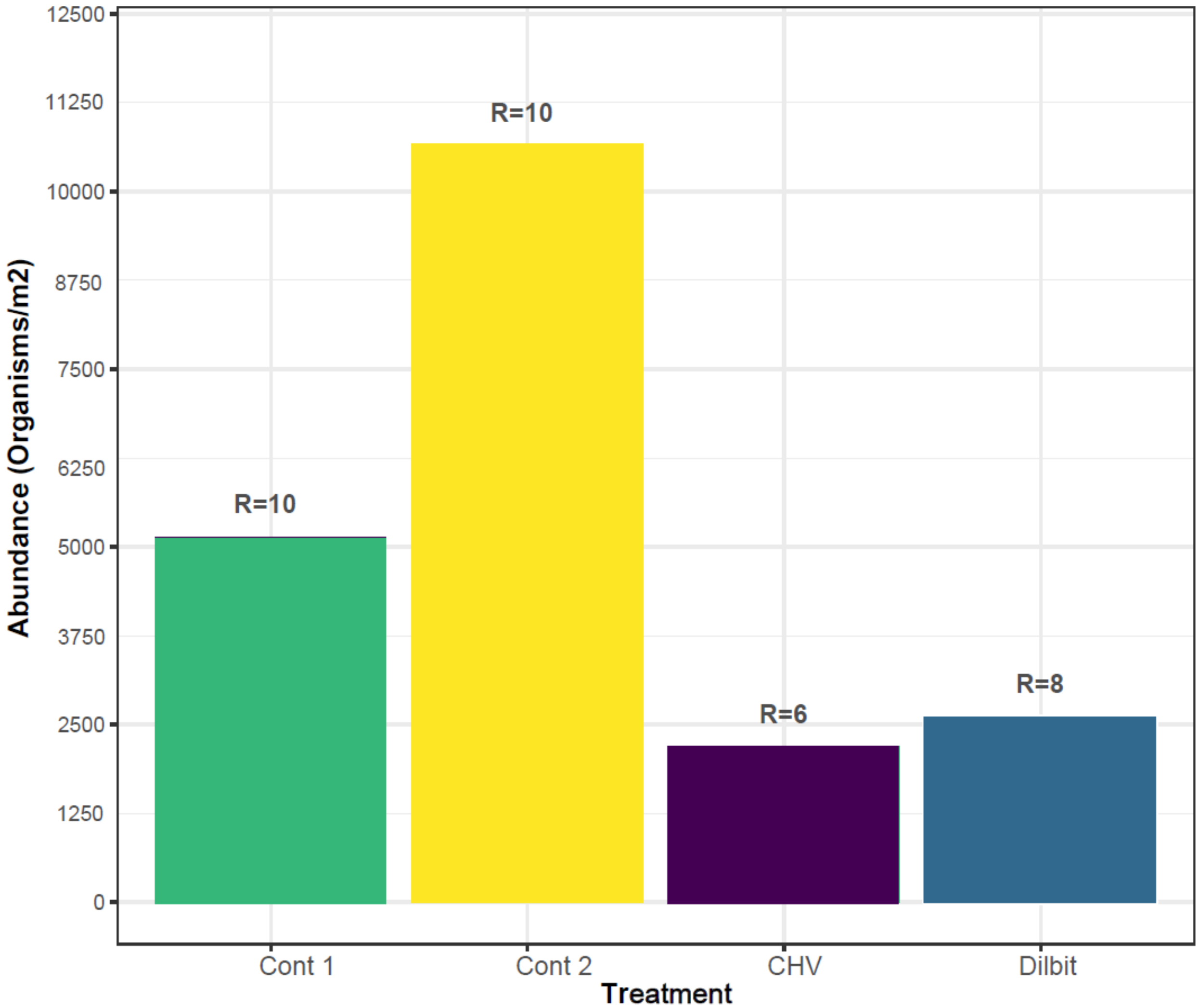

Figure 2.6: Total emergent insect abundance (organisms/m^2) in enclosures treated with dilbit or heavy crude (CHV) relative to the control enclosures. Total family richness (R) is reported above each treatment column.

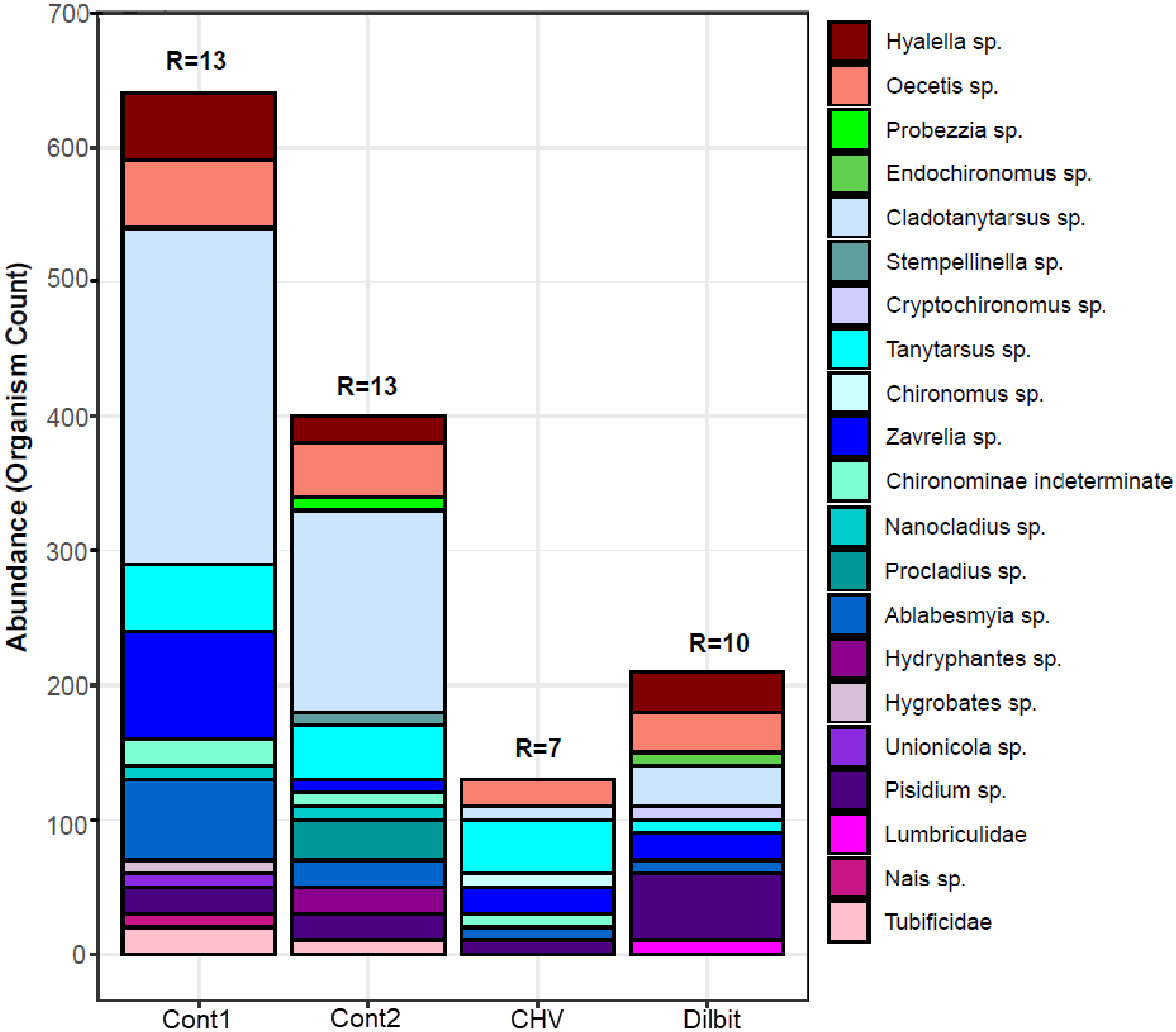

Figure 2.7: Total and individual taxa abundance (individual organisms) of benthic invertebrates in each enclosure at the end of the sampling season (Sept 4, 2018) via kick net sampling. Taxa richness (R) is reported above each treatment column.

2.5 Discussion

This study examined the effects of CHV and dilbit on aquatic emerging insects and benthic invertebrates. Overall, insect emergence appeared to be strongly suppressed in both treatments, with >50% reductions in total abundance and lower taxa richness relative to control enclosures. A similar trend was observed in the benthic survey, with >50% reduction in abundance and lower overall taxa richness relative to the control enclosures. Neither emergence nor benthic sampling exhibited evidence of recovery throughout the 83-day study. While this pilot study lacked replication, the results indicate that even following industry standard physical oil product recovery efforts, both CHV and dilbit treatments displayed similarly dramatic suppression of emergent insects and benthic invertebrates. This supports our hypothesis (H1), that oil does have an adverse impact to the macroinvertebrate community. While both the physical mechanisms of effects such as oil sheen and chemical drivers through polycyclic aromatic compounds (PACs) toxicity likely contributed to the observed declines, it is the former that is likely the most significant, as elaborated upon below.

2.5.1 Chemical Impacts

Since both the CHV and dilbit was weathered prior to addition to the treatment enclosures, the focus of the study was not on the acute toxicity of BTEX and monoaromatics that deplete rapidly during weathering (Barron et al., 2018). Rather, our study was intended to characterize residual effects of CHV and dilbit after an initial bulk spill cleanup. Weathered oils are substantially less toxic to aquatic organisms than fresh oils (Steen et al., 1999) and alkylated PACs are considered the most bioaccumulative and chronically toxic compounds (Palace et al., 2019; Madison et al., 2015). Although dilbit contains higher concentrations of asphaltenes and lower levels of saturates, it is expected to have similar short-term toxicity to that of crude oil (Barron et al., 2018). The acute toxicity (LC50) of aromatic hydrocarbons for *Chironomus dilutus* and *Chironomous attenuatus* were 15 mg/L and 2.8 mg/L water soluble fraction (WSF), respectively (Steen et al., 1999). The chronic toxicity of PACs to freshwater organisms has been reported to occur at concentrations between ~2-80 ug/L (Palace et al., 2021; Lee et al. 2015). The highest concentration of PACs observed in this study were phenanthrenes and dibenzothiophenes that contributed most to elevated water concentrations was ~0.47 µg/L (CHV) and ~0.028 µg/L (dilbit), which is lower than these reported toxicity values. Alkylated PACs were found in the water column and the sediment in this study. C2 and C3 dibenzothiophenes and C4 phenanthrenes had stronger toxicity to the macroinvertebrates (*C. tentans*) than water column species (Klerks et al., 2004; Steen et al., 1999). This is similar to a previous study by Klerks et al. (2004) that determined water column and sediment dwelling species were affected by different PAHs. Additionally, chironomids can also ingest sediments accumulating hydrocarbons from both sediment intake and interstitial water (Klerks et al., 2004), which may have contributed to their reduction in abundance in the treatment enclosures.

Within the sediments, PAC concentration were highest two months post spill for 3-ring alkylated PACs with 3.45 µg/g for CHV and 0.52 µg/g for dilbit. A concern for benthic invertebrates is exposure to residual oil remaining in sediments. Molting of invertebrates during larval development may also be affected following exposure to sediment-bound PAHs (Black, 2019; Oberdorster et al, 1999; Song et al, 2017). During the Talmadge Creek Kalamazoo dilbit incident, it was determined that Total Polycyclic Aromatic Hydrocarbons (tPAH) in heavily oiled areas ranging from 32-168 µg/g had chronic impacts (survival, growth, and biomass) to *C. dilutus and Hyalella azteca* (Fitzpatrick, 2012; GLEC, 2012). The results also suggest that impacts to *H. azteca* were correlated to Total Extractable Hydrocarbons (TEH) while impacts to *C. dilutus* resulted from a combination of sediment characteristics (chemical and physical) as well as residual oils (Dew et al., 2016; Fitzpatrick 2012). Since our study was designed as a light oiling, the PACs in the sediment were significantly lower and would likely not be chronically toxic for either oil treatment. Impacts were likely caused from physical impacts causing toxicological effects such as the molting of invertebrates during larval development following exposure to sediment-bound PAHs (Black, 2019; Oberdorster et al, 1999; Song et al, 2017).

Despite the implementation of mechanical clean up of the oil reducing concentrations in both treatment enclosures from 0.05 L/m^2 to 0.03 L/m^2; and the low concentrations of tPACs, there was evidence of large adverse impacts to the macroinvertebrate community within the treated enclosures. This suggests that laboratory test species may not be representative of an entire benthic community as some species may have greater sensitivity than others. Barsdate et al. (1980) demonstrated a range of sensitivities across species. Previous field experiments with application rates of crude oil at 10 L/m^2 and 0.24 L/m^2 have resulted in declines in certain species of Chironomidae (*Tanytarsini sp)* and Trichoptera (Mozley et al., 1978), while some other species of Chironomidae are very tolerant (Lacerda et al., 2014). In our study, we found that certain Chironomidae, specifically *Cladotanytarsus sp.,* were highly sensitive to both oil treatments as compared to *Tanytarsus sp*., which showed no impacts to CHV.

2.5.2 Physical Impacts

Oil slicks affect the oil-water interface impacting insects trying to pass through as adults, as well as their oviposition (Black, 2019). There was an oil sheen observed in the enclosures after cleanup, primarily along the shoreline. However, residual oil was crusted around the emergence trap and the agitation of the trap during sampling would agitate the crusted oil, therefore emergence data may be biased because of sampling artifacts. No emergent insects were observed in the sheen, suggesting that the cause of the declines in emergence within the treatments did not directly result from mortality while insects were attempting to cross the air-water interface. Since the chemical concentrations were generally below chronic toxicity levels for freshwater macroinvertebrates it is likely that the cause of impacts to the macroinvertebrate community were due to physical impacts (primarily a limitation in oviposition and fecundity) from both dilbit and CHV. The oil slick in this study allotted for a 1 mm slick from the addition of 1.25 L (nominal thickness 0.05 L/m^2) in the dilbit and CHV enclosures prior to cleanup that was reduced to a nominal thickness of 0.03 L/m^2 in both treatment enclosures after cleanup. The sheen however was not evenly spread and was likely thicker around the emergent trap and along the shoreline that could have affected emergence as well, oviposition, and fecundity in our sampling area. According to Black (2019) a nominal oil thickness of 0.07 L/m^2 would have significant impacts to insect emergence with a reduction of 50% throughout a 90 day period. While the nominal thickness is less than half of the Black (2019) study, similar effects were apparent. In a laboratory study by Mozley and Butler, (1978), hatching of *Chironomus, Procladius*. and *Tanytarsini* were not affected by oil exposure, but pupae were trapped by oil slicks thicker than 0.5 mm. In experimental ponds, it was observed that the more sensitive species (i.e., Ephemeroptera, Trichoptera, and Plecoptera or EPT) could be similarly trapped in the oil on plant stems (Bardate et al., 1980).

While Ephemeroptera, Trichoptera, and Plecoptera (EPT) numbers were too low to discern treatment differences, the vegetation in the littoral zone was not cleaned, affecting their emergence. Additional physical impacts from the cleanup itself may have further reduced macroinvertebrates. Oil would have been agitated from working near the shoreline, as well additional damage to the sediments and shoreline vegetation that had oil on it may have entered the water again (Lee et al., 2015; Fitzpatrick, 2012). Not only would this impact emergent insects but the agitation of oil as well as impacted vegetation and other surfaces may limit oviposition and fecundity. In the Black (2019) BOREAL study, the benthic communities did not exhibit a clear response to dilbit compared to the control enclosures even though oil was observed to be physically embedded in the sediment. The BOREAL study limnocorrals were deeper with depths varying from 1.5-2 m, where the FOReSt emergence traps were set over a depth of 1 m and this may account for some of the differences in observed responses.

2.5.3. Community Composition

In the two treatment enclosures, Chironomidae and Ceratopogonidae accounted for the majority (98%) of the emergent insects, with only a very few other Dipteran families present, Chaoboridae and Culicidae. Very few additional taxa were present. Trichoptera were present from the families Leptoceridae and Hydroptilidae and limited Ephemeroptera and Odonata in the control enclosures and one Hymenoptera individual in the dilbit enclosure. For benthic taxa, Chironomidae and Ceratopogonidae accounted for 43% (dilbit) and 77% (CHV) of abundances, with low counts of the amphipod *Hyalella sp*, the Trichoptera *Oecetis sp*. and Veneroida *Pisidium sp.* Lake 260 has low productivity and is naturally dominated by Chironomidae, followed by Ceratopogonidae and Trichoptera (Kidd et al., 2014). In the BOREAL study, also conducted on Lake 260 in 2018, the same dominant taxa were identified (Black, 2020). Similar to other studies, invertebrate communities demonstrated reduced species diversity and abundance following exposure to oil (Steen et al., 1999). Sensitive taxa, such as Trichoptera and some crustacea, are considered indicator species while more tolerant species, such as Diptera and chironomids, tend to gain dominance after an oil release (Steen et al., 1999). Although chironomids are the dominant taxa in this lake, impacts were substantial in both emergent insects and macrobenthos with reductions <50%. There was no increase to chironomid dominance observed. Of the eleven taxa of Chironomidae identified, only eight were found in treatment enclosures. *Cladotanytarsus sp., Zavrelia sp., Tanytarsus sp.* and *Ablabesmyia sp. were* the only chironomid genus found in all enclosures. *Tanytarsus sp.* was not impacted by the CHV but did decrease in abundance with dilbit. The only increase in abundance observed in treatments was *Pisidium sp.* in the dilbit treatment only. Additional studies would be required to verify these trends, as the other families were too low in abundance to discern impacts by treatments.

2.5.4 Trophic Interactions

Trophic level effects can also play a role in aquatic invertebrate abundance and richness. Trophic interactions are connected to these communities addressed with the physical impacts from the treatments added and the alterations to the water chemistry as a result; as well the role of primary producers and consumers in the food web. As previously mentioned, while the highest concentration of dilbit and CHV was low and unlikely to cause acute impacts the PAC concentrations did increase in sediments, which may have impacted the benthic communities. The physical impacts, primarily oil sheen collecting around the shorelines and emergent trap impacted insects to emerge and would have caused a reduction in oviposition on contaminated waters/shorelines.

While the oil treatments had no seemingly significant impact on nutrients, observed lower alkalinity and dissolved inorganic carbon (DIC) and calcium concentrations, coupled with peaks of chlorophyll-a (Chla) would suggest primary productivity may have been enhanced in the oil treated enclosures relative to the control enclosures (Palace et al., 2021; Khan et al. 2020). Additionally, the addition of oil may have depleted oxygen due to increased respiration of organic carbon and lower concentrations of soluble silica may have altered diatom production (Palace et al., 2021). Previous studies have also shown a positive correlation between P, N and chlorophyll-a and chironomids community structure by increasing food availability (Saether, 1979 and Davies, 1980). While Chl-a increased and there were no significant impacts to nutrients it would be expected to see an increase in chironomids. However, direct physical impacts from the oil to chironomids did not allow this correlation to be observed.

Unfortunately, no results on zooplankton community are available, and periphyton and phytoplankton were not assessed in this study. Therefore, the ability to examine indirect trophic interactions is limited. There were no significant effects on fish development; mortality in fathead minnows (*Pimephales promelas)* was slightly higher in CHV and deformities were greater in dilbit exposed fish (Palace et al., 2021). Thus, the declines in benthic invertebrate communities in the treatments are unlikely to result from increased grazing pressure (i.e. top-down effects) from the fish community The large decrease in invertebrates due to the physical impacts would likely have a bottom up effect on fish as these are a main food source. Further assessment of the zooplankton and phytoplankton communities would be required to see if they increased with primary productivity and offset a reduced macroinvertebrate food source (Finlay et al., 2007).

No significant effects on riparian vegetation health indices were observed, however the riparian area was impacted by human activity from cleanup and sample collection for riparian vegetation did score significantly lower in the treatment enclosures (Palace et al., 2021). Physical agitation of shoreline vegetation and the oil collected there likely contributed to the reduction in macroinvertebrate densities. Trichoptera and Ephemeroptera may have been particularly adversely impacted as littoral vegetation is their preferred habitat. Vegetation was not wiped clean of oil; each time the shoreline was agitated more oil was likely released into the enclosures.

Overall, it is seems that the impacts on abundance and richness are primarily related to the physical impacts of oil treatments or perhaps indirect effects from other trophic levels or other mechanisms (e.g., oil sheen from residual oil impairing emergence and egg laying) and not due to direct toxicity of the oil treatments themselves.

2.5.5 Limitations

There was no replication in the treatment enclosures and consequently no statistical analysis was possible. Increased replication of the control enclosures would have been beneficial as well due to the high variation in abundance observed. The natural variation observed limits the statistical power of such studies, so adequate replication is imperative in order to determine the significance of any changes observed and the likelihood they resulted from a treatment effect.

The placement of the traps within enclosures may influence the insect assemblage collected. Diptera and Trichoptera tend to emerge via the water column while other orders like Ephemeroptera, Odonata, and Plecoptera are more commonly found to emerge along shorelines (Malison et al., 2010). This study was limited to one emergence trap per enclosure in the center of the enclosure, approximately 5 m from the shoreline and over a depth of ~1 m to avoid impacts of enclosure walls. The addition of a second emergence trap closer to the shoreline (i.e. directly over emergent vegetation) to monitor the area between terrestrial and aquatic habitats that act as a bridge or impede migration of larvae or nymphs (Davies, 1984) may have provided a better insight into effects of other emergent insects such as the Ephemeroptera, Odonata, and Plecoptera. However, for this study it was decided that adding an additional emergence trap closer to the shoreline may affect shoreline and cleaning efforts and moving of the trap could impact oil and cleanup effects. As well, the study would have benefited by incorporating a submerged emergence trap would have been beneficial to measure direct impacts from the oil sheen.

It would have been beneficial to review the aquatic macrophyte assemblage and debris under each emergent trap, which was not performed in this study. Mozley and Butler (1978) incorporated two emergence traps per study area for replication; however, the test ponds were larger than this study's enclosures. Additional approaches should be considered while sampling macroinvertebrates to determine which lifecycle stage such as colonization samplers to better determine partition effects to exposure in the sediments, water column, or surface at emergence throughout the study.

2.6 Conclusion

There were observable effects of the two treatments (CHV and dilbit) on emergent insect and macroinvertebrate communities. CHV and dilbit reduced the taxa abundance and richness of emerging insects and benthic invertebrate communities compared to the control enclosures. Throughout the study, no recovery of the emergent insect community was observed. Further research is needed to determine recovery over multiple years as many macroinvertebrates are univoltine therefore recovery may not occur until following years. Due to the low concentrations of PACs in the water and sediment it is unlikely the effects were driven from chemical impacts and were likely caused by physical impacts of the sheen and shoreline agitation during cleanup efforts.

Further consideration of other trophic level effects in future studies may help to determine which populations and communities have direct and indirect effects from the added oil.

Chapter 3: Response of boreal littoral macrobenthos and emergent insects to a simulated diluted bitumen spill followed by chemical and biological cleanup methods

3.1 Abstract

The effects of chemical and biological cleanup methods for oil spills in freshwater ecosystems have not been well characterized. To help address this uncertainty, the Freshwater Oil Remediation Study (FOReSt) was undertaken at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA). It evaluated the efficacy of two secondary remediation methods on the fate and behavior of diluted bitumen and freshwater food webs under natural conditions. As a subcomponent of the broader FOReST project, we examined the effects to the emergent insect and aquatic macroinvertebrate communities following a simulated spill of weathered diluted bitumen (dilbit), mechanical clean up, and two different secondary cleanup methods: a shoreline washing agent (SWA) and enhanced monitored natural recovery (EMNR). The effects of the oil addition, mechanical and secondary treatments were evaluated within shoreline enclosures in a boreal lake across two shoreline and lake bottom types: rocky shorelines with rock-cobble lake bottoms and peat dominated shorelines with organic lake bottoms. In 2019 controlled releases in contained littoral enclosures were performed, with approximately ~2.5 L of weathered dilbit were undertaken within each treatment enclosure (nominal thickness 0.05 L/m^2). Dilbit additions were followed by mechanical recovery 72 hours after oil addition, and secondary treatment was implemented 96 hours after oil addition. The emergent insect and benthic macroinvertebrates communities were monitored using insect emergence traps through

the experiment and standard kick net sampling at the end of the experiment (Day 95), respectively. In general, for both treatments (EMNR and SWA) no significant effects on the total abundance or richness of emergent insects or benthic macroinvertebrates were detected regardless of shoreline type relative to control enclosures that had no added oil. However, there was a decrease in the diversity of emergent insects within the EMNR treatment relative to control enclosures for peat organic shorelines. Our results indicate minimal adverse impacts to the emergent insect and aquatic macroinvertebrate communities exposed to a dilbit release with the addition of both treatments compared to the observations from the 2018 study with a release of dilbit alone with no secondary treatment applied..

3.2 Introduction

Canada has the third largest oil reserves in the world and is a major exporter of oil to other nations (NRCan, 2014). Oil in a variety of forms is transported using many different methods; of which pipelines are the most efficient, transporting 85% of Canada's oil production (NRCan, 2017; CER, 2019). Pipelines, which often extend over long distances (i.e. 1000's of kilometers), often traverse or run adjacent to surface water (i.e. lakes, rivers, ponds, and wetlands). Since pipeline ruptures can occur, there is potential for oil to enter surface water either directly or indirectly via overland flow or groundwater transport (Alsaadi et al., 2017). For example, in 2010, a pipeline release occurred releasing ~3,320 m^3 into a tributary of the Kalamazoo River in Marshall Michigan (NOAA, 2021). Release of oil can impact the environment and last months to years depending on the location, product released, environmental effects, and cleanup efforts implemented (Lee et al., 2015).

Bitumen is currently the most economically important of Canada's petroleum products and prior to transportation must be diluted with natural gas condensates (Dupuis et al., 2015) for pipeline transport (Dew et al., 2016). The addition of condensates to bitumen results in a less viscous product known as diluted bitumen or 'dilbit', which is an unconventional crude oil developed to facilitate pipeline transportation across North America (Black et al., 2021a). Dilbit can rapidly weather with loss of diluent components, increasing density, viscosity, adhesion, and asphaltene content compared to conventional oil (Barron et al., 2017). There are seven different blends of dilbit (Dew et al., 2016) and they are variable in the percent of diluent added making the properties of dilbit within each oil release unique (Barron et al., 2017). To date, little is known on fate and effects of dilbit releases in terrestrial and freshwater environments and research has been recommended to better understand whole ecosystem effects (Lee et al., 2015).

Oil releases in marine, freshwater, and terrestrial ecosystems create concerns regarding impacts to the environment and the potential adverse effects of cleanup operations (Vandermeulen et al., 1994; Lee et al., 2015). Prior to the Exxon Valdez oil release on the west coast of Alaska, there was limited information available for constructing risk assessment models to predict ecological impacts of oil spills (Peterson et al., 2003). In response to the observed chronic effects characterized at the Exxon Valdez site, the Shoreline Cleanup and Assessment Techniques (SCAT) surveys were developed for both marine and freshwater releases (IPECA, 2014). The information derived from the SCAT surveys support the Net Environmental Benefit Analysis (NEBA) used to determine the least impactful cleanup response (IPECA, 2015). While much information is available for marine shoreline cleanup, there are limited available data on fate, transport and ecological effects of releases to inland freshwaters. A current drawback of existing recommendations for freshwater oil release response is that they focus heavily on mechanical removal and light oils. There is insufficient information on medium and heavy oils and the impacts of utilizing chemical and biological response methods are poorly understood (API, 1994; API, 2016).

For all large-scale responses to spills on water, booming, and skimming are utilized to contain and collect bulk oil from the water surface. However, such efforts do not recover all oil and sheening problems often persist and oil will eventually reach shorelines. Common methods to remove oil from shorelines include pressure washing, raking, use of oleophilic sorbents, vegetation removal, or soil and debris removal; all of which can cause additional adverse impacts (Reid, 2015; API, 1994). Further, traditional cleanup methods based on physical recovery of oil slicks on water surfaces are ineffective for spilled oil once it becomes submerged in oil particle aggregates and transferred to the water column and benthic environment (Fitzpatrick et al., 2015). While mechanical removal of oil is the most common form of cleanup, it may not be the least impactful to the environment and may not be as useful if the oil starts to sink.

Shoreline washing agents (SWA) also known as chemical cleaners are an alternative to traditional mechanical cleanup methods and allow stranded oil to be washed from surfaces such as rocks and shorelines to facilitate the recovery of oil using traditional mechanical methods (Bhattacharyya et al., 2003; Stroski et al., 2019). Shoreline washing agents are approved for use in marine water in Canada and the US as marine environments have sufficient wave energy and water circulation (Lee et al., 2015). However, shoreline washing agents for freshwater releases have seldom been applied in the because of the potential for consequent contamination to the environment and to potable water sources (Vandermuelen et al., 1994). For a shoreline washing agent to work effectively, minimal to no released oil should be dispersed, unlike that of a dispersant product and the washing agent should be used on habitats where flooding and flushing is applicable to facilitate recovery (API, 1994). While shoreline washing agents can aid in cleanup, there are still concerns of dispersion of oil product to the water column, as well as an increase of total hydrocarbons as hydrocarbon-based solvents such as shoreline cleaners may themselves adversely impact macroinvertebrate communities (Black et al., 2021; Foicco et al., 1991). Therefore, if the addition of chemicals to an oil release is being considered, it must be determined that the added chemical to the oil product and the synergistic effects are less toxic to the environment than the oil itself (Scott et al., 1984). For a chemical to be utilized in Canada for inland freshwater releases, regulatory approvals are required prior to use. If further studies can demonstrate that oil in combination with a chemical cleaner is less toxic than the oil itself, then it could be used as a cleanup method and as an alternative to mechanical cleanup. Corexit 9580 is a Surface Washing Agent (SWA), also known as a shoreline cleaner, formulated from biodegradable surfactants in a low toxicity, highly refined hydrocarbon solvent (Fiocco et al., 1991). Corexit EC9580-A (formally Corexit 9580) should be used in concentrations of 1 gal/100sqft (0.4075L/m^2) as per US EPA recommendations (EPA, 2013). Oil and cleaner should be left to presoak for 15-30 min prior to washing (Fiocco et al., 1991). This product has been used on marine oil spills; however, little research has been conducted on its effectiveness and potential toxicity for freshwater spills (Bhattacharyya et al., 2003). In a laboratory trial, Corexit 9580 outperformed 43 other test products during a one-minute settling assay, removing 26% of applied oil and dispersing only 7% (Fiocco et al., 1991). However, a study on toxic effects of Corexit 9580 in lab and field microcosm exposures indicated that for all three test species (daphnid; *Daphnia pulex*, Japanese medaka; *Oryzias latipes*, and chironomids; *Chironomus tentans*) the toxicity of Corexit 9580-treated oil was greater than oil alone, with no reduction in toxicity detected over the 6-month study duration (Bhattacharyya et al., 2003). In a more recent study by Black et al., (2021), the addition of both dilbit and Corexit EC9580-A (previously called Corexit 9580) caused immediate immobility in water striders, whereas tests with only dilbit took 24 hours to demonstrate immobility. This shows that dilbit and Corexit EC9580-A can be more toxic than the dilbit itself for certain pleuston species. It should be noted that both studies (Bhattacharyya et al., 2003; Black et al., 2021) were based on worst case scenarios, using concentrations based on the entire spill, not considering containment and initial mechanical response would take place, therefore and are not reflective of realistic residual oil cleanup scenarios.

Bioremediation has been used as a cleanup approach in marine spills but not as often in freshwater (Vandermuelen et al., 1994). A form of bioremediation is nutrient enrichment, also referred to as enhanced monitored natural recovery (EMNR), which aims to speed up rates of natural attenuation by stimulating the microbial community and microbial degradation of oil products through nitrogen and/or phosphorus addition (API, 1994). Nutrients including nitrogen, phosphate and iron are essential to biological processes, and crude oils are naturally deficient in these nutrients elements (Atlas et al., 2011). Adding nutrients or growth enhancing co-substrates can improve habitat quality and stimulate the growth of indigenous oil degrading bacteria, increasing degradation rates (Lee et al., 2001). Nutrient enrichment is best used after bulk oil cleanup is completed and shorelines are left lightly oiled, as its less effective on thick oil residues with high molecular weight and slow degrading components (API, 1994). Furtheer, EMNR represents a potentially useful option for areas with limited access or natural nutrient deficiencies. However, there is a potential for nutrient overloading in small waterbodies (AP1, 1994).

Few studies have been conducted on the use of shoreline washing agents or nutrient enrichment as cleanup methods for oil spills in freshwater environments. As previous tests have indicated that benthic invertebrates are susceptible to impacts from oil spills and cleanup methods, The objective of this study was to evaluate the effects of two different secondary cleanup methods (EMNR, SWA) on the abundance, richness and diversity of emergent insects and benthic macroinvertebrates under natural conditions. The effects of the two treatments were evaluated within shoreline enclosures, over two shoreline and lake bottom types (rocky shorelines with cobble lake bottom, peat shorelines dominated by organic substrata) in a boreal lake with natural invertebrate communities..

We hypothesized that benthic invertebrate and emergent insect communities would be impacted by secondary treatment methods in both shoreline types, as compared to unoiled reference conditions. We predicted that the total abundance of benthic invertebrate and emergent insects would decrease with the addition of weathered dilbit and secondary treatment shoreline washing agent (Corexit EC9580-A) in both shoreline types and increase with the addition of enhanced monitored natural recovery (EMNR) treatment in both shoreline types.

3.3 Methods:

3.3.1 Study Location

This 95-day study simulated a freshwater oil spill, mechanical cleanup, and secondary remediation methods to determine, in part, the impacts on the aquatic macroinvertebrate community. The study was conducted at Lake 260, at the IISD-Experimental Lakes Area, Northwestern Ontario, Canada. Additional information on Lake 260 is provided in Chapter 2, Section 2.3.1. The design of each enclosure was similar to those used during the 2018 pilot study with the exception that each enclosure had no divider. Sixteen shoreline enclosures were deployed (15 m x 5 m x 1.5 m) in the littoral zone of the lake (Figure 3.1). Of the 15 m in length (perpendicular to the shoreline), 5 m extended onto land with the remaining 10 m extending out into the lake to a depth of ~ 1.5 m. The water volume of each enclosure was estimated to be ~37.5 m^3. Additional enclosure design details are found in Chapter 2, Section 2.3.2

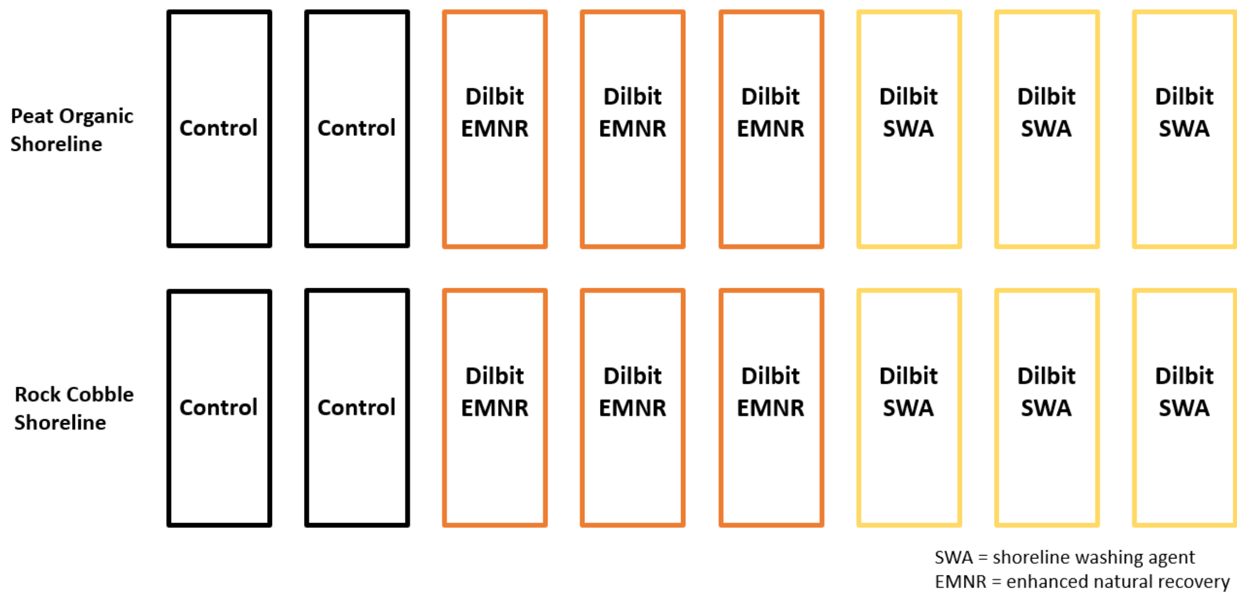

Figure 3.1: Schematic of a shoreline enclosure, Treatments were randomized (order not shown) along each shoreline type. Control (control enclosures), EMNR (enhanced monitored natural recovery), and SWA (shoreline washing agent) (image adapted from Perry, 2021).

3.3.2 Enclosure Design and Treatment Application

Of the sixteen enclosures, eight were situated on a Peat Organic type shoreline, with soft organic sediment while the remaining eight were situated on Rock Cobble shoreline, all treatments were randomly assigned to enclosures (Perry, 2021) (Figure 3.2). Within each shoreline type, six enclosures were treatment enclosures and received oil addition, mechanical clean up, and one of two secondary clean up methods, enhanced monitored natural recovery (EMNR) and shoreline washing agent (SWA) (Figure 3.3). The remaining two enclosures were defined as controls and received no oil addition, mechanical clean up, or secondary treatment. However, shoreline rinsing using enclosure water was performed on both treatment and control enclosures. Prior to oil addition, fish were removed from the enclosures using cage traps and nets. A total of 10 free-swimming fish *(Pimephales promelas)* were then added to each enclosure prior to the oil addition (June 20-21, 2019 for Rock Cobble and June 17-21, 2019 for Peat Organic) to ensure similar predation pressure among treatments and controls. Enclosures were installed and allowed to acclimate for two weeks prior to treatment.

Figure 3.2: Satellite imagery of Lake 260 located at the IISD-ELA in Northwestern Ontario, Canada. The coloured letters indicate the location of study enclosures. Green POs represent the Peat Organic enclosures, while orange RC's represent the Rock Cobble enclosures. Details of this nomenclature can be found in the legend of Appendix Table A.3.

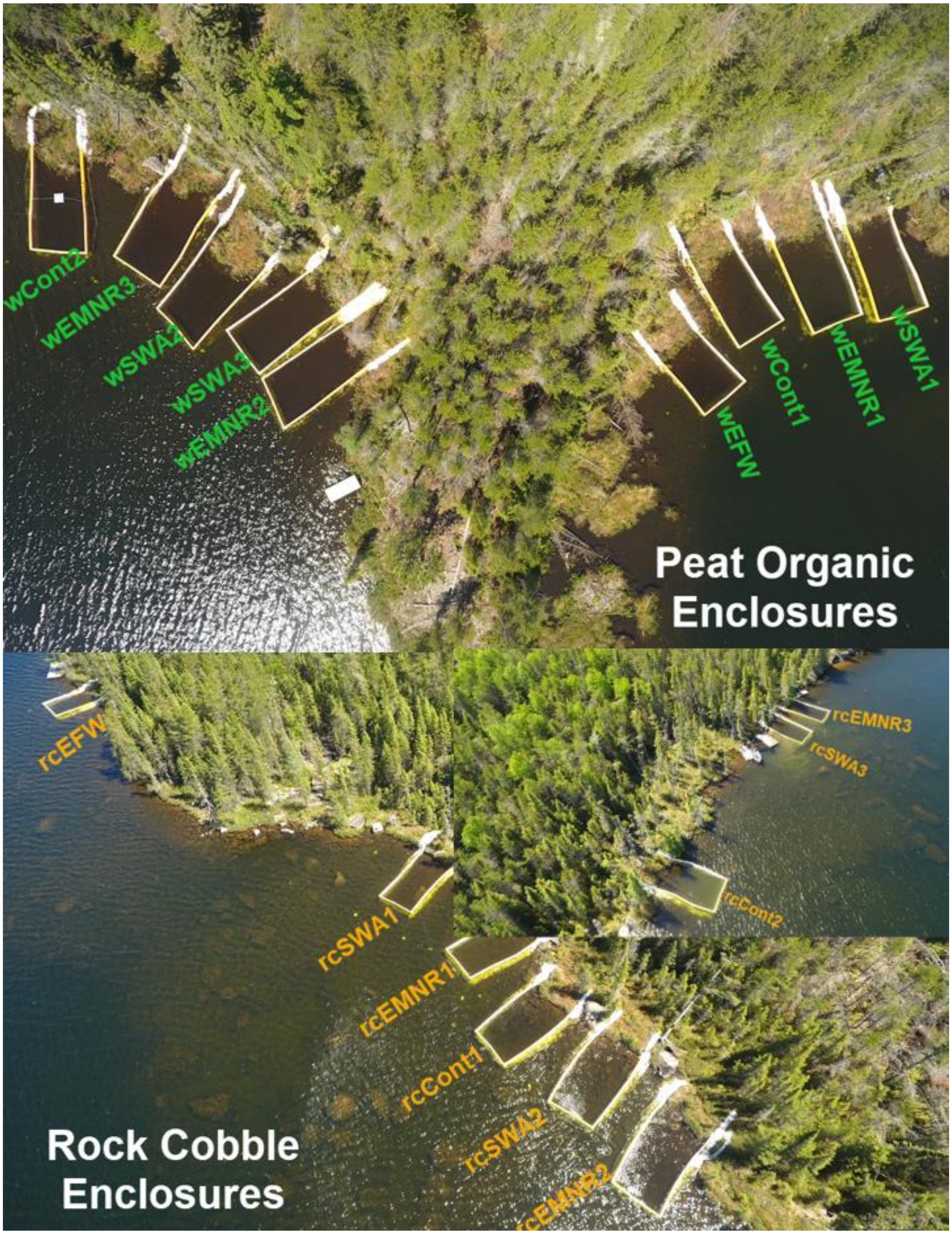

Figure 3.3: Drone images of all enclosures in both shoreline types and labelling convention for treatment enclosures (Drone image from Tyler Black).

Prior to application, the dilbit was naturally weathered outdoors for 36 hours in water from Lake 260 in stainless steel evaporation pans exposed to air and sunlight to simulate the residual oil left over from a spill. After oil was weathered, it was collected, weighed, and transported in glass jars to the lake. A total of 2.5 L of weathered dilbit was added to the enclosures; if spread evenly this would create a 0.1 cm-thick sheen on the entire enclosure (nominal thickness 0.05 L/m^2). The addition of weathered dilbit to each treatment enclosure occurred within 50 cm of the shoreline of each treatment enclosure (targeted 1 mm-thick coating for entire 5 m of linear shoreline). Natural lake conditions such as wind and waves affecting the oil dispersion were simulated upon application. Waves were simulated using a leaf blower and by moving the enclosure collar up and down (Perry, 2021). The Peat Organic enclosures were dosed with the dilbit June 21, 2019 while the Rock Cobble enclosures were dosed on June 22, 2019 and both days are referred to as Day 0 of the experiment for each respective shoreline type. Additional information on enclosure installation and product weathering and application can be found in Chapter 2 Section 2.3.2.

Primary cleanup of the dilbit occurred after 72 hours, (i.e. Day 3 of the experiment), which is representative of industry response times for cleanup (WCMRC, 2022). The Peat Organic enclosures were cleaned from, June 24, 2019 to June 25, 2019 (i.e. Days 3 to 4 post-treatment) while the Rock Cobble enclosures were cleaned June 25, 2019 to June 26, 2019 (i.e. Days 3 to 4 post-treatment). Details on mechanical (primary) oil cleanup were the same as the 2018 pilot study and can be found Chapter 2, Section 2.3.1. Application of the secondary treatments were applied 24 hours after mechanical cleanup (Day 5 for Peat Organic; Day 6 for Rock Cobble). The EMNR treatment simulating natural microbial recovery was undertaken by a one-time addition of 10 g of granular nutrient fertilizer (Scott's Osmocote Pro™ 19:6:9 N:P:K by weight) to each EMNR treatment enclosure within 1 m of the shoreline. This treatment was expected to increase nitrogen by ~13% and phosphorous by ~64%, based on background nutrient concentrations in each enclosure (Perry, 2021). The SWA used was Corexit EC9580-A™. Approximately 250 g (~9 mg/L) of SWA was applied to treatment enclosures using a pesticide grade hand-held applicator and was distributed for ~45 seconds per enclosure on the shoreline vegetation and riparian zone (water depth <3 m) (Perry, 2021). The shoreline cleaner was left on the oiled shoreline for 30 minutes, (as recommended by the products technical bulletin (EPA, 2013)) prior to flushing with freshwater and applying secondary cleanup treatments using sorbent pads to collect mobilized oil.

3.3.3 General Water, Oil Chemistry, and Sediment Sampling

Surface water samples were collected from each enclosure at 11 different time points: Day -3 (Peat Organic) and Day -4 (Rock Cobble), both prior to oiling, and then on Days 1, 2, 3, 4, 5, 6, 20, 66, and 87 post-oil application. Water samples were collected using the same methods as the 2018 pilot study (see Chapter 2, Section 2.3.2). Total petroleum hydrocarbons (TPH), saturates, aromatics (including polycyclic aromatic hydrocarbons (PAH and alkylated PAHs) were analyzed in 1 L surface water samples using gas chromatography mass spectrometry (GC/MS) (Perry, 2021). Routine water quality measurements, including pH, temperature, dissolved oxygen, and conductivity, nutrients and additional biological parameters were broken up into three time periods (Day 0 to Day 89, Day 0 to Day 33, and Day 47 to Day 89) and analyzed separately throughout the duration of the study (Perry, 2021). A radiotracer tritium was added to all treatment enclosures to track potential water exchange between enclosures and Lake 260 (Perry, 2021). Water quality analyses were completed at the IISD-ELA chemistry lab under the direction of Dr. Sonya Havens-Higgins following the protocols of Stainton et al., (1977). See Perry (2021) for additional details on water quality analysis. The sediment sampling and analysis followed the methods in Xia et al., (2021).

3.3.4 Emergent Insect and Macroinvertebrate Sampling

The emergent insect and macroinvertebrate communities were assessed via: floating emergence traps throughout the duration of the study; and by kicknet sampling at the end of the study, as described below.

3.3.4.1 Insect Emergence Traps

The floating emergence traps used were the same design as the 2018 pilot study and additional information on design can be found in Chapter 2, Section 2.3.4.1. Each enclosure contained one trap deployed on June 14th, 2019 (Day -7 for PO and Day -8 for RC). The traps were deployed in the same way as the 2018 study, attached to the brackets with rope and centered in the middle of the enclosure where water depth was ~1 m. During weekly inspections, traps were inspected for holes, ensuring sample bottle and preservative were intact, presence of predators (i.e., spiders and dragonflies), and larval colonization as these factors can alter data collected (Davies, 1984). There were some predators identified in some of the traps, which were removed upon inspection (see Section 3.4.2). Samples were taken pre- and post-dosing of dilbit, at intervals, (Day -1, 12, 19, 26, 33, 40, 47, 54, 61, and 68) to determine a baseline impact of the contaminant and secondary cleanup strategies. Samples were enumerated and counted in the same process as the pilot study, by H. Kajpust see chapter 2 Section 2.3.4.1.

3.3.4.2 Benthic Sampling

Benthic macroinvertebrate sampling using the kicknet method was conducted on September 23rd and 24th, 2019 (Day 95), respectively in each of the Peat Organic and Rock Cobble enclosures. The sampling method, collection, protocols, and sample analysis used were the same as the 2018 pilot and can be found in Chapter 2, Section 2.3.4.2. The kick-and-sweep path was defined before entering the enclosure. For the Peat Organic enclosures, benthos was dislodged from the substratum by gently tapping the bottom of the kicknet on substrate, as well as gently swiping feet along the substrate (Environment Canada, 2019). For Rock Cobble enclosures, benthos was dislodged from the substratum through the vigorous twisting and kicking of feet (Environment Canada, 2012).

As per CABIN protocol (Environment Canada 2014), each sample was split into 100 equal cells using a Marchant box (Marchant, 1989). To avoid confounding taxonomic richness calculations (total number of taxa observed), the number of cells subsampled was standardized so that search efforts remained consistent; 10% of each of the samples from the enclosures over the Peat Organic substrata was sorted (i.e. 10 of 100 cells) and 5% of each sample from the enclosures over the Rock Cobble substrata was sorted (i.e. 5 of 100 cells). For control samples of Peat Organic and Rock Cobble enclosures this resulted in approximately 100 and 300 enumerated organisms, respectively. For the benthic identification, the taxonomist who identified the samples was Dr. Michael White.

3.3.5 Statistical Analysis

All data were compiled in Microsoft Excel, and statistical analyses were conducted using Sigma Plot and R version 3.5.3 and R Studio version 1.1.456 with the packages vegan version 2.5-5 and *ggplot2* for data visualization. Raw data sets are included in **Appendix A.** Both emerging insects and benthic invertebrates were converted to abundances based on the sampling method employed. Since the experimental design of the broader FOReST project included unequal replication (treatment n = 3, control n = 2 for each shoreline type) one-way ANOVAs, which are resilient to unequal replication, were chosen to assess potential treatment effects. Normality (Shapiro-Wilkes) and equal variance (Brown-Forsythe) were assessed prior to analyses and log10 or square root transformations where used where necessary. If transformed data still did not pass normality or equal variance, a non-parametric Kruskal-Wallis test on ranks was used to assess potential treatment effects If the P value from the ANOVA was significant ($p<0.05$), a multiple comparison test versus the control group using Dunnett's method was completed. Due to variation between replicates, a power analysis was completed to determine if the sampling program collected sufficient information for the decisions to be made, using the method outlined in Environment Canada, (2012B). Univariate biodiversity metrics and community composition assessments included determination of taxa richness (total number of taxa observed) and the Inverse Simpson Index ($1/\lambda$) which takes into account the number of taxa present, as well as the relative abundance of each taxa and was used to quantify average proportional abundance of taxa in the dataset of interest.

As reported by Perry (2021) the results from the tritium radiotracer indicated significant water exchange between the enclosure and lake by ~ Day 30 in enclosures situated over the Peat Organic substrata. As a result, water and oil chemistry and primary producers were assessed in three different time periods to more accurately quantify effects related to actual oil exposure in the water column, 1) Day 0 to Day 94; 2) the first portion of the experiment prior to complete mixing of enclosure water with surrounding lake water, Day 0 to Day 38; and 3) the final period of the experiment, Day 52 to Day 94. Due to rapid water exchange within the first few days (Day 3) in the Rock Cobble enclosures the data was not subdivided into three time periods, only the full study duration (Day-1 to Day 68). The emergent samples for the Peat Organic enclosures were assessed for the study duration (Day-1 to Day 68) and the portion of the experiment prior to completed mixing (Day -1 to Day 33, based on emergence sample days). The final period was not assessed as emergent insects are not as mobile as other communities such as zooplankton and many are univoltine (one brood per season) and would have been exposed in the initial part of the experiment.

Non-metric multidimensional scaling (NMDS) was used in evaluating benthic invertebrate communities and assist in visualizing potential differences in community structure. NMDS was chosen as it can efficiently represent abundance data relative to a variety of treatments, in this case SWA, EMNR, and Control treatments. NMDS reduces a complex data matrix into a 2-dimensional scale ordination of the data that is more easily interpreted (Black, 2019). Absolute distances between objects (i.e. taxa or treatments) indicate similarities among them. NMDS functions by placing objects (i.e. taxa or treatments) on a 2-dimensional plane several times until the stress (measure of how poorly objects are positioned) is reduced (Black, 2019). Stress values below 0.1 are optimal, whereas a value >0.2 is poor and indicates risks in interpretation. The Bray Curtis index was used to determine dissimilarity among taxa. Bray Curtis dissimilarity is the community ordination standard, therefore is the prime choice for abundance or count data, as with the benthic data analyzed (Black, 2019). No rare taxa were dropped; however, members of the genus Chironomidae were combined into a subfamily. It should be noted that the control enclosures were only plotted as there are only two replicates and a minimum of three are required to form a polygon to help illustrate how taxa cluster based on the treatments.

3.4 Results:

3.4.1 General Oil Chemistry, Water Quality, and Nutrients

After initial cleanup using sorbent pads, the average primary percent recovery of oil for the Peat Organic enclosures was 5.8% and for the Rock Cobble it was 6.1% (Vince Palace, personal communication 2021). Secondary recovery of oil in enclosures treated with Corexit EC9580-A was 13.5% for the Peat Organic enclosures and 61.4% for the Rock Cobble enclosures (Vince Palace, personal communication 2021). The difference of recovery is likely due to shoreline material where oil on rock shorelines should be easier to remove than peat organic shorelines where it may adhere to organic material or vegetation. Total polychlorinated aromatic compounds (tPACs) were elevated above control levels in all treatment enclosures in each type of shoreline. In the Peat Organic enclosures, the highest concentration of tPACs in the EMNR treatment was 7.54 µg/L and for the SWA treatment 9.36 µg/L. In the Rock Cobble enclosures, the highest concentration of tPACs in the EMNR treatment was 9.32 µg/L and for the SWA treatment was 10.91 µg/L (Vince Palace, personal communication 2021). It was observed in the Rock Cobble enclosures that PAC concentrations were close to those in control enclosures by Day 20. By the end of the sample period (Day 66) all treatment enclosures for both shoreline types had tPAC concentrations that were comparable to the control enclosures (Perry, 2021).

Based on radiotracer tritium added on Day 1,the Rock Cobble enclosures had rapid water exchange with the lake, reaching background levels (300Bq/L) within the first few days of the study (Perry, 2021). The Peat Organic enclosures also had significant water exchange, reaching background levels (300 Bq/L) by ~Day 30 (Perry, 2021).

In the Rock Cobble enclosures, no significant differences ($p>0.05$) were observed in the nutrient and water chemistry between treatments and controls throughout the entire study period (Day 0 to Day 94). This was probably due to the high rates of water exchange in the enclosures (Vince Palace, personal communication; Perry, 2021). For the Peat Organic enclosures, there were significant decreases in the EMNR treatments for alkalinity (Alk) for sample period 1 and 2 and calcium (Ca) for sample period 1, and significant increases of sulfate (SO_4), for sample period 1 and 3compared to the controls (Perry, 2021). In SWA treatments, chlorine (Cl), potassium

(K) for sample period 1, chlorophyll-a (Chl-a) in sample period 1 and 2, ammonia (NH_3), nitrogen dioxide (NO_2), for sample period 1 and 3 and suspended phosphorous (SUS-P), for sample period 1 and 2 were significantly higher than the control. Dissolved inorganic carbon (DIC) was significantly lower in both treatments for sample period 1 and 3 and for sample period 2 for EMNR only (Perry, 2021). In the EMNR treated enclosures there was no measurable difference in total nitrogen and total phosphorous, which were added with an expectation of an increase of ~ 13% N and ~ 64% P per enclosure based on background nutrient concentrations (Perry, 2021). The added nutrients were likely utilized by periphyton, triggering growth or collected in sediments (Perry, 2021). Detailed water chemistry, oil data, and their interpretations can be reviewed in Perry (2021).

3.4.2 Insect Emergence Response

Rock Cobble

Total mean abundance of emerging insects in the Rock Cobble enclosures (sum over all sampling days) increased in EMNR (135%), and SWA (13.3%) compared to the control enclosures but these increases were not statistically significant ($p>0.05$ ANOVA), nor were there statistically significant differences at each time point (Figure 3.4). Since there was a lack of significance for the endpoints and large variance, one way ANOVAs were also completed for each date for all three endpoints. Variation between enclosures can be found in Appendix Figure B.4. Similar to the pilot study, abundance was dominated by chironomids averaging 83% for the control, 93% EMNR and 92% SWA enclosures. The next most abundant family was *Ceratopogonidae* (4%

for control enclosures and ~3% in both EMNR and SWA treatments). Very few Trichoptera were present from the Families Leptoceridae and Hydroptilidae. The total percent of Trichoptera were 1.7% for control enclosures, 2% in EMNR, and ~4% in SWA. There were rare observations of other Diptera, Ephemeroptera, and Hymenoptera orders. Due to the dominance of Chironomidae, no further analysis was conducted as the remaining groups were too low in number to distinguish treatment-related impacts. Emergent rate was also determined and can be reviewed in Appendix Figure B.5.

The total family richness was not statistically significant from the control enclosures ($p>0.05$ ANOVA). The family richness at Day 26 and Day 61 for EMNR was statistically greater ($p<0.05$ ANOVA followed by a Dunnett's test) from control enclosures (Figure 3.5). Diversity as measured by Inverse Simpson Indices, was not statistically significant between treatments and the control enclosures for total diversity over time as well at each time point ($p>0.05$) ANOVA, (Figure 3.6 and Appendix Figure B.6). Family richness across enclosures ranged from 8 to 13 taxa, with inverse Simpson diversity varying from 5.12 to 9.26. For statistical analysis outputs refer to Appendix A Table 6.

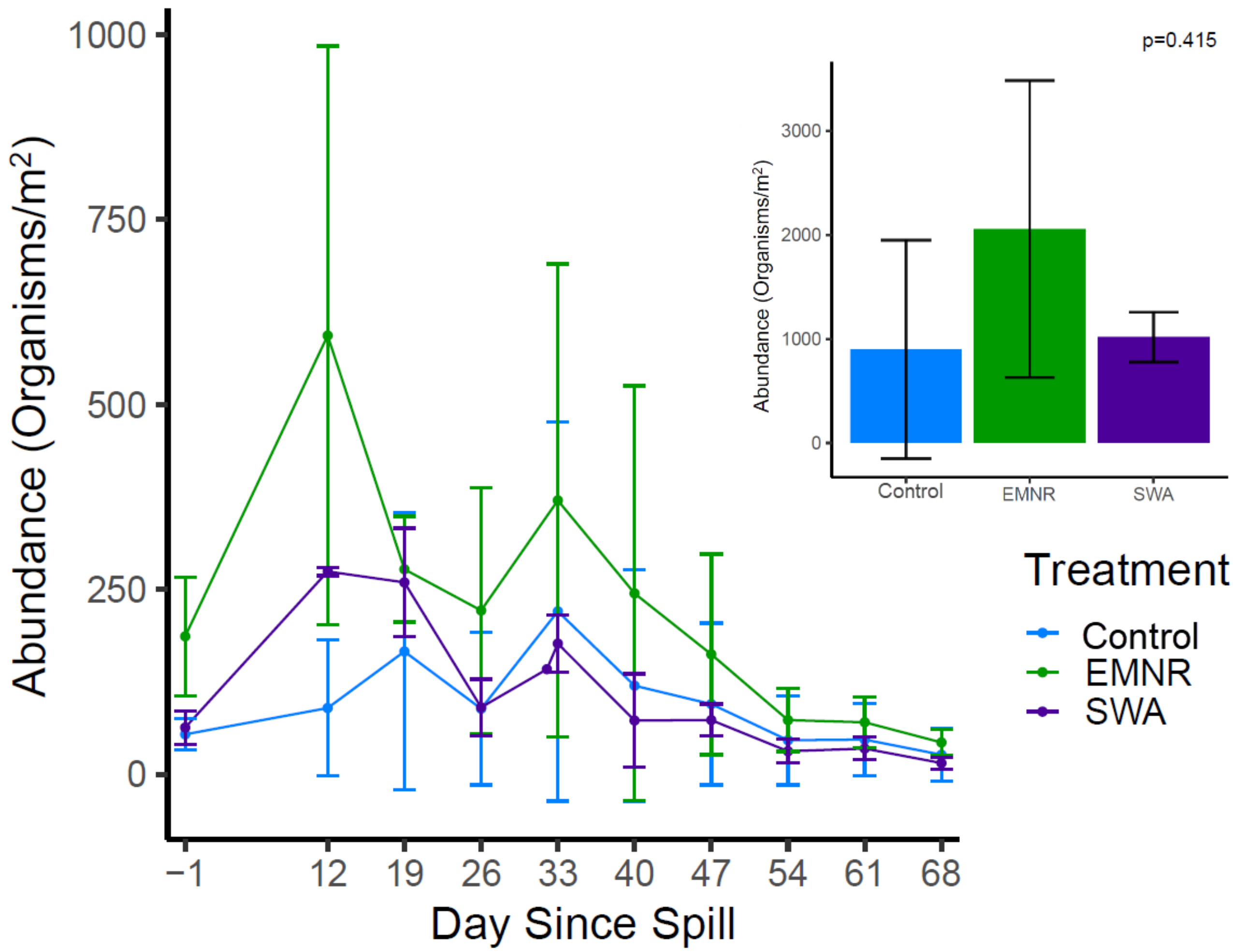

Figure 3.4: Abundance (organism/m^2) of all insect families within emergent traps in the Rock Cobble enclosures. The enclosures were sampled from June 22, 2019 (Day -1) to August 29, 2019 (Day 68). Each point is the mean and standard deviation (n=2 control, n=3 treatments). Treatments EMNR and SWA were not statistically significant from control using a one way ANOVA (p=0.415) (Day12-Day 68). Inset graph is the sum of the mean abundance over all post-exposure days from July 3, 2019 (Day 12) to August 28, 2019 (Day 68) error bars represent the standard deviation.

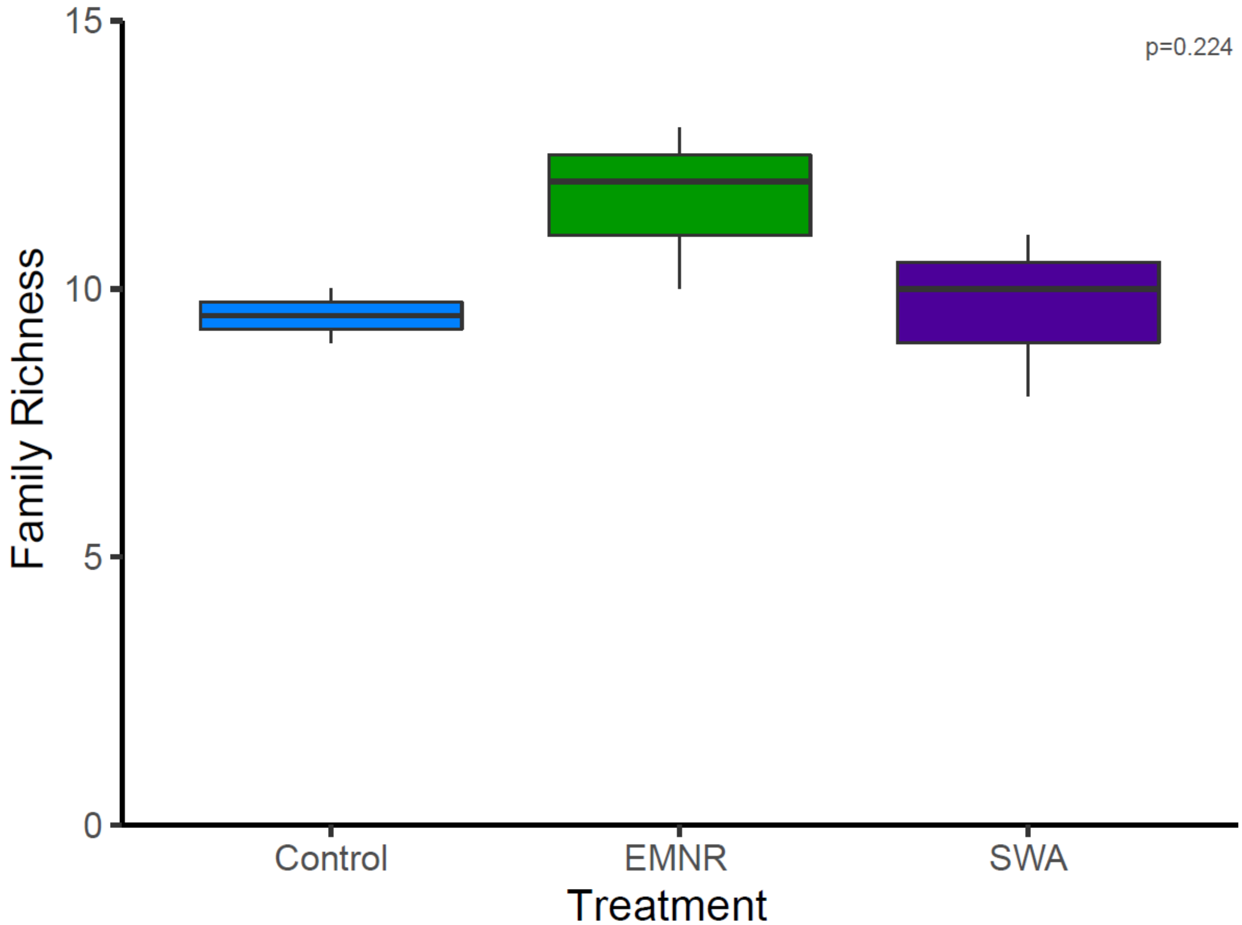

Figure 3.5: Total family richness (sum of all sampling days) in emergence traps on the Rock Cobble enclosures. The horizontal line in the box is the median, while the whiskers go from each quartile to the minimum or maximum of the sample set. The enclosures were sampled from June 22, 2019 (Day -1) to August 29, 2019 (Day 68). Treatments EMNR and SWA were not statistically significant from control for post-exposure sampling (Day 12-Day 68) using a one way ANOVA (p=0.224).

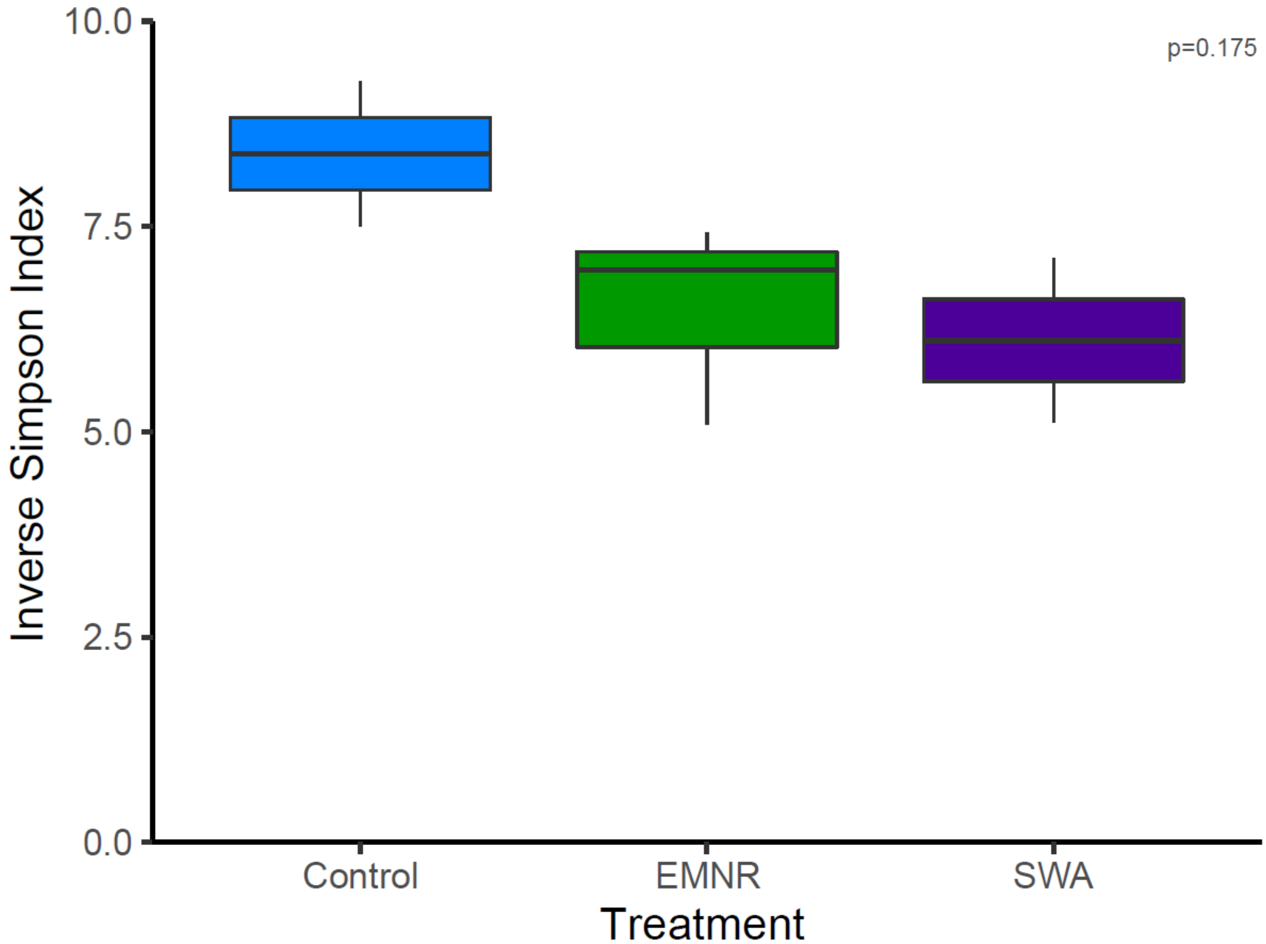

Figure 3.6: Inverse Simpson indices (sum of all sampling days) in emergence traps on the Rock Cobble enclosures. The horizontal line in the box is the median, while the whiskers go from each quartile to the minimum or maximum of the sample set. The enclosures were sampled from June 22, 2019 (Day -1) to August 29, 2019 (Day 68). Treatments EMNR and SWA were not statistically significant from control for post-exposure sampling (Day 12-Day 68) using a one way ANOVA (p=0.175)

Peat Organic

For the Peat Organic enclosures, total mean abundance of emerging insects (sum over all sampling days) increased in EMNR (46%) and SWA (32%) as compared to the control enclosures, but these differences were not statistically significant ($p>0.05$ ANOVA) nor were there statistical significance at each time point (Figure 3.7). Since there was a lack of significance, yet large variances between replicates, statistical tests were also completed for each date for all three endpoints. Variation between enclosures can be referenced in Appendix Figure B.7. Similar to the pilot study, abundance was dominated by chironomids averaging 84% for the control, 91% EMNR and 95% SWA enclosures. The next most abundant family was *Ceratopogonidae* (~2% for control enclosures, ~2% in EMNR, and 1.7% in SWA). Very few Trichoptera were present from the families Leptoceridae and Hydroptilidae. The total percent of Trichoptera were (~2% for control enclosures, 1.5% in EMNR, 1.7% in SWA). There were rare observations of other Diptera, Ephemeroptera, Hymenoptera and Odonata. Due to the dominance of Chironomidae, no further analysis was conducted, as the remaining groups were too low in number to distinguish treatment-related impacts. Emergent rate was also determined and can be reviewed in Appendix Figure B.8.

Total family richness in treatment enclosures was not statistically significantly different from the control enclosures ($p>0.05$ Kruskall Wallis). The family richness at Day 47 was statistically different ($p<0.05$ ANOVA with Dunnett's test) for both treatments compared to the control enclosures (Figure 3.8). Diversity as measured by Inverse Simpson Indices was statistically significant (increased) for the EMNR treatment ($p<0.05$ ANOVA with Dunnett's test) compared to the control enclosures, due to the increased relative abundance of chironomids. There was no statistical significance for either treatment at any timepoint for diversity ($p>0.05$) (Figure 3.9 and Appendix Figure B.9). Family richness across enclosures ranged from 7 to 11 taxa, with inverse Simpson diversity varying from 5.22-8.55. For the time period of Day 12 to Day 33, there were no statistical significance for abundance, diversity or richness for either treatment compared to the control (Appendix B Figures 10-12). For statistical analysis outputs, refer to Appendix A Table 6.

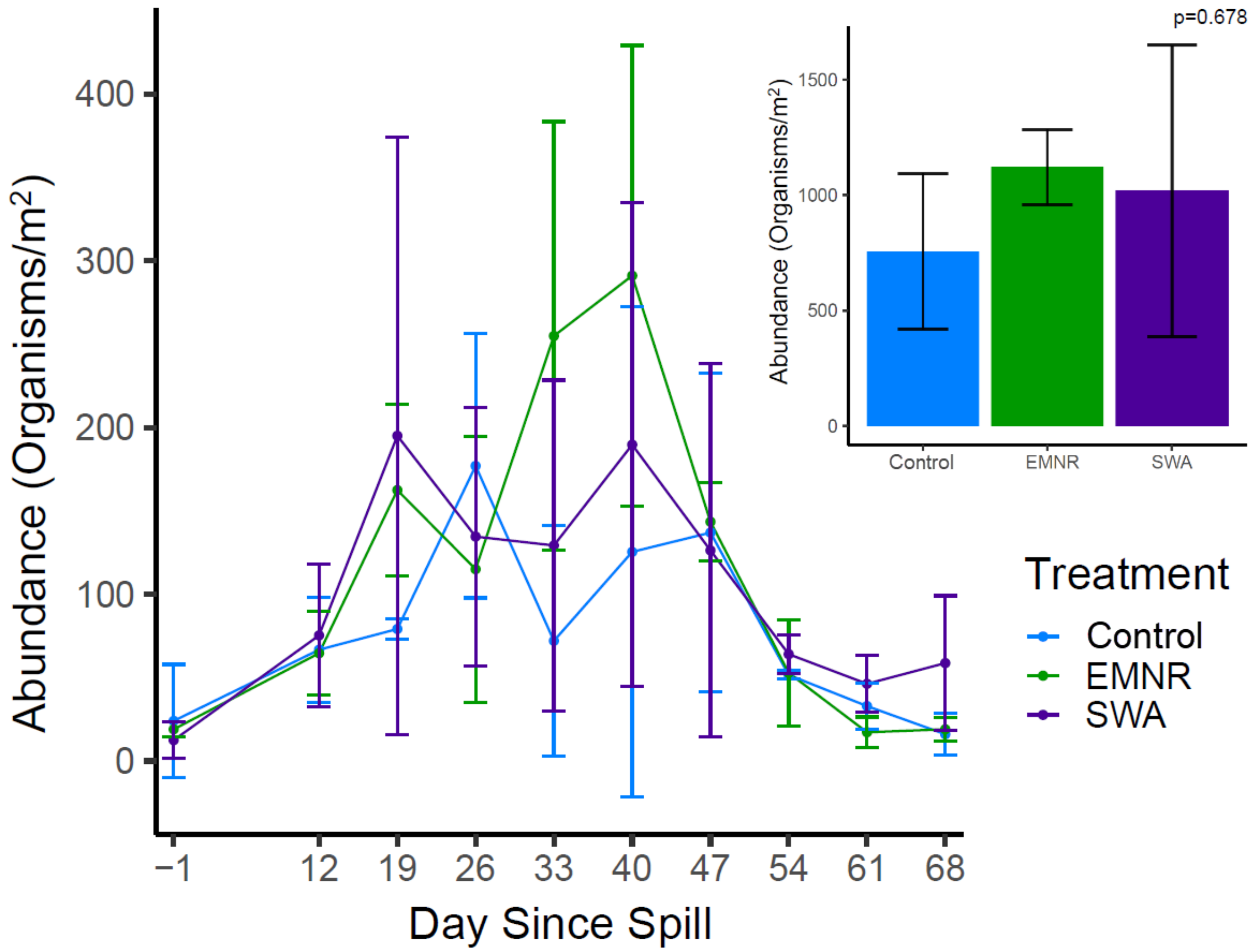

Figure 3.7: Abundance (organism/m^2) of all emergent insects identified in the Peat Organic enclosures. The enclosures were sampled from June 21nd, 2019 (Day -1) to August 28, 2019 (Day 68). Each point is the mean and standard deviation (n=2 control, n=3 treatments). Treatments EMNR and SWA were not statistically significant from control using a one way ANOVA ($p=0.678$) (Day12-Day 68). Inset graph is the sum of abundance over all post-exposure days from July 3, 2019 (Day 12) to August 28, 2019 (Day 68) error bars represent the standard deviation.

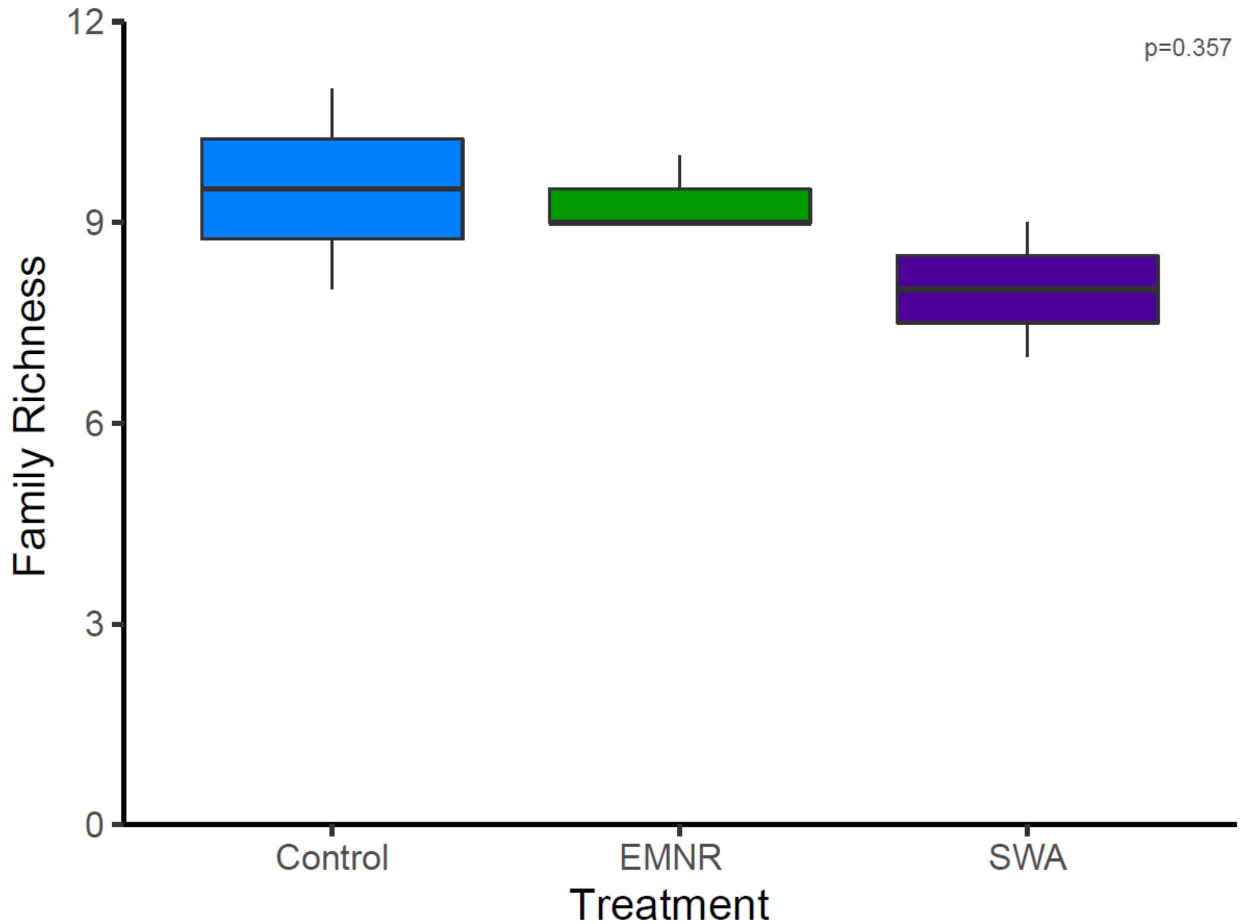

Figure 3.8: Total family richness (sum over all sampling days) in emergence traps on the Peat Organic enclosures. The horizontal line in the box is the median, while the whiskers go from each quartile to the minimum or maximum of the sample set. The enclosures were sampled from June 21, 2019 (Day -1) to August 28, 2019 (Day 68). Treatments EMNR and SWA were not statistically significant from control for post-exposure sampling (Day 12-Day 68) using a Kruskal Wallis test (p=0.357).

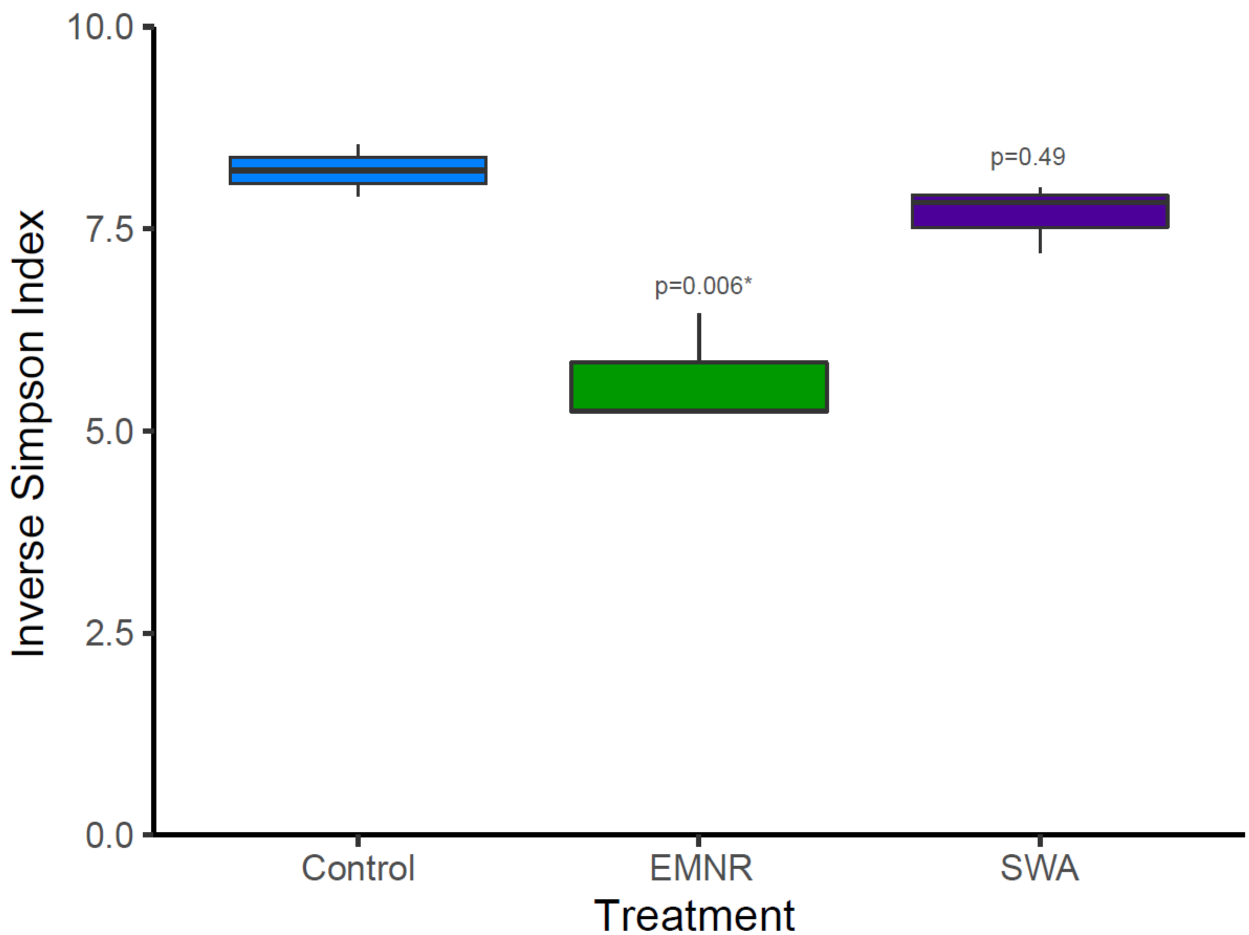

Figure 3.9: Inverse Simpson diversity (sum over all sampling days) in emergence traps on the Peat Organic enclosures. The horizontal line in the box is the median, while the whiskers go from each quartile to the minimum or maximum of the sample set. The enclosures were sampled from June 21, 2019 (Day -1) to August 28, 2019 (Day 68). Treatment EMNR was statistically different from control for post-exposure sampling (Day 12-Day 68) using a one way ANOVA with a Dunnett's test (data not transformed, $p<0.05$).

3.4.3 Benthic Macroinvertebrates Response

Rock Cobble

For the Rock Cobble enclosures, average abundance was not significantly different compared to the control enclosures ($p>0.05$ ANOVA), (Figure 3.10 and Figure 3.15). There were general decreases in the enclosures with the SWA (-15.3%) and EMNR (-26%) treatments compared to the control enclosures. Similarly, richness, and diversity (Inverse Simpson Index) on both family and lowest practical level (LPL, genus or family if it could not be identified) were not significant ($p>0.05$ ANOVA except Diversity (LPL) Kruskall Wallis), for either treatment compared to the control enclosures (Figure 3.11 and 3.12). Taxa richness across enclosures ranged from 9 to 21 taxa, with inverse Simpson diversity varying from 6.73 to 14.28.

The Chironomidae were the most abundant invertebrate group in all enclosures.. The average abundance was 67% for the control enclosures, 72% EMNR and 69% SWA enclosures. Three subfamilies Chironominae, Orthocladinae, and Tanypodinae were present (Figure 3.13); of the three subfamilies the most abundant genera were *Tanytarsus sp., Parakiefferiella sp., Cladotanytarsus sp*., and *Chironomus sp.* At the Rock Cobble sites, Chironominae was the most dominant subfamily in the control enclosures, Tanypodinae was the second out of the three subfamilies with Orthocladinae having the smallest proportion of abundance (Figure 3.13). The SWA and EMNR had a greater abundance of Orthocladinae than Tanypodinae. Both treatments had a decrease in other non-Chironomidae families (Figure 3.16). *Hyalella* sp. as well as the order Trichoptera and Ephemeroptera were decreased in the EMNR treatment. The average abundance of three orders, Ephemeroptera, Trichoptera, and Odonata was 12% for the control, 3% EMNR and 10% SWA enclosures (Figure 3.14). The Order Tubificida did not see any discernable impacts. In the SWA treatments the largest decrease was in chironomids. Figure 3.10, visualizes the difference in lowest possible level identified for the taxa, there are some taxa of chironomids more sensitive than others. Variation between enclosures can be referenced in Appendix Figure B.13

Non-metric multidimensional scaling (NMDS) was used to examine family and site scores using data for all taxonomic groups (Figure 3.17). A stress value of 0.043 and an R^2 of 0.98 indicated a strong ordination following scaling using 1,000 NMDS iterations. Total Chironomidae (broken down to subfamilies Chironominae, Orthocladinae, and Tanypodinae), showed no distinction among treatments and appeared diffuse throughout the ordination. A few taxa only appeared in certain treatment enclosures (Philopotamidae and Phryganeidae in SWA, Lumbriculidae in EMNR and Planorbidae in the control). Beyond these low-abundance taxa, the remaining taxa showed no trends in proportions in the enclosures based on treatment.

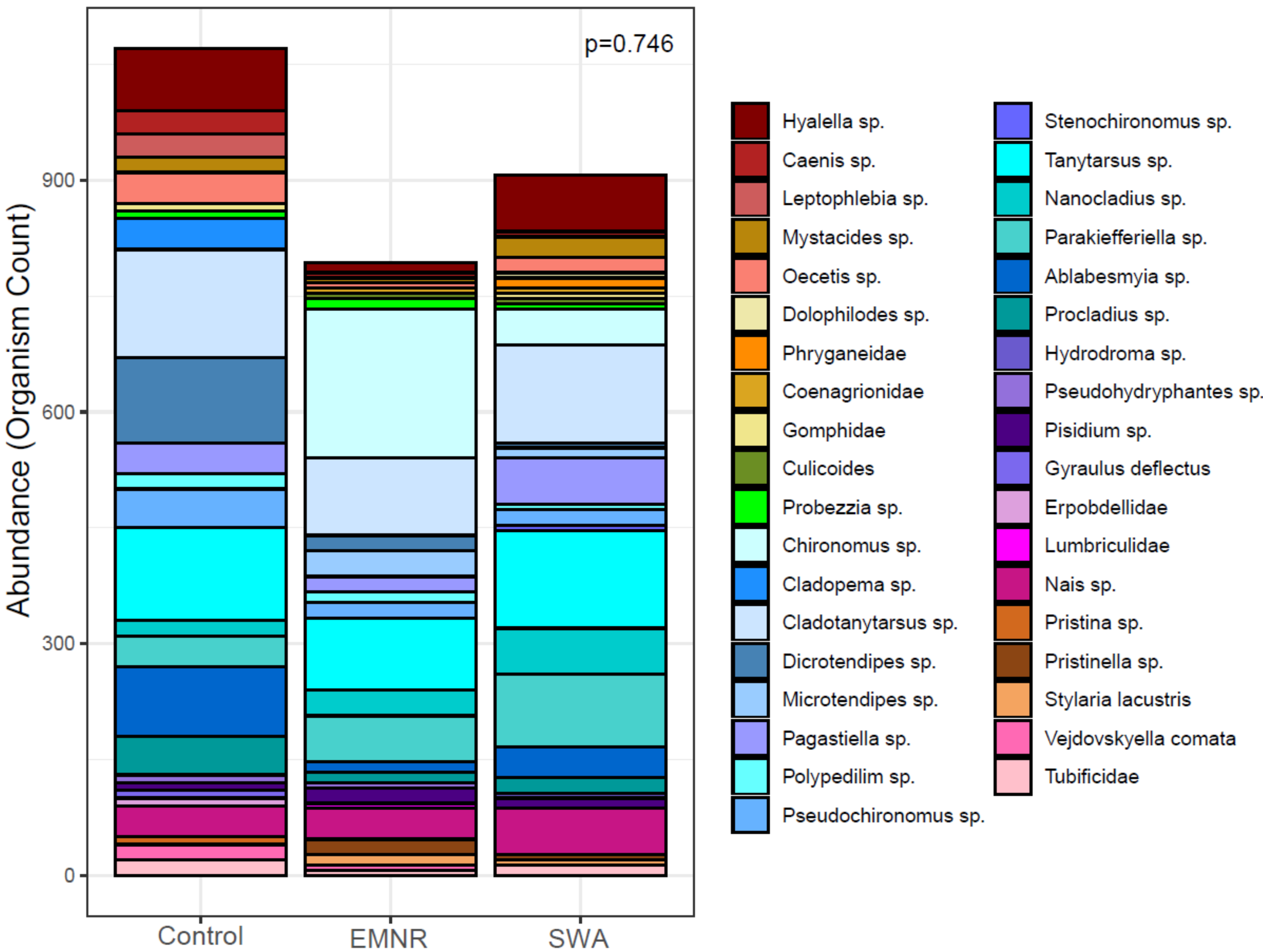

Figure 3.10: Mean abundance (individual organism counts) and organism breakdown from benthic kick net sampling on the Rock Cobble enclosures. The enclosures were sampled on September 24, 2019 (Day 95). Treatment abundances for EMNR and SWA were not statistically significant from control using a one way ANOVA (p=0.746).

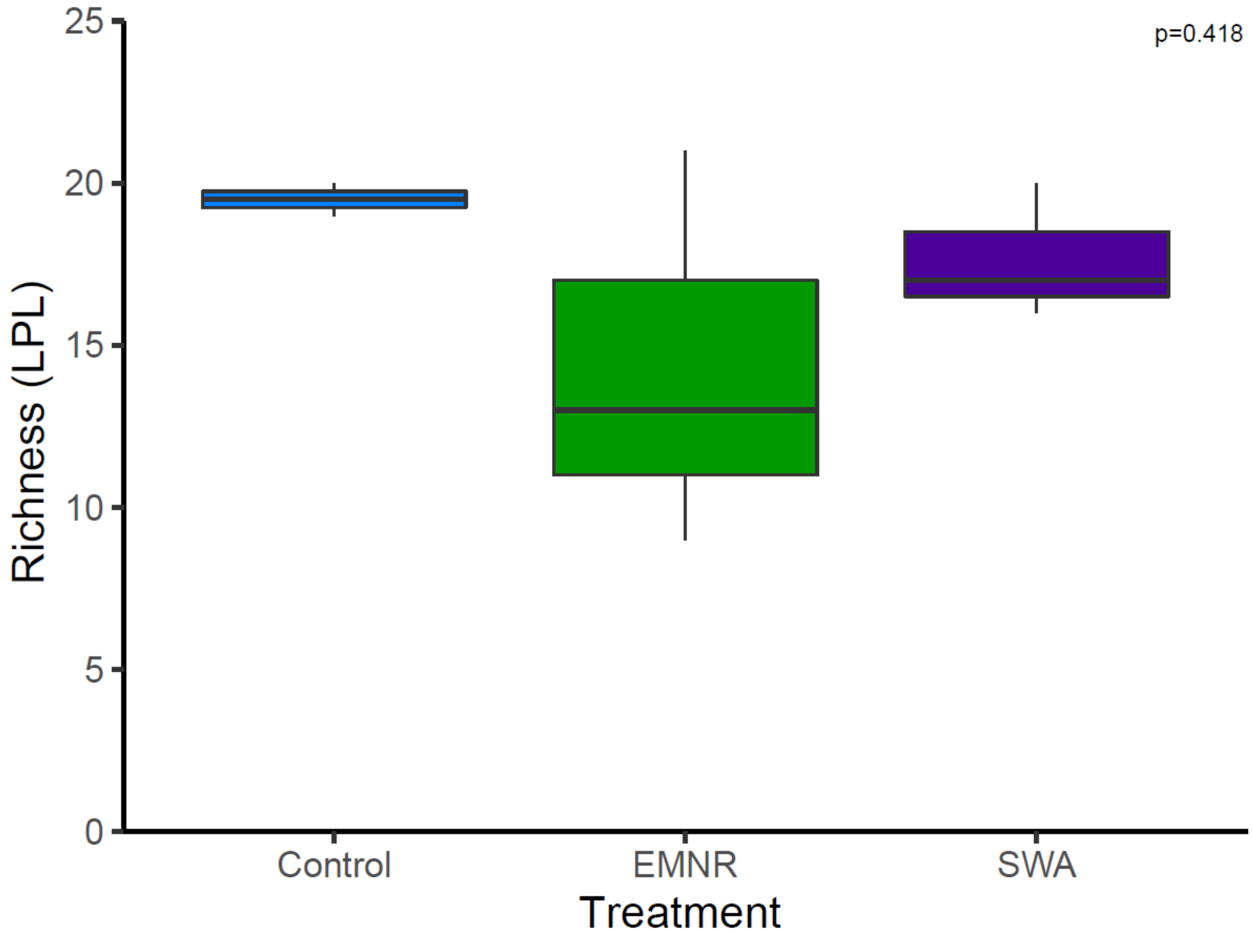

Figure 3.11: Average richness to lowest possible level (genus or family if could not be identified) of benthic kick net sampling on the Rock Cobble enclosures. The horizontal line in the box is the median, while the whiskers go from each quartile to the minimum or maximum of the sample set. The enclosures were sampled on September 24, 2019 (Day 95). Treatments EMNR and SWA were not statistically significant from control using a one way ANOVA (p=0.418).

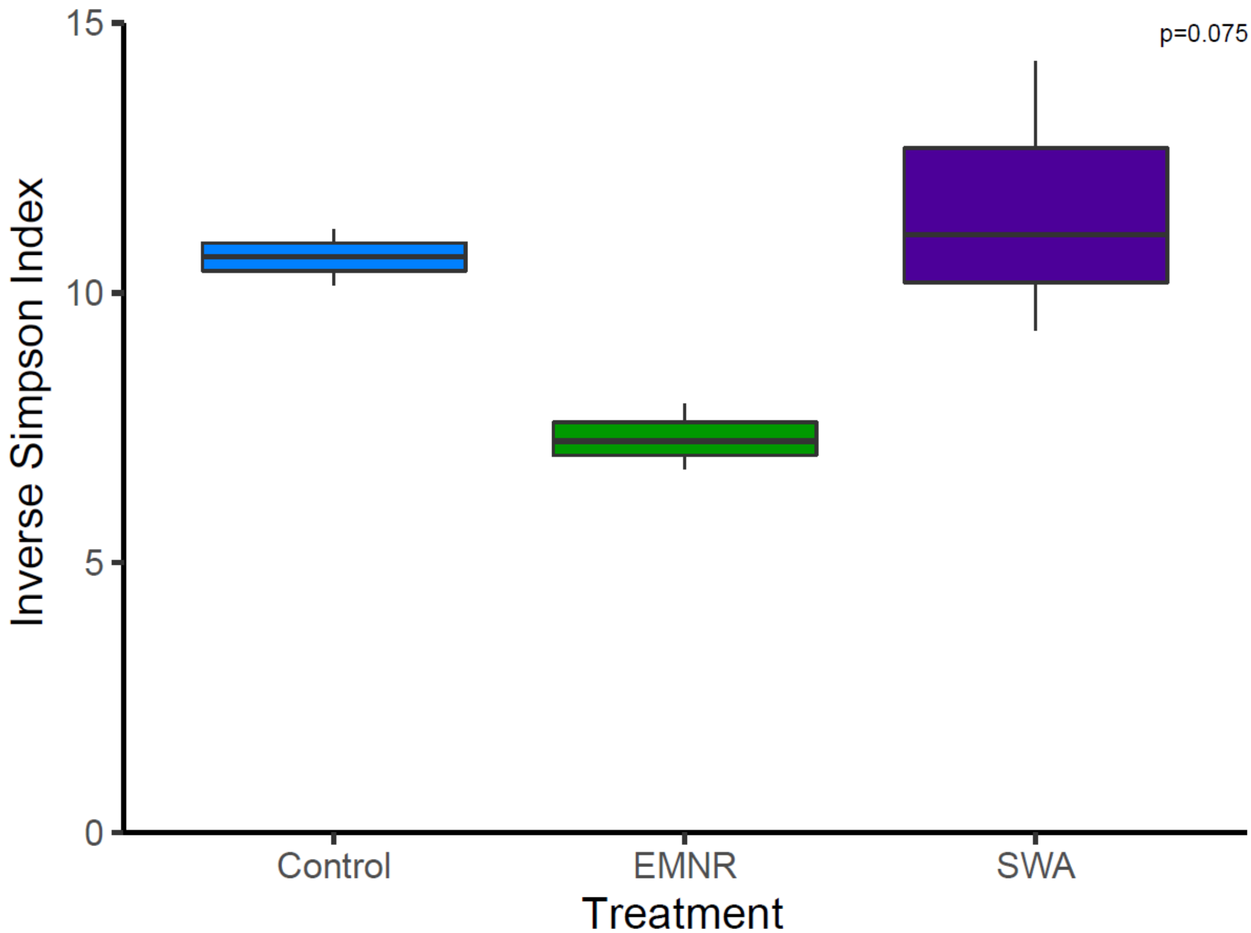

Figure 3. 12: Inverse Simpson to lowest taxa level (genus or family if could not be identified) in benthic kick net on the Rock Cobble enclosures. The horizontal line in the box is the median, while the whiskers go from each quartile to the minimum or maximum of the sample set. The enclosures were sampled on September 24, 2019 (Day 95). Treatments EMNR and SWA were not statistically significant from control using a Kruskal-Wallis test (p=0.075).

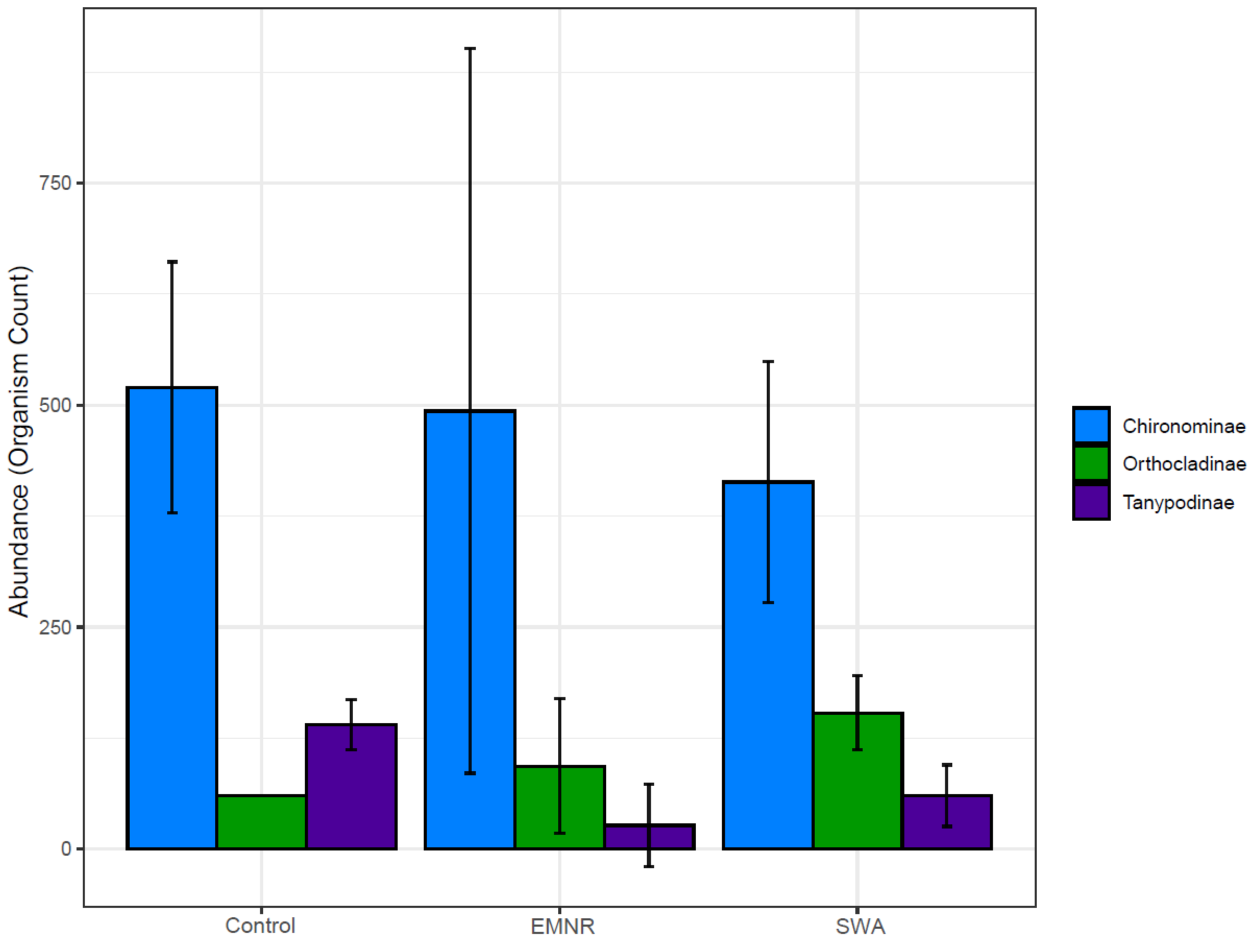

Figure 3. 13: Mean Chironomidae subfamily composition in benthic kick net sampling on the Rock Cobble enclosures. Error bars represent the standard deviation. The enclosures were sampled on September 24, 2019 (Day 95). Total sum of abundance of chironomids in each treatment was not statistically different by one way ANOVA ($p=0.942$) nor for each subfamily ($p>0.05$ ANOVA).

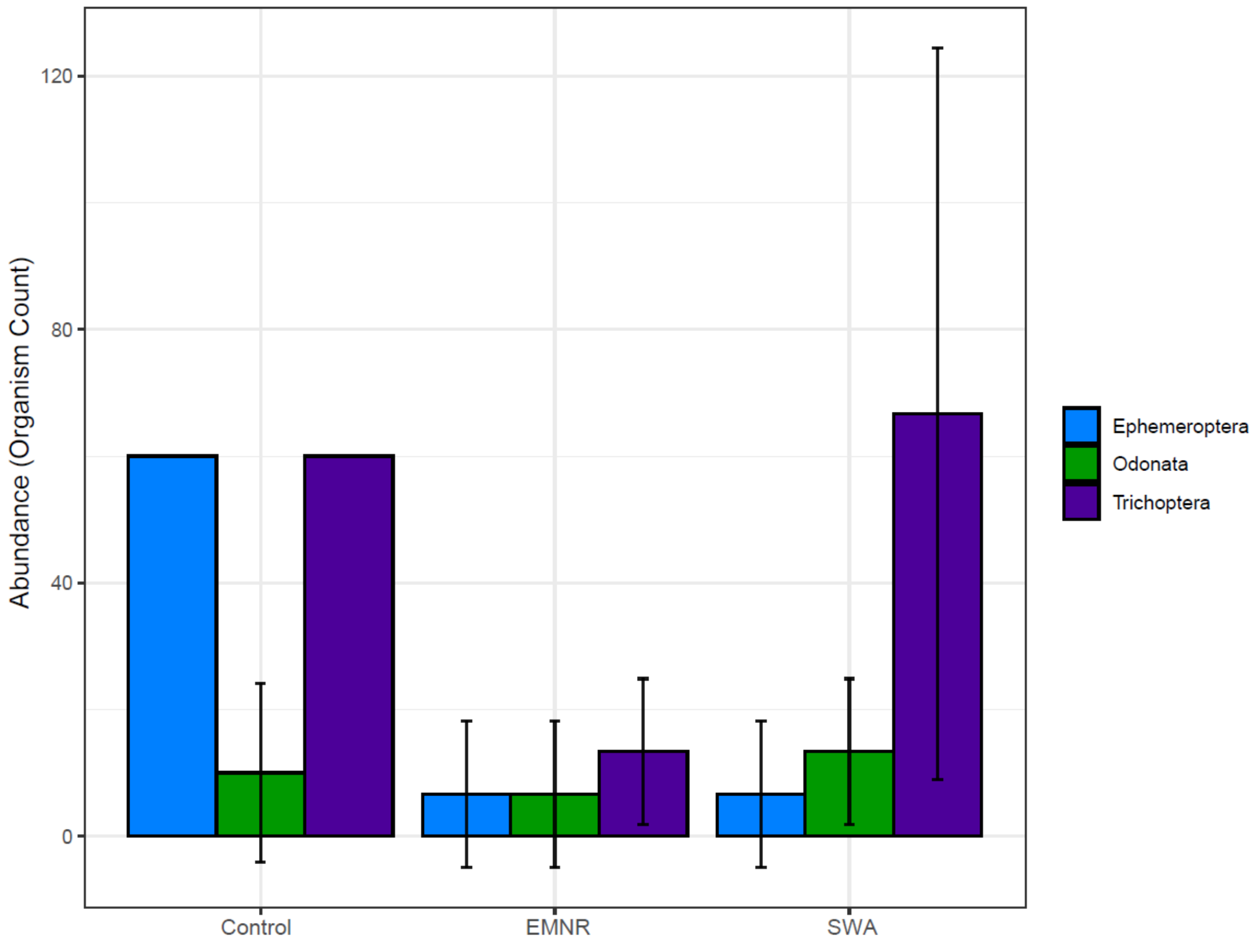

Figure 3.14: Ephemeroptera, Trichoptera and Odonata (ETO) composition in benthic kick net sampling on the Rock Cobble enclosures, the error bars represent the standard deviation. The enclosures were sampled on September 24, 2019 (Day 95). Total sum of abundance of ETO in each treatment was not statistically significant using a one way ANOVA ($p=0.165$) nor was there a statistical significance between each order ($p>0.050$) using an ANOVA for Odonata and Trichoptera and Kruskal Wallis for Ephemeroptera.

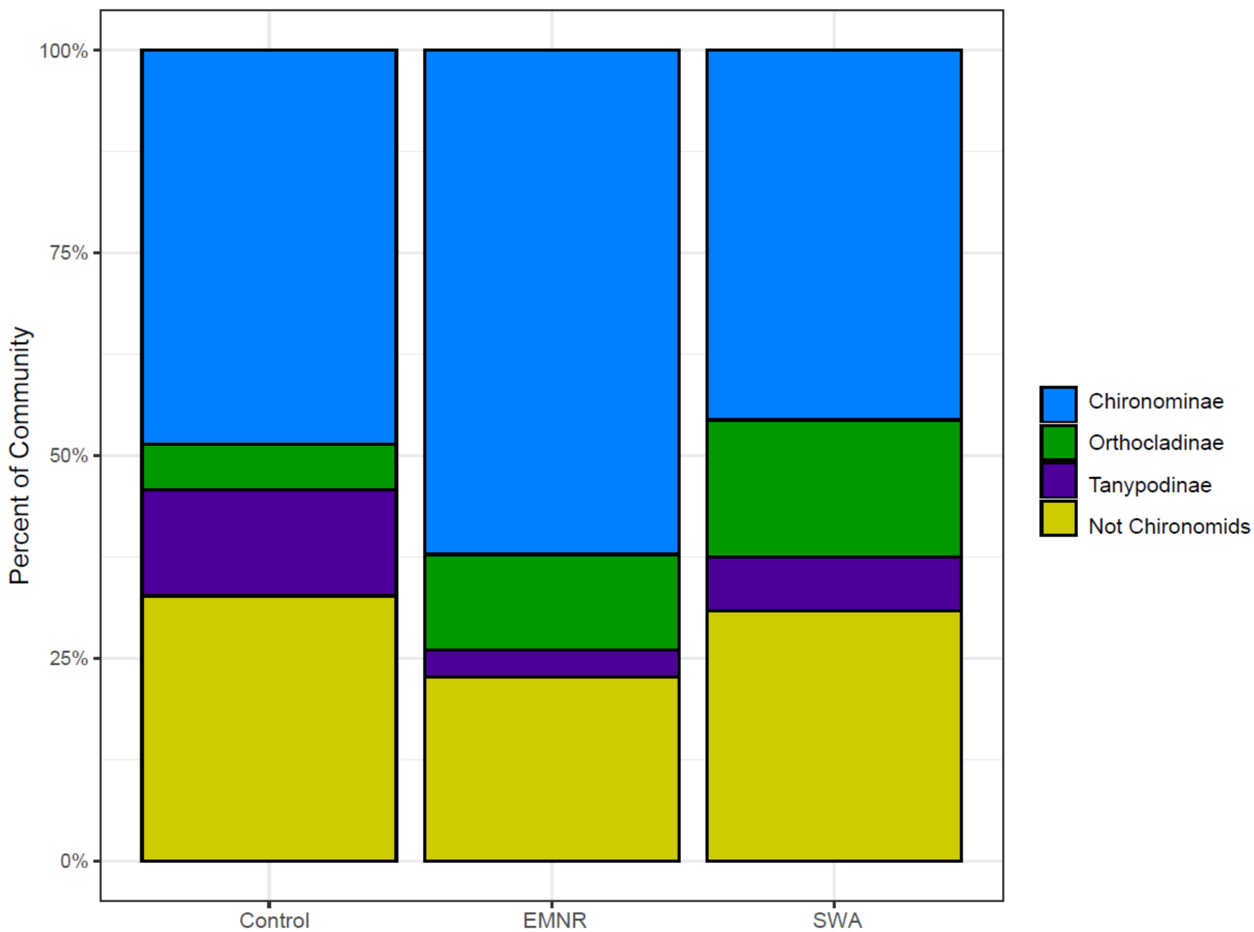

Figure 3.15: Chironomidae subfamily composition and remaining taxa grouped as "not chironomids" in benthic kick net sampling on the Rock Cobble enclosures. The enclosures were sampled on September 24, 2019 (Day 95).

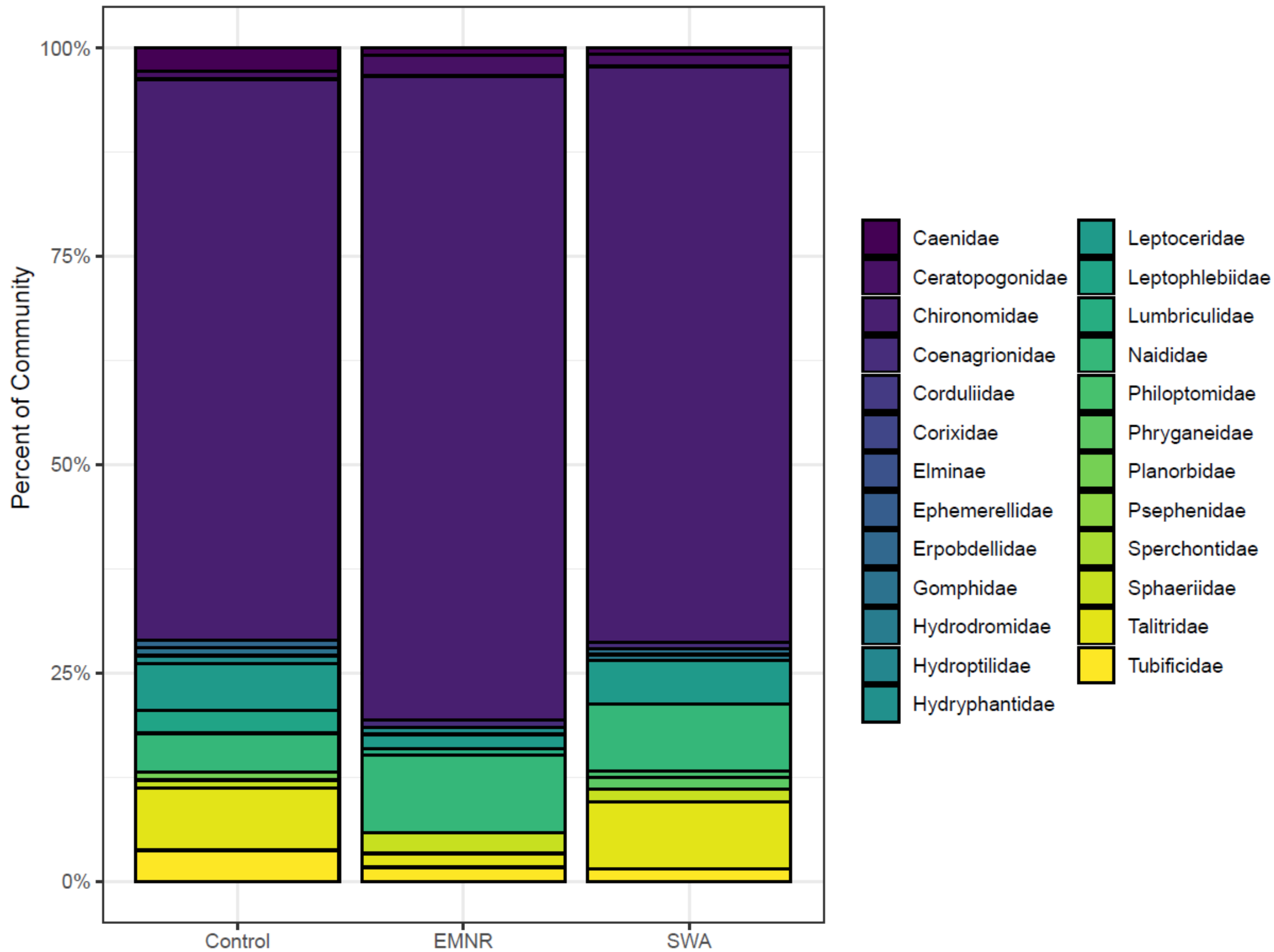

Figure 3.16: Percent stacked counts based on family composition in benthic kick net sampling on the Rock Cobble enclosures. The enclosures were sampled on September 24, 2019 (Day 95).

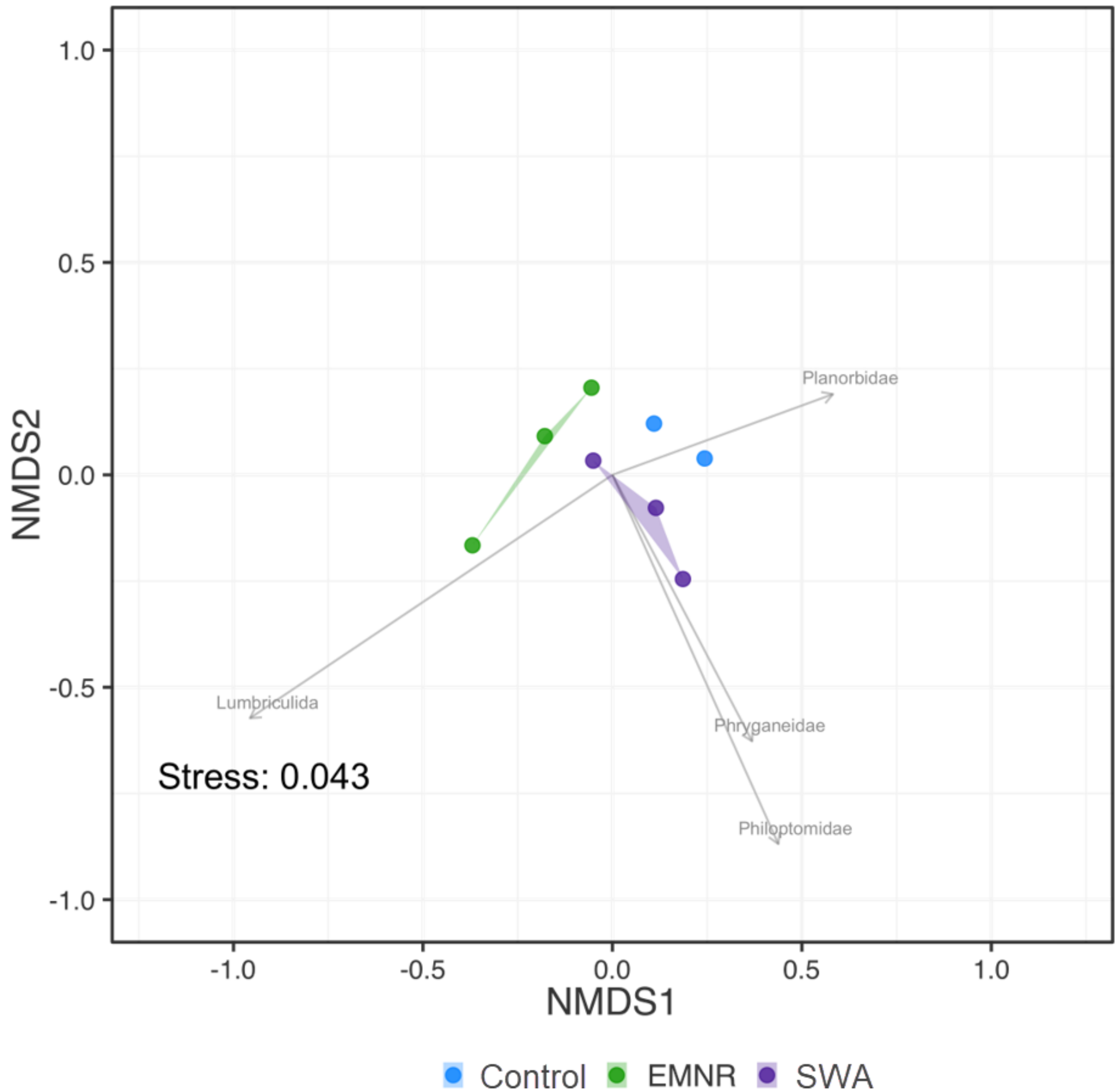

Figure 3. 17: Non-metric multidimensional scaling (NMDS) for benthic invertebrate families (with exception of Chironomidae, where subfamilies were used) using Bray Curtis dissimilarity index on the Rock Cobble shoreline enclosures. The enclosures were sampled on September 24, 2019 (Day 95). The taxa scores are represented as arrows, and only arrows that have an absolute score more than 0.5 on either axis are plotted. Stress value of 0.043 indicates a strong fit of the enclosures and families – this corresponds with a strong linear fit ($R^2 = 0.98$) based on observed dissimilarity of ordination distances.

Peat Organic

For the Peat Organic enclosures, the average abundances were not significantly different as compared to the control ($p>0.05$ ANOVA). There were general decreases in abundance for SWA (-60%) and EMNR (-51%) compared to the control enclosures (Figure 3.18 and Figure 3.24). Similarly, richness and diversity (Inverse Simpson Index) on both family and lowest practical level (LPL) were not significantly different ($p>0.05$ ANOVA), for either treatment compared to the control enclosures (Figure 3.19 and Figure 3.20). Taxa richness across enclosures ranged from 8 to 19 taxa, with inverse Simpson diversity varying from 5.97 to 8.68.

The Chironomidae were the most abundant invertebrate group in all enclosures, which was also observed for the emergence data and the 2018 data. The average abundance was 78% for the control enclosure, 51% EMNR and 76% SWA enclosures. Three subfamilies Chironominae, Orthocladinae, and Tanypodinae were present; of the three subfamilies the most abundant genera were *Tanytarsus sp., Procladius sp., Cladotanytarsus sp., Chironomus sp.,* and *Microtendipes sp.* At the Peat Organic control sites, Chironominae were the dominant subfamily; Tanypodinae was the second out of three subfamilies with Orthocladinae having the lowest abundance (Figure 3.21). This was similar for both treatments; however none of the treatment enclosures had any Orthocladinae present. All three treatments had an increase in relative abundance in other non-Chironomidae families, with a significant impact ($p<0.05$) observed in the *Chironminae sp.* (Figure 3.23) in both treatments compared to the control enclosures. Overall, there was a decrease in relative abundance in chironomids, and an increase in Ephemeroptera in the EMNR treatments while there was a decrease in Ephemeroptera in SWA. For total Ephemeroptera, Trichoptera, and Odonata abundance the average abundance was 12% for the control, 29% EMNR and 11% SWA enclosures (Figure 3.22). Variation between enclosures can be referenced in Appendix Figure B.14.

Non-metric multidimensional scaling (NMDS) was used to examine family and site scores using data for all taxonomic groups (Figure 3.25). A stress value of 0.043 and an R^2 of 0.98 indicated a strong ordination following scaling using 1,000 NMDS iterations. Total Chironomidae (broken down to subfamilies Chironominae, Orthocladinae, Tanypodinae), showed no distinction among treatments and appeared diffuse throughout the ordination. A few taxa only appeared in certain treatment enclosures (*Philopotamidae* and *Phryganeidae* in SWA, *Sericostomatidae, Polycentropodidae* and *Phryganeidae* in EMNR and *Planorbidae* and *Talitridae* in the control). The Ephemeroptera family, Caenidae as previously mentioned was much higher in the enclosures in the located further to the north (Figure 3.3). Leptoceridae and Lumbriculidae were only observed in low counts in a few enclosures. Beyond these low abundance taxa, the remaining taxa no trends in proportions in the enclosures based on treatment. EMNR enclosure demonstrating slightly more dissimilarity compare to the control and SWA enclosures.

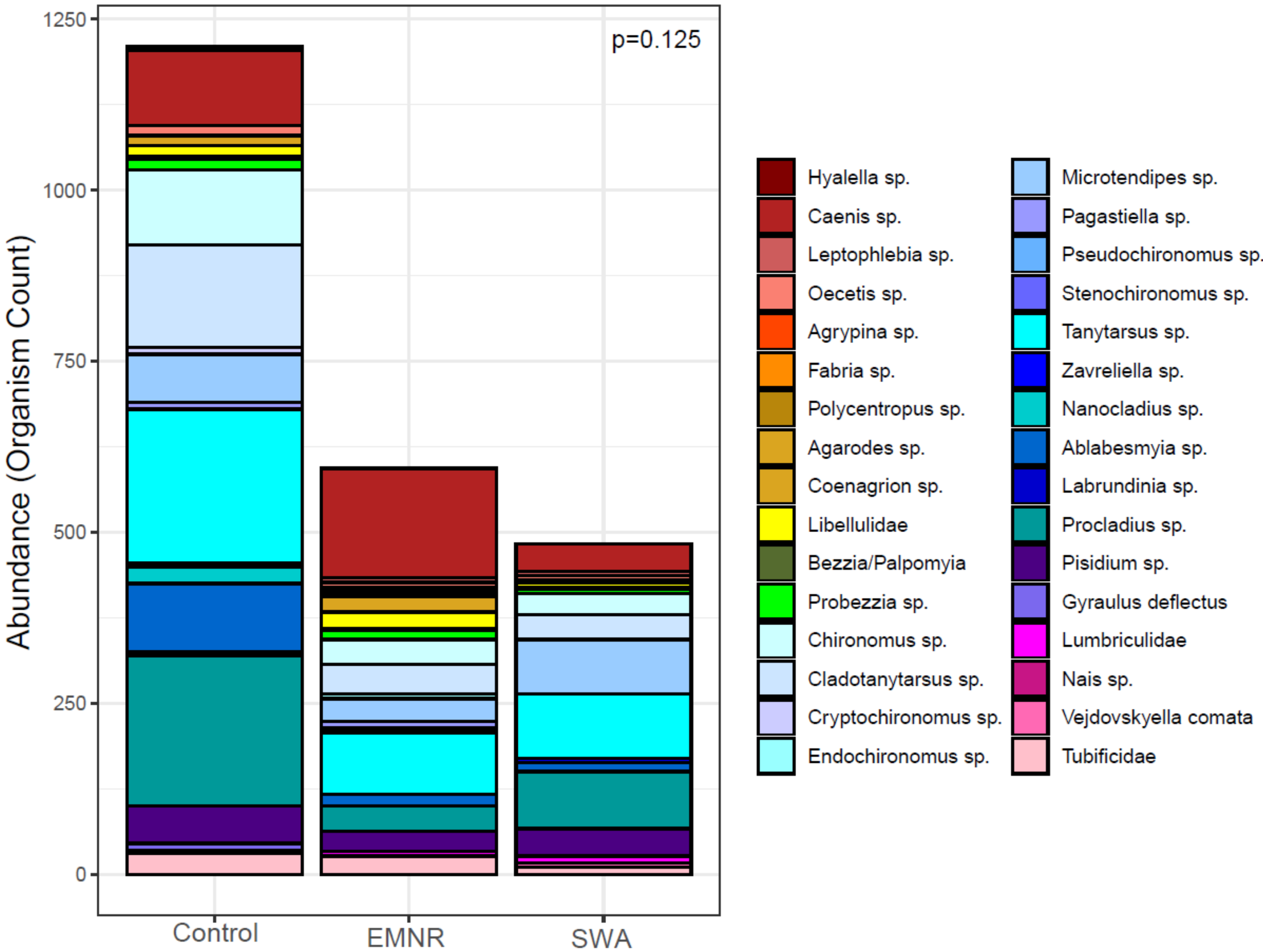

Figure 3.18: Mean abundance (individual organism counts) and organism breakdown from benthic kick net samples on the Peat Organic enclosures. The enclosures were sampled on September 23, 2019 (Day 95). Treatment abundances for EMNR and SWA were not statistically significant from control using a one way ANOVA (p=0.125).

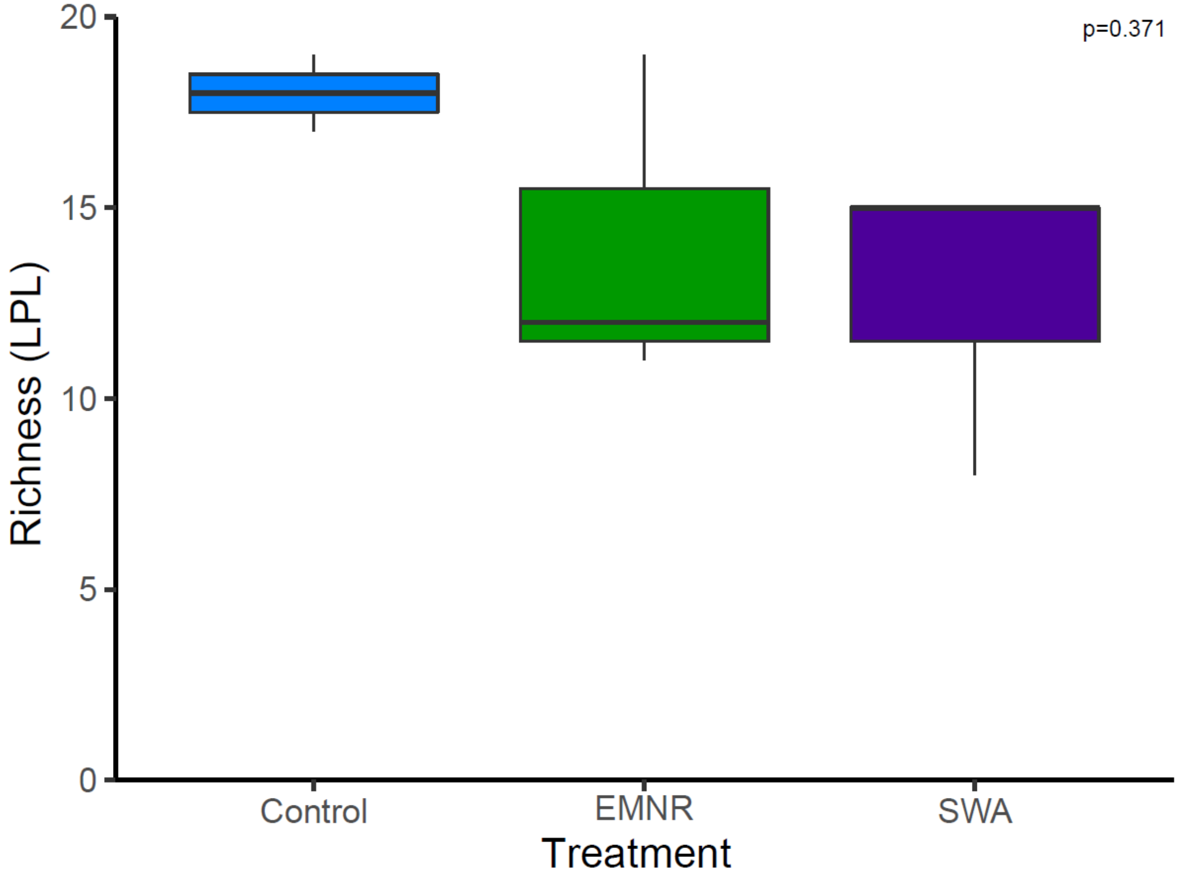

Figure 3.19: Average richness to lowest possible level (genus or family if could not be identified) of benthic kick net sampling on the Peat Organic enclosures. The horizontal line in the box is the median, while the whiskers go from each quartile to the minimum or maximum of the sample set. The enclosures were sampled on September 23, 2019 (Day 95). Treatments EMNR and SWA were not statistically significant from control using a one way ANOVA (p=0.371).

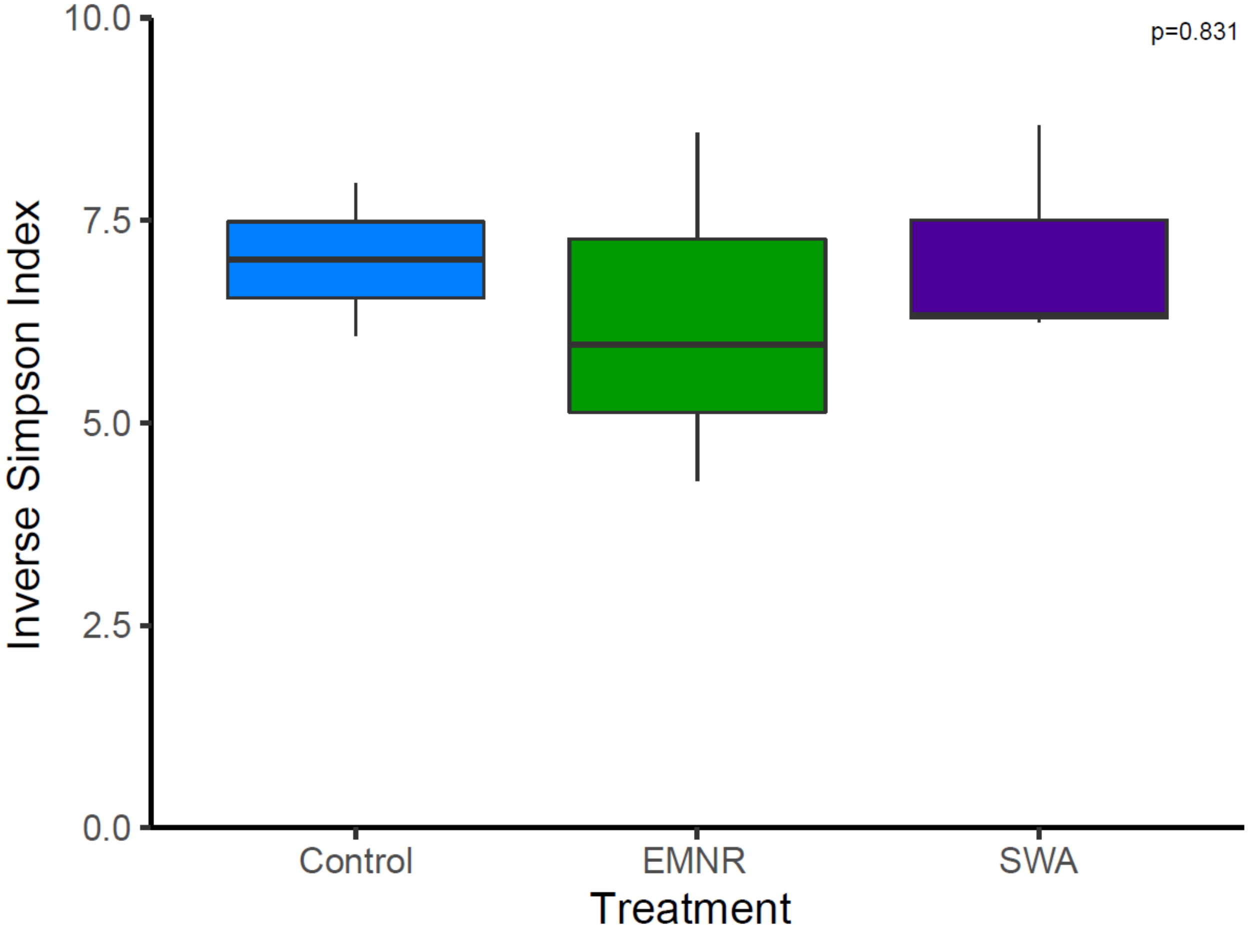

Figure 3.20: Inverse Simpson to lowest taxa level (genus or family if could not be identified) in benthic kick net on the Peat Organic enclosures. The horizontal line in the box is the median, while the whiskers go from each quartile to the minimum or maximum of the sample set. The enclosures were sampled on September 23, 2019 (Day 95). Treatments EMNR and SWA were not statistically significant from control using a one way ANOVA (p=0.831).

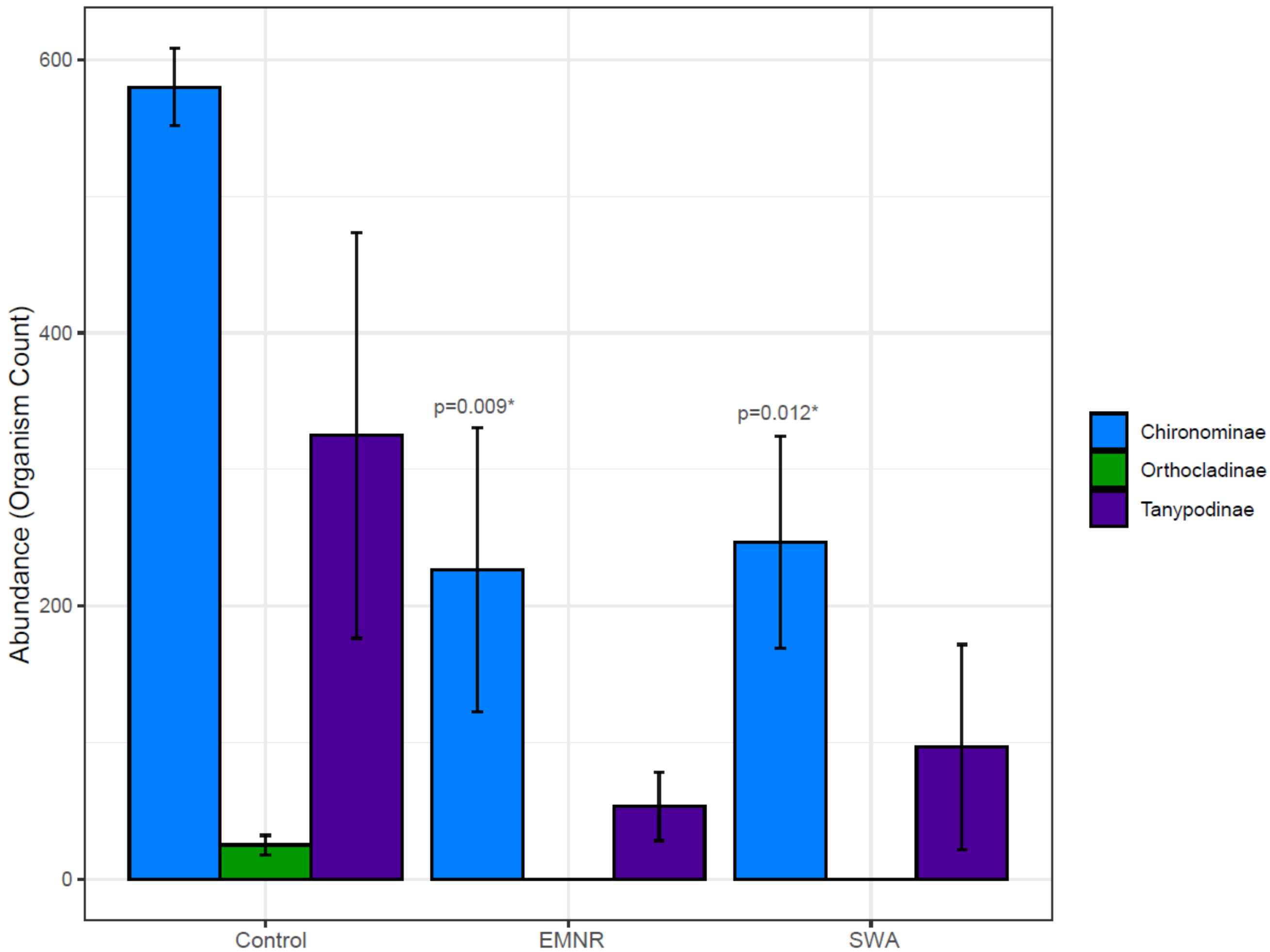

Figure 3.21: Mean Chironomidae subfamily composition in benthic kick net sampling on the Peat Organic enclosures, the error bars represent the standard deviation. The enclosures were sampled on September 23, 2019 (Day 95). Total sum of abundance of chironomids in each treatment were statistically different as well the Subfamily Chironominae were statistically different from the control (p=0.012 EMNR and p=0.009 SWA using ANOVA with a Dunnett's test)

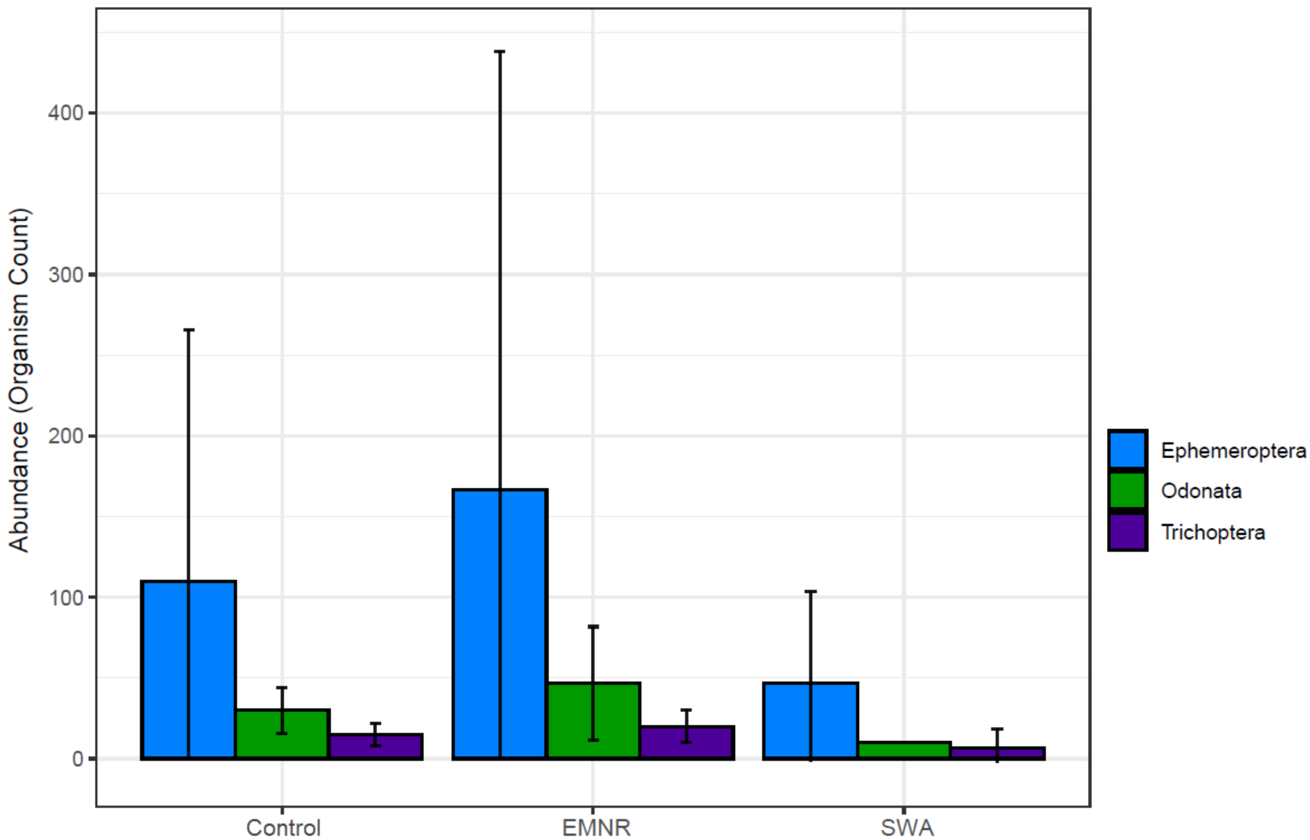

Figure 3. 22: Ephemeroptera, Trichoptera and Odonata (ETO) composition in benthic kick net sampling on the Peat Organic enclosures, the error bars represent the standard deviation. The enclosures were sampled on September 23, 2019 (Day 95). Total sum of abundance of ETO in each treatment was not statistically significant compared to the control ($p=0.646$ ANOVA); abundance of specific orders were also not significant compared to the control ($p>0.05$ ANOVA) for Odonata and Trichoptera and Kruskal Wallis for Ephemeroptera).

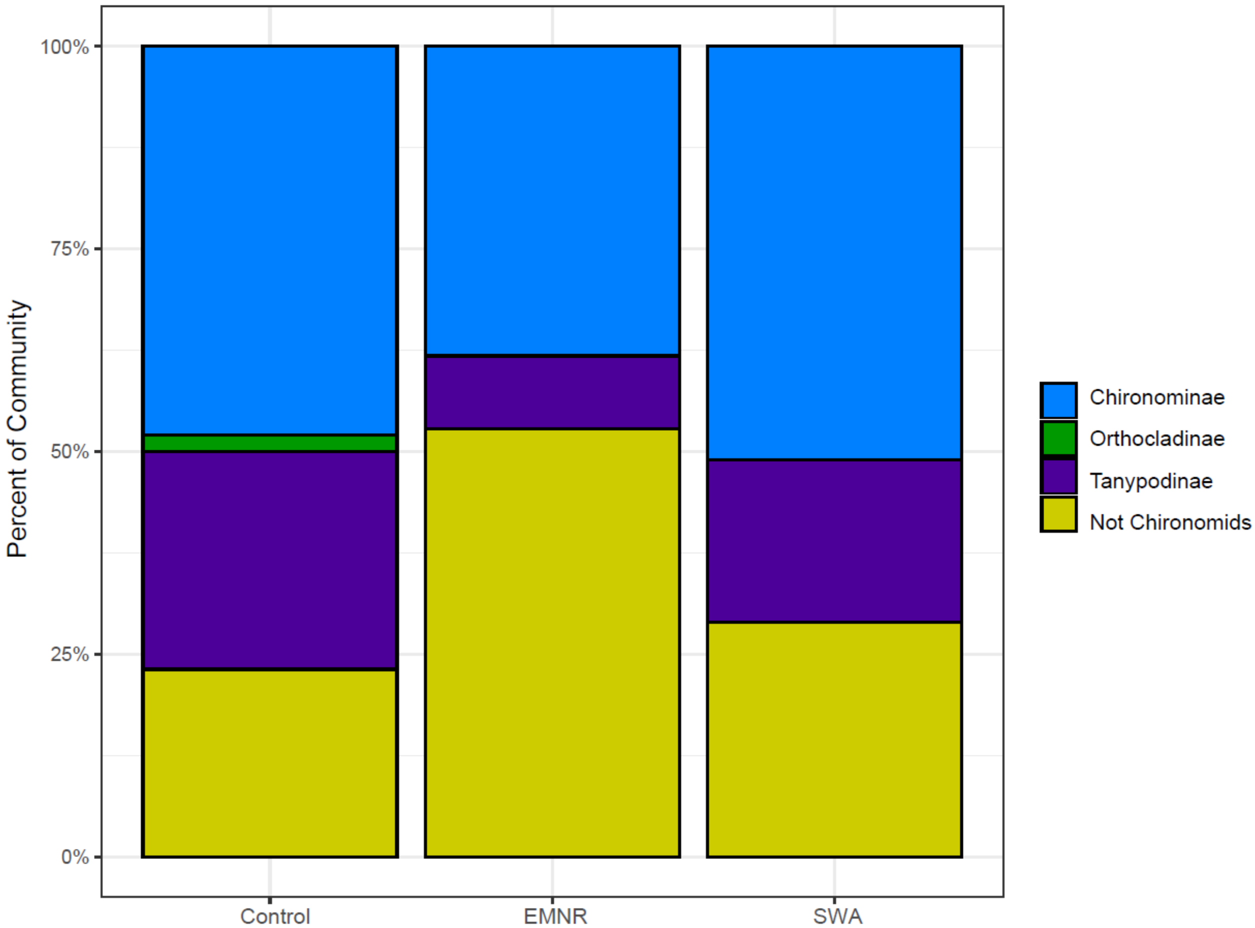

Figure 3.21: Chironomidae subfamily composition and remaining taxa grouped as “not chironomids” in benthic kick net sampling on the Peat Organic enclosures. The enclosures were sampled on September 23, 2019 (Day 95).

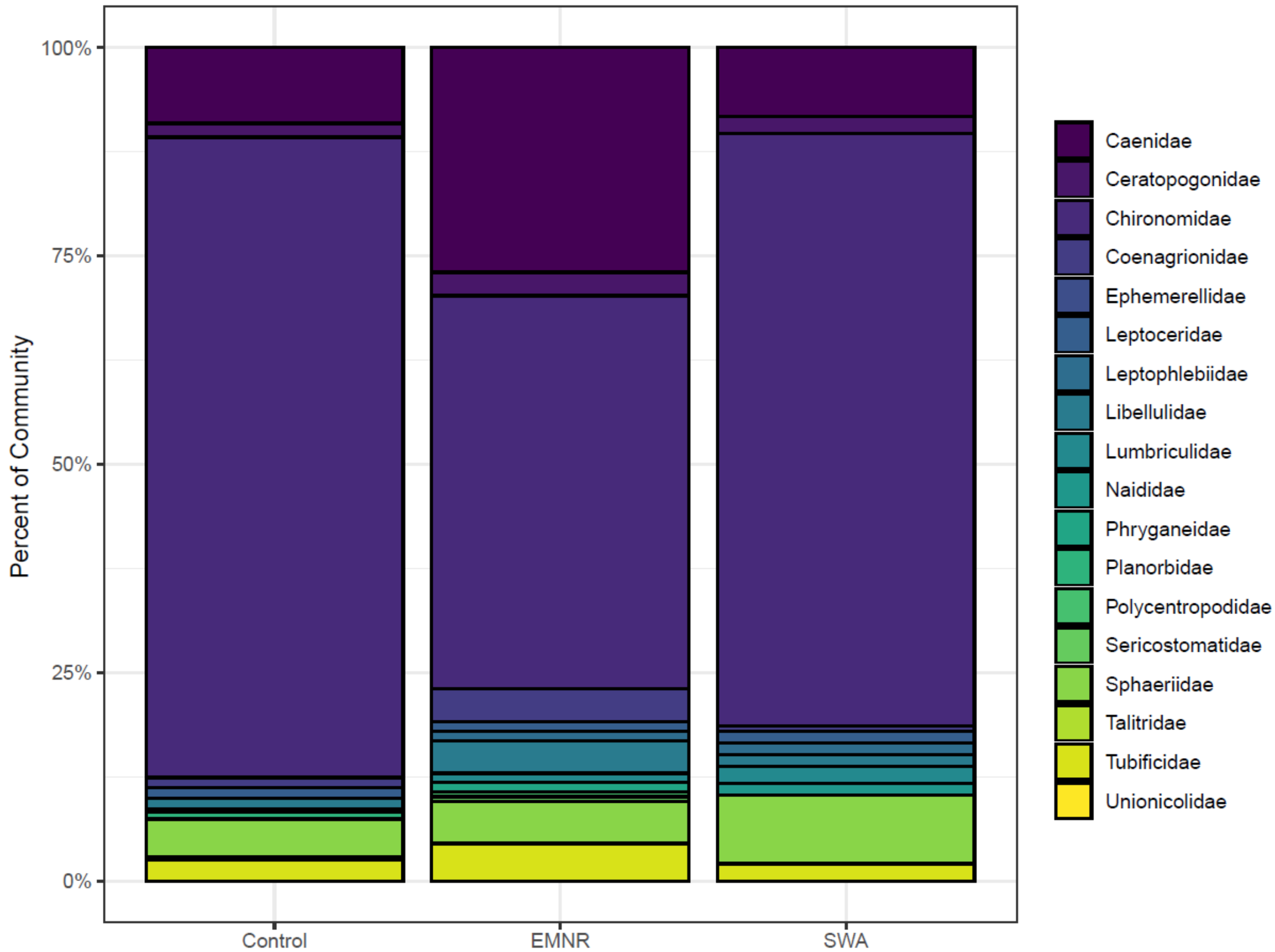

Figure 3.22: Percent stacked counts based on family composition in benthic kick net sampling on the Peat Organic enclosures. The enclosures were sampled on September 23, 2019 (Day 95).

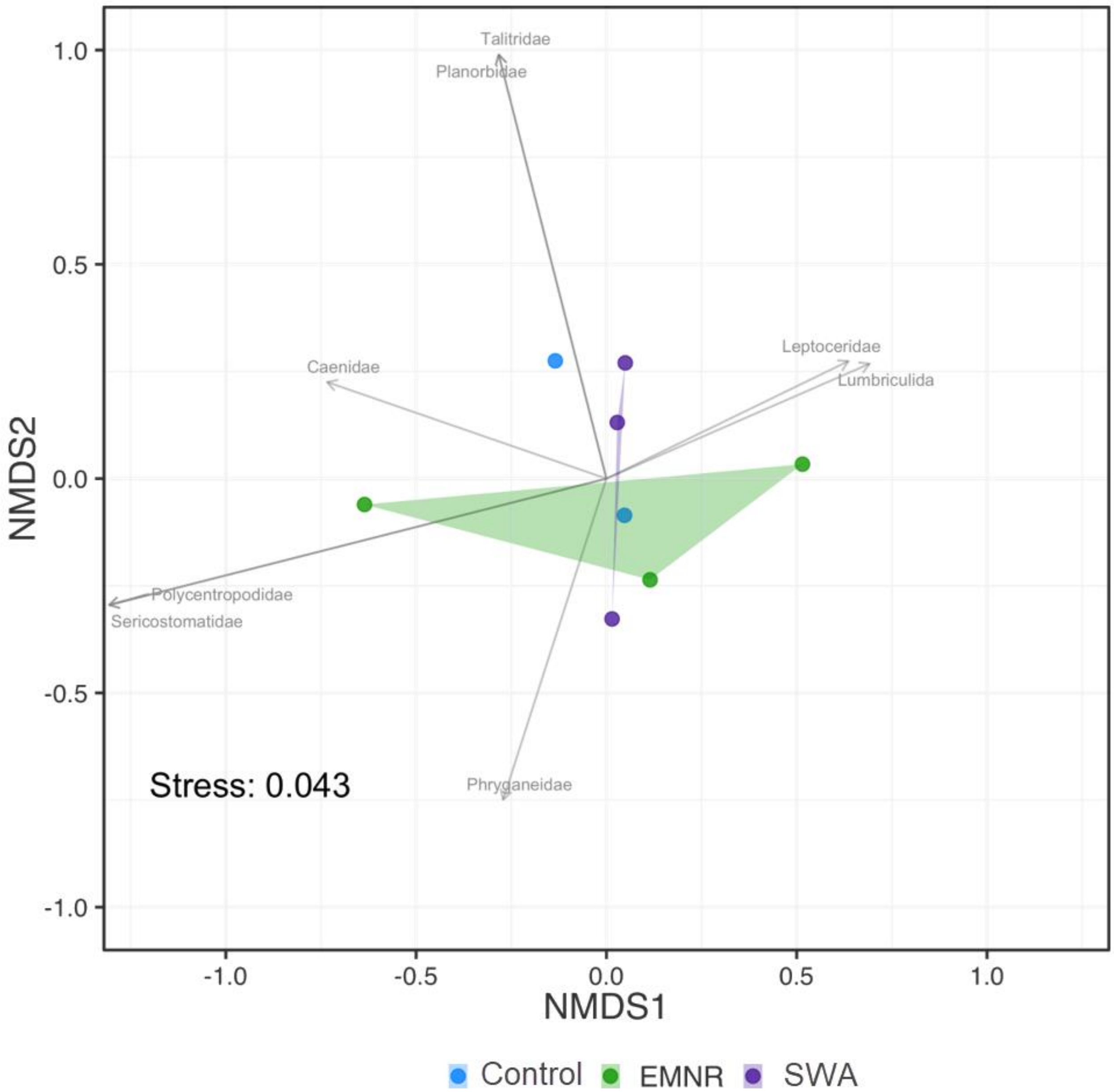

Figure 3. 23: Non-metric multidimensional scaling (NMDS) for benthic invertebrate families (with exception Chironomidae subfamilies) using Bray Curtis dissimilarity index on the Peat Organic shoreline. The enclosures were sampled on September 23, 2019 (Day 95). The taxa scores are represented as arrows, and only arrows that have an absolute score more than 0.5 on either axis are plotted. Stress value of 0.043 indicates a good fit of the enclosures and families – this corresponds with a strong linear fit (R^2 = 0.98) based on observed dissimilarity of ordination distances.

3.4.4 Power Analysis

Large variation among replicates, due to a combination of natural variation, differing shoreline locations within the lake and treatment related variability was observed. Since variability has a major impact on power and sample size requirements, a power analysis was completed (Table 3.1), to determine if the study could detect a ecologically meaningful significant difference between control and treatment means. Statistical power is a function of the sample size, the variability and the target difference between areas. The coefficient of variation (COV), expressed as a percentage of the control mean (COV = SD / control mean x 100) is used as a measure of variability in sample size calculations (Environment Canada, 2012B).

A statistical difference in the mean abundance between controls and treatments exceeding 25% are ecologically meaningful/relevant (Environment Canada, 2010). Additionally, many researchers agree that a power of 80% (detecting an effect size of 20% or greater from the control) or higher as credible enough for determining the actual effects of research studies (Bezeau and Graves 2001). Review of the minimum sample size required to statistically detect a 25% difference between sample means reveals that the sample size of the study (n ~ 3) was not sufficient to detect an ecologically meaningful difference. Future studies should consider increasing the number of replicates in efforts to reduce risk of a Type II error since a sample size of n~3 did not have enough statistical power to detect effects due to high variability. The greater the sample size, the less chance you will have in making a Type II error.

Table 3. 1: Power analysis table for each endpoint in each shoreline type to determine minimum sample size required to be ecologically meaningful.

Endpoint	Sample Size		Model	Treatment with most different mean	Mean		MSE	Magnitude of Difference (%)	Minimum effect size detectable (%)		Minimum Sample Size
Response	Control	Treatment			Control	Treatment			Increase	Decrease	Increase 25%, decrease 20%
PO Total Abundance Emergence	2	3	ANOVA	EMNR	781.333	1138.963	47353.8478	45.772	140.723	-140.723	22
PO Total Richness Emergence	2	3	ANOVA	SWA	9.5	8.333	2.3888	-12.284	82.203	-82.203	8
PO Total Diversity Emergence	2	3	ANOVA	EMNR	8.705	5.833	0.8433	-32.993	53.302	-53.302	4
RC Total Abundance Emergence	2	3	ANOVA	EMNR	935.11	2243.517	1856057.12	139.920	736.134	-736.134	583
RC Total Richness Emergence	2	3	ANOVA[1]	EMNR	9	12	3.3333	33.333	102.499	-102.499	12
RC Total Diversity Emergence	2	3	ANOVA	SWA	8.28	6.753	0.4764	-18.442	42.119	-42.119	3
PO Total Abundance Emergence (no pre exposure)	2	3	ANOVA	EMNR	757.331	1120	192317.719	47.888	292.582	-292.582	93
PO Total Richness Emergence (no pre exposure)	2	3	ANOVA[1]	SWA	9.5	8	1.43333	-15.789	63.676	-63.676	6
PO Total Diversity Emergence (no pre exposure)	2	3	ANOVA	EMNR	8.225	5.64	0.308	-31.429	34.093	-34.093	2
RC Total Abundance Emergence (no pre exposure)	2	3	ANOVA	EMNR	900.389	2056.888	1056720.25	128.444	576.864	-576.864	358
RC Total Richness Emergence (no pre exposure)	2	3	ANOVA	EMNR	9.5	11.667	1.967	22.811	74.594	-74.594	7
RC Total Diversity Emergence (no pre exposure)	2	3	ANOVA	SWA	8.36	6.117	1.3	-26.830	68.911	-68.911	6

Endpoint	Sample Size		Model	Treatment with most different mean	Mean		MSE	Magnitude of difference (%)	Minimum effect size detectable (%)		Minimum Sample Size
Response	Control	Treatment			Control	Treatment			Increase	Decrease	Increase 25%, decrease 20%
PO Total Abundance Benthics	2	3	ANOVA	SWA	1210	483.333	75822.2222	-60.055	114.984	-114.984	15
PO Total Richness Benthics (LPL)	2	3	ANOVA	SWA	18	12.667	11.5555	-29.628	95.421	-95.421	11
PO Total Richness Benthics (Family)	2	3	ANOVA	SWA	9	7.333	12.2222	-18.522	196.271	-196.271	43
PO Total Diversity Benthics (LPL)	2	3	ANOVA	EMNR	7.015	6.28	3.6982	-10.478	138.513	-138.513	22
PO Total Diversity Benthics (Family)	2	3	ANOVA	EMNR	1.625	2.747	0.196	69.046	137.657	-137.657	22
RC Total Abundance Benthics	2	3	ANOVA	EMNR	1070	793.333	236422.222	-25.857	229.606	-229.606	58
RC Total Richness Benthics (LPL)	2	3	ANOVA	EMNR	19.5	14.333	25.0555	-26.497	129.700	-129.700	19
RC Total Richness Benthics (Family)	2	3	ANOVA[2]	EMNR	1	0.778	0.0206	-40.021	431.128	-81.172	39
RC Total Diversity Benthics (LPL)	2	3	ANOVA[1]	EMNR	10.66	7.3	0.4019	-31.520	30.049	-30.049	2
RC Total Diversity Benthics (Family)	2	3	ANOVA	EMNR	2.178	1.846	0.1588	-15.243	92.447	-92.447	10
(DAY-1-DAY 33) PO Abundance	2	3	ANOVA	EMNR	418.667	615.704	20578.152	47.063	173.124	-173.124	33
(DAY-1-DAY 33) PO Diversity	2	3	ANOVA	EMNR	4.26	3.617	0.302	-15.094	65.180	-65.180	6
(DAY-1-DAY 33) PO Richness	2	3	ANOVA[2]	EMNR	0.772	0.864	0.00431	23.595	114.641	-53.411	9
(DAY12-DAY 33) PO Abundance (no pre exposure)	2	3	ANOVA	EMNR	394.666	596.737	53374.819	51.201	295.775	-295.775	95
(DAY12-DAY 33) PO Diversity (no pre exposure)	2	3	ANOVA	SWA	3.825	3.477	0.24	-9.098	64.714	-64.714	6

Endpoint	Sample Size		Model	Treatment with most different mean	Mean			Magnitude of difference (%)	Minimum effect size detectable (%)		Minimum Sample Size
Response	Control	Treatment			Control	Treatment	MSE		Increase	Decrease	Increase 25%, decrease 20%
(DAY12-DAY 33) PO Richness (no pre exposure)	2	3	ANOVA	EMNR	6	7.333	3.067	22.217	147.479	-147.479	25
PO ETO Benthic Abundance	2	3	ANOVA	SWA	155	63.3333333	11905.5555	-59.140	355.686	-355.686	137
PO Chironomid Benthic Abundance	2	3	ANOVA	EMNR	930	280	15133.3333	-69.892	66.836	-66.836	6
RC ETO Benthic Abundance	2	3	ANOVA	EMNR	130	26.6666667	422.222222	-79.487	79.864	-79.864	8
RC Chironomid Benthic Abundance	2	3	ANOVA	EMNR	720	613.333333	194755.555	-14.815	309.696	-309.696	104

ANOVA[1]= RC Total Abundance Emergence and RC Total Diversity Benthics (LPL) originally ran via Kruskal Wallis, however for this test used ANOVA

ANOVA[2]= RC total Richness Benthics (family) and (DAY-1-DAY 33) PO Richness was transformed to log10

$n=2(t_{\alpha}|+t_{\beta})^2(COV/CES)^2$

n=sample size

t_{α}=value of Students t statistic (two tailed) with (n-1) degrees of freedom at a significance level of α (0.05)

t_{β}= value of Students t statistic (one tailed) with (n-1) degrees of freedom at a significance level of β (0.1)

COV= coefficient of variation (expressed as a percentage)

CES= critical effect size, represented in the measurement units of the responsible variable

3.5 Discussion

This study aimed to characterize the effects of two forms of secondary cleanup methods (EMNR and SWA) following dilbit release on macroinvertebrates in freshwater littoral habitats (Peat Organic and Rock Cobble). For the Rock Cobble habitats, we observed no significant adverse effects ($p > 0.05$) for emergent insects or benthic invertebrates in abundance, diversity or richness for both SWA and EMNR compared to the controls that received no oil. Therefore, my hypothesis that SWA treatments following dilbit release will have a negative impact on emergent insects or benthic invertebrates for the Rock Cobble enclosures is not supported and that EMNR treatments will have an equal impact on emergent insects or benthic invertebrates compared to the control was supported. For the Peat Organic enclosures, we observed no significant adverse effects ($p > 0.05$) on emergent insects or benthic invertebrates in abundance or richness for both SWA and EMNR compared to the control. However, diversity for EMNR was significantly decreased ($p<0.05$) compared to the control due to high abundance of chironomids relative to other taxa (may have been a results of a Type 1 Error). Therefore, our hypothesis that SWA treatments following dilbit release will have a negative impact on emergent insects or benthic invertebrates for the Peat Organic enclosures is not supported, but there is some evidence of adverse impacts of EMNR, but are considered minor, as discussed below. These results indicate that the addition of chemical and biological treatments in response to a dilbit release in freshwater has no significant adverse impacts to aquatic macroinvertebrates, however large sample variation and results from the power analysis did not meet ecological relevance. It must be noted that the results from the Rock Cobble enclosures have significant uncertainty associated with them due to the enclosure leakage and should be interpreted cautiously. Despite this, the relatively sessile nature of most benthic organisms means those found in the enclosures likely did not migrate in from outside, and vice versa, meaning there would have been some exposure to both dilbit and spill responses agents to organisms at least initially.

3.5.3 Comparison to dilbit only treatments from 2018 Pilot Study (Chapter 2)

This study was designed to assess the effect of oil releases into natural freshwater environments; one that would have bulk oil removed mechanically via boom, skimmers, and sorbents with residual amounts of oil to potentially collect along shorelines (2018 pilot study, Chapter 2) and the effects of secondary treatments on the residual oil (2019 FOReSt). As demonstrated in the pilot study (Chapter 2), residual dilbit after primary cleanup methods are employed can negatively affect macroinvertebrates. The 2018 pilot study did not include replication of treatments or controls, therefore it is challenging to statistically compare the results between the plot study and the 2019 study; however, some generalizations can be made. In the pilot study, a large decrease in abundance and richness of emergent insects and benthic macroinvertebrates occurred in the dilbit treatment relative to the control enclosures. Additionally, no recovery was observed for macroinvertebrates over the duration the 2018 study. This contrasts with the 2019 study that did not observe statistically significant impacts from both dilbit and SWA and dilbit and EMNR treatments on emergent insects and benthic macroinvertebrates in both shoreline types, relative to unoiled controls.

To contrast the two studies, the dilbit only treatment (2018) had total abundance supressed by 68% (emerging insects) and 60% (benthic invertebrates) in the dilbit enclosures. Rather than detecting declines in emergent insects in response to oil additions and treatments, the 2019 study found the average total abundance of the emerging insects was increased in EMNR (135%) and SWA (13%) for Rock Cobble and increased in EMNR (46%) and SWA (32%) in the Peat Organic treatment relative to the control enclosures (Figure 3.24 and 3.25). However, while not statistically significant the direction of effect for benthic macroinvertebrates in the 2019 study was similar to the 2018 pilot study. In the 2019 study the average total abundance of benthic macroinvertebrates was suppressed by 15% (SWA) and 26% (EMNR) for the Rock Cobble treatments and by 60% (SWA) and 51% (EMNR) for Peat Organic treatments relative to control enclosures (Figure 3.24 and 3.25). While these effects were not found to be statistically significant, as our power analysis indicated that our sample size was potentially insufficient, we cannot discount the potential for effects of the treatments on the benthic macroinvertebrate community. The dilbit only treatment (2018) richness (Family) measures were supressed by 20% (emerging insects) and 23% (benthic invertebrates) in the dilbit enclosure. In the 2019 study, the average richness of emerging insects increased in EMNR (28%), and SWA (10%) for Rock Cobble and decreased in EMNR (2%) and SWA (13%) for Peat Organic compared to control enclosures (Figure 3.24 and 3.25). For benthic invertebrates, average richness (LPL) was suppressed by 10% (SWA) and 30% (EMNR) for the Rock Cobble and by 36% (SWA) and 25% (EMNR) for Peat Organic enclosures compared to control enclosures, again indicating some impact, though not statistically significantly different (Figure 3.24

& 3.25). The two studies had the same amount of oil applied (0.05 L/m^2) and differences in total abundance results between the two studies are stark but richness metrics are similar, with the exception of Rock Cobble richness in benthic inverebrates. This 2019 experiment demonstrated that in a scenario where residual oils remain, the addition of the tested biological (EMNR) and chemical treatments (SWA-Corexit EC9850-A) may not increase toxicity to the macroinvertebrate community over the effects of oil alone (i.e. 2018 pilot study). Rather the secondary treatments seemingly reduced the impacts of the addition of dilbit alone, unlike that of previous tests using these alternative methods to treat worst-case scenario releases (using these secondary treatments as the primary treatment, not for the residual oil left from initial containment and recovery) , at least for total abundance. Further work is recommended to understand the impacts on diversity as there is no comparison to the 2018 data.

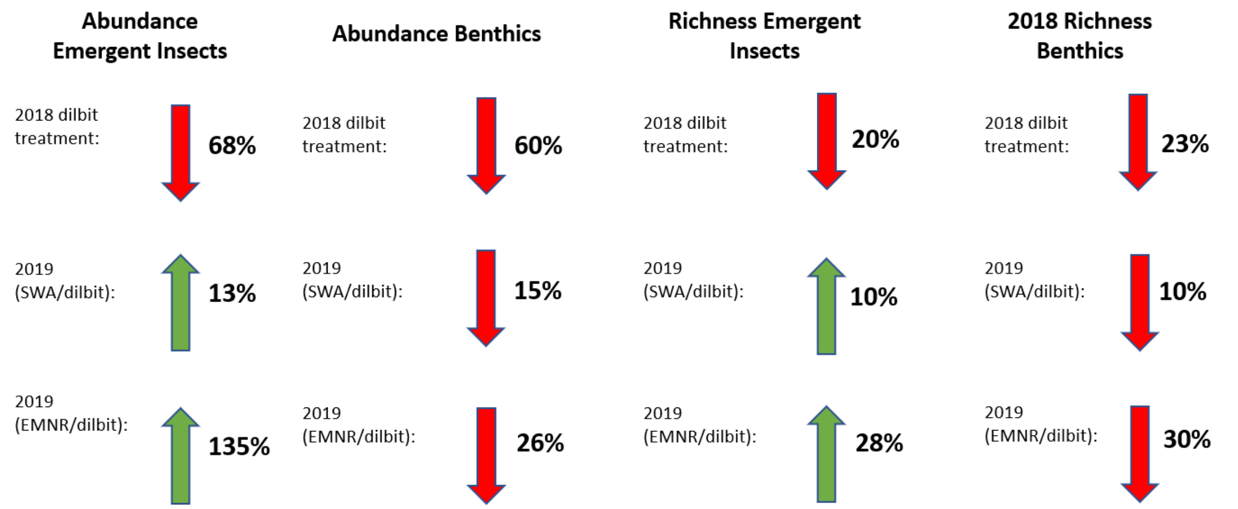

Figure 3. 24: Rock cobble abundance and richness comparison between 2018 and 2019 treatment studies. Arrow direction indicates direction of change.

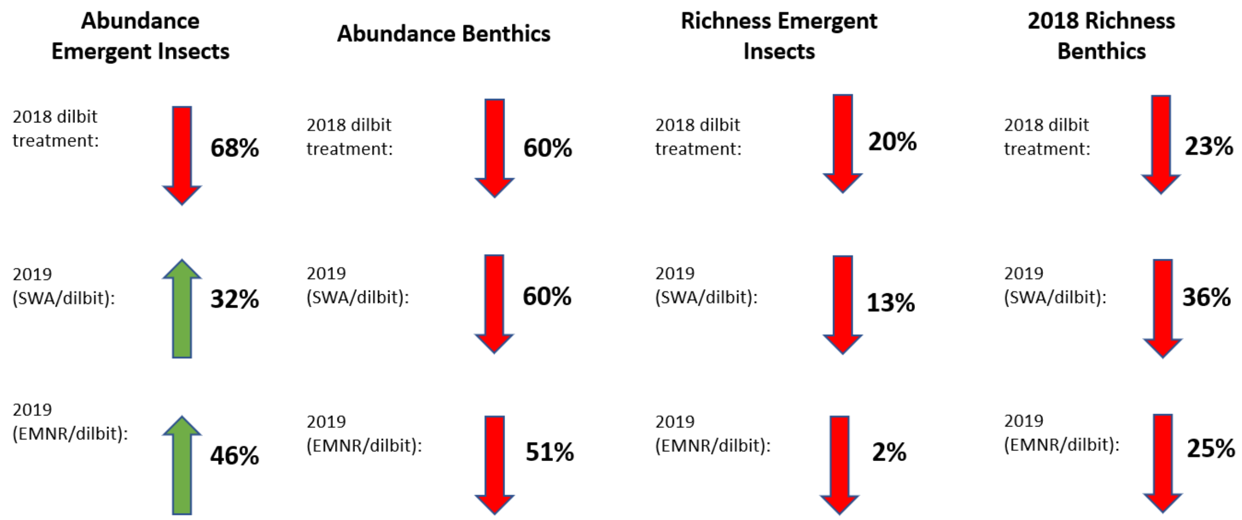

Figure 3. 25: Peat Organic abundance and richness comparison between 2018 and 2019 treatment studies. Arrow direction indicates direction of change.

3.5.4 Chemical Impacts

The 2.5 L of dilbit used in this study was weathered prior to addition; therefore the light end hydrocarbons were depleted, which would be expected to have reduced acute toxicological effects. Initial cleanup occurred 72 hours (Day 3) after dosing. Primary recovery of the initial oil added for the Peat Organic EMNR enclosures were 5.8% and for the 6.1% for the Rock Cobble (Vince Palace, personal communication). Secondary treatments (SWA and EMNR) were applied an additional 24 hours afterwards (Day 4). Once the SWA lifted the oil off shoreline surfaces it was collected within the enclosures using sorbent pads, which improved the total recovery to 13.5% of the dilbit added in the Peat Organic enclosures and 61.4% of the dilbit added to the Rock Cobble enclosures.

All treatment enclosures had elevated levels of tPACs relative to the control. The greatest concentrations of tPAC for Peat Organic were 7.5 µg/L in EMNR and 9.3 µg/L in SWA. For Rock Cobble, the highest concentrations were 9.3 µg/L in EMNR and 10.9 µg/L in SWA between Day 1 and Day 20. These concentrations are higher than what was observed in the pilot study (with primary recovery of oil being 18.5%) which had a highest concentration of ~0.03 µg/L of tPACs. The tPAC concentrations in the water samples declined to levels comparable to the control enclosures by Day 60 (<1 µg/L). This was similar to the 2018 dilbit only treatment where tPAC reached background levels by Day 80). These concentrations are all below those reported to cause chronic toxicity in freshwater organisms (~2-80 µg/L) (Palace et al., 2021). Concentrations in the 2019 study were within the range of chronic toxicity for freshwater organisms (Palace et al., 2021).

In both shoreline types following secondary treatment, an increase in tPACs were observed on Days 4 to 5. In the Peat Organic enclosures, average tPAC concentrations increased from 2 µg/L to 5 µg/L in the EMNR treatments and 1.7 µg/L to 4.7 µg/L in the SWA treatment. In the Rock Cobble enclosures, tPAC concentrations increased from 0.5 µg/L to 2.5 µg/L in the EMNR treatment and 0.5 µg/L to 2.6 µg/L in the SWA treatment. While tPAC concentrations increased, and continued to increase until Day 20, they were still below acute toxicity for certain chironomid species *C. dilutes* (15 mg/L) and *C. attenuatus* (2.8 mg/L WSF) (Steen et al. 1999). Chironomids were the dominant family of both emergent and benthic invertebrates and there is a consensus that they are more pollution tolerant than other taxa. This is likely why no significant impacts on overall abundance was observed while ability to detect an effect was low (Black et al., 2021a; Arimoro et al., 2008; Mozely et al., 1978).

The EMNR treatment aided in breaking down oil and hence made the oil more bioavailable, which could have temporarily increased the toxicity to aquatic organisms. In a previous study by Lee et al., (2001), the addition of nutrients to oil found *H. azteca* (20-100% mortality) was more sensitive than *Daphnia magna* (0-20% mortality), indicating nutrients may collect in the sediments. This suggests that benthic species may have a greater response to oil from their contact with the sediment compared to that of water column species. This may have been a factor aiding in the declines in abundance, while not significant, of benthic invertebrates in our study. At the time of this thesis, the sediment data had not been compiled to verify any accumulation of tPACs in the sediment.

The SWA is itself a hydrocarbon-based solution that may increase acute toxicity. Offsetting this, the SWA would increase oil break-down due to more rapid weathering of light end hydrocarbons (EPA, 2013; Lee et al., 2015). There have been very limited studies on the effects of SWA on macroinvertebrates following a dilbit spill. With bulk oil, it has been shown that when using the recommended concentration SWA has minor impacts on the macroinvertebrate community. In a laboratory study conducted by Bhattacharyya et al., (2003), the addition of a shoreline washing agent Corexit 9580 to crude oil was more toxic than the oil itself on chironomid species *Chironomous tentans*. When the concentration of Corexit 9580 was reduced by 50% (20 mL) (original concentration 600 mL water, 200 mL oil (SLC) and 40 mL Corexit 9580) the toxicity was relatively minor to *C. tentans* after 7-days (Bhattacharyya et al., 2003). Since my study was based on residual sheen remaining after an initial cleanup, the concentration of Corexit 9580A required was limited compared to that of a release that did not utilize primary cleanup methods first. Using low concentrations of SWA as a secondary treatment, rather than higher concentrations required for its use as a primary treatment method is likely the reason for the observations of no significant adverse effects on emergent insects and benthic invertebrates.

The addition of the secondary treatments aided in further degradation of the dilbit for the EMNR and for the SWA aided in lifting it off surfaces and allowing more oil to be removed. The application of the two treatments likely agitated the sheen that may have assisted in its breakdown and allowed the oil to become more bioavailable in the water column. Additionally, as seen in the increased recovery percentages (61% for Rock Cobble and 13% for Peat Organic), Corexit EC9580-A successfully facilitated further recovery of oil by washing it off shoreline surfaces. The EMNR treatment may have increased degradation rates by stimulating growth of indigenous oil degraders (Lee et al., 2001). The faster degradation and additional cleanup likely reduced the sheen to a degree where the impact on emergent insects at the water/surface interface was minimized, as well improving the ability for oviposition to occur. The fact that tPAC concentrations were reduced to background levels and that no population was extirpated implies that any impacts would be temporary and recovery of impacted taxa is possible. However, further studies are needed to verify if rare taxa are not present in this study, as well, most taxa would typically take a year to recover so longer study durations would be beneficial to see impacts to any univoltine taxa.

The exchange of water in the enclosures would have had an impact on tPAC concentrations. However, many benthic macroinvertebrates are univoltine and are not as mobile as other biotic groups such as fish, phytoplankton and zooplankton. Therefore, the initial impact from the release and the cleanup methods would probably have impacted the benthic community as increases in tPAC concentrations were observed for the first 20 days in the Peat Organic enclosures and for the first 7 days in the Rock Cobble. Although the Rock Cobble water transfer into the enclosure was rapid, and within the first few days tPACs reached background levels and the Peat Organic reached background levels by ~Day 30 there were no significant impacts to abundance richness or diversity during the time frame of Day -1 to Day 33 (see Appendix Figure B 10-12).

Sediment data for oil constituents for the 2019 study were not available at the time of writing this thesis. There was no significant impact observed in the benthic invertebrate communities, which implies that the concentrations of tPACs in sediment were not sufficient to cause adverse effects, though there were declines relative to the controls in abundance and diversity measures. Once sediment data are available it will be useful to compare with the 2018 pilot study, which may assist in understanding the potential effectiveness of the secondary treatments in relation to effects (or lack thereof) on benthic invertebrate communities.

3.5.5 Physical Impacts

Previous field studies conducted at Lake 260 on freshwater oil spills such as the 2018 Pilot study (Chapter 2) and the 2018 Boreal Lake Oil Release Experiment by Additions to Limnocorrals (BOREAL) study (Black 2019; Stoyanovich et al., 2019) determined that physical impacts from the oil sheen at the air-water interface were the primary driver of adverse impacts for macroinvertebrates. Black (2019) determined a nominal sheen of 0.07 L/m^2 based on surface area of the enclosure had significant adverse impacts on insect emergence and oviposition, reducing fecundity and the abundance of benthic macroinvertebrates.

In this 2019 study, dosing resulted in a nominal oil thickness of 0.05 L/m^2; however, most of the oil migrated to the shoreline. For the Peat Organic enclosures, EMNR had 0.048 L/m^2 and SWA had 0.04 L/m^2 of dilbit remaining. For the Rock Cobble enclosures, EMNR had 0.046 L/m^2 and SWA had 0.02L/m^2 of dilbit. With the exception of the Rock Cobble SWA cleanup, the remaining cleanup efforts were similar to that of the 2018 pilot study that was reduced to 0.04 L/m^2 of dilbit. The estimates of surface sheen thickness for both of the 2018 and 2019 studies accounted for additional oil that may have adhered to the curtains and collar of the enclosures; thus the surface sheen calculations are likely over estimates. While none of these exceeded the 0.07 L/m^2 thickness reported for the BOREAL study (Black, 2019), the actual thickness was not measured and was observed to migrate to the shoreline as well, surrounding the emergent traps at the beginning of the study (Appendix Figure B.15). The EMNR treatment had an increased amount of oil remaining for both shoreline types compared to that of the 2018 study. This indicates that EMNR likely aided in the breakdown of oil into the water column as the emergent insects were not suppressed compared to the control enclosures. The SWA treatment in the Peat Organic enclosures had the same oil remaining in the enclosure as 2018 study and for the Rock Cobble enclosures there was a decreased amount of oil compared to the dilbit only in 2018; likely due to a rock cobble shoreline being easier to wash and lift oil off the surfaces. This indicates that SWA did successfully aid in lifting oil off surfaces to be cleaned., As emergent insects were not suppressed in the SWA treatments for either shoreline, this implies that the treatment would also help the recovery of benthic communities from the effects of the sheen on oviposition and fecundity.

While significant impacts to emergent insect abundance were not observed in our study, others have shown that SWA, specifically Corexit EC9580-A, can increase toxicity when applied to oil spills. Black et al. (2021) found that an application of oil at 0.0026-0.2070 µm (sheen thickness), treatments with Corexit EC9580-A at an application rate of 0.006 L/m^2 caused 100% immobility in water striders within 5 minutes. This demonstrates that estimates of toxic chemical impacts cannot be utilized alone and that physical impacts also need to be considered. Black et al.’s study was based on a worst case oil release scenario. In our study, impacts to the macroinvertebrate community were not considered significantly adverse.

3.5.6 Community Composition and Variability

The emergent insect and benthic invertebrate communities across enclosures were heavily dominated (>90%) by the family Chironomidae. Chironomidae are commonly found in softer substrates, i.e. sand and silt (Zack, 2018) and are the dominant taxa at ELA. (Black et al. 2021a).

Both shoreline types had very similar emergent insect community composition and had little variation between control and treatments. In the Rock Cobble enclosures, neither diversity (Inverse Simpson Index) nor taxa richness impacts were significant ($p>0.05$) compared to the controls. The enclosures averaged 89% (+/-5.5%) Chironomidae*,* 3% (+/-0.5%) Ceratopogonidae*,* and 2.5% (+/-1.2%) Trichoptera were present from the Families Leptoceridae and Hydroptilidae. While changes in abundance of emergent insects were not statistically significant compared to the control there were large variation among replicates (Appendix Figure B.4). The largest variation between treatment replicates were between EMNR1 and EMNR3 (104%) and may be due to the placement of the enclosures (Figure 3.2). Although the enclosures were all within the same lake, the shorelines in different sections of the lake can vary in community composition. EMNR3 was the furthest south of all the Rock Coble enclosures whereas EMNR1 and EMNR2 were close in proximity ~20m apart.

Emerging insects in the Peat Organic enclosures also did not vary much in community composition; differences in taxa richness were not significant ($p>0.05$) compared to the control, however diversity, only for the EMNR treatment were significantly lower ($p>0.05$) compared to the control. The enclosures averaged 90% (+/- 5.5%) Chironomidae*,* 2% (+/-0.2%) Ceratopogonidae*,* and 1.7% (+/-0.2%) Trichoptera were present from the Families Leptoceridae and Hydroptilidae. While abundance of emergent insects was not statistically significantly different compared to the controls, there were large variation among replicates (Appendix Figure B.7). The largest variation in abundance was between SWA2 and SWA3 (110%). No colonization or eggs were observed on the trap and no shift in community structure was observed, only an increased abundance of the four families previously mentioned (Figure 3.2). Family composition was slightly altered at the three enclosures located on the north side of the Peat Organic enclosures (EMNR1, SWA1 and Cont1) as they did have an increase in Hymenoptera compared to the other enclosures. Although the data are reflective of a typical boreal lake community, further research on the rarer taxa is required as they were too low in abundance to discern impacts.

Both shoreline types had very similar benthic invertebrate composition and had little variation between control enclosures and treatments, neither diversity (Inverse Simpson Index), abundance or richness impacts were significant ($p>0.05$) compared to the control. The Rock Cobble enclosures averaged 69% (+/-2.5%) for Chironomidae abundance (dominant taxa) and 8% (+/-4.7%) for the combined orders, Ephemeroptera, Trichoptera, and Odonata. There was variation between the Rock Cobble EMNR enclosures; EMNR2 had very low abundance and EMNR1 had high abundance yielding a 131% difference between enclosures. Enclosures (SWA1) and (EMNR1) had the highest abundance and were located beside each other (Figure 3.2) suggesting that variation in community composition was affected by location along the shoreline.

Enclosure (EMNR2) was on the furthest south of the enclosure set; (SWA3), (EMNR3) and (Cont2) were in different locations (Figure 3.2).

The Peat Organic enclosures averaged 68% (+/-15%) for Chironomidae abundance (dominant taxa) and 17% (+/-10%) for the combined orders, Ephemeroptera, Trichoptera, and Odonata. The Ephemeroptera genus *Caenis sp*. Was more abundant in enclosures Ref1, EMNR1 and SWA1, all of which were located on the northwest side of the peninsula (Appendix Figure B.15). The highest abundance of *Caenis sp.* was in the EMNR treatment (46% of the total abundance), which increased average ETO abundance for this treatment. The difference in *Caenis* abundance between EMNR1 and EMNR3 was 116% and for SWA1 and SWA2 was 105%. Similar to the emergent abundance, enclosures (Cont1), (SWA1) and (EMNR1) all had increased abundance of Ephemeroptera (*Caenis sp.*) compared to the remaining Peat Organic enclosures. Enclosure (SWA2) similar to (EMNR3) had the least abundance (Figure 3.2).

While impacts were not statistically significant; with the exception of Peat Organic emergence diversity; there were general increases and decreases observed among replicate enclosures. As discussed in Sections 3.4.2 and 3.4.3, natural variation is expected; however having limited and uneven replicates (control enclosures had two replicates, treatments had three) posed difficulty to determine treatment vs. natural variation effects. Additionally, although all shorelines were within the same lake splitting them into two different locations for each shoreline type (Figure 3.3) may increase the natural variation observed.

3.5.7 Trophic Interactions

There were no significantly detectable direct adverse impacts of the secondary treatments on macroinvertebrate community abundance and richness; however there may be some indirect trophic interactions occurring that caused a significant decrease in emergence diversity for the Peat Organic EMNR treatment enclosures. Trophic interactions may also act to suppress responses through density dependence or compensation. Due to the rapid and significant leakage of the Rock Cobble enclosures, discussion of trophic interactions will focus solely on the Peat Organic enclosures. This is due to the likely significant migration of zooplankton and phytoplankton into Rock Cobble enclosures.

In the Peat Organic SWA treatment enclosures, there were significant observed increases in suspended phosphorous, chlorophyll-a, and phytoplankton biomass and decreases in zooplankton and periphyton compared to the control (Perry, 2021). The decrease in zooplankton may have reduced grazing pressure on phytoplankton, which is their main food source (Perry, 2021). While there was a decrease in periphyton, which chironomids and other grazer and collectors use as a food source (Mahdy et al., 2015), likely a result from increased competition reduced nutrients and light associated with increased phytoplankton (Perry, 2021). The lack of competition on phytoplankton from zooplankton likely aided in masking a possible significant impact in the macroinvertebrates as high phytoplankton production yields high emergence (Davies, 1980). Previous studies have also shown a positive correlation between phosphorous, nitrogen, and chlorophyll-a and chironomid community structure, by increasing food availability (Saether, 1979 and Davies, 1980). In the EMNR treated enclosures, there was no significant difference in phytoplankton or zooplankton biomass compared to the controls; however periphyton biomass was significantly higher (Perry, 2021). The increased nutrients and periphyton-enhanced available food sources for the grazer/gatherer/collector macroinvertebrates (Wallace and Webster, 1996) primarily chironomids. This increase in emerging chironomids could be an indirect trophic level impact from an increased food source. The abundance of Trichoptera and Ephemeroptera also increased within th EMNR treatments relative to the control, perhaps due to increased periphyton, and Odonata increased, likely due to increased prey taxa. As the counts for these groups were low in all enclosures, the larger ecological implications are uncertain.

For both treatments the fish analysis observed significant bycatch, likely due to the leaking of the enclosures. Of the 160 tagged fish (10/enclosure) deployed, only 11 were retrieved. Preliminary results on fish analysis suggest that there were no noticeable differences in the mortality between treatment and control enclosures (Perry, 2021).

3.5.8 Limitations

Not having the same number of replicates in the treatments compared to the control enclosures impacted statistical analysis. It is recommended that sample replicated be equal for reach treatment (including the control). As per the power analysis increasing the number of replicates would have aided in assuming the difference between variance of enclosures is ecologically meaningful. Leaking in the enclosures was an issue and an alternative method to seal the enclosures should be considered. The current study did not identify the macroinvertebrate community to species level and therefore effects on individual species were evaluated. Additional studies observing impacts at the species level would prove beneficial to better understand entire benthic community impacts. In efforts to reduce agitation of dilbit we were limited to one emergence trap. Since this study was limited to one emergent trap per enclosure, we may have not assessed the entire emergent insect community. Different placement of the emergent trap will influence the insect assemblage collected and more in depth impacts to those species could have been impacted and will be able to better determine community based trophic level effects, for example a trap closer to the shoreline, with emergent vegetation directly below the trap may have had greater EPT species.

3.6 Conclusions

The impacts of treatments (oil + primary and two different secondary cleanup methods) to the macroinvertebrate abundance or richness were not detected statistically. EMNR treatment in the Peat Organic enclosures resulted in a significant decrease in diversity, while the SWA treatment and both treatments in the Rock Cobble enclosures had no statistically detectable effects. This result strongly contrasts the results of the pilot study (Chapter 2), where effects on macroinvertebrate abundance and diversity were detected. The main methodological difference between the two studies was the inclusion of only primary cleanup (sorbent pads) within the pilot study; the two secondary cleanup methods (SWA, EMNR) that were used in the 2019 study were excluded from the 2018 pilot study. While speculative, the comparison of the two studies suggests that both secondary cleanup methods reduced the toxicity of the oil. If used in the recommended concentrations should not cause cascading damage to the function and structure of a boreal ecosystem. This field study, along with data from previous laboratory studies will aid in the determination of the best spill cleanup method for freshwater oil releases based on a risk based approach. Cleanup methods have many factors to be considered, some of which are the location of the release, the type of waterbody, season, the shoreline type, trajectory modelling, the type of oil released and general access to the location. This study successfully addressed a number of gaps addressed in the Royal Society of Canada Report (2015) on impacts on freshwater ecosystems from oil releases. While this information is beneficial, the utilization of these results in other systems such as eutrophic lakes or wetlands and rivers may illicit different results. Additional studies on other systems and the information found in previous laboratory studies will help better understand if there are changes in response to these cleanup methods.

Chapter 4: Synthesis and Conclusions

There are recognized and significant data gaps for invertebrate responses to oil exposure and cleanup methods in freshwater ecosystems. The FOReSt research program at the IISD-ELA was developed to address these with a whole ecosystem approach for boreal lakes. This thesis specifically attempted to characterize impacts to macroinvertebrates and effects from diluted bitumen (dilbit) and conventional heavy crude (CHV) alone, and dilbit releases with secondary chemical and biological treatments on two different shoreline type, Rock Cobble and Peat Organic. Previous studies have used opportunistic oil spills or laboratory based studies often on worst-case scenarios. The FOReSt study examined multiple trophic levels to understand the complexity of responses in a freshwater environment. in Chapter 2 we determined that even small amounts of residual oils (dilbit and CHV) in the littoral zone can be detrimental to the aquatic macroinvertebrate community. The addition of biological treatment (enhanced monitored natural recovery; EMNR) to dilbit releases resulted in slight decreases in diversity on emergent insects, likely due to the increase of food sources for chironomids; and no significant impacts to benthic macroinvertebrates relative to the control enclosures. The addition of chemical treatment (Corexit EC9850-A: SWA) to dilbit releases had no significant impact on the emergent insects or macroinvertebrates in the treatment enclosures relative to the control enclosures.

It must be noted that the enclosures on both substrates had significant leakage identified via tritium tracing. Rock Cobble enclosures had significant leakage immediately evident by Day 2, while Peat Organic had a slower decline to near complete flushing by Day 30. Still, this leaking was less likely to impact macroinvertebrate community composition directly (e.g., migration in and out) as they are not very mobile and many are univoltine (as compared to zooplankton or phytoplankton); and the organisms within the enclosures were likely exposed from the beginning. Conclusions drawn from these experimental units have significant uncertainty and therefore further discussion will not include Rock Cobble enclosures, as was also done in with primary producer responses (Perry, 2021). This study along with the Boreal Lake Oil Release Experiment by Additions to Limnocorrals (BOREAL) study (Black et al., 2021a) is one of the first to look at effects on macroinvertebrates from oil in natural systems in an experimental fashion. Overall, these data will help response teams supporting oil spills on freshwater determine which cleanup method will be least impactful to the environment.

4.1 Pilot Scale Study

The 2018 pilot study assessed how both conventional heavy crude oil and diluted bitumen impact the aquatic macroinvertebrate community in the littoral zone of a freshwater boreal lake. Emerging insects were monitored during the 83 day study while benthic invertebrates were characterized at the end of the study period. The objective was to characterize the response of macroinvertebrate communities to experimental dilbit and CHV oil spills under field conditions and to provide additional information for the following year's full-scale study. We hypothesized that oil has an adverse impact on the macroinvertebrate community, and our hypothesis was confirmed.

Overall, we found that emerging insects and benthic invertebrate abundance and richness were reduced in both the CHV and dilbit enclosures, with CHV causing slightly greater declines than dilbit. The reductions were drastic, similar in proportion to other studies such as the Black et al., (2021) limnocorral study and Robidoux et al., (2018) laboratory study. Chironomids were the dominant taxa in the enclosures, and while being considered pollutant tolerant still demonstrated significant declines in abundance. The total polycyclic aromatic compounds (tPACs) concentrations in the enclosures were lower than the acute toxicity for many freshwater species. This leads us to believe that physical impacts of oil such as sheen or shoreline disturbance played a significant role in emergent insects and benthic invertebrate impacts.

There were limitations to this study; there was no replication therefore enclosure/trap issues (predators/damage) may have impacted counts. For example, there may have been colonization occurring on the traps, which may have resulted in the increase in abundance between control enclosures. Additionally, depending on the density of aquatic macrophytes under the location of the emergence traps may have yielded higher abundance and richness from change in habitat. Another issues was that the enclosures were not randomized, therefore treatments and controls were likely to have similar community composition. Only one trap per enclosure was added to reduce agitation to the oil and trap location can be limiting to certain taxa based on distance from shoreline. Additionally, only having one trap may have contributed to issues with variability within traps. Similarly, to reduce agitation there were no water column traps that may have better identified impacts from the surface sheen. Kick net sampling was not done throughout the study, only at the end therefore estimation of changes in benthic abundance and richness over time was not possible. Despite these limitations, the direct impacts to the macroinvertebrate community were prominent (Figure 4.1).

Unfortunately, at the time of this thesis the results of zooplankton were not yet analyzed and periphyton and phytoplankton were not assessed. As a result, determination of indirect effects from bottom up interactions were not possible. Still, there were no significant effects on fish development however mortality in CHV was slightly higher and deformities were greater in dilbit exposed fish (Palace et al., 2021), which may indicate both direct (deformities) and indirect (mortality due to food loss) effects in fish from oil spills.

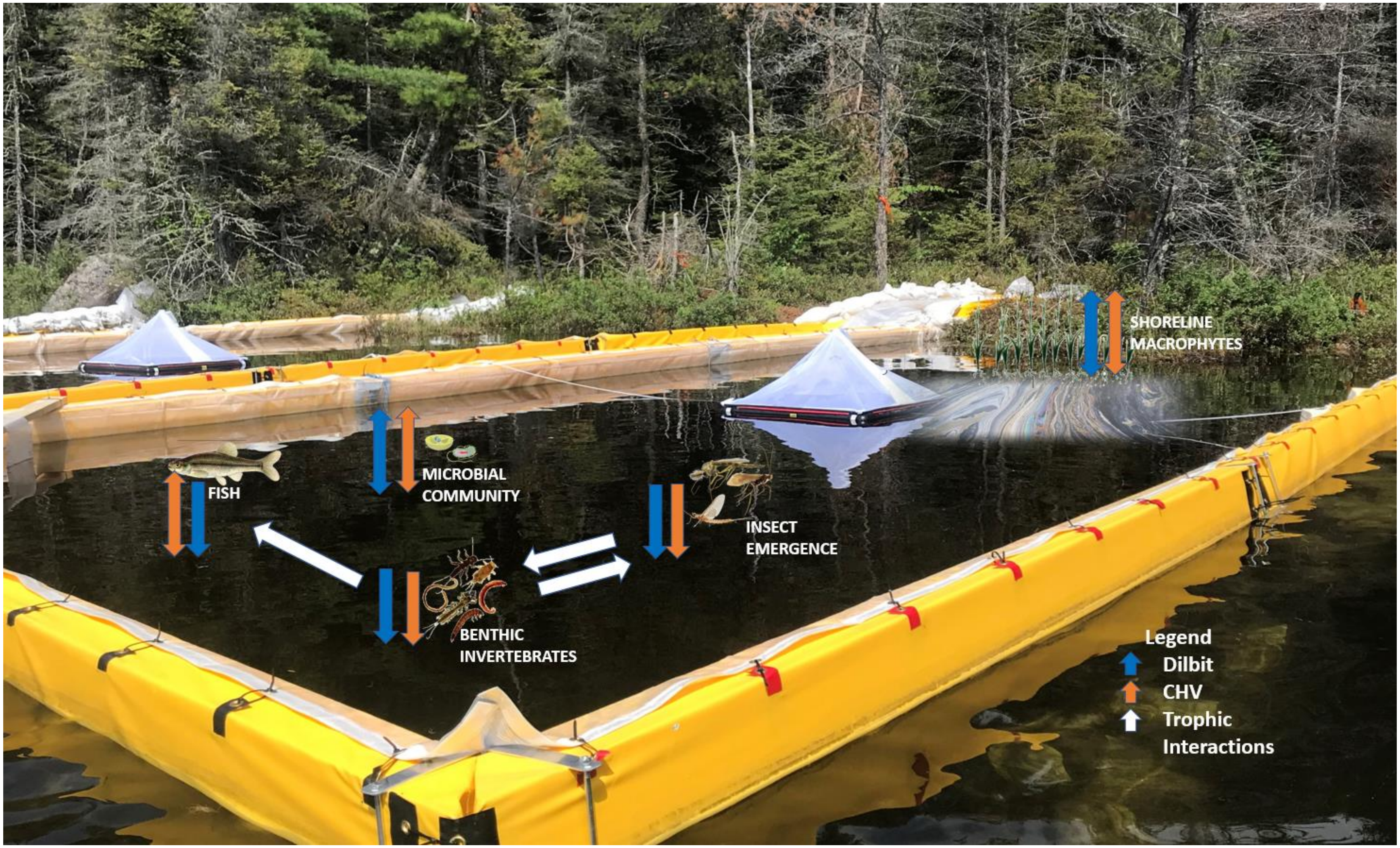

Figure 4.1: Conceptual model of trophic level response to dilbit in the littoral zone of a boreal lake. The coloured arrows represent the direct effects on communities from the dilbit, while the white arrows represent the indirect effects between trophic levels. The arrows with tips on both ends represent no effect.

4.2 Full Scale Study

This study assessed the impacts of dilbit and alternative cleanup methods; shoreline washing agent (SWA) and enhanced natural monitored recovery (EMNR) on benthic invertebrates and insect emergence. The objective of this study was to characterize the response of aquatic macroinvertebrates following an experimental dilbit spill and two treatment methods, SWA and EMNR, on two shoreline types, Rock Cobble and Peat Organic. Due to significant water transfer in the Rock Cobble enclosures this shoreline type will not be discussed further. We hypothesized that emergent insect abundances of control enclosures would be greater than the abundance in the dilbit/SWA enclosures and this was not supported. However, we hypothesized that the abundance in the control enclosures would be equal or less than that of dilbit/EMNR enclosure and this was supported. For the benthics; our hypothesis for decreased abundance in dilbit/SWA was proven, however our hypothesis for increased abundance in the dilbit/EMNR was not proven.

Overall, we found that there were no significant impacts on total abundance, diversity, and richness of benthic invertebrates on either shoreline with either secondary treatment. For emergent insects there were no significant impacts on total abundance or by each timepoint, the total richness had no significant impacts although a couple of the timepoints in each shoreline type did have statistically significant impacts compared to the control (for Peat Organic Day 48 both SWA and EMNR were statistically significant). Diversity was decreased and was statistically significant in the Peat Organic enclosure only for the EMNR treatment relative to the control enclosure, due to a high increase in chironomids. In both treatments for both shoreline types the tPAC concentrations were comparable to that of the control enclosure and pretreatment concentrations by Day 66.

Indirect effects may have been affecting the results in the enclosures. In the Peat Organic enclosures, phytoplankton biomass, suspended phosphorous and chlorophyll-a were significantly higher in the SWA treatment while periphyton and zooplankton biomass were significantly lower than the control enclosure (Perry, 2021). This top down effect of decreasing zooplankton, leading to an increase in its food source phytoplankton seemingly had minimal influence on the macroinvertebrate community, as the dominant taxa (chironomids) do not utilize either as the primary food source. Additionally while there was a decrease in periphyton which chironomids and some other grazers use as a food source (Mahdy et al., 2015), the depletion in zooplankton that can also feed on periphyton and phytoplankton, therefore lessening the exploitative competition (Bronmark e al., 1997). This may explain why there were no significant impacts to macroinvertebrate abundance. Similar to the zooplankton, the shoreline agitation and the SWA treatment may have reduced reproductive output (fecundity) that may have led to the decrease in abundance, diversity, and richness at the end of the season; however, there was no significant impact compared to the control enclosure, and the slight impact seen may have been result of the lack of disturbed shoreline in the control enclosure from first and secondary treatments, or by chance as statistics imply (Figure 4.2).

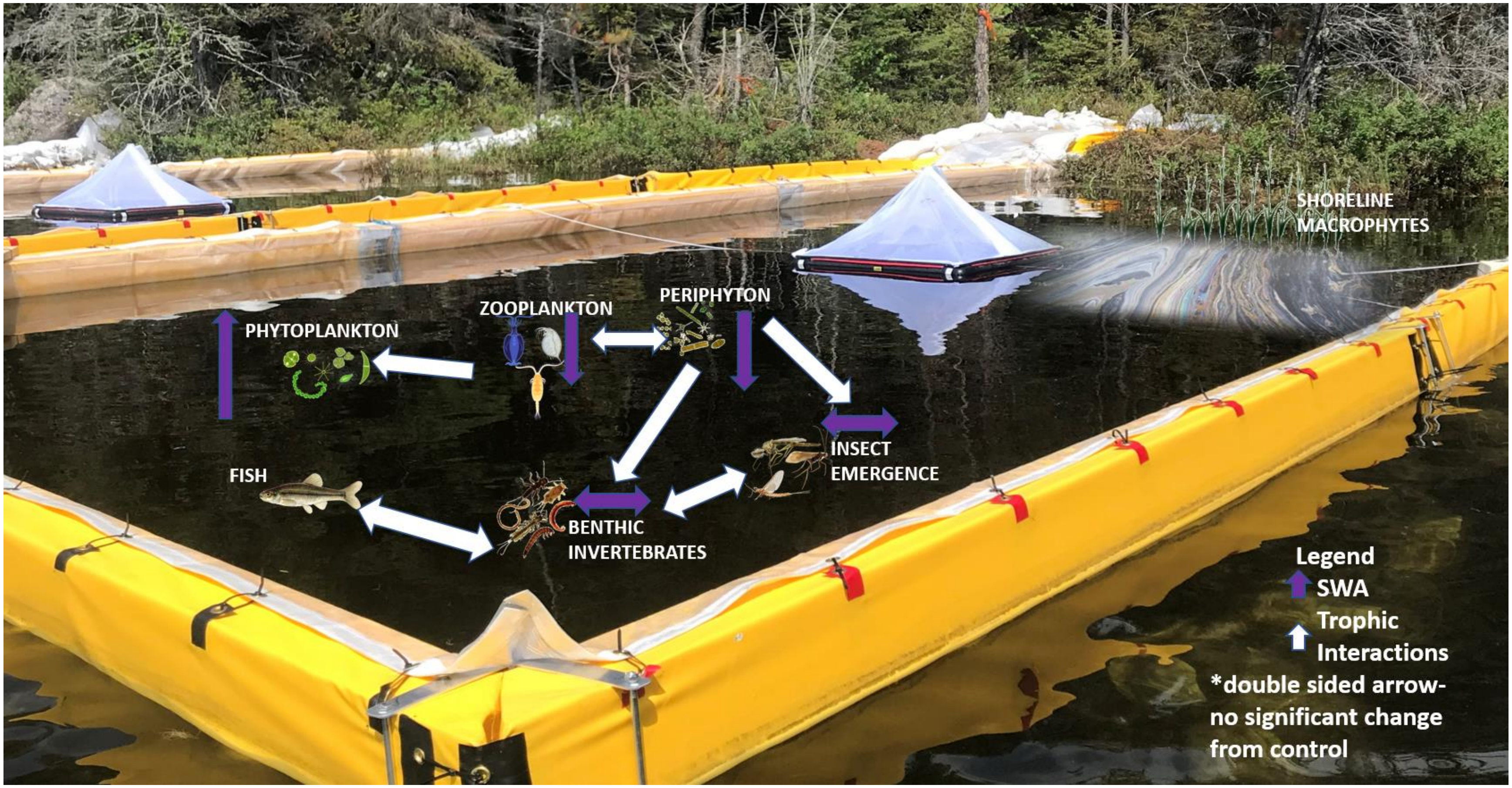

Figure 4.2: Conceptual model of trophic level response to dilbit and secondary treatment shoreline washing agent (SWA) in the littoral zone of a boreal lake. The coloured arrows represent the direct effects on communities from the dilbit and SWA treatment, while the white arrows represent the indirect effects between trophic levels. The arrows with tips on both ends represent no effect.

In the EMNR treated enclosures, there was no difference in phytoplankton growth or zooplankton biomass, however periphyton biomass was significantly higher than in the control enclosures (Perry, 2021). The increased nutrients and periphyton increased food sources for the grazer/gatherer/collector macroinvertebrates (Wallace and Webster, 1996) primarily chironomids in the emergent traps. Chironomids can have higher removal rates of periphyton than certain zooplankton species (Mahdy et al., 2015). This increase in chironomids in the emergent traps may have resulted in the significant impact to diversity. Similar to the SWA enclosures while there were general decreases in abundance they were not significant relative to the control enclosures. The decreases were likely from shoreline agitation and agitation of any oil along the shorelines, which may impact oviposition and limit fecundity in benthic communities (Black et al., 2021a). There was a slight change in community composition in the benthic samples, specifically an increase the scraper functional groups such as Trichoptera and Ephemeroptera as well, likely from the increase in periphyton and nutrients (Figure 4.3).

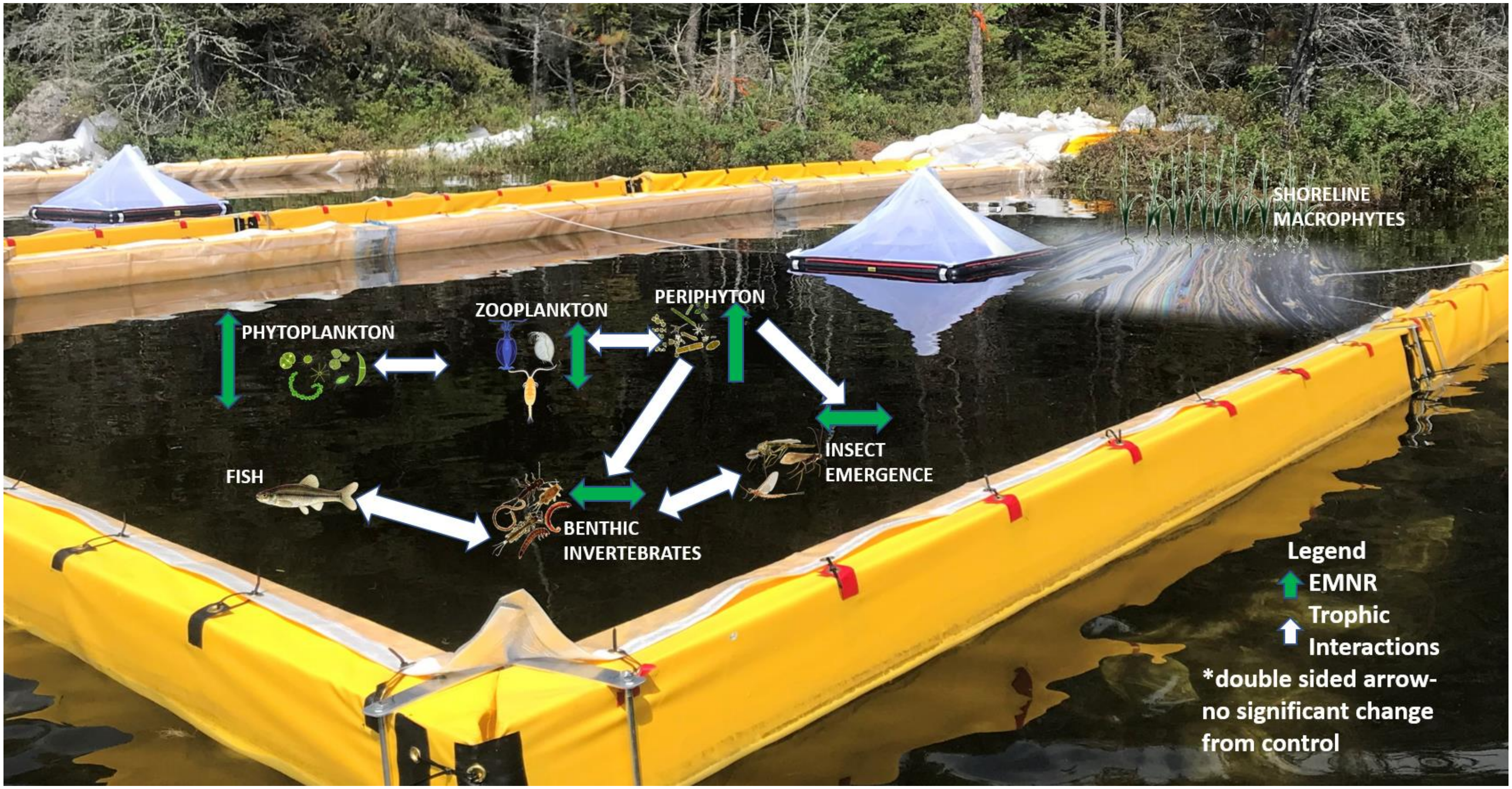

Figure 4.3: Conceptual model of trophic level response to dilbit and secondary treatment enhanced monitored natural recovery (EMNR) in the littoral zone of a boreal lake. The coloured arrows represent the direct effects on communities from the dilbit and EMNR treatment, while the white arrows represent the indirect effects between trophic levels. The arrows with tips on both ends represent no effect.

Preliminary results for fish analysis suggest there were no significant difference in the mortality between treatment and control enclosures. In both treatments, fish rely on chironomids as a main diet source; approximately 48% of their diet consist of chironomids (Wallace and Webster, 1996; Wagner et al., 2012), as well there was no complete elimination of macroinvertebrate functional groups, richness or abundance; the decrease in diversity due to high chironomids likely had no impact as its one of their dominant food sources. Therefore, there would be no bottom up trophic cascade impacts from macroinvertebrate to fish species or terrestrial vertebrate consumers i.e. turtles or waterfowl (Covich et al., 1999). If the oil and secondary treatments did have a direct impact to fish species, the loss of predation may have had a top down effect to the aquatic macroinvertebrates, but we did not observe this in the field. While the FOReSt study observed minimal impacts to the macroinvertebrate community and a successful reduction of tPACs over time; to properly evaluate recovery after exposure, additional studies that would be multi-year would prove beneficial to see how secondary production is impacted and how long it takes for all trophic levels to recover to background levels.

4.3 Improvements to Spill Cleanup Efforts

Both studies were comparable in oil concentrations/enclosure size. The 2018 study had an enclosure size of 18.75 m^3 and the 2019 study had an enclosure size of 37.5 m^3; the pilot study had 1.25 L or oil added to the enclosure and the 2019 study had 2.5 L; double the amount added and the size of the enclosure also doubled. Both studies had clear trends in response to selected treatments; the pilot study showed that both dilbit and conventional heavy crude alone can drastically decrease the abundance and diversity of emerging and benthic macroinvertebrates with no obvious recovery in the study period for both treatments. The full-scale study, focused on dilbit and alternative cleanup methods, Surface Washing Agent (SWA) and Enhanced Monitored Natural Recovery (EMNR) demonstrated that all treatments (dilbit with a secondary cleanup method) had less of an impact on the macroinvertebrate community compared to the pilot study of the dilbit only release. While there were still declines, compared to the control with no treatment recovery to control levels was observed.

A strength of this study was that it did not just focus on certain test species, but assessed responses reflective of a natural freshwater boreal lake community. Boreal systems are relevant to study as there are far more boreal lakes than any other type and the boreal region is crossed by several pipelines. In boreal oligotrophic lakes, there tends to be lower species diversity and richness that can lead to a stronger effect on trophic cascades compared to that of a eutrophic lake; however the littoral zone generally has the highest complexity (Bronmark et al., 1997). Therefore, this study should show the greatest range of species sensitivity relative to the whole lake as the highest abundance of macroinvertebrates are within the littoral zone (Davies, 1980). Macroinvertebrates are a main component of freshwater littoral ecosystems and the evidence of this community have limited impacts and evidence of recovery will help in minimizing higher trophic level impacts.

Overall, the intent of this study was to address research gaps identified in the Royal Canada Society Report of 2015 on the need for additional studies on freshwater oil releases, their impacts to the macroinvertebrate communities, whole lake ecosystems and efficacy of alternative cleanup methods. The two secondary methods used in this study, enhanced monitored natural recovery (EMNR), a form of bioremediation or biostimulation and a Surface Washing Agent (SWA; Corexit EC9580-A), a chemical agent, have had limited studies conducted in freshwater. The findings of both the pilot study and the full-scale study provide some insights. While dilbit alone caused significant impacts to the macroinvertebrate community, the addition of both secondary treatments, EMNR and SWA on a Peat Organic shoreline significantly reduced the adverse impacts of the oil itself. FOReSt evaluated biological effects of oil and treatment methods on primary producers periphyton and phytoplankton, primary consumers, zooplankton, macroinvertebrate, fish, and vegetation and some possible trophic level cascades were proposed. We recommend these findings be implemented into the American Petroleum Institute (API) “Options for Minimizing Environmental Impacts of Inland Spills”. As well as the Shoreline Cleanup Assessment Technologies (SCAT) handbooks to outline the impacts of the biological and chemical treatment method in the environment studied (Peat Organic shorelines) on diluted bitumen.

The data from this study will aid in the decision making process when completing a Net Environmental Benefit Analysis (NEBA) for spill response. Factors that should be considered when determining what type of cleanup method should be used on a release would be oil type, recovery time frame based on impacts to sensitive receptors, scale and location of release, accessibility to site and season. The broader understanding of the two alternative cleanup strategies evaluated in this study will support the compilation of data available based on the factors mentioned above and the potential cleanup methods available. It will assist in the prediction of outcomes, such as impacts to the freshwater ecosystem compared to that of mechanical removal options and will support in balancing the ecological benefits and drawbacks to each cleanup method to determine the best response option to minimize any additional impacts to the environment (IPIECA, 2015). Since these data are the first of their kind for freshwater ecosystems, it will greatly improve the accuracy in the assessment of risk for a NEBA. The improved data will not only benefit spill response efforts but will likely improve relationships with industry regulators, stakeholders and improve public perception as there is now thorough data exploring all options of cleanup; mechanical, chemical and biological, specific to freshwater oil releases.

Additionally, this research supports the utilization of using these cleanup methods as a means of secondary response where bulk oil would be collected using a primary cleanup method, such as on water recovery (containment and recovery). This is important information for dosing as previous laboratory studies have shown secondary treatments for worst case scenarios can contribute toxicity not reduce it. The information collected from this study will allow, if approved, for a site-specific scenario, an alternative cleanup option for shorelines that will be less detrimental than mechanical removal (Dew et al., 2016 and API, 2016) that often increases environmental impacts as shorelines are sensitive species habitat and we want to minimize impacts as best as possible.

4.4 Limitations and Recommendations

There were limitations in this study within the scope and methods. While it was beneficial to have the pilot study solely look at impacts of dilbit and CHV, it would have been beneficial to have control enclosures in the 2019 study with only dilbit to directly compare to the enclosures with secondary treatments. The change in year and size of the enclosure allows for other factors, either environmental or project driven to generate different results. It would be beneficial to have equal numbers of control enclosures (n=2) as treatment enclosures (n=3) to allow for more accurate statistical analysis. Additionally, increasing the number of enclosures would be recommended for future studies due to the high variation among replicates. The results from this study are specific to the littoral zone of a boreal lake and may vary in alternate fresh waterbodies with varying community structures. Additional studies of the same magnitude should be executed on different fresh waterbody types and varying seasons/climates. Enclosure design had some issues, as mentioned there was leakage in some of the enclosures. Alternative ways to seal enclosure curtains to the lake bottom should be considered, such as attaching heavy chains to the bottom of the curtain to keep it in place or use an enclosure with a closed bottom and add substrate into the enclosure. The two secondary treatments tested were only used on dilbit product and it would have been beneficial to see impacts of these alternative cleanup methods on the CHV as well a synbit.

Lake samples were taken throughout the study to verify the effects of the enclosure itself has on an ecosystem. The enclosures can limit water transfer and reduce wave action and movement throughout the lake; therefore, some natural effects can be isolated. While wind and wave action were simulated during treatment application, the remainder of the study did not include such efforts. In the event a similar release would occur in a lake it would be expected that the results would cause less of an impact due to the increased movement within the lake as well the movement of biota through the system allows reintroduction of communities to come repopulate impacted areas that is hindered by enclosure walls. A combination of field and laboratory are beneficial to accurately determine toxicity and effectiveness of the products, oil and secondary treatments to determine ecosystem health.

4.5 Conclusion

Impacts of secondary treatments (dilbit + EMNR and SWA) to the macroinvertebrate community in the littoral zones of a boreal lake proved to cause less adverse impacts than that of a dilbit only release. The results of this study help address the significant gaps in our understanding of how to response to oil releases in freshwater ecosystems. The findings of this study can be utilized in real life release scenarios when conducting a Net Environmental Benefit Analysis. While there are still many unknowns around impacts to oil releases in freshwater ecosystems, this thesis adds to our knowledge around how to appropriately implement oil spill cleanup in freshwater ecosystems.

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A.1 2018 Insect Emergence Data

Appendix Table A. 1 FOReST pilot study emergent insects with Order, Family, taxa counts, and total abundance of each sample.

Sample ID	Order	Family	Counts	Total Abundance
Sample Name: enclsure 1 (ref 1) **Enclosure:** #1 **Date Collected:** June 22, 2018 **Time Collected:** 16:15	Diptera	chironomidae	106	126
	Diptera	ceratopogonidae	8	
	Diptera	chaoboridae	4	
	Diptera	culicidae	2	
	Trichoptera	hydroptilidae	5	
	Odonata	Coenagrionidae	1	
Sample Name: enclosure 2 (ref 2) **Enclosure:** #2 **Date Collected:** June 22, 2018 **Time Collected:** 16:24	Diptera	chironomidae	158	187
	Diptera	ceratopogonidae	17	
	Diptera	chaoboridae	3	
	Diptera	culicidae	1	
	Trichoptera	hydroptilidae	7	
	Trichoptera	limnephilidae	1	
Sample Name: enclosure 3 heavy crude **Enclosure:** #3 **Date Collected:** June 22, 2018 **Time Collected:** 16:31	Diptera	chironomidae	132	148
	Diptera	ceratopogonidae	3	
	Diptera	chaoboridae	4	
	Diptera	culicidae	2	
	Trichoptera	hydroptilidae	7	
Sample Name: enclosure 4 dilbit **Enclosure:** #4 **Date Collected:** June 22, 2018 **Time Collected:** 16:30	Diptera	chironomidae	140	153
	Diptera	ceratopogonidae	5	
	Diptera	chaoboridae	2	
	Diptera	culicidae	1	
	Trichoptera	hydroptilidae	4	
	Hymentoptera	diapriidae	1	
Sample Name: FOREST-M1-CON-ET-2018-02 **Enclosure:** #1 **Date Collected:** June 27, 2018 **Time Collected:** 16:30	Diptera	chironomidae	108	121
	Diptera	ceratopogonidae	11	
	Diptera	culicidae	1	
	Diptera	syrphidae	1	
Sample Name: FOREST-M2-DBN-ET-2018-02 **Enclosure:** #2 **Date Collected:** June 27, 2018 **Time Collected:** 16:27	Diptera	chironomidae	75	80
	Diptera	ceratopogonidae	2	
	Diptera	culicidae	1	
	Trichoptera	hydroptilidae	2	

Sample ID	Order	Family	Counts	Total Abundance
Sample Name: FOREST-M3-COW-ET-2018-02 **Enclosure:** #3 **Date Collected:** June 27, 2018 **Time Collected:** 16:18	Diptera	chironomidae	40	43
	Diptera	ceratopogonidae	1	
	Diptera	culicidae	1	
	Trichoptera	hydroptilidae	1	
Sample Name: FOREST-M4-DBW-ET-2018-02 **Enclosure:** #4 **Date Collected:** June 27, 2018 **Time Collected:** 16:12	Diptera	chironomidae	91	102
	Diptera	ceratopogonidae	4	
	Diptera	culicidae	5	
	Trichoptera	hydroptilidae	2	
Sample Name: FOREST-M1-CON-ET-2018-03 **Enclosure:** #1 **Date Collected:** July 04, 2018 **Time Collected:** 14:14	Diptera	chironomidae	176	189
	Diptera	ceratopogonidae	10	
	Trichoptera	hydroptilidae	2	
	Trichoptera	leptoceridae	1	
Sample Name: FOREST-M2-DBN-ET-2018-03 **Enclosure:** #2 **Date Collected:** July 04, 2018 **Time Collected:** 14:16	Diptera	chironomidae	237	248
	Diptera	ceratopogonidae	9	
	Diptera	dolichopodidae	1	
	Odonata	coenagrionidae	1	
Sample Name: FOREST-M3-COW-ET-2018-03 **Enclosure:** #3 **Date Collected:** July 04, 2018 **Time Collected:** 14:20	Diptera	chironomidae	106	114
	Diptera	ceratopogonidae	2	
	Diptera	culicidae	6	
Sample Name: FOREST-M4-DBW-ET-2018-03 **Enclosure:** #4 **Date Collected:** July 04, 2018 **Time Collected:** 14:23	Diptera	chironomidae	161	171
	Diptera	ceratopogonidae	7	
	Trichoptera	hydroptilidae	2	
	Trichoptera	leptoceridae	1	
Sample Name: FOREST-M1-CON-ET-2018-04 **Enclosure:** #1 **Date Collected:** July 11, 2018 **Time Collected:** 14:07	Diptera	chironomidae	485	516
	Diptera	ceratopogonidae	27	
	Trichoptera	leptoceridae	4	
Sample Name: FOREST-M2-DBN-ET-2018-04 **Enclosure:** #2 **Date Collected:** July 11, 2018 **Time Collected:** 14:14	Diptera	chironomidae	751	772
	Diptera	ceratopogonidae	21	
Sample Name: FOREST-M3-COW-ET-2018-04 **Enclosure:** #3 **Date Collected:** July 11, 2018 **Time Collected:** 14:22	Diptera	chironomidae	118	121
	Diptera	Ceratopogonidae	3	

Sample ID	Order	Family	Counts	Total Abundance
Sample Name: FOREST-M4-DBW-ET-2018-04 **Enclosure:** #4 **Date Collected:** July 11, 2018 **Time Collected:** 14:34	Diptera	chironomidae	231	238
	Diptera	ceratopogonidae	6	
	Diptera	chaoboridae	1	
Sample Name: FOREST-M1-CON-ET-2018-05 **Enclosure:** #1 **Date Collected:** July 18, 2018 **Time Collected:** 09:48	Diptera	chironomidae	974	1023
	Diptera	ceratopogonidae	44	
	Trichoptera	leptoceridae	5	
Sample Name: FOREST-M2-DBN-ET-2018-05 **Enclosure:** #2 **Date Collected:** July 18, 2018 **Time Collected:** 09:52	Diptera	chironomidae	1589	1710
	Diptera	ceratopogonidae	119	
	Trichoptera	hydroptilidae	1	
	Trichoptera	leptoceridae	1	
Sample Name: FOREST-M3-COW-ET-2018-05 **Enclosure:** #3 **Date Collected:** July 18, 2018 **Time Collected:** 10:01	Diptera	chironomidae	227	240
	Diptera	ceratopogonidae	13	
Sample Name: FOREST-M4-DBW-ET-2018-05 **Enclosure:** #4 **Date Collected:** July 18, 2018 **Time Collected:** 10:11	Diptera	chironomidae	233	240
	Diptera	ceratopogonidae	4	
	Trichoptera	leptoceridae	2	
	Coleoptera		1	
Sample Name: FOREST-M1-CON-ET-2018-06 **Enclosure:** #1 **Date Collected:** July 25, 2018 **Time Collected:** 10:27	Diptera	chironomidae	448	474
	Diptera	ceratopogonidae	21	
	Trichoptera	leptoceridae	4	
	Ephemeroptera	baetidae	1	
Sample Name: FOREST-M2-DBN-ET-2018-06 **Enclosure:** #2 **Date Collected:** July 25, 2018 **Time Collected:** 10:30	Diptera	chironomidae	1939	2090
	Diptera	ceratopogonidae	149	
	Diptera	sciaridae	1	
	Trichoptera	leptoceridae	1	
Sample Name: FOREST-M3-COW-2018-06 **Enclosure:** #3 **Date Collected:** July 25, 2018 **Time Collected:** 10:34	Diptera	chironomidae	204	207
	Diptera	ceratopogonidae	3	
Sample Name: FOREST-M4-DBW-2018-06 **Enclosure:** #4 **Date Collected:** July 25, 2018 **Time Collected:** 10:43	Diptera	chironomidae	213	226
	Diptera	ceratopogonidae	10	
	Trichoptera	leptoceridae	3	

Sample ID	Order	Family	Counts	Total Abundance
Sample Name: FOREST-M1-CON-ET-2018-07 **Enclosure:** #1 **Date Collected:** August 01, 2018 **Time Collected:** 15:31	Diptera	chironomidae	188	199
	Diptera	ceratopogonidae	6	
	Trichoptera	hydroptilidae	3	
	Trichoptera	leptoceridae	1	
	Ephemeroptera	baetidae	1	
Sample Name: FOREST-M2-DBN-ET-2018-07 **Enclosure:** #2 **Date Collected:** August 01, 2018 **Time Collected:** 15:33	Diptera	chironomidae	520	542
	Diptera	ceratopogonidae	19	
	Trichoptera	leptoceridae	2	
	Trichoptera	limnephilidae	1	
Sample Name: FOREST-M3-COW-ET-2018-07 **Enclosure:** #3 **Date Collected:** August 01, 2018 **Time Collected:** 15:35	Diptera	chironomidae	105	115
	Diptera	ceratopogonidae	8	
	Trichoptera	hydroptilidae	2	
Sample Name: FOREST-M4-DBW-ET-2018-07 **Enclosure:** #4 **Date Collected:** August 01, 2018 **Time Collected:** 15:33	Diptera	chironomidae	106	107
	Diptera	ceratopogonidae	1	
Sample Name: FOREST-M1-CON-2018-08 **Enclosure:** #1 **Date Collected:** August 08, 2018 **Time Collected:** 15:03	Diptera	chironomidae	109	117
	Diptera	ceratopogonidae	3	
	Trichoptera	hydroptilidae	4	
	Ephemeroptera	baetidae	1	
Sample Name: FOREST-M2-DBN-2018-08 **Enclosure:** #2 **Date Collected:** August 08, 2018 **Time Collected:** 15:10	Diptera	chironomidae	118	130
	Diptera	ceratopogonidae	11	
	Trichoptera	hydroptilidae	1	
Sample Name: FOREST-M3-COW-2018-08 **Enclosure:** #3 **Date Collected:** August 08, 2018 **Time Collected:** 15:12	Diptera	chironomidae	57	63
	Diptera	ceratopogonidae	2	
	Trichoptera	hydroptilidae	1	
	Trichoptera	leptoceridae	3	
Sample Name: FOREST-M4-DBW-2018-08 **Enclosure:** #4 **Date Collected:** August 08, 2018 **Time Collected:** 15:09	Diptera	chironomidae	50	52
	Diptera	ceratopogonidae	2	

Sample ID	Order	Family	Counts	Total Abundance
Sample Name: *FOREST-M1-CON-2018-09* ***Enclosure:*** *#1* ***Date Collected:*** *August 15, 2018* ***Time Collected: not collected***			*not collected*	0
Sample Name: FOREST-M2-DBN-2018-09 **Enclosure:** #2 **Date Collected:** August 15, 2018 **Time Collected:** 16:42	Diptera	chironomidae	50	55
	Diptera	ceratopogonidae	4	
	Coleoptera		1	
Sample Name: FOREST-M3-COW-2018-09 **Enclosure:** #3 **Date Collected:** August 15, 2018 **Time Collected:** 16:46	Diptera	chironomidae	44	48
	Diptera	ceratopogonidae	3	
	Trichoptera	leptoceridae	1	
Sample Name: FOREST-M4-DBW-2018-09 **Enclosure:** #4 **Date Collected:** August 15, 2018 **Time Collected:** 16:50	Diptera	chironomidae	61	62
	Diptera	ceratopogonidae	1	
Sample Name: FOREST-M1-CON-2018-10 **Enclosure:** #1 **Date Collected:** August 22, 2018 **Time Collected:** 09:11	Diptera	chironomidae	36	41
	Diptera	ceratopogonidae	4	
	Trichoptera	hydroptilidae	1	
Sample Name: FOREST-M2-DBN-2018-10 **Enclosure:** #2 **Date Collected:** August 22, 2018 **Time Collected:** 09:14	Diptera	chironomidae	34	40
	Diptera	ceratopogonidae	6	
Sample Name: FOREST-M3-COW-2018-10 **Enclosure:** #3 **Date Collected:** August 22, 2018 **Time Collected:** 09:18	Diptera	chironomidae	42	43
	Diptera	ceratopogonidae	1	
Sample Name: FOREST-M4-DBW-2018-10 **Enclosure:** #4 **Date Collected:** August 22, 2018 **Time Collected:** 09:22	Diptera	chironomidae	28	31
	Diptera	ceratopogonidae	3	

Sample ID	Order	Family	Counts	Total Abundance
Sample Name: FOREST-M1-CON-2018-11 **Enclosure:** #1 **Date Collected:** August 29, 2018 **Time Collected:** 15:45	Diptera	chironomidae	28	31
	Diptera	ceratopogonidae	2	
	Trichoptera	leptoceridae	1	
Sample Name: FOREST-M2-DBN-2018-11 **Enclosure:** #2 **Date Collected:** August 29, 2018 **Time Collected:** 15:49	Diptera	chironomidae	66	69
	Diptera	ceratopogonidae	3	
Sample Name: FOREST-M3-COW-2018-11 **Enclosure:** #3 **Date Collected:** August 29, 2018 **Time Collected:** 15:53	Diptera	chironomidae	51	54
	Diptera	ceratopogonidae	3	
Sample Name: FOREST-M4-DBW-2018-11 **Enclosure:** #4 **Date Collected:** August 29, 2018 **Time Collected:** 15:57	Diptera	chironomidae	43	46
	Diptera	ceratopogonidae	3	
Sample Name: FOREST-M1-CON-2018-12 **Enclosure:** #1 **Date Collected:** Sept. 6, 2018 **Time Collected:** 13:45	Diptera	chironomidae	43	45
	Diptera	ceratopogonidae	2	
Sample Name: FOREST-M2-DBN-2018-12 **Enclosure:** #2 **Date Collected:** Sept. 6, 2018 **Time Collected:** 13:47	Diptera	chironomidae	70	72
	Diptera	ceratopogonidae	2	
Sample Name: FOREST-M3-COW-2018-12 **Enclosure:** #3 **Date Collected:** Sept. 6, 2018 **Time Collected:** 13:48	Diptera	chironomidae	26	28
	Diptera	ceratopogonidae	2	
Sample Name: FOREST-M4-DBW-2018-12 **Enclosure:** #4 **Date Collected:** Sept. 6, 2018 **Time Collected:** 13:50	Diptera	chironomidae	29	33
	Diptera	ceratopogonidae	4	
Sample Name: FOREST-M4-CON-ET-2018-13 **Enclosure:** #1 **Date Collected:** Sept. 12, 2018 **Time Collected:** 11:26	Diptera	chironomidae	19	21
	Diptera	ceratopogonidae	1	
	Thysanoptera		1	

Sample ID	Order	Family	Counts	Total Abundance
Sample Name: FOREST-M3-DBN-ET-2018-13 **Enclosure:** #2 **Date Collected:** Sept. 12, 2018 **Time Collected:** 11:24	Diptera	chironomidae	10	11
	Diptera	ceratopogonidae	1	
Sample Name: FOREST-M2-COW-ET-2018-13 **Enclosure:** #3 **Date Collected:** Sept. 12, 2018 **Time Collected:** 11:23	Diptera	chironomidae	14	15
	Diptera	ceratopogonidae	1	
Sample Name: FOREST-M1-DBW-ET-2018-13 **Enclosure:** #4 **Date Collected:** Sept. 12, 2018 **Time Collected:** 11:22	Diptera	chironomidae	10	11
	Diptera	ceratopogonidae	1	

A.2 2018 Benthic Macroinvertebrate Data

Appendix Table A. 2 FOReST pilot study benthic macroinvertebrate analysis with Order, Family, lowest level taxa counts and total abundance of each sample

Major Group	Order	Family	Subfamily	Lowest Practical Level	lake ref	Ref 1	ref 2	Dilbit	Crude
Crustacea	Amphipoda	Talitridae	-	*Hyalella sp.*	110	50	20	30	-
Insecta	Ephemeroptera	Caenidae	-	*Caenis sp.*	-	10	-	-	-
Insecta	Trichoptera	Leptoceridae	-	*Oecetis sp.*	10	50	40	30	20
Insecta	Trichoptera	Hydroptilidae	-	*Oxythira sp.*	10	-	-	-	-
Insecta	Odonata	Gomphidae	-	Immature	10	-	-	-	-
Insecta	Diptera	Ceratopogonidae	-	*Probezzia sp.*	-	-	10	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Endochironomus sp.*	-	-	-	10	-
Insecta	Diptera	Chironomidae	Chironominae	*Cladotanytarsus sp.*	20	250	150	30	10
Insecta	Diptera	Chironomidae	Chironominae	*Stempellinella sp.*	-	-	10	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Cryptochironomus sp.*	-	-	-	10	-
Insecta	Diptera	Chironomidae	Chironominae	*Tanytarsus sp.*	-	50	40	10	40
Insecta	Diptera	Chironomidae	Chironominae	*Chironomus sp.*	-	-	-	-	10
Insecta	Diptera	Chironomidae	Chironominae	*Glyptotendipes sp.*	10	-	-	-	-
Major Group	Order	Family	Subfamily	Lowest Practical Level	lake ref	Ref 1	ref 2	Dilbit	Crude
Insecta	Diptera	Chironomidae	Chironominae	*Zavrelia sp.*	20	80	10	20	20
Insecta	Diptera	Chironomidae	Chironominae	indeterminate	-	20	10	-	10
Insecta	Diptera	Chironomidae	Orthocladinae	*Nanocladius sp.*	-	10	10	-	-
Insecta	Diptera	Chironomidae	Tanypodinae	*Procladius sp.*	20	-	30	-	-
Insecta	Diptera	Chironomidae	Tanypodinae	*Clinotanypus sp.*	10	-	-	-	-
Insecta	Diptera	Chironomidae	Tanypodinae	*Ablabesmyia sp.*	10	60	20	10	10
Acari	Acariformes	Hydryphantidae	-	*Hydryphantes sp.*	-	-	20	-	-
Acari	Acariformes	Hygrobatidae	-	*Hygrobates sp.*	-	10	-	-	-
Acari	Acariformes	Unionicolidae	-	*Unionicola sp.*	-	10	-	-	-
Acari	Acariformes	Sperchonidae	-	*Sperchon sp.*	10	-	-	-	-
Bivalvia	Veneroida	Sphaeriidae	-	*Pisidium sp.*	30	20	20	50	10
Oligocheata	Lumbriculida	Lumbriculidae	-	indeterminate	-	-	-	10	-
Oligocheata	Tubificida	Naididae		*Nais sp.*	-	10	-	-	-

Oligocheata	Tubificida	Tubificidae	-	Immature without hairs	-	20	-	-	-
Oligocheata	Tubificida	Tubificidae	-	Immature with hairs	10	-	10	-	-
Total Abundance					**280**	**650**	**400**	**210**	**130**
Count					13	14	14	10	8
Redundant					0	1	1	0	1
Richness (LPL)					**13**	**13**	**13**	**10**	**7**
Counted but not included in analysis (CABIN protocol)									
Porifera	Spongillida	Spongillidae	-	indeterminate	57	73	32	17	5
Nemertea	-	-	-	indeterminate	-	1	-	-	1
Crustacea	Cyclopoida	-	-	indeterminate	-	-	1	-	1
Crustacea	Ostracoda	-	-	indeterminate	-	1	2	1	-
Crustacea	Cladocera	-	-	indeterminate	1	5	1	9	-
Nemata	-	-	-	indeterminate	-	1	-	1	-
					280	**650**	**400**	**210**	**130**

A.3 2019 Insect Emergence Data: Rock Cobble and Peat Organic

Appendix Table A. 3 FOReST full scale study emergent insect analysis with Order, Family, taxa counts, and total abundance of each sample.

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
WL1	20/06/2019	Diptera	Chironomidae	27	27	27	1
WL2	20/06/2019	Diptera	Chironomidae	11	11	11	1
WL3	20/06/2019	Diptera	Chironomidae	2	3	2	2
WL3	20/06/2019	Coleoptera	-	1			
WL4	20/06/2019	Diptera	Chironomidae	14	14	14	1
WL5	20/06/2019	Diptera	Chironomidae	13	13	13	1
WL6	20/06/2019	Diptera	Chironomidae	5	6	6	2
WL6	20/06/2019	Diptera	Dolichopodidae	1			
WL7	20/06/2019			0	0	0	0
WL8	20/06/2019	Diptera	Chironomidae	8	8	8	1
WL9	20/06/2019	Diptera	Chironomidae	4	4	4	1
RC9	21/06/2019	Diptera	Chironomidae	390	397	396	6
RC9	21/06/2019	Diptera	Ceratopogonidae	1			
RC9	21/06/2019	Diptera	Chaoboridae	2			
RC9	21/06/2019	Thysanoptera	-	1			
RC9	21/06/2019	Trichoptera	Hydroptilidae	2			
RC9	21/06/2019	Ephemeroptera	Baetidae	1			
RC8	21/06/2019	Diptera	Chironomidae	43	45	45	3
RC8	21/06/2019	Diptera	Ceratopogonidae	1			
RC8	21/06/2019	Trichoptera	Hydroptilidae	1			
RC7	21/06/2019	Diptera	Chironomidae	113	117	117	2
RC7	21/06/2019	Trichoptera	Hydroptilidae	4			
RC6	21/06/2019	Diptera	Chironomidae	39	39	39	1
RC5	21/06/2019	Diptera	Chironomidae	19	21	21	2
RC5	21/06/2019	Trichoptera	Hydroptilidae	2			
RC4	21/06/2019	Diptera	Chironomidae	52	55	55	2
RC4	21/06/2019	Trichoptera	Hydroptilidae	3			
RC3	21/06/2019	Diptera	Chironomidae	21	22	22	2
RC3	21/06/2019	Diptera	Ceratopogonidae	1			
RC2	21/06/2019	Diptera	Chironomidae	39	41	40	3
RC2	21/06/2019	Thysanoptera	-	1			
RC2	21/06/2019	Trichoptera	Hydroptilidae	1			
RC1	21/06/2019	Diptera	Chironomidae	135	143	143	3

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
RC1	21/06/2019	Diptera	Simuliidae	1			
RC1	21/06/2019	Trichoptera	Hydroptilidae	7			
WL1	03/07/2019	Diptera	Chironomidae	44	50	50	3
WL1	03/07/2019	Diptera	Ceratopogonidae	2			
WL1	03/07/2019	Trichoptera	Hydroptilidae	4			
WL2	03/07/2019	Diptera	Chironomidae	23	32	32	6
WL2	03/07/2019	Diptera	Ceratopogonidae	1			
WL2	03/07/2019	Diptera	Dolichopodidae	1			
WL2	03/07/2019	Diptera	Sciaridae	1			
WL2	03/07/2019	Trichoptera	Hydroptilidae	5			
WL2	03/07/2019	Trichoptera	Leptoceridae	1			
WL3	03/07/2019	Diptera	Chironomidae	46	52	52	2
WL3	03/07/2019	Trichoptera	Hydroptilidae	6			
WL4	03/07/2019	Diptera	Chironomidae	56	60	60	2
WL4	03/07/2019	Trichoptera	Hydroptilidae	4			
WL5	03/07/2019	Diptera	Chironomidae	49	52	52	4
WL5	03/07/2019	Diptera	Chaoboridae	1			
WL5	03/07/2019	Trichoptera	Hydroptilidae	1			
WL5	03/07/2019	Trichoptera	Phryganeidae	1			
WL6	03/07/2019	Diptera	Chironomidae	68	69	69	2
WL6	03/07/2019	Trichoptera	Hydroptilidae	1			
WL7	03/07/2019	Diptera	Chironomidae	24	25	24	2
WL7	03/07/2019	Coleoptera	-	1			
WL8	03/07/2019	Diptera	Chironomidae	24	25	25	2
WL8	03/07/2019	Trichoptera	Phryganeidae	1			
WL9	03/07/2019	Diptera	Chironomidae	15	15	15	1
wLR	27/06/2019	Diptera	Chironomidae	63	70	70	3
wLR	27/06/2019	Diptera	Ceratopogonidae	5			
wLR	27/06/2019	Diptera	Chaoboridae	2			
wLR	03/07/2019	Diptera	Chironomidae	106	125	125	4
wLR	03/07/2019	Diptera	Ceratopogonidae	16			
wLR	03/07/2019	Diptera	Chaoboridae	1			
wLR	03/07/2019	Trichoptera	Hydroptilidae	2			
RC1	04/07/2019	Diptera	Chironomidae	508	587	587	6
RC1	04/07/2019	Diptera	Ceratopogonidae	61			
RC1	04/07/2019	Diptera	Dolichopodidae	1			
RC1	04/07/2019	Trichoptera	Hydroptilidae	13			

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
RC1	04/07/2019	Trichoptera	Leptoceridae	2			
RC1	04/07/2019	Ephemeroptera	Baetidae	2			
RC2	04/07/2019	Diptera	Chironomidae	137	153	152	7
RC2	04/07/2019	Diptera	Ceratopogonidae	5			
RC2	04/07/2019	Diptera	Chaoboridae	2			
RC2	04/07/2019	Arachnida	-	1			
RC2	04/07/2019	Trichoptera	Hydroptilidae	5			
RC2	04/07/2019	Trichoptera	Leptoceridae	2			
RC2	04/07/2019	Ephemeroptera	Ephemeridae	1			
RC3	04/07/2019	Diptera	Chironomidae	8	14	13	4
RC3	04/07/2019	Diptera	Ceratopogonidae	4			
RC3	04/07/2019	Arachnida	-	1			
RC3	04/07/2019	Trichoptera	Hydroptilidae	1			
RC4	04/07/2019	Diptera	Chironomidae	200	224	224	6
RC4	04/07/2019	Diptera	Ceratopogonidae	10			
RC4	04/07/2019	Hymenoptera	Diapriidae	1			
RC4	04/07/2019	Trichoptera	Phryganeidae	1			
RC4	04/07/2019	Trichoptera	Hydroptilidae	9			
RC4	04/07/2019	Ephemeroptera	Ephemeridae	3			
RC5	04/07/2019	Diptera	Chironomidae	134	152	152	4
RC5	04/07/2019	Diptera	Ceratopogonidae	15			
RC5	04/07/2019	Diptera	Dolichopodidae	1			
RC5	04/07/2019	Trichoptera	Hydroptilidae	2			
RC6	04/07/2019	Diptera	Chironomidae	76	87	87	2
RC6	04/07/2019	Diptera	Ceratopogonidae	11			
RC7	04/07/2019	Diptera	Chironomidae	183	190	189	5
RC7	04/07/2019	Diptera	Ceratopogonidae	2			
RC7	04/07/2019	Coleoptera	-	1			
RC7	04/07/2019	Trichoptera	Hydroptilidae	3			
RC7	04/07/2019	Ephemeroptera	Baetidae	1			
RC8	04/07/2019	Diptera	Chironomidae	148	158	157	5
RC8	04/07/2019	Diptera	Ceratopogonidae	2			
RC8	04/07/2019	Arachnida	-	1			
RC8	04/07/2019	Trichoptera	Hydroptilidae	6			
RC8	04/07/2019	Ephemeroptera	Baetidae	1			
RC9	04/07/2019	Diptera	Chironomidae	154	184	183	6
RC9	04/07/2019	Diptera	Ceratopogonidae	19			

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
RC9	04/07/2019	Arachnida	-	1			
RC9	04/07/2019	Hymenoptera	Diapriidae	1			
RC9	04/07/2019	Trichoptera	Hydroptilidae	8			
RC9	04/07/2019	Ephemeroptera	Ephemeridae	1			
rcLR	28/06/2019	Diptera	Chironomidae	561	602	602	8
rcLR	28/06/2019	Diptera	Ceratopogonidae	17			
rcLR	28/06/2019	Diptera	Culicidae	6			
rcLR	28/06/2019	Diptera	Chaoboridae	1			
rcLR	28/06/2019	Diptera	Dolichopodidae	1			
rcLR	28/06/2019	Hymenoptera	Diapriidae	1			
rcLR	28/06/2019	Trichoptera	Hydroptilidae	14			
rcLR	28/06/2019	Ephemeroptera	Baetidae	1			
rcLR	04/07/2019	Diptera	Chironomidae	195	274	274	7
rcLR	04/07/2019	Diptera	Ceratopogonidae	56			
rcLR	04/07/2019	Diptera	Chaoboridae	2			
rcLR	04/07/2019	Diptera	Culicidae	10			
rcLR	04/07/2019	Diptera	Sciaridae	2			
rcLR	04/07/2019	Diptera	Mycetophilidae	1			
rcLR	04/07/2019	Trichoptera	Hydroptilidae	8			
WL2	10/07/2019	Diptera	Chironomidae	49	58	58	6
WL2	10/07/2019	Diptera	Ceratopogonidae	3			
WL2	10/07/2019	Diptera	Chaoboridae	1			
WL2	10/07/2019	Trichoptera	Hydroptilidae	3			
WL2	10/07/2019	Trichoptera	Leptoceridae	1			
WL2	10/07/2019	Ephemeroptera	Ephemeridae	1			
WL1	10/07/2019	Diptera	Chironomidae	37	42	42	3
WL1	10/07/2019	Hymenoptera	Diapriidae	1			
WL1	10/07/2019	Trichoptera	Hydroptilidae	4			
WL8	10/07/2019	Diptera	Chironomidae	105	108	108	3
WL8	10/07/2019	Hymenoptera	Indeterminate	1			
WL8	10/07/2019	Odonata	Coenagrionidae	2			
WL7	10/07/2019	Diptera	Chironomidae	27	47	47	4
WL7	10/07/2019	Diptera	Ceratopogonidae	2			
WL7	10/07/2019	Hymenoptera	Indeterminate	16			
WL7	10/07/2019	Trichoptera	Hydroptilidae	2			
WL9	10/07/2019	Diptera	Chironomidae	36	39	38	4
WL9	10/07/2019	Hymenoptera	Platygastridae	1			

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
WL9	10/07/2019	Trichoptera	Hydroptilidae	1			
WL9	10/07/2019	Coleoptera	-	1			
WL4	10/07/2019	Diptera	Chironomidae	218	225	225	3
WL4	10/07/2019	Diptera	Ceratopogonidae	6			
WL4	10/07/2019	Trichoptera	Leptoceridae	1			
WL3	10/07/2019	Diptera	Chironomidae	61	65	65	3
WL3	10/07/2019	Diptera	Mycetophilidae	1			
WL3	10/07/2019	Trichoptera	Hydroptilidae	3			
WL6	10/07/2019	Diptera	Chironomidae	159	166	166	6
WL6	10/07/2019	Diptera	Dolichopodidae	3			
WL6	10/07/2019	Hymenoptera	Platygastridae	1			
WL6	10/07/2019	Hymenoptera	Diapriidae	1			
WL6	10/07/2019	Ephemeroptera	Ephemeridae	1			
WL6	10/07/2019	Trichoptera	Hydroptilidae	1			
wLREF	10/07/2019	Diptera	Chironomidae	89	105	104	4
wLREF	10/07/2019	Diptera	Ceratopogonidae	14			
wLREF	10/07/2019	Diptera	Chaoboridae	1			
wLREF	10/07/2019	Coleoptera	-	1			
WL5	10/07/2019	Diptera	Chironomidae	104	108	108	4
WL5	10/07/2019	Diptera	Tachinidae	1			
WL5	10/07/2019	Trichoptera	Dipseudopsidae	1			
WL5	10/07/2019	Trichoptera	Hydroptilidae	2			
RC3	11/07/2019	Diptera	Chironomidae	15	19	17	4
RC3	11/07/2019	Diptera	Dolichopodidae	1			
RC3	11/07/2019	Trichoptera	Phryganeidae	1			
RC3	11/07/2019	Coleoptera	-	2			
RC6	11/07/2019	Diptera	Chironomidae	155	168	168	4
RC6	11/07/2019	Diptera	Ceratopogonidae	7			
RC6	11/07/2019	Diptera	Sciaridae	1			
RC6	11/07/2019	Trichoptera	Hydroptilidae	5			
RC4	11/07/2019	Diptera	Chironomidae	102	114	114	6
RC4	11/07/2019	Diptera	Ceratopogonidae	5			
RC4	11/07/2019	Diptera	Chaoboridae	1			
RC4	11/07/2019	Hymenoptera	Platygastridae	1			
RC4	11/07/2019	Trichoptera	Hydroptilidae	4			
RC4	11/07/2019	Ephemeroptera	Ephemeridae	1			
RC5	11/07/2019	Diptera	Chironomidae	155	176	176	4

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
RC5	11/07/2019	Diptera	Ceratopogonidae	15			
RC5	11/07/2019	Trichoptera	Leptoceridae	2			
RC5	11/07/2019	Trichoptera	Hydroptilidae	4			
RC8	11/07/2019	Diptera	Chironomidae	155	163	162	4
RC8	11/07/2019	Diptera	Ceratopogonidae	3			
RC8	11/07/2019	Trichoptera	Hydroptilidae	4			
RC8	11/07/2019	Coleoptera	-	1			
RC9	11/07/2019	Diptera	Chironomidae	166	191	190	8
RC9	11/07/2019	Diptera	Ceratopogonidae	8			
RC9	11/07/2019	Diptera	Mycetophilidae	1			
RC9	11/07/2019	Hymenoptera	Platygastridae	1			
RC9	11/07/2019	Trichoptera	Phryganeidae	1			
RC9	11/07/2019	Trichoptera	Leptoceridae	1			
RC9	11/07/2019	Trichoptera	Hydroptilidae	12			
RC9	11/07/2019	Coleoptera	-	1			
RC2	11/07/2019	Diptera	Chironomidae	95	99	99	3
RC2	11/07/2019	Diptera	Ceratopogonidae	1			
RC2	11/07/2019	Trichoptera	Hydroptilidae	3			
RC1	11/07/2019	Diptera	Chironomidae	169	194	194	4
RC1	11/07/2019	Diptera	Ceratopogonidae	17			
RC1	11/07/2019	Diptera	Chaoboridae	2			
RC1	11/07/2019	Trichoptera	Hydroptilidae	6			
RC7	11/07/2019	Diptera	Chironomidae	154	160	160	5
RC7	11/07/2019	Diptera	Ceratopogonidae	3			
RC7	11/07/2019	Trichoptera	Hydroptilidae	1			
RC7	11/07/2019	Trichoptera	Leptoceridae	1			
RC7	11/07/2019	Hymenoptera	Platygastridae	1			
rcLREF	11/07/2019	Diptera	Chironomidae	195	251	250	8
rcLREF	11/07/2019	Diptera	Ceratopogonidae	49			
rcLREF	11/07/2019	Diptera	Mycetophilidae	1			
rcLREF	11/07/2019	Diptera	Sciaridae	1			
rcLREF	11/07/2019	Trichoptera	Hydroptilidae	2			
rcLREF	11/07/2019	Hymenoptera	Diapriidae	1			
rcLREF	11/07/2019	Ephemeroptera	Baetidae	1			
rcLREF	11/07/2019	Coleoptera	-	1			
wLREF	17/07/2019	Diptera	Chironomidae	73	74	74	2
wLREF	17/07/2019	Trichoptera	Leptoceridae	1			

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
WL1	17/07/2019	Diptera	Chironomidae	63	68	68	3
WL1	17/07/2019	Diptera	Ceratopogonidae	2			
WL1	17/07/2019	Trichoptera	Hydroptilidae	3			
WL2	17/07/2019	Diptera	Chironomidae	27	29	29	3
WL2	17/07/2019	Diptera	Ceratopogonidae	1			
WL2	17/07/2019	Trichoptera	Hydroptilidae	1			
WL3	17/07/2019	Diptera	Chironomidae	41	42	42	2
WL3	17/07/2019	Trichoptera	Hydroptilidae	1			
WL4	17/07/2019	Diptera	Chironomidae	121	125	125	4
WL4	17/07/2019	Diptera	Ceratopogonidae	1			
WL4	17/07/2019	Diptera	Chaoboridae	2			
WL4	17/07/2019	Trichoptera	Hydroptilidae	1			
WL5	17/07/2019	Diptera	Chironomidae	113	115	115	2
WL5	17/07/2019	Diptera	Ceratopogonidae	2			
WL7	17/07/2019	Diptera	Chironomidae	128	131	129	3
WL7	17/07/2019	Trichoptera	Leptoceridae	1			
WL7	17/07/2019	Coleoptera	-	2			
WL9	17/07/2019	Diptera	Chironomidae	57	60	60	4
WL9	17/07/2019	Diptera	Ceratopogonidae	2			
WL9	17/07/2019	Hymenoptera	Platygastridae	1			
WL8	17/07/2019	Diptera	Chironomidae	25	50	49	
WL8	17/07/2019	Diptera	Dolichopodidae	2			
WL8	17/07/2019	Hymenoptera	Indeterminate	22			
WL8	17/07/2019	Coleoptera	-	1			
WL6	17/07/2019	Diptera	Chironomidae	169	170	170	2
WL6	17/07/2019	Trichoptera	Hydroptilidae	1			
RC4	18/07/2019	Diptera	Chironomidae	88	96	93	5
RC4	18/07/2019	Hymenoptera	Platygastridae	1			
RC4	18/07/2019	Trichoptera	Hydroptilidae	2			
RC4	18/07/2019	Trichoptera	Leptoceridae	2			
RC4	18/07/2019	Coleoptera	-	3			
RC7	18/07/2019	Diptera	Chironomidae	43	49	47	4
RC7	18/07/2019	Diptera	Ceratopogonidae	1			
RC7	18/07/2019	Trichoptera	Hydroptilidae	3			
RC7	18/07/2019	Coleoptera	-	2			
RC6	18/07/2019	Diptera	Chironomidae	86	91	90	3
RC6	18/07/2019	Trichoptera	Hydroptilidae	4			

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
RC6	18/07/2019	Coleoptera	-	1			
RC2	18/07/2019	Diptera	Chironomidae	26	30	30	3
RC2	18/07/2019	Trichoptera	Hydroptilidae	3			
RC2	18/07/2019	Hymenoptera	Platygastridae	1			
rcLREF	18/07/2019	Diptera	Chironomidae	121	144	144	7
rcLREF	18/07/2019	Diptera	Ceratopogonidae	9			
rcLREF	18/07/2019	Diptera	Chaoboridae	1			
rcLREF	18/07/2019	Diptera	Sciaridae	6			
rcLREF	18/07/2019	Trichoptera	Hydroptilidae	4			
rcLREF	18/07/2019	Trichoptera	Leptoceridae	1			
rcLREF	18/07/2019	Ephemeroptera	Baetidae	2			
RC3	18/07/2019	Diptera	Chironomidae	8	9	8	2
RC3	18/07/2019	Coleoptera	-	1			
RC8	18/07/2019	Diptera	Chironomidae	46	50	49	4
RC8	18/07/2019	Diptera	Ceratopogonidae	1			
RC8	18/07/2019	Trichoptera	Hydroptilidae	2			
RC8	18/07/2019	Coleoptera	-	1			
RC5	18/07/2019	Diptera	Chironomidae	66	73	70	4
RC5	18/07/2019	Diptera	Ceratopogonidae	2			
RC5	18/07/2019	Trichoptera	Hydroptilidae	2			
RC5	18/07/2019	Coleoptera	-	3			
RC1	18/07/2019	Diptera	Chironomidae	214	229	225	6
RC1	18/07/2019	Diptera	Ceratopogonidae	5			
RC1	18/07/2019	Trichoptera	Hydroptilidae	3			
RC1	18/07/2019	Trichoptera	Leptoceridae	2			
RC1	18/07/2019	Hymenoptera	Platygastridae	1			
RC1	18/07/2019	Coleoptera	-	4			
RC9	18/07/2019	Diptera	Chironomidae	148	151	151	3
RC9	18/07/2019	Diptera	Ceratopogonidae	2			
RC9	18/07/2019	Trichoptera	Hydroptilidae	1			
WL2	24/07/2019	Diptera	Chironomidae	100	112	112	4
WL2	24/07/2019	Diptera	Ceratopogonidae	10			
WL2	24/07/2019	Trichoptera	Hydroptilidae	1			
WL2	24/07/2019	Trichoptera	Leptoceridae	1			
WL4	24/07/2019	Diptera	Chironomidae	90	91	91	2
WL4	24/07/2019	Hymenoptera	Indeterminate	1			
WL7	24/07/2019	Diptera	Chironomidae	36	68	68	3

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
WL7	24/07/2019	Diptera	Sciaridae	1			
WL7	24/07/2019	Hymenoptera	Indeterminate	31			
WL1	24/07/2019	Diptera	Chironomidae	11	13	13	3
WL1	24/07/2019	Diptera	Ceratopogonidae	1			
WL1	24/07/2019	Trichoptera	Leptoceridae	1			
WL9	24/07/2019	Diptera	Chironomidae	107	117	117	2
WL9	24/07/2019	Diptera	Ceratopogonidae	1			
WL9	24/07/2019	Diptera	Dolichopodidae	1			
WL9	24/07/2019	Trichoptera	Leptoceridae	1			
WL9	24/07/2019	Hymenoptera	Indeterminate	7			
WL5	24/07/2019	Diptera	Chironomidae	91	92	92	2
WL5	24/07/2019	Trichoptera	Hydroptilidae	1			
wLREF	24/07/2019	Diptera	Chironomidae	107	108	108	2
wLREF	24/07/2019	Diptera	Ceratopogonidae	1			
WL6	24/07/2019	Diptera	Chironomidae	205	209	209	2
WL6	24/07/2019	Diptera	Ceratopogonidae	4			
WL3	24/07/2019	Diptera	Chironomidae	10	10	10	1
WL8	24/07/2019	Diptera	Chironomidae	224	226	226	2
WL8	24/07/2019	Trichoptera	Leptoceridae	2			
RC5	25/07/2019	Diptera	Chironomidae	109	115	115	6
RC5	25/07/2019	Diptera	Ceratopogonidae	1			
RC5	25/07/2019	Trichoptera	Hydroptilidae	2			
RC5	25/07/2019	Trichoptera	Leptoceridae	1			
RC5	25/07/2019	Ephemeroptera	Baetidae	1			
RC5	25/07/2019	Ephemeroptera	Ephemeridae	1			
RC9	25/07/2019	Diptera	Chironomidae	197	198	198	2
RC9	25/07/2019	Ephemeroptera	Ephemeridae	1			
RC6	25/07/2019	Diptera	Chironomidae	218	226	226	6
RC6	25/07/2019	Diptera	Ceratopogonidae	2			
RC6	25/07/2019	Diptera	Chaoboridae	1			
RC6	25/07/2019	Trichoptera	Hydroptilidae	2			
RC6	25/07/2019	Trichoptera	Leptoceridae	2			
RC6	25/07/2019	Ephemeroptera	Baetidae	1			
RC1	25/07/2019	Diptera	Chironomidae	397	409	407	6
RC1	25/07/2019	Diptera	Ceratopogonidae	4			
RC1	25/07/2019	Diptera	Sciaridae	1			
RC1	25/07/2019	Trichoptera	Hydroptilidae	2			

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
RC1	25/07/2019	Trichoptera	Leptoceridae	3			
RC1	25/07/2019	Coleoptera	-	2			
RC3	25/07/2019	Diptera	Chironomidae	19	22	20	3
RC3	25/07/2019	Hymenoptera	Diapriidae	1			
RC3	25/07/2019	Arachnida	-	2			
RC8	25/07/2019	Diptera	Chironomidae	81	84	84	4
RC8	25/07/2019	Trichoptera	Hydroptilidae	1			
RC8	25/07/2019	Trichoptera	Leptoceridae	1			
RC8	25/07/2019	Hymenoptera	Diapriidae	1			
RC2	24/07/2019	Diptera	Chironomidae	73	80	80	5
RC2	24/07/2019	Diptera	Ceratopogonidae	2			
RC2	24/07/2019	Diptera	Chaoboridae	1			
RC2	24/07/2019	Trichoptera	Hydroptilidae	3			
RC2	24/07/2019	Trichoptera	Leptoceridae	1			
RC7	25/07/2019	Diptera	Chironomidae	59	62	62	3
RC7	25/07/2019	Diptera	Ceratopogonidae	2			
RC7	25/07/2019	Trichoptera	Leptoceridae	1			
rcLREF	25/07/2019	Diptera	Chironomidae	267	286	286	7
rcLREF	25/07/2019	Diptera	Ceratopogonidae	11			
rcLREF	25/07/2019	Diptera	Chaoboridae	1			
rcLREF	25/07/2019	Diptera	Sciaridae	1			
rcLREF	25/07/2019	Trichoptera	Hydroptilidae	2			
rcLREF	25/07/2019	Trichoptera	Phryganeidae	1			
rcLREF	25/07/2019	Ephemeroptera	Baetidae	3			
RC4	25/07/2019	Diptera	Chironomidae	147	154	153	7
RC4	25/07/2019	Diptera	Ceratopogonidae	2			
RC4	25/07/2019	Diptera	Chaoboridae	1			
RC4	25/07/2019	Trichoptera	Hydroptilidae	1			
RC4	25/07/2019	Trichoptera	Leptoceridae	1			
RC4	25/07/2019	Hymenoptera	Indeterminate	1			
RC4	25/07/2019	Coleoptera	-	1			
wLREF	31/07/2019	Diptera	Chironomidae	157	158	158	2
wLREF	31/07/2019	Diptera	Ceratopogonidae	1			
WL9	31/07/2019	Diptera	Chironomidae	95	111	111	2
WL9	31/07/2019	Diptera	Ceratopogonidae	2			
WL9	31/07/2019	Hymenoptera	Indeterminate	14			
WL3	31/07/2019	Diptera	Chironomidae	21	23	21	2

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
WL3	31/07/2019	Coleoptera	-	2			
WL6	31/07/2019	Diptera	Chironomidae	282	286	286	4
WL6	31/07/2019	Diptera	Ceratopogonidae	2			
WL6	31/07/2019	Diptera	Chaoboridae	1			
WL6	31/07/2019	Trichoptera	Leptoceridae	1			
WL4	31/07/2019	Diptera	Chironomidae	182	186	186	3
WL4	31/07/2019	Diptera	Ceratopogonidae	3			
WL4	31/07/2019	Trichoptera	Leptoceridae	1			
WL5	31/07/2019	Diptera	Chironomidae	76	81	80	4
WL5	31/07/2019	Diptera	Ceratopogonidae	3			
WL5	31/07/2019	Trichoptera	Hydroptilidae	1			
WL5	31/07/2019	Coleoptera	-	1			
WL1	31/07/2019	Diptera	Chironomidae	11	12	11	2
WL1	31/07/2019	Arachnida	-	1			
WL7	31/07/2019	Diptera	Chironomidae	103	129	129	3
WL7	31/07/2019	Diptera	Ceratopogonidae	3			
WL7	31/07/2019	Hymenoptera	Indeterminate	23			
WL2	31/07/2019	Diptera	Chironomidae	224	235	235	4
WL2	31/07/2019	Diptera	Ceratopogonidae	7			
WL2	31/07/2019	Trichoptera	Leptoceridae	3			
WL2	31/07/2019	Ephemeroptera	Baetidae	1			
WL8	31/07/2019	Diptera	Chironomidae	174	175	175	2
WL8	31/07/2019	Hymenoptera	Indeterminate	1			
RC4	01/08/2019	Diptera	Chironomidae	68	70	70	3
RC4	01/08/2019	Diptera	Ceratopogonidae	1			
RC4	01/08/2019	Trichoptera	Leptoceridae	1			
RC2	01/08/2019	-	-		0	0	0
RC7	01/08/2019	Diptera	Chironomidae	25	25	25	1
RC8	01/08/2019	Diptera	Chironomidae	62	63	63	2
RC8	01/08/2019	Diptera	Dolichopodidae	1			
RC5	01/08/2019	Diptera	Chironomidae	58	60	60	3
RC5	01/08/2019	Diptera	Ceratopogonidae	1			
RC5	01/08/2019	Trichoptera	Phryganeidae	1			
RC6	01/08/2019	Diptera	Chironomidae	127	130	130	2
RC6	01/08/2019	Diptera	Ceratopogonidae	3			
rcLREF	01/08/2019	Diptera	Chironomidae	142	151	151	5
rcLREF	01/08/2019	Diptera	Ceratopogonidae	2			

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
rcLREF	01/08/2019	Diptera	Sciaridae	5			
rcLREF	01/08/2019	Trichoptera	Hydroptilidae	1			
rcLREF	01/08/2019	Ephemeroptera	Baetidae	1			
RC9	01/08/2019	Diptera	Chironomidae	124	126	126	3
RC9	01/08/2019	Diptera	Chaoboridae	1			
RC9	01/08/2019	Trichoptera	Phryganeidae	1			
RC1	01/08/2019	Diptera	Chironomidae	296	318	318	6
RC1	01/08/2019	Diptera	Ceratopogonidae	13			
RC1	01/08/2019	Diptera	Chaoboridae	2			
RC1	01/08/2019	Trichoptera	Hydroptilidae	4			
RC1	01/08/2019	Trichoptera	Leptoceridae	1			
RC1	01/08/2019	Ephemeroptera	Baetidae	2			
RC3	01/08/2019	Diptera	Chironomidae	4	5	4	2
RC3	01/08/2019	Arachnida	-	1			
wLREF	07/08/2019	Diptera	Chironomidae	115	118	118	
wLREF	07/08/2019	Diptera	Ceratopogonidae	2			
wLREF	07/08/2019	Diptera	Chaoboridae	1			
WL2	07/08/2019	Diptera	Chironomidae	94	96	96	3
WL2	07/08/2019	Diptera	Chaoboridae	1			
WL2	07/08/2019	Trichoptera	Leptoceridae	1			
WL9	07/08/2019	Diptera	Chironomidae	33	49	49	2
WL9	07/08/2019	Hymenoptera	Indeterminate	16			
WL4	07/08/2019	Diptera	Chironomidae	131	142	142	2
WL4	07/08/2019	Diptera	Ceratopogonidae	11			
WL6	07/08/2019	Diptera	Chironomidae	263	269	269	2
WL6	07/08/2019	Diptera	Ceratopogonidae	6			
WL8	07/08/2019	Diptera	Chironomidae	71	72	72	2
WL8	07/08/2019	Diptera	Ceratopogonidae	1			
WL3	07/08/2019	Diptera	Chironomidae	21	22	22	2
WL3	07/08/2019	Trichoptera	Hydroptilidae	1			
WL7	07/08/2019	Diptera	Chironomidae	82	115	115	4
WL7	07/08/2019	Diptera	Ceratopogonidae	2			
WL7	07/08/2019	Diptera	Culicidae	1			
WL7	07/08/2019	Hymenoptera	Indeterminate	30			
WL1	07/08/2019	Diptera	Chironomidae	32	39	36	7
WL1	07/08/2019	Diptera	Ceratopogonidae	1			
WL1	07/08/2019	Diptera	Chaoboridae	1			

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
WL1	07/08/2019	Trichoptera	Leptoceridae	1			
WL1	07/08/2019	Hymenoptera	Indeterminate	1			
WL1	07/08/2019	Arachnida	-	2			
WL1	07/08/2019	Coleoptera	-	1			
WL5	07/08/2019	Diptera	Chironomidae	73	74	74	2
WL5	07/08/2019	Trichoptera	Hydroptilidae	1			
RC8	08/08/2019	Diptera	Chironomidae	42	44	44	3
RC8	08/08/2019	Diptera	Chaoboridae	1			
RC8	08/08/2019	Trichoptera	leptoceridae	1			
RC7	08/08/2019	Diptera	Chironomidae	27	28	28	2
RC7	08/08/2019	Diptera	Ceratopogonidae	1			
RC5	08/08/2019	Diptera	Chironomidae	50	52	52	2
RC5	08/08/2019	Diptera	Ceratopogonidae	2			
RC6	08/08/2019	Diptera	Chironomidae	88	97	97	4
RC6	08/08/2019	Diptera	Ceratopogonidae	7			
RC6	08/08/2019	Diptera	Chaoboridae	1			
RC6	08/08/2019	Diptera	Dolichopodidae	1			
RC4	08/08/2019	Diptera	Chironomidae	63	70	70	4
RC4	08/08/2019	Diptera	Ceratopogonidae	5			
RC4	08/08/2019	Trichoptera	Hydroptilidae	1			
RC4	08/08/2019	Ephemeroptera	Ephemeridae	1			
RC1	08/08/2019	Diptera	Chironomidae	158	176	176	4
RC1	08/08/2019	Diptera	Ceratopogonidae	16			
RC1	08/08/2019	Trichoptera	Hydroptilidae	1			
RC1	08/08/2019	Ephemeroptera	Ephemeridae	1			
RC3	08/08/2019	Diptera	Chironomidae	2	10	2	2
RC3	08/08/2019	Arachnida	-	8			
RC9	08/08/2019	Diptera	Chironomidae	66	72	72	3
RC9	08/08/2019	Diptera	Ceratopogonidae	5			
RC9	08/08/2019	Diptera	Dolichopodidae	1			
RC2	08/08/2019	Diptera	Chironomidae	24	28	28	3
RC2	08/08/2019	Diptera	Ceratopogonidae	3			
RC2	08/08/2019	Trichoptera	Leptoceridae	1			
rcLREF	08/08/2019	Diptera	Chironomidae	81	106	106	5
rcLREF	08/08/2019	Diptera	Ceratopogonidae	16			
rcLREF	08/08/2019	Diptera	Chaoboridae	2			
rcLREF	08/08/2019	Diptera	Sciaridae	4			

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
rcLREF	08/08/2019	Ephemeroptera	Baetidae	3			
WL1	14/08/2019	Diptera	Chironomidae	27	30	29	3
WL1	14/08/2019	Diptera	Ceratopogonidae	2			
WL1	14/08/2019	Arachnida	-	1			
WL6	14/08/2019	Diptera	Chironomidae	140	159	159	4
WL6	14/08/2019	Diptera	Ceratopogonidae	11			
WL6	14/08/2019	Hymenoptera	Indeterminate	7			
WL6	14/08/2019	Trichoptera	Leptoceridae	1			
WL5	14/08/2019	Diptera	Chironomidae	9	10	10	2
WL5	14/08/2019	Hymenoptera	Indeterminate	1			
WL2	14/08/2019	Diptera	Chironomidae	34	34	34	1
wLREF	14/08/2019	Diptera	Chironomidae	107	109	109	3
wLREF	14/08/2019	Diptera	Ceratopogonidae	1			
wLREF	14/08/2019	Hymenoptera	Indeterminate	1			
WL3	14/08/2019	Diptera	Chironomidae	27	30	30	4
WL3	14/08/2019	Diptera	Ceratopogonidae	1			
WL3	14/08/2019	Diptera	Chaoboridae	1			
WL3	14/08/2019	Hymenoptera	Indeterminate	1			
WL7	14/08/2019	Diptera	Chironomidae	24	28	28	2
WL7	14/08/2019	Hymenoptera	Indeterminate	4			
WL4	14/08/2019	Diptera	Chironomidae	40	43	42	3
WL4	14/08/2019	Diptera	Ceratopogonidae	2			
WL4	14/08/2019	Thysanoptera	-	1			
WL8	14/08/2019	Diptera	Chironomidae	42	45	45	3
WL8	14/08/2019	Diptera	Ceratopogonidae	2			
WL8	14/08/2019	Hymenoptera	Indeterminate	1			
WL9	14/08/2019	Diptera	Chironomidae	23	35	35	3
WL9	14/08/2019	Diptera	Ceratopogonidae	3			
WL9	14/08/2019	Hymenoptera	Indeterminate	9			
RC4	15/08/2019	Diptera	Chironomidae	26	27	27	2
RC4	15/08/2019	Trichoptera	Phryganeidae	1			
RC5	15/08/2019	Diptera	Chironomidae	13	14	14	2
RC5	15/08/2019	Diptera	Dolichopodidae	1			
rcLREF	15/08/2019	Diptera	Chironomidae	39	43	43	3
rcLREF	15/08/2019	Diptera	Sciaridae	3			
rcLREF	15/08/2019	Trichoptera	Hydroptilidae	1			
RC9	15/08/2019	Diptera	Chironomidae	32	32	32	1

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
RC1	15/08/2019	Diptera	Chironomidae	69	69	69	1
RC6	15/08/2019	Diptera	Chironomidae	49	50	50	2
RC6	15/08/2019	Trichoptera	Hydroptilidae	1			
RC7	15/08/2019	Diptera	Chironomidae	24	28	27	4
RC7	15/08/2019	Diptera	Chaoboridae	1			
RC7	15/08/2019	Thysanoptera	-	1			
RC7	15/08/2019	Trichoptera	Leptoceridae	2			
RC3	15/08/2019	Arachnida	-	2	2	0	1
RC2	15/08/2019	Diptera	Chironomidae	11	11	11	1
RC8	15/08/2019	Diptera	Chironomidae	25	28	28	3
RC8	15/08/2019	Diptera	Chaoboridae	2			
RC8	15/08/2019	Trichoptera	Leptoceridae	1			
WL5	21/08/2019	Diptera	Chironomidae	3	4	4	2
WL5	21/08/2019	Hymenoptera	Indeterminate	1			
WL4	21/08/2019	Diptera	Chironomidae	37	37	37	1
WL2	21/08/2019	Diptera	Chironomidae	14	14	14	1
WL7	21/08/2019	Diptera	Chironomidae	8	13	13	2
WL7	21/08/2019	Hymenoptera	Indeterminate	5			
WL6	21/08/2019	Diptera	Chironomidae	98	115	114	3
WL6	21/08/2019	Hymenoptera	Indeterminate	16			
WL6	21/08/2019	Coleoptera	-	1			
WL3	21/08/2019	Diptera	Chironomidae	19	21	20	3
WL3	21/08/2019	Diptera	Sciaridae	1			
WL3	21/08/2019	Coleoptera	-	1			
WL8	21/08/2019	Diptera	Chironomidae	10	11	11	2
WL8	21/08/2019	Diptera	Ceratopogonidae	1			
WL9	21/08/2019	Diptera	Chironomidae	10	20	19	3
WL9	21/08/2019	Hymenoptera	Indeterminate	9			
WL9	21/08/2019	Arachnida	-	1			
wLREF	21/08/2019	Diptera	Chironomidae	24	24	24	1
WL1	21/08/2019	Diptera	Chironomidae	21	24	23	4
WL1	21/08/2019	Diptera	Sciaridae	1			
WL1	21/08/2019	Diptera	Scathophagidae	1			
WL1	21/08/2019	Arachnida	-	1			
RC5	22/08/2019	Diptera	Chironomidae	29	29	29	1
RC6	22/08/2019	Diptera	Chironomidae	43	46	46	3
RC6	22/08/2019	Diptera	Ceratopogonidae	2			

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
RC6	22/08/2019	Diptera	Sciaridae	1			
RC9	22/08/2019	Diptera	Chironomidae	11	12	12	2
RC9	22/08/2019	Diptera	Ceratopogonidae	1			
RC8	22/08/2019	Diptera	Chironomidae	10	12	12	2
RC8	22/08/2019	Trichoptera	Leptoceridae	2			
RC7	22/08/2019	Diptera	Chironomidae	16	20	20	4
RC7	22/08/2019	Diptera	Chaoboridae	1			
RC7	22/08/2019	Diptera	Mycetophilidae	1			
RC7	22/08/2019	Trichoptera	Hydroptilidae	2			
RC2	22/08/2019	Diptera	Chironomidae	18	18	18	1
RC4	22/08/2019	Diptera	Chironomidae	38	41	40	3
RC4	22/08/2019	Diptera	Chaoboridae	2			
RC4	22/08/2019	Thysanoptera	-	1			
RC1	22/08/2019	Diptera	Chironomidae	53	58	54	3
RC1	22/08/2019	Diptera	Tachinidae	1			
RC1	22/08/2019	Thysanoptera	-	4			
RC3	22/08/2019	Diptera	Chironomidae	2	7	3	3
RC3	22/08/2019	Diptera	Syrphidae	1			
RC3	22/08/2019	Arachnida	-	4			
rcLREF	22/08/2019	Diptera	Chironomidae	18	20	20	3
rcLREF	22/08/2019	Diptera	Chaoboridae	1			
rcLREF	22/08/2019	Hymenoptera	Indeterminate	1			
WL5	28/08/2019	Diptera	Chironomidae	6	6	6	1
WL7	28/08/2019	Diptera	Chironomidae	2	4	4	2
WL7	28/08/2019	Hymenoptera	Indeterminate	2			
wLREF	28/08/2019	Diptera	Chironomidae	27	29	29	2
wLREF	28/08/2019	Hymenoptera	Indeterminate	2			
WL6	28/08/2019	Diptera	Chironomidae	43	54	54	2
WL6	28/08/2019	Hymenoptera	Indeterminate	11			
WL1	28/08/2019	Diptera	Chironomidae	14	14	14	1
WL4	28/08/2019	Diptera	Chironomidae	56	59	59	2
WL4	28/08/2019	Diptera	Ceratopogonidae	3			
WL3	28/08/2019	Diptera	Chironomidae	17	17	17	21
WL8	28/08/2019	Diptera	Chironomidae	11	13	13	3
WL8	28/08/2019	Diptera	Chaoboridae	1			
WL8	28/08/2019	Hymenoptera	Indeterminate	1			
WL2	28/08/2019	Diptera	Chironomidae	12	13	13	2

Treatment	Date Collected	Order	Family	Counts	Total Abundance	Total Abundance subtracting non emergence	Family Richness
WL2	28/08/2019	Hymenoptera	Indeterminate	1			
WL9	28/08/2019	Diptera	Chironomidae	21	23	23	2
WL9	28/08/2019	Hymenoptera	Indeterminate	2			
RC1	29/08/2019	Diptera	Chironomidae	32	32	32	1
RC7	29/08/2019	Diptera	Chironomidae	13	13	13	1
RC9	29/08/2019	Diptera	Chironomidae	13	15	15	3
RC9	29/08/2019	Diptera	Ceratopogonidae	1			
RC9	29/08/2019	Trichoptera	Phryganeidae	1			
RC3	29/08/2019	Diptera	Chironomidae	1	1	1	1
RC8	29/08/2019	Diptera	Chironomidae	8	9	9	2
RC8	29/08/2019	Diptera	Ceratopogonidae	1			
rcLREF	29/08/2019	Diptera	Chironomidae	2	2	2	1
RC6	29/08/2019	Diptera	Chironomidae	24	29	29	3
RC6	29/08/2019	Diptera	Ceratopogonidae	4			
RC6	29/08/2019	Trichoptera	Limnephilidae	1			
RC4	29/08/2019	Diptera	Chironomidae	27	28	28	2
RC4	29/08/2019	Diptera	Ceratopogonidae	1			
RC2	29/08/2019	Diptera	Chironomidae	4	4	4	1
RC5	29/08/2019	Diptera	Chironomidae	11	13	13	3
RC5	29/08/2019	Diptera	Ceratopogonidae	1			
RC5	29/08/2019	Diptera	Culicidae	1			

Legend:

Peat Organic (Wetland) Environment
wR1 – Wetland Reference #1 (control)
wR2 - Wetland Reference #2 (control)
wEMNR1 – Wetland Enhanced MNR #1
wEMNR2 - Wetland Enhanced MNR #2
wEMNR3 - Wetland Enhanced MNR #3
wSC1 – Wetland Shoreline Cleaner (SWA) #1
wSC2 - Wetland Shoreline Cleaner (SWA) #2
wSC3 - Wetland Shoreline Cleaner (SWA) #3
wEFW – Wetland Engineered Floating Wetland
wLR- Wetland Lake Reference

Rock/Cobble Environment
rR1 – Rock Cobble Reference #1 (control)
rR2 - Rock Cobble Reference #2 (control)
rEMNR1 – Rock Cobble Enhanced MNR #1
rEMNR2 - Rock Cobble Enhanced MNR #2
rEMNR3 - Rock Cobble Enhanced MNR #3
rSC1 – Rock Cobble Shoreline Cleaner (SWA) #1
rSC2 - Rock Cobble Shoreline Cleaner (SWA) #2
rSC3 - Rock Cobble Shoreline Cleaner (SWA) #3
rEFW – Rock Cobble Engineered Floating Wetland
rLR- Rock Cobble Lake Reference

A.4 2019 Benthic Macroinvertebrate Data- Rock Cobble

Appendix Table A. 4 FOReST full scale study benthic macroinvertebrates- Rock Cobble with Order, Family, lowest level taxa counts, and total abundance of each sample.

Major Group	Order	Family	Subfamily	Lowest Practical Level	RC-Lake-1	RC-Lake-2	RC-R-1	RC-R-2	RC-EFW	RC-SC-1	RC-SC-2	RC-SC-3	RC-EMNR-1	RC-EMNR-2	RC-EMNR-3
Crustacea	Amphipoda	Talitridae	-	*Hyalella sp.*	320	160	100	60	60	60	80	80	20	-	20
Insecta	Coleoptera	Elmidae	-	*Dubiraphia sp.*	-	140	-	-	-	-	-	-	-	-	-
Insecta	Coleoptera	Psephenidae	-	*Ectopria sp.*	-	20	-	-	-	-	-	-	-	-	-
Insecta	Ephemeroptera	Caenidae	-	Caenis sp.	80	-	20	40	-	-	20	-	20	-	-
Insecta	Ephemeroptera	Ephemerellidae	-	*Eurylophella sp.*	180	160	-	-	-	-	-	-	-	-	-
Insecta	Ephemeroptera	Leptophlebiidae	-	*Leptophlebia sp.*	60	20	40	20	20	-	-	-	-	-	-
Insecta	Trichoptera	Hydroptilidae	-	Immature	20	-	-	-	-	-	-	-	-	-	-
Insecta	Trichoptera	Leptoceridae	-	*Mystacides sp.*	-	-	20	20	-	60	20	-	0	-	20
Insecta	Trichoptera	Leptoceridae	-	*Oecetis sp.*	20	20	40	40	20	20	40	-	20	-	-
Insecta	Trichoptera	Philopotamidae	-	*Dolophilodes sp.*	-	-	-	-	-	-	20	-	-	-	-
Insecta	Trichoptera	Phryganeidae	-	Immature	-	-	-	-	-	20	20	-	-	-	-
Insecta	Odonata	Coenagrionidae	-	Immature	-	20	-	-	-	-	20	-	-	-	20
Insecta	Odonata	Corduliidae	-	Immature	20	-	-	-	-	-	-	-	-	-	-
Insecta	Odonata	Gomphidae	-	Immature	20	-	20	-	-	20	-	-	-	-	-
Insecta	Odonata	Gomphidae	-	*Dromogomphus sp.*	-	20	-	-	-	-	-	-	-	-	-
Insecta	Diptera	Ceratopogonidae	-	*Bezzia/Palpomyia*	-	-	-	-	20	-	-	-	-	-	-
Insecta	Diptera	Ceratopogonidae	-	*Culicoides*	20	-	-	-	-	20	-	-	20	-	-
Insecta	Diptera	Ceratopogonidae	-	*Mallochohelea*	20	-	-	-	-	-	-	-	-	-	-
Insecta	Diptera	Ceratopogonidae	-	*Probezzia sp.*	-	-	-	20	-	20	-	-	20	20	-

Major Group	Order	Family	Subfamily	Lowest Practical Level	RC-Lake-1	RC-Lake-2	RC-R-1	RC-R-2	RC-EFW	RC-SC-1	RC-SC-2	RC-SC-3	RC-EMNR-1	RC-EMNR-2	RC-EMNR-3
Insecta	Diptera	Chironomidae	Chironominae	*Chironomus sp.*	-	20	-	0	300	80	40	20	340	40	200
Insecta	Diptera	Chironomidae	Chironominae	*Cladopema sp.*	-	-	-	80	20	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Cladotanytarsus sp.*	300	120	80	200	-	160	40	180	260	-	40
Insecta	Diptera	Chironomidae	Chironominae	*Dicrotendipes sp.*	20	20	200	20	20	-	20	-	-	60	-
Insecta	Diptera	Chironomidae	Chironominae	*Microtendipes sp.*	-	-	-	-	100	-	40	-	20	20	60
Insecta	Diptera	Chironomidae	Chironominae	*Pagastiella sp.*	20	40	40	40	-	60	20	100	20	20	20
Insecta	Diptera	Chironomidae	Chironominae	*Polypedilim sp.*	-	60	40	-	-	-	-	20	40	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Pseudochironomus sp.*	100	20	60	40	20	-	-	60	60	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Stenochironomus sp.*	-	-	-	-	-	-	-	20	-	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Tanytarsus sp.*	140	100	-	240	160	160	100	120	200	-	80
Insecta	Diptera	Chironomidae	Orthocladinae	*Nanocladius sp.*	20	40	-	40	-	100	40	40	40	-	60
Insecta	Diptera	Chironomidae	Orthocladinae	*Parakiefferiella sp.*	180	-	60	20	20	100	80	100	140	40	-
Insecta	Diptera	Chironomidae	Tanypodinae	*Ablabesmyia sp.*	-	20	80	100	40	40	60	20	40	-	-
Insecta	Diptera	Chironomidae	Tanypodinae	*Procladius sp.*	40	-	40	60	20	40	20	-	40	-	-
Insecta	Hemiptera	Corixidae	-	*Hesperocorixa sp.*	20	-	-	-	-	-	-	-	-	-	-
Acari	Acariformes	Hydrodromidae	-	*Hydrodroma sp.*	-	-	-	-	-	-	-	20	-	-	-
Acari	Acariformes	Hydryphantidae	-	*Pseudohydryphantes sp.*	20	-	20	-	-	-	-	-	20	-	-
Acari	Acariformes	Sperchonidae	-	*Sperchon sp.*	-	40	-	-	-	-	-	-	-	-	-
Bivalvia	Veneroida	Sphaeriidae	-	*Pisidium sp.*	120	-	-	20	40	-	20	20	60	-	-
Gastropoda	Basommatophora	Planorbidae	-	*Gyraulus deflectus*	-	40	20	-	-	-	-	-	-	-	-
Gastropoda	Basommatophora	Ancylidae	-	*Ferrissia sp.*	20	-	-	-	20	-	-	-	-	-	-
Hirudinea	Arhynchobdellida	Erpobdellidae	-	Immature	-	-	-	20	-	-	-	-	-	-	-

Major Group	Order	Family	Subfamily	Lowest Practical Level	RC-Lake-1	RC-Lake-2	RC-R-1	RC-R-2	RC-EFW	RC-SC-1	RC-SC-2	RC-SC-3	RC-EMNR-1	RC-EMNR-2	RC-EMNR-3
Oligocheata	Lumbriculida	Lumbriculidae	-	indeterminate	-	-	-	-	-	-	-	-	-	20	-
Oligocheata	Tubificida	Naididae	-	*Nais sp.*	20	20	20	60	40	60	80	40	20	60	40
Oligocheata	Tubificida	Naididae	-	*Pristina sp.*	20	-	20	-	-	-	-	-	-	-	-
Oligocheata	Tubificida	Naididae	-	*Pristinella sp.*	-	-	-	-	-	-	-	20	20	20	20
Oligocheata	Tubificida	Naididae	-	*Stylaria lacustris*	-	-	-	-	-	-	-	20	-	-	40
Oligocheata	Tubificida	Naididae	-	*Vejdovskyella comata*	-	-	-	40	-	-	-	-	-	-	20
Oligocheata	Tubificida	Tubificidae	-	Immature without hairs	80	-	40	-	40	20	20	-	20	-	-
Oligocheata	Tubificida	Tubificidae	-	Immature with hairs	-	80	-	-	-	-	-	-	-	-	-
Total Abundance					**1880**	**1180**	**960**	**1180**	**960**	**1040**	**800**	**880**	**1440**	**300**	**640**
Richness (LPL)					**25**	**21**	**19**	**20**	**17**	**17**	**20**	**16**	**21**	**9**	**13**
Redundant					9	8	8	10	8	8	9	11	13	5	8
Family Richness					**16**	**13**	**11**	**10**	**9**	**9**	**11**	**5**	**8**	**4**	**5**
Counted but not included in analysis (CABIN protocol)															
Porifera	Spongillida	Spongillidae	-	indeterminate	440	180	140	120	60	100	380	420	1020	180	660
Crustacea	Cyclopoida	-	-	indeterminate	20	-	-	100	40	120	40	100	60	40	80
Crustacea	Ostracoda	-	-	indeterminate	-	20	-	-	-	-	20	-	20	0	-
Crustacea	Cladocera	-	-	indeterminate	200	120	280	80	160	20	20	120	360	80	80
Nemata	-	-	-	indeterminate	-	-	-	-	-	-	-	-	20	20	-
Cnidaria	Anthoathecata	Hydridae	-	*Hydra sp.*	-	-	-	-	-	-	-	-	-	20	-

A.5 2019 Benthic Macroinvertebrate Data- Peat Organic

Appendix Table A.5 FOReST full scale study benthic macroinvertebrate analysis- Peat Organic with Order, Family, lowest level taxa counts, and total abundance of each sample.

Major Group	Order	Family	Subfamily	Lowest Practical Level	W-Lake-1	W-Lake-2	W-R-1	W-R-2	W-EFW	W-SC-1	W-SC-2	W-SC-3	W-EMNR-1	W-EMNR-2	W-EMNR-3
Crustacea	Amphipoda	Talitridae	-	*Hyalella sp.*	40	60	10	-	10	-	-	-	-	-	-
Insecta	Ephemeroptera	Caenidae	-	*Caenis sp.*	-	310	220	-	70	100	-	20	470	10	-
Insecta	Ephemeroptera	Ephemerellidae	-	*Eurylophella sp.*	-	10	-	-	-	-	-	-	-	-	-
Insecta	Ephemeroptera	Leptophlebiidae	-	*Leptophlebia sp.*	10	60	-	-	-	10	-	10	10	-	10
Insecta	Trichoptera	Leptoceridae	-	*Oecetis sp.*	-	20	10	20	-	20	-	-	-	-	20
Insecta	Trichoptera	Phryganeidae	-	*Agrypina sp.*	-	-	-	-	-	-	-	-	10	-	-
Insecta	Trichoptera	Phryganeidae	-	*Fabria sp.*	-	-	-	-	-	-	-	-	-	10	-
Insecta	Trichoptera	Polycentropodidae	-	*Polycentropus sp.*	-	20	-	-	-	-	-	-	10	-	-
Insecta	Trichoptera	Sericostomatidae	-	*Agarodes sp.*	-	-	-	-	-	-	-	-	10	-	-
Insecta	Odonata	Coenagrionidae	-	*Coenagrion sp.*	-	10	20	10	-	-	10	-	50	10	10
Insecta	Odonata	Libellulidae	-	Immature	10	20	20	10	-	10	-	10	30	40	-
Insecta	Diptera	Ceratopogonidae	-	*Bezzia/Palpomyia*	-	-	-	10	-	-	-	10	10	-	-
Insecta	Diptera	Ceratopogonidae	-	*Probezzia sp.*	-	-	20	10	-	20	-	-	-	40	-
Insecta	Diptera	Chironomidae	Chironominae	*Chironomus sp.*	-	10	160	60	80	30	40	20	40	50	20
Insecta	Diptera	Chironomidae	Chironominae	*Cladotanytarsus sp.*	-	70	220	80	30	40	30	40	80	50	-
Insecta	Diptera	Chironomidae	Chironominae	*Cryptochironomus sp.*	-	-	20	-	-	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Endochironomus sp.*	-	-	-	-	-	-	-	-	20	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Microtendipes sp.*	-	50	30	110	30	100	40	100	80	-	20
Insecta	Diptera	Chironomidae	Chironominae	*Pagastiella sp.*	-	-	10	10	-	-	-	-	30	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Phaenopsectra sp.*	-	10	-	-	-	-	-	-	-	-	-

Major Group	Order	Family	Subfamily	Lowest Practical Level	W-Lake-1	W-Lake-2	W-R-1	W-R-2	W-EFW	W-SC-1	W-SC-2	W-SC-3	W-EMNR-1	W-EMNR-2	W-EMNR-3
Insecta	Diptera	Chironomidae	Chironominae	*Pseudochironomus sp.*	-	-	-	-	-	-	-	-	10	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Stenochironomus sp.*	-	-	-	-	-	-	-	-	-	-	10
Insecta	Diptera	Chironomidae	Chironominae	*Tanytarsus sp.*	20	120	120	330	90	100	40	140	50	160	60
Insecta	Diptera	Chironomidae	Chironominae	*Tribelos sp.*	10	-	-	-	-	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	Chironominae	*Zavreliella sp.*	-	-	-	10	20	-	10	10	-	-	-
Insecta	Diptera	Chironomidae	Orthocladinae	*Nanocladius sp.*	-	-	20	30	-	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	Tanypodinae	*Ablabesmyia sp.*	-	70	110	90	-	20	-	20	30	-	20
Insecta	Diptera	Chironomidae	Tanypodinae	*Labrundinia sp.*	-	-	-	10	10	-	-	-	-	-	-
Insecta	Diptera	Chironomidae	Tanypodinae	*Procladius sp.*	30	100	320	120	90	80	20	150	50	50	10
Insecta	Diptera	Chironomidae	Tanypodinae	*Thienemannimyia sp.*	-	10	-	-	-	-	-	-	-	-	-
Acari	Acariformes	Unionicolidae	-	*Unionicola sp.*	-	10	-	-	-	-	-	-	-	-	-
Bivalvia	Veneroida	Sphaeriidae	-	*Pisidium sp.*	10	10	70	40	80	90	-	30	20	40	30
Gastropoda	Basommatophora	Planorbidae	-	*Gyraulus deflectus*	-	-	20	-	-	-	-	-	-	-	-
Gastropoda	Basommatophora	Planorbidae	-	*Helisoma anceps*	-	-	-	-	10	-	-	-	-	-	-
Oligocheata	Lumbriculida	Lumbriculidae	-	indeterminate	-	-	-	-	-	10	-	20	-	-	20
Oligocheata	Tubificida	Naididae	-	*Nais sp.*	-	10	10	-	40	-	-	-	-	-	-
Oligocheata	Tubificida	Naididae	-	*Vejdovskyella comata*	-	-	-	-	10	10	-	10	-	-	-
Oligocheata	Tubificida	Tubificidae	-	Immature without hairs	-	-	-	30	10	-	-	-	10	-	20
Oligocheata	Tubificida	Tubificidae	-	Immature with hairs	10	20	30	-	20	10	10	10	10	20	20
					0										
Total Abundance					**140**	**1000**	**1440**	**980**	**600**	**650**	**200**	**600**	**1030**	**480**	**270**
Count					8	20	19	17	15	15	8	15	20	11	13
Redundant					0	0	0	0	0	0	0	0	0	0	0
Richness (LPL)					**8**	**20**	**19**	**17**	**15**	**15**	**8**	**15**	**20**	**11**	**13**

Major Group	Order	Family	Subfamily	Lowest Practical Level	W-Lake-1	W-Lake-2	W-R-1	W-R-2	W-EFW	W-SC-1	W-SC-2	W-SC-3	W-EMNR-1	W-EMNR-2	W-EMNR-3
Counted but not included in analysis (CABIN protocol)															
Porifera	Spongillida	Spongillidae	-	indeterminate	40	40	100	160	80	140	40	10	100	50	60
Crustacea	Cyclopoida	-	-	indeterminate	-	60	10	30	20	20	-	-	20	-	-
Crustacea	Ostracoda	-	-	indeterminate	-	20	100	40	20	80	10	20	40	50	30
Crustacea	Cladocera	-	-	indeterminate	30	60	80	80	90	40	30	10	50	80	20
Platyhelminthes	Tricladida	-	-	indeterminate	-	10	-	-	-	-	-	-	-	-	-

Appendix Table A. 6: Summary statistical table 2019 data displaying the normality and equal variance, statistical test, and p value for each endpoint

Sample day	Data	Data Transformation	Normality Test (Shapiro Wilk):		Equal Variance Test (Brown-Forsythe)		Statistical Test	P Value	
			Pass/Fail	P Value	Pass/Fail	P Value			
-1	PO Abundance Emergence	Log10	Passed	(P = 0.898)	Passed	(P = 0.118)	One Way ANOVA	0.758	
12			Passed	(P = 0.349)	Passed	(P = 0.724)	One Way ANOVA	0.925	
19			Passed	(P = 0.318)	Passed	(P = 0.309)	One Way ANOVA	0.588	
26			Passed	(P = 0.550)	Passed	(P = 0.312)	One Way ANOVA	0.642	
33			Passed	(P = 0.657)	Passed	(P = 0.697)	One Way ANOVA	0.234	
40			Passed	(P = 0.183)	Passed	(P = 0.383)	One Way ANOVA	0.476	
47			Passed	(P = 0.765)	Passed	(P = 0.361)	One Way ANOVA	0.969	
54			Passed	(P = 0.730)	Passed	(P = 0.117)	One Way ANOVA	0.765	
61			Passed	(P = 0.110)	Passed	(P = 0.845)	One Way ANOVA	0.117	
68			Passed	(P = 0.349)	Passed	(P = 0.377)	One Way ANOVA	0.203	
-1	PO Diversity Emergence	Log10	Failed	(P < 0.050)	-	-	Kruskal-Wallis	0.871	
12			Passed	(P = 0.368)	Passed	(P = 0.593)	One Way ANOVA	0.658	
19			Passed	(P = 0.763)	Passed	(P = 0.078)	One Way ANOVA	0.17	
26			Passed	(P = 0.077)	Passed	(P = 0.388)	One Way ANOVA	0.5	
33			Passed	(P = 0.572)	Passed	(P = 0.179)	One Way ANOVA	0.059	
40			Passed	(P = 0.409)	Passed	(P = 0.099)	One Way ANOVA	0.234	
47			Passed	(P = 0.347)	Passed	(P = 0.421)	One Way ANOVA	0.131	
54			Passed	(P = 0.324)	Passed	(P = 0.464)	One Way ANOVA	0.455	
61			Passed	(P = 0.325)	Passed	(P = 0.619)	One Way ANOVA	0.753	
68			Passed	(P = 0.941)	Failed	(P < 0.050)	Kruskal-Wallis	0.946	
-1			Passed	(P = 0.324)	Passed	(P = 1.000)	One Way ANOVA	0.26	

Sample day	Data	Data Transformation	Normality Test (Shapiro Wilk): Pass/Fail	P Value	Equal Variance Test (Brown-Forsythe) Pass/Fail	P Value	Statistical Test	P Value	
12	PO Richness Emergence	Log_{10}	Passed	(P = 0.391)	Passed	(P = 0.090)	One Way ANOVA	0.175	
19			Passed	(P = 0.523)	Passed	(P = 0.184)	One Way ANOVA	0.534	
26			Passed	(P = 0.093)	Failed	(P < 0.050)	Kruskal-Wallis	1	
33			Passed	(P = 0.338)	Passed	(P = 0.160)	One Way ANOVA	0.964	
40			Passed	(P = 0.099)	Passed	(P = 1.000)	One Way ANOVA	0.548	
47			Passed	(P = 0.499)	Passed	(P = 1.000)	One Way ANOVA	0.027	
					REF vs. EMNR		Dunnett's	0.032	
					REF vs. SWA		Dunnett's	0.022	
54		Log_{10}	Passed	(P = 0.872)	Passed	(P = 0.132)	One Way ANOVA	0.262	
61			Passed	(P = 0.210)	Passed	(P = 1.000)	One Way ANOVA	0.426	
68			Failed	(P < 0.050)	-	-	Kruskal-Wallis	0.968	
-1	RC Abundance Emergence		Passed	(P = 0.475)	Passed	(P = 0.195)	One Way ANOVA	0.059	
12			Passed	(P = 0.137)	Passed	(P = 0.411)	One Way ANOVA	0.166	
19			Passed	(P = 0.802)	Passed	(P = 0.305)	One Way ANOVA	0.531	
26			Passed	(P = 0.865)	Passed	(P = 0.278)	One Way ANOVA	0.379	
33			Passed	(P = 0.274)	Passed	(P = 0.200)	One Way ANOVA	0.285	
40			Passed	(P = 0.528)	Passed	(P = 0.477)	One Way ANOVA	0.578	
47			Passed	(P = 0.910)	Passed	(P = 0.208)	One Way ANOVA	0.568	
54			Passed	(P = 0.454)	Passed	(P = 0.664)	One Way ANOVA	0.474	
61		Log_{10}	Passed	(P = 0.939)	Passed	(P = 0.090)	One Way ANOVA	0.498	
68			Passed	(P = 0.852)	Passed	(P = 0.256)	One Way ANOVA	0.315	
-1	RC Diversity Emergence		Passed	(P = 0.280)	Passed	(P = 0.745)	One Way ANOVA	0.281	
12			Passed	(P = 0.324)	Failed	(P < 0.050)	Kruskal-Wallis	0.286	
19			Passed	(P = 0.924)	Passed	(P = 0.462)	One Way ANOVA	0.4	
26			Passed	(P = 0.052)	Passed	(P = 0.968)	One Way ANOVA	0.74	

Sample day	Data	Data Transformation	Normality Test (Shapiro Wilk): Pass/Fail	Normality Test (Shapiro Wilk): P Value	Equal Variance Test (Brown-Forsythe): Pass/Fail	Equal Variance Test (Brown-Forsythe): P Value	Statistical Test	P Value	
33			Passed	(P = 0.847)	Passed	(P = 0.288)	One Way ANOVA	0.278	
40		Square Root	Passed	(P = 0.110)	Passed	(P = 0.517)	One Way ANOVA	0.435	
47			Passed	(P = 0.245)	Passed	(P = 0.863)	One Way ANOVA	0.399	
54			Passed	(P = 0.691)	Passed	(P = 0.311)	One Way ANOVA	0.622	
61			Passed	(P = 0.487)	Passed	(P = 0.114)	One Way ANOVA	0.355	
68			Passed	(P = 0.439)	Passed	(P = 0.196)	One Way ANOVA	0.443	
-1	**RC Richness Emergence**		Passed	(P = 0.235)	Passed	(P = 1.000)	One Way ANOVA	0.199	
12			Passed	(P = 0.858)	Passed	(P = 0.388)	One Way ANOVA	0.124	
19			Passed	(P = 0.629)	Failed	(P < 0.050)	Kruskal-Wallis	0.286	
26		Log_{10}	Passed	(P = 0.081)	Passed	(P = 0.065)	One Way ANOVA	0.039	
					REF vs. EMNR		Dunnett's	0.025	
					REF vs. SWA		Dunnett's	0.152	
33			Passed	(P = 0.522)	Passed	(P = 0.544)	One Way ANOVA	0.875	
40			Passed	(P = 0.861)	Passed	(P = 0.050)	One Way ANOVA	0.559	
47			Passed	(P = 0.210)	Passed	(P = 1.000)	One Way ANOVA	0.745	
54			Passed	(P = 0.926)	Passed	(P = 0.192)	One Way ANOVA	0.76	
61		Log_{10}	Passed	(P = 0.195)	Passed	(P = 1.000)	One Way ANOVA	0.015	
					REF vs. EMNR		Dunnett's	0.03	
					REF vs. SWA		Dunnett's	0.894	
68			Passed	(P = 0.228)	Passed	(P = 1.000)	One Way ANOVA	0.663	
PO Total Abundance Emergence			Passed	(P = 0.676)	Passed	(P = 0.245)	One Way ANOVA	0.688	
PO Total Richness Emergence			Passed	(P = 0.402)	Passed	(P = 1.000)	One Way ANOVA	0.543	
PO Total Diversity Emergence			Passed	(P = 0.330)	Passed	(P = 0.748)	One Way ANOVA	0.018	

Sample day	Data	Data Transformation	Normality Test (Shapiro Wilk): Pass/Fail	Normality Test (Shapiro Wilk): P Value	Equal Variance Test (Brown-Forsythe): Pass/Fail	Equal Variance Test (Brown-Forsythe): P Value	Statistical Test	P Value	
					REF vs. EMNR		Dunnett's	0.015	
					REF vs. SWA		Dunnett's	0.404	
	RC Total Abundance Emergence		Passed	(P = 0.530)	Passed	(P = 0.488)	One Way ANOVA	0.365	
	RC Total Richness Emergence		Passed	(P = 0.857)	Failed	(P < 0.050)	Kruskal-Wallis	0.243	
	RC Total Diversity Emergence		Passed	(P = 0.522)	Passed	(P = 0.350)	One Way ANOVA	0.246	
	(DAY-1-DAY 33) PO Abundance		Passed	(P = 0.678)	Passed	(P = 0.469)	One Way ANOVA	0.673	
	(DAY-1-DAY 33) PO Diversity		Passed	(P = 0.450)	Passed	(P = 0.704)	One Way ANOVA	0.586	
	(DAY-1-DAY 33) PO Richness	Log_{10}	Passed	(P = 0.988)	Passed	(P = 0.070)	One Way ANOVA	0.504	
	PO Total Abundance Emergence (no pre exposure)		Passed	(P = 0.769)	Passed	(P = 0.256)	One Way ANOVA	0.678	
	PO Total Richness Emergence (no pre exposure)		Passed	(P = 0.926)	Failed	(P < 0.050)	Kruskal-Wallis	0.357	
	PO Total Diversity Emergence (no pre exposure)		Passed	(P = 0.168)	Passed	(P = 0.848)	One Way ANOVA	0.007	
					REF vs. EMNR		Dunnett's	0.006	
					REF vs. SWA		Dunnett's	0.49	
	RC Total Abundance Emergence (no pre exposure)		Passed	(P = 0.682)	Passed	(P = 0.430)	One Way ANOVA	0.415	
	RC Total Richness Emergence (no pre exposure)		Passed	(P = 0.182)	Passed	(P = 0.281)	One Way ANOVA	0.224	
	RC Total Diversity Emergence		Passed	(P = 0.182)	Passed	(P = 0.870)	One Way ANOVA	0.175	
	(DAY12-DAY 33) PO Abundance (no pre exposure)		Passed	(P = 0.658)	Passed	(P = 0.522)	One Way ANOVA	0.653	

Sample day	Data	Data Transformation	Normality Test (Shapiro Wilk): Pass/Fail	Normality Test (Shapiro Wilk): P Value	Equal Variance Test (Brown-Forsythe) Pass/Fail	Equal Variance Test (Brown-Forsythe) P Value	Statistical Test	P Value	
(DAY12-DAY 33) PO Diversity (no pre exposure)			Passed	(P = 0.318)	Passed	(P = 0.623)	One Way ANOVA	0.707	
(DAY12-DAY 33) PO Richness (no pre exposure)			Passed	(P = 0.947)	Passed	(P = 0.081)	One Way ANOVA	0.523	
PO Total Abundance Benthics			Passed	(P = 0.491)	Passed	(P = 0.706)	One Way ANOVA	0.125	
PO Total Richness Benthics (LPL)			Passed	(P = 0.919)	Passed	(P = 0.236)	One Way ANOVA	0.371	
PO Total Richness Benthics (Family)			Passed	(P = 0.276)	Passed	(P = 0.754)	One Way ANOVA	0.803	
PO Total Diversity Benthics (LPL)			Passed	(P = 0.556)	Passed	(P = 0.522)	One Way ANOVA	0.831	
PO Total Diversity Benthics (Family)			Passed	(P = 0.643)	Passed	(P = 0.528)	One Way ANOVA	0.16	
PO ETO Abundance			Passed	(P = 0.401)	Passed	(P = 0.608)	One Way ANOVA	0.646	
PO Chirnomid Benthic Abundance			Passed	(P = 0.367)	Passed	(P = 0.453)	One Way ANOVA	0.007	
					REF vs. EMNR		Dunnett's	0.005	
					REF vs. SWA		Dunnett's	0.009	
PO Benthics Ephemeroptera Abundance		Log_{10}	Passed	(P = 0.153)	Passed	(P = 0.355)	One Way ANOVA	0.904	
PO Benthics Trichoptera Abundance			Passed	(P = 0.373)	Failed	(P < 0.050)	Kruskal-Wallis	0.368	
PO Benthics Odonata Abundance		Log_{10}	Passed	(P = 0.397)	Passed	(P = 0.348)	One Way ANOVA	0.184	

Sample day	Data	Data Transformation	Normality Test (Shapiro Wilk):		Equal Variance Test (Brown-Forsythe)		Statistical Test	P Value	
			Pass/Fail	P Value	Pass/Fail	P Value			
PO Benthics Chironominae Abundance			Passed	(P = 0.409)	Passed	(P = 0.382)	One Way ANOVA	0.011	
					REF vs. EMNR		Dunnett's	0.009	
					REF vs. SWA		Dunnett's	0.012	
PO Benthics Orthocladinae Abundance			Failed	(P < 0.050)	-	-	Kruskal-Wallis	0.164	
PO Benthics Tanypodinae Abundance		Log_{10}	Passed	(P = 0.757)	Passed	(P = 0.346)	One Way ANOVA	0.118	
RC Total Abundance Benthics			Passed	(P = 0.410)	Passed	(P = 0.151)	One Way ANOVA	0.746	
RC Total Richness Benthics (LPL)			Passed	(P = 0.569)	Passed	(P = 0.115)	One Way ANOVA	0.418	
RC Total Richness Benthics (Family)		Log_{10}	Passed	(P = 0.325)	Passed	(P = 0.051)	One Way ANOVA	0.339	
RC Total Diversity Benthics (LPL)			Passed	(P = 0.433)	Failed	(P < 0.050)	Kruskal-Wallis	0.075	
RC Total Diversity Benthics (Family)			Passed	(P = 0.617)	Passed	(P = 0.466)	One Way ANOVA	0.726	
RC ETO Benthic Abundance			Passed	(P = 0.393)	Passed	(P = 0.353)	One Way ANOVA	0.165	
RC Chironomid Benthic Abundance			Passed	(P = 0.522)	Passed	(P = 0.235)	One Way ANOVA	0.942	
RC Benthics Ephemeroptera Abundance			Failed	(P < 0.050)	-	-	Kruskal-Wallis	0.164	

Sample day	Data	Data Transformation	Normality Test (Shapiro Wilk):		Equal Variance Test (Brown-Forsythe)		Statistical Test	P Value	
			Pass/Fail	P Value	Pass/Fail	P Value			
RC Benthics Trichoptera Abundance			Passed	(P = 0.097)	Passed	(P = 1.000)	One Way ANOVA	0.265	
RC Benthics Odonata Abundance			Passed	(P = 0.235)	Passed	(P = 1.000)	One Way ANOVA	0.805	
RC Benthics Chironominae Abundance			Passed	(P = 0.725)	Passed	(P = 0.172)	One Way ANOVA	0.903	
RC Benthics Orthocladinae Abundance			Passed	(P = 0.357)	Passed	(P = 0.397)	One Way ANOVA	0.242	
RC Benthics Tanypodinae Abundance			Passed	(P = 0.458)	Passed	(P = 0.175)	One Way ANOVA	0.32	

P=<0.050

tests did not meet normality or equal variance for raw data, Log10 and Square Root transformations

a. Tests ran in Log10 or Square Root, did not meet normality or equal variance with raw data, transformation was required

Appendix Table A. 7: Benthic Macroinvertebrate percent difference from the two treatments, SWA and EMNR compared to the control to lowest taxa level identified for Rock Cobble Enclosures

Rock Cobble			Treatment/ AvgTaxa Count			% Difference from Control	
Family	**Subfamily**	**Lowest Practical Level**	**Control**	**SWA**	**EMNR**	**SWA**	**EMNR**
Talitridae	-	*Hyalella sp.*	80	73.33	13.33	-8.70%	-142.86%
Caenidae	-	Caenis sp.	30	6.67	6.67	-127.27%	-127.27%
Leptophlebiidae	-	*Leptophlebia sp.*	30	0.00	0.00	-200.00%	-200.00%
Leptoceridae	-	*Mystacides sp.*	20	26.67	6.67	28.57%	-100.00%
Leptoceridae	-	*Oecetis sp.*	40	20.00	6.67	-66.67%	-142.86%
Philopotamidae	-	*Dolophilodes sp.*	0	6.67	0.00	200.00%	0.00%
Phryganeidae	-	Immature	0	13.33	0.00	200.00%	0.00%
Coenagrionidae	-	Immature	0	6.67	6.67	200.00%	200.00%
Gomphidae	-	Immature	10	6.67	0.00	-40.00%	-200.00%
Ceratopogonidae	-	*Culicoides*	0	6.67	6.67	200.00%	200.00%
Ceratopogonidae	-	*Probezzia sp.*	10	6.67	13.33	-40.00%	28.57%
Chironomidae	Chironominae	*Chironomus sp.*	0	46.67	193.33	200.00%	200.00%
Chironomidae	Chironominae	*Cladopema sp.*	40	0.00	0.00	-200.00%	-200.00%
Chironomidae	Chironominae	*Cladotanytarsus sp.*	140	126.67	100.00	-10.00%	-33.33%
Chironomidae	Chironominae	*Dicrotendipes sp.*	110	6.67	20.00	-177.14%	-138.46%
Chironomidae	Chironominae	*Microtendipes sp.*	0	13.33	33.33	200.00%	200.00%
Chironomidae	Chironominae	*Pagastiella sp.*	40	60.00	20.00	40.00%	-66.67%
Chironomidae	Chironominae	*Polypedilim sp.*	20	6.67	13.33	-100.00%	-40.00%
Chironomidae	Chironominae	*Pseudochironomus sp.*	50	20.00	20.00	-85.71%	-85.71%
Chironomidae	Chironominae	*Stenochironomus sp.*	0	6.67	0.00	200.00%	0.00%
Chironomidae	Chironominae	*Tanytarsus sp.*	120	126.67	93.33	5.41%	-25.00%
Chironomidae	Orthocladinae	*Nanocladius sp.*	20	60.00	33.33	100.00%	50.00%
Chironomidae	Orthocladinae	*Parakiefferiella sp.*	40	93.33	60.00	80.00%	40.00%
Chironomidae	Tanypodinae	*Ablabesmyia sp.*	90	40.00	13.33	-76.92%	-148.39%

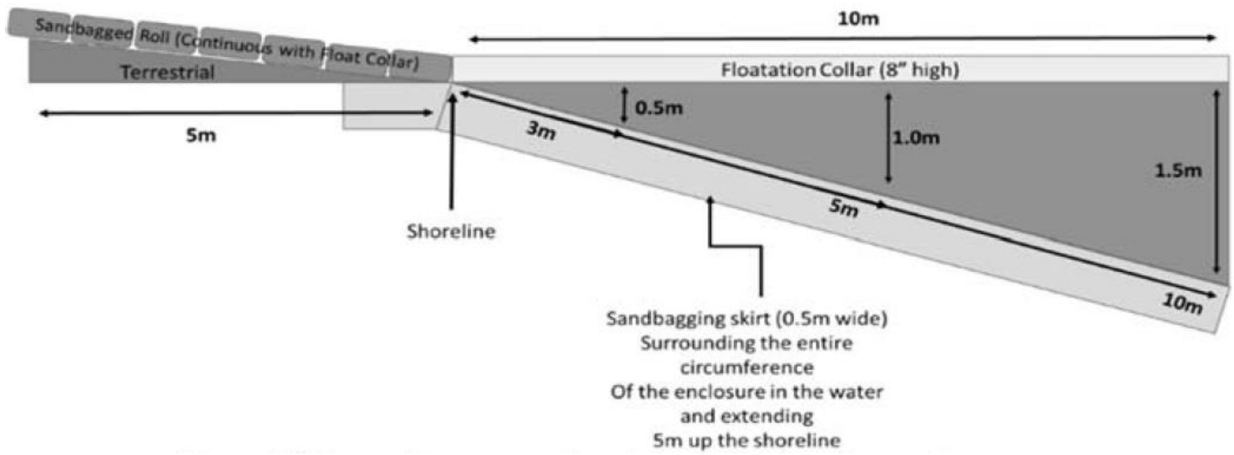
Rock Cobble			Treatment/ AvgTaxa Count			% Difference from Control	
Family	**Subfamily**	**Lowest Practical Level**	**Control**	**SWA**	**EMNR**	**SWA**	**EMNR**
Chironomidae	Tanypodinae	*Procladius sp.*	50	20.00	13.33	-85.71%	-115.79%
Hydrodromidae	-	*Hydrodroma sp.*	0	6.67	0.00	200.00%	0.00%
Hydryphantidae	-	*Pseudohydryphantes sp.*	10	0.00	6.67	-200.00%	-40.00%
Sphaeriidae	-	*Pisidium sp.*	10	13.33	20.00	28.57%	66.67%
Planorbidae	-	*Gyraulus deflectus*	10	0.00	0.00	-200.00%	-200.00%
Erpobdellidae	-	Immature	10	0.00	0.00	-200.00%	-200.00%
Lumbriculidae	-	indeterminate	0	0.00	6.67	0.00%	200.00%
Naididae	-	*Nais sp.*	40	60.00	40.00	40.00%	0.00%
Naididae	-	*Pristina sp.*	10	0.00	0.00	-200.00%	-200.00%
Naididae	-	*Pristinella sp.*	0	6.67	20.00	200.00%	200.00%
Naididae	-	*Stylaria lacustris*	0	6.67	13.33	200.00%	200.00%
Naididae	-	*Vejdovskyella comata*	20	0.00	6.67	-200.00%	-100.00%
Tubificidae	-	Immature without hairs	20	13.33	6.67	-40.00%	-100.00%

Appendix Table A. 8: Benthic Macroinvertebrate percent difference from the two treatments, SWA and EMNR compared to the control to lowest taxa level identified for Peat Organic Enclosures

Peat Organic			Treatment/ AvgTaxa Count			% Difference from Control	
Family	**Subfamily**	**Lowest Practical Level**	**Control**	**SWA**	**EMNR**	**SWA**	**EMNR**
Talitridae	-	*Hyalella sp.*	5	0.00	0.00	-200.00%	-200.00%
Caenidae	-	*Caenis sp.*	110	40.00	160.00	-93.33%	37.04%
Leptophlebiidae	-	*Leptophlebia sp.*	0	6.67	6.67	200.00%	200.00%
Leptoceridae	-	*Oecetis sp.*	15	6.67	6.67	-76.92%	-76.92%
Phryganeidae	-	*Agrypina sp.*	0	0.00	3.33	0.00%	200.00%
Phryganeidae	-	*Fabria sp.*	0	0.00	3.33	0.00%	200.00%
Polycentropodidae	-	*Polycentropus sp.*	0	0.00	3.33	0.00%	200.00%
Sericostomatidae	-	*Agarodes sp.*	0	0.00	3.33	0.00%	200.00%
Coenagrionidae	-	*Coenagrion sp.*	15	3.33	23.33	-127.27%	43.48%
Libellulidae	-	Immature	15	6.67	23.33	-76.92%	43.48%
Ceratopogonidae	-	*Bezzia/Palpomyia*	5	3.33	3.33	-40.00%	-40.00%
Ceratopogonidae	-	*Probezzia sp.*	15	6.67	13.33	-76.92%	-11.76%
Chironomidae	Chironominae	*Chironomus sp.*	110	30.00	36.67	-114.29%	-100.00%
Chironomidae	Chironominae	*Cladotanytarsus sp.*	150	36.67	43.33	-121.43%	-110.34%
Chironomidae	Chironominae	*Cryptochironomus sp.*	10	0.00	0.00	-200.00%	-200.00%
Chironomidae	Chironominae	*Endochironomus sp.*	0	0.00	6.67	0.00%	200.00%
Chironomidae	Chironominae	*Microtendipes sp.*	70	80.00	33.33	13.33%	-70.97%
Chironomidae	Chironominae	*Pagastiella sp.*	10	0.00	10.00	-200.00%	0.00%
Chironomidae	Chironominae	*Pseudochironomus sp.*	0	0.00	3.33	0.00%	200.00%
Chironomidae	Chironominae	*Stenochironomus sp.*	0	0.00	3.33	0.00%	200.00%
Chironomidae	Chironominae	*Tanytarsus sp.*	225	93.33	90.00	-82.72%	-85.71%

Peat Organic			Treatment/ AvgTaxa Count			% Difference from Control	
Family	**Subfamily**	**Lowest Practical Level**	**Control**	**SWA**	**EMNR**	**SWA**	**EMNR**
Chironomidae	Chironominae	*Zavreliella sp.*	5	6.67	0.00	28.57%	-200.00%
Chironomidae	Orthocladinae	*Nanocladius sp.*	25	0.00	0.00	-200.00%	-200.00%
Chironomidae	Tanypodinae	*Ablabesmyia sp.*	100	13.33	16.67	-152.94%	-142.86%
Chironomidae	Tanypodinae	*Labrundinia sp.*	5	0.00	0.00	-200.00%	-200.00%
Chironomidae	Tanypodinae	*Procladius sp.*	220	83.33	36.67	-90.11%	-142.86%
Sphaeriidae	-	*Pisidium sp.*	55	40.00	30.00	-31.58%	-58.82%
Planorbidae	-	*Gyraulus deflectus*	10	0.00	0.00	-200.00%	-200.00%
Lumbriculidae	-	indeterminate	0	10.00	6.67	200.00%	200.00%
Naididae	-	*Nais sp.*	5	0.00	0.00	-200.00%	-200.00%
Naididae	-	*Vejdovskyella comata*	0	6.67	0.00	200.00%	0.00%
Tubificidae	-	Immature without hairs	15	0.00	10.00	-200.00%	-40.00%
Tubificidae	-	Immature with hairs	15	10.00	16.67	-40.00%	10.53%

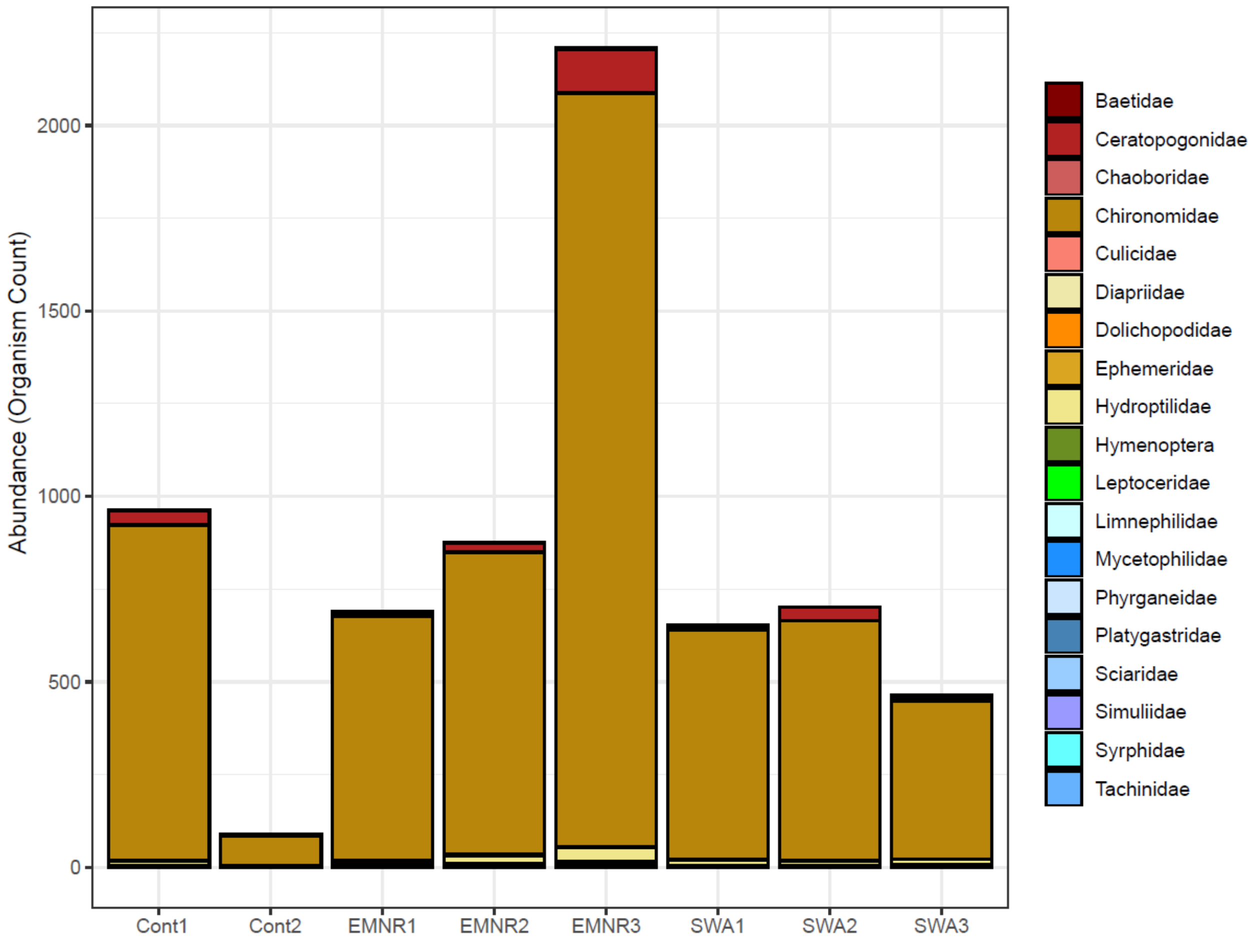
Appendix B Supplemental Figures



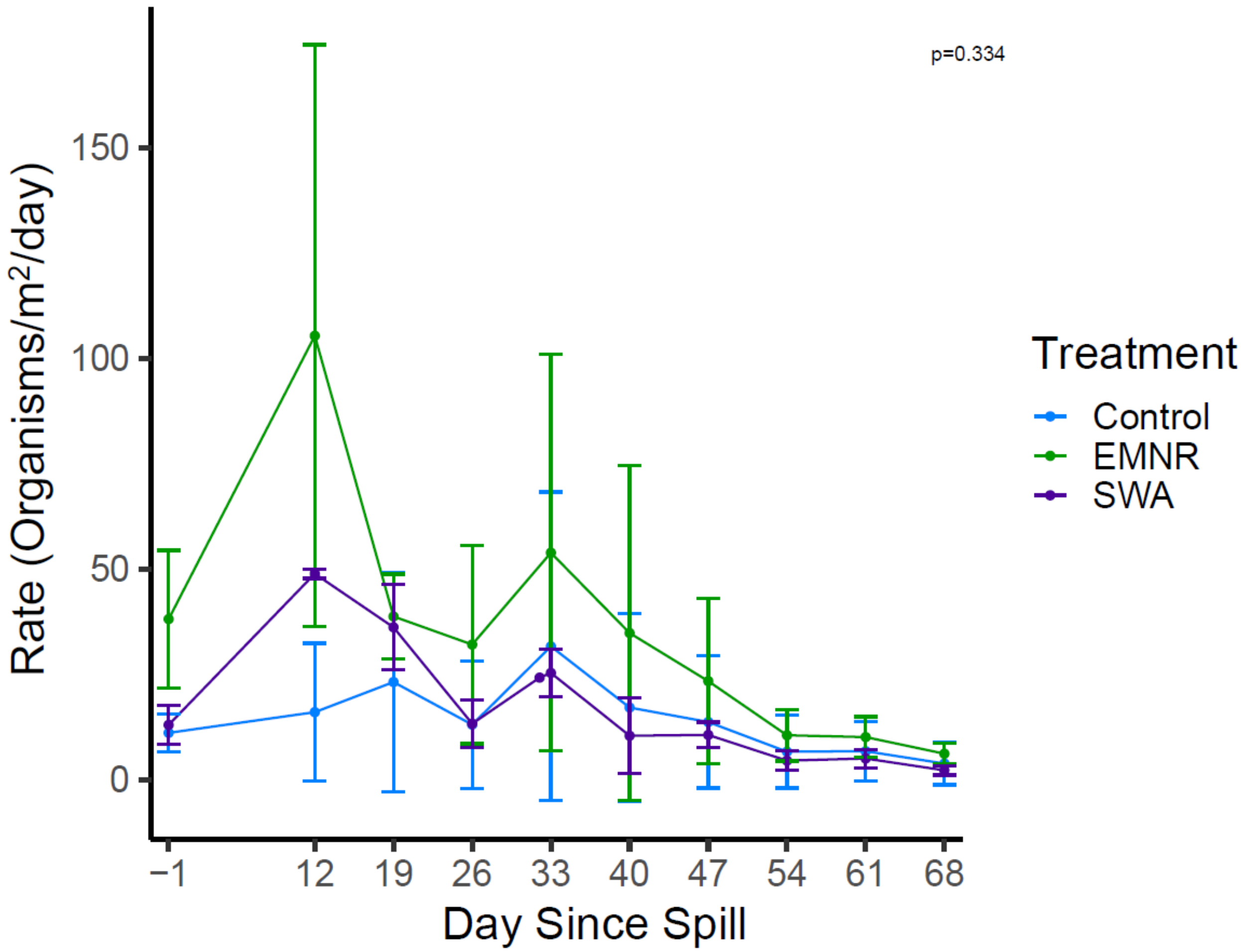
Appendix Figure B.1: Schematic cross-sectional view of a shoreline enclosure (taken from Palace et al., 2021).

Appendix Figure B.2: Image of littoral enclosures taken June 16th, 2019 (photo by Holly Kajpust).

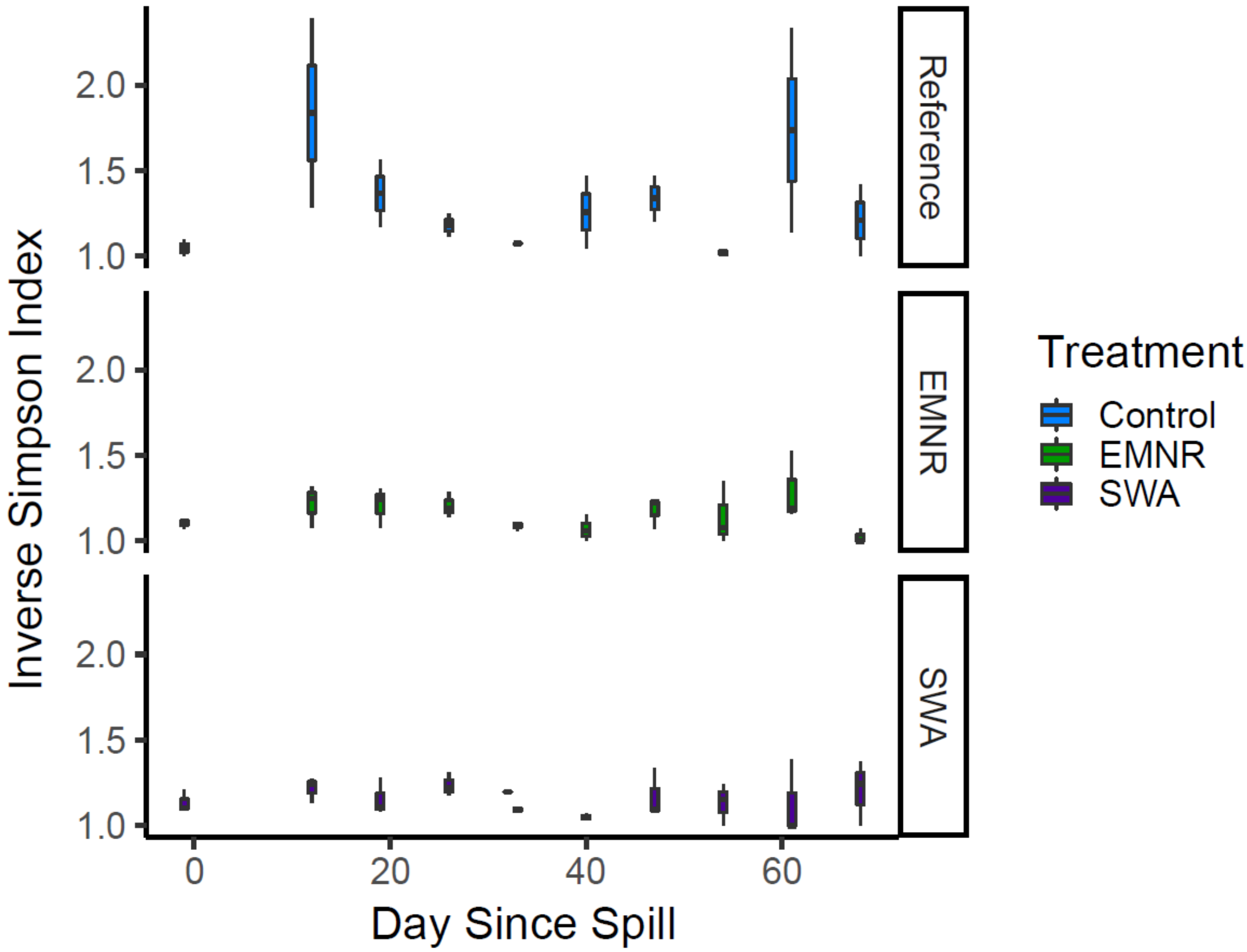
Appendix Figure B.3: Image of emergence trap deployed in an enclosure taken June 16th, 2019 (photo by Holly Kajpust).



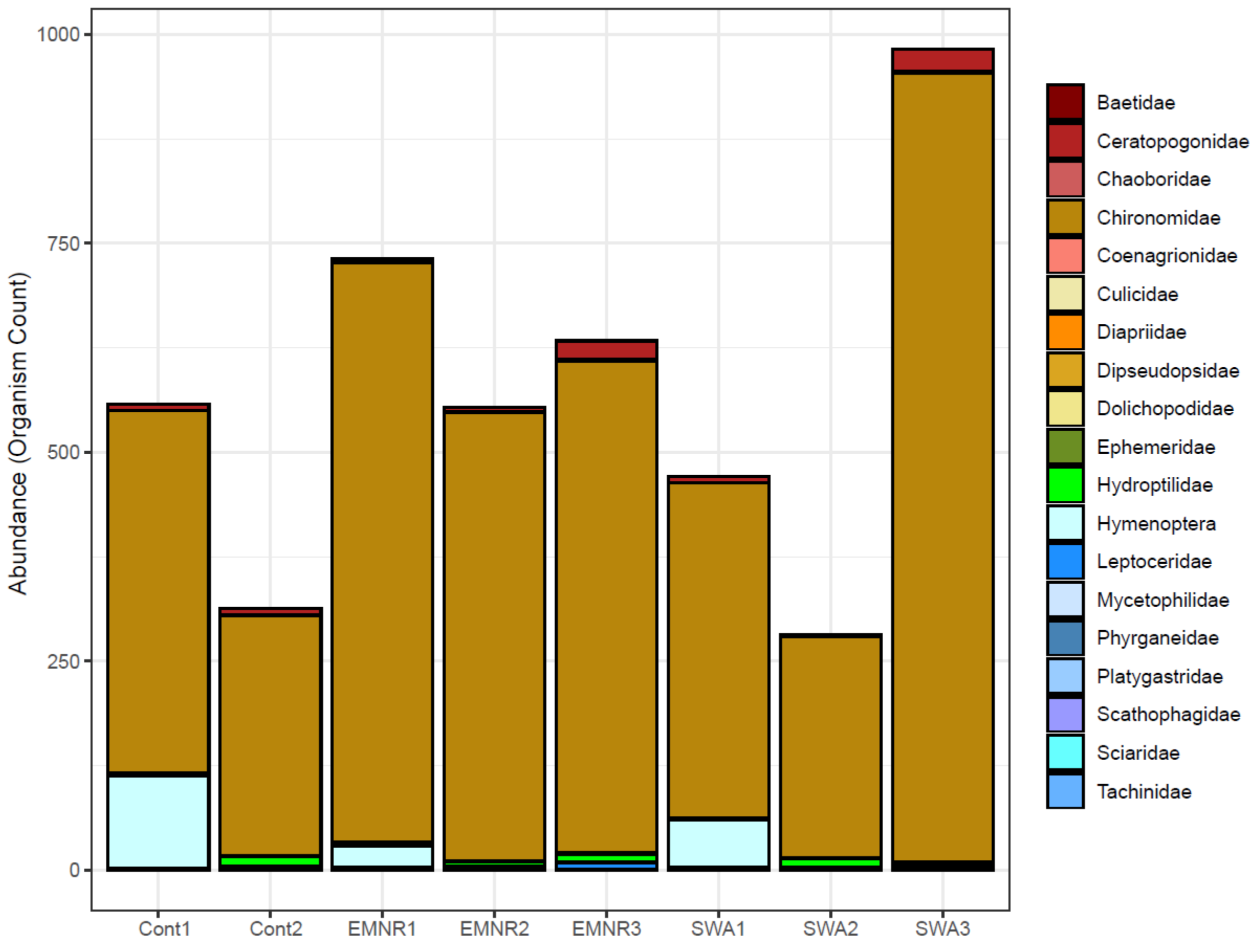
Appendix Figure B. 4: Rock Cobble enclosure variation among replicates and family abundance collected in emergent traps.



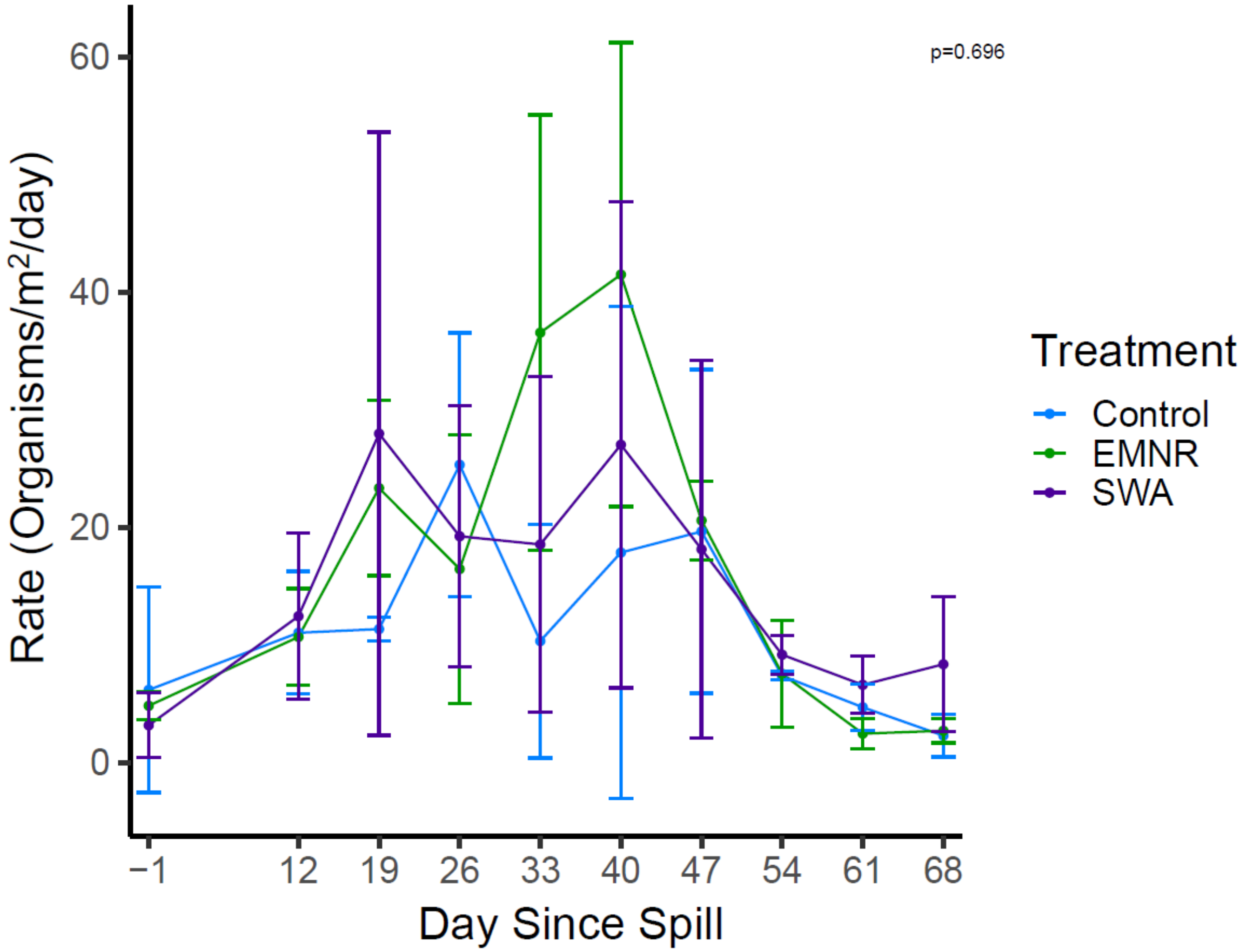
Appendix Figure B. 5: Changes in rate of emergence (organism/m^2/day) over time on the Rock Cobble enclosures. The enclosures were sampled from June 22nd, 2019 (Day -1) to August 29, 2019 (Day 68). Treatments EMNR and SWA were not statistically significant from control using a one way ANOVA (p=0.334).



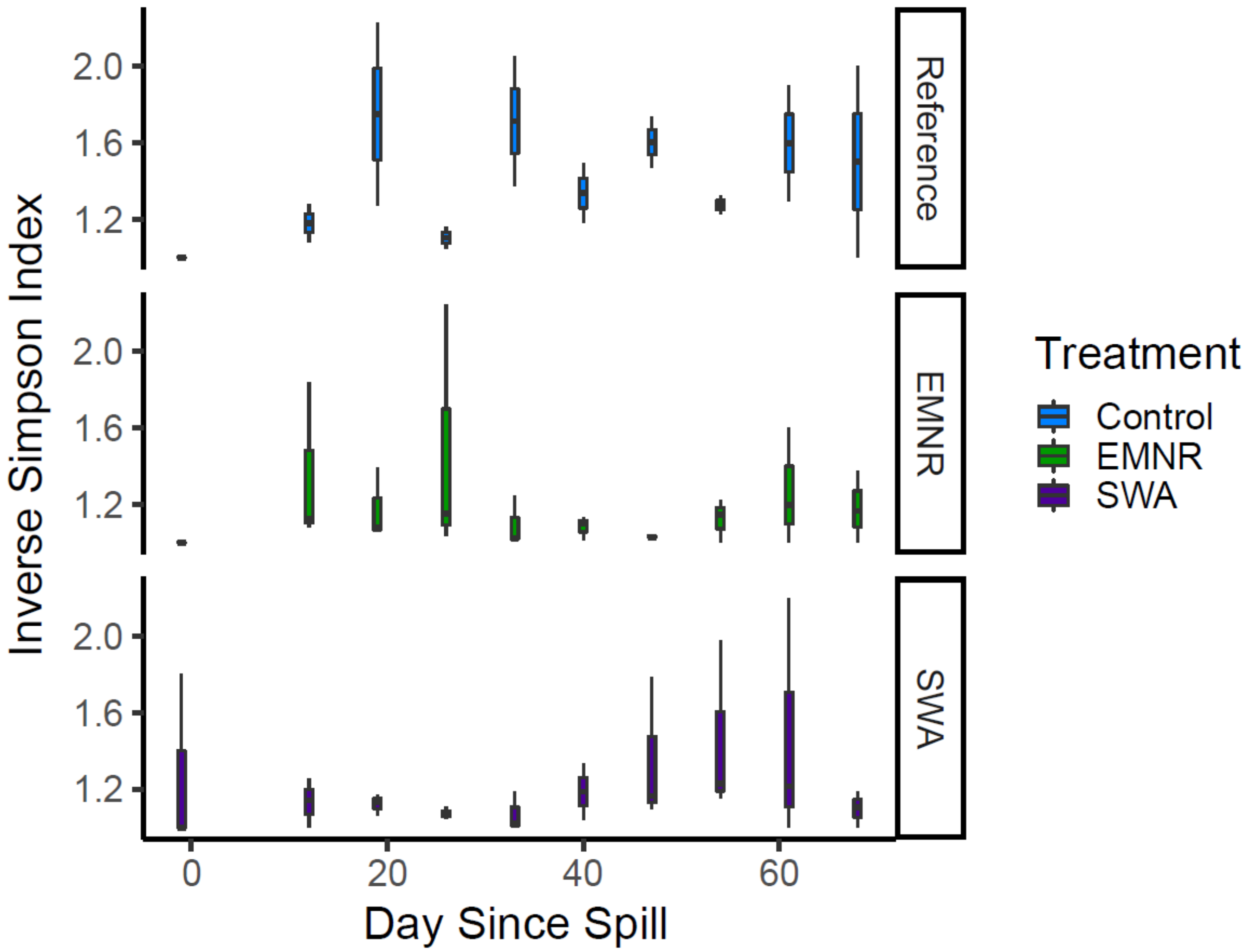
Appendix Figure B. 6: Inverse Simpson diversity over time in emergence traps on the Rock Cobble enclosures. The enclosures were sampled from June 22nd, 2019 (Day -1) to August 29, 2019 (Day 68).



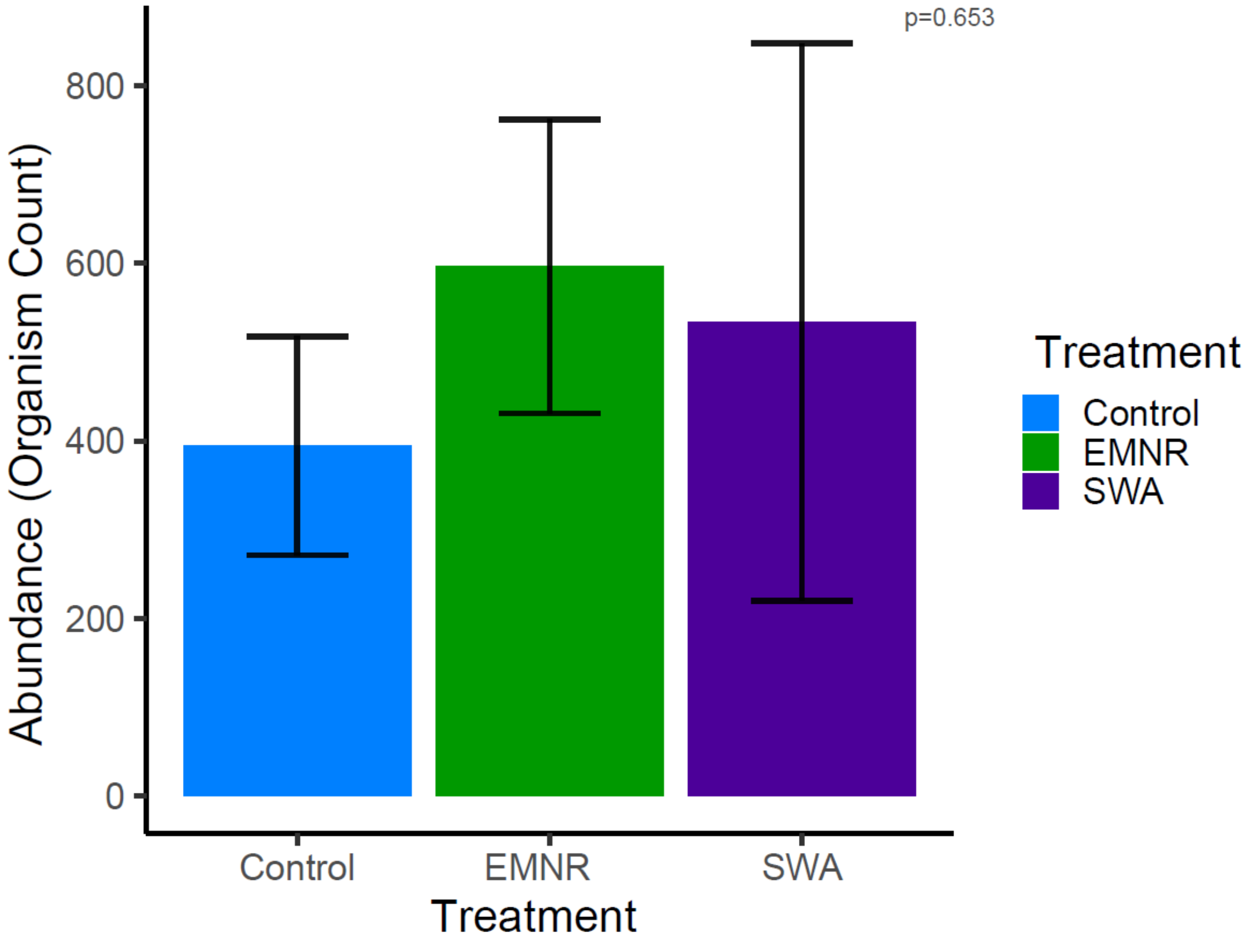
Appendix Figure B. 7; Peat Organic enclosure variation among replicates and all family abundance collected in emergent traps.



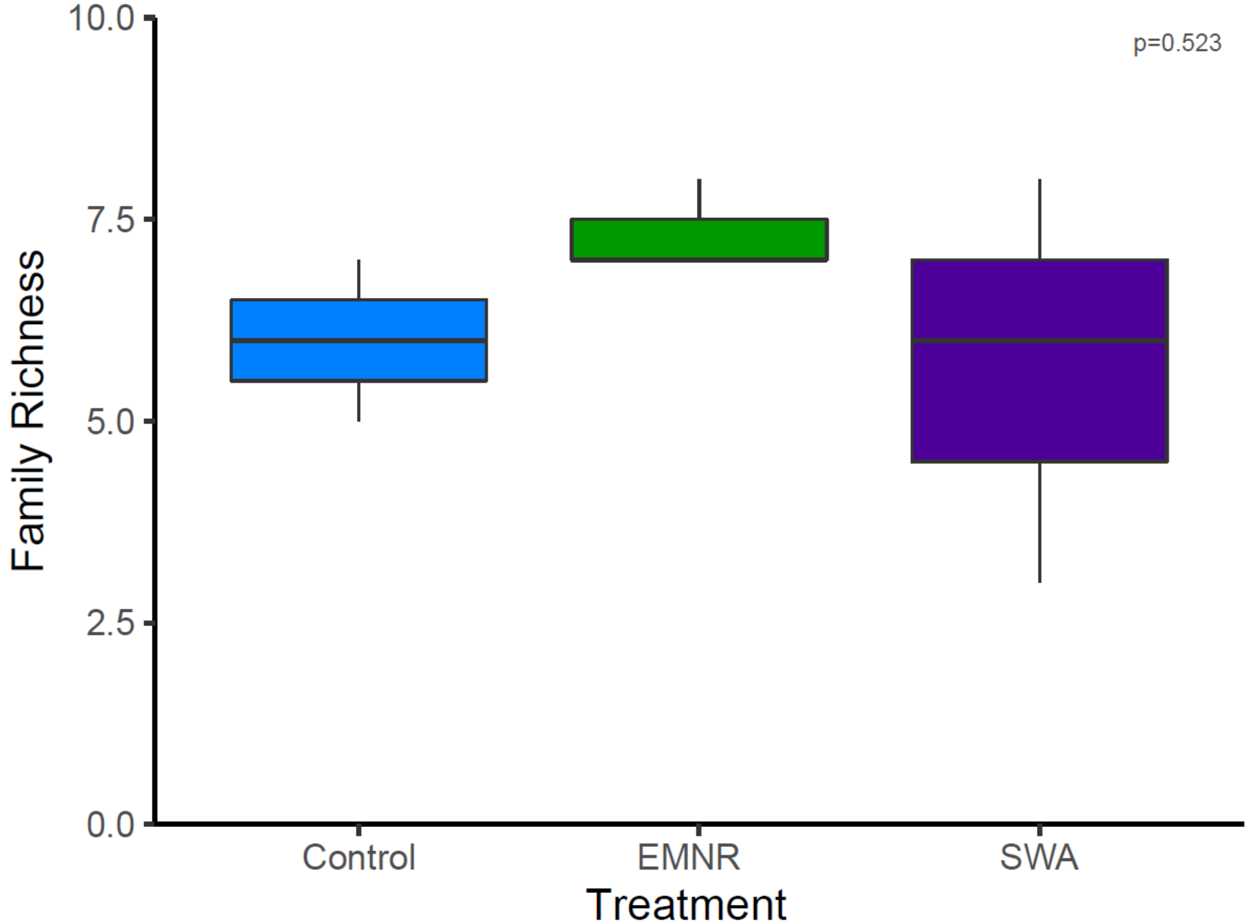
Appendix Figure B. 8: Changes in rate of emergence (organism/m^2/day) over time on the Peat Organic enclosures. The enclosures were sampled from June 21nd, 2019 (Day -1) to August 28, 2019 (Day 68). Treatments EMNR and SWA were not statistically significant from control using a one way ANOVA (p=0.696).



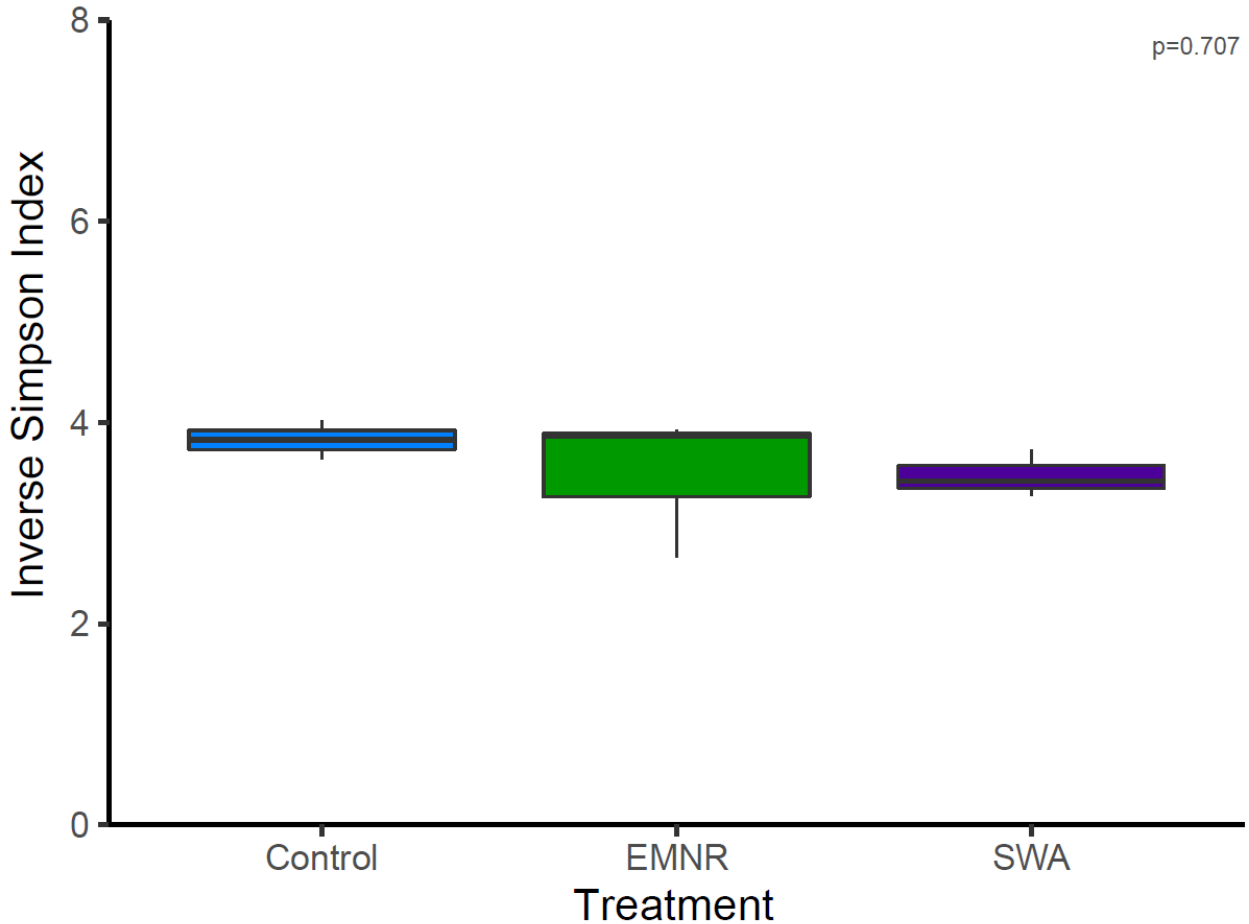
Appendix Figure B. 9: Inverse Simpson diversity over time in emergence traps on the Peat Organic shoreline. The enclosures were sampled from June 21nd, 2019 (Day -1) to August 28, 2019 (Day 68).



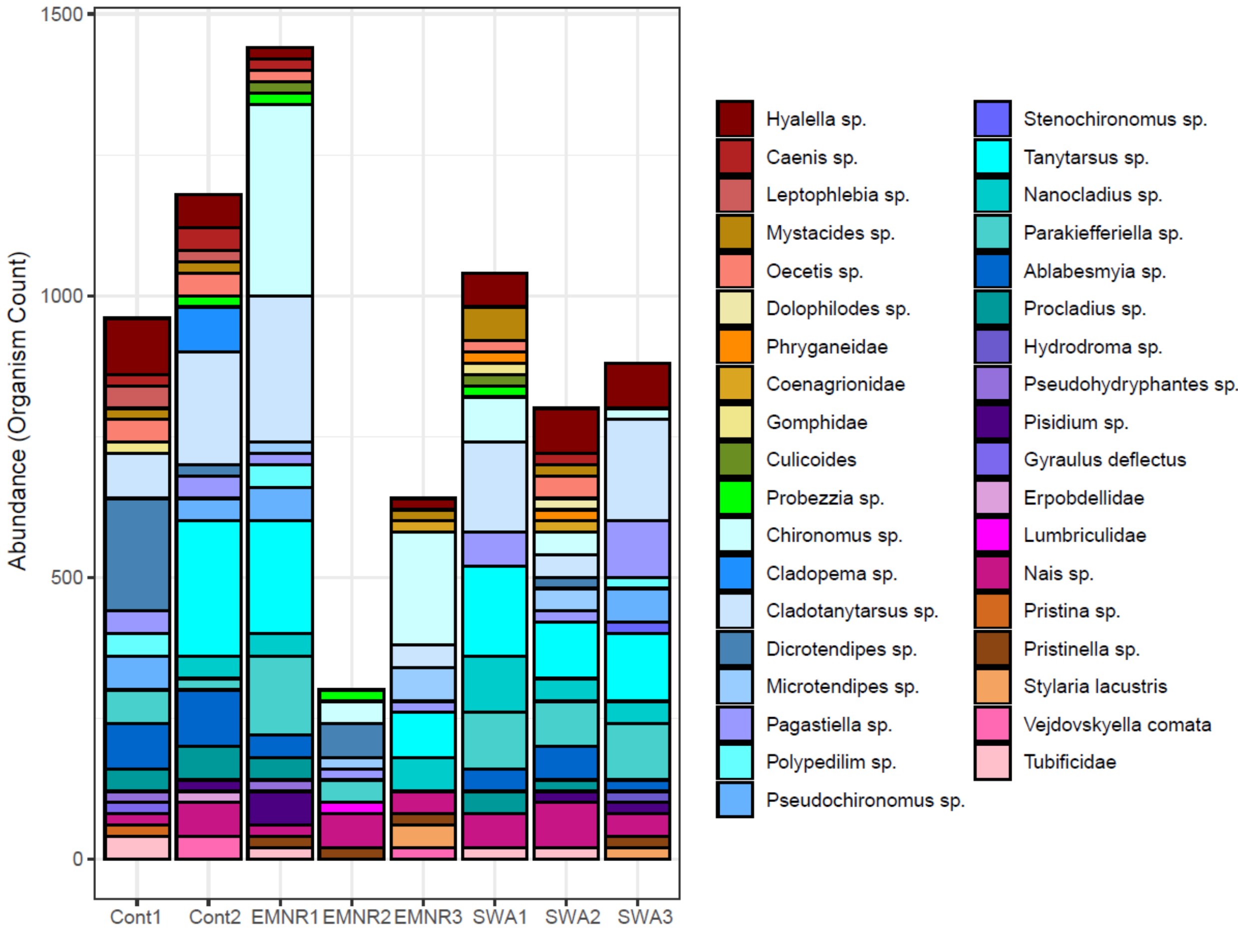
Appendix Figure B. 10: Sum of abundance (organism/m^2) over all days (Day 12 to Day 33) of all families identified over time on the Pear Organic enclosures. Treatments EMNR and SC were not statistically significant from control using a one way ANOVA (p=0.653).The error bars represent the standard deviation.



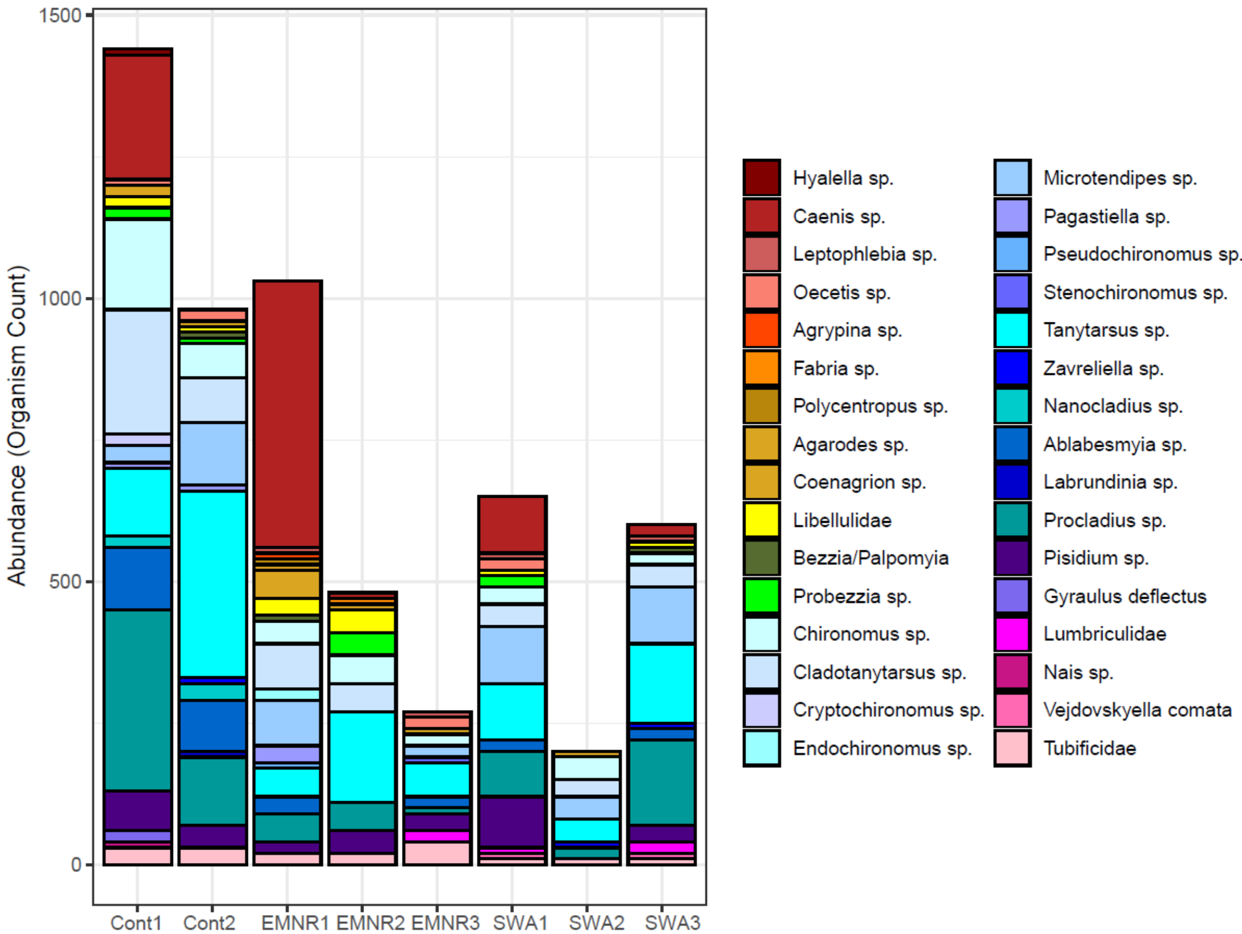
Appendix Figure B. 11: **Total family richness (sum over all sampling days) in emergence traps on the Peat Organic enclosures. The horizontal line in the box is the median, while the whiskers go from each quartile to the minimum or maximum of the sample set. The enclosures were sampled from Day 12 to Day 33. Treatments EMNR and SWA were not statistically significant from control using a one way ANOVA (p=0.523).**



Appendix Figure B. 12: Inverse Simpson diversity (sum over all sampling days) in emergence traps on the Peat Organic enclosures. The horizontal line in the box is the median, while the whiskers go from each quartile to the minimum or maximum of the sample set. The enclosures were sampled from Day 12 to Day 33. Treatments EMNR and SWA were not statistically significant from control using a one way ANOVA ($p=0.707$).



Appendix Figure B. 13: Rock cobble enclosure variation among replicates and taxa abundance collected in kick net sampling.



Appendix Figure B. 14: Peat Organic enclosure variation among replicates and taxa abundance collected in kick net sampling.

Appendix Figure B. 15: Image from time-lapse video or peat organic enclosures and oil sheen (June 26th, 2019) (FOReSt Google Drive).