A Mesocosm-Scale Study of Chemical and Ecological Response to Different Types of Oil Sands Tailings and Process Water (2019 - 2021)

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EXECUTIVE SUMMARY

The use of pit lakes (PL) to reclaim pits at the end of mine life is common in metal and coal extraction operations. Oil sands mine operators of Canada's Oil Sands Innovation Alliance (COSIA) Demonstration Pit Lake project (DPL) Joint Industry Project (JIP) have undertaken a series of mesocosm-scale projects to provide information to support the design of operational pit lakes in the closure landscapes.

Mesocosms are simplified and replicated physical models of 'real world' ecosystems and contain many of the same structural and functional components. In the spring of 2019, a study funded by the COSIA DPL JIP was undertaken to investigate the chemical and ecological effects of different types of oil sands tailings and process water on simplified aquatic ecosystems. The DPL JIP project aims to address research questions that are not covered by other regional pit lake research and address knowledge gaps at a mesocosm scale. This project is the second in a series of mesocosm-based studies that commenced in May 2017.

Why was this study done?
The 2019-2021 mesocosm research project had three main goals. The first was to evaluate the physical and chemical properties of the water cap, which was potentially affected by the test materials (tailings and associated pore water, as well as Oil Sands Process affected Water (OSPW)). The second was to determine how different tailing types and OSPW alter the biological and toxicological properties of the water cap. The third was to provide information on early biological establishment rates and water quality trajectories.

The mesocosms were allocated to six treatment groups defined by sediment type (either filter sand or tailings), Athabasca River water (ARW) and/or OSPW (Table S1). No additional OSPW or tailings materials were added to the mesocosms after the initial material installation. A broad range of physical, chemical, biological and toxicological parameters was assessed to investigate how tailings alone, or in combination with OSPW, would affect these parameters. In addition, the effect of tailings type, and OSPW in the presence of tailings, was tested. Four years of data (2017 - 2020) were also examined using multivariate analysis to improve our understanding of how treatment materials influenced chemical and biological parameters during the mesocosm experiments.

Special considerations
The current report documents a two-year study, executed from May 2019 through October 2020. Within this report, the term "significance" and its derivatives should be read as meaning statistically significant. It should be understood that statistically significant may not be biologically significant, nor necessarily denote an exceedance of the Canadian water quality guideline values for the protection of aquatic life. In addition, when examining results caution should be taken against over-extrapolation in terms of deriving regulatory acceptable concentrations. Furthermore, our aquatic mesocosms are models of lentic ecosystems, and how well data from our experiments could be extrapolated to natural lotic systems is debatable. Mesocosm-based experiments can be used to examine patterns of response and trends over time in test parameters; however, the absolute values of these responses should not be extrapolated to the scale of operational PL.

Table S1. Control and treatment groups with prescribed factors and levels.

Experimental group	Water Cap	Sediment	Treatment Material Source	Number of mesocosms (n)
CTL	100% ARW	Filter Sand (FS)	N/A	5
TRT 1	100% ARW	Coarse Sand Tailings (CST)	Imperial	4
TRT 2	100% ARW	Thickened Tailings (TT)	Imperial	4
TRT 3	100% ARW	Tailings Solvent Recovery Unit Tailings (TSRU)	Imperial	4
TRT 4	100% ARW	Fluid Fine Tailings (FFT)	Imperial	4
TRT 5	100% OSPW	Fluid Fine Tailings (FFT)	Imperial	4
TRT 6	50% OSPW and 50% ARW	Densified Fluid Fine Tailings (dFFT)	Suncor	5
Total of 7 Experimental Groups				**30**

Note: CTL denotes Control, and TRT denotes Treatment; ARW = Athabasca River Water; OSPW = Oil Sands Process affected Water.

What was measured?

This study utilized thirty 15 m^3 mesocosms (Figure S1), which are simplified and replicated aquatic ecosystems, as small-scale models of pit lakes. Various datasets were collected, including water quality (physical and chemistry), toxicity (rainbow trout, fathead minnow, biomimetic extraction via solid-phase microextraction [BE-SPME]), and biological (phytoplankton, vascular plants, faunal community). The sampling program is briefly summarized in Table S2.

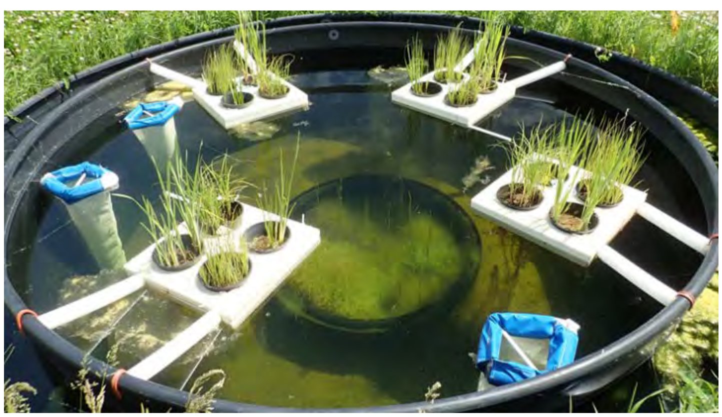


Figure S1. Image of an aquatic mesocosm showing a common internal configuration, including floating shelves, mesh socks, soil trough, and installed and adventitious vegetation. (Photo take in June 2020).

Table S2. Sampling programs conducted in 2019 and 2020 open water season.

Data category	Sub-category	Sample collection	Parameters	Frequency
Water quality	Field measurement	Data sonde (surface and bottom)	pH, conductivity, DO, temperature, and turbidity	Once every 14 days
	Laboratory analysis	Surface sample	Major ions, naphthenic acids, PAHs, DOC, metals, BTEX, phenol, COD, etc.	3 times per year
	Loggers	~1 m below surface	DO and conductivity	One replicate of each group
Toxicity	Rainbow trout	Surface sample	96-hour survival	Twice in 2019, once in 2020
	Fathead minnow	Surface sample	7-day survival/biomass	Once per year
	BE-SPME	Submit surface samples to ExxonMobil	Screening test	3 times per year
Phytoplankton	Total algae sensor	Data sonde (surface and bottom)	Chlorophyll fluorescence	Once every 14 days
	Quantification in laboratory	Surface sample	Chlorophyll a	3 times per year
	Visual estimation	Imaging	Coverage estimation	Monthly
Vascular plants	Plant growth	Emergent and floating plants	Leaf length (emergent plants), wet weight (free-floating plants)	Every 3 weeks
	Biomass	Above-ground portion of emergent plants	Dry biomass	End of each year
	Tissue analysis	Above-ground portion of emergent plants	PAHs and metals	End of 2019
Faunal community	Zooplankton	Traps	Taxonomic analysis	4 times per year
	Macroinvertebrate	Activity traps and H-D samplers	Taxonomic analysis	3 times per year

What we have learned: Objective 1 - Evaluate the physical and chemical properties of the water cap, potentially affected by tailing types and the resulting pore water, as well as Oil Sands Process affected Water (OSPW)

- pH is known to impact some ecological components, influence chemical processes, and play a role in modifying toxicity. The pH in all treatment groups (including CTL) was in the neutral to alkaline range (7.5 – 9.5). The pH was usually lowered by treatment materials, but typically no more than 1 pH unit from the CTL mesocosms.
- Dissolved oxygen (DO) is necessary to support all forms of life in water. DO levels were initially reduced following the introduction of tailings and OSPW. DO levels did not meet the acute guideline for surface water in Alberta (5 mg/L) for the first few weeks in each open water season in mesocosms with 50% OSPW and dFFT. However, these conditions did not persist, and DO concentrations in all treatment groups (including CTL) converged later in the growing season in each year (Figure S2).

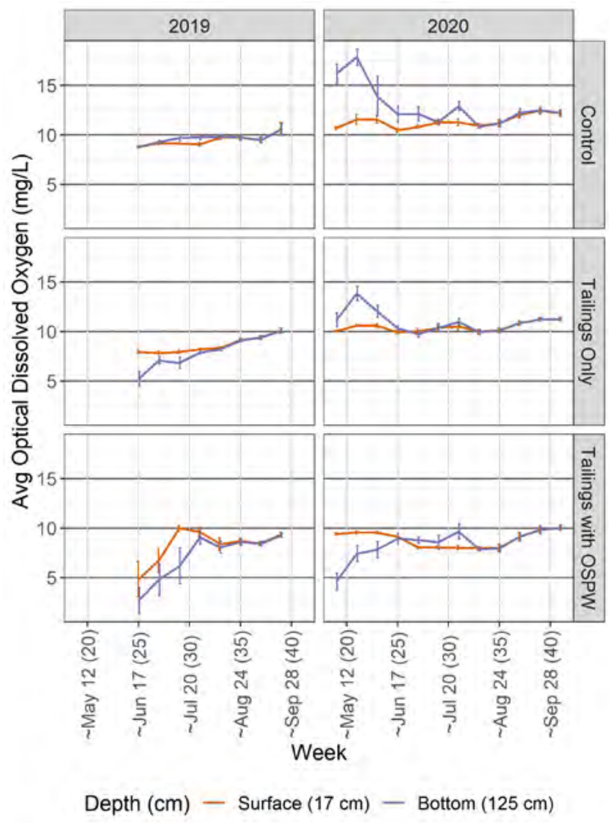


Figure S2 Mean dissolved oxygen by experimental groups in 2019 and 2020. Error bars at each point denote ± standard error across the mesocosms measured at that time point. Note that data points have been combined as Tailings Only (TRTs 1, 2, 3, 4) and Tailings with OSPW (TRT 5 and 6). Refer to Table S1 for details on treatment groups.

- Conductivity is a measure of salinity. Overall, the treatments with tailings alone had relatively similar conductivity to the CTL mesocosms. However, OSPW groups exhibited higher specific conductivity than CTL units (Figure S3). Experimental groups rarely exhibited any depth-related differences within treatments and weeks in 2019. Early in 2020, specific conductivity was significantly higher at the bottom than the surface in all mesocosms. Differences in the length of time these depth-related conductivity differences persisted could be related to degree of salinity of the test materials i.e., differences between surface and bottom water lasted longer for treatments containing OSPW, as they were more saline.

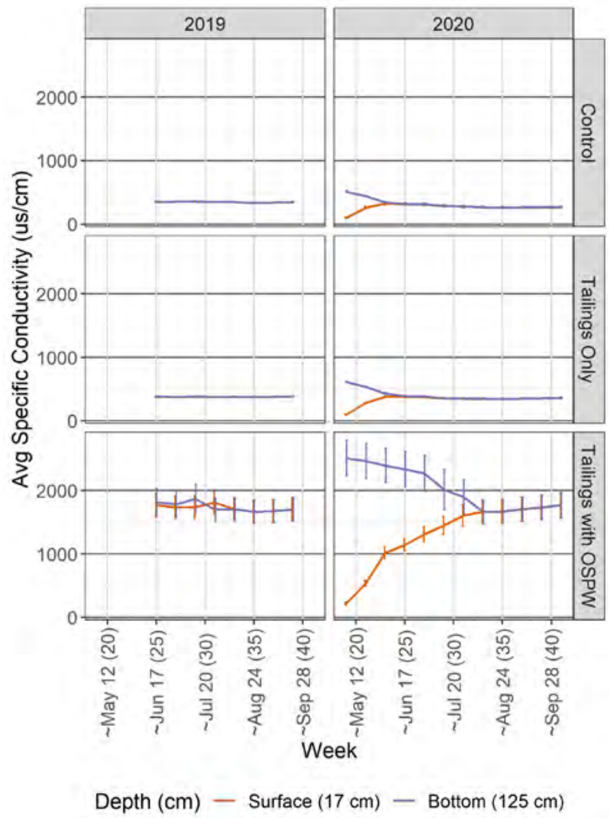


Figure S3 Mean specific conductivity by experimental groups in 2020. Error bars at each point denote ± standard error across the mesocosms measured at that time point. Note that data points have been combined as Tailings Only (TRTs 1, 2, 3, 4) and Tailings with OSPW (TRT 5 and 6). Refer to Table S1 for details on treatment groups

- After installation, the high turbidity levels decreased over time and remained low in all treatment groups.
- Treatments with both OSPW and tailings (TRT 5 and TRT 6) were associated with higher concentrations of most elements and compounds.
- The concentrations of some analytes exhibited a subtle increase over the two study years, with this increase usually being more evident in tailings-only groups (e.g., time-related increases in boron concentration in TRT 4 (FFT)).

- Mesocosms containing both OSPW and tailings (TRT 5 and 6) showed significantly higher levels of naphthenic acids (NAs) relative to CTL mesocosms. However, there was a statistically significant decrease in NAs for both TRT 5 and 6 mesocosms over two years (Figure S4). In spring of 2020 (indicated by red arrow in Figure S4) a chemocline artificially lowered relative NAs concentrations at the surface, but not absolute concentrations across the entire water column. The high variability around the mean NA levels in the Tailings with OSPW group (Figure S4) reflects the fact that two different sources of tailings are included within this group.

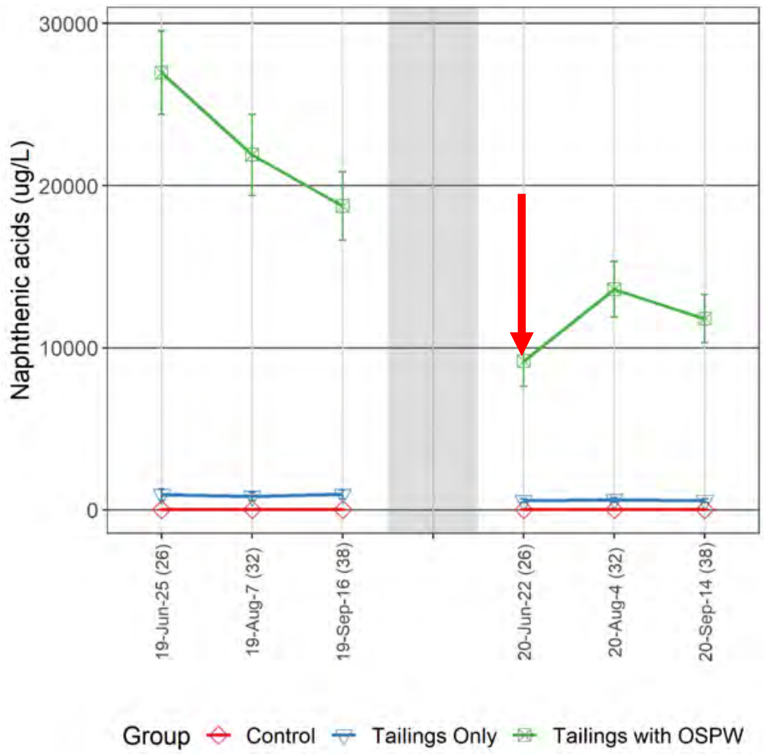


Figure S4 Mean concentrations of total naphthenic acids. The red arrow indicates the first sampling point in 2020, where the formation of a chemocline over winter. Error bars at each point denote ± standard error across the mesocosms measured at that time point. Note that data points have been combined as Tailings Only (TRTs 1, 2, 3, 4) and Tailings with OSPW (TRT 5 and 6). Refer to Table S1 for details on treatment groups. Error bars show concentration range.

- Brine rejection substantially affected the water quality parameters analyzed (as the chemocline described above), and this effect was relatively pronounced in TRT 5 and TRT 6. For example, molybdenum showed a significant mid-study decrease in TRT 5 and TRT 6, while no change was observed in CTL and tailings-alone mesocosms. Since water samples for laboratory analysis were only collected at the surface, some of the significant mid-study decreases observed may be an artefact of the heterogenous vertical distribution of analytes following the overwintering period, since water samples were not collected at depth.

- Concentrations of hydrocarbons associated with OSPW and tailings treatments, such as BTEX (Benzene, Toluene, Ethylbenzene, Xylenes), PAHs (Polycyclic Aromatic Hydrocarbons, Figure S5) and phenols, were generally low, and most of these compounds were undetectable with current commercially available analytical methods in the second year of the experiment. Detection limits and non-detects of analytes are listed in Section A.5 of Appendix A.

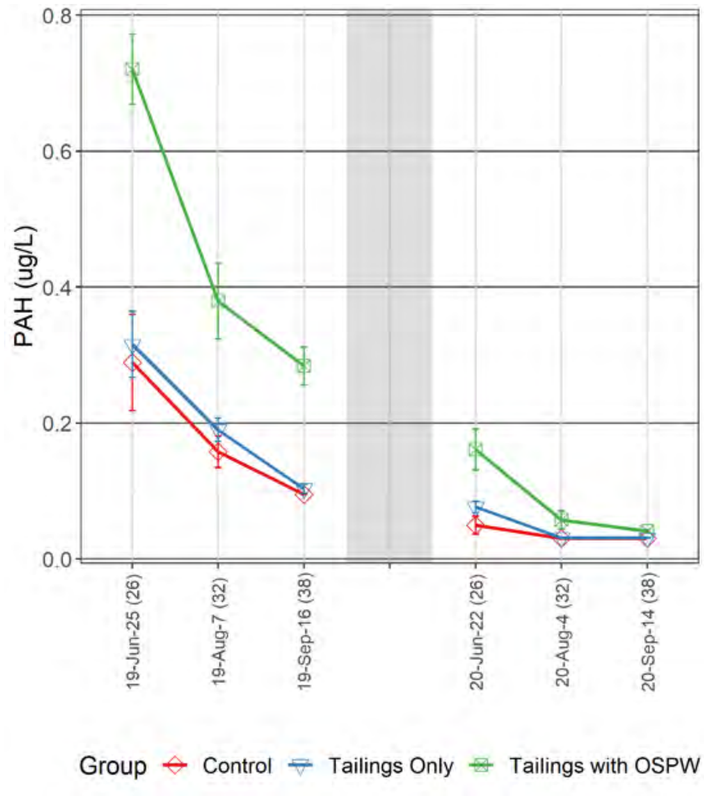


Figure S5 Polycyclic Aromatic Hydrocarbons (PAH) across time points. Error bars at each point denote ± standard error across the mesocosms measured at that time point. Note that data points have been combined as Tailings Only (TRTs 1, 2, 3, 4) and Tailings with OSPW (TRT 5 and 6). Refer to Table S1 for details on treatment groups.

- The addition of tailings alone to the mesocosms was not associated with exceedance of CCME guidelines for the long-term and short-term freshwater protection of aquatic life based on the mean concentrations of most compounds; the only exception was for fluoride (Table S3, TRTs 1, 2, 3, and 4). Though fluoride concentration exceeded the long term CCME guideline (120 µg/L) - mostly in treatments with tailings alone - fluoride concentrations were only statistically significantly higher in the presence of FFT (TRT 4) compared to CTL at the beginning of 2019. In 2020, the concentration of fluoride was statistically significantly higher in the presence of TT (TRT 2), TSRU (TRT 3), and FFT (TRT 4) when compared to CTL.
- Besides fluoride, selenium concentrations were higher than the CCME water quality guideline (1 µg/L) during the last two sampling points in TRT 5 in 2020. Mercury levels exceeded long term

CCME guideline values for the protection of freshwater aquatic life (0.026 µg/L) mainly in 2019. However, the reporting limit of mercury in this study is 0.02 µg/L.

- In TRT6 (50% Suncor OSPW with dFFT), chloride and molybdenum exceeded the CCME chronic guidelines in 2019 and 2020. Arsenic and boron also slightly exceeded the chronic guidelines (Table S3). Several guidelines for the protection of aquatic life vary with hardness, and TRT 6 exhibited lower hardness (62 - 99 mg/L $CaCO_3$) compared to CTL (84 – 140 mg/L $CaCO_3$).

Table S3. Mean (n = 4 or 5) concentration values at the beginning of 2019 and end of 2020 sampling season. Note that highlighted values exceed CCME guidelines for acute and/or chronic exposure.

Constituent (total)	CCME limit		Time	Control	Tailings only				Tailings and OSPW	
	Acute	Chronic		CTL	TRT 1	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6
Hardness (mg/L)			Start	123.6	127.3	119.8	124.8	115.8	135	92.2
			End	84.2	104.5	88.3	97.3	117.2	127	82.8
pH		6.5-9.0	Start	8.24	7.96	8.23	8.02	8.45	8.69	7.97
			End	9.37	8.84	9.02	9	8.91	8.76	8.77
DOC (mg/L)			Start	11.4	11.1	11.8	11.8	13.7	43.8	45
			End	11.1	11.3	12.6	10.9	14.6	39.6	43.6
Naphthenic acids (mg/L)			Start	0.021	0.111	0.943	0.258	2.49	33.2	22
			End	0.019	0.151	0.51	0.384	1.27	14.5	9.62
Arsenic (µg/L)	NA	5	Start	1.1	1.07	1.2	1.09	1.13	2.73	5.8
			End	1.25	1.23	1.29	0.67	1.06	1.86	4.9
Boron (mg/L)	29	1.5	Start	0.034	0.048	0.05	0.058	0.135	1.04	1.84
			End	0.032	0.072	0.101	0.11	0.219	1.04	2.16
Chloride (mg/L)	640	120	Start	15.2	15.5	15	14.6	15	36.5	359.4
			End	14.3	13.6	14.4	13.7	14.1	32.8	356.6
Copper (µg/L)	Equation	7	Start	2.97	2.84	2.51	2.02	1.79	2.11	1.64
			End	1.81	1.65	1.66	1.23	1.48	1.24	1.26
Fluoride (µg/L)		120	Start	110	110	160	160	540	4300	1500
			End	180	150	320	310	690	4080	1450
Mercury (µg/L)		0.026	Start	0.01	0.01	0.01	0.01	0.01	0.0625	0.14
			End	0.01	0.01	0.01	0.01	0.01	0.01	0.038
Molybdenum (µg/L)		73	Start	0.99	0.98	1.65	1.97	4.84	57.8	173
			End	1.47	1.01	2.8	2.53	6.37	51.9	115
Nickel (µg/L)	NA	Equation	Start	1.2	4.7	2.1	2.5	3	7	5.6
			End	1.5	3.5	3	2.2	3.9	6.6	4.7
Selenium (µg/L)		1	Start	0.18	0.22	0.15	0.15	0.2	0.68	3.74
			End	0.4	0.3	0.3	0.28	0.32	1	4.26
Vanadium (µg/L)	NA	NA	Start	0.24	0.49	0.91	3.17	5.63	14.82	7.65
			End	0.52	0.48	0.77	0.45	1.34	3.2	5.78

Notes:
1. Water Quality Guidelines for the Protection of Aquatic Life Freshwater (https://ccme.ca/en/summary-table). Concentrations were rounded off as per CCME guideline levels.
2. The chlorine data represent chloride concentrations.
3. For below-reporting-limit results (i.e., mercury and selenium), a value half-way between the reporting limit (0.02 µg/L for mercury and 0.2 µg/L for selenium) and zero was assigned to those samples.

What we have learned: Objective 2 - To determine how different tailing types alter the water column in terms of biological and toxicological properties.

- Phytoplankton is an important component of the food web and has been used as an indicator of water quality for decades. From the mid- to end-point of 2020 sampling season, phytoplankton coverage in CTL mesocosms was significantly higher than that observed in TRT mesocosms. Algal mats were only observed in TRT 6 mesocosms in the spring of 2020.
- In general, tailings alone and OSPW with FFT did not affect the growth of the emergent plants (ratroot, water sedge, awned sedge, and cattail) most of the time over two years of exposure with few exceptions, suggesting that these plants were tolerant of the various constituents associated with these materials. However, TRT 6 had a significant stimulatory effect on the growth of all four emergent species. Emergent plants growing in Suncor OSPW (50%) with dFFT were significantly

taller. The aboveground biomass of all four emergent species was significantly greater in TRT 6 than CLT mesocosms in both years.

- Tailings alone groups negatively affected hornwort in 2019, but this effect only persisted into 2020 for FFT mesocosms. However, tailings with OSPW negatively impacted hornwort in both years, which did not survive in mesocosms containing OSPW with tailings treatments in 2020. These findings align with the AESRD *Criteria and Indicators Framework for Oil Sands Mine Reclamation Certification*, which references a decrease in relative cover of hornwort as an indicator of stress for open water marsh zones in reclaimed oil sands mining landscapes.
- There were no statistically significant differences between TRTs3, 5, 6 and CTL in terms of uptake of PAHs. Compared to CTL mesocosms, statistically significant accumulation of trace metals was rarely documented in the above-ground tissues of all four species of emergent plants by the end of the first sampling season, although concentrations of metals in tissues could be influenced by different OSPW and tailings treatments. Overall, differences in metals uptake by emergent plants were more evident in the presence of both OSPW and tailings (TRTs 5 and 6) than tailings alone (TRT3).
- The presence of treatment materials occasionally affected zooplankton (Figure S6) and macroinvertebrate (Figure S7) communities. Occasional reduced zooplankton and macroinvertebrate indices, including richness, abundance, and diversity, in TRTs relative to CTL suggest that even tailings alone can negatively affect the zooplankton community, although the negative effects tend to be short-lived (mainly in 2019).
- The acute 96-hour LC_{50} (median lethal concentration) for rainbow trout, and the chronic 7-day LC_{50} and IC_{25} (inhibition concentration; concentration of a test item that causes a 25% reduction in a parameter such as weight) for fathead minnow, tests were included for estimating toxicity of the treatment materials. In general, both the trout and fathead minnow assays detected "non-lethal" in the presence of tailings alone. TRT 5 (FFT + 100% Imperial OSPW), and one TRT 6 mesocosm (dFFT + 50% Suncor OSPW) had rainbow trout LC_{50} values less than 100% (~55% and 82%, respectively) at the beginning of the 2019 sampling season (Table S4). In 2020, some level of mortality was also observed in TRT 5 mesocosms. Limited biomass effects were observed early in 2019, for one mesocosm in each of the TRT 4, TRT 5, and TRT 6 mesocosms (Table S4).

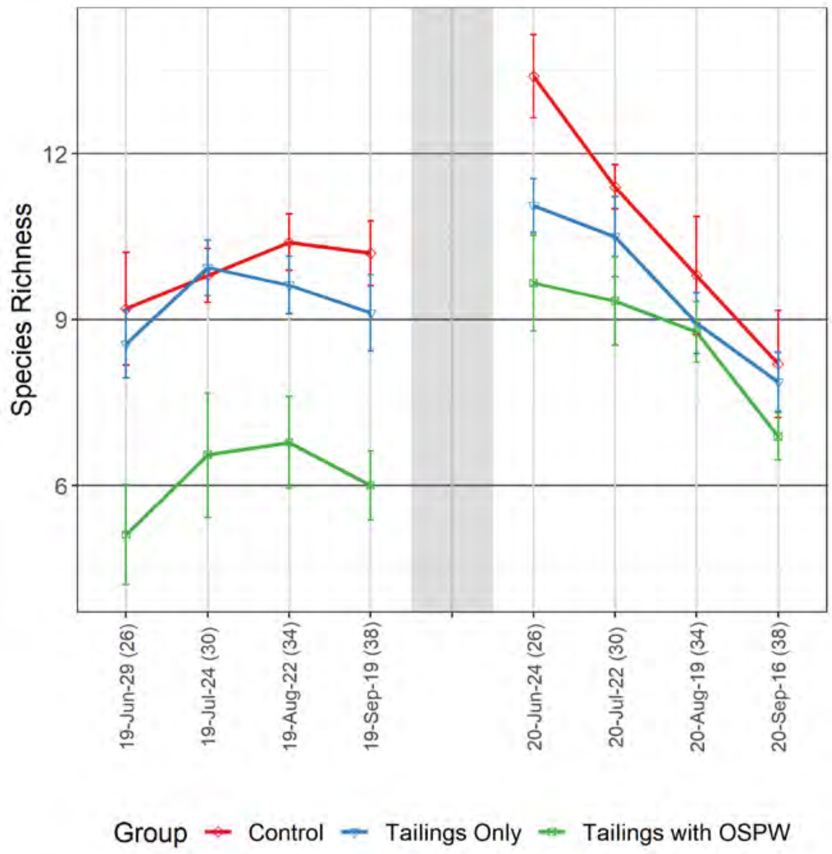


Figure S6. Zooplankton taxa richness in 2019 and 2020. Error bars at each point denote ± standard error across the mesocosms measured at that time point. Note that data points have been combined as Tailings Only (TRTs 1, 2, 3, 4) and Tailings with OSPW (TRT 5 and 6). Refer to Table S1 for details on treatment groups.

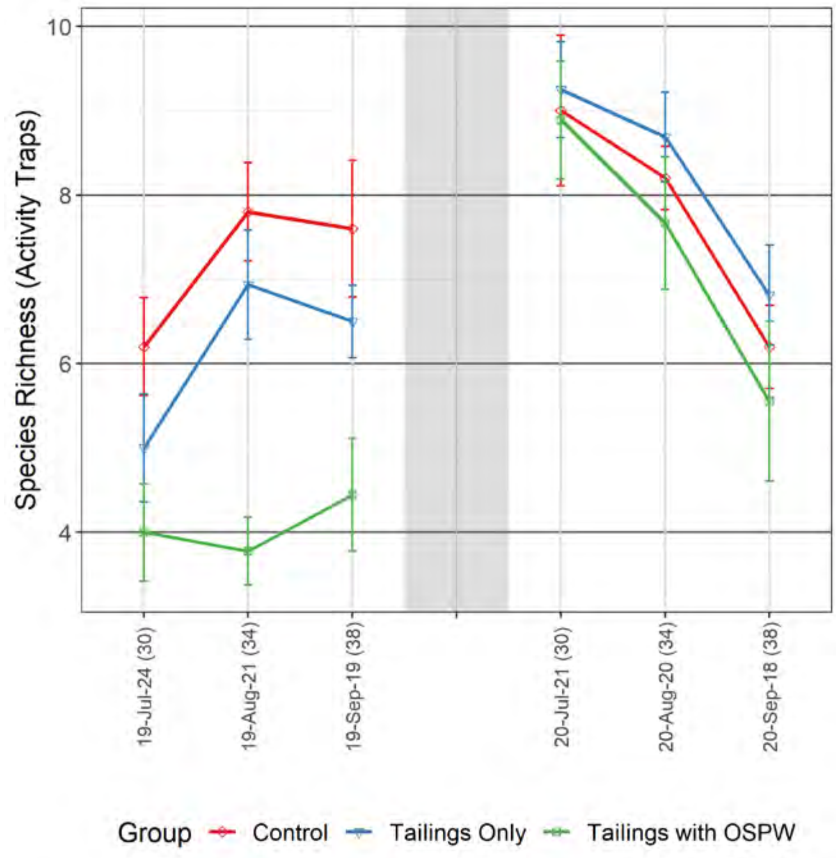


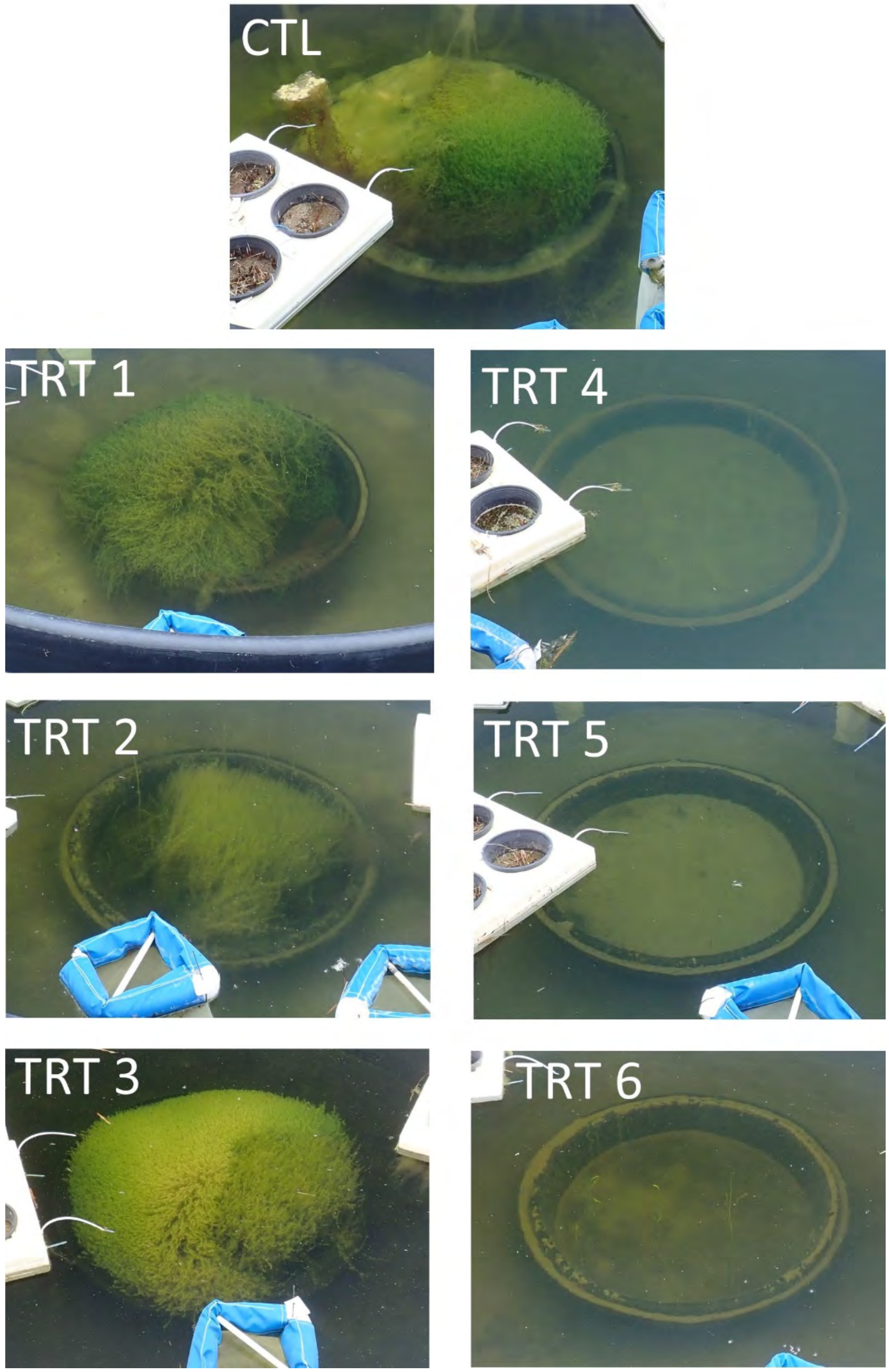
Figure S7. Macroinvertebrate species richness in samples collected by activity traps in 2019 and 2020. Error bars at each point denote ± standard error across the mesocosms measured at that time point. Note that data points have been combined as Tailings Only (TRTs 1, 2, 3, 4) and Tailings with OSPW (TRT 5 and 6). Refer to Table S1 for details on treatment groups

Table S4. LC_{50} and IC_{25} values of < 100% across all experimental groups for water samples collected in 2019 and 2020. All LC_{50} and IC_{25} values not listed from each experimental group were returned as >100 % ("non-lethal").

Rainbow trout 96-h survival			**Fathead minnow 7-d growth**		
Group	**Week**	**LC_{50} (% v/v)**	**Group**	**Week**	**Biomass IC_{25} (%v/v)**
TRT5	2019.W.26	56.2	TRT4	2019.W.26	81.0
TRT5	2019.W.26	53.6	TRT5	2019.W.26	83.1
TRT5	2019.W.26	53.6	TRT6	2019.W.26	87.8
TRT6	2019.W.26	82.0			
TRT5	2019.W.38	82.0			
TRT5	2020.W.38	62.8			

What we have learned: Objective 3 - To provide information on early biological establishment rates and water quality trajectories

- Adventitious plants/algae were expected to create additional habitat and act as food for aquatic organisms. By the end of two years of exposure, vascular plants had colonized installed submerged soil in CTL, and TRT 1, 2, 3 mesocosms, but colonization was limited in mesocosms containing FFT and OSPW (TRT 4, 5, and 6) (Figure S8).
- There was rarely any indication of growth inhibition of installed emergent plants over the two years of the study. For TRT 6, the growth and flowering rates of emergent plants were higher than in other treatments or the controls. No statistically significant effect on emergent plant growth was observed in TRT 5 (which also contained OSPW and FFT) compared to CTL mesocosms.
- Trout and minnow survival was unaffected by the presence of tailings alone. Overall, toxicity to trout declined over time in OSPW-containing groups (TRT 5 and TRT 6) throughout 2019, while fathead minnow mortality results stayed mainly unchanged in both of these treatments. Some biomass reductions were observed in TRTs 4, 5, and 6 in 2019.
- Zooplankton are small invertebrates that represent a substantial portion of the food web in aquatic systems. There is some indication that time influenced the structure of the zooplankton community in both CTL and TRTs mesocosms. Zooplankton richness was initially low in 2019, during the establishment of ecological communities within the mesocosm tanks. Richness values increased into the spring of 2020, when they peaked, thereafter declining.
- Macroinvertebrates play a substantial role in the food webs of aquatic ecosystems, with some members of this group being the top predators in the aquatic mesocosms used in this study. All mesocosms were found to support populations of macroinvertebrates in both years. Macroinvertebrate species richness appeared to increase or exhibit little change in 2019 but decreased during 2020 (Figure S7). The similarity in the pattern of species richness across all experimental groups suggests the influence of an environmental factor common to all mesocosms (e.g., overwintering and time) and not a specific treatment effect.
- Some level of risk to zooplankton and aquatic macroinvertebrates related to the experimental materials was indicated. The presence of OSPW and tailings together significantly impacted the zooplankton and macroinvertebrate communities during some sampling periods; the majority of these effects were detected in 2019. For example, TRT5 and 6 mesocosms had significantly lower zooplankton/macroinvertebrate species richness and abundance compared to CTL at least once in 2019. Our results suggest the overall ecological structure of the mesocosm community changed over time, and it is possible that the zooplankton and macroinvertebrate community might recover from the effect of treatment materials over time.
- The Spearman Rank correlations (Appendix F) indicated some measures of zooplankton and macroinvertebrate species richness and/or abundance exhibited a negative relationship with increased concentrations of metals (i.e., boron, molybdenum, nickel, vanadium), metalloids (i.e., selenium), naphthenic acids, specific conductivity, and chloride, and with decreased dissolved oxygen levels.



- Figure S8. Photos showing TRT 3 mesocosms, and low adventitious colonization in TRT 4, TRT 5, and TRT 6 mesocosms. Pictures taken on October 5, 2020.

Keywords: Aquatic mesocosms, end pit lake, OSPW (Oil Sands Process (affected) Water), tailings, water chemistry, aquatic plants, toxicity, zooplankton, macroinvertebrates, phytoplankton

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1.0 INTRODUCTION

1.1 Demonstration Pit Lake Joint Industry Project

Canada's Oil Sands Innovation Alliance (COSIA) focuses on accelerating the pace of improvement in environmental performance in Canada's oil sands through collaborative action and innovation (www.cosia.ca). The use of pit lakes (PL) to treat tailings at the end of mine life is common in metal and coal mining operations. To date, only one such lake - Base Mine Lake (Syncrude) - has been constructed at a commercial scale for the oil sands mining sector. Oil sands mine operators of COSIA's Water Environmental Priority Area (EPA) are undertaking a Demonstration Pit Lake project (DPL) to provide information to support the design of commercial pit lakes in the closure landscapes. The planned Joint Industry Partnership (JIP) DPL project will help to address research questions that are not covered by Syncrude's Base Mine Lake (BML) commercial demonstration and help address knowledge gaps at a mesocosm scale (McCullough and Vandenberg, 2020).

The 2019 – 2021 aquatic mesocosm program was managed by Imperial Resources Limited (Imperial). This program was funded by the following oil sand mining operators: Imperial, Suncor, Canadian Natural Resources Limited, and Teck, with input from Syncrude.

1.2 Aquatic Mesocosms

Mesocosms are simplified and replicated physical models of 'real world' ecosystems and contain many of the same structural and functional components. As simplified models of natural ecosystems, mesocosms represent a balance between the control of bench-scale experimentation and the realism of field studies. More details about the aquatic mesocosms facility at InnoTech Alberta can be found in Section 2.2 of the 2017 study (Davies, 2018).

Aquatic mesocosms are known as outdoor artificial systems that simulate food webs and mimic industrial processes to understand natural environmental conditions (Boone and James, 2005). The use of aquatic mesocosms for environmental risk assessment has been conducted for decades (Caquet, 2002). For example, the periphyton community under zinc stress, identified as one of the COCs in OSPW (McQueen et al., 2017), was evaluated using mesocosm experiments in the 1980s (Genter et al., 1987). Mesocosm studies could confirm or disconfirm laboratory experiments while providing some extent of realistic responses to oil sands materials (Cappello and Yakimov, 2010).

Mesocosm testing was identified as an active research need in OSPW toxicity as a nonstandard test (Tanna et al., 2019). Greenhouse mesocosm experiments were run to determine how plants and microbial communities reacted to OSPW (Rezanezhad et al., 2012). Stream mesocosm studies were performed to examine the composition of the benthic macroinvertebrate community in the presence of OSPW (Howland, 2019). Mesocosms constructed from oil sands process materials were used to evaluate zooplankton and aquatic vegetation development (Dings-Avery, 2019), aquatic invertebrate communities (Randell, 2019), amphibian development (Patterson, 2019), and track tailings' biogeochemical evolution (Reid, 2019).

Recently, the opportunities and limitations of mesocosm-scale experiments have been reviewed in mine pit lake research (McCullough and Vandenberg, 2020). Mesocosm-associated microorganism studies in Alberta were also briefly reviewed focusing on the effect of hydrocarbons (Richardson and Dacks, 2019). Riverside mesocosm investigations were recommended to support the Joint Oil Sands Monitoring Plan to gauge the response of macroinvertebrate to OSPM (Culp et al., 2018). More mesocosms studies are planned or are expected to have started to assess the ecotoxicity of OSPW (Hatfield Consultants, 2019).

higher

1.3 Study Purpose

The 2019-2021 mesocosm research project had three main goals. The first was to evaluate the physical and chemical properties of tailings types and the resulting pore water. The second was to determine how different tailings types alter the water column in terms of physicochemical, biological, and toxicological properties. The third was to provide information on early biological establishment rates and water quality trajectories.

2.0 MATERIALS AND METHODS

This section is intended to summarize the experiment design, schedule, materials, sampling, and data analysis methods. More detail can be found in Sections 1 and 2 of Appendix C and D.

2.1 Study Design

The 2019-2021 experiment used the full 30 mesocosm array. These mesocosms were divided into one control group and six treatment groups with sediment (either filter sand or tailings), Athabasca River water (ARW) and/or OSPW as outlined in Table 1. Each mesocosm contains a 0.5 cubic meter dish with homogenized soil, four floating rafts to support emergent plants and sampling devices, and three mesh socks to contain floating and submerged vegetation (Figure 1). In the fall of 2019, all the mesocosms were allowed to freeze without making any meaningful changes to their contents.

Table 1 Control and treatment groups with prescribed factors and levels.

Experimental group[1]	Water Cap	Sediment	Treatment Material Source	Number of mesocosms (n)
CTL	100% ARW	Filter Sand (FS)	N/A[2]	5
TRT 1	100% ARW	Coarse Sand Tailings (CST) [3]	Imperial	4
TRT 2	100% ARW	Thickened Tailings (TT) [3]	Imperial	4
TRT 3	100% ARW	Tailings Solvent Recovery Unit Tailings (TSRU) [3]	Imperial	4
TRT 4	100% ARW	Fluid Fine Tailings (FFT) [3]	Imperial	4
TRT 5	100% OSPW[3]	Fluid Fine Tailings (FFT) [3]	Imperial	4
TRT 6	50% OSPW[4] and 50% ARW	Densified Fluid Fine Tailings (dFFT) [4]	Suncor	5
Total of 7 Experimental Groups				**30**

[1] Note: CTL denotes Control, and TRT denotes Treatment.

[2] InnoTech Alberta sourced the filter sand from Target Products Ltd. (Morinville, Alberta).

[3] Sourced from Imperial.

[4] Sourced from Suncor.

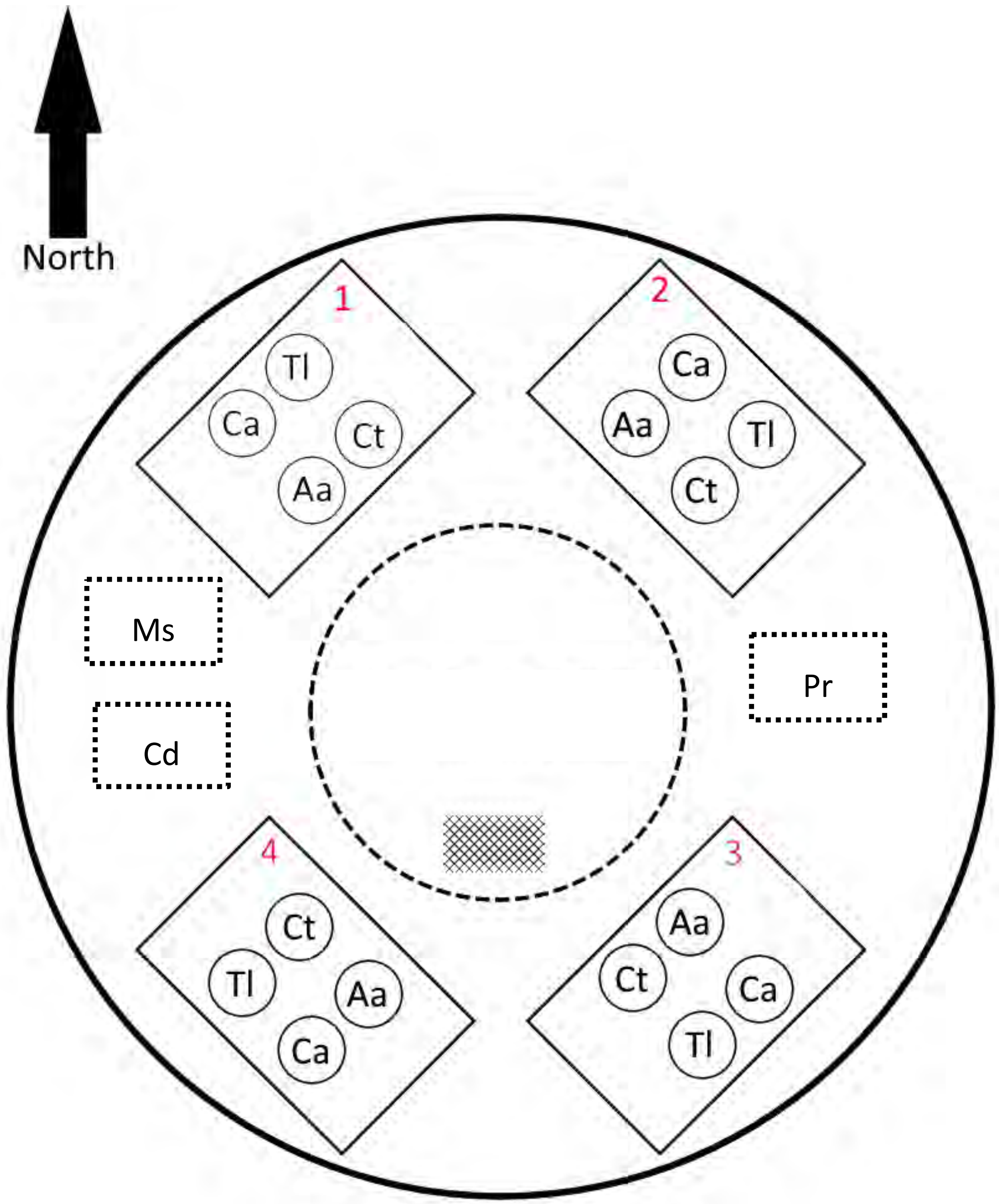

Figure 1. Mesocosm layout.
The large outer circle represents the wall of the inner tank (outer tank not shown). Rectangles with solid lines represent the installed floating rafts. Small circles with solid lines represent pots containing emergent plants and conditioned topsoil. The rectangles with dotted lines represent the floating mesh socks (tend to move during the spring melt). The rectangle with diagonal cross-hatching indicates the logger placement (when present, see section 2.7.4). The large circle with dotted lines is the edge of the soil trough. Red numbers indicate the replicate. Plant species: Aa = *Acorus americanus*, Ca = *Carex aquatilis*, Ct = *Carex atherodes*, Cd = *Ceratophyllum demersum*, Tl = *Typha latifolia*, Ms= *Myriophyllum sibiricum*, Pr = *Potamogeton richardsonii*.

2.2 Experimental Groups, Materials, and Configuration

2.2.1 Control Group (CTL)

There was one control group with 5 replicates in this study. Approximately 1 750 kg (~ 1 m^3) filter sand (10-20 mesh), which volumetric equivalent to an average depth of 20 cm layer, was added to each control mesocosm floor in 2019 (Table 1).

2.2.2 Tailings (All TRTs)

Imperial Oil and Suncor supplied tailings in May 2019. For clarity, there has been no mixing of different tail types or suppliers. Tailings were installed into each treatment mesocosms (Table 1) volumetrically, with a target of approximately 2 m^3 of tails material (liquid and solid) per mesocosm. This volume of tailings results in a solids layer approximately 10 to 30-cm deep at installation; the variation in depth is due to differences in solids content between different tailing types.

2.2.3 OSPW (TRT 5, 6)

Imperial Oil and Suncor supplied OSPW in 2019. OSPW from each supplier was housed and homogenized separately and installed in mesocosms as per Table 1. For clarity, there was no mixing of OSPW between suppliers. The approximate exposure levels of OSPW are set at 50% (TRT 6) and 100% (TRT 5) by volume. OSPW was introduced into TRT 5 (100%) and TRT 6 (50%) mesocosms volumetrically between June 10 and 14, 2019.

2.3 Mesocosm Contents

Materials required to execute the 2019-2021 study are outlined in Table 2 of Appendix D, Study Plan. Much of it was supplied by InnoTech Alberta as part of the mesocosm facility.

2.3.1 Internal Structures

Several different structures are contained within each mesocosm to support the installation and monitoring of plants (Figure 3 and 4 of Appendix D). It is understood that internal structures added to internal surface area within the mesocosms and may provide additional habitat for surface-attached (periphytic) biota, which may become predominant within the mesocosms (Perceval et al., 2009). However, the amount of additional surface occupied by equipment used during the experiment is uniform such that any influence of surface area is constant across all mesocosms.

2.3.2 Dilution Water

Water used to fill the mesocosms prior to treatment, and that contained within the CTL mesocosms throughout the experiment is referred to as "dilution water". For the 2019 – 2021 study, dilution water was collected from the Athabasca River by Imperial and transported to Vegreville in the spring of 2019 where it was stored temporarily in open-top tanks before transfer to the mesocosms. Dilution water was present in the mesocosms prior to commissioning (Section 2.7.1) and circulated between mesocosms during the establishment period to ensure homogeneity before installing the test items (Section 2.8) in 2019. No potable water was added to mesocosms in 2019 or 2020.

2.3.3 *Conditioned and Unconditioned Topsoil*

In this study, two types of topsoil have been utilized: unconditioned and conditioned. In this context, conditioning refers to the process of conferring characteristics commonly observed in wetland sediment by housing topsoil underwater for at least 3 months (OECD, 2006). Unconditioned topsoil is material that has been housed underwater for less than 3 months and is not expected to exhibit sediment-like properties. Topsoil should not be confused with tailings (Section 2.2.2) or the small volume of wetland sediment used to inoculate the mesocosms.

In the fall of 2018, pots were filled with unconditioned topsoil, and individual emergent rooted plants were planted in each pot and placed in the shallow supply pond. In 2019, conditioned topsoil was used in pots that supported emergent plants' growth and submerged rooting plants in the tapered socks. For the 2019 – 2021 study, 0.3 m^3 of unconditioned topsoil was added to a 0.5 m^3 round polypropylene trough installed into each mesocosm described in Appendix C. A seed population of zooplankton, phytoplankton, and macroinvertebrates was provided to all mesocosms in the spring of 2019 via an inoculation of 4 L of homogenized sediment collected from a nearby wetland. In order to avoid confounding results, no additional wetland sediment was added in 2020.

2.3.4 *Installed Plants*

Installed plant species were selected based on their natural occurrence in the Athabasca Oil Sands Region (AOSR), their relevance to oil sands reclamation practices, their significance to regional indigenous peoples, and their commercial availability. Indigenous significance was assessed by Alberta Environment and Sustainable Resource Development (AESRD) (Alberta Environment and Sustainable Resource Development, 2013), and five species were identified. Two of them (i.e., *T. latifolia* and *A. americanus*) were included in the current study based on availability. Selected plants include the emergent, submerged rooted, and free-floating growth forms, which represents multiple zones within a freshwater aquatic ecosystem (e.g., lake).

Existing information on the chosen species suggests they may exhibit a range of sensitivities to oil sands materials (See Section 1.3.2.4 of Appendix C for more details). Plants were purchased from Bearberry Creek Greenhouses (Sundre, Alberta) in 2019 and overwintered in the mesocosms for 2020 (The "above-ground" portion of emergent plants was harvested as described in section 2.13.10). Four emergent plants of each species, including *Acorus americanus* (Ratroot), *Carex aquatilis* (Water sedge), *Carex atherodes* (Awned sedge), *Typha latifolia* (Common cattail), were placed in each mesocosm. One of each submergent and floating species, including *Myriophyllum sibiricum* (Northern watermilfoil), *Potamogeton richardsonii* (Richardson's pondweed), and *Ceratophyllum demersum* (Hornwort), were contained within mesh socks and deployed in each mesocosm.

2.4 Test Items

2.4.1 *Tailings (CST, TT, TSRU, FFT, dFFT)*

In 2019, tailings materials were included in the experimental design (refer to Table 1 for details). No additional tailings were added in 2020. In 2019, tailings were delivered to the mesocosm facility in Vegreville in steel barrels (CST, TT, TSRU, and dFFT) and totes (FFT). Subsample collection took place during the treatment material installation phase, and the physical and chemical characteristics of each tailing type were determined by laboratory analysis (Appendix C). Imperial Oil provided Coarse Sand Tailings (CST), Thickened Tailings (TT), Tailings Solvent Recovery Unit Tails (TSRU) and Fluid Fine tails (FFT). Suncor Energy provided densified Fluid Fine Tails (dFFT), which are also known as coagulated-flocculated Mature

Fine Tails (cfMFT) (Omotoso et al., 2019). Note, for consistency and to avoid confusion, densified Fluid Fine Tails are referenced as dFFT in this report.

2.4.1.1 Formulation of Tailings

Imperial Oil's tailings were collected from each production site where they had undergone prescribed post-production treatment before packaging and transport to Vegreville.

CST refers to primary separation tailings produced using conventional tailings technologies, predominantly sand saturated with tailings pond water (TPW) (COSIA and OSTC, 2012). In this study, Coarse Sand Tailings (CST) is predominantly the underflow of the Primary Separation Cell, primarily sand. It was used to construct tailings dykes and also used as a capping material (Personal communication, James Guthrie at Imperial, December 17, 2021).

TT is another engineered tailing product with the addition of a substance (typically a polymer flocculant) to promote binding of the active minerals for rapid settlement (COSIA and OSTC, 2012). In this study, Thickened Tailings (TT) was produced at Kearl by treating mixtures of flotation tailings directly from the extraction plant and FFT recycled from the tailings pond with thickeners and inline injection of flocculant (polyacrylamide). It is a type of treated tailings (Personal communication, James Guthrie at Imperial, December 17, 2021).

TSRU is created in a distillation unit that is used to recover solvent from the froth treatment tailings stream, where light components (e.g. hydrocarbon solvent) are separated from heavier components (e.g. asphaltenes and minerals) in the solvent dilute tailings (Ansari, 2013). In this study, Tailings Solvent Recovery Unit (TSRU) Tailings was generated from paraffinic froth treatment and contain asphaltenes in addition to water, sand, and clay. It has high fines content (Personal communication, James Guthrie at Imperial, December 17, 2021).

Fluid fine tails (FFT) refers to a liquid suspension of oil sands fines in water where solids content is greater than 2%, but less than the solids content corresponding to the Liquid Limit, with that Liquid Limit being the boundary between a liquid and a solid as they pertain to soil mechanics (COSIA and OSTC, 2012). In this study, Fluid Fine Tailings (FFT) is a subset of the fluid tailings at Kearl. As per Canada's Oil Sands Innovation Alliance (COSIA, 2014), the fluid in the tailings area lying between the mudline (typically around 5% solids content) and the pond bottom (typically around 5 kPa) (Personal communication, James Guthrie at Imperial, December 17, 2021).

Suncor's tailings were transported from their production site to Coanda (Coanda Research and Development Corporation, Edmonton, AB), where densification treatment was completed before transport to Vegreville in steel drums. The densification process involves injecting a flocculent and a coagulant into untreated FFT. Over a few minutes, the fluid FFT condenses into a toothpaste-like material, releasing most of its pore-water.

2.4.1.2 Tailings Analysis

At the commencement of the 2019 study, tailings samples were collected from steel drums or totes during the addition of tailings to the mesocosms (Section 2.12). These samples were sent to the Environmental and Analytical Testing Services (EAS) department (now Monitoring, Analytics & Informatics Services division - MAIS) at InnoTech Vegreville or ALS Labs for analysis. The panel of analyses conducted on the composite samples is provided in Section A.5.3 of Appendix A.

2.4.1.3 Tailings Storage, Containment, and Fate

In 2019, tailings were stored at the Vegreville mesocosm facility for up to 8 weeks until added to the mesocosms. Emptied/open barrels and totes of tailings material were stored on tarps, duck ponds, or temporary berms with raised edges to contain any minor spillage. Any residual tailings, including CST, TT, TSRU, FFT, or dFFT (barrels and totes), have been collected from the Vegreville site by the DPL JIP participants that initially supplied the tailings (Imperial Oil or Suncor). In 2020, no tailings were transported for disposal.

2.4.2 OSPW

Imperial Oil and Suncor Energy provided OSPW. OSPW refers to water used in the bitumen extraction process. OSPW typically contains suspended solids, dissolved organics (e.g., naphthenic acids and surfactants), and various types and amounts of inorganic ions. The dominant inorganic ions are generally sodium, chloride, bicarbonate, and sulphate, with a small amount of divalent cations such as calcium, magnesium, and potassium (COSIA and OSTC, 2012).

OSPW can show significant variation in composition across operators, ore sources, ages, seasons, and even locations within a single tailings pond. A range of OSPW constituents, including metals, salts, naphthenic acids, and polycyclic aromatic hydrocarbons (PAHs), could impact the ecological state of a PL. OSPW from each supplier was housed and homogenized separately, as per the experimental design (see Appendix C and D).

2.4.2.1 Reception and Homogenization of OSPW

OSPW was received in the spring of 2019. After all of the OSPW had been received, the OSPW from each supplier was circulated across all storage vessels, within source type. OSPW sources were not mixed.

2.4.2.2 OSPW Analysis

After homogenization, sample of OSPW from each source was collected and submitted to InnoTech Alberta's EAS department for analysis.

2.4.2.3 OSPW Storage, Containment, and Fate

OSPW (60 m^3 from Imperial and 40 m^3 from Suncor) was shipped to the Vegreville site in the spring of 2019 in bulk and stored in the wastewater storage tanks before addition to mesocosms. OSPW was added to selected mesocosms in the spring of 2019. In 2020, no OSPW transportation was undertaken.

2.5 Study Schedule

Table 2 and Table 3 provide a graphical outline of the major periods and events in the execution of the 2019 and 2020 studies, respectively. The execution of the 2021 study can be found in Appendix I. Calendar dates/weeks are used in the current report. Due to the time requirements for establishing mesocosms and sampling events, groups have been divided into two cohorts; these cohorts were generally sampled/monitored sequentially over two consecutive days. Each experimental group was represented with two or three replicates within each cohort to minimize the impact of sampling date on statistical comparison. The cohorts were discussed in more detail in Section 2.8 of Appendix D.

Table 2 Outline of mesocosm sampling/monitoring activities during 2019

2019		W18	W19	W20	W21	W22	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32	W33	W34	W35	W36	W37	W38	W39	W40	W41			
		May					**June**				**July**					**August**				**September**					**October**		**November**	**December**
No.	Task Name	29-3	6-10	13-17	20-24	27-31	3-7	10-14	17-21	24-28	1-5	8-12	15-19	22-26	29-2	5-9	12-16	19-23	26-30	2-6	9-13	16-20	23-27	30-4	7-11			
1	**Receive material and commissioning**	x	x	x	x																							
2	**Establishment and homogenization**			x	x	x	x																					
3	**Water quality (data sonde)**					x	x		x		x		x		x		x		x		x		x					
4	**Material characterization**							x																				
5	**Lab chemistry sampling (including BE-SPME and FTIR)**									x						x						x						
6	**Plant height measurements (emergent)**							x		x		x		x			x			x			x					
7	**Plant mass record (floating)**											x		x			x			x			x					
8	**Acute toxicity sampling**									x												x						
9	**Chronic toxicity sampling**									x																		
10	**Phytoplankton estimation/picture**									x		x		x		x		x		x		x		x				
11	**Hester-Dendy samplers' deployment**									x				x				x										
12	**Hester-Dendy samplers' collection**															x				x				x				
13	**Macroinvertebrate activity traps deployment**											x				x				x								
14	**Macroinvertebrate activity traps collection**													x				x				x						
15	**Zooplankton traps deployment**									x				x				x				x						
16	**Zooplankton traps collection**									x				x				x				x						
17	**Depth measurement**			x		x		x		x		x		x			x		x		x		x					
18	**Data loggers install and check**								x				x			x				x				x				
19	**Plants harvest (biomass)**																								x			
20	**Data analysis***																									x	x	x

*Data analysis continued into 2020

Table 3 Outline of mesocosm sampling/monitoring activities during 2020

2020		W18	W19	W20	W21	W22	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32	W33	W34	W35	W36	W37	W38	W39	W40	W41			
		May					June				July					August				September					October		November	December
No.	Task Name	27-1	4-8	11-15	18-22	25-29	1-5	8-12	15-19	22-26	29-3	6-10	13-17	20-24	27-31	3-7	10-14	17-21	24-28	31-4	7-11	14-18	21-25	28-2	5-9			
1	**Water quality (data sonde)**		x		x		x		x		x		x		x		x		x		x		x		x			
2	**Install plants (if needed)**																											
3	**Lab chemistry sampling (including BE-SPME and FTIR)**									x						x						x						
4	**Plant height measurements (emergent)**					x		x			x			x			x			x			x					
5	**Plant mass record (floating)**							x			x			x			x			x			x					
6	**Acute toxicity sampling**																					x						
7	**Chronic toxicity sampling**																					x						
8	**Phytoplankton estimation/picture**	x		x		x		x		x		x			x		x		x		x		x		x			
9	**Hester-Dendy samplers' deployment**									x				x				x										
10	**Hester-Dendy samplers' collection**															x				x				x				
11	**Macroinvertebrate activity traps deployment**											x				x				x								
12	**Macroinvertebrate activity traps collection**													x				x				x						
13	**Zooplankton traps deployment**									x				x				x				x						
14	**Zooplankton traps collection**									x				x				x				x						
15	**Depth measurement**	x		x		x		x		x		x			x		x		x		x		x		x			
16	**Data loggers install and check**		x				x					x				x				x					x			
17	**Plants harvest (biomass)**																							x				
18	**Data analysis***																									x	x	x
19	**2020 Draft report****																										x	x

*Data analysis continued into August of 2021

**Draft report is expected to be delivered in November 2021

2.6 Reception of Materials

A full accounting of the reception of materials to the mesocosms can be found in Appendix C.

2.7 Mesocosm Commissioning

Commissioning refers to the installation of internal structures and materials, including shelving/rafts, mesh socks, plants, troughs, filter sand, and conditioned/unconditioned topsoil. Commissioning began in 2019 when the mesocosms were filled with dilution water. All installed structures and materials were left in place over the winter of 2019/20. No new items were installed in 2020.

Commissioning was followed by a period known as Establishment, a short period during which simple food webs stabilize and biotic/abiotic elements of the mesocosm start to interact (see Section 2.4 of Appendix C for more details).

2.7.1 Installation of Dilution Water and Soil Trough (All mesocosms)

Dilution water and a soil trough were installed in all mesocosms during the commissioning phase. Each mesocosm was inoculated with a small volume of sediment collected from a nearby wetland to establish a more natural invertebrate community. Roughly 120 L of sediment was collected and mixed thoroughly before roughly four 1 L aliquots of the mixed sediment were distributed to each mesocosm.

2.7.2 Installation of Floating Rafts and Emergent Plants

In 2019, four floating rafts were used to support emergent vegetation and sampling apparatuses (Figure 1). These were installed after dilution water was added to each mesocosm. These rafts are composed of closed-cell food-grade (modified) polystyrene foam. Emergent plants were installed at the same time as floating rafts.

During the commissioning period, dead above-ground plant material was trimmed from all emergent plants except *T. latifolia*, because observations in 2017 and 2018 found that such trimming earlier in the season caused excess stress for this species and often resulted in mortality. For *T. latifolia*, only detrital material was removed from the pots, but the main stem was not altered.

2.7.3 Installation of Mesh Socks and Floating Plants

Both socks were designed to allow the plants in question to grow and move freely but also to contain them so they could be monitored during the study with minimal disturbance. *C demersum*, *M. sibiricum*, and *P. richardsonii* were left in the mesocosms over winter. The position of socks was largely undisturbed although the socks tend to move during the spring melt in early 2020.

Mesh socks were cylindrical or tapered cylindrical nets composed of UV-resistant polyester suspended from a foam ring float (See Figure 4 of Appendix D); socks allow submerged and free-floating plants to make vertical movements within the sock throughout the study.

2.7.4 Data Loggers

In both years, autonomous data loggers were included in one replicate of each experimental group to maintain continuous monitoring. These data loggers measured water temperature and conductivity (Aqua Troll 100, In-Situ Incorporated, Fort Collins, Colorado, USA) and dissolved oxygen (HOBO U26, Onset Computer Corporation, Cape Cod, Massachusetts, USA) over the open water season. Both types of data loggers were attached to a stand composed of PVC pipe and placed on the surface of the soil trough at a depth of around 100 cm (See Figure 8 of Appendix C).

2.8 Establishment and Homogenization

The establishment period allows the formation of simplified food webs within the mesocosms and stabilizes water chemistry and nektonic communities across all mesocosms (OECD, 2006). This period began when vegetation and wetland sediment were installed into the mesocosms and ended at the treatment phase. Due to logistical limitations, a relatively short establishment period (25 days) was adapted in the current study. Extended establishment periods on the order of 1 to 9 months are recommended when the study timeline allows for future studies (SETAC, 1992), and may be influenced by the test system and the variables being investigated.

During the 2018/19 overwintering period, mesocosms were filled with surface water from a local wetland. While that water was replaced by dilution water in the commissioning phase in spring of 2019, the mesocosms were not emptied completely, nor were the walls cleaned of periphytic growth, allowing some residual biota to remain. Since the mesocosms were largely undisturbed since the spring of 2019, whatever communities and food webs were established at that time, and which persisted through the winter were expected to be present during the 2020 field season.

Homogenization minimizes the chemical and biotic differences between mesocosms before the introduction of test items. By minimizing these differences at the beginning of the experiment, within-group variation is reduced, thereby increasing the statistical power of the study. Such procedures are standard practice in mesocosm-based research (OECD, 2006). Homogenization began after all the mesocosms had been filled with dilution water (Section 2.4.1 in Appendix C).

2.9 Allocation to Group

In the spring of 2019, the 30-mesocosm array was divided into five experimental groups, which contained 4 mesocosms each (TRT 1 – TRT 5) and two experimental groups, which contained 5 mesocosms each (CTL and TRT 6) as described in Table 1. Within each group, mesocosms were randomly assigned to two cohorts (A or B, Figure 2). No statistical comparison of cohorts was conducted.

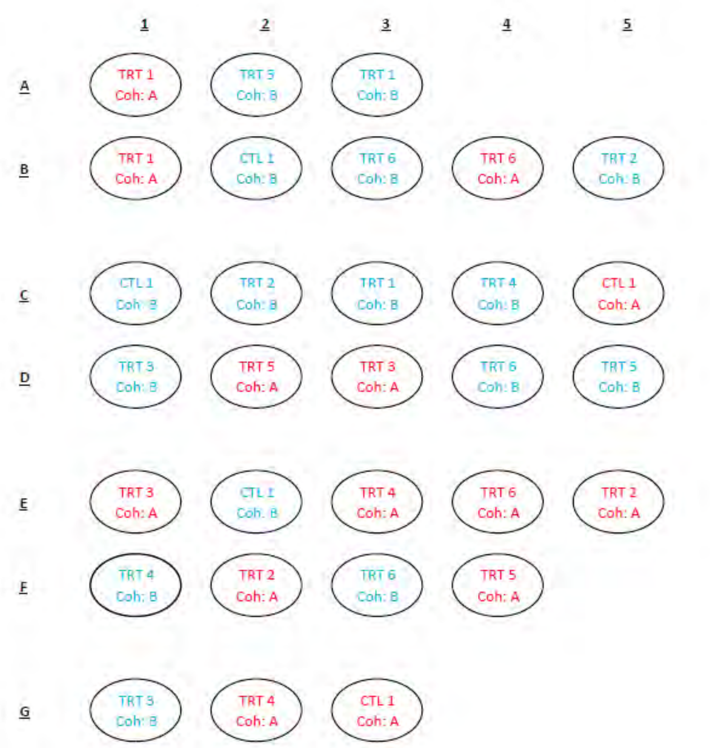

Figure 2. Allocation of mesocosms to experimental group.

2.10 Plants and Conditioned Topsoil

Installed plants and conditioned topsoil were present in equal amounts and configurations in all mesocosms.

2.11 Source Material Characterization

Samples were collected for material for characterization prior to, or at the time of, installation into the mesocosms. Selected analytes measured in the original materials before they were distributed to the mesocosms were summarized in Table 4 and Table 5. Selection was made based on commonly concerned bitumen-related water quality constituents (e.g., naphthenic acids), toxicity-modifying factors (e.g., pH and hardness), and potential stressors that might contribute to variations in biological responses. Detailed material characterization data can be found in Appendix A, section A.5.3.

Table 4 The quantity of selected analytes measured in the original water source installed in the mesocosms in 2019

Source Material	Unit	ARW	Imperial OSPW	Suncor OSPW
Conductivity (25°C)	µS/cm	345	1260	3940
pH	pH units	7.61	8.26	7.92
Hardness (calc)	mg/L $CaCO_3$	128	135	59.3
DOC	mg/L	10.5	41.7	92.7
COD	mg/L	25	142	315
Naphthenic Acids, Total	µg/L	29.9	24900	39600
Arsenic, Total	µg/L	1.18	2.69	1.76
Boron, Total	µg/L	31.8	1130	3770
Chloride, Dissolved	mg/L	15.1	41.6	709
Cobalt, Total	µg/L	0.11	3.45	2.43
Copper, Total	µg/L	2.18	2.57	1.72
Fluoride, Dissolved	mg/L	0.10	5.09	2.51
Mercury, Total	µg/L	< 0.02	0.05	0.27
Molybdenum, Total	µg/L	0.871	62.2	401
Nickel, Total	µg/L	1.97	8.83	11.4
Selenium, Total	µg/L	0.2	0.9	7.9
Vanadium, Total	µg/L	0.614	17.2	8.48

2.12 Tailings, OSPW and Filter sand Installation

All tailings, OSPW, and filter sand were installed in 2019. The details of this installation process are outlined in section 2.8 of Appendix C.

2.13 Exposure Period

The exposure period began at treatment and ended with mesocosm decommissioning in 2021. The exposure period denotes that time during which the mesocosms are exposed to tailings and/or OSPW.

2.13.1 Water Quality – Field Measurement

Field measurement data (i.e., those data collected with the YSI EXO2 data sonde) were collected once every 14 days at two depths per mesocosm: just below the surface of the water, and just above the filter sand/tailings layer (at a depth of ~125 cm). Data (pH, conductivity, dissolved oxygen, temperature, chlorophyll, and turbidity) were collected using the sonde and stored on the EXO2 handheld device.

Table 5 The quantity of selected analytes measured in the original substrates source installed in the mesocosms in 2019

Source Material (Total, µg/g)	Soil (Trough)	Filter Sand	CST	TT	TSRU	FFT	dFFT
Calcium	21600	19700	701	1780	8680	6460	5820
Naphthenic Acids	41.6	20.5	447	872	205	599	1130
Arsenic	3.89	2.66	1.25	1.75	11.5	2.88	4.39
Boron	33.3	7.95	3.66	20.7	441	104	127
Chlorine	< 39	< 39	53	70	< 39	< 39	484
Cobalt	6.05	2.49	1.13	2.78	17.9	8.76	12.3
Copper	13.5	1.4	1.3	3.5	19.8	8.0	13.5
Mercury	0.031	0.005	0.003	0.007	0.161	0.026	0.039
Molybdenum	0.433	0.367	0.077	0.27	11.5	1.19	2.85
Nickel	13.5	5.06	1.67	5.94	60	22.9	41.6
Selenium	0.46	0.14	0.24	0.14	0.84	0.25	0.62
Vanadium	53.9	11.2	5.64	24	181	102	126

2.13.2 Water Quality – Laboratory Measurement

Water samples were collected from the mesocosms at the surface at the beginning, middle, and end of each open-water season. Samples and their corresponding analyses were described in Table 6 of Appendix D. The collection schedule can be found in Table 2 and Table 3 (Section 2.5).

Analysis and reporting of water samples was conducted according to MAIS Standard Operating Procedures (SOPs). Observations from the 2019 study indicated that most PAHs, BTEX (Benzene, Toluene, Ethylbenzene, Xylenes), and phenols were reported to be near or below the detection limit (Appendix A, Section A.5.4). Therefore, PAHs, BTEX, and phenols were sampled from only 3 replicates per treatment group in 2020 (instead of 4 or 5, as was done in 2019).

Additional water samples from each mesocosm were collected in 480 mL amber glass bottles and sent to Dr. Gamal's lab at the University of Alberta for analysis, including naphthenic acid analysis by Fourier Transform Infrared Spectroscopy (FTIR) and time-of-flight mass spectrometry (TOFMS). Only those duplicate samples collected during 2019 and the last time point of 2020 sampling season were sent to Syncrude for similar analyses. This course of action was taken because a significantly reduced capacity of Syncrude's lab due to COVID-19 restrictions.

2.13.3 Plant Growth

Plant growth measurements were taken every 3 weeks, starting the week of treatment (June 10) in 2019 and approximately three weeks after the mesocosms thawed (May 25) in 2020. At the same time, plant condition was also observed to help interpreting growth data (e.g., a dead plant can be distinguished from a live plant that does not grow).

2.13.3.1 Emergent plants – *A. americanus*, *C. aquatilis*, *C. atherodes* and *T. latifolia*

The length of the longest leaf from the surface of the soil to the tip was measured in centimetres using a ruler, tape measure, or meter stick. If any emergent plants produced a flower stem, its presence was noted.

2.13.3.2 Free floating plants – *C. demersum*

Prior to removal from the mesocosm, metaphyton found floating inside a mesh sock, was removed into the mesocosm to avoid weighing errors. Plants were collected and any excess water or non-plant material (e.g., snails) was gently shaken or plucked the plant material. Plant material was then transferred to a plastic bag. Using a spring scale, the weight of the bag and its contents were determined and recorded. The plant material was then returned to the sock, and then the weight of the bag plus any residual water was recorded. Net wet weight of *C. demersum* was calculated by subtracting the weight of the bag and any residual water from the gross weight of the bag, residual water, and plant material.

2.13.3.3 Submerged rooting plants – *M. sibiricum* and *P. richardsonii*

Submerged rooted plants were left undisturbed (i.e., unmeasured) in both years.

2.13.4 Toxicity

The toxicity of water samples drawn from the mesocosms can be assessed using a range of standard acute toxicity and sublethal tests. The acute toxicity test included in this study was EPS 1/RM/13, 4-day LC_{50} for rainbow trout (*Oncorhynchus mykiss*) (Environment Canada, 2000), conducted by ALS laboratories.

The sublethal toxicity test included in the study is EPS 1/RM/22; 7-day survival and biomass of fathead minnow (*Pimephales promelas*)(Environment Canada, 2011), conducted by Bureau Veritas Laboratories (Maxxam Analytics).

In addition to traditional toxicity testing, Imperial Oil is validating a method of biomimetic extraction via solid phase-micro extraction (BE–SPME) as a new toxicity screening test. Duplicate samples were collected at three equally spaced time points throughout the open-water season in both years in concert with the water chemistry samples. These samples were submitted to ExxonMobil Biomedical Sciences, Inc. (NJ, USA), a laboratory designated by Imperial Oil for analysis and method validation. It should be noted that the analysis of BE-SPME is not included in the original scope of this study. These results are included in Appendix H, Section H.4.3 with further analysis and interpretation in Appendix F.

2.13.5 Zooplankton

Zooplankton are small invertebrates that occupy a substantial portion of the food web in aquatic systems. Zooplankton were collected in traps consisting of a semi-opaque translucent screw-top 1 L HDPE sample bottle into which a polypropylene funnel has been inserted (See Figure 9 of Appendix D). Traps (2 per mesocosm) were zip-tied to a PVC pipe attached to the inner (i.e., most central) edge of a floating raft (Figure 3). Traps were deployed for 24-hour periods to accommodate the diurnal migration of zooplankton (Harding et al., 1986).

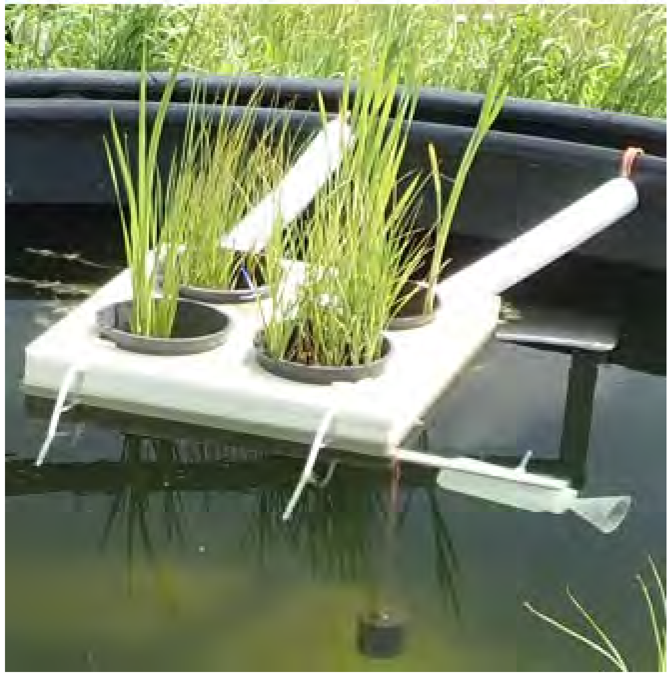

Figure 3. Deployment scheme of zooplankton activity trap and Hester Dendy sampler. Picture was taken on June 24, 2020, of the TRT 2 mesocosm (E5).

Samples were collected from all mesocosms monthly over the open water season of both years. Samples were sent to Biologica Environmental Services (Victoria, BC, Canada) for taxonomic analysis.

2.13.6 Macroinvertebrates

Macroinvertebrates play a substantial role in the food webs of aquatic ecosystems, with some members of this group being the top predators in the aquatic mesocosms used in this study. Macroinvertebrate communities were assayed using Hester-Dendy samplers (Figure 3) and activity traps (Figure 4).

Hester Dendy samplers were deployed at the beginning, middle, and end of the open water season, with recovery occurring 6 weeks after each deployment as per Table 2 and Table 3.

All methods have biases, and the Hester Dendy traps have a strong bias towards macroinvertebrates that 'hide' in the sampler (Macanowicz et al., 2013). Thus, macroinvertebrate sampling also included the deployment of activity traps to gain a more complete picture of the overall macroinvertebrate community. A vertical orientation was selected because it was more sensitive to temporal change and had higher detection rates than a horizontal orientation (Muscha et al., 2001). These traps were deployed two weeks after the Hester-Dendy trap and left in place for two weeks before being retrieved.

Figure 4. Deployment macroinvertebrate trap.
Picture was taken on September 9, 2020, in TRT 2 mesocosm (B5).

Since the Hester Dendy and activity traps were used to profile different portions of the macroinvertebrate community (i.e., surface-dwelling vs. nektonic) over different deployment periods (i.e., six weeks vs. two weeks), samples were analyzed separately according to collection method, rather than as composite samples. Macroinvertebrate samples from the Hester Dendy sampler and activity traps were submitted to Biologica Environmental Services (Victoria, BC, Canada) separately for taxonomic analysis to the family level.

2.13.7 Data Loggers

As described in Section2.7.4, loggers were attached to the fixed frame and placed at the center of the mesocosms to minimize any disturbance by other sampling activities. Data loggers were pulled from the mesocosms and cleaned of periphyton monthly so that the metabolism of adherent biota does not bias the data.

2.13.8 Phytoplankton

Phytoplankton are free-floating photosynthetic organisms that play an important role in aquatic ecosystems. They are drivers of primary production in wetland ecosystems and can be responsible for a significant portion of community metabolism. These organisms potentially influence water chemistry in the mesocosms because of their impact on diel dissolved oxygen fluctuations and nutrient uptake. Three methods were used to estimate the relative abundance of phytoplankton in the aquatic mesocosms.

The first method used to estimate phytoplankton abundance was to include a total algae/chlorophyll fluorescence sensor on the YSI data sonde (Section 2.13.1). This sensor records fluorescence measurements associated with chlorophyll and phycocyanin (a proxy for phytoplankton abundance). Chlorophyll/total algae were measured using this approach on a bi-weekly basis, as per Water Quality – Field Measurement (Section 2.1.14.1).

The second method for estimating relative phytoplankton abundance involved collection of a water sample during the water chemistry sampling sessions (Section 2.1.14.2). This sample was submitted to MAIS for quantification of chlorophyll a. This method was used to verify data collected using the sensor on the data sonde; it is possible that hydrocarbons in the treatment tanks could fluoresce in the same wavelength range as chlorophyll a, resulting in an overestimation of chlorophyll if the YSI data sonde were used without this laboratory validation.

The third method for estimating relative phytoplankton abundance includes the visual estimation of metaphyton/periphyton cover, recorded as a percent value. This descriptive measure was included to assess the relative size of the phytoplankton community near the surface of the water (0-5 cm depth), which other methods may miss. Visual estimates can be biased, so a range of techniques were applied to protect against distorted assessments and are outlined in Section 2.9.1.8 of Appendix D.

2.13.9 Imaging

Images provided a visual record of changes in the mesocosms throughout the study. Images were captured as part of other protocols (Sections 2.13.8) and on an as-needed basis to provide a visual record of observations, to document unexpected or unusual observations, to provide a visual record of a procedure, or external factors causing a mesocosm disturbance, (e.g., emergent plants grazed by caterpillar). All images are included in Appendices G and H.

2.13.10 Plant Harvest and Dry Biomass

Emergent plant material above the topsoil was cut from each pot and placed in a separate labelled paper bag. This “above-ground” biomass represents growth within the calendar year (i.e., growth occurring in 2019 or 2020, but not both), except *T. latifolia* at the beginning of 2019 Observations in 2017 and 2018 found that trimming earlier in the season caused excess stress for this species and often resulted in mortality. Once the drying was complete, the plant material was weighed, and that weight recorded.

2.13.11 Additional Investigations

A satellite project was conducted to analyze emergent plant tissue related to the uptake of polycyclic aromatic hydrocarbons (PAHs) and metals (See section B.1 of Appendix B). An analysis of plant tissues collected from 2019 was investigated. The methods, results, and interpretations of this satellite project are reported in their entirety in Appendix B.

2.14 Maintenance

In addition to the sampling planned, InnoTech Alberta also provided ongoing maintenance to the facility, including managing groundwater levels, mesocosm water levels and routine site upkeep.

The water level in the mesocosms varied during the exposure period due to evaporation, evapotranspiration, precipitation, snowfall, and sampling activities. The water level was checked bi-weekly using a simple depth gauge to maintain a water depth of approximately 150 – 155 cm. No potable water was added in either year. When water levels approached the rim of the inner tank, extra volume was removed and stored in suitable vessels until disposal.

2.15 Mesocosm Decommissioning

Mesocosm decommissioning refers to the process of collecting final samples and refurbishing the tanks for a new study. The only destructive sampling undertaken was the harvest of emergent plant material for dry biomass determination (Section 2.13.10). The mesocosms were not decommissioned in the autumn of 2020 but were maintained until the fall of 2021. The refurbishment was also conducted in the fall of 2021.

2.16 Data Analysis

2.16.1 General

A more detailed description of the statistical analyses can be found in Section A.1 of the Data Analysis report (Appendix A). Marginal means are provided in Appendix A for each tested parameter. Statistical testing focussed on the differences between experimental groups and depths (in data sonde results). Trends over time were also examined, and interpretation of statistical testing results can be found in Section A.1.2.

The words "significance" and "appear" are used with specific meanings within this report. The term "significance" and its derivatives explicitly indicate statistical significance when $\alpha \leq 0.05$. The lack of a significant difference between groups or depths may signify the absence of an effect or the presence of an effect that is too small to be distinguished from random variation. It should be noted that the distinction between the "absence of significance" and the "absence of difference" is critical when comparing highly variable data from small experimental groups or samples. Such circumstances are not uncommon in mesocosm-based experiments. Therefore, one cannot reliably distinguish between the actual absence of difference and the presence of a difference, which is too small to be discerned. In the interests of minimizing of a Type 2 errors (the risk of accepting the null hypothesis while it is false), the authors have elected to articulate their perceptions of the data. As a result, the term "appear," and its derivatives should be read as signifying the authors' perceptions, irrespective of statistical support. In addition, it should be understood that statistically significant may not be biologically significant, nor necessarily denote exceeding guidelines for the protection of aquatic life.

2.16.2 Trends

Within each experimental group, changes over time are articulated as ‘trends’. Six trends have been defined in such a way as to account for the time-series data presented in this report: increasing, decreasing, mid-study increase, mid-study decrease, no changes, or fluctuation. These trends are defined below.

Admittedly, this simplified and arbitrary category (i.e., trend) has the potential to obscure important information. However, this approach was considered to be an effective means of balancing brevity with comprehensiveness. When appropriate, within-year comparisons were conducted qualitatively, largely based on graphs. For those unwilling to accept this level of ambiguity in the interpretation of data, mean values and standard errors of each parameter at each time point are provided. Furthermore, the report detailing all statistical analyses is provided in Appendix A.

2.16.2.1 Increasing

An increasing trend was declared when the value of a given variable recorded the end of the 2020 season was greater than the value of the same variable recorded at the beginning of the 2019 season. In addition, the values of the variable recorded during the intervening period had to fall between those two extremes. The presence of the word ‘significant’ or its derivatives when referencing an increasing trend indicates the positive statistical significance of linear contrasts.

2.16.2.2 Decreasing

A decreasing trend is the inverse of the increasing trend. The value of a variable recorded the beginning of the 2019 season is greater than the value of the same variable at the end of the 2020 season. Meanwhile, the values of the variable recorded during the intervening period had to fall between those two extremes. The word 'significant' or its derivatives indicates that the negative linear contrasts are statistically significant.

2.16.2.3 Mid-Study Increase

A mid-study increase was defined as a trend wherein at least one value at any time point other than the first and last record values was higher than that of both the first recorded value and the last recorded value. The presence of the word 'significant' or its derivatives indicates a negative statistical significance of quadratic contrasts.

2.16.2.4 Mid-Study Decrease

A mid-study decrease was defined as a trend in which at least one value at any time point other than the first and last record values was lower than both the first recorded value and the last recorded value. The presence of the word 'significant' or its derivatives indicates a positive statistical significance of quadratic contrasts.

2.16.2.5 No Changes

Not any of the aforementioned trends could be declared. Meanwhile, any statistical significance of contrasts was not detected.

2.16.2.6 Fluctuation

The fluctuation was used to describe statistical significance of cubic contrasts.

2.16.3 Differences Between Groups

Statistical testing for group-wise differences (as Table 1) within time points focussed on describing effects associated with the following situations (Table 6):

- The effects associated with the presence of a treatment material;
- The effects associated with the treatment of tailings (TT vs. FFT); and
- The effect associated with the presence of FFT with and without 100% Imperial OSPW.

Table 6 Outline of differences between groups comparison (within time points)

vs.	CTL (ARW+FS)	TRT1 (ARW+CST)	TRT2 (ARW+TT)	TRT3 (ARW+TSRU)	TRT4 (ARW+FFT)	TRT5 (100%OSPW+FFT)	TRT6 (50%OSPW+dFFT)
CTL (ARW+FS)							
TRT1 (ARW+CST)	X						
TRT2 (ARW+TT)	X				X		
TRT3 (ARW+TSRU)	X						
TRT4 (ARW+FFT)	X					X	
TRT5 (100%OSPW+FFT)	X						
TRT6 (50%OSPW+dFFT)	X						

2.16.3.1 Effects associated with the presence of a treatment material (CTL vs. each TRT)

Effects associated with treatment material were identified using three approaches. The first was comparison of each treatment group which included only tailings (TRTs 1, 2, 3, and 4) to the control group, (i.e., TRT 1 vs. CTL, TRT 2 vs. CTL, TRT 3 vs. CTL, and TRT 4 vs. CTL). These experimental groups differed only in the type of tailings (CST/TT/TSRU/FFT) or filter sand (CTL) they contained. This was done in an attempt to isolate the effect of tailings on the mesocosms. The second approach was to compare TRT 5 vs. CTL, where combined effects of 100% Imperial OSPW and FFT were identified. The third approach was to compare TRT 6 with CTL, where combined effects associated with the presence of 50% Suncor OSPW and dFFT were identified.

2.16.3.2 Effect of treatment of tailings (TRT 2 vs. TRT 4)

The effects of fluid fine tailings (FFT) were compared to thickened tailings (TT) by comparing TRT 2 to TRT 4. Since TT is dewatered FFT (COSIA, 2014), this comparison shows the effect of dewatering.

2.16.3.3 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

The effects with OSPW in the presence of FFT were identified by comparing TRT 4 to TRT 5.

2.16.4 Line Graphs and Scatter Plots

Unlike box-and-whiskers plots presented in the 2017 and 2018 studies, traditional means-and-error scatter plots are used to facilitate visualization and communication as per DPL's request. For both n=4 (i.e., TRT 1 to TRT 5) and n=5 (i.e., CTL and TRT 6) experimental groups, scatter plots were constructed with lines and the error bars defined as ± standard error.

2.16.5 Special considerations

2.16.5.1 Correlation & PCA (principal component analyses)

Many of the parameters contained within the Water Quality – Laboratory Measurement dataset were expected to be highly correlated. Correlation plots and principal component analyses were used to guide data grouping (e.g., PAHs). Since PCA and analysis of correlation were conducted in Appendix F, correlation and PCAs are not emphasized in Appendix A.

2.16.5.2 Naphthenic acids

Classically, naphthenic acids have been defined as organic carboxylic acids with the molecular formula $C_nH_{2n+z}O_2$, where n is the carbon number and z are a negative even integer that refers to hydrogen deficiency. To analyze data obtained from the Orbitrap mass spectrometer, however, naphthenic acids were defined as water-soluble compounds demonstrating the following characteristics:

- Detected under negative ionization mode using the Orbitrap mass spectrometer,
- Contain between 6 and 22 carbon atoms,
- Contain two oxygen atoms,
- Contain no nitrogen or sulphur atoms, and
- Exhibit between 1 and 11 double bond equivalents (DBEs).

131 molecular formulae accounted for compounds matching the above criteria; however, naphthenic acids are a complex mixture of compounds with structural diversity far beyond this number (Grewer et al., 2010). A single value was generated by summing the peak areas for all of the appropriate formulae, representing the aggregate amount of naphthenic acid present in the sample. This univariate measure of naphthenic acid content was calculated at the start, middle and end of each study year. A profile based on the peak area, carbon number, and DBE was used to describe the naphthenic acid species present in each sample. It is understood that Orbitrap analyses are rich in data that may be exploited by sophisticated analyses in subsequent studies, such as untargeted data mining analysis.

For this report, a family of naphthenic acids is defined by its "Z number" and DBE (Zhao et al., 2012). These two values change in lockstep as per Table 8 of Davies (2018) and can be considered structural complexity indicators. That is, higher Z numbers and DBE's imply more structurally complex molecules. For the sake of brevity, each family is referred to only by its Z number. Estimates of each naphthenic acid (NA) family's absolute concentration were made to understand some of the patterns observed in total naphthenic acids and changes in each NA family's proportion. However, caution should be exercised in reviewing these results and their interpretation because of the diversity of compounds designated as NAs when using the criteria defined above.

$$\frac{\text{Peak area of each compound}}{\text{Peak area of standard}} \text{ X Concentration of standard (ug/L)} = \text{Concentration (ug/L)}$$

$$\frac{\text{Peak area of each compound}}{\text{Peak are of total NA compounds}} \text{ X } 100\% = \text{Percentage (\%)}$$

Further, MAIS's standard quantification method uses a laboratory standard mixture of naphthenic acids. This mixture shares the common molecular backbone to NAs but may not capture the full diversity of structure present in OSPW (or related) samples. As such, there is significant uncertainty around the absolute concentration of these compounds. As a result, estimates of absolute NA family concentrations should be considered estimates, with some expected yet poorly defined error levels.

2.16.6 Reporting limits

Some water samples had to be diluted to facilitate analysis. This dilution effect on the sensitivity of measurement (i.e., the method detection limit) is communicated as the reporting limit. Because some samples required more dilution than others, the reporting limit is often provided as a range of values attached to graphs depicting the data (e.g., Figure A.5.61 in Appendix A).

2.16.7 Rainbow Trout and Fathead Minnow Toxicity

Observed toxicity levels in rainbow trout and fathead minnow assays were sufficiently low that the planned analyses were not possible (Section 3.4). Instead, some non-standard measures were evaluated as a replacement for LC_{50} and IC_{25} (biomass). As a result, only biomass of fathead minnows in 100% mesocosm water (without dilution) were tested statistically, while all the toxicity data are presented graphically. Fathead minnow tests were only conducted at the beginning and end of this two-year study. Between-year comparisons are conducted to provide some quantitative assessment of changes in sublethal toxicity over time.

2.16.8 The Focus of Results

Additional comparisons to those described above are included in the figures of Appendix A but are not featured prominently in the Results (Section 3) or Discussion (Section 4) sections. As agreed during virtual DPL meeting on January 21, 2021, comparisons between certain tailing types were not conducted to avoid misleading regarding operator capabilities. Confounding comparisons, such as comparisons between TRT 5 and TRT 6, were typically omitted from the main report. A cautious approach is recommended for any reader attempting to evaluate these comparisons due to confounded treatment materials (e.g., different tailing types, different OSPW dilution, and different sources of OSPW). Furthermore, the properties of OSPW and dFFT in 2017/2018 are remarkably different than those in 2019-2021. Hence, comparisons of the current study results to previous mesocosms results (2017/2018) are not provided in this report.

3.0 RESULTS

In this section, treatment (TRT) groups will be identified by their number (i.e., TRT 1, TRT 2, etc.). Consequently, the reader is advised to print or memorize Table 1 (Section 2.1) to facilitate his/her understanding of the Results section. In addition, considering the static model feature of the mesocosm study, interpretation of the data in terms of absolute values must be approached with caution.

3.1 Water Quality – Field Measurement

3.1.1 Temperature

Figures and tables describing mesocosm water temperature can be found in Section A.3.3.1 of Appendix A.

3.1.1.1 Effect of depth

Water temperature tended to be lower at the bottom of the mesocosms than near the surface, especially during the first half of each year (Figure 5). However, this temperature gradient diminished over time in each year. Water temperature did not vary significantly between depths for any experimental group after Week 35 in 2019 and Week 31 in 2020, respectively. In 2019, the surface temperature was significantly higher than that at the bottom on most sampling dates until Week 31. Exceptions include CTL (Week 25) and TRT 1 (Weeks 27 and 31. During Week 33 and 35, TRT 3 (only Week 35). TRT 4, TRT 5 (only Week 33) and TRT 6 (only Week 35) demonstrated significantly lower water temperature at the bottom.

Similarly, the temperature difference between the bottom and surface was significantly different on almost all sampling dates in 2020 until Week 31. Exceptions include Week 27 for TRT 1 (CST) and TRT 3 (TSRU), and Week 29 for all the experimental groups.

3.1.1.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

Tailings-only treatments were not associated with reduced water temperature at the surface in 2019 and 2020. In 2019, mesocosms receiving CST (TRT 1) and TSRU (TRT 3) demonstrated significantly lower initial (Week 25) water temperatures at the bottom. However, these reduced water temperatures resolved quickly. TRT 4 (FFT) mesocosms were significantly cooler than CTL mesocosms at the bottom from Weeks 25 to 31, 2019 (Figure 6).

In 2020, there were no significant differences in temperature when comparing CTL to TRTs 1, 2, 3, and 4, with one exception. The bottoms of CTL mesocosms were significantly cooler than those of TRT 3 (TSRU) mesocosms from Week 19 to 25, 2020 (Figure 6).

3.1.1.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

The bottoms of TRT 5 mesocosms were significantly cooler than the bottoms of CTL mesocosms from Week 27 to 31, 2019.

3.1.1.2 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

As with TRT 5 mesocosms, mesocosms receiving dFFT with 50% Suncor OSPW (TRT 6) showed a significantly lower temperature at the bottom until Week 31 of 2019 when compared to CTL. During Week 19 to 23, 2020, the bottoms of TRT 6 mesocosms were significantly cooler than those of CTL mesocosms.

A statistically significant inverse effect in water temperature (warmer in TRT 6 at the bottom) was also observed at Week 29, 2020.

3.1.1.3 Effect of treatment of tailings (TRT 2 vs. TRT 4)

There were no significant differences in surface temperatures when comparing TRT 2 (TT) to TRT 4 (FFT), with one exception. TRT 2 (TT) was significantly cooler at the surface than TRT 4 (FFT) at Week 25, 2020. Contrastly, the bottoms of TRT4 (FFT) mesocosms were significantly cooler than the bottoms of TRT 2 (TT) mesocosms from Weeks 25 to 31, 2019.

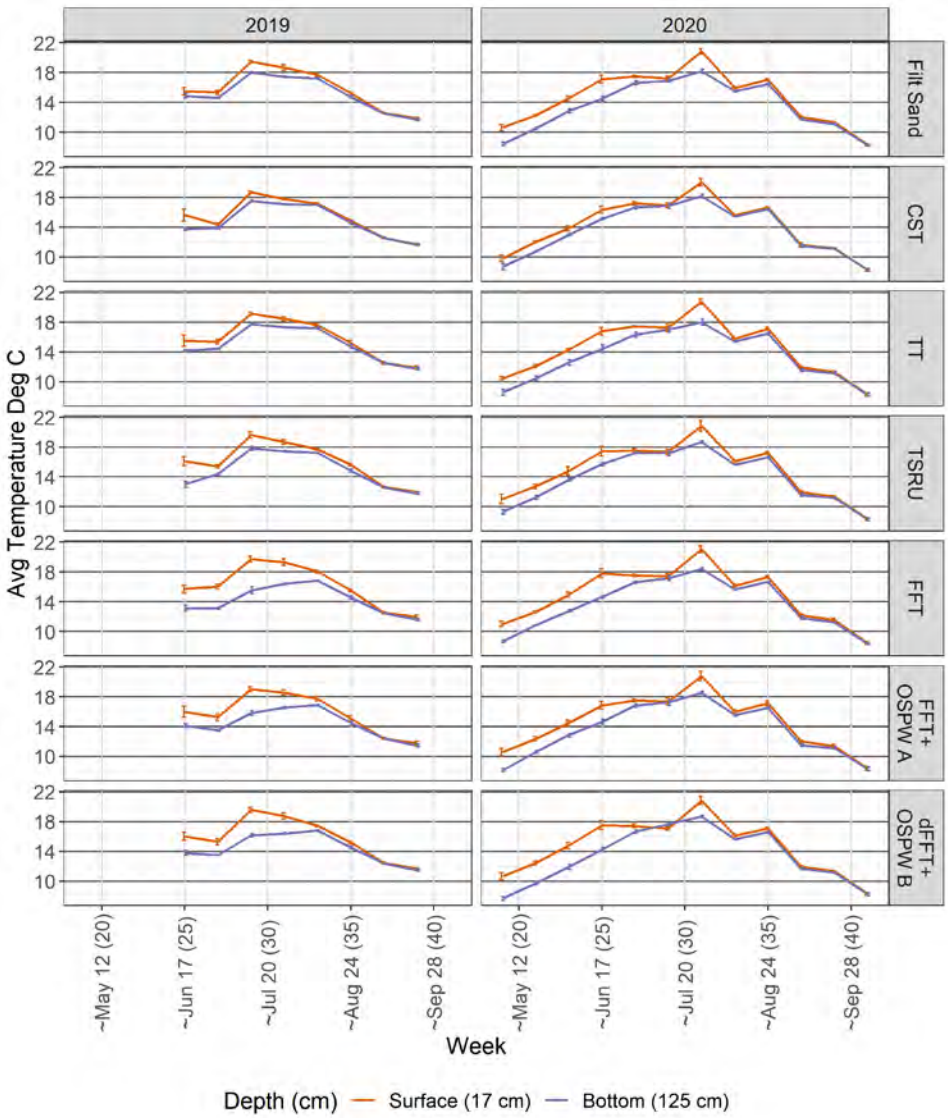

Figure 5. Mean surface and bottom water temperature by experimental group. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+FFT; TRT 6: 50% Suncor OSPW B+dFFT.

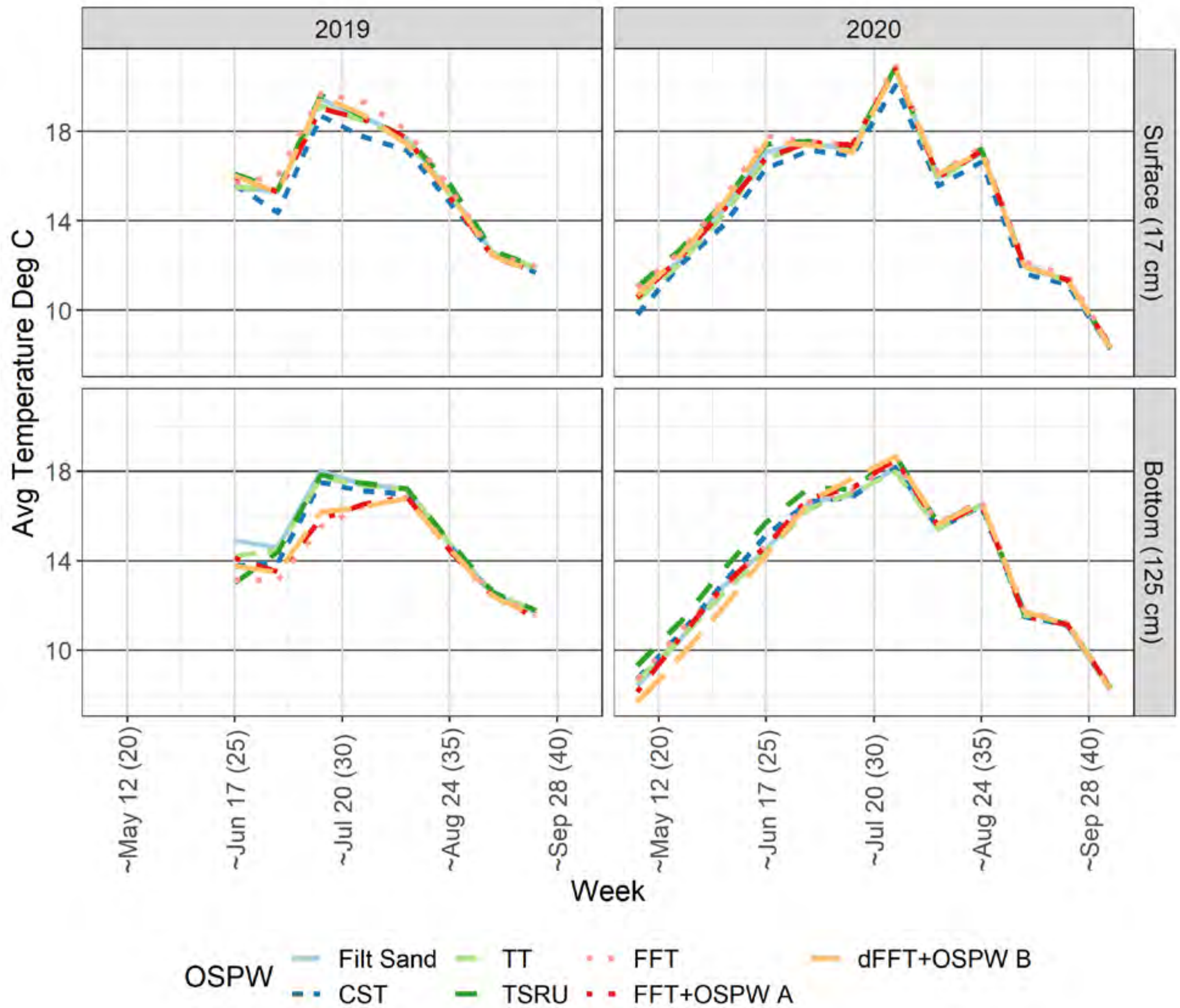

Figure 6. Mean surface and bottom water temperature in 2019 and 2020.
Standard errors of each value can be found in Table A.3.4 of Appendix A. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPWA+FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.1.1.4 Effect of time

Mesocosm water temperature was subject to seasonal changes in ambient conditions. In 2019, the water temperature at the surface increased until Week 29 and then decreased. Water temperature at both depths tended to converge in all groups over time. This thermal homogeneity was maintained until the end of 2019.

In 2020, temperatures rose in the first half of the year after surface waters were clear of ice and fell during the second half.

3.1.2 pH

Figures and tables describing pH levels in the mesocosms can be found in Section A.3.3.2 of Appendix A.

3.1.2.1 Effect of depth

pH did not differ significantly between different depths for CTL (FS, 8.14 - 9.43), TRT 1 (CST, 7.80 – 9.19) or TRT 2 (TT, 7.9 - 9.29) in 2019 and 2020 (Figure 7). At the beginning of 2019, lower pH was associated with increasing depths for the rest of the experimental groups, being more evident in TRT 4 (FFT, 7.77 – 8.91) and TRT 6 (dFFT+50% Suncor OSPW, 7.47 - 8.77) than TRT 3 (TSRU, 7.54 – 9.02) and TRT 5 (FFT+100% Imperial OSPW, 7.94 - 8.77). After Week 31 in 2019, no experimental group showed any significant depth-wise differences in pH.

In TRT 3 (TSRU), higher pH was recorded at the surface during Week 27, 2019 and Week 21, 2020. For FFT (TRT 4), the pH at the bottom was significantly lower than at the surface through the first three-time points in 2019. However, in the beginning of 2020, significantly higher pH was recorded at the bottom. Significant differences between depths were present in TRT 5 (FFT + 100% Imperial OSPW) at Week 25, 2019, and from Weeks 23 to 27 of 2020. Significantly higher pH at the surface was present in TRT 6 (dFFT + 50% Suncor OSPW) from Weeks 25 to 31 of 2019, and Weeks 21 to 31 of 2020. Differences in pH between depths were not significantly different for any experimental groups after Week 31 of 2020.

3.1.2.2 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

In 2019, the presence of tailings materials had almost no significant effect on pH at the water surface. During Week 27 of 2019, only TRT 3 (TSRU) demonstrated a significantly lower surface pH compared to CTL. However, at the bottom of the mesocosms, significantly lower pH levels were recorded at least once in TRT 1 (CST), TRT 3 (TSRU), and TRT 4 (FFT) when compared to CTL.

In 2020, there were significant differences in pH within-depths for tailings treatments. Mesocosms with CST (TRT 1) had significantly lower pH at the surface (Weeks 37 to 41) and bottom (prior to Week 23 and after Week 33) than CTL. The pH at the bottom of TRT 2 (TT) mesocosms was significantly lower than CTL at Weeks 19, 39, and 41 of 2020. TSRU (TRT 3) mesocosms had significantly lower-than-CTL pH at the surface and bottom throughout 2020. TRT 4 (FFT) produced a significantly lower pH than CTL at the surface and bottom after Week 25, 2020.

3.1.2.3 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

100% Imperial OSPW with FFT treatment was associated with significantly higher pH at the surface and the bottom compared to CTL until Week 33 of 2019 (except for Week 25 at the bottom). In contrast, the same mesocosms had significantly lower pH levels than CTL in 2020. The pH differences at the surface were evident after Week 21 of 2020 and at the bottom for the entire open water season in 2020 (Figure 8).

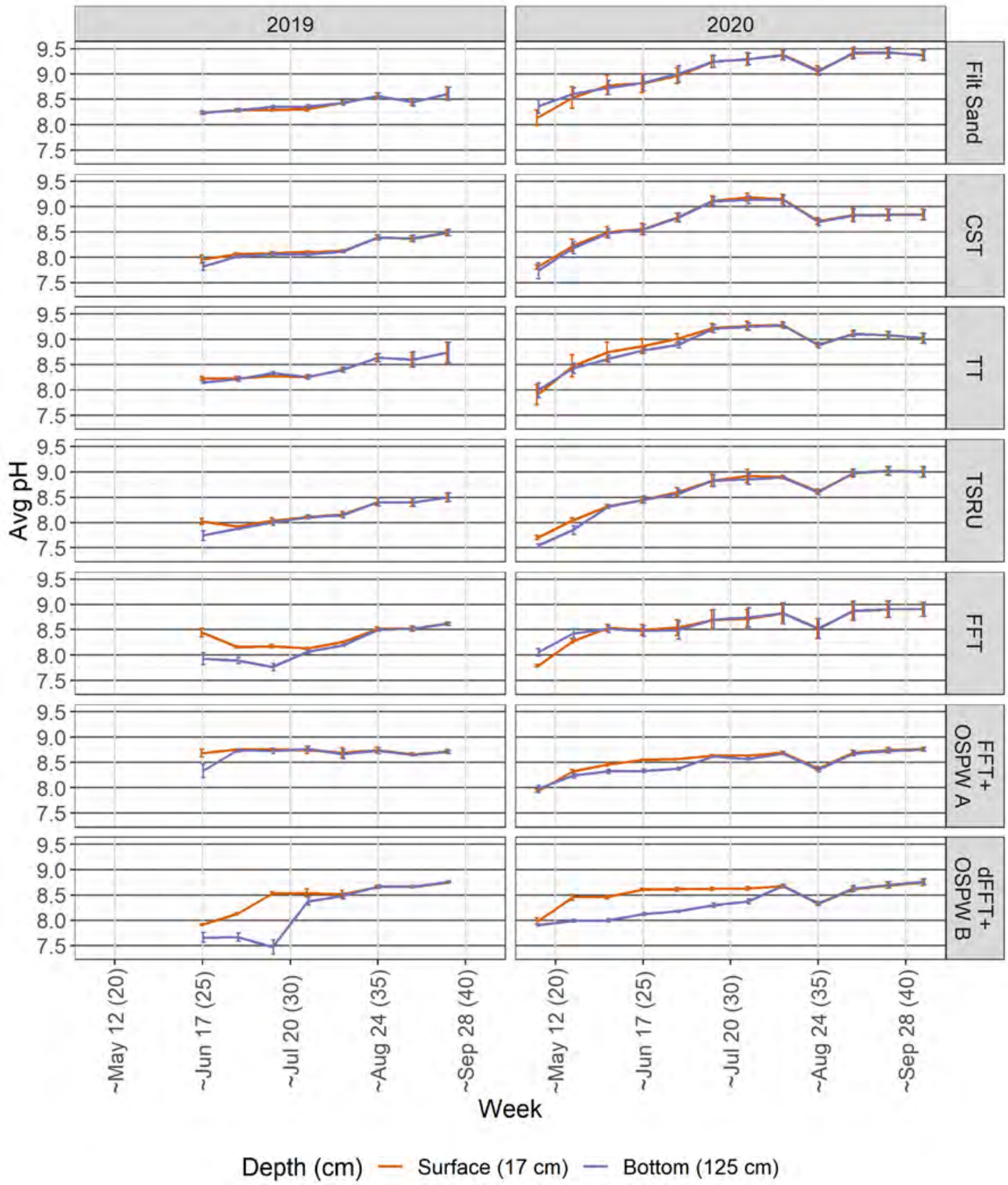

Figure 7. Mean pH measurement by depth in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Sunor OSPW B+dFFT.

3.1.2.4 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

Interestingly, the presence of 50% Suncor OSPW with dFFT (TRT 6) was not associated with surface pH values that were significantly different in 2019, except during Week 25 of 2019. However, from Week 25 to Week 29 in 2019, significantly lower pH was measured in the bottom of TR6 compared to CTL. In 2020, significantly lower pH was measured in TRT 6 compared to CTL at all time points and depths except at the surface during Week 19, 21, and 25.

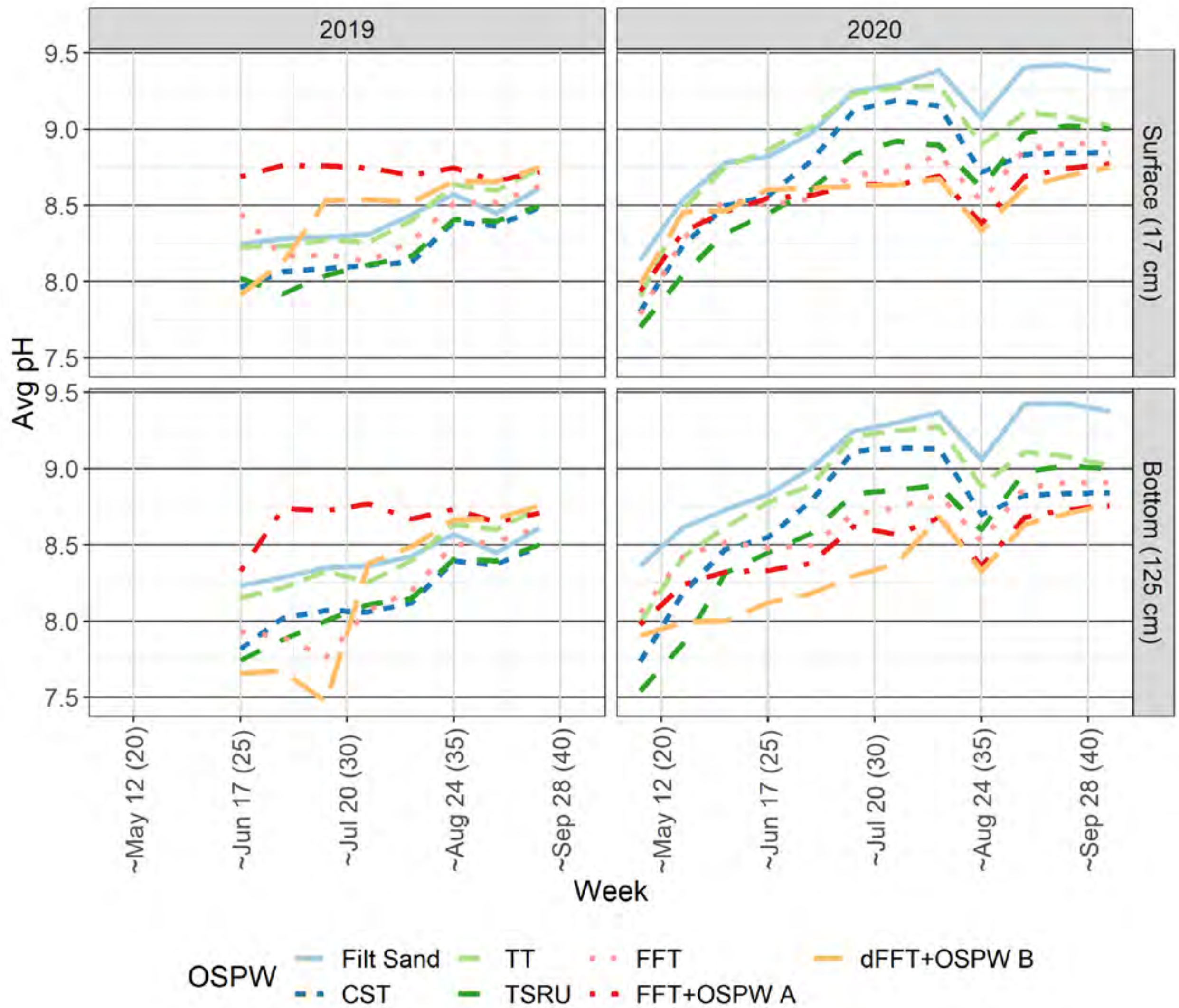

Figure 8. Mean pH readings at the surface and bottom in 2019 and 2020.
Standard errors of each value can be found in Table A.3.22 of Appendix A. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.1.2.5 Effect of treatment of tailings (TRT 2 vs. TRT 4)

Different tailing types were only rarely associated with significant pH changes in water in 2019, especially at the surface. TRT 2 (TT) produced significantly higher pHs (0.1 – 0.3) at the bottom than TRT 4 (FFT) in Weeks 27 and 29, 2019. In 2020, mesocosms with TT (TRT 2) had a significantly higher pH at the surface and bottom than FFT (TRT 4) from Week 25s to 35, 2020.

3.1.2.6 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

Compared to TRT 4 (FFT), 100% Imperial OSPW with FFT (TRT 5) was associated with significant higher pH levels at both depths prior to Week 33 of 2019, with the exception surface measurements taken during Week 25. In 2020, there were no significant differences in pH between these two groups at either depth.

3.1.2.7 Effect of time

Overall, pH measured at both depths in the mesocosms increased over time. The exception to this was in TRT 5 where the pH was relatively stable, especially in 2019. It was also noticed that the pH measured at the surface in TRT 3 (TSRU) and TRT 4 (FFT) dropped slightly at the beginning of 2019 until Week 27. Within the 2019 season, the highest pH values measured at the bottom occurred at the end of 2019 except TRT 5 (FFT + 100% Imperial OSPW). It was also noted that pH decreased at the bottom of TRT 4 (FFT) and TRT 6 (dFFT+50% Suncor OSPW) in the initial weeks of 2019 (until Week 29).

In 2020, pH levels measured at both depths in all mesocosms increased over time.

3.1.3 Turbidity

Figures and tables describing mesocosm turbidity can be found in Section A.3.3.3 of Appendix A.

3.1.3.1 Effect of Depth

In general, turbidity was not significantly different at the surface or bottom of mesocosms in 2019. When compared to the surface, significantly higher turbidities were recorded at the bottom of TRT 2 (TT), TRT 3 (TSRU), and TRT 5 (FFT + 100% Imperial OSPW) in Week 25 of 2019, TRT 4 (FFT) in Week 25 and 29 of 2019), and TRT 6 from Week 25 to Week 29 of 2019). After Week 29, 2019, no significant differences existed between depths in any experimental group.

In 2020, there were little differences in turbidity observed between depths for all mesocosms. Where there was a difference in turbidity measurements, the bottom was more turbid than the surface. Experimental groups where the difference in turbidity between depths was significantly different included: CTL (FS, Week 21, 2020), TRT 2 (TT, Week 27, 2021), and TRT 5 (FFT + 100% Imperial OSPW, Week 19 and 21, 2020).

3.1.3.2 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

The introduction of CST (TRT 1), TT (TRT 2), TSRU (TRT 3) was not associated with increases in turbidity at either depth in 2019, except for the first week of 2019. TRT 4 (FFT) mesocosms produced significantly higher turbidity values than CTL during the entire 2019 season at both depths (Figure 9). In 2020, turbidity levels measured at both depths in TRT 1, 2, 3, and 4 did not differ significantly from CTL.

3.1.3.3 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

In 2019 turbidity measured at the surface and bottom in TRT 5 mesocosms was significantly higher compared to CTL. In 2020, turbidity levels were only significantly higher in TRT 5 at the surface from Week 33 to Week 37 and at the bottom from Week 19 to Week 25.

3.1.3.4 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

During the first eight weeks of 2019, turbidity levels measured at the surface and bottom of TRT 6 mesocosms were significantly higher than CTL. Differences in turbidity levels measured in TRT 6 and CTL mesocosms were insignificant after Week 31 in 2019. In 2020, higher turbidity levels measured at the surface of TRT 6 compared to CTL were only statistically significant for Week 21, 2020.

3.1.3.5 Effect of treatment of tailings (TRT 2 vs. TRT 4)

In 2019, turbidity levels measured at the surface and bottom of TRT 4 (FFT) mesocosms were significantly higher than the TRT 2 (TT). However, in 2020, differences in turbidity levels measured at both depths in TRT 4 (FFT) and TRT 2 (TT) mesocosms were not significant.

3.1.3.6 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

During the first few weeks of 2019, the water in TRT 4 (FFT) mesocosms was significantly more turbid than TRT 5 mesocosms. At the end of 2019, significantly higher turbidity levels were measured at both depths in the FFT and OSPW mesocosms (TRT 5), compared to FFT alone (TRT 4). In 2020, there were no significant differences in turbidity levels measured at both depths between treatments.

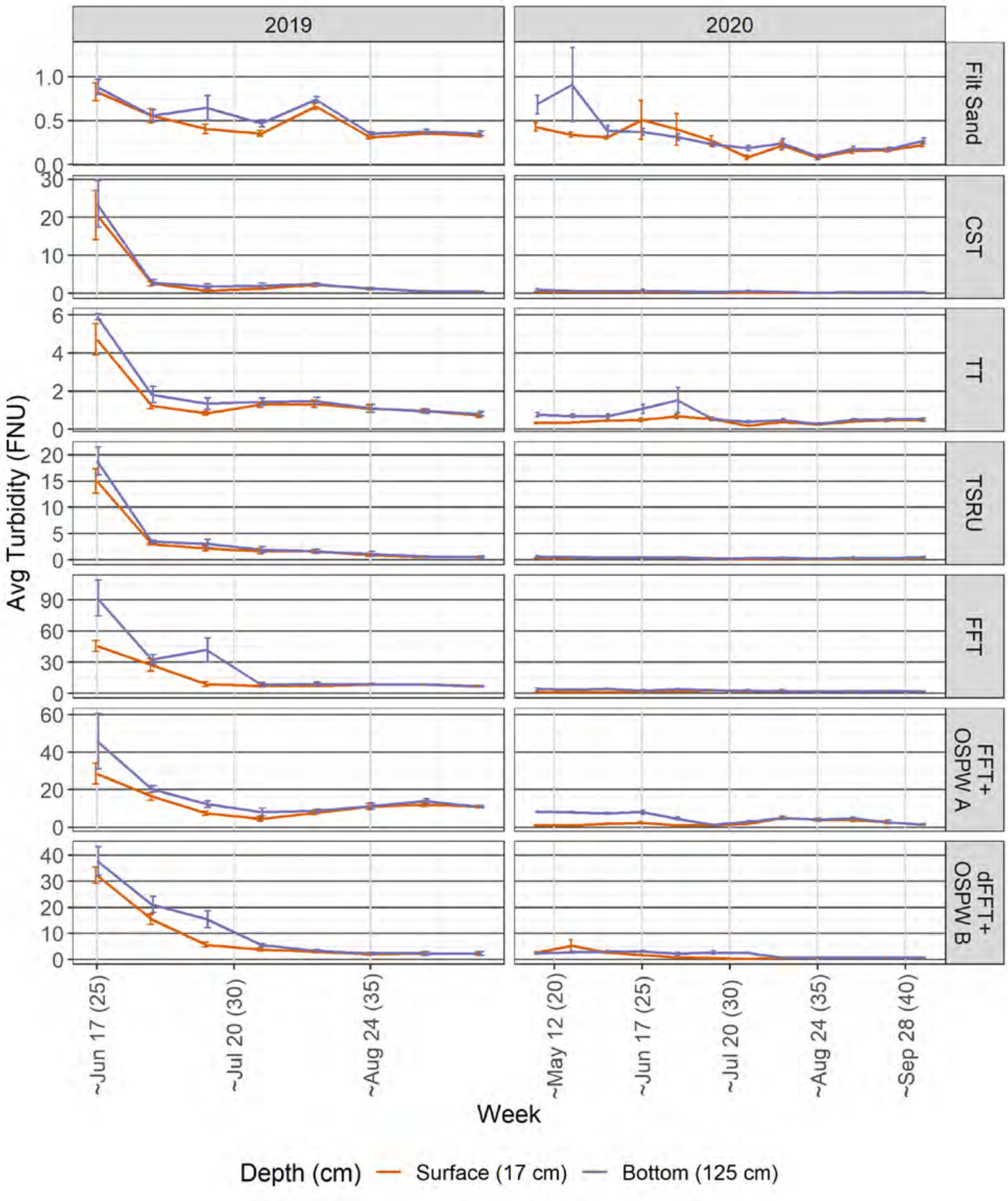

Figure 9. Mean turbidity measurement by depth in 2019 and 2020.
Note that the y-axis scales change across each experimental group. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.1.3.7 Effect of Time

In all treatment groups, turbidity levels measured at both depths decreased over the 2019 sampling season (Figure 10). However, in 2020, there were no apparent changes in turbidity levels over time. Over the two-year study, turbidity levels decreased at both the surface and bottom of the treatment mesocosms, while the turbidity levels in the CTL did not change. Overall, turbidity levels measured at the bottom of the mesocosms decreased in all treatments, except TRT 2 (TT) where the turbidity was relatively stable.

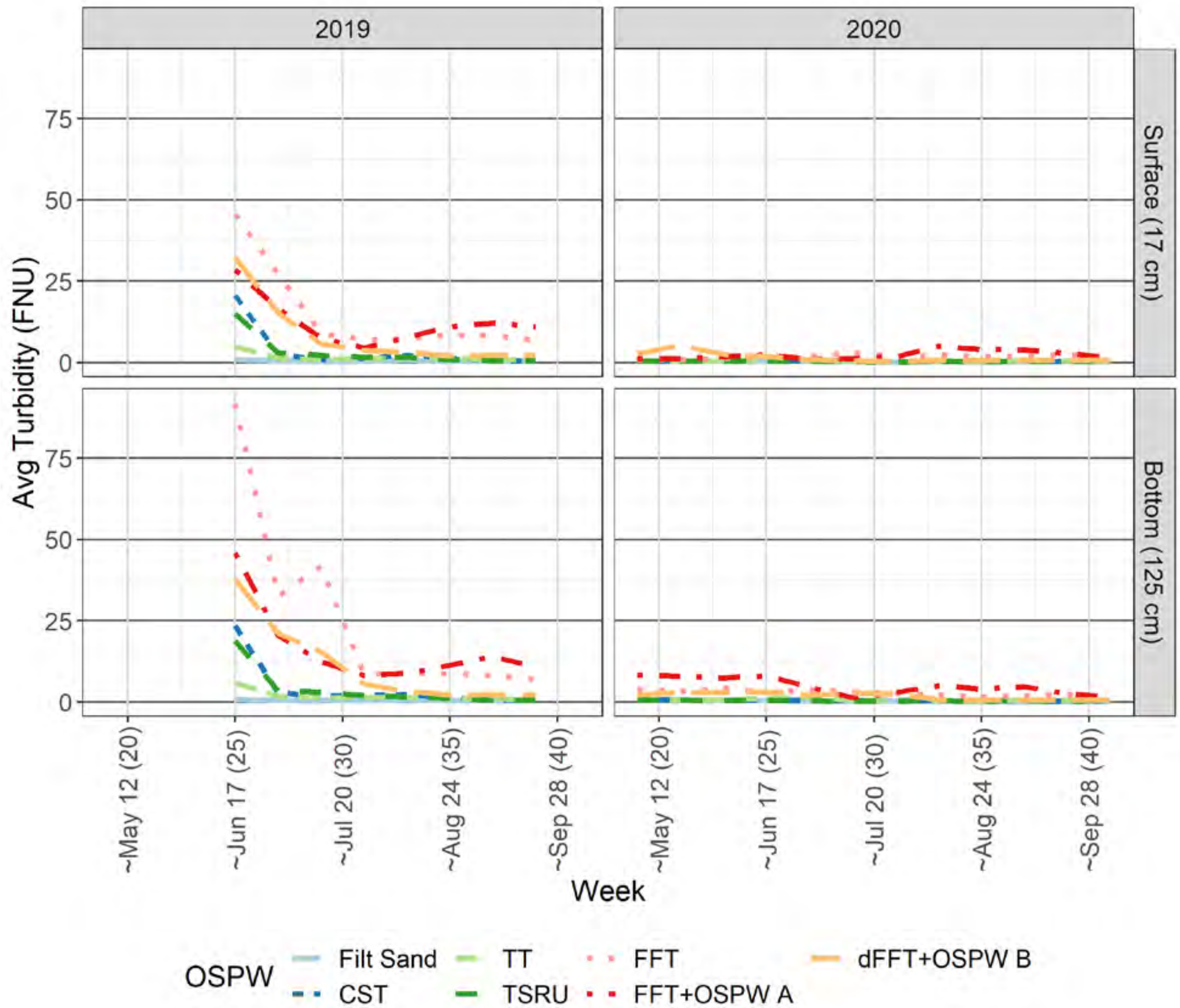

Figure 10. Mean turbidity measurement at the surface bottom in 2019 and 2020. Standard errors of each value can be found in Table A.3.40 of Appendix A. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+FFT; TRT 6, 50% Suncor OSPW B+dFFT.

3.1.4 Specific Conductivity

Figures and tables describing specific conductivity can be found in Section A.3.3.4 of Appendix A.

3.1.4.1 Effect of depth

Experimental groups did not exhibit any significant depth-related differences within treatments and weeks in specific conductivity for most of 2019. When compared to surface values, significantly higher specific conductivities were observed at the bottom of TRT 3 (TSRU) mesocosms at Week 25, 2019 and TRT 6 (dFFT+50% Suncor OSPW) at Weeks 25 and 29, 2019. TRT 5 (FFT+100% Imperial OSPW) was the only treatment where conductivity measured at the surface was higher than the bottom during Week 31, 2019.

In the first few weeks of 2020, specific conductivity was significantly less at the surface than at the bottom in all mesocosms (Figure 11). This difference was observed in:

- TRT 3 (TSRU) until Week 21,
- CTL, TRT 1 (CST), and TRT 4 (FFT) until Week 23,
- TRT 2 (TT) and TRT 5 (FFT + 100% Imperial OSPW) until Week 27, and
- TRT 6 (dFFT + 50% Suncor OSPW) until Week 31.

3.1.4.2 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

In 2019, no significant differences in specific conductivity could be detected between TRT 1 (CST), TRT 2 (TT), and CTL mesocosms. Conversely, significantly higher specific conductivity values were measured in TRT 4 (FFT) mesocosms compared to CTL mesocosms at both depths. Specific conductivity values were significantly higher in the presence of TSRU than filter sand at the surface at Week 39, 2019, and at the bottom at Weeks 25 and 39, 2019.

In 2020, specific conductivity values were significantly higher for mesocosms with tailings at both the bottom and surface for most time points (as compared to CTL), especially during the second half of 2020. In 2020, there were no significant differences in specific conductivity measured at the surface for TRT 1 (CST) during Weeks 19 and 21 for TRT 2 (TT) from Week 19 to Week 29, for TRT 3 (TSRU) at Week 19, and for TRT 4 (FFT) during Weeks 19 and 21. Week 29 was the only sampling point in in 2020 where no significant differences in conductivity were detected at the bottom in between TRT 2 (TT) and CTL mesocosms.

3.1.4.3 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

TRT 5 (100% Imperial OSPW with FFT) mesocosms exhibited significantly higher-than-CTL specific conductivities at both depths in 2019 and 2020. The only exception was in Week 19, 2020 where there were no significant differences in specific conductivity measured at the surface.

3.1.4.4 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT6)

Like TRT 5, the presence of 50% Suncor OSPW with dFFT (TRT 6) was associated with significantly higher specific conductivity in both years when compared to CTL.

3.1.4.5 Effect of treatment of tailings (TRT 2 vs. TRT 4)

In 2019, specific conductivity values measured in TRT 4 (FFT) mesocosms were significantly higher than TRT 2 (TT) at both depths. A simlar pattern was observed for most of 2020. The only exception was observed during Weeks 19 and 21, 2020, where no significant differences in specific conductivity measured at the surface could be detected.

3.1.4.6 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

Specific conductivity rose significantly at both depths and years when OSPW was added to FFT. The only exception observed was during Week 19, 2020, where no significant differences in specific conductivity measured at the surface was detected between TRT 4 and TRT 5.

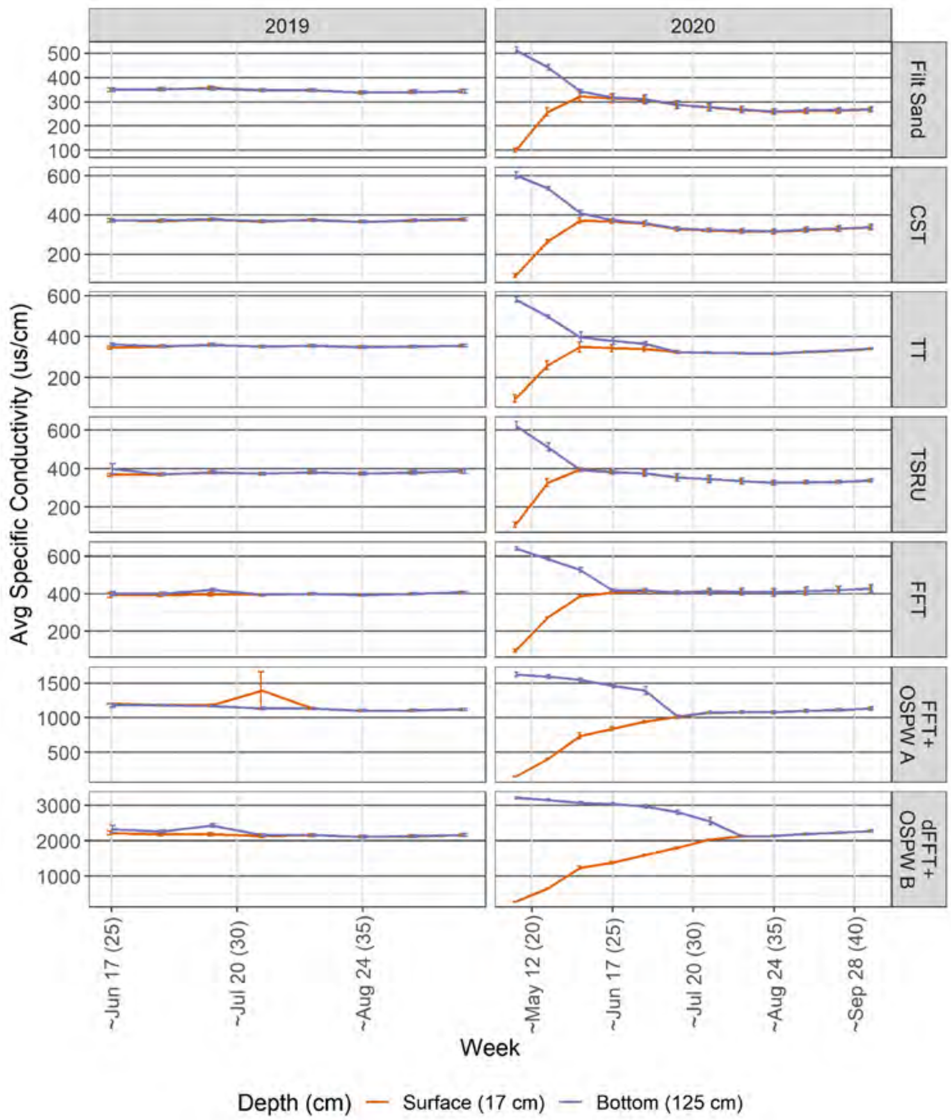

Figure 11. Mean specific conductivity by experimental groups in 2019 and 2020.
Note that the y-axis scales change across each experimental group. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.1.4.7 Effect of time

There were no apparent time-related trends for most experimental groups in 2019. At the start of the 2020 study season, specific conductivity levels near the surface appeared to increase, while those taken the bottom decreased (Figure 12). These trends occurred in all mesocosms, but there were some variations in individual experimental group responses. Through 2020 open water season, the values at the surface and the bottom converged, as described in Section 3.1.4.1.

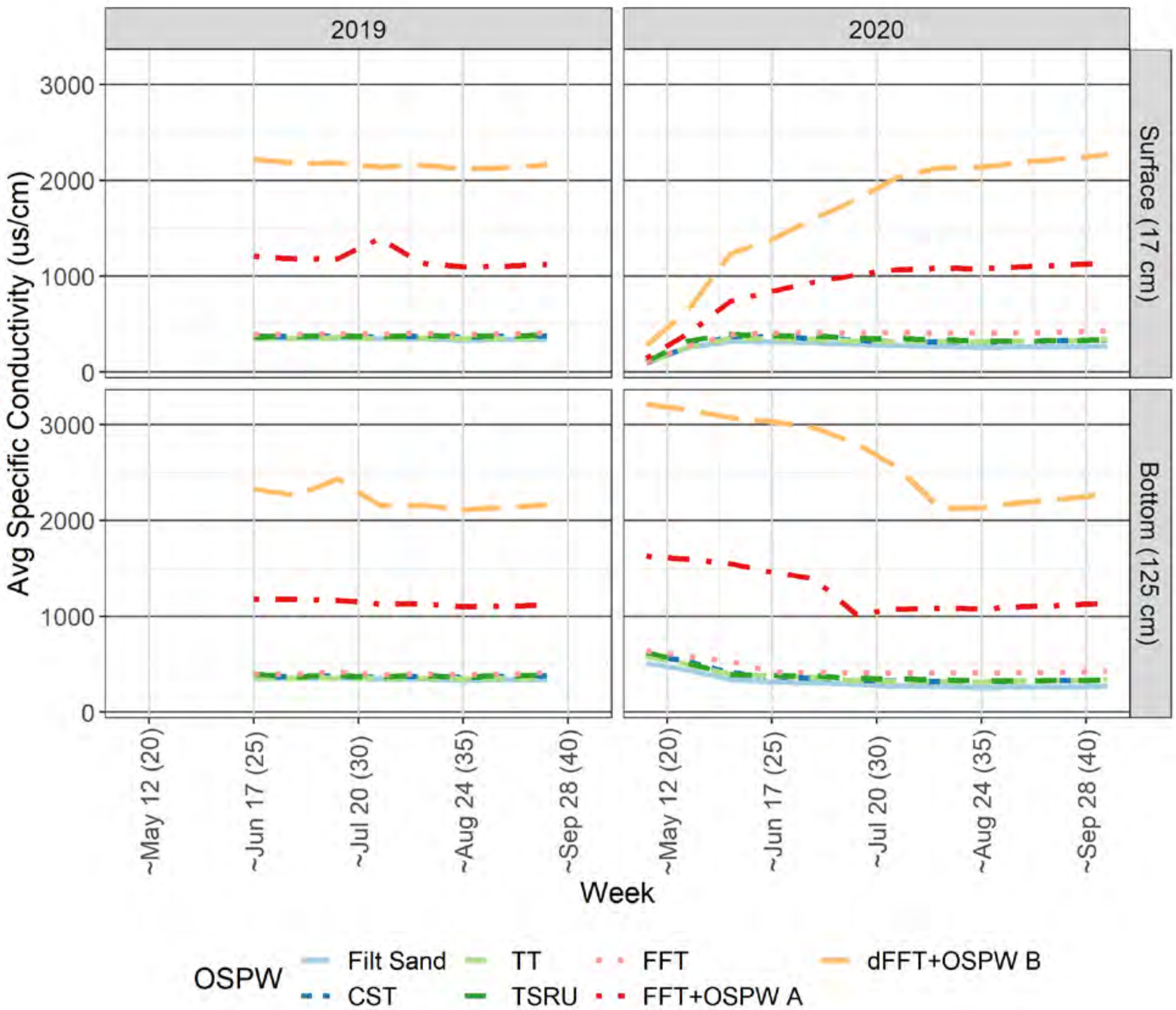

Figure 12. Mean specific conductivity at the surface and bottom in 2019 and 2020. Standard errors of each value can be found in Table A.3.58 of Appendix A. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.1.5 Optical Dissolved Oxygen

Figures and tables describing optical dissolved oxygen (henceforth referred to as dissolved oxygen) content in the mesocosms can be found in Section A.3.3.5 of Appendix A.

3.1.5.1 Effect of depth

At the beginning of 2019, lower dissolved oxygen concentrations tended to be associated with increasing depths, except in CTL. This effect was prolonged in TRT 4 (FFT) and TRT 6 (dFFT + 50% Suncor OSPW), compared to other experimental groups. No significant difference in dissolved oxygen between depths was seen in any experimental group after Week 29 of 2019.

In 2020, the effects of depth on dissolved oxygen results were more variable (Figure 13). In CTL, dissolved oxygen measured at the surface compared to the bottom was significantly lower between Weeks 19 and 25, 2020. Similarly, dissolved oxygen measurements taken at the surface were significantly lower the bottom in TRT 1 (CST) and TRT 4 (FFT) from Weeks 19 to Week 23, in TRT 2 (TT) during Weeks 19 and 21, and in TRT 6 (dFFT+50% Suncor OSPW) during Week 31, 2020. However, dissolved oxygen was significantly higher near the surface in TRT 3 (TSRU) and TRT 5 (FFT+100% Imperial OSPW) during Week 19 and in TRT 6 (dFFT+50% Suncor OSPW) until Week 23, 2020. After Week 33 of 2020, there were no significant differences in dissolved oxygen between depths in any mesocosms (Figure 13).

3.1.5.2 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

For the most part, CST (TRT 1) and TT (TRT 2) appeared to have little effect on dissolved oxygen concentrations at either depth in 2019. Dissolved oxygen levels measured in TRT 3 (TSRU) and TRT 4 (FFT) mesocosms were significantly lower than CTL at both depths for most time points prior to Week 33 of 2019.

On occasion, surface dissolved oxygen concentrations were significantly lower in TRT 1 (CST), TRT 2 (TT), and TRT 3 (TSRU) than CTL in 2020. From Weeks 27 to 39 of 2020, the presence of FFT in TRT 4 mesocosms was associated with significantly lower surface dissolved oxygen concentrations than what was observed in CLT. Prior to Week 31, 2020, dissolved oxygen concentrations measured in the bottom of the TRT 2 (TT), TRT 3 (TSRU), and TRT 4 (FFT) mesocosms were significantly lower than CTL during most weeks.

3.1.5.3 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

Compared to CTL, OSPW with FFT (TRT 5) mesocosms had significantly lower dissolved oxygen concentrations at the surface from Weeks 35 to 39, of 2019, while lower dissolved oxygen levels measured at the bottom were only observed during Week 25 of 2019. Interestingly, dissolved oxygen measured at the surface in TRT 5 were higher than CTL during Weeks 25 and 27, 2019.

During Weeks 21 to 41 of 2020, surface dissolved oxygen concentrations were significantly lower in TRT 5 than in CTL. Near the bottom, dissolved oxygen concentrations in TRT 5 were significantly lower than CTL except for Weeks 39 and 41, 2020 (Figure 14).

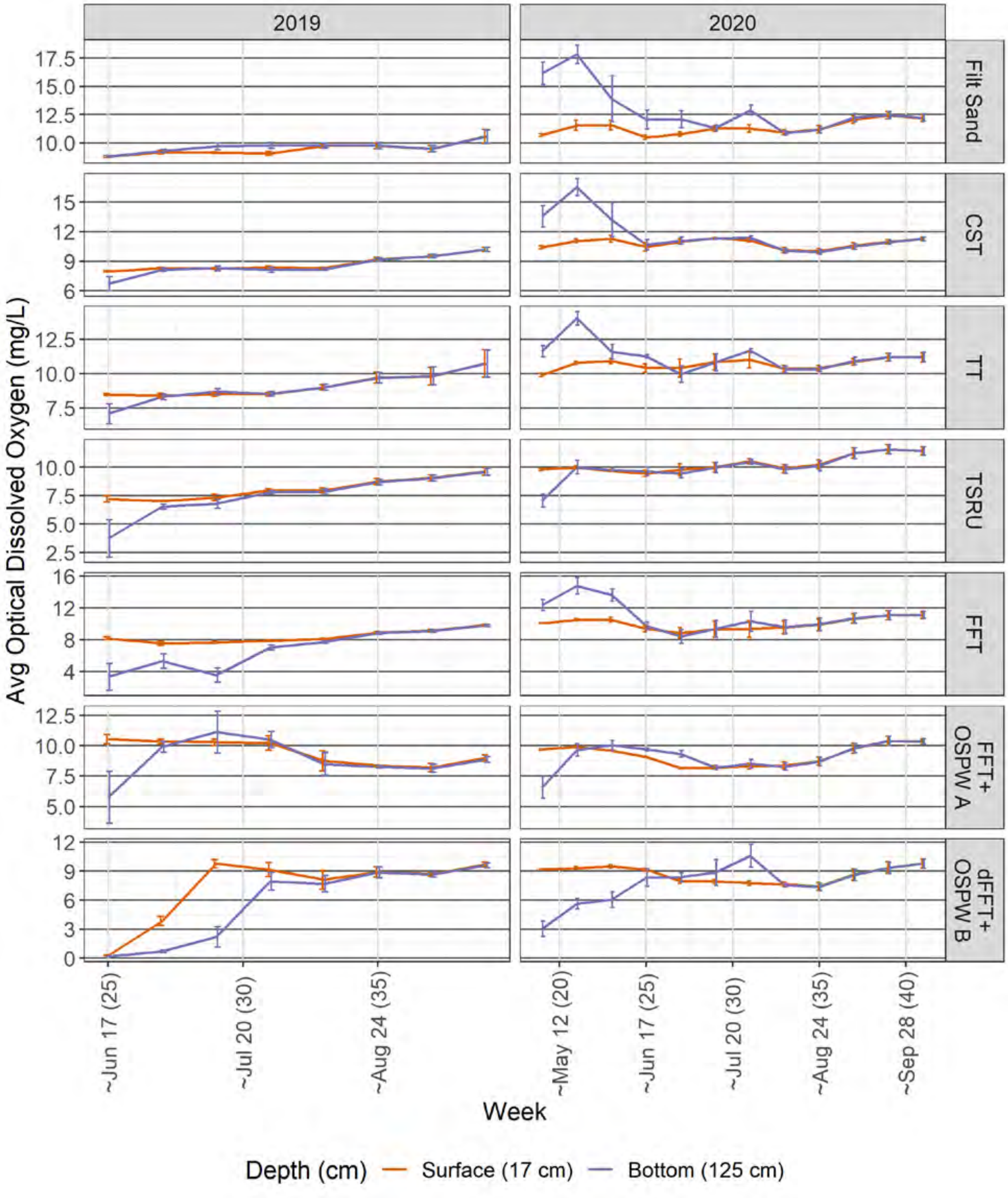

Figure 13. Mean dissolved oxygen by experimental groups in 2019 and 2020.
Note that the y-axis scales change across each experimental group. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.1.5.4 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

When compared to CTL, the presence of 50% Suncor OSPW with dFFT (TRT6) mesocosms was associated with significantly reduced dissolved oxygen concentrations at the surface and bottom until Week 33 and 29 of 2019, respectively.

Overall, in 2020, dissolved oxygen concentrations were significantly lower in TRT 6 compared to CTL (Figure 14).

3.1.5.5 Effect of treatment of tailings (TRT 2 vs. TRT 4)

In 2019, dissolved oxygen concentrations measured at the surface did not differ significantly between of mesocosms with different tailing types (i.e., FFT vs. TT). However, at the bottom, dissolved oxygen concentrations were significantly lower in TRT 4 (FFT) compared to TRT 2 (TT) until Week 29 of 2019. However, this effect was not significant for the rest of 2019.

In 2020, dissolved oxygen concentrations measured at the surface were significantly lower in TRT 4 (FFT) than TRT 2 (TT) from Weeks 27 to 31. However, at the bottom, there were no significant differences between these experimental groups, except for Week 23, 2020, when TRT 2 (TT) was significantly lower than TRT 4 (FFT).

3.1.5.6 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

Until Week 31 of 2019, TRT 5 mesocosms had significantly higher dissolved oxygen levels at the surface and bottom compared to TRT 4 mesocosms. However, in 2020, the addition of OSPW to FFT (TRT 5) was associated with reduced dissolved oxygen concentrations at the surface during Weeks 29, 33, and 35 and at the bottom until Week 23, 2020.

3.1.5.7 Effect of time

In 2019, dissolved oxygen concentrations measured at the surface appeared to increase, except in TRT 5 (FFT+100% Imperial OSPW). Dissolved oxygen concentrations appeared to decrease at the surface in TRT 5 (FFT+100% Imperial OSPW) after Week 31. Whereas TRT 6 (dFFT+50% Suncor OSPW) demonstrated a pronounced increase in surface dissolved oxygen concentrations. Dissolved oxygen concentrations measured in the bottom of all experimental groups appeared to increase over time. However, by the end of 2019, dissolved oxygen levels measured at both depths tended to converge across experimental groups.

Dissolved oxygen concentrations measured at the surface did not change much through the 2020 season. However, dissolved oxygen concentrations measured near the bottom of the mesocosms was varied.

Over two years, an overall increase in dissolved oxygen concentrations measured at the surface and bottom of the mesocosms was observed across experimental groups. One exception is that an overall mid-study decrease in surface dissolved oxygen levels in TRT 5 (FFT + 100% Imperial OSPW) was observed.

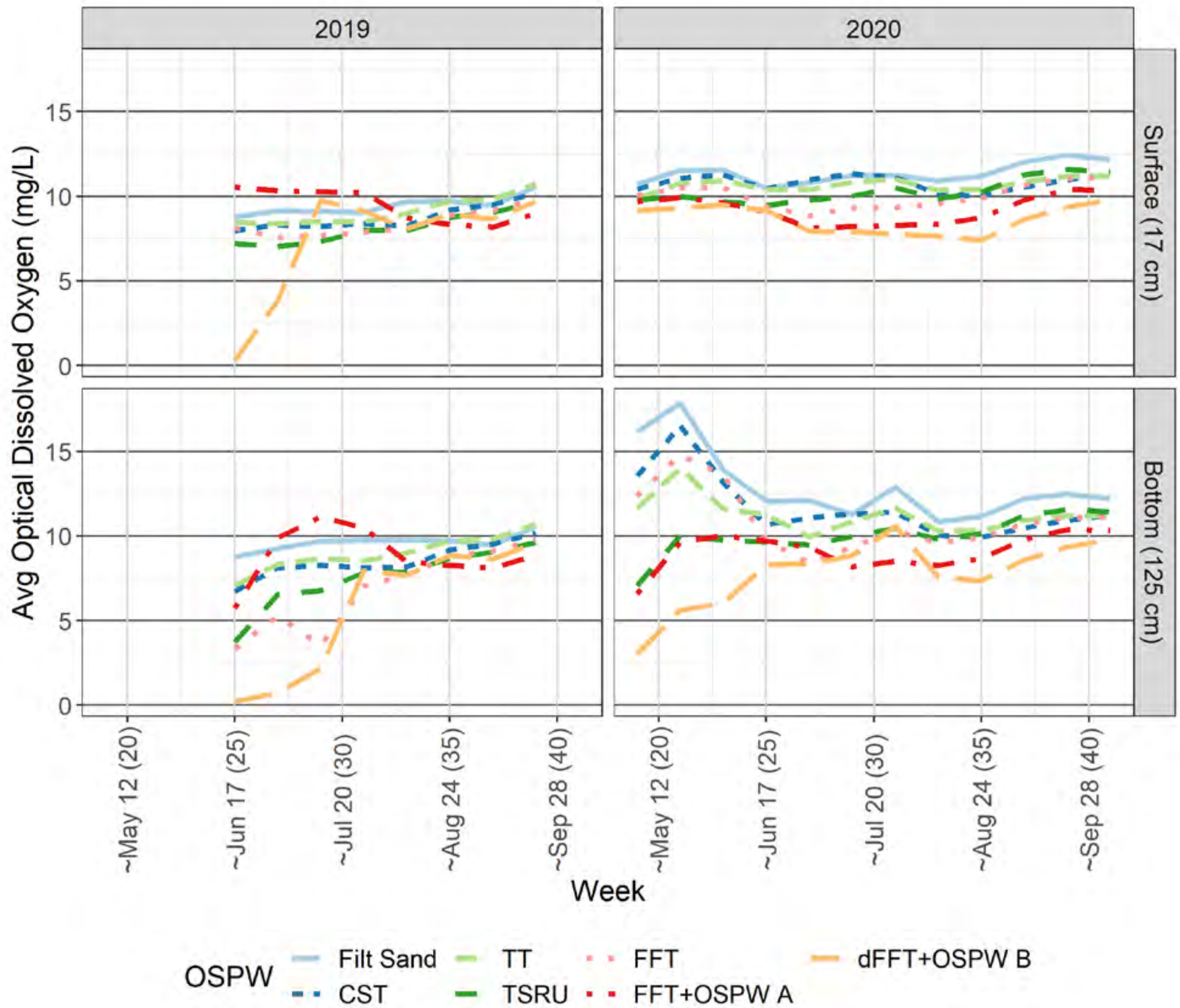

Figure 14. Mean dissolved oxygen at the surface and bottom in 2019 and 2020. Standard errors of each value can be found in Table A.3.76 of Appendix A. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.1.6 Total Algae (Chlorophyll and Blue-green Algae)

Two data sets were generated from a dual-channel fluorescence sensor of data sonde: chlorophyll and blue-green algae (cyanobacteria). Figures and tables describing chlorophyll and blue-green algae (BGA) measured by data sonde can be found in Section A.3.3.6 and A.3.3.7 of Appendix A.

3.1.6.1 Effect of depth

Chlorophyll values were significantly higher at the bottom of the mesocosms than near the surface from time to time in both years (Figure 15). Significant differences in chlorophyll and blue-green algae were observed in all treatment groups (except CTL) at least once in 2019, mainly prior to Week 29 (Figure 16).

Prior to Week 35 of 2020, significantly higher values of both chlorophyll and BGA recorded at the bottom were found at least once in CTL, TRT 1 (CST), TRT 2 (TT), and TRT 3 (TSRU).

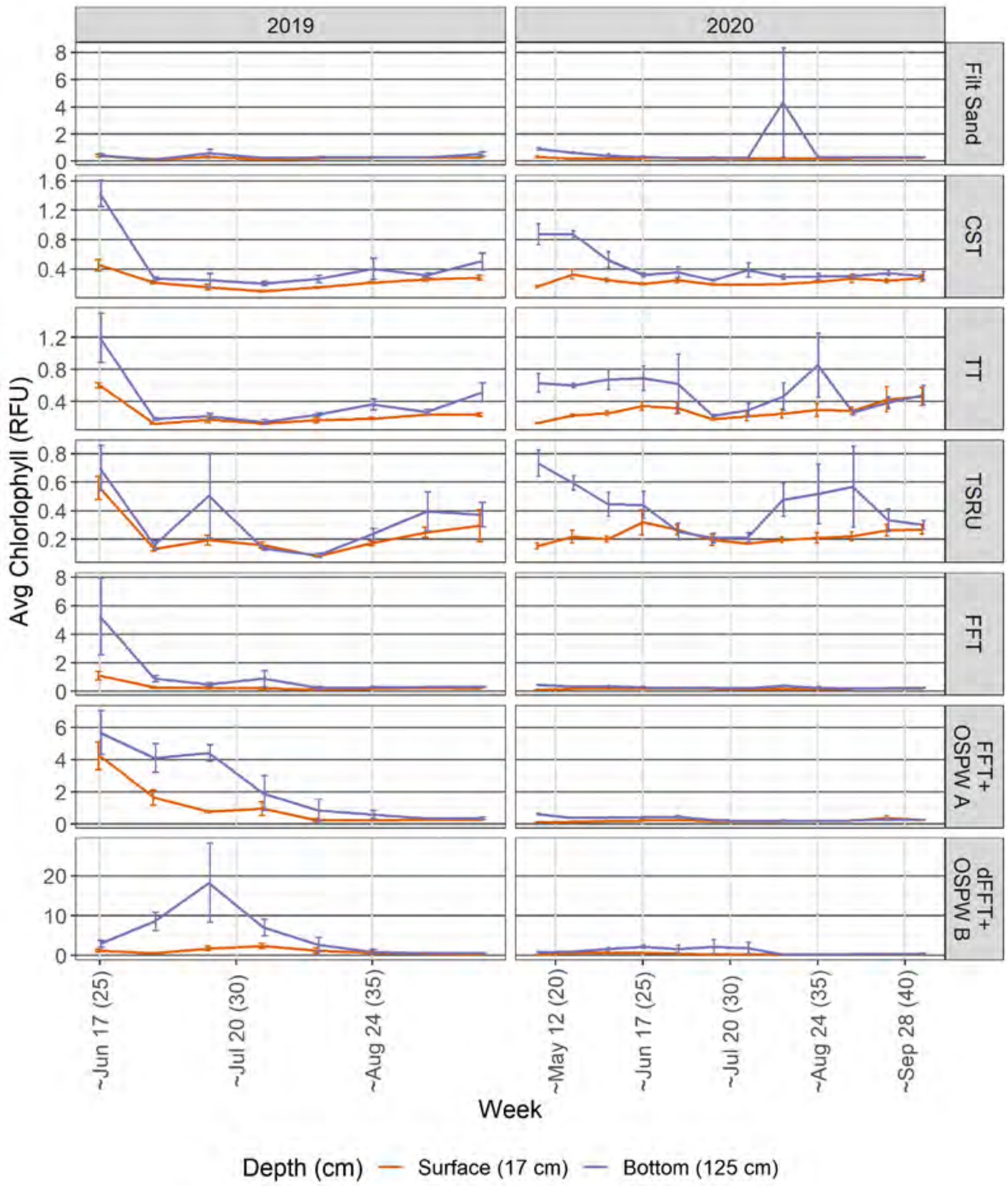

Figure 15. Mean Chlorophyll (RFU) by experimental groups in 2019 and 2020.
Note that the y-axis scales change across each experimental group. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

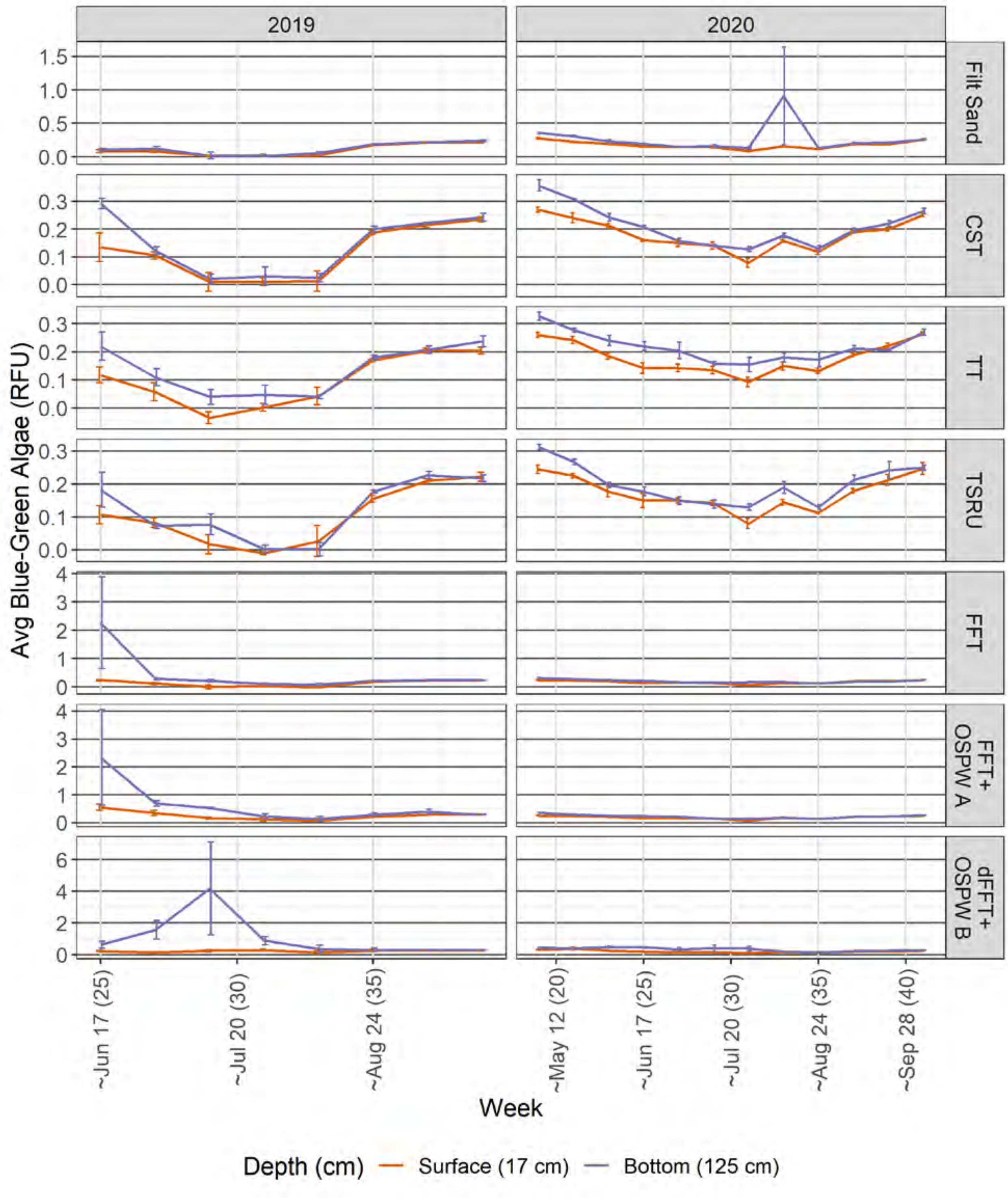

Figure 16. Mean blue-green algae (RFU) by experimental groups in 2019 and 2020. Note that the y-axis scales change across each experimental group. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.1.6.2 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

In 2019, no significant differences in chlorophyll (RFU) were observed at either depth, when comparing tailings-only treatments to CTL. A few exceptions were noticed in the beginning of 2019 (Week 25), where significantly higher chlorophyll measures were recorded at both depths in TRT 4 (FFT) compared to CTL, and at the surface of TRT 2 (TT) compared to CTL. BGA readings were not significantly different in the presence of tailings compared to the CTL mesocosms, except for the higher recordings taken at both depths in TRT 4 (FFT) during first week of 2019.

In 2020, on a few occasions, there were significant differences in both chlorophyll and BGA (RFU) levels when comparing TRTs 1, 2, 3, and 4 to CTL. In Week 19, chlorophyll (RFU) values taken at the surface in TRT 2 (TT) and TRT 4 (FFT) were significantly lower than CTL. Whereas in Weeks 39 and 41, CTL chlorophyll (RFU) values recorded in in TRT 2 (TT) were significantly higher than CTL. During Week 33, Chlorophyll and BGA values measured at the bottom of TRTs 1, 2, 3, and 4 mesocosms were significantly lower than CTL.

3.1.6.3 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

Until Week 31 of 2019, chlorophyll values recorded at the surface of TRT 5 were significantly higher than CTL. During the first sampling week of 2019, BGA values were also significantly higher in TRT 5 at the bottom. In 2020, neither chlorophyll or BGA values were significantly different between TRT 5 and CTL mesocosms.

3.1.6.4 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

In the first half of 2019, chlorophyll readings measured at both depths were significantly higher in TRT 6 compared to CTL. However, differences in BGA readings at the bottom were not significantly different, except during Week 29. However, after Week 33, no significant differences in chlorophyll or BGA were observed between CTL and TRT 6. In 2020, neither RFU values were significantly different in TRT 6 compared to CTL, except during Week 21, and for BGA measured at the surface.

3.1.6.5 Effect of treatment of tailings (TRT 2 vs. TRT 4)

During Weeks 25 and 27 of 2019, chlorophyll and BGA levels recorded at the surface of the TRT 4 mesocosm were significantly higher than TRT 2 (FFT). However, bottom measurements of chlorophyll and BGA were only higher during Week 25. Conversely, in Week 33, BGA values recorded at the surface of TRT 4 mesocosms were significantly lower than TRT 2 mesocosms.

During Weeks 25, 39, and 41 of 2020, chlorophyll values measured at the surface of TRT 2 were significantly higher than TRT 4 (FFT).

3.1.6.6 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

In early 2019, chlorophyll values recorded at both depths were significantly higher in TRT 5 than in TRT 4. However, as the season progressed, these differences in chlorophyll concentration were only significant from Weeks 25 to 31 (at the surface) and Weeks 27 to 29 (at the bottom). Prior to Week 33, significantly higher readings of BGA in TRT 5 were only recorded at the surface.

In 2020, no significant differences in either RFU values were observed at either depth.

3.1.6.7 Effect of time

Overall, chlorophyll RFU measurements demonstrated no clear trend at either depth for CTL and TRTs 1, 2, and 3 mesocosms. There was a significant decrease over time in chlorophyll of TRT 4 (FFT) at the surface. Although not significant, a decrease in TRT 4 (FFT) appeared at the bottom as well. TRT 5 (FFT+100% Imperial OSPW) displayed a decrease in chlorophyll levels at the surface until Week 33, 2019. Similarly, in TRT 6 (dFFT + 50% Suncor OSPW), there was an overall decrease in chlorophyll levels over the study period. Chlorophyll values settled at 0.2 to 0.5 RFU at all experimental groups at Week 41 of 2020 (Figure 17).

In both years, a mid-season increase in BGA levels near the surface was noticed for CTL, TRTs 1, 2, and 3, with the highest values recorded during Week 19, 2020. BGA values recorded near the mesocosm floor showed no clear trend over time for CTL, TRTs 1, 2, and 3. For TRT 4 (FFT), BGA levels recorded at the surface increased, but a decrease was recorded at the bottom. BGA levels recorded in TRT 5 (FFT+100% Imperial OSPW) and TRT 6 (dFFT+50% Suncor OSPW) decreased at both depths (Figure 18).

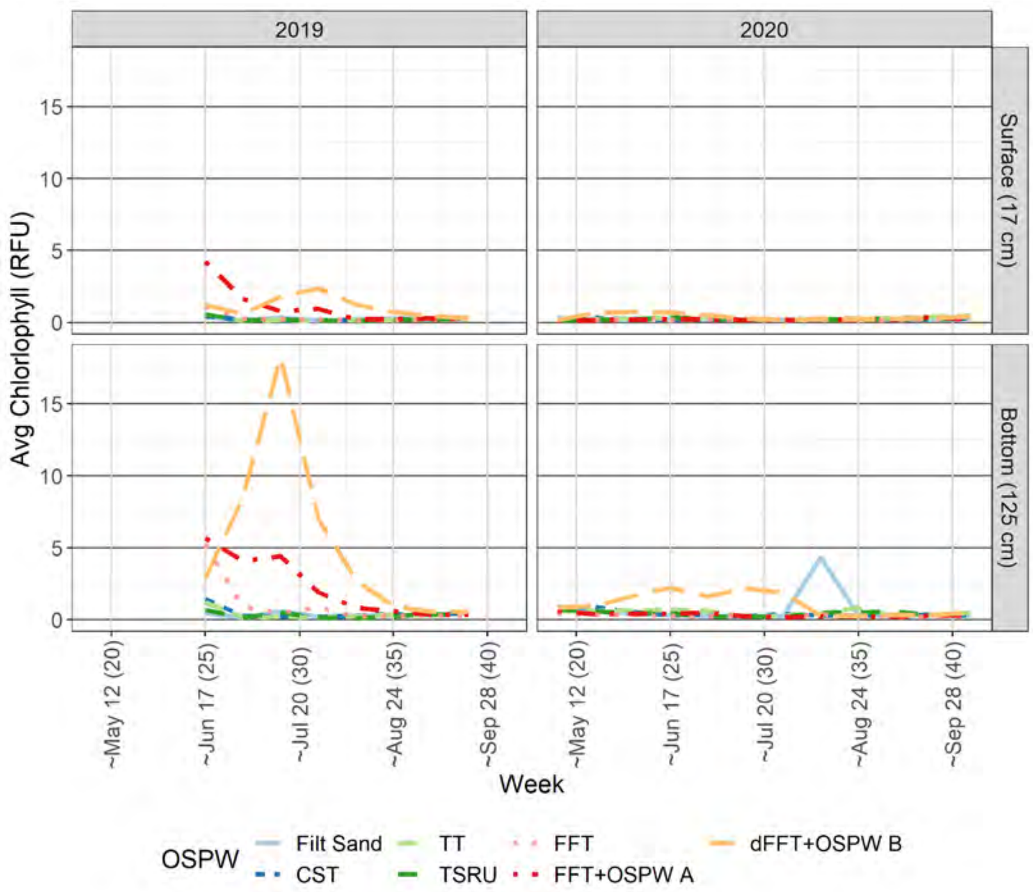

Figure 17. Mean chlorophyll (RFU) at the surface and bottom in 2019 and 2020.
Standard errors of each value can be found in Table A.3.94 of Appendix A. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT

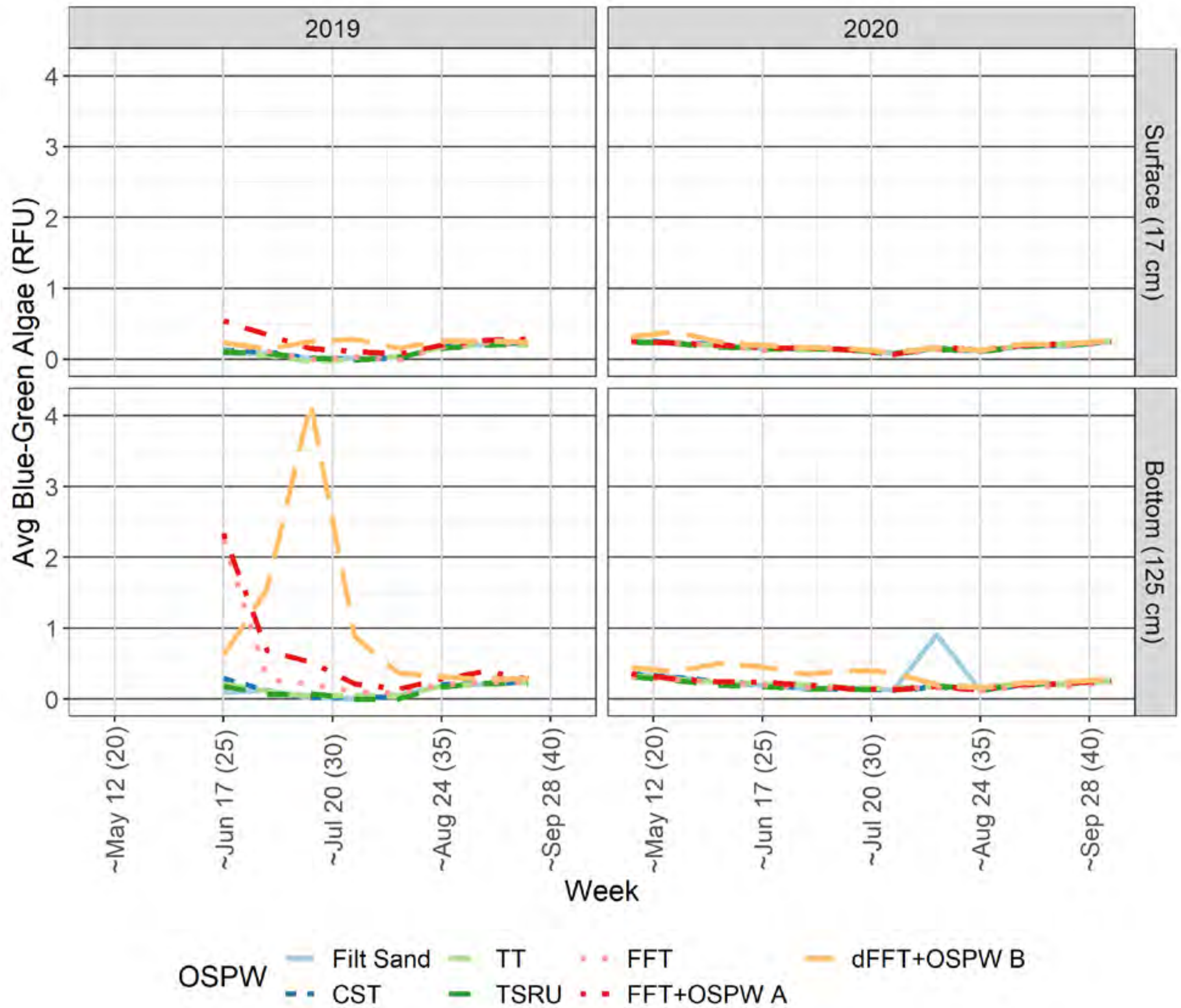

Figure 18. Mean blue-green algae (BGA)at the surface and bottom in 2019 and 2020. Standard errors of each value can be found in Table A.3.112 of Appendix A. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.2 Water Quality – Laboratory Measurement

3.2.1 Naphthenic Acids – Total

Figures and tables describing total naphthenic acids (NAs) concentration in mesocosm waters can be found in Section A.5.8.43 of Appendix A. Mean NA (total) concentrations for the:

- CTL mesocosms ranged from 13 to 25 µg/L,
- TRTs 1, 2, 3, and 4 groups ranged from 110 to 2,491 µg/L, and
- TRT 5 and 6 mesocosms ranged from 5,656 to 33,200 µg/L (Figure 19).

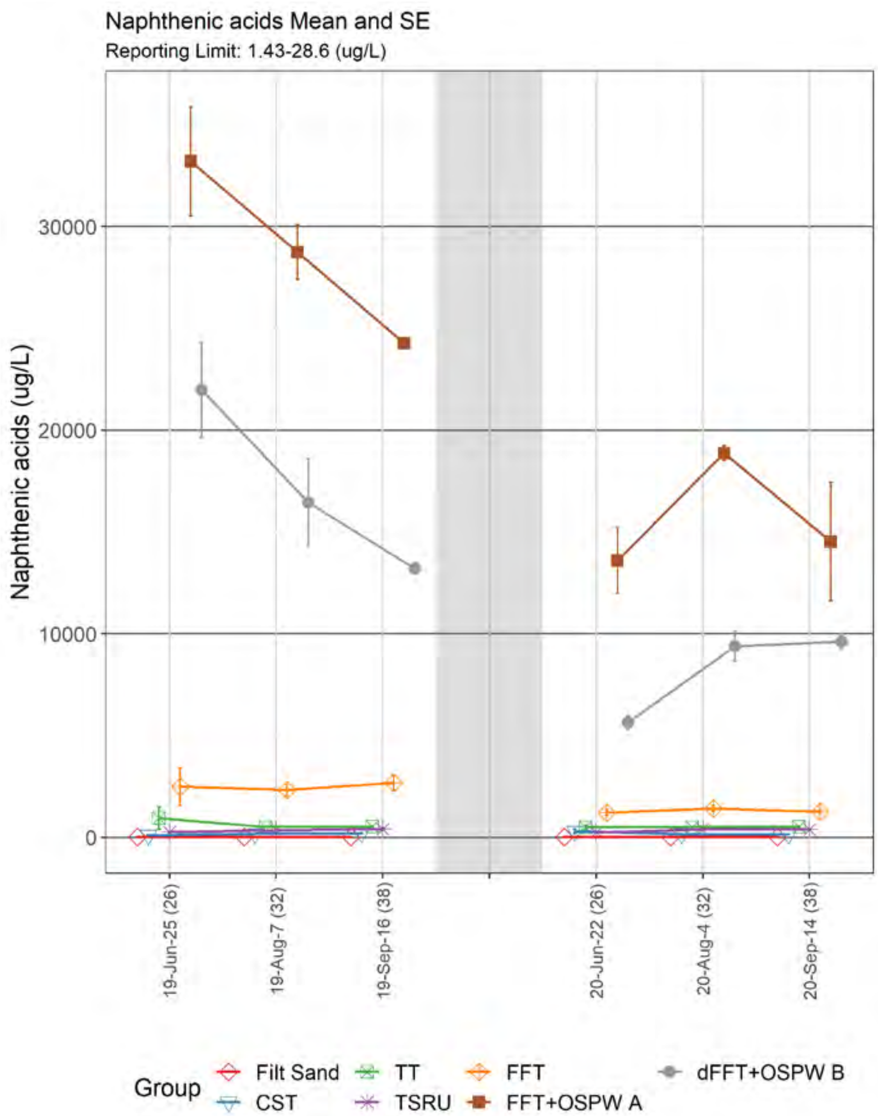

Figure 19. Mean concentrations of total naphthenic acids.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.2.1.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

Significantly higher NA concentrations were measured in TRT 4 (FFT) compared to CTL at all six sampling points in 2019 and 2020. The mean NA concentration in TRT 4 had a range from 1,216 to 2,688 µg/L. Higher NA concentrations in TRT 2 (TT) occurred only during Week 26 of 2019.

3.2.1.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT5)

In 2019 and 2020, the presence of 100% Imperial OSPW with FFT (TRT 5) was associated with higher NA concentrations relative to CTL.

3.2.1.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT6)

In both 2019 and 2020, dFFT with 50% Suncor OSPW (TRT 6) was associated with significantly higher total NA values compared to CTL.

3.2.1.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

The NA concentrations measured in TRT 4 (FFT) were significantly higher than TRT 2 (TT) in both years.

3.2.1.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

The total NA concentration was significantly higher when both FFT and OSPW were included in the mesocosms (TRT 5) compared to the inclusion of only FFT (TRT 4) in both years.

3.2.1.6 Effect of time

NA concentrations measured in the tailing materials (prior to placement in the mescosms) were 10-40 times higher than filter sand (See Section A.5.3 of Appendix A). Given the low concentrations of NAs in ARW (measured at 30 µg/L) and the fact that ~2 m^3 of tails material was placed, the total NA concentration in TRT 1 (CST), TRT 2 (TT), TRT 3 (TSRU), and TRT 4 (FFT) water were anticipated to be higher than CTL water, mainly considering the NA levels in tailings. Total NA concentration measured in TRT 1 water was measured at 110 ± 5 µg/L at the beginning of 2019, compared to CTL water (measured at 21 ± 1 µg/L). However, total NAs in the TRT 1 mesocosms appeared to approximate their anticipated level more closely over time (measured at 150 – 299 µg/L). Taking into consideration:

- NA concentrations measured in OSPW (prior to the study) were 24,900 µg/L for Imperial OSPW and 39,600 µg/L for Suncor OSPW
- and dilution (50% in TRT 6),
- as well as the addition of FFT and dFFT (see Section Table A.5.3 of Appendix A),

the total NA concentration in TRT 5 and 6 were expected to be at least 25,000 µg/L and 20,000 µg/L for TRT 5 and TRT 6, respectively. In Week 26 of 2019, NA concentrations recorded in the experimental groups receiving OSPW (TRT 5 and TRT 6) were similar to what was expected; TRT 5 and 6 total NA concentrations were at 33,200 ± 2,670 µg/L and 21,980 ± 2,335 µg/L, respectively.

During the study, total NA concentration did not change significantly over time in CTL and TRTs 1, 2, 3, or 4 mesocosms, although NA concentrations measured in TRT 4 were lower in 2020 (1,216 – 1,428 µg/L), compared to what was measured in 2019 (2,325 – 2,688 µg/L). It should be noted that the total NA concentration in CTL and TRTs 1, 2, 3, or 4 mesocosms were relatively low compared to that in TRTs 5 and 6, where analytical variability can be as high as a temporal change that might have happened. However, over the two years, total NA concentrations decreased significantly in TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW). A significant mid-study decrease was also noticed for both treatments, as evidenced by the considerably lower NA levels measured in Week 26 of 2020 compared to Week 38 of 2019.

3.2.2 Naphthenic Acids – Proportion by Family

Detailed figures and tables for the proportion of NA families can be found in Sections A.5.6 of Appendix A. Correlations and Principal Component Analyses (PCA's) of the NA family data as a percentage of total analyzed NAs based on current methodology can be found in Section A.5.4.7 of Appendix A. The proportion of mesocosms below the reporting limit is presented in Table A.5.2 of Appendix A. Note that the results in this Section are based on proportion only, and comparisons of absolute concentrations are provided in the following Sections.

3.2.2.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

Statistical comparisons between naphthenic acid family proportions (%) associated with tailings only (TRTs 1, 2, 3,4) compared to control treatments for 2019 are summarized below in Table 7. In 2019, the effect of tailings alone on the proportions of NA families compare to CTL was generally similar, except family Z-10. In terms of proportion, CTL mesocosms were richer in Z-0, Z-2 (except TRT 1) over 2019, and Z-8 at Week 26. In contrast, CTL was impoverished for the rest of the families compared to TRT 1, 2, 3, and 4. For Z-10, however, CTL was enriched compared to TRT 3 (TSRU, Weeks 26 and 38) and TRT 4 (FFT, Week 38), but significantly depleted compared to TRT 1 (CST, Week 32 and 38) and TRT 2 (TT, Week 32).

Similarly, in 2020, the proportion of smaller naphthenic acids (Z-0 and Z-2) was significantly higher in CTL, compared to TRT 1, 2, 3, and 4 for all time points in 2020 except for Z -2 in TRT 1 (CST) at Week 26 as shown in Table 6 below. TRT 1 (CST) was significantly higher in its proportion of Z-10, Z-12, and Z-14 at all three time points, as well as Z-16 (Week 32 and 38) and Z-18 (Week 32) compared to CTL. TT (TRT 2), TSRU (TRT 3), and FFT (TRT 4) have significant effects on the proportion of Z-6/Z-12/Z-14 at all three-time points in 2020. TRT 2 (TT) mesocosms were richer in Z-10 (Week 28), Z-16 (Week 32 and 38), and Z-18 (Week 32). TSRU (TRT 3) was richer in Z-10 compared to CTL (Week 28), as well as Z-16 at all three time points. Besides, TRT 4 (FFT) was significantly higher than CTL in the proportion of Z-4 NAs over 2020 (Table 8). In contrast, the proportions of Z-10 in TRT 3 (TSRU) and TRT 4 (FFT) were lower at Week 32.

3.2.2.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

In 2019, CTL mesocosms contained higher proportions of Z-0 (all three time points), Z-2 (Weeks 26 and 38), Z-8 (Week 26), and Z-10 (Week 38) than TRT 5 mesocosms. Z-4, Z-6, and Z-12 to Z-18 were present in higher proportions in the TRT 5 than in the CTL mesocosms in 2019.

In 2020, there were some exceptions to this pattern. CTL was enriched compared to TRT 5 for Z-0 (all three time points), Z-2 (Week 32 and 38), and Z-10 (Week 32). TRT 5 was significantly higher in its proportion for Z-12 (Week 26 and 38), besides Z-4, Z-6, and Z-14 at all three time points. No significant difference was detected for Z-8, Z-16 and Z-18 at any time in 2020 (Table 9).

Table 7. Statistical comparisons between naphthenic acid family proportions (%) associated with tailings only presence in 2019.

Fraction%	TRT 1 (CST): CTL			TRT 2 (TT): CTL			TRT 3 (TSRU): CTL			TRT 4 (FFT): CTL		
	2019 W26	2019 W32	2019 W38	2019 W26	2019 W32	2019 W38	2019 W26	2019 W32	2019 W38	2019 W26	2019 W32	2019 W38
Z-0	<	<	<	<	<	<	<	<	<	<	<	<
Z-2	=	=	=	<	=	<	<	=	<	<	=	<
Z-4	>	>	>	>	>	=	=	>	>	>	>	>
Z-6	>	>	=	>	>	>	>	>	>	>	>	>
Z-8	<	=	=	<	=	=	<	=	=	<	=	=
Z-10	=	>	>	=	>	=	<	=	<	=	=	<
Z-12	=	>	>	>	>	>	>	>	>	>	>	>
Z-14	=	>	>	>	>	>	>	>	>	>	>	>
Z-16	=	>	>	>	>	>	>	>	>	>	=	=
Z-18	>	>	>	>	>	>	>	>	>	>	>	=

> indicates TRTs contains a statistically significant higher proportion of that NA family than does CTL (TRT 1, 2, 3, or 4 > CTL)

< indicates TRTs contains a statistically significant lower proportion of that NA family than does CTL. (TRT 1, 2, 3, or 4 < CTL)

= indicates TRTs is not significantly different from CTL in that NA family (TRT 1, 2, 3, or 4 = CTL)

Table 8. Statistical comparisons between naphthenic acid family proportions (%) associated with tailings only presence in 2020.

Fraction%	TRT 1 (CST): CTL			TRT 2 (TT): CTL			TRT 3 (TSRU): CTL			TRT 4 (FFT): CTL		
	2020 W26	2020 W32	2020 W38	2020 W26	2020 W32	2020 W38	2020 W26	2020 W32	2020 W38	2020 W26	2020 W32	2020 W38
Z-0	<	<	<	<	<	<	<	<	<	<	<	<
Z-2	=	<	<	<	<	<	<	<	<	<	<	<
Z-4	=	=	=	=	=	=	=	=	=	>	>	>
Z-6	=	=	=	>	>	>	>	>	>	>	>	>
Z-8	=	=	=	=	=	=	=	=	=	=	=	=
Z-10	>	>	>	=	=	>	=	<	>	=	<	=
Z-12	>	>	>	>	>	>	>	>	>	>	>	>
Z-14	>	>	>	>	>	>	>	>	>	>	>	>
Z-16	=	>	>	=	>	>	>	=	>	=	=	=
Z-18	=	>	=	=	>	=	>	>	>	=	=	=

> indicates TRTs contains a statistically significant higher proportion of that NA family than does CTL (TRT 1, 2, 3, or 4 > CTL)

< indicates TRTs contains a statistically significant lower proportion of that NA family than does CTL (TRT 1, 2, 3, or 4 < CTL)

= indicates TRTs is not significantly different from CTL in that NA family (TRT 1, 2, 3, or 4 = CTL)

3.2.2.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

The effect of adding 50% Suncor OSPW to a dFFT layer on the proportions of NA families was broadly similar to what was observed in TRT 5. TRT 6 produced a high proportion of Z-4, Z-6 (except Week 26, 2020), Z-10 (only at Week 38, 2020), Z-12, Z14 (except Week 26, 2020), Z-16 (all three times points in 2019 and Week 26 of 2020), and Z-18 (only in 2019) compared to CTL. The proportion of smaller naphthenic acids, including Z-0 (all six time points) and Z-2 (Week 38 of 2019 and Weeks 32 and 38 of 2020), as well as Z-8 (Week 26 and 38 of 2019 and Week 32 of 2020) were significantly lower in the presence of OSPW and tailings (TRT 6). However, there was no significant difference in the proportions of Z-18 throughout 2020.

Table 9. Statistical comparisons between naphthenic acid family proportions (%) associated with tailings and OSPW presence in 2019 and 2020.

Fraction%	TRT 5 (100% Imperial OSPW+FFT): CTL						TRT 6 (50% Suncor OSPW+dFFT): CTL					
	2019	2019	2019	2020	2020	2020	2019	2019	2019	2020	2020	2020
	W26	W32	W38	W26	W32	W38	W26	W32	W38	W26	W32	W38
Z-0	<	<	<	<	<	<	<	<	<	<	<	<
Z-2	<	=	<	=	<	<	=	=	<	=	<	<
Z-4	>	>	>	>	>	>	>	>	>	>	>	>
Z-6	>	>	>	>	>	>	>	>	>	=	>	>
Z-8	<	=	=	=	=	=	<	=	<	=	<	=
Z-10	=	=	<	=	<	=	=	=	<	=	=	>
Z-12	>	>	>	>	=	>	>	>	>	>	>	>
Z-14	>	>	>	>	>	>	>	>	>	=	>	>
Z-16	>	>	>	=	=	=	>	>	>	>	=	=
Z-18	>	>	>	=	=	=	>	>	>	=	=	=

> indicates TRTs contains a statistically significant higher proportion of that NA family than does CTL (TRT 5 or 6 > CTL)

< indicates TRTs contains a statistically significant lower proportion of that NA family than does CTL (TRT 5 or 6 < CTL)

= indicates TRTs is not significantly different from CTL in that NA family (TRT 5 or 6 = CTL)

3.2.2.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

Table 8 summarizes the differences between TRT 2 (TT) and TRT 4 (FFT). On a proportional basis, TRT 2 (TT) mesocosms were significantly richer in Z-10 to Z-18 at Week 32 and 38 of both years. TRT 4 (FFT) was significantly higher in the proportion of Z-4 after Week 32 and Z-6 after Week 38, 2019. No significant difference could be discerned for Z-0, Z-2, and Z-8.

3.2.2.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

Table 10 summarizes the differences between TRT 4 (FFT) and TRT 5 (FFT+100% Imperial OSPW). In 2019, 100 % OSPW with FFT (TRT 5) significantly higher the proportion of Z-14 to Z-18 NAs compared to FFT alone (TRT 4), except in the case of Z-16 and Z-18 in the first week of the study. OSPW in the presence of FFT (TRT 5) had no significant effect on Z-14 to Z-18 of NA family proportion at any point in 2020. However, TRT 5 was significantly higher in the proportion of Z-4 and Z-6 (except for the Week 26) in 2020. TRT 4 (FFT) tended to be proportionally higher in Z-0, Z-2, Z-8 (Week 38 of 2019 and Week 26 of 2020), and Z-10 (Week 26 and 32 of 2020). Interestingly, the pattern was mixed for Z-4 and Z-12 in different years. For Z-4, TRT 4 was proportionally higher during Week 32 of 2019 but lower at all three time points in 2020.While for Z-12, TRT 4 (FFT) was proportionally lower in this family during Week 26 and 32 in 2019 but higher at Week 32 and 38 in 2020.

Table 10. Statistical comparisons between naphthenic acid family proportions (%) associated with TT and FFT in 2019 and 2020.

Fraction%	TRT 2 (TT): TRT 4 (FFT)						TRT 4 (FFT): TRT 5 (FFT+100% OSPW-I)					
	2019 W26	2019 W32	2019 W38	2020 W26	2020 W32	2020 W38	2019 W26	2019 W32	2019 W38	2020 W26	2020 W32	2020 W38
Z-0	=	=	=	=	=	=	>	>	>	=	>	=
Z-2	=	=	=	=	=	=	>	>	=	=	=	>
Z-4	=	<	<	<	<	<	=	>	=	<	<	<
Z-6	=	=	<	<	<	<	=	=	=	=	<	<
Z-8	=	=	=	=	=	=	=	=	>	>	=	=
Z-10	=	>	>	=	>	>	=	=	=	>	>	=
Z-12	=	>	>	=	>	>	<	<	=	=	>	>
Z-14	=	>	>	=	>	>	<	<	<	=	=	=
Z-16	=	>	>	=	>	>	=	<	<	=	=	=
Z-18	=	>	>	=	>	>	=	<	<	=	=	=

> indicates TRT 2 or 4 contains a statistically significant higher proportion of that NA family than does TRT 4 or 5 (TRT 2 > TRT 4 or TRT 4 > TRT 5)

< indicates TRT 2 or 4 contains a statistically significant lower proportion of that NA family than does TRT 4 or 5 (TRT 2 < TRT 4 or TRT 4 < TRT 5)

= indicates TRT 2 or 4 is not significantly different from TRT 4 or 5 in that NA family (TRT 2 = TRT 4 or TRT 4 = TRT 5)

3.2.2.6 Effect of time

The changes in NA family proportions over time are summarized in Table 11. The effect of time varies according to the NA family and experimental group. As stated in Section 2.16.2 and introduced in Section A.1.2 in Appendix A, more than one trend could occur simultaneously. For example, the trend of Z-4 in TRT 6 (dFFT + 50% Suncor OSPW) could be described as both significantly increasing (a significant positive "linear") and mid-study increase (a significant negative "quadratic"). For simplicity when more than one trend over time was significant, the most perceivable trend was summarized below. More details of trends overtime can be found in Section A.5.6 of Appendix A.

Most NA fraction proportions (%) in the CTL mesocosms did not change significantly or fluctuate ("cubic" pattern). The family of Z-2 showed a significant mid-study decrease, and Z-16 showed a significant decrease for CTL mesocosms. TRT 1 (CST) showed a significant decrease in the percentage of Z-2 and Z-4 across both years, but conversely, it showed a significant increase in the percentage of Z-10 to Z-14. In TRT 2 (TT) mesocosms, the proportions of Z-4 and Z-6 significantly decreased, while Z-10 to Z-14 increased throughout two years. The proportion of Z-16 and 18 in TRT 2 (TT) showed a significant mid-study decrease, which mainly occurred over winter.

In TRT 3 (TSRU), the proportion of Z-4 and Z-6 showed a significant mid-study increase. The NA family Z-10 to Z-14 significantly increased, but showed a significant mid-study decrease for Z-16 and Z-18. In TRT 4 (FFT), NA Z-4 and Z-16 families decreased over time while the Z-12 family significantly increased over time. TRT 4 (FFT) mesocosms also showed a significant mid-study increase in the percentage of Z-10, however there was a statistically significant mid-study decrease in the Z-18 family.

In TRT 5 (FFT + 100% Imperial OSPW), the proportions of Z-6 and Z-8 showed a significant increase over two years, while there was a significant mid-study decrease for Z-16 and Z-18. In TRT 6 (dFFT + 50% Suncor OSPW) mesocosms, Z-6 and Z-12 NA families increased significantly over time, whereas-14 showed a significant decrease. The Z-4 family showed a significant mid-study increase.

In TRT 6 (dFFT + 50% Suncor OSPW), the proportion of Z-16 and Z-18 NAs showed a significant mid-study decrease.

Table 11. Changes in the proportion (%) of NA family over time across two years.

Fraction%	CTL	TRT1	TRT2	TRT3	TRT4	TRT5	TRT6
Z-0	↕	↔	↔	↔	↔	↔	↔
Z-2	ᵛ	↘	↔	↔	↔	↔	↔
Z-4	↕	↘	↘	^	↘	↔	^
Z-6	↕	↔	↘	^	↔	↗	↗
Z-8	↕	↔	↔	↔	↔	↗	↔
Z-10	↕	↗	↗	↗	^	↔	↔
Z-12	↕	↗	↗	↗	↗	↔	↗
Z-14	↕	↗	↗	↗	↕	↔	↘
Z-16	↘	↔	ᵛ	ᵛ	↘	ᵛ	ᵛ
Z-18	↔	↔	ᵛ	ᵛ	ᵛ	ᵛ	ᵛ

CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT. ↗: Increasing; ↘: Decreasing; ^: Mid-Study Increase; ᵛ: Mid-Study Decrease; ↔: No trend; ↕: Fluctuation

3.2.3 Naphthenic Acids – Concentration by Family

Although absolute concentration estimates (µg/L) are presented in this Section, such values are considered less reliable than the percentages (%), as described in Section 2.16.5.2. NAs in the samples were extracted by solid phase extraction and analyzed by liquid-chromatography coupled with high-resolution Orbitrap mass spectrometer. The composition of the NAs is quantitatively analyzed based on the peak area of each compound in the chromatogram, which are directly from chromatogram of each sample (Personal communication, Eric Ruan at InnoTech Alberta). With this caveat in mind and considering the variability of material sources (tailings and OSPW), these results should be reviewed with caution; concentrations of NA families in µg/L may represent a range of calculation based on current analytical method and calibration standards.

Figures and tables describing NA family concentration in mesocosm waters can be found in Section A.5.5 of Appendix A. Correlations and Principal Component Analyses (PCA's) of the NA family data as absolute concentrations can be found in Section A.5.4.6 of Appendix A.

3.2.3.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

There were no significant differences in NA family concentrations when comparing CST (TRT 1) and TSRU (TRT 3) against CTL. One exception was for Z-0 at Week 26, 2020, where CST demonstrated higher concentrations than CTL.

In Week 26 of 2019, TRT 2 (TT) mesocosms consistently demonstrated higher NA concentrations for all families compared to CTL, except for Z-0. In 2020, TRT 2 (TT) mesocosms often showed no significant differences in NA family concentration compared to CTL, with a few exceptions. The exceptions include Z-0 at Week 26 and Z-10 to Z-14 at Weeks 32 and 38, 2020, where TRT 2 (TT) demonstrated higher concentrations than CTL.

The TRT 4 (FFT) mesocosms consistently demonstrated higher concentrations of all NA families compared to CTL at all three sampling time points in 2019 and 2020. The exceptions (no significant differences) included Z-0, Z-16 and Z-18 at all three 2020 sampling times.

3.2.3.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

Mesocosms with FFT and 100% Imperial OSPW (TRT 5) had higher concentrations of most NA families compared to CTL mesocosms. Exceptions where no significant differences were found include the concentration of Z-0 at Week 32 of 2020, and Z-16, and Z-18 families at all three time points in 2020.

3.2.3.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

Trends in NA families for mesocosms with 50% OPSW and dFFT (TRT 6) were similar to those described above to TRT 5, with a few exceptions. There were no significant differences in the concentration of Z-14 family at Week 26 of 2020, or Z-0, Z-16, and Z-18 families at all three time points in 2020.

3.2.3.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

The TRT 4 (FFT) mesocosms consistently demonstrated significantly higher NA family concentrations than TRT 2 (TT). The exceptions where no significant differences were found include Z-16 and Z-18 at Week 32, 2019, Z-0 at Weeks 26 and Week 38 of 2020, Z-2 at Week 26 of 2020, Z-10 at Week 38, 2020, and Z-18 all three-time points in 2020.

3.2.3.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

In both 2019 and 2020, TRT 5 (FFT + 100% OSPW) mesocosms consistently demonstrated significantly higher NA family concentrations than TRT 4 (FFT). Exceptions where there were no significant differences between TRTs 4 and 5 include: Z-0 at Week 26, Z-16 at Week 32 and Week 28, and Z-18 at all three-time points in 2020.

3.2.3.6 Effect of time

When comparing changes over time in NA concentration by family, there were no significant trend for CTL and TRTs 1 (CST), 2 (TT), and 3 (TSRU). However, In TRT 4 (FFT) mesocosms, only Z-0 showed an overall decrease over two years. All NA families decreased significantly over time in both TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW) mesocosms

3.2.4 Metals

For the sake of brevity and comprehensibility, this section focuses on statistically significant results. It should be understood that statistical significance in itself, does not necessarily denote importance. Results for a few highly concerned metals, where the magnitude of the differences between TRT groups (especially TRT 5 and TRT 6) and CTL was relatively large, are discussed in the following sections (3.2.5, 3.2.6, and 3.2.7). These sections mainly focus on comparing TRTs 5 and 6 to CTL. More specific details can be found in Section A.5.8 of Appendix A. In addition, average metal concentrations are summarized in Table 6 of Appendix F with their CCME water quality guidelines (where available).

The results of Correlations and Principal Component Analyses (PCA) completed for the metals data can also be found in Section A.5.4.4 (dissolved) and A.5.4.5 (total) of Appendix A. Excluded data is summarized in Section A.5.2 of Appendix A. Metals removed from the subsequent summaries include beryllium (dissolved), bismuth (dissolved and total), lead (dissolved), mercury (dissolved) and tin (dissolved and total), due to largely non-detected in more than 90% of the samples at each sampling point. Note that metalloids and elements that behave like metals (e.g., selenium) are summarized below in subsequent subsections.

3.2.4.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

2019

The results of statistical comparisons between metal concentrations (in water samples) in tailings and control treatments for 2019 are summarized in Table 12. Elements where no significant differences were detected in any of the sampling points were not included in this summary table. Tailings only treatments (i.e., in the absence of OSPW) were not associated with changes in the concentration of many elements. However, several metal concentrations were significantly higher in TRT groups compared to CTL.

In 2019, dissolved and total nickel concentrations were significantly higher in TRT 1 (CST) compared to CTL at all three time points. TRT 2 (TT) was associated with a significant higher dissolved and total concentrations of boron, lithium, molybdenum, nickel, and sodium for at least two-time points (Weeks 32 and 38 of 2019). Total and dissolved vanadium was more concentrated in TRT 2 than CTL during Weeks 26 and 32.

TRT 3 (TSRU) mesocosms had significantly higher dissolved and total barium, boron, lithium (except Week 26), molybdenum, nickel, silicon (except Week 26), sodium (except Week 26), strontium, and vanadium at all three time points in 2019. TRT 4 (FFT) mesocosms demonstrated significantly higher dissolved and total concentrations of barium, boron, lithium, molybdenum, nickel, potassium, silicon, sodium, strontium and vanadium at all three time points in 2019.

However, in 2019, concentrations of a few elements were significantly lower in tailings only treatments compared to CTL. For example, in TRT 1 (CST) and TRT 2 (TT), only metal uranium (dissolved and total at Week 32 and 38) was lower than CTL. For TRT 3 (TSRU), dissolved and total copper significantly reduced at all three time points, while thallium (Weeks 26 and 32) and uranium (Weeks 32 and 38) concentrations were significantly reduced at two time points in 2019, compared to CLT. In TRT 4 (FFT), only the concentration of copper (total and dissolved) was significantly lower than CTL at all three time points in 2019.

2020

Differences observed in 2020 were similar to 2019 (Table 13), with a few exceptions. In addition to nickel, TRT 1 (CST) had dissolved and total metal concentrations that were significantly higher than CTL at all three time points. These elements included boron, lithium, magnesium, sodium and strontium.

In addition to the metals that were higher in 2019, TRT 2 (TT) had significantly higher potassium at all three time points. Conversely, total and dissolved concentrations of vanadium did not differ significantly in the presence of TT (TRT 2) compared to filter sand (CTL).

In addition to the metals that were higher in 2019, TRT 3 (TSRU) had significantly higher levels of magnesium and potassium, at all three time points in 2019 (except for the dissolved fraction at Week 26). However, unlike 2019, concentrations of silicon and vanadium were not significantly different between TRT 3 and CTL.

In addition to the metals that were higher in 2019, TRT 4 (FFT) had significantly higher dissolved and total magnesium concentrations compared to CTL during Weeks 32 and 38. Silicon concentrations were not significantly different in the presence of FFT (TRT 4) compared to filter sand (CTL).

In 2020, a few of the elements exhibited significantly lower concentrations for tailings-only treatments compared to CTL (filter sand), with a few exceptions. In addition to the metals that were higher in 2019, TRT 3 (TSRU) had significantly lower dissolved and total arsenic compared to CTL at all three time points. Unlike in 2019, copper concentrations did not differ significantly between TRT 4 (FFT) and CTL in 2020. Also contrary to 2019 results, FFT (TRT 4) produced significantly lower uranium concentrations (total and dissolved) than filter sand (CTL) at all three time points.

Table 12. Statistically significant comparisons of metal analytes associated with tailings only in 2019 (F: Fraction; D: Dissolved; T: Total).

Element	F	TRT1 (CST): CTL			TRT2 (TT): CTL			TRT 3 (TSRU): CTL			TRT4 (FFT): CTL		
		2019 W26	2019 W32	2019 W38	2019 W26	2019 W32	2019 W38	2019 W26	2019 W32	2019 W38	2019 W26	2019 W32	2019 W38
Aluminum	T	>	=	=	=	=	=	>	=	=	>	>	>
Arsenic	D	=	=	=	=	=	=	=	=	<	=	=	=
Arsenic	T	=	=	=	=	=	=	=	=	<	=	=	=
Barium	D	=	=	=	=	=	=	>	>	>	>	>	>
Barium	T	=	=	=	=	=	=	>	>	>	>	>	>
Beryllium	T	>	=	=	=	=	=	>	=	=	>	>	>
Boron	D	=	=	=	=	>	>	>	>	>	>	>	>
Boron	T	=	=	=	=	>	>	>	>	>	>	>	>
Calcium	T	=	=	=	=	=	=	=	=	=	<	=	<
Chromium	T	=	=	=	=	=	=	=	=	=	>	>	>

Cobalt	D	>	=	=	>	>	>	=	=	=	>	>	=
Cobalt	T	>	=	=	>	=	=	=	=	=	>	=	=
Copper	D	=	=	=	=	=	=	<	<	<	<	<	<
Copper	T	=	=	=	=	=	=	<	<	<	<	<	<
Iron	D	=	=	=	=	=	=	=	=	=	<	<	=
Iron	T	>	=	=	=	=	=	=	=	=	>	=	=
Lead	T	=	=	=	=	=	=	=	=	=	>	=	=
Lithium	D	=	=	>	=	>	>	=	>	>	>	>	>
Lithium	T	=	=	=	=	>	>	=	>	>	>	>	>
Manganese	T	>	=	=	=	=	=	=	=	=	>	=	=
Molybdenum	D	=	=	=	=	>	>	>	>	>	>	>	>
Molybdenum	T	=	=	=	=	>	>	>	>	>	>	>	>
Nickel	D	>	>	>	=	>	>	>	>	>	>	>	>
Nickel	T	>	>	>	=	>	>	>	>	>	>	>	>
Potassium	D	=	=	=	=	=	=	=	=	=	>	>	>
Potassium	T	=	=	=	=	=	=	=	=	=	>	>	>
Selenium	T	=	=	=	=	=	=	=	=	<	=	=	=
Silicon	D	=	>	=	=	>	=	=	>	>	>	>	>
Silicon	T	=	>	=	=	>	=	=	>	>	>	>	>
Silver	T	=	=	=	=	=	=	=	=	=	>	>	>
Sodium	D	=	=	=	=	>	>	=	>	>	>	>	>
Sodium	T	=	=	=	=	>	>	=	>	>	>	>	>
Strontium	D	=	=	=	=	=	>	>	>	>	>	>	>
Strontium	T	=	=	=	=	=	>	>	>	>	>	>	>
Thallium	D	=	=	=	=	<	=	<	<	=	<	=	=
Thallium	T	>	=	=	=	=	=	<	<	=	=	=	=
Thorium	T	>	=	=	=	=	=	=	=	=	>	>	>
Titanium	D	=	>	=	>	>	=	=	=	=	=	>	=
Titanium	T	>	=	=	=	=	=	=	=	=	>	>	=
Uranium	D	=	<	<	=	<	<	=	<	<	>	=	=
Uranium	T	=	<	<	=	<	<	=	<	<	>	=	=
Vanadium	D	=	=	=	>	>	=	>	>	>	>	>	>
Vanadium	T	=	=	=	>	>	=	>	>	>	>	>	>

Zinc	D	=	=	=	=	=	=	=	=	=	<	=	=

> indicates TRTs contains a statistically significant higher metal concentration than does CTL (TRT 1, 2, 3, or 4 > CTL)

< indicates TRTs contains a statistically significant lower metal concentration than does CTL (TRT 1, 2, 3, or 4 < CTL)

= indicates TRTs is not significantly different from CTL in that metal concentration (TRT 1, 2, 3, or 4 = CTL)

Table 13. Statistically significant comparisons of metal analytes associated with tailings only in 2020 (F: Fraction; D: Dissolved; T: Total).

Element	F	TRT1 (CST): CTL			TRT2 (TT): CTL			TRT3 (TSRU): CTL			TRT4 (FFT): CTL		
		2020 W26	2020 W32	2020 W38	2020 W26	2020 W32	2020 W38	2020 W26	2020 W32	2020 W38	2020 W26	2020 W32	2020 W38
Aluminum	D	=	=	=	=	=	=	=	=	<	=	=	=
Aluminum	T	=	=	=	=	=	=	=	=	=	=	>	>
Antimony	D	=	=	=	=	=	=	=	<	<	=	=	=
Antimony	T	=	=	=	=	=	=	=	=	<	=	=	=
Arsenic	D	=	=	=	=	=	=	<	<	<	=	=	<
Arsenic	T	=	=	=	=	=	=	<	<	<	=	=	=
Barium	D	=	=	=	=	=	=	>	>	=	>	>	>
Barium	T	=	=	=	=	=	=	>	=	=	>	>	>
Boron	D	>	>	>	>	>	>	>	>	>	>	>	>
Boron	T	>	>	>	>	>	>	>	>	>	>	>	>
Cadmium	T	=	=	<	=	=	<	=	=	<	=	=	=
Calcium	D	=	=	=	=	=	=	=	=	=	=	>	>
Calcium	T	=	=	=	=	=	=	=	=	=	=	>	>
Chromium	D	=	=	=	=	=	=	=	=	>	=	=	=
Cobalt	D	=	>	>	>	>	>	=	=	=	=	=	=
Copper	D	=	=	=	=	=	=	<	<	=	=	=	=
Copper	T	=	=	=	=	=	=	<	<	=	=	=	=
Lead	T	=	=	=	=	>	=	=	=	=	=	=	>
Lithium	D	>	>	>	>	>	>	>	>	>	>	>	>
Lithium	T	>	>	>	>	>	>	>	>	>	>	>	>
Magnesium	D	=	>	>	=	=	=	=	>	>	=	>	>
Magnesium	T	=	>	>	=	=	=	>	>	>	=	>	>
Manganese	D	=	=	=	=	=	=	=	=	=	=	=	<
Molybdenum	D	=	=	=	>	>	>	>	>	>	>	>	>
Molybdenum	T	=	=	=	>	>	>	>	>	>	>	>	>
Nickel	D	>	>	>	>	>	>	>	>	=	>	>	>
Nickel	T	>	>	>	>	>	>	>	=	=	>	>	>
Potassium	D	=	=	>	>	>	>	=	>	>	>	>	>
Potassium	T	=	=	>	>	>	>	>	>	>	>	>	>

Selenium	D	=	=	=	=	=	=	=	=	<	=	=	=
Silver	D	=	=	=	=	=	=	=	=	<	=	=	=
Sodium	D	>	>	>	>	>	>	>	>	>	>	>	>
Sodium	T	>	>	>	>	>	>	>	>	>	>	>	>
Strontium	D	>	>	>	>	>	>	>	>	>	>	>	>
Strontium	T	>	>	>	>	>	>	>	>	>	>	>	>
Thallium	D	=	=	=	<	=	=	<	=	=	<	=	=
Thallium	T	=	=	=	<	=	=	<	=	=	<	=	=
Titanium	T	=	=	=	=	=	=	=	=	=	=	>	>
Uranium	D	<	<	<	<	<	<	<	<	<	<	<	<
Uranium	T	<	<	<	<	<	<	<	<	<	<	<	<
Vanadium	D	=	=	=	=	=	=	=	=	=	>	>	>
Vanadium	T	=	=	=	=	=	=	=	=	=	>	>	>
Zinc	T	=	=	=	=	>	=	=	>	=	=	=	=

> indicates TRTs contains a statistically significant higher metal concentration than does CTL (TRT 1, 2, 3, or 4 > CTL)

< indicates TRTs contains a statistically significant lower metal concentration than does CTL (TRT 1, 2, 3, or 4 < CTL)

= indicates TRTs is not significantly different from CTL in that metal concentration (TRT 1, 2, 3, or 4 = CTL)

3.2.4.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

For most elements, the presence of 100% OSPW with FFT (TRT 5) was associated with an increase in concentration, both for the dissolved and total fractions (Table 14). There were, however, some exceptions to this pattern. In 2019, metal concentrations where there was no significant difference between TRT 5 and CTL at any of the three time points included: manganese (dissolved), sliver (dissolved), thallium (total), and thorium (dissolved). In 2020, element concentrations that did not differ significantly between TRT 5 and CTL included all those identified for 2019, as well as:

- aluminum (dissolved and total)
- arsenic (dissolved)
- beryllium (total)
- chromium (dissolved)
- iron (dissolved)
- lead (total)
- manganese (total)

- mercury (total)
- silver (total)
- thorium (total)

In 2019, metals significantly less concentrated in TRT 5 (100% Imperial OSPW with FFT) than CTL were:

- aluminum (dissolved, Week 26),
- calcium (dissolved and total, except for dissolved at Week 26),
- copper (dissolved and total, except for Week 38),
- iron (dissolved, except for Week 38),
- thallium (dissolved, except for Week 38).

In 2020, only the concentrations of calcium (Week 26), copper (Week 26 and 32), and thallium (Week 26) were lower in TRT 5 than CTL.

Table 14. Statistically significant comparisons of metal analytes associated with tailings and OSPW in 2019 and 2020 (F: Fraction; D: Dissolved; T: Total).

Element	Fraction	TRT5 (100% Imperial OSPW+FFT): CTL						TRT6 (50% Suncor OSPW+dFFT): CTL					
		2019 W26	2019 W32	2019 W38	2020 W26	2020 W32	2020 W38	2019 W26	2019 W32	2019 W38	2020 W26	2020 W32	2020 W38
Aluminum	D	<	=	=	=	=	=	=	=	>	>	=	=
Aluminum	T	>	>	>	=	=	=	=	=	=	=	=	=
Antimony	D	>	>	>	>	>	>	>	>	>	>	>	>
Antimony	T	>	>	>	>	>	>	>	>	>	>	>	>
Arsenic	D	>	>	>	=	=	=	>	=	>	>	>	>
Arsenic	T	>	>	>	=	>	=	>	=	>	>	>	>
Barium	D	>	>	>	>	>	>	>	>	>	>	>	>
Barium	T	>	>	>	>	>	>	>	>	>	>	>	>
Beryllium	T	>	>	>	=	=	=	>	=	=	=	=	=
Boron	D	>	>	>	>	>	>	>	>	>	>	>	>
Boron	T	>	>	>	>	>	>	>	>	>	>	>	>
Cadmium	D	>	>	>	>	>	>	>	>	>	>	>	>
Cadmium	T	>	>	>	>	>	=	>	>	>	>	>	>
Calcium	D	=	<	<	<	>	>	<	<	<	<	=	=
Calcium	T	<	<	<	<	>	>	<	<	<	<	=	=
Chromium	D	=	>	=	=	=	=	=	>	>	=	>	=
Chromium	T	>	>	>	=	>	=	=	>	>	=	=	=
Cobalt	D	>	>	>	>	>	>	>	>	>	=	>	>
Cobalt	T	>	>	>	>	>	>	>	>	>	=	>	>
Copper	D	<	<	=	<	<	=	<	=	=	<	=	=
Copper	T	<	<	=	<	<	=	<	=	=	<	=	=
Iron	D	<	<	=	=	=	=	=	<	=	=	>	>

Iron	T	>	=	>	=	>	>	>	=	=	=	=	=
Lead	T	>	=	>	=	=	=	>	=	=	=	=	=
Lithium	D	>	>	>	>	>	>	>	>	>	>	>	>
Lithium	T	>	>	>	>	>	>	>	>	>	>	>	>
Magnesium	D	>	>	>	>	>	>	=	=	=	<	=	=
Magnesium	T	>	>	>	>	>	>	=	=	=	<	=	=
Manganese	D	=	=	=	=	=	=	>	=	=	=	=	=
Manganese	T	>	=	=	=	=	=	>	=	>	=	>	>
Mercury	T	>	>	>	=	=	=	>	>	>	=	=	=
Molybdenum	D	>	>	>	>	>	>	>	>	>	>	>	>
Molybdenum	T	>	>	>	>	>	>	>	>	>	>	>	>
Nickel	D	>	>	>	>	>	>	>	>	>	>	>	>
Nickel	T	>	>	>	>	>	>	>	>	>	>	>	>
Potassium	D	>	>	>	>	>	>	>	>	>	>	>	>
Potassium	T	>	>	>	>	>	>	>	>	>	>	>	>
Selenium	D	>	>	>	>	>	>	>	>	>	>	>	>
Selenium	T	>	>	>	>	>	>	>	>	>	>	>	>
Silicon	D	>	>	>	>	>	>	>	>	>	=	=	=
Silicon	T	>	>	>	>	>	>	>	>	>	=	=	=
Silver	D	=	=	=	=	=	=	>	>	>	=	=	>
Silver	T	>	>	>	=	=	=	>	>	>	=	=	>
Sodium	D	>	>	>	>	>	>	>	>	>	>	>	>
Sodium	T	>	>	>	>	>	>	>	>	>	>	>	>
Strontium	D	>	>	>	>	>	>	>	>	>	>	>	>
Strontium	T	>	>	>	>	>	>	>	>	>	>	>	>
Thallium	D	<	<	=	<	=	=	<	<	<	<	=	=
Thallium	T	=	=	=	<	=	=	<	<	<	<	=	=
Thorium	D	=	=	=	=	>	>	=	=	=	=	=	=
Thorium	T	>	>	>	=	=	=	>	=	=	=	=	=
Titanium	D	>	>	=	=	>	>	>	>	=	=	>	>
Titanium	T	>	>	>	>	>	>	>	=	=	=	>	>
Uranium	D	>	>	>	>	>	>	>	>	>	>	>	>
Uranium	T	>	>	>	>	>	>	>	>	>	>	>	>
Vanadium	D	>	>	>	>	>	>	>	>	>	>	>	>
Vanadium	T	>	>	>	>	>	>	>	>	>	>	>	>
Zinc	D	<	>	=	=	>	>	<	=	=	=	>	=
Zinc	T	=	>	>	=	>	>	=	=	=	=	>	>

> indicates TRTs contains a statistically significant higher metal concentration than does CTL (TRT 5 or 6 > CTL)

< indicates TRTs contains a statistically significant lower metal concentration than does CTL (TRT 5 or 6 < CTL)

= indicates TRTs is not significantly different from CTL in metal concentration (TRT 5 or 6 = CTL)

3.2.4.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

50% Suncor OSPW with dFFT (TRT 6) was associated with a significant elevation in most metal concentrations compared to CTL (Table 12). Metal concentrations that were not significantly different at any point in either year were: aluminum (total) and thorium (dissolved). Metals associated with a significantly reduced concentration at least one-time point were:

- calcium (total and dissolved) at all three time points in 2019 and Week 26 of 2020,
- copper (total and dissolved) during Week 26 of 2019 and 2020
- magnesium (total and dissolved) during Week 26 of 2020
- thallium (total and dissolved) at all three time points in 2019 and Week 26 of 2020,
- and zinc (dissolved) during Week 26 of 2019.

3.2.4.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

TRT 4 (FFT) was associated with a significant elevation in most metal concentrations compared to TRT 2 (TT) (Table 15). Metal concentrations that were not significantly different between TRTs 2 and 4 at any time point were:

- antimony (dissolved and total)
- cadmium (total)
- mercury (total)
- selenium (dissolved and total)
- silver (dissolved)
- thallium (dissolved and total)
- titanium (dissolved)
- zinc (total)

Metals that were significantly reduced in TRT 4 (FFT) compared to TRT 2 (TT) in at least one-time point were:

- aluminum (dissolved) during Weeks 32 and 38 of 2019
- arsenic (dissolved and total) at Week 38 of 2020
- chromium (dissolved) at Week 38 of 2020
- cobalt (dissolved)during Week 38 if 2019 and all three time points of 2020
- copper (dissolved and total) at all three time points of 2019

- iron (dissolved)during Weeks 26 and 32 of 2019 and Week 26 of 2020
- lead (total)during Week 32 of 2020
- manganese (dissolved)during Week 38 of 2020
- zinc (dissolved)during Weeks 26 and 38 of 2020

Table 15. Statistically significant comparisons of metal analytes associated with treatment of tailings and 100% Imperial OSPW in the presence of FFT (F: Fraction; D: Dissolved; T: Total).

Element	Fraction	TRT2 (TT): TRT4 (FFT)						TRT4 (FFT): TRT5 (FFT+100% Imperial OSPW)					
		2019 W26	2019 W32	2019 W38	2020 W26	2020 W32	2020 W38	2019 W26	2019 W32	2019 W38	2020 W26	2020 W32	2020 W38
Aluminum	D	=	>	>	=	=	=	=	=	=	=	=	>
	T	<	<	<	=	<	<	<	=	<	=	=	=
Antimony	D	=	=	=	=	=	=	<	<	<	<	<	<
	T	=	=	=	=	=	=	<	<	<	<	<	<
Arsenic	D	=	=	=	=	=	>	<	<	<	<	<	<
	T	=	=	=	=	=	>	<	<	<	<	<	<
Barium	D	<	<	<	<	<	<	<	<	<	=	<	<
	T	<	<	<	<	<	<	<	<	<	=	<	<
Beryllium	D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T	<	<	<	=	=	=	=	=	<	=	=	=
Boron	D	<	<	<	<	<	<	<	<	<	<	<	<
	T	<	<	<	<	<	<	<	<	<	<	<	<
Cadmium	D	=	<	<	=	=	=	<	<	<	<	<	<
	T	=	=	=	=	=	=	<	<	<	<	<	<
Calcium	D	=	=	=	=	<	<	=	=	=	>	=	=
	T	=	=	=	=	<	<	=	=	=	>	=	=
Chromium	D	=	=	=	=	=	>	=	<	=	=	=	=
	T	<	<	<	=	=	=	<	<	<	=	=	=
Cobalt	D	=	=	>	>	>	>	<	<	<	<	<	<
	T	<	=	=	=	=	=	<	<	<	=	<	<
Copper	D	>	>	>	=	=	=	<	=	<	>	=	=
	T	>	>	>	=	=	=	<	=	<	>	=	=
Iron	D	>	>	=	>	=	=	=	>	=	=	=	=
	T	<	=	=	=	=	=	<	=	<	=	<	=
Lead	D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T	<	=	=	=	>	<	=	=	=	=	=	=
Lithium	D	<	<	<	<	<	<	<	<	<	<	<	<
	T	<	<	<	<	<	<	<	<	<	<	<	<
Magnesium	D	<	<	=	<	<	<	<	<	<	=	<	<

	T	=	<	=	<	<	<	<	<	<	=	<	<
Manganese	D	=	=	=	=	=	>	=	=	=	=	=	=
	T	<	=	=	=	=	=	<	=	=	=	=	=
Mercury	D	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	T	=	=	=	=	=	=	<	<	<	=	<	=
Molybdenum	D	<	<	<	<	<	<	<	<	<	<	<	<
	T	<	<	<	<	<	<	<	<	<	<	<	<
Nickel	D	<	=	=	=	<	<	<	<	<	<	<	<
	T	<	=	=	=	<	<	<	<	<	<	<	<
Potassium	D	<	<	<	<	<	<	<	<	<	<	<	<
	T	<	<	<	<	<	<	<	<	<	<	<	<
Selenium	D	=	=	=	=	=	=	<	<	<	<	<	<
	T	=	=	=	=	=	=	<	<	<	<	<	<
Silicon	D	<	<	<	=	=	=	<	<	<	<	<	<
	T	<	<	<	=	=	=	<	<	<	<	<	<
Silver	D	=	=	=	=	=	=	=	=	=	=	=	=
	T	<	=	<	=	=	=	=	=	<	=	=	=
Sodium	D	<	<	<	<	<	<	<	<	<	<	<	<
	T	<	<	<	<	<	<	<	<	<	<	<	<
Strontium	D	<	<	<	<	<	<	<	<	<	<	<	<
	T	<	<	<	<	<	<	<	<	<	<	<	<
Thorium	D	=	=	=	=	<	=	=	=	=	=	=	=
	T	<	<	<	=	=	=	=	=	<	=	=	=
Titanium	D	=	=	=	=	=	=	<	<	=	<	<	=
	T	<	<	=	=	<	<	=	=	=	=	=	=
Uranium	D	<	<	<	<	<	<	<	<	<	<	<	<
	T	<	<	<	<	<	<	<	<	<	<	<	<
Vanadium	D	<	<	<	<	<	<	<	<	<	<	<	<
	T	<	<	<	<	<	<	<	<	<	<	<	<
Zinc	D	>	=	>	=	=	=	<	<	<	=	<	<
	T	=	=	=	=	=	=	<	<	<	=	<	<

> indicates TRT 2 or 4 contains a statistically significant higher metal concentration than does TRT 4 or 5 (TRT 2 > TRT 4 or TRT 4 > TRT 5)

< indicates TRT 2 or 4 contains a statistically significant lower metal concentration than does TRT 4 or 5 (TRT 2 < TRT 4 or TRT 4 < TRT 5)

= indicates TRT 2 or 4 is not significantly different from TRT 4 or 5 in that metal concentration (TRT 2 = TRT 4 or TRT 4 = TRT 5)

3.2.4.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

100% Imperial OSPW in the presence of FFT (TRT 5) was associated with a significant elevation in most metal concentrations compared to TRT 4 (FFT) (Table 12). Metal concentrations that were not significantly different between TRT 4 and TRT 5 in any time points were:

- lead (total)
- manganese (dissolved)
- silver (dissolved)
- thallium (dissolved and total)
- thorium (dissolved)
- titanium (dissolved).

Metals associated with a significant reduction in TRT 5 (FFT + 100% Imperial OSPW) compared to TRT 4 (FFT) at least one-time point were:

- aluminum (dissolved) at Week 38 of 2020
- calcium (dissolved and total) at Week 26 of 2020
- copper (dissolved and total) at Week 26 of 2020
- iron (dissolved)at Week 32 of 2019

3.2.4.6 Effect of time

The effects of time are summarized in Table 16. Due to the various metal concentration trends, results are summarized broadly across two years. The trends are generally characterized as three broad trends (increase or mid-study increase, decrease or mid-study decrease, no change or fluctuation). Some deviation from the general summary should be expected in some perception, e.g., decrease, mid-year decrease, and fluctuation might occur simultaneously. More details are available in Appendix A.

Table 16. Changes in the concentration of metals associated with time.

Element	Fraction	CTL	TRT 1	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6
Aluminum	D	↔	↔	↔	↘	↗	↗	^
Aluminum	T	↔	↔	↔	↔	v	↕	↘
Antimony	D	↔	↔	↔	↘	↔	↘	↘
Antimony	T	↔	↔	↔	↘	↔	↘	↘
Arsenic	D	↔	↔	↔	↔	↔	↘	↕
Arsenic	T	↔	↔	↔	↔	↔	↘	↕
Barium	D	↘	↘	↘	↘	↘	v	↘
Barium	T	↘	↘	↘	↘	↘	v	↘
Beryllium	T	↔	↘	↔	↘	↘	↕	↘

Boron	D	↔	↔	↔	↔	↗	˅	↕
Boron	T	↔	↔	↔	↔	↗	˅	↕
Cadmium	D	↔	↔	↔	↔	↕	˄	˄
Cadmium	T	↗	↔	↔	↔	↔	˄	˄
Calcium	D	˄	˄	˄	˄	↘	˅	˅
Calcium	T	˄	˄	˄	˄	˄	˅	˅
Chromium	D	↔	↔	↔	↗	↔	↕	˄
Chromium	T	↔	↔	↔	↔	↘	↕	↘
Cobalt	D	↕	↔	↕	↔	↕	↘	↕
Cobalt	T	↔	↕	↔	↔	˅	↘	˅
Copper	D	↘	↘	↘	↘	↔	↕	↕
Copper	T	↘	↘	↘	↘	↘	↕	↕
Iron	D	↕	↕	↕	↕	↕	↕	↕
Iron	T	↕	˅	↕	↕	˅	˅	↕
Lead	T	↔	↘	↕	↔	˅	↘	↕
Lithium	D	↔	↔	↗	↔	↗	˅	˅
Lithium	T	↔	↔	↗	↔	↗	˅	↕
Magnesium	D	˄	↗	˄	↗	↗	↕	↕
Magnesium	T	˄	↗	˄	˄	↗	↕	↕
Manganese	D	↔	↔	↔	↔	↔	↔	↕
Manganese	T	↔	˅	↔	↔	↘	˅	˅
Mercury	T	↔	↔	↔	↔	↔	↕	↕
Molybdenum	D	↔	↔	↔	↔	↔	˅	˅
Molybdenum	T	↔	↔	↔	↔	↔	˅	˅
Nickel	D	˄	˄	˄	˄	˄	↕	˅
Nickel	T	˄	˄	˄	˄	↗	↕	˅
Potassium	D	↘	↕	↕	↘	↔	˅	˅
Potassium	T	↘	↘	↕	˄	↔	˅	˅
Selenium	D	↗	↔	↔	↔	↔	↗	↕
Selenium	T	↔	↔	↔	↔	↔	↗	↕
Silicon	D	˅	˅	˅	˅	↕	↘	↕
Silicon	T	˅	˅	˅	˅	↕	↘	↕
Silver	D	↔	↔	↔	↔	↔	↔	˅

Silver	T	˅	˅	↔	↔	↕	↕	˅
Sodium	D	↔	↔	↗	↗	↗	˅	˅
Sodium	T	↔	↔	↗	↔	↗	˅	˅
Strontium	D	↘	↘	↘	˄	↔	˅	˅
Strontium	T	↘	↘	˄	˄	↔	˅	˅
Thallium	D	˄	↘	↘	↔	↔	↔	↔
Thallium	T	↘	↘	↘	↔	↘	↘	↔
Thorium	D	↔	↔	↔	↔	↔	↗	↔
Thorium	T	↔	↔	↔	↔	↘	˅	˅
Titanium	D	˅	↘	˅	˅	˅	˅	˅
Titanium	T	↔	↘	˅	˅	˅	˅	˅
Uranium	D	↗	↔	↔	↘	↔	↘	˅
Uranium	T	↗	↔	↔	↘	↔	↘	↘
Vanadium	D	↔	↔	↕	˅	↘	↘	˅
Vanadium	T	↔	↔	↕	˅	↘	↘	˅
Zinc	D	↘	↘	↘	˄	˄	↕	↗
Zinc	T	↘	↘	↘	˄	˄	↔	˄

CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT. ↗: Increasing; ↘: Decreasing; ^: Mid-Study Increase; ˅: Mid-Study Decrease; ↔: No trend; ↕: Fluctuation; D: Dissolved; T: Total

Elements that exhibited a significant increase or mid-study increase in CTL include:

- cadmium (total)
- calcium (dissolved and total)
- magnesium (dissolved and total)
- nickel (dissolved and total)
- selenium (dissolved)
- thallium (dissolved)
- and uranium (dissolved and total).

The elements that exhibited a significant decrease or mid-study decrease in CTL were:

- barium (dissolved and total)
- copper (dissolved and total)

- potassium (dissolved and total)
- silicon (dissolved and total)
- silver (total)
- strontium (dissolved and total)
- thallium (total)
- titanium (dissolved)
- and zinc (dissolved and total).

All the other elements showed no consistent change or fluctuation over time in CTL.

Three elements showed a significant increase or mid-study increase in TRT 1 (CST) throughout the study: calcium (dissolved and total), magnesium (dissolved and total), and nickel (dissolved and total). Elements with a significant decrease or mid-study decrease profile in TRT 1 (CST) were:

- barium (dissolved and total)
- beryllium (dissolved)
- copper (dissolved and total)
- lead (total)
- manganese (total)
- potassium (total)
- silicon (dissolved and total)
- silver (total)
- strontium (dissolved and total)
- thallium (dissolved and total)
- titanium (dissolved and total)
- and zinc (dissolved and total).

All the other elements showed no consistent change over time in TRT 1 (CST).

Elements that exhibited a significant increase or mid-study increase in TRT 2 (TT) were:

- calcium (dissolved and total)
- lithium (dissolved and total)
- magnesium (dissolved and total)
- nickel (dissolved and total)
- sodium (dissolved and total)
- and strontium (total).

Elements with a significant decrease or mid-study decrease profile in TRT 2 (TT) were:

- barium (dissolved and total),
- copper (dissolved and total),
- silicon (dissolved and total),
- strontium (dissolved),
- thallium (dissolved and total),
- titanium (dissolved and total),
- and zinc (dissolved and total).

All the other elements showed no consistent change or fluctuation over time in TRT 2 (TT).

Elements that exhibited a significant increase or mid-study increase in TRT 3 (TSRU) include:

- calcium (dissolved and total)
- chromium (dissolved)
- magnesium (dissolved and total)
- nickel (dissolved and total)
- potassium (total)
- sodium (dissolved)
- strontium (dissolved and total)
- and zinc (dissolved and total).

Elements that exhibited a significant decrease or mid-study decrease in TRT 3 (TSRU) were:

- aluminum (dissolved)
- antimony (dissolved and total)
- barium (dissolved and total)
- beryllium (total)
- copper (dissolved and total)
- potassium (dissolved)
- silicon (dissolved and total)
- titanium (dissolved and total)
- uranium (dissolved and total)
- and vanadium (dissolved and total).

All the other elements showed no consistent change or fluctuation over time in TRT 3 (TSRU).

Elements that exhibited a significant increase or mid-study increase in TRT 4 (FFT) include:

- aluminum (dissolved)
- boron (dissolved and total)

- calcium (total)
- lithium (dissolved and total)
- magnesium (dissolved and total)
- nickel (dissolved and total)
- sodium (dissolved and total)
- and zinc (dissolved and total).

Elements that exhibited a significant decrease or mid-study decrease in TRT 4 (FFT) were:

- aluminum (total),
- barium (dissolved and total),
- beryllium (total),
- calcium (dissolved),
- chromium (total),
- cobalt (total),
- copper (total),
- iron (total),
- lead (total),
- manganese (total),
- thallium (total),
- thorium (total),
- titanium (dissolved and total),
- and vanadium (dissolved and total).

All the other elements showed no consistent change or fluctuation over time in TRT 4 (FFT).

Elements that exhibited a significant increase or mid-study increase in TRT 5 (100%OSPW+FFT) include:

- aluminum (dissolved),
- cadmium (dissolved and total),
- selenium (dissolved and total),
- thorium (dissolved).

The elements that exhibited a no change or fluctuation in TRT 5 (100%OSPW+FFT) were:

- aluminum (total),
- beryllium (total),
- chromium (dissolved and total),
- copper (dissolved and total),

- iron (dissolved),
- magnesium (dissolved and total),
- mercury (total),
- nickel (dissolved and total),
- silver (dissolved and total),
- thallium (dissolved),
- zinc (dissolved and total).

All the other elements showed a significant decrease or mid-year decrease over time in TRT 5 (100% OSPW+FFT).

As with TRT 5, TRT 6 (50% OSPW with dFFT) was associated with a significant decrease or mid-study decrease in most metal concentrations (Table 14). Metal concentrations that did not changed or fluctuate were:

- arsenic (dissolved and total),
- boron (dissolved and total),
- cobalt (dissolved),
- copper (dissolved and total),
- iron (dissolved and total),
- lead (total),
- lithium (total),
- magnesium (dissolved and total),
- manganese (dissolved),
- mercury (total),
- selenium (dissolved and total),
- silicon (dissolved and total),
- thallium (dissolved and total),
- and thorium (dissolved).

3.2.5 Potassium and Sodium

Potassium and sodium concentrations are presented as total concentrations in this section (see Section 3.2.4 for details). Sodium concentration was significantly higher by one order of magnitude in the presence of both OSPW and tailings (TRT 5 and TRT 6, Figure 20), compared to AWR and filter sand (CTL). However, potassium concentrations were significantly higher, but of a lesser magnitude (Figure 21).

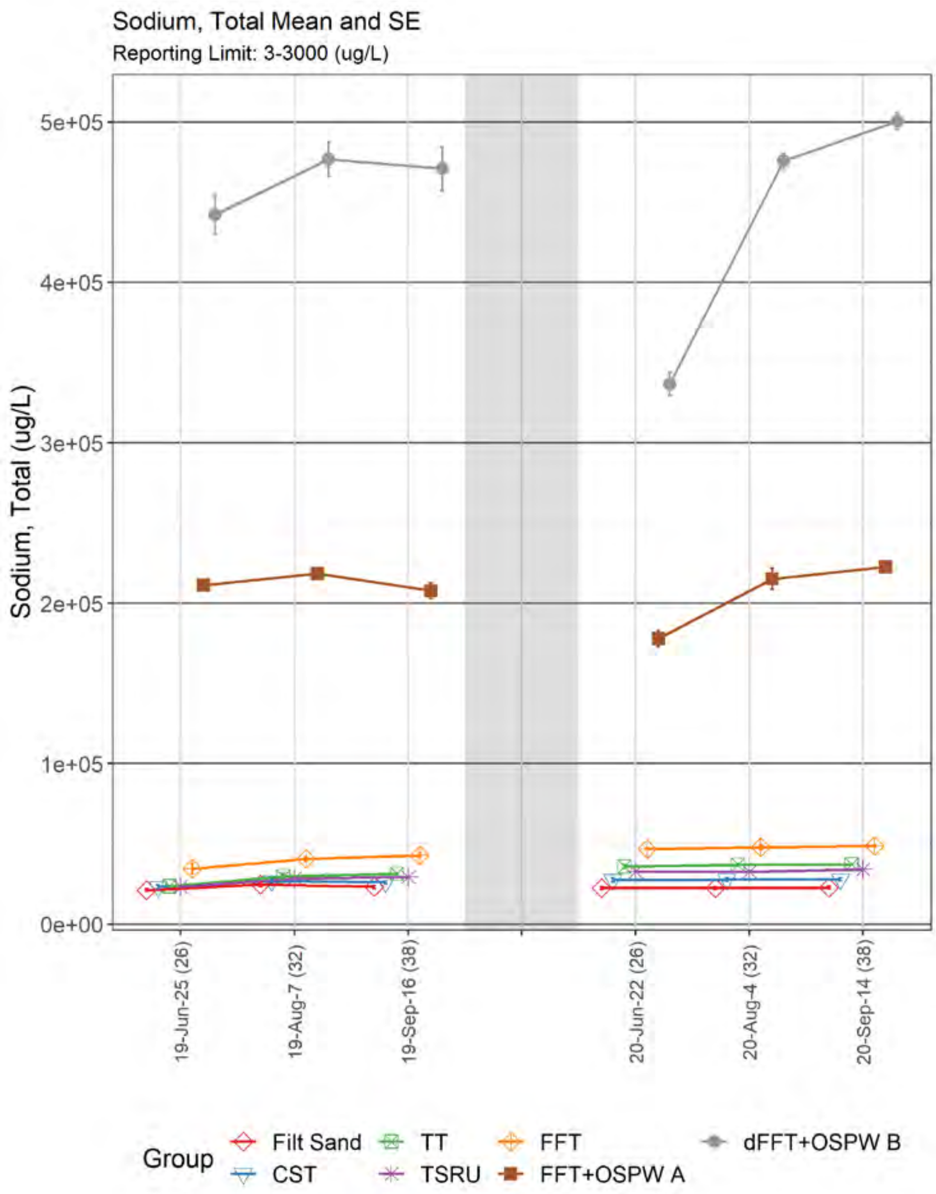

Figure 20. Total sodium concentration across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

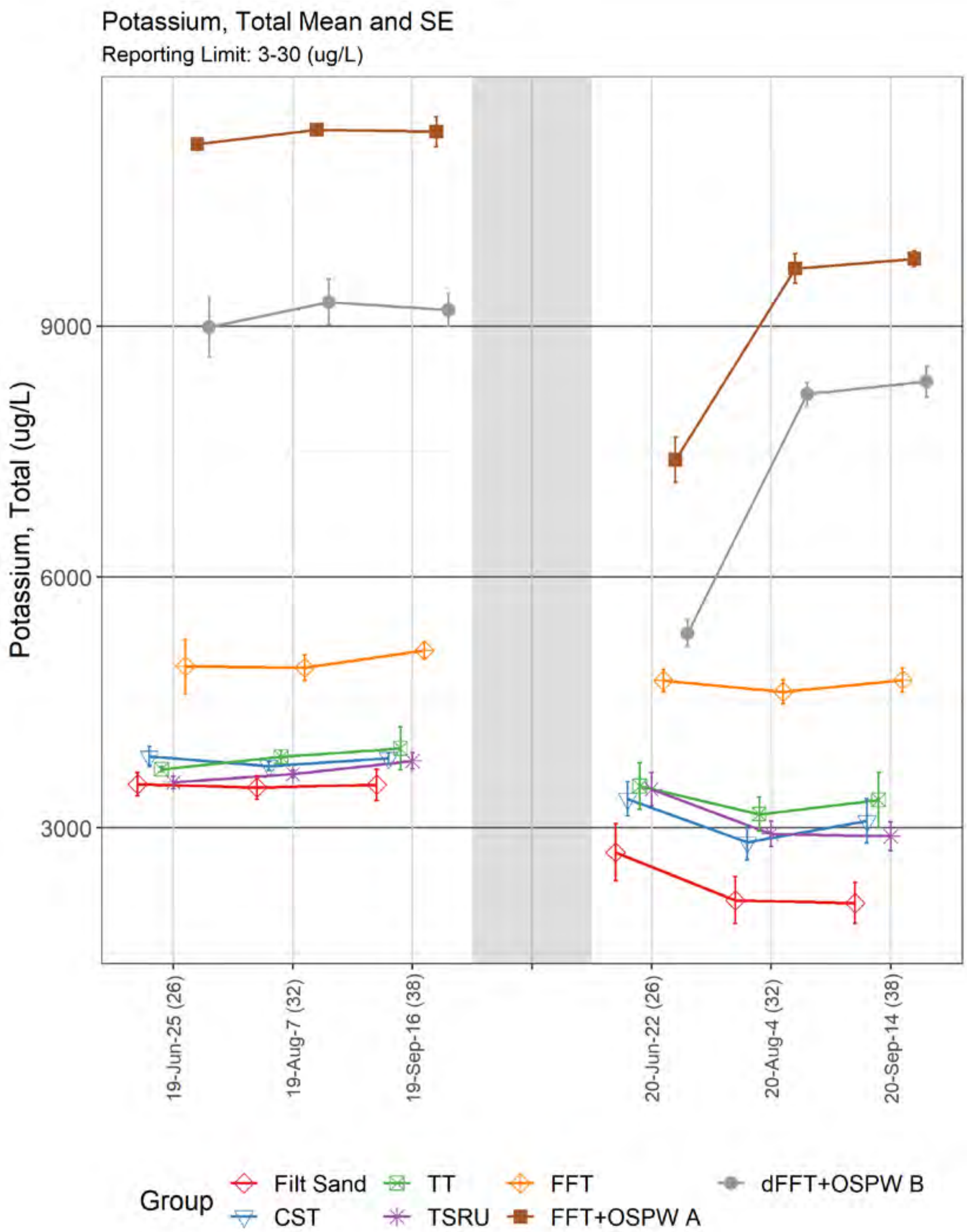

Figure 21. Total potassium concentration across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.2.6 Boron, calcium, and molybdenum

Detailed changes over time and differences between groups for both the dissolved and total boron, calcium, and molybdenum concentrations can be found in Section 3.2.4. Briefly, boron and molybdenum concentration in the OSPW-containing groups were more than one order of magnitude greater than in the CTL (Figure 22 and Figure 23). Calcium is noteworthy in that its concentration in TRTs 5 and 6 was significantly less than CTL in 2019 (Figure 24).

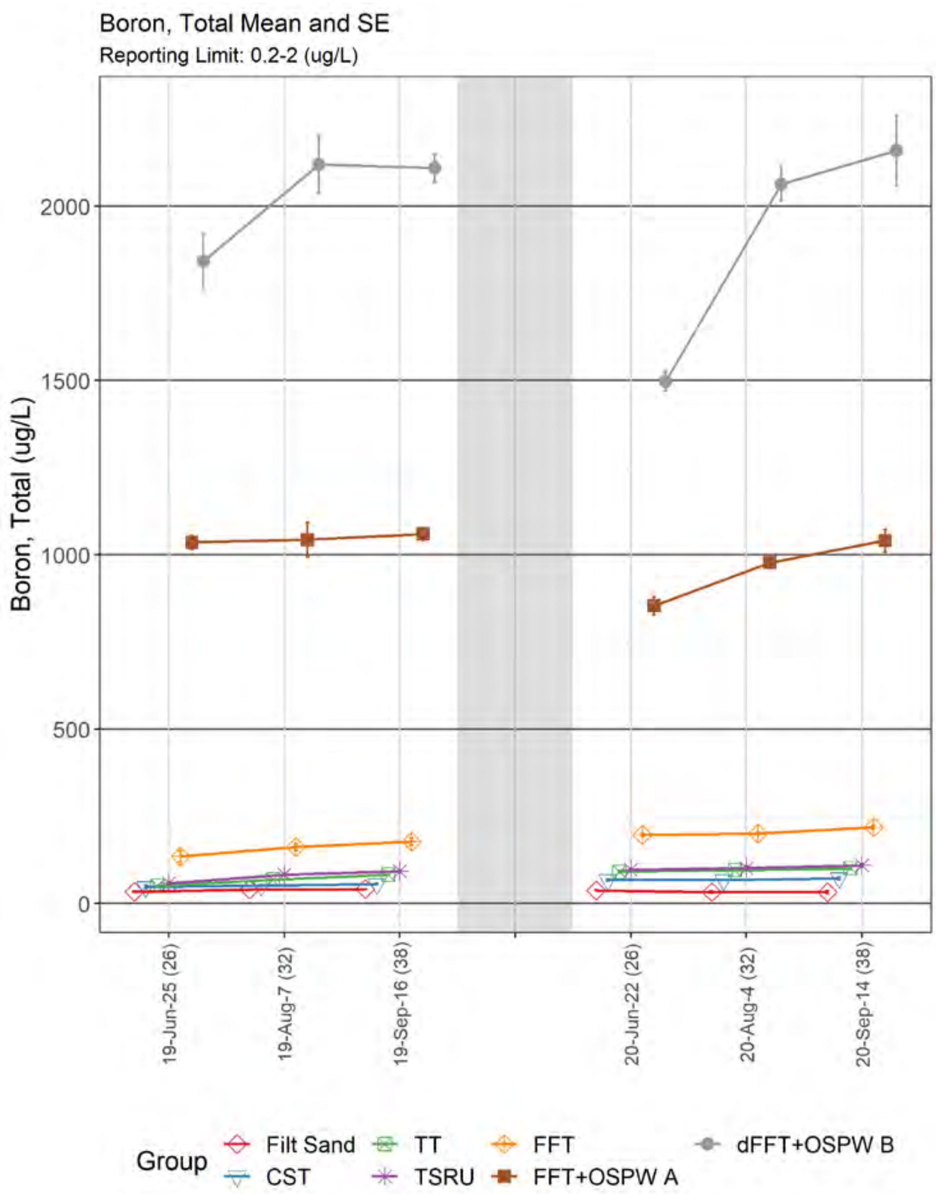

Figure 22. Total boron concentration across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

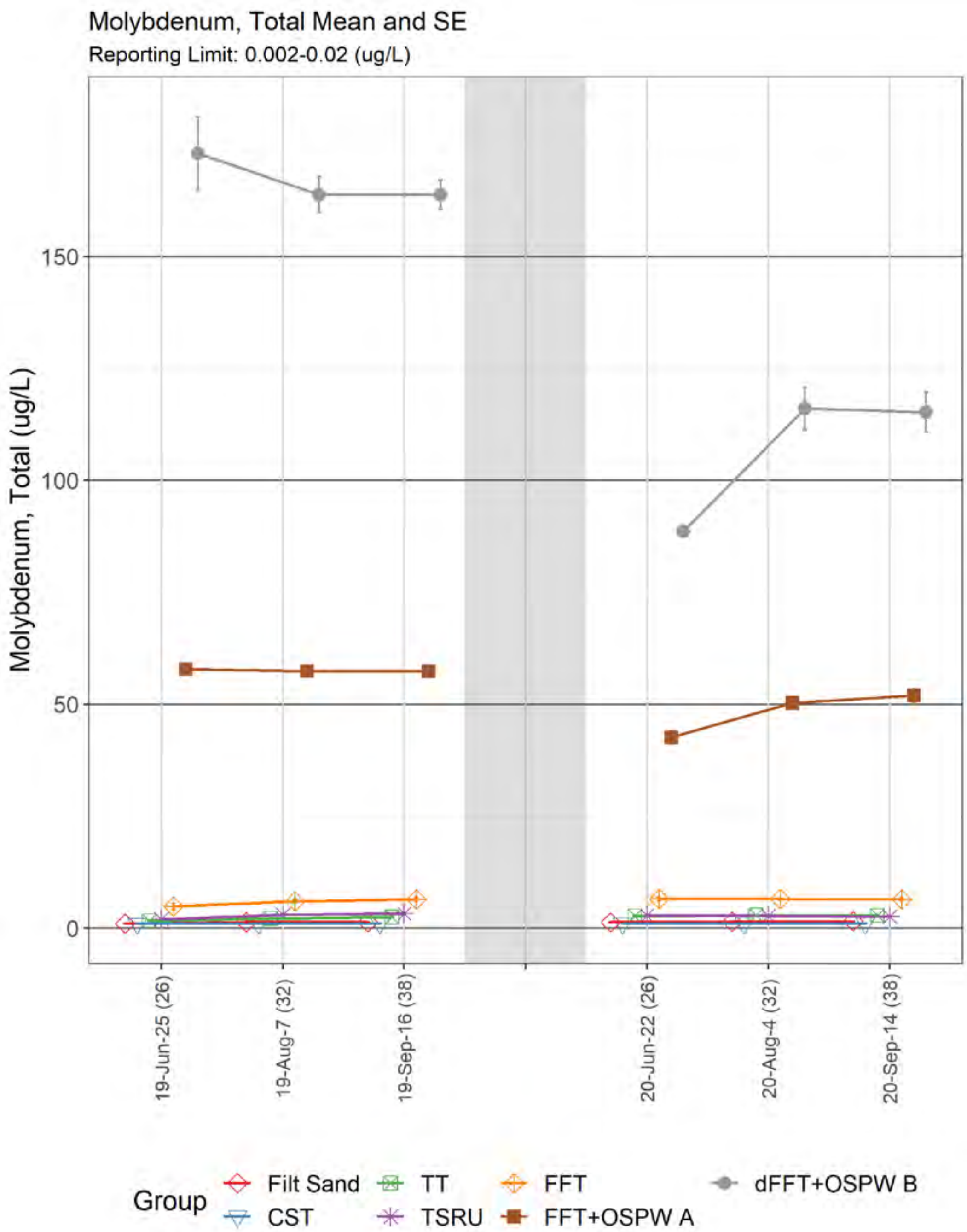

Figure 23. Total molybdenum concentration across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

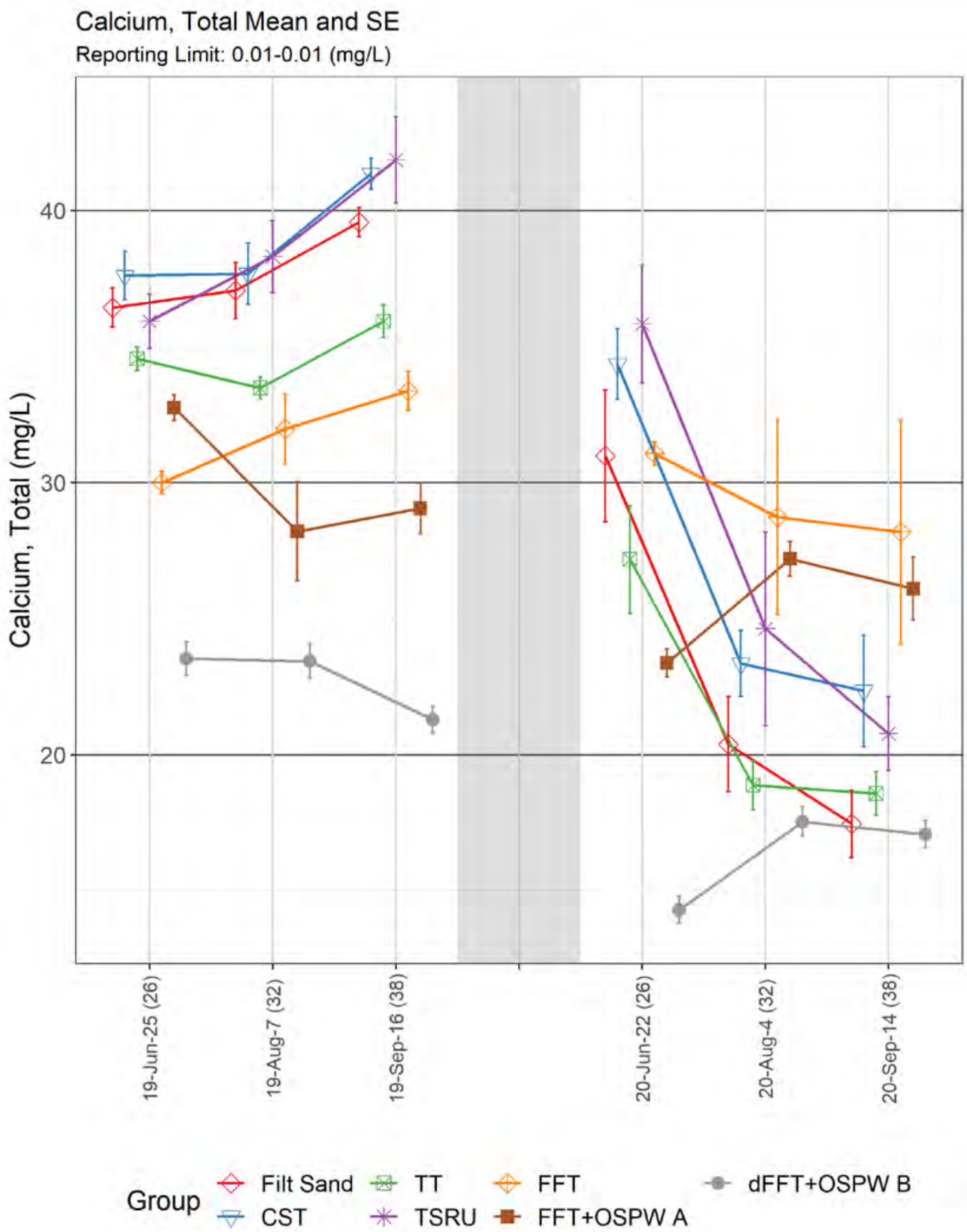

Figure 24. Total calcium concentration across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.2.7 Lithium, nickel, and uranium

Lithium concentrations in TRTs 5 and 6 were roughly an order of magnitude greater than those in the CTL (Figure 25). While the concentration of nickel and uranium in TRTs 5 and 6 were still significantly higher than CTL, the discrepancy was less than what was observed for lithium. More results pertaining to lithium, nickel, and uranium are summarized in Section 3.2.4.

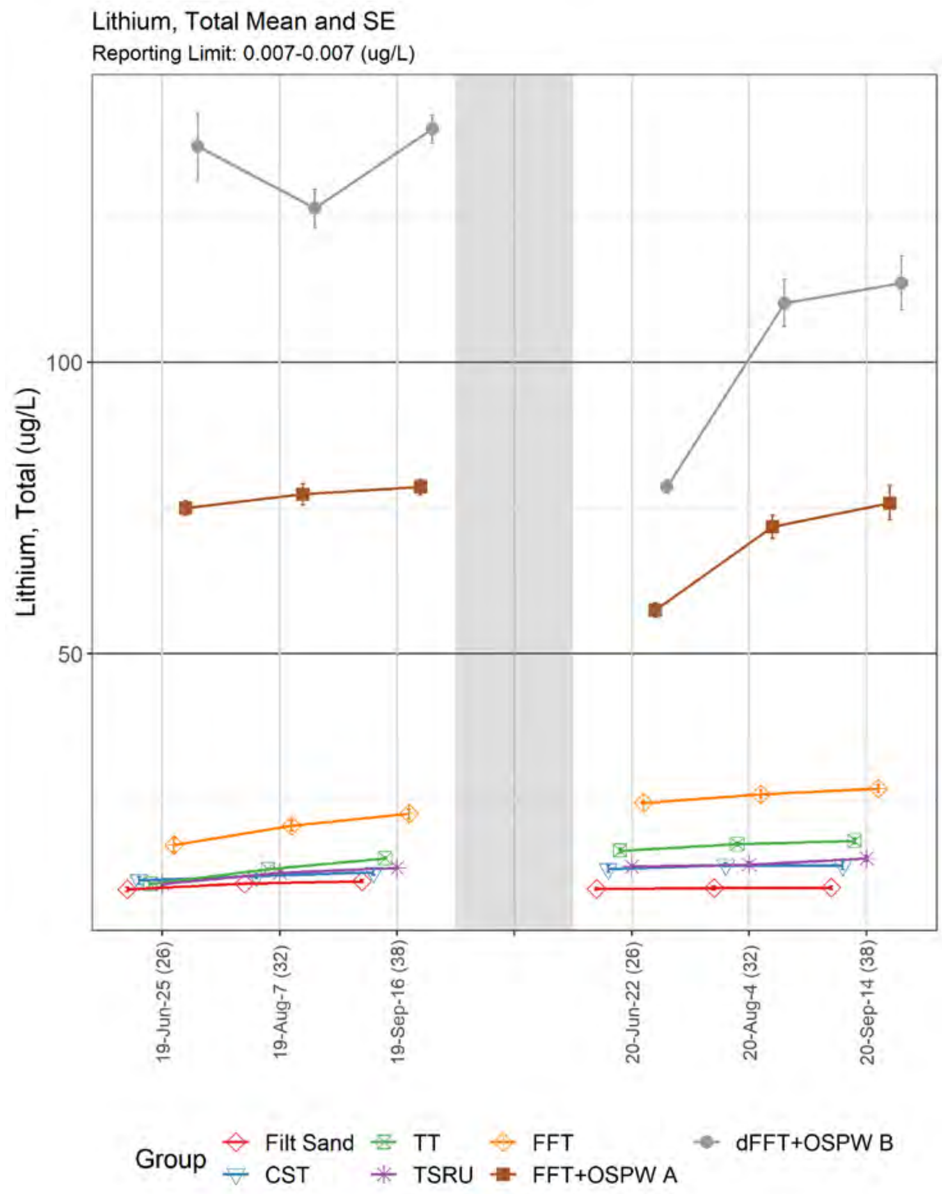

Figure 25. Total lithium concentration across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.2.8 Non-metals and compounds

Summary trends are shown in Table 17, including compound ions (nitrate, silica, sulfate) and non-metals (chloride, fluoride, phosphorus and sulfur). Chlorine is highly soluble, so both total and dissolved fractions values are similar. Previous mesocosm study found that dissolved chloride concentrations were highly correlated with dissolved chlorine concentrations (r2 = 0.9997) and indicated the presence of both chloride (measured by ion chromatography) and chlorine (measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was redundant. Therefore, chlorine concentrations are presented. More details are explained in the following sections (Section 3.2.9 to 3.2.13).

Table 17. Changes in the concentration of non-metals and compound ions associated with time.

Element	Fraction	**CTL**	**TRT1**	**TRT2**	**TRT3**	**TRT4**	**TRT5**	**TRT6**
Chlorine	D	↔	↔	↔	↔	↔	↔	↕
Chlorine	T	↔	↔	↔	↔	↔	↔	↕
Fluoride	D	↗	↔	↗	↗	↗	˅	˅
Nitrate	D	↔	↔	↔	↔	↕	↕	↕
Phosphorus	D	↔	↔	˅	↔	↔	↔	↗
Phosphorus	T	↔	˅	˅	↔	˅	˅	˅
Silica		↘	↘	↘	↘	↘	↘	↘
Sulfate	D	^	↗	↗	↗	↗	˅	˅
Sulfur	D	^	^	^	^	^	˅	↕
Sulfur	T	^	^	^	^	^	˅	↕

CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT. ↗: Increasing; ↘: Decreasing; ^: Mid-Study Increase; ˅: Mid-Study Decrease; ↔: No trend; ↕: Fluctuation

3.2.9 Chlorine

Chlorine concentrations were measured by ICP-MS, and both dissolved and total fractions are reported. Figures and tables detailing chlorine values can be found in Sections A.5.8.18 and A.5.8.19 of Appendix A. Chloride (dissolved) was measured by ion chromatography. Previous mesocosm project showed both values are highly correlated, making the presentation of both chlorine and chloride values redundant.

3.2.9.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

Tailings-only and CTL mesocoms did not differ significantly in chlorine concentrations.

3.2.9.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

The chlorine concentration (both dissolved and total) in TRT 5 (FFT + 100% Imperial OSPW, < 50 mg/L) was significantly higher compared to CTL in 2019. In 2020 significant differences were only detected during Weeks 32 and 38 for dissolved concentrations (Figure 21).

3.2.9.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

Chlorine concentration was significantly higher (by an order of magnitude) in TRT 6 compared to CTL in both years.

3.2.9.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

TT (TRT 2) did not significantly affect chlorine concentrations compared to FFT (TRT 4).

3.2.9.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

Chlorine concentrations were higher in 100% OSPW and FFT mesocosms (TRT 5) in both years.

3.2.9.6 Effect of time

There was no change for chlorine concentrations observed in CTL and TRTs 1, 2, 3, and 4 mesocosms. There was no change observed in TRT 5 mesocosms over time either. In TRT 6 mesocosms, chlorine concentrations fluctuated across two years; chlorine appeared to increase in 2019, decreased mid-study as evidenced by the considerably low values recorded during Week 26, 2020.

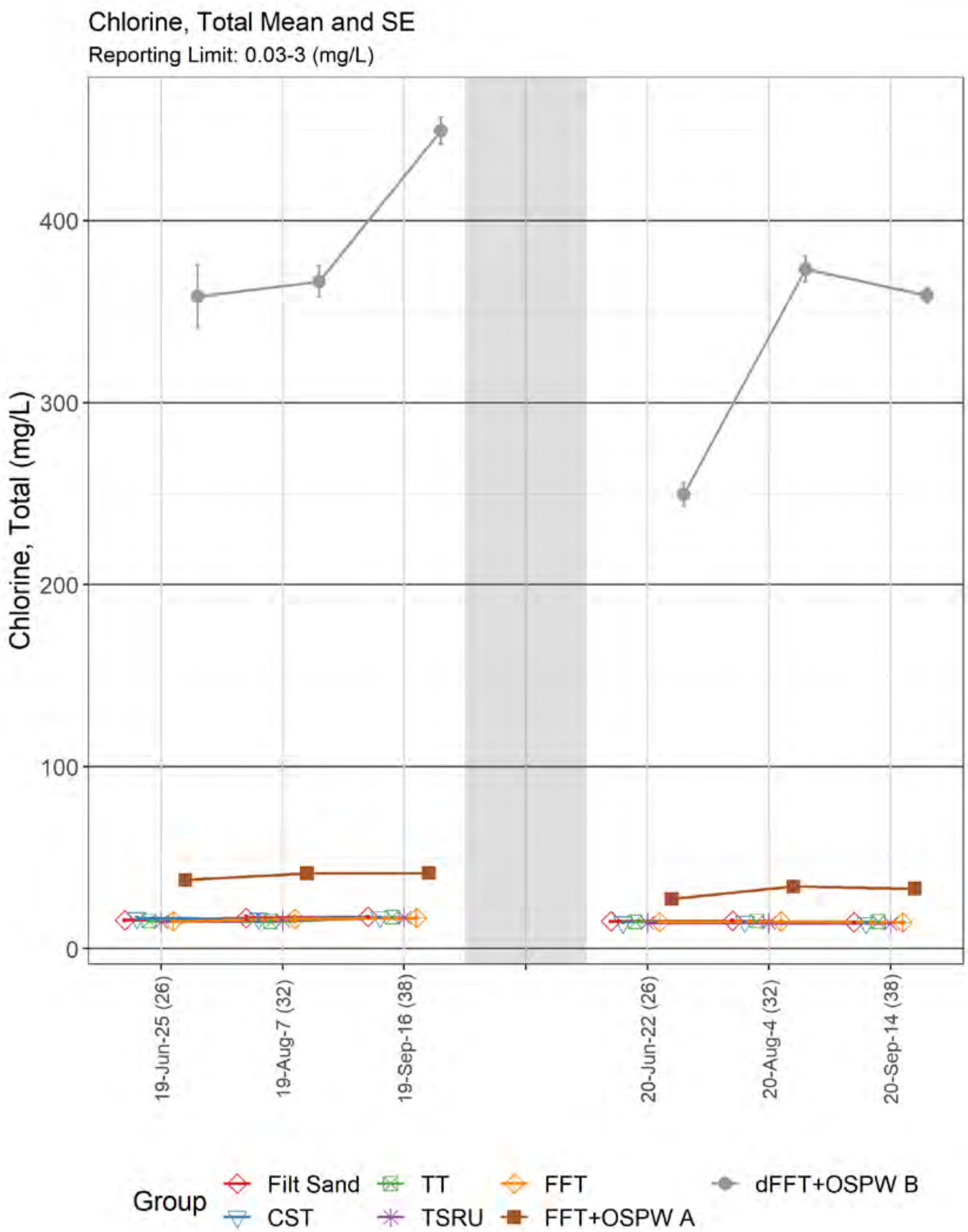

Figure 26. Total chlorine concentration across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.2.10 Fluoride

Figures and tables describing mesocosm fluoride concentrations can be found in Section A.5.8.28 of Appendix A.

3.2.10.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

In 2019, fluoride concentrations were significantly higher in the presence of FFT (TRT 4) compared to CTL. For TT (TRT 2), fluoride concentrations were significantly higher during Week 38 of 2019. In 2020, the concentration of fluoride was seen to rise significantly in the presence of TT (TRT 2), TSRU (TRT 3), and FFT (TRT 4) when compared to CTL.

3.2.10.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

The presence of 100% Imperial OSPW and FFT (TRT 5) was associated with higher fluoride concentration in both years when compared to CTL (Figure 27).

3.2.10.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

Fluoride concentration was significantly higher in the presence of 50% Suncor OSPW with dFFT compared to CTL.

3.2.10.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

Fluoride concentration was significantly higher in FFT (TRT 4) compared to TT (TRT 2).

3.2.10.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

Fluoride concentration was significantly higher in the presence of both OSPW and FFT (TRT 5) compared to FFT alone (TRT 4) in 2019 and 2020.

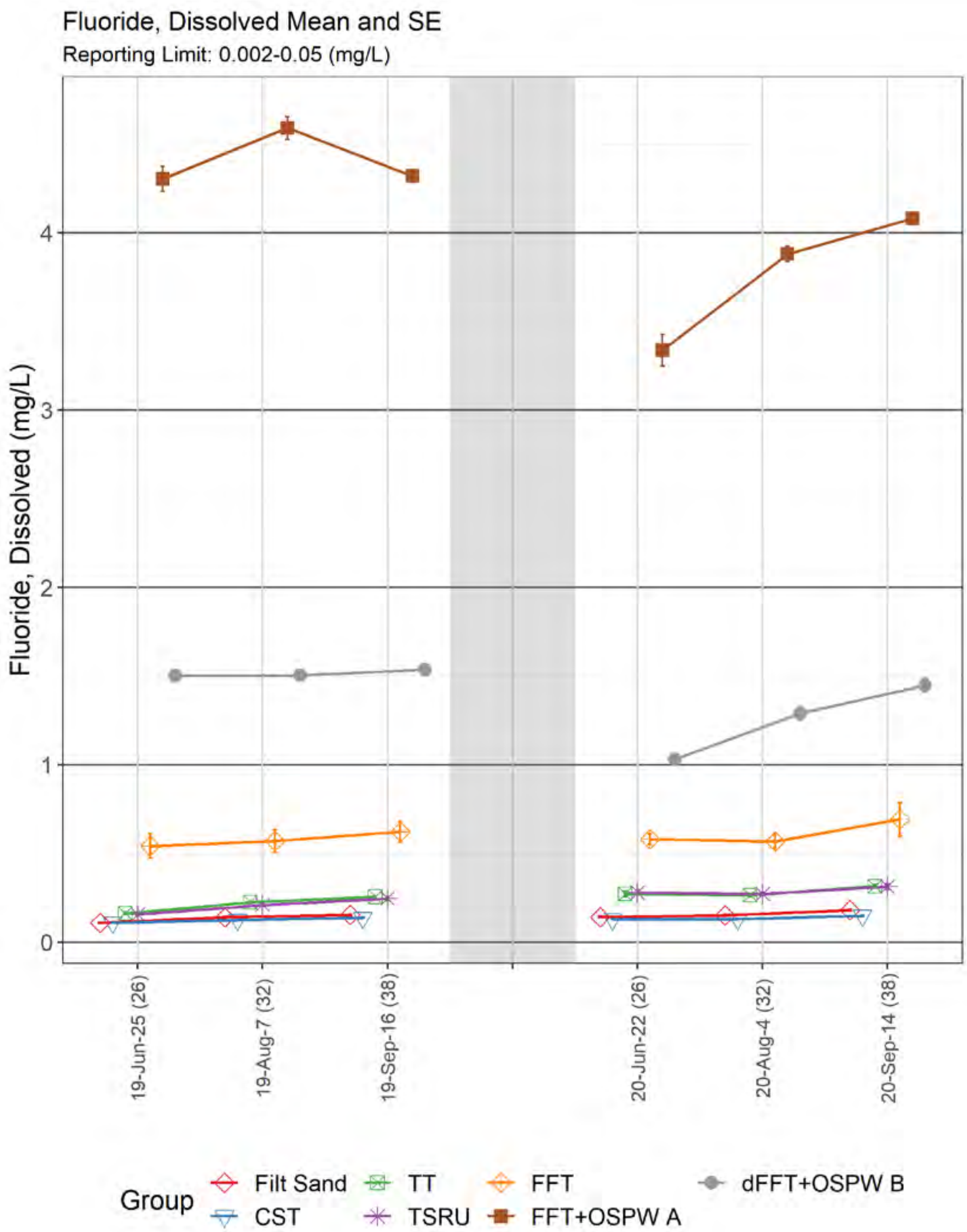

Figure 27. Fluoride (dissolved) concentration across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.2.10.6 Effect of time

Fluoride concentrations significantly increased in CTL and TRTs 2, 3, and 4 mesocosms over a two-year period. In TRT 1 mesocosms, there were no apparent changes. In TRTs 5 and 6 mesocosms, fluoride concentrations showed a significant mid-study decrease, mainly due to the considerably lower values recorded at Week 26, 2020.

3.2.11 Nitrate

Figures and tables detailing nitrate values can be found in Sections A.5.8.46 of Appendix A. Note that nitrite (dissolved) data were excluded, because analytical results were below the detection limit (Section A.5.2).

3.2.11.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

Tailings alone had no significant effect on nitrate (dissolved) concentrations in 2019 and 2020.

3.2.11.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

During Week 26 of 2019, TRT 5 (100% Imperial OSPW with FFT) mesocosms produced higher nitrate (dissolved) concentration than CTL.

3.2.11.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

TRT 6 showed significantly higher nitrate (dissolved) values compared to CTL, but only at Week 32 of 2019.

3.2.11.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

There were no significant differences in nitrate (dissolved) concentrations when comparing TRT 2 (TT) to TRT 4 (FFT).

3.2.11.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

TRT 5 (FFT+100% Imperial OSPW) showed significantly higher nitrate (dissolved) values than TRT 4 (FFT) during Week 26 of 2019.

3.2.11.6 Effect of time

The presence of filter sand (CTL), as well as CST, TT, and TSRU (TRT 1, 2, and 3) had no identifiable effect on nitrate (dissolved) concentration over time. The fluctuation ("cubic" pattern) was exhibited in TRT 4, 5, and 6 mesocosms. Concentrations of nitrate (dissolved) decreased significantly in TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW) mesocosms as well.

3.2.12 Sulfur and Sulfate

Figures and tables detailing sulfur and sulfate values can be found in Sections A.5.8.63, A.5.8.64, and A.5.8.65 of Appendix A. Sulfur was analyzed by ICP-MS, and both dissolved and total fractions are reported. Sulphate was analyzed using ion chromatographic procedures, and only the dissolved fraction is reported.

3.2.12.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

TRT 1 (CST), TRT 3 (TSRU), and TRT 4 (FFT) (tailings-only treatments) were associated with significantly higher sulfate concentrations relative to CTL over both years, except during Week 26 of 2019 for TRT 1 (CST) and TRT 3 (TSRU). TT (TRT 2) was not significantly different from CTL in terms of sulfate (dissolved) concentration in 2019 but was significantly higher in 2020.

In 2019, there were no significant effects on overall sulfur concentrations for most of the tailings treatments, compared to CTL. Exceptions included significantly higher sulfur in TRT 3 (dissolved and total) and TRT 4 (dissolved sulfur only) at Week 38, 2019. However, TRTs 1, 2, 3, and 4 mesocosms had significantly higher sulfur concentrations than CTL at all time points in 2020, except for TRT 2 (TT) at Week 26.

3.2.12.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

TRT 5 (100% Imperial OSPW with FFT) mesocosms had significantly higher sulphate and sulfur concentrations than CTL mesocosms over the entire study.

3.2.12.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

TRT 6 mesocosms had significantly higher sulphate and sulfur concentrations than CTL mesocosms over the entire study.

3.2.12.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

Sulfur and sulfate concentrations were often significantly higher in TRT 4 (FFT) compared to TRT 2(TT), with a few exceptions. There was no significant difference in: sulfate at Week 38, 2020, dissolved sulfur at Week 32 of 2019, and total sulfur at Weeks 32 and 38, 2019.

3.2.12.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

During both years, sulfur and sulfate concentrations were significantly higher in TRT 5 (with 100% Imperial OSPW + FFT) as compared to TRT 4 (FFT).

3.2.12.6 Effect of time

Sulfate (dissolved) concentrations showed a significant mid-study increase in CTL mesocosms. Sulfate (dissolved) concentrations rose significantly in TRTs 1, 2, 3, and 4 (tailings-only treatments), with the largest increases observed in 2019. Sulfate (dissolved) concentrations showed a significantly mid-study decrease in TRTs 5 and 6 (OSPW in the presence of tailings) over the two-year study. Dissolved and total sulfur had a significant mid-study increase in CTL and TRTs 1, 2, 3, and 4 (tailings-alone treatments), while a significant mid-study decrease was noted in TRT 5 (FFT + 100% Imperial OSPW). Dissolved and total sulfur concentration showed fluctuation ("cubic" pattern) in TRT 6 (dFFT + 50% Suncor OSPW) mesocosms, although this overall increase stabilized in 2020 (Figure 28).

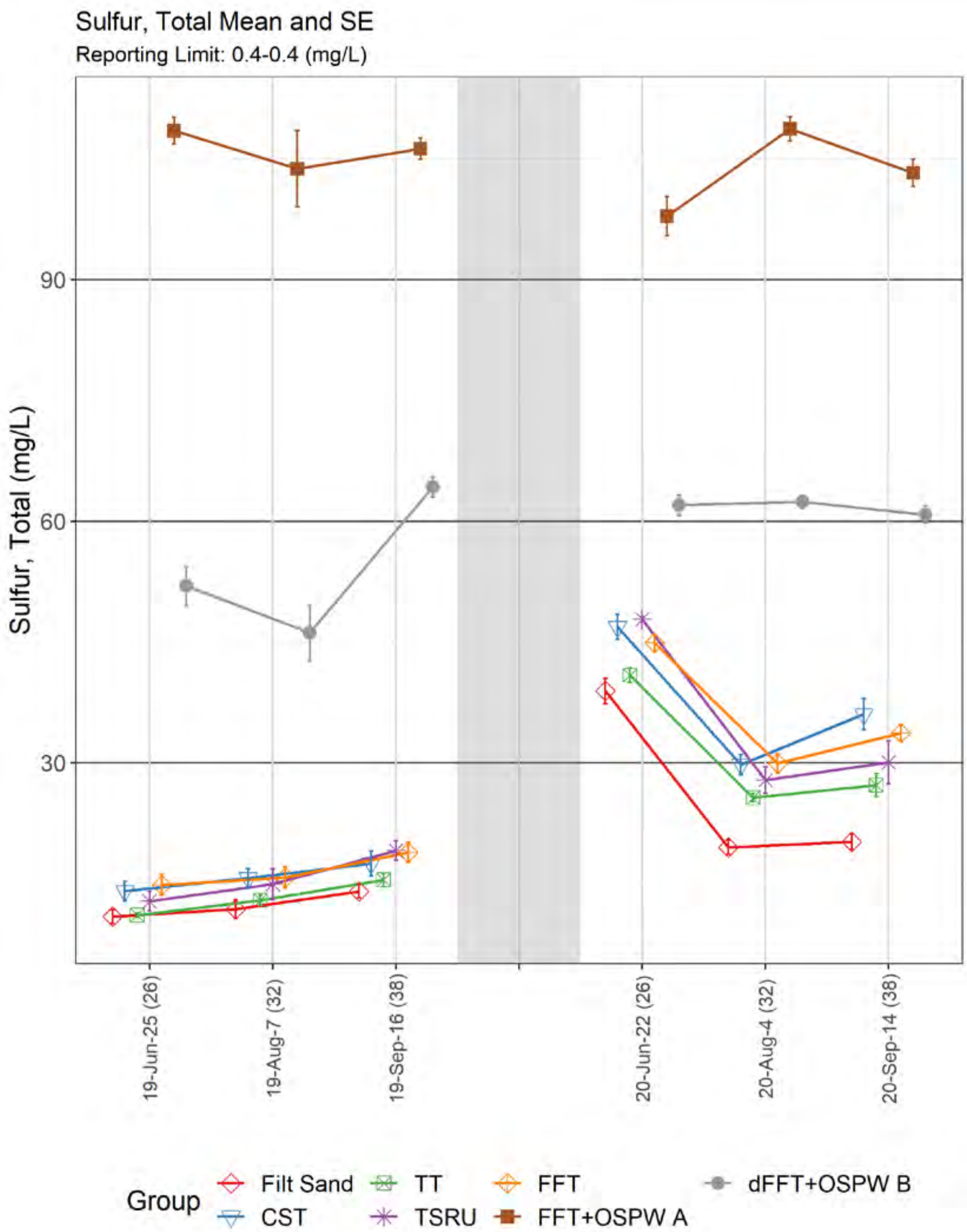

Figure 28. Total sulfur concentration across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.2.13 Phosphorus

Phosphorus was analyzed by ICP-MS, and both dissolved and total fractions are reported. Figures and tables detailing phosphorus values can be found in Sections A.5.8.48 and A.5.8.49 of Appendix A.

3.2.13.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

Tailings did not have a significant effect on phosphorus concentrations in general. Exceptions included significantly higher dissolved and total phosphorus concentrations in TRT 2 (TT) and total phosphorus in TRT 4 (FFT) at Week 26, 2019.

3.2.13.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

At Week 38 of 2019, dissolved phosphorus was significantly higher in TRT 5 compared to CTL. However, total phosphorous was significantly higher at Weeks 26 and 38 of 2019. At Week 38 of 2020, both fractions were significantly higher.

3.2.13.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

Phosphorus (dissolved and total) was significantly higher in TRT 6 (50% Suncor OSPW with dFFT) compared to CTL at all time points in both years, except for the dissolved fraction (no significant difference) at Week 26 of 2019.

3.2.13.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

Dissolved phosphorus was significantly higher in the presence of TT (TRT 2) compared to FFT (TRT 4) in Week 26, 2019. No significant difference was observed in total or dissolved phosphorus concentrations at any other time.

3.2.13.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

Phosphorus (dissolved fraction) was significantly higher in TRT 5 compared to TRT 4 at Week 38, 2019 and Week 38, 2020.

3.2.13.6 Effect of time

There were no apparent trends in phosphorus concentrations (either dissolved or total) in CTL or TRT 3 (TSRU) mesocosms. TRT 1 (CST) or TRT 4 (FFT) also showed no apparent trends, but in the dissolved fraction only. Phosphorus (dissolved and total) showed a significant mid-study decrease in TRT 2 (TT). The total fraction of phosphorus also showed a significant mid-study decrease with time in TRT 1 (CST) and TRT 4 (FFT). For TRT 5 (FFT + 100% Imperial OSPW), the total fraction of phosphorus showed a significant mid-study decrease. However, the dissolved fraction was found no significant change over two years, although dissolved phosphorus appeared increasing over the course of 2020. In TRT 6 (dFFT + 50% Suncor OSPW) mesocosms, the dissolved fraction of phosphorus increased significantly, while the total fraction produced a significant mid-study decrease.

3.2.14 Biological Oxygen Demand

This section includes results of Biological Oxygen Demand (BOD). Figures and tables describing BOD in mesocosm waters can be found in Section A.5.8.11 of Appendix A.

3.2.14.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

None of the tailings-only treatments had any significant effects on BOD.

3.2.14.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

These two groups were not significantly different in BOD levels at any time point during the study.

3.2.14.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

CTL mesocosms produced lower BOD values than TRT 6 mesocosms at all three-time points in 2019. In 2020, the only significant difference in BOD concentrations was at Week 26, where TRT 6 concentrations were higher than CTL.

3.2.14.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

TRT 2 (TT) mesocosms were significantly higher in BOD than TRT 4 (FFT) mesocosms at Weeks 26 and 32, 2020. No significant differences were observed between the two tailing types at any other time.

3.2.14.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

Overall, TRT 4 (FFT) did not significantly differ from TRT 5 (100% Imperial OSPW with FFT) in terms of BOD, although a significant difference was only detected during Week 32, 2019, where TRT 5 produced significantly higher BOD values than TRT 4.

3.2.14.6 Effect of time

There were no apparent time trends in CTL and TRT 1 (CST), TRT 4 (FFT), and TRT 5 (FFT + 100% Imperial OSPW). A significant increase in BOD levels was identified in TRT 2 (TT) and TRT 3 (TSRU) over two years. Significant time-related and mid-study decreases in BOD were identified in TRT 6 mesocosms (dFFT + 50% Suncor OSPW).

3.2.15 Chemical Oxygen Demand

Figures and tables describing Chemical Oxygen Demand (COD) in mesocosm waters can be found in Section A.5.8.25 of Appendix A.

3.2.15.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

The only significant difference in COD relative to the CTL was observed in TRT 1 (CST), where the value was higher than CTL during Week 26, 2019.

3.2.15.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

COD in OSPW with FFT mesocosms (TRT 5) was significantly higher than CTL across all time points in 2019 and 2020.

3.2.15.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

COD values were significantly higher in TRT 6 (50% OSPW with dFFT) compared to CTL at all time points in 2019 and 2020.

3.2.15.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

Tailings formulation had no significant effects on COD when comparing TRT 2 (TT) to TRT 4 (FFT) in 2019 and 2020.

3.2.15.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

TRT 4 (FFT) produced significantly lower COD values than TRT 5 (FFT + 100% Imperial OSPW) at all time points across both years.

3.2.15.6 Effect of time

COD was stable in all experimental groups throughout the study.

3.2.16 Dissolved Organic Carbon

Figures and tables describing Dissolved Organic Carbon (DOC) in mesocosm waters can be found in Section A.5.8.47 of Appendix A.

3.2.16.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

A significant difference in DOC concentration relative to the CTL (9 – 11 mg/L) was observed for TRT 4 (FFT, 12– 14 mg/L), where the concentration was higher across all Weeks in both 2019 and 2020. DOC concentrations in TRT 2 (TT) were also significantly higher than CTL, but only in Week 32 of 2019, and Weeks 26 and 32 of 2020.

3.2.16.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

Significantly higher DOC values were detected in TRT 5 mesocosms compared to CTL mesocosms throughout both years (Figure 29).

3.2.16.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

Significantly higher DOC concentrations were associated with the presence of OSPW with dFFT across both 2019 and 2020.

3.2.16.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

DOC concentrations in TRT 4 (FFT) mesocosms were significantly higher than TRT 2 (TT) at Weeks 26 and 38, 2019, as well as during Week 38, 2020.

3.2.16.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

Significantly higher DOC values were detected in TRT 5 (100% Imperial OSPW with FFT) mesocosms compared to TRT4 (FFT) mesocosms in both years.

3.2.16.6 Effect of time

In CTL mesocosms, DOC concentrations showed a significantly mid-study decrease. In TRTs 1 (CST), 2 (TT), 3 (TSRU), and 4 (FFT) mesocosms, there were no changes in DOC over time. DOC concentrations exhibited a significant mid-study decrease in TRT 5 (FFT + 100% Imperial OSPW) and TRT6 (dFFT + 50% Suncor OSPW) over time, mainly due to the much lower initial level (Week 26) in 2020 compared to the last time point of 2019.

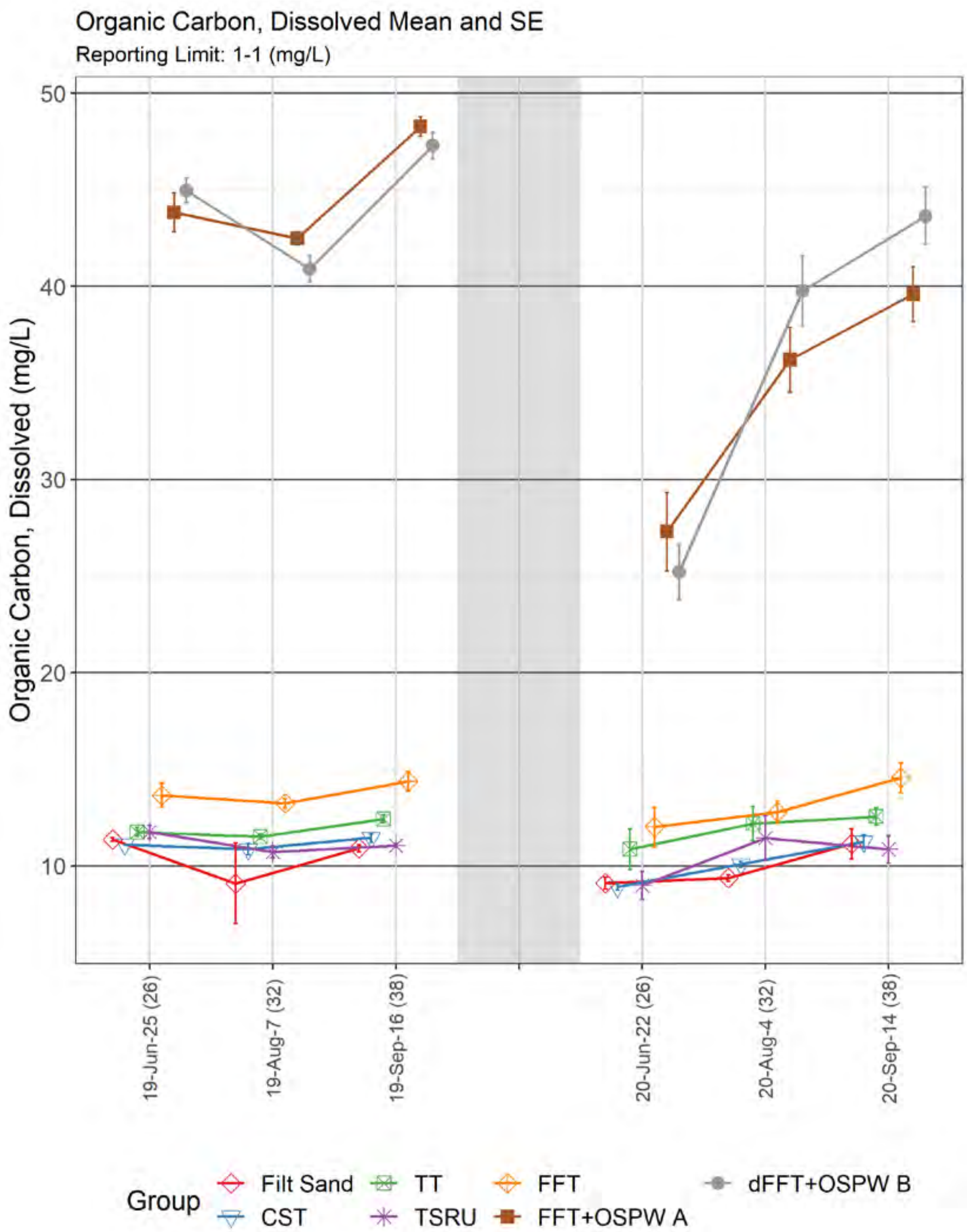

Figure 29. Dissolved Organic Carbon (DOC) across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.2.17 Polycyclic Aromatic Hydrocarbons

Figures and tables describing the results from statistical analyses (e.g., correlations and Principal Component Analyses (PCA's)) for the PAH data can be found in Section A.5.4.1 and A.5.7 of Appendix A. It should be noted that of the 40 alkylated and non-alkylated PAH compounds planned for analysis, only 5 (Chrysene, C3 Naphthalene, C3 Fluorene, C2 Dibenzothiophene, and C1 Dibenzothiophene) were ever detected at levels above their respective reporting limits. Of these 5 compounds, only the concentrations of the two Dibenzothiophenes were highly correlated (r=0.79).

The percentage of PAHs of all 40 compounds below reporting limit was 67%, 71%, and 85% across all mesocosms at the three sampling points in 2019, respectively. All PAHs were below the detection limit in 90% of the mesocosms by the end of 2020.

3.2.17.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

There were no significant differences in PAH levels for any tailings-only treatment, compared to CTL in both 2019 and 2020.

3.2.17.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

The presence of OSPW with FFT was associated with significantly higher PAH levels relative to CTL mesocosms in 2019 but exhibited no significant differences in 2020.

3.2.17.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

TRT 6 (50% Suncor OSPW with dFFT) mesocosms produced significantly higher PAH concentrations compared to CTL in 2019, but not in 2020.

3.2.17.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

PAH concentrations in TRT 4 (FFT) mesocosms were significantly higher than TRT 2 (TT) mesocosms in during Week 26, 2019.

3.2.17.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

PAH concentrations in TRT 5 (FFT+100% OSPW) mesocosms were significantly higher than TRT 4 (FFT) mesocosms during Weeks 26 and 38, 2019.

3.2.17.6 Effect of time

All treatment groups, including CTL, showed significantly decreased PAH concentrations over time, and this decrease was perceived more apparent in 2019 (Figure 30).

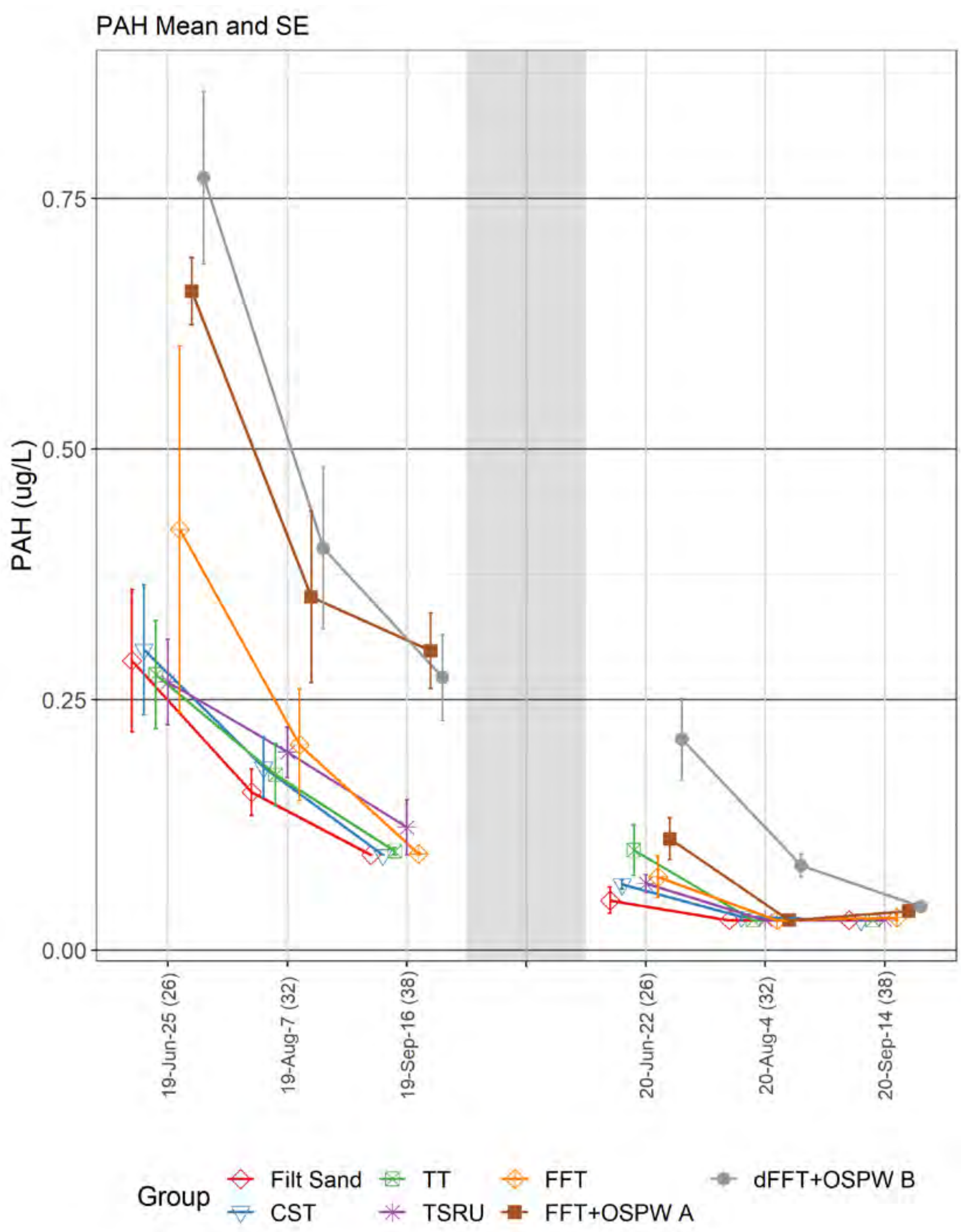

Figure 30. Polycyclic Aromatic Hydrocarbons (PAH) across time points.
Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.2.18 Phenols and BTEX

Figures and tables describing phenol concentrations in mesocosm waters can be found in Section A.5.2 of Appendix A. It should be noted that the overall concentration of phenolic compounds was low, and statistically significant differences could rarely be detected between experimental groups or time points. Even when detected, many of the phenol concentrations recorded in 2019 were very close to the analytical laboratory's reporting limit (0.001 mg/L). The proportion of mesocosms with phenol concentrations below the reporting limit was 87% at Week 26, 2019. This proportion then increased to 97% and 100% in Week 32 and 38 of 2019, respectively. In 2020, phenolic compounds were below the detection limit in all samples.

As shown in Table A.5.8 of Appendix A, BTEX compounds were rarely detected in mesocosms. At Week 26, 2019, 87% of samples were below the reporting limit. After Week 26, 2019, only o-xylene were analyzed. Similar trends were observed in 2020, where BTEX compounds were almost completely undetectable in all of the mesocosms. At Week 26, all samples were below reporting limit. By Weeks 32 and 38, 95% and 86% respectively of samples were below detection limit. Most of the BTEX concentrations recorded were very close to the analytical laboratory's reporting limit (See Section 2.16.6 for the description of reporting limit).

3.2.19 Other Derivative Measures

Some water quality measures were calculated from their components (e.g., hardness is calculated from the degree of dissolved calcium and magnesium content in water). These values included alkalinity, hardness, ion balance, sum anions, sum cations, total dissolved solids (TDS), and silica (reactive). These parameters are discussed only briefly below since direct measurements of their component analytes have already been presented or were not included as dependent variables in the study plan (Appendices C and D).

3.2.19.1 Alkalinity (pH 4.5)

Alkalinity was measured at pH 8.3 and 4.5 by the analytical laboratory and expressed as mg/L $CaCO_3$. Statistical analyses were completed on the alkalinity values measured at pH 4.5 only, because the contributing alkalinity sources are the same for both methods (i.e., pH's 8.3 and 4.5).

No significant differences in alkalinity could be detected between TRT 1 (CST) and CTL over the course of the study. TRT 2 (TT) had significantly higher alkalinity than CTL during Week 38 of 2020. No significant differences could be detected between TRT 3 (TSRU) and CTL in 2019. However, alkalinity was significantly higher in TRT 3 (TSRU) mesocosms than in CTL mesocosms at all three time points in 2020. After Week 32, 2019, the alkalinity measured in TRT 4 (FFT) was significantly higher than that of CTL. The presence of OSPW with FFT (TRT 5) or dFFT (TRT 6) was associated with an increase in alkalinity compared to CTL in both years at all six time points. After Week 32, 2019, alkalinity in TRT 2 mesocosms was significantly lower than that of TRT 4 mesocosms. Alkalinity was significantly higher in TRT 5 (FFT + 100% Imperial OSPW) mesocosms than in TRT 4 mesocosms in 2019 and 2020.

Alkalinity in CTL and TRTs 1, 2, and 3 showed a significant mid-study increase. The highest alkalinity values appeared at the end of 2019 (Week 38) and the beginning of 2020 (Week 26). Alkalinity increased significantly over two years in TRT 4 (FFT). In TRT 5 (FFT + 100% Imperial OSPW), significant mid-study decrease was detected over time. Alkalinity in TRT 6 (dFFT + 50% Suncor OSPW) showed an overall increase and a significant mid-study decrease, mainly due to the first time point of 2020. Over the course of the study, TRT 6 increased in alkalinity. Over the 2019/20 winter, there appeared to be a decrease. The appearance of a decrease is the result of a low value recorded during Week 26 of 2020, compared to values at Week 38, 2019 and Week 32, 2020.

3.2.19.2 Hardness

Figures and tables describing water hardness can be found in Section A.5.8.29 of Appendix A.

TRT 1 (CST, 103– 149 mg/L $CaCO_3$) and TRT 3 (TSRU, 97 – 148 mg/L $CaCO_3$) mesocosms produced significantly higher hardness values than CTL (84 – 140 mg/L $CaCO_3$) at Weeks 38 and 26 of 2020, respectively. Hardness in the presence of FFT (TRT 4, 117 – 129 mg/L $CaCO_3$) and FFT with 100% Imperial OSPW (TRT 5, 103 - 135 mg/L $CaCO_3$) was significantly higher than CTL during the last two collection dates. Interestingly in Week 26 of 2020, hardness measured in TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW, 62 - 99 mg/L $CaCO_3$) was significantly lower compared to CTL. Hardness measured in TRT 6 was significantly lower than CTL at all time points of 2019, as well as Week 26 of 2020. Hardness values in TRT 4 mesocosms were significantly higher than TRT 2 (TT, 88 - 129 mg/L $CaCO_3$) in Weeks 32 and 38, 2019. Hardness was also significantly higher in the presence of OSPW (TRT 5 vs. TRT 4) during the first collection dates in 2019. However, the reverse (i.e., TRT 4 > TRT 5) was observed during the first collection dates in 2020.

In CTL and TRTs 1, 2, and 3 mesocosms, hardness significantly decreased over two years; a significant mid-year increase was also observed. There appeared to be an initial increase in 2019, followed by an overwinter decrease (detected in 2020). A significant mid-study increase trend was observed in TRT 4 (FFT) mesocosms. While in TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW), an overall decrease in hardness was detected. Over the 2019/20 winter there appeared to be a decrease, and significant mid-study decrease were also observed.

3.2.19.3 Ion balance

Ion balance summary serves as a quality check of the laboratory analysis, and an indicator of any missing ions. Figures and tables describing ion balance can be found in Section A.5.8.30 of Appendix A. Ion balance check returned the values between ± 5%, except for one mesocosm in TRT 1 (CST) at Week 26 and TRT 2 (TT) at Week 38 of 2019. It was considered acceptable for the mean values within ± 10% range at all time points.

3.2.19.4 Sum of Anions and Cations

Figures and tables can be found for Sum of Anions and Sum of Cations in Section A.5.8.66 and A.5.8.67 of Appendix A. TRTs 1, 2, and 3 had no significant differences in the sum of anions or cations compared to CTL in 2019. However, the sum of anions and cations in TRTs 1, 2, and 3 were significantly higher in at least two sampling time points in 2020. Exceptions noted include no significant differences in the sum of anions for TRT 1 compared to CTL during Week 32 of 2020, and no significant differences in the sum of anions and cations for TT (TRT 2) compared to CTL, during the week of Week 26 of 2020.

Significantly higher sum of cations of TRT 4 (FFT) compared to CTL was detected during the last sampling time period of 2019. In the presence of FFT (TRT 4), the sum of anions and cations were both significantly higher at all three time points in 2020. TRTs 5 (100% Imperial OSPW+FFT) and 6 (50% Suncor OSPW+dFFT) were always significantly higher in sum of anions and cations compared CTL. The sum of anions and cations in TRT 4 (FFT) were significantly higher compared to TRT 2 (TT). OSPW had a significant effect (higher) on the sum of anions and cations in the presence of FFT (TRT 4 vs. 5) during 2019 and 2020.

The sum of anions and cations decreased overtime in CTL and TRT 1 (CST). There was a significant mid-study increase in CTL and TRTs 1, 2, and 3. TRT 4 (FFT) showed a significant increase. However, a mid-study decrease was observed in TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW). Similar to alkalinity trends, this decrease can be attributed to a lower value during Week 26 of 2020, compared to values at Week 38, 2019 and Week 32, 2020.

3.2.19.5 TDS (total dissolved solids)

Figures and tables describing TDS values in the mesocosms can be found in Section A.5.8.74 of Appendix A.

No significant difference in TDS was found between TRTs 1 (CST), 2 (TT), and 3 (TSRU) mesocosms and CTL in 2019, except for a significant higher value in TRT 3 (TSRU) at Week 38 of 2019. However, TRT 4 (FFT) mesocosms produced significantly higher TDS values than CTL across all time points in 2019. In 2020, all treatment mesocosms produced significantly higher TDS values than CTL. TRT 4 mesocosms produced significantly consistently higher TDS values than TRT 2 (TT) in both years. TDS of all treatments that did not contain OSPW were below 260 mg/L. In both 2019 and 2020, OSPW with FFT (TRT 5) was associated with a significant elevation in TDS compared to FFT only (TRT 4) across all time points.

TRTs 1, 2, 3 and CTL mesocosms demonstrated a significant mid-study increase in TDS. TDS increased significantly in TRT 4 (FFT) over two years. However, TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW) mesocosms demonstrated a significant mid-study decrease, which can be likely attributed to a low value recorded during Week 26 of 2020, compared to values at Week 38 of 2019 and Week 32 of 2020.

3.2.19.6 Silica (reactive)

Figures and tables detailing silica values can be found in Section A.5.8.54 of Appendix A. In 2019, the presence of treatment materials significantly higher silica concentration above CTL during Week 32 in TRT 1 (CST) and TRT 2 (TT), Weeks 32 and 38 in TRT 3 (TSRU), and at all three time points in TRT 4 (FFT) mesocosms. However, differences between treatment groups and CTL became less distinct in 2020. Significant higher silica values above CTL were only observed in TRT 5 (FFT + 100% Imperial OSPW). Over 2019, TRT 4 mesocosms produced significantly higher silica values than TRT 2, but not in 2020. TRT 5 mesocosms produced significantly higher silica values than TRT 4 over all time points in both years. Silica decreased significantly over time in all groups. A significant mid-study decease was also observed in most experimental groups, except in TRT 5.

3.3 Installed Vegetation

Figures and tables describing the growth and biomass of installed vegetation in the mesocosms can be found in Section A.8 of Appendix A.

3.3.1 Survival

All emergent and floating plants survived over winter, and no new plants were installed in 2020. The status of *C. demersum* in the spring of 2020 is described in Section 3.9 Anecdotal Observations below.

A total of eight *P. richardsonii* plants were considered dead at Week 27, 2020, including:

- one from CTL,
- three from TRT 5 (FFT + 100% Imperial OSPW), and

- four from TRT 6 (dFFT + 50% Suncor OSPW).

At Week 39, 2020, three more were considered dead: one from TRT 2 (TT), one from TRT 3 (TSRU), and one more from TRT 6 (dFFT + 50% Suncor OSPW). The mortality in TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW) suggests that the survival rate of *P. richardsonii* was related to the presence of OSPW or dFFT.

Four *M. sibiricum* plants died in the spring of 2020; one in CTL, one in TRT 2 (TT), one in TRT 4 (FFT), and one in TRT 5 (FFT + 100% Imperial OSPW). Surprisingly, more than half of *M. sibiricum* disappeared by the end of 2020, including:

- three from TRT 1, TRT 2, and TRT 5,
- two from TRT 3 (TSRU), and TRT 4 (FFT), and
- one from TRT 6 (dFFT + 50% Suncor OSPW).

The more-or-less even distribution across TRT groups and CTL suggests that the *M. sibiricum* mortality was not related to the presence of treatment materials.

3.3.2 Emergent Plants Growth

3.3.2.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

For each emergent plant species, the presence of tailings only had no statistically significant effect on growth most of the time. Exceptions to this include the heights of *T.latifolia* in TRT 3 (TSRU) and *A.americanus* in TRT 4 (FFT) were significantly less than CTL at Week 39 of 2019 and Week 26 of 2019, respectively. It was also observed that the flowering rate of *T.latifolia* was lower than CTL in TRT 1 (CST) at Week 30 of 2020, and in TRT 3 from Weeks 30 to 39 of 2020.

3.3.2.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

For each emergent plant species, the presence of 100% Imperial OSPW with FFT had no significant effect on plant growth. However, the flowering rate of *T.latifolia* was lower than CTL from Weeks 30 to 39, 2020.

3.3.2.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

The presence of 50% Suncor OSPW with dFFT (TRT 6) was associated with a significant effect on plant growth. In general, TRT 6 mesocosms had significantly taller plants compared to CTL during the later season of 2019 (Figure 31). In 2020, Suncor OSPW (50%) with dFFT was also associated with significantly taller emergent plants, although the growth effect was more apparent for *T. latifolia* and *C. atherodes*. By the end of 2020, the flowering rates of emergent plants (except *A.americanus*) were also significantly higher for TRT 6 than that in CTL.

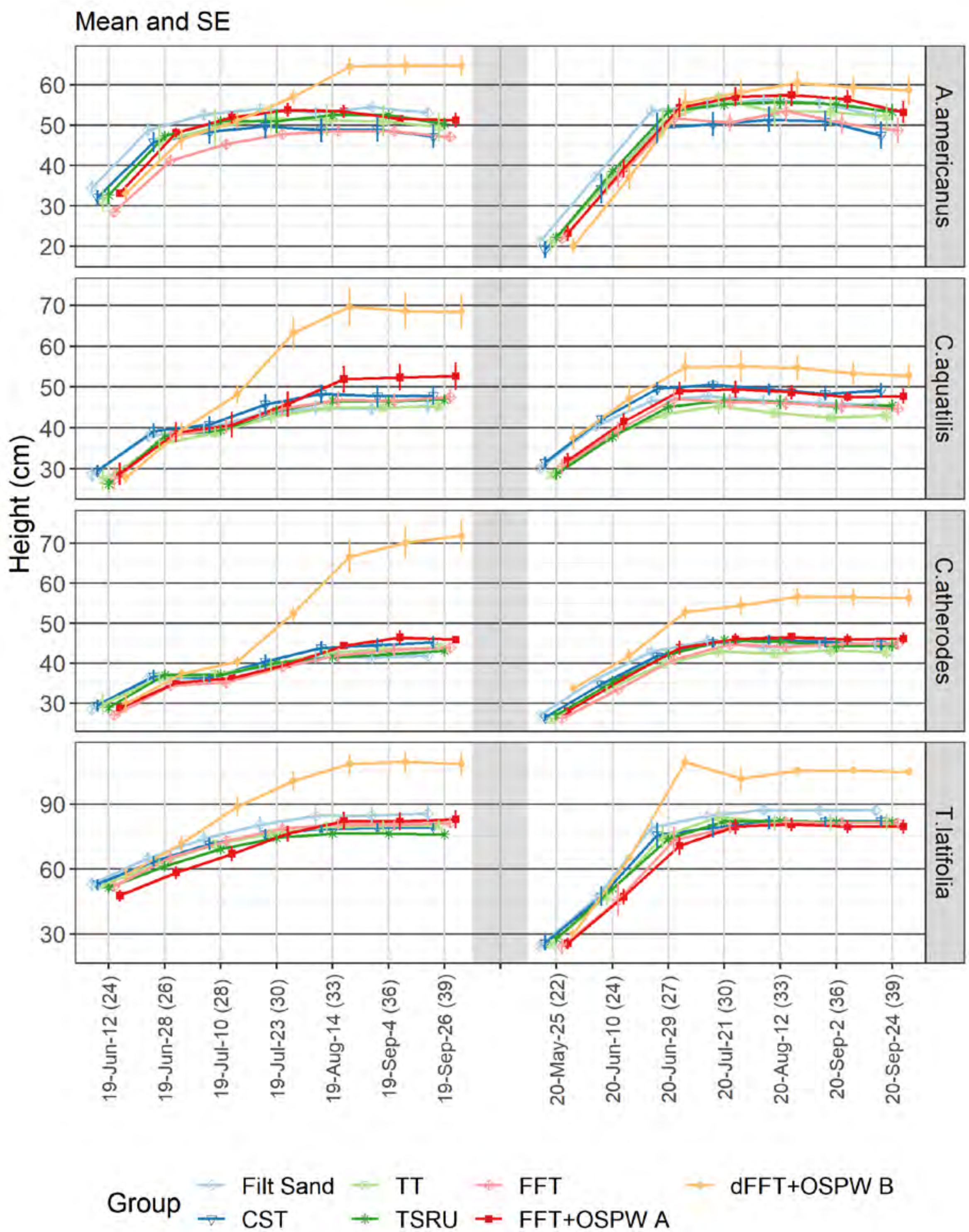

Figure 31. Mean emergent plant heights during 2019 and 2020 aquatic mesocosm studies. Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.3.2.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

When comparing TRT 2 (TT) to TRT 4 (FFT), no statistically significant differences in emergent plant growth was exhibited in either year.

3.3.2.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

No significant difference between TRT 4 (FFT) and TRT 5 (FFT + 100% Imperial OSPW) was identified in the study, with a few exceptions. TRT 5 produced significantly taller *A. americanus* and *C. atherodes* at least one measuring point in each year. Interestingly, a significantly higher flowering rate was observed for *C. aquatilis* in TRT 5 at Week 22 of 2020, while a significantly lower rate was observed for *T. latifolia* from Weeks 27 to 39 of 2020.

3.3.2.6 Effect of time

The trends were apparent that, in each year, all four species grew over time in the spring and reached their plateau for the longest leaf's length in the fall. *A. americanus* flowers were only observed in 2019, whereas flowers of all four species were observed in 2020.

3.3.3 Floating Plants Growth

3.3.3.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

In general, tailings only were observed to significantly affect the weight of *C. demersum* after a few weeks of exposure during the 2019 open water season. In 2020, TRT 1(CST), TRT 2(TT), TRT 3 (TSRU) were not observed to have a significant effect on *C. demersum* weight. However, *C. demersum* wet weights were significantly higher in CTL than in TRT 4 (FFT) mesocosms at all weeks in 2020.

3.3.3.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

C. demersum wet weights were significantly higher in CTL than TRT 5 mesocosms after Week 26 in 2019. *C. demersum* died in almost all TRT 5 (100% Imperial OSPW with FFT) mesocosms. The mortalities of *C. demersum* in each mesocosm was difficult to determine due to the presence of excessive metaphyton (Figure 32).

3.3.3.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

Similar to TRT 5, *C. demersum* wet weights in TRT 6 mesocosms were lower than those of CTL mesocosms except during the first sampling week. *C. demersum* plants almost all died in 2020.

3.3.3.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

No significant differences in floating plant weight were detected between TRT 2 (TT) and TRT 4 (FFT) mesocosms in 2019. In 2020, *C. demersum* wet weights were significantly higher in TRT 2 mesocosms than in TRT 4 mesocosms from Weeks 27 to 33, 2020.

3.3.3.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

As shown in Figure 32, the presence of OSPW resulted in reduced floating plant weight. This lower wet weight in TRT 5 was significant from Week 28, 2019 onwards.

3.3.3.6 Effect of time

Overall, *C. demersum* decreased significantly in biomass in all experimental groups. However, a significant drop in *C. demersum* biomass in TRT 5 (FFT+100% Imperial OSPW) and TRT 6 (dFFT+50% Suncor OSPW) was apparent and this species almost died off in 2020. The effect of adherent metaphyton could not be eliminated due to entanglement, although all efforts were made to avoid metaphyton burden. The presence of metaphyton might contribute to the within-group variability of weight values in 2020. In 2020, there was an apparent decrease in all *C. demersum* mass in CTL and TRTs 1, 2, 3, and 4 (tailings alone), and recovery of TRT 5 and TRT was not noticeable.

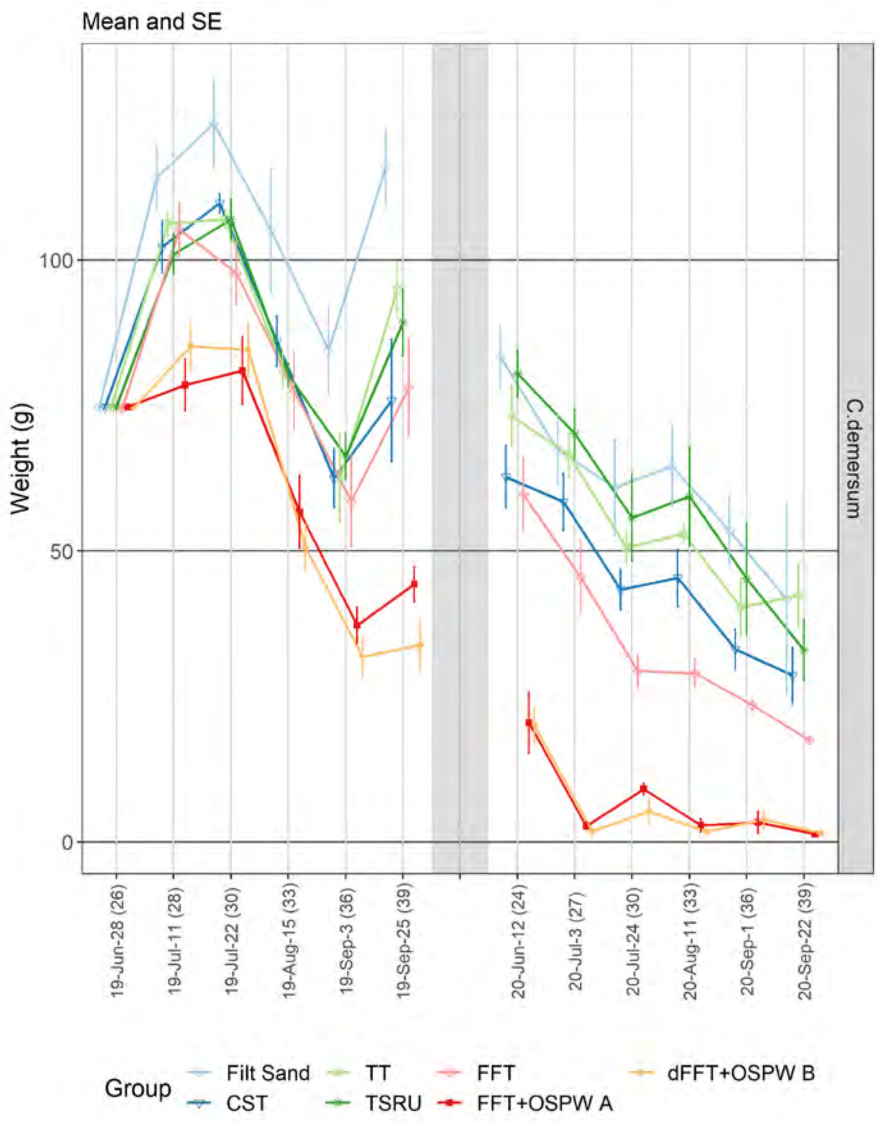

Figure 32. Mean *C. demersum* (hornwort) weight during 2019 and 2020 aquatic mesocosm studies. Note that data points have been jittered horizontally. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.3.4 Aboveground Dry Biomass

Figures and tables describing the aboveground biomass (dry) of emergent plants can be found in Section A.8.5 of Appendix A.

3.3.4.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

There were no significant differences in aboveground dry biomass between CTL and TRTs 1, 2, 3, and 4, with a few exceptions. Aboveground dry biomass of *C. atherodes* was significantly higher in in TRT 1 (CST) relative to CTL in both years. The aboveground dry biomass of *T. latifolia* in TRT 2 (TT) and TRT 4 (FFT) was significantly reduced, compared to CTL in 2020.

3.3.4.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

There were no significant differences in aboveground dry biomass between CTL and TRT 5 (100% Imperial OSPW with FFT) mesocosms.

3.3.4.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

The aboveground biomass of emergent plants was significantly greater in TRT 6 than CLT mesocosms in both years (Figure 33).

3.3.4.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

No other significant differences in the aboveground biomass of emergent plants (for any species) were found between TRT 2 (TT) and TRT 4 (FFT) in either year.

3.3.4.5 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

Higher aboveground dry biomass was found in TRT 5 (FFT + 100% Imperial OSPW) relative to TRT 4 (FFT) for *C. aquatilis* (2019 and 2020), *C. atherodes* (2019), and *A. americanus* (2020). No significant differences in aboveground dry biomass were observed between TRTs 4 and 5 for any other emergent plant species included in the study.

3.3.4.6 Effect of time

Overall, the aboveground dry biomass of emergent plants in 2020 was significantly increased compared to that in 2019.

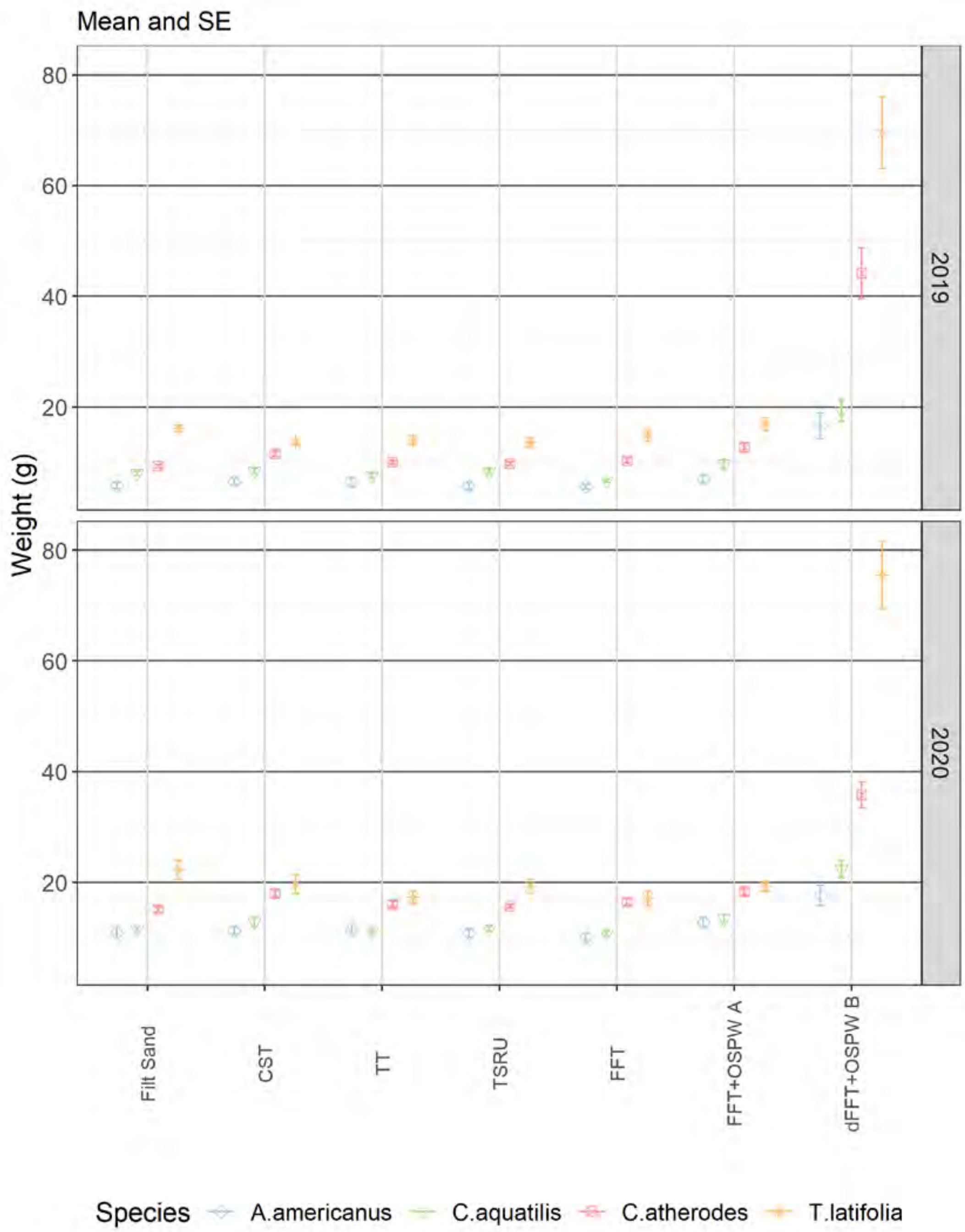

Figure 33. Aboveground dry biomass of aquatic emergent plants in 2019 and 2020. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.4 Toxicity

3.4.1 Rainbow Trout

Tables and figures describing the 96-hour acute toxicity of mesocosm water to rainbow trout can be found in Section A.6.2 of Appendix A. Overall, observed levels of toxicity in rainbow trout were low in both years. For all the samples collected in 2019 and 2020, 90% of LC_{50} values were reported as "non-lethal" or ">100%" by the analytical laboratories. Figure 34 shows all mortality across all experimental groups in 2019 and 2020.

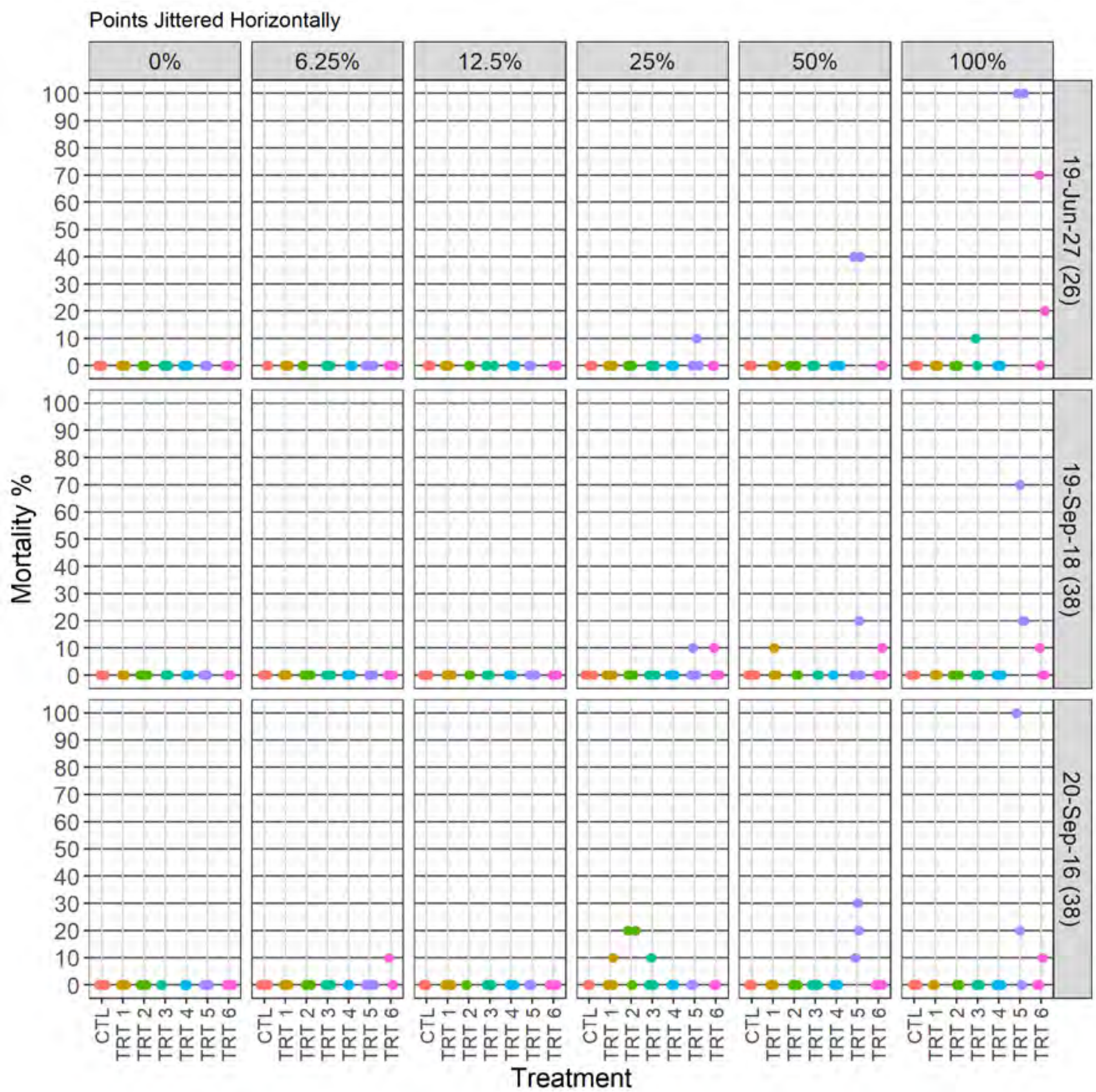

Figure 34. Rainbow trout mortality across all experimental groups for water samples collected in2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.4.1.1 Effect of time

Rainbow trout toxicity appeared to decrease over time in 2019, but not in 2020. At Week 26, 2019, an LC_{50} value could only be determined for water collected from all three of the TRT 5 (FFT + 100% Imperial OSPW, 56.2%, 53.6%, 53.6%) mesocosms, as well as one TRT 6 (dFFT + 50% Suncor OSPW) mesocosm (82%). At Week 38 of 2019 and 2020, all mesocosms reported LC_{50} values of >100% (or non-lethal), except one TRT 5 (FFT + 100% Imperial OSPW) mesocosm each year (82% and 67%). At Week 26, 2019, a total of 39 rainbow trout died in the water collected from OSPW associated mesocosms with no dilution (TRTs 5 and 6), plus one from a TRT 3 (TSRU) mesocosm. At Week 38, 2019, only 12 deaths occurred in water from TRT 5 and TRT 6 (without dilution). By Week 38, 2020, 13 fish died in water (100% concentration) collected from TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW) mesocosms. Some stressed fish were observed over the three sampling periods. However, no noticeable change was shown over time in general.

3.4.1.2 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

In general, it appears that rainbow trout survival was not affected by the tailing materials alone (CST, TT, TSRU, and FFT) (i.e., in the absence of OSPW). No mortalities occurred in water collected from CTL and TRTs 1, 2, 3, and 4 mesocosms, except one fish was found dead in the water from TRT 3 (TSRU) mesocosm at Week 26, 2019. It is noted that limited deaths were found at diluted mesocosms water (e.g., two fish dead at 25% dilution of one TRT 2 (TT) mesocosm on Week 38, 2020). However, because no mortality was found at 0% or 100% mesocosm water, this mortality pattern at the mid-range dilutions was considered acceptable for live-organism assays, since live-organism assays have an allowance for some mortality even under highly controlled conditions.

3.4.1.3 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

More mortalities occurred in TRT 5 mesocosms compared to CTL. It is perceivable that the most stressed trout was found in the water (100% concentration) of TRT 5 mesocosms at Week 38, 2019, where a total of 17 fish stressed. In 2020, some level of mortality was also observed in TRT 5 mesocosms.

3.4.1.4 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

Nine fish died in water collected from TRT 6 mesocosms at Week 26, 2019, one fish died at Week 38, 2019, and there was single mortality in water collected at Week 38, 2020.

3.4.1.5 Effect of treatment of tailings (TRT 2 vs. TRT 4)

No rainbow trout mortalities occurred in water collected from TRT 2 (TT) or TRT 4 (FFT) mesocosms at any point in the study.

3.4.1.6 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

It appears that trout survival was adversely affected by 100% Imperial OSPW in the presence of FFT. At Week 28, 2019, trout mortalities appeared to increase with the proportion of TRT 5 mesocosm water. For example, four fish died at 50% dilution of TRT 5 water on average, while ten fish died without dilution over 96 hours. However, none of the mortalities occurred in any of the dilution rates using TRT 4 (FFT) water. The perceived dose-response relationship was not apparent at Week 38 of 2019 and 2020, since mortalities were so few, although this dose-effect was somewhat perceivable by stressed fish observed in TRT 5 (FFT + 100% Imperial OSPW) water during Week 38 of 2020.

3.4.2 Fathead minnow

Figures and tables describing the 7-day toxicity test results of mesocosm water and fathead minnow can be found in Section A.6.3 of Appendix A. Like the trout toxicity data, toxicity levels measured by the survival bioassay of fathead minnow were too small to calculate LC_{50} values. All samples collected in both 2019 and 2020 were reported as LC_{50} >100 (%v/v). Limited biomass (average dry weight per fish) effects, expressed as IC_{25}, could be observed in 2019. All other IC_{25} values from each experimental group were returned as >100 (%v/v).

3.4.2.1 Effect of time

No apparent changes in mortality levels of fathead minnow were observed between 2019 and 2020. In terms of the growth (biomass) endpoint of larvae minnow, toxicity appeared to decrease over time. IC_{25} was observed in one of each mesocosm of TRT 4 (FFT, 81.0%), TRT 5 (FFT + 100% Imperial OSPW, 83.1%), and TRT 6 (dFFT + 50% Suncor OSPW, 87.8%) on Week 26, 2019. By Week 38, 2020, all IC_{25} values returned a result of >100 (%v/v).

3.4.2.2 Effect of treatment material presence (CTL vs. each TRT)

There was no apparent difference in mortality from treatment materials in both years (Figure 35). In 2019, a significant elevation in fathead minnow biomass was observed in TRT 1 (CST) and TRT 3 (TT) assays with no dilution compared to CTL. However, significant biomass reductions were observed in TRT 5 (FFT+100% Imperial OSPW) and TRT 6 (dFFT + Suncor 50% OSPW) mesocosms. In 2020, the biomass of fathead minnow in the water collected from treatment mesocosms did not differ significantly from CTL, with the exception that TRT 4 (FFT) mesocosms had fathead minnows with significantly lower biomass compared to CTL.

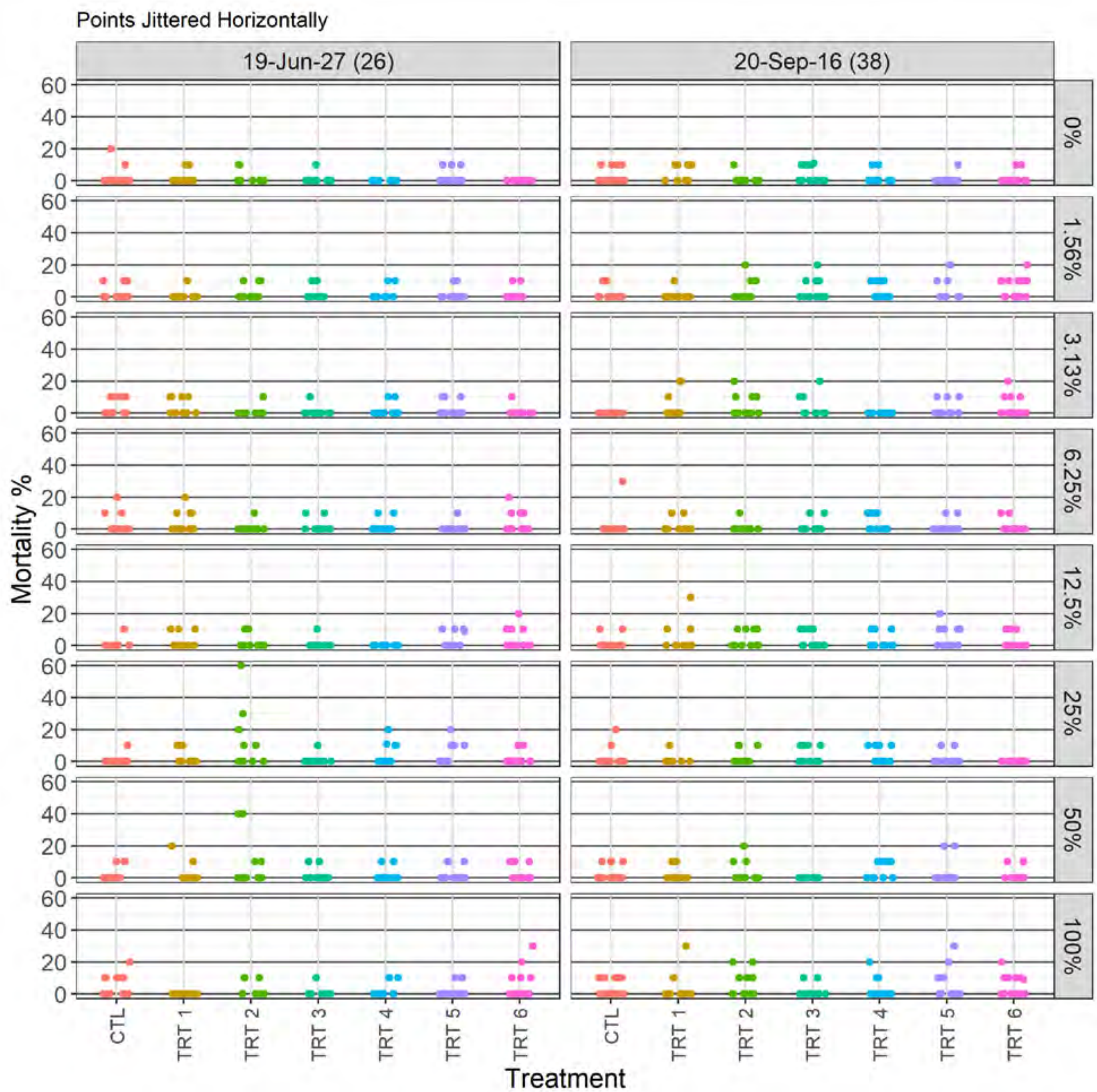

Figure 35. Fathead minnow mortality across all experimental groups for water samples collected in2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.4.2.3 Effect of treatment of tailings (TRT 2 vs. TRT 4)

For mortality, no apparent difference was observed in both 2019 and 2020. Biomass (100% without dilution) in TRT 2 (TT) was found to be significantly higher than TRT 4 (FFT) in both years.

3.4.2.4 Effect of 100% Imperial OSPW in the presence of FFT (TRT 4 vs. TRT 5)

There were no apparent effects on mortality or biomass associated with OSPW in the presence of FFT in both years.

3.5 Zooplankton

Figures and tables describing the zooplankton community can be found in Section A.10 of Appendix A. Taxa richness (Figure 36), abundance (Figure 37), diversity (as measured by the Shannon-Weiner Diversity Index, Figure 38), and biomass (calculated, Figure 39) were statistically analyzed. Relative abundance and biomass estimation of each Order group were plotted without statistical analyses.

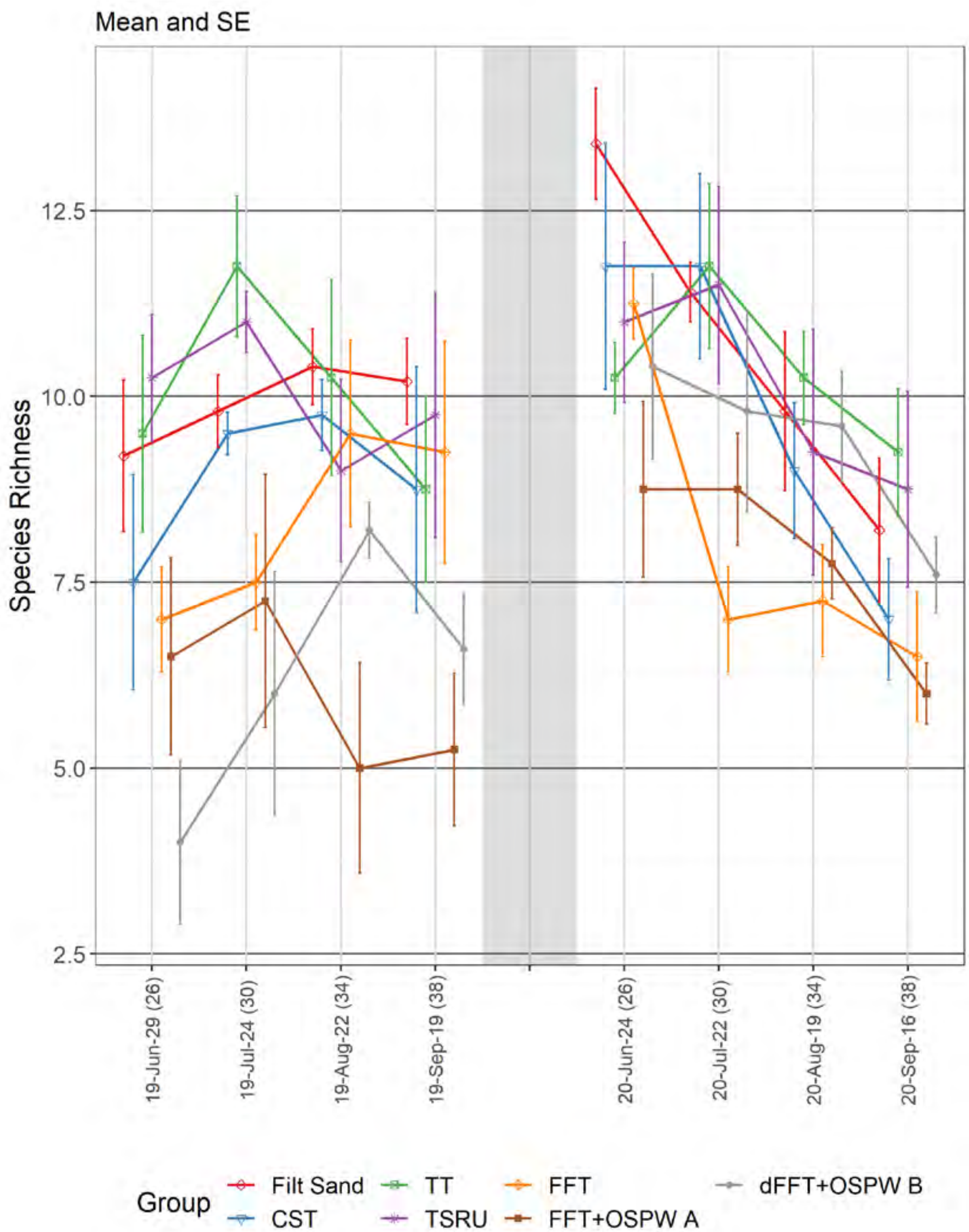

Figure 36. Zooplankton taxa richness in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

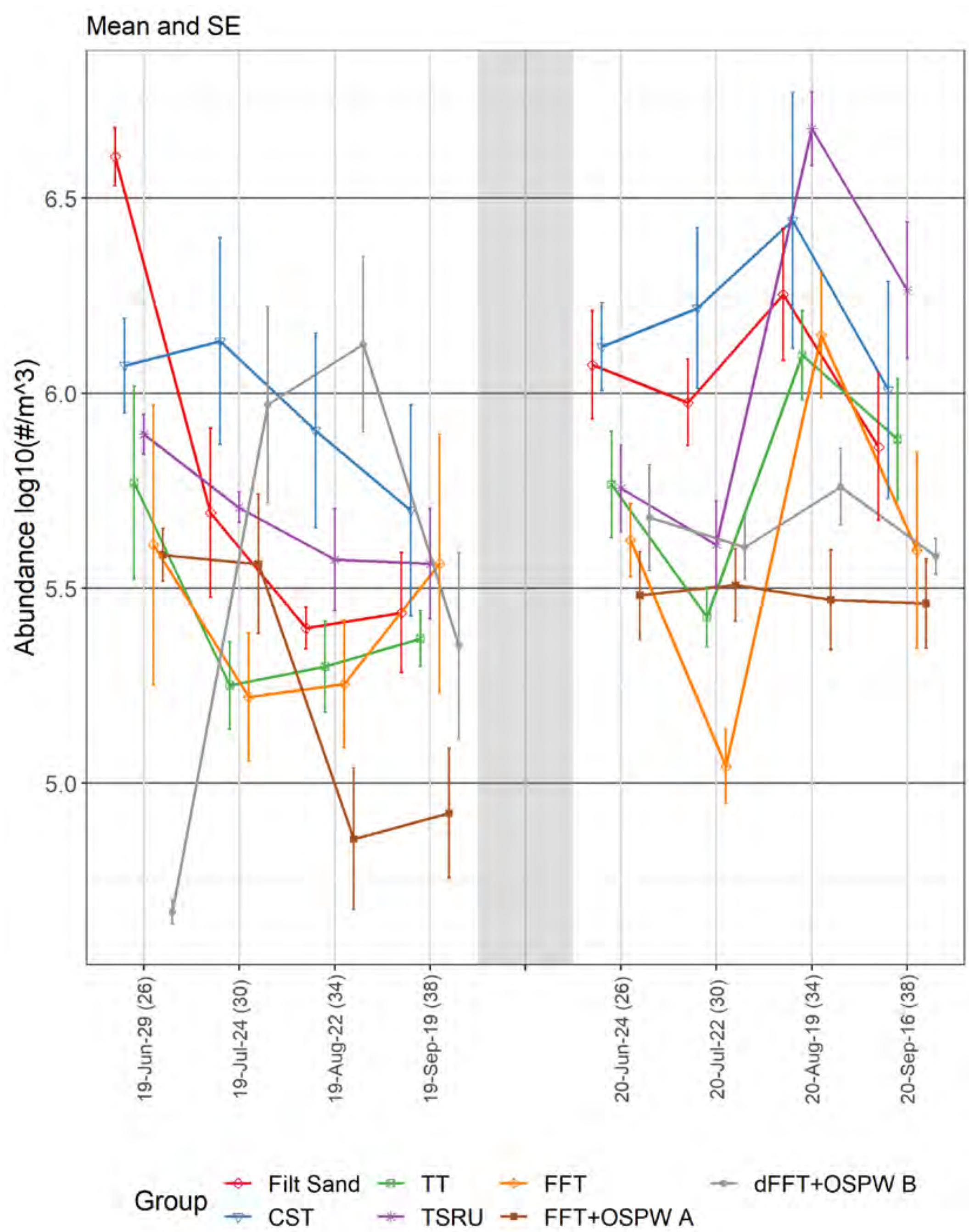

Figure 37. Zooplankton abundance in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

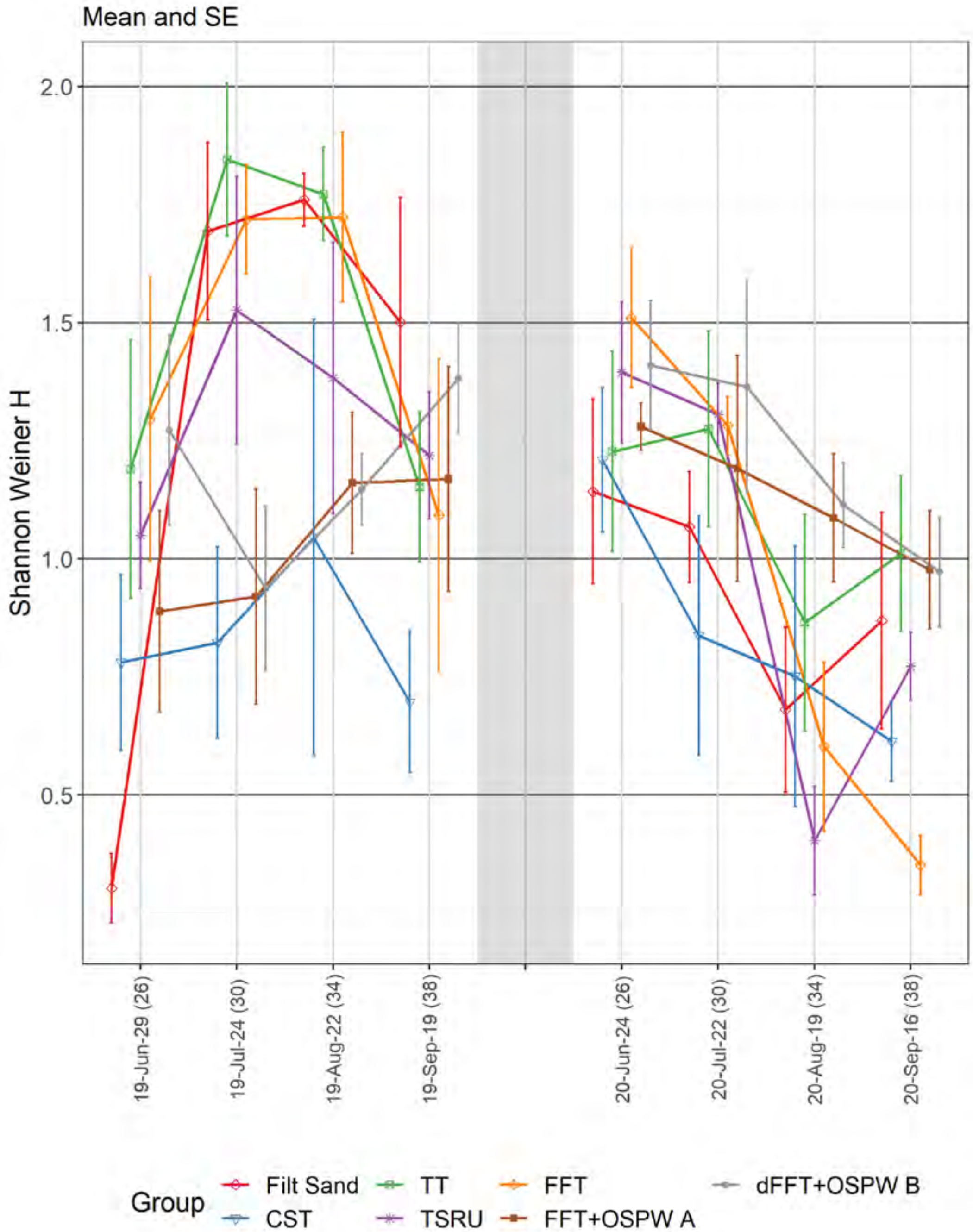

Figure 38. Zooplankton diversity in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

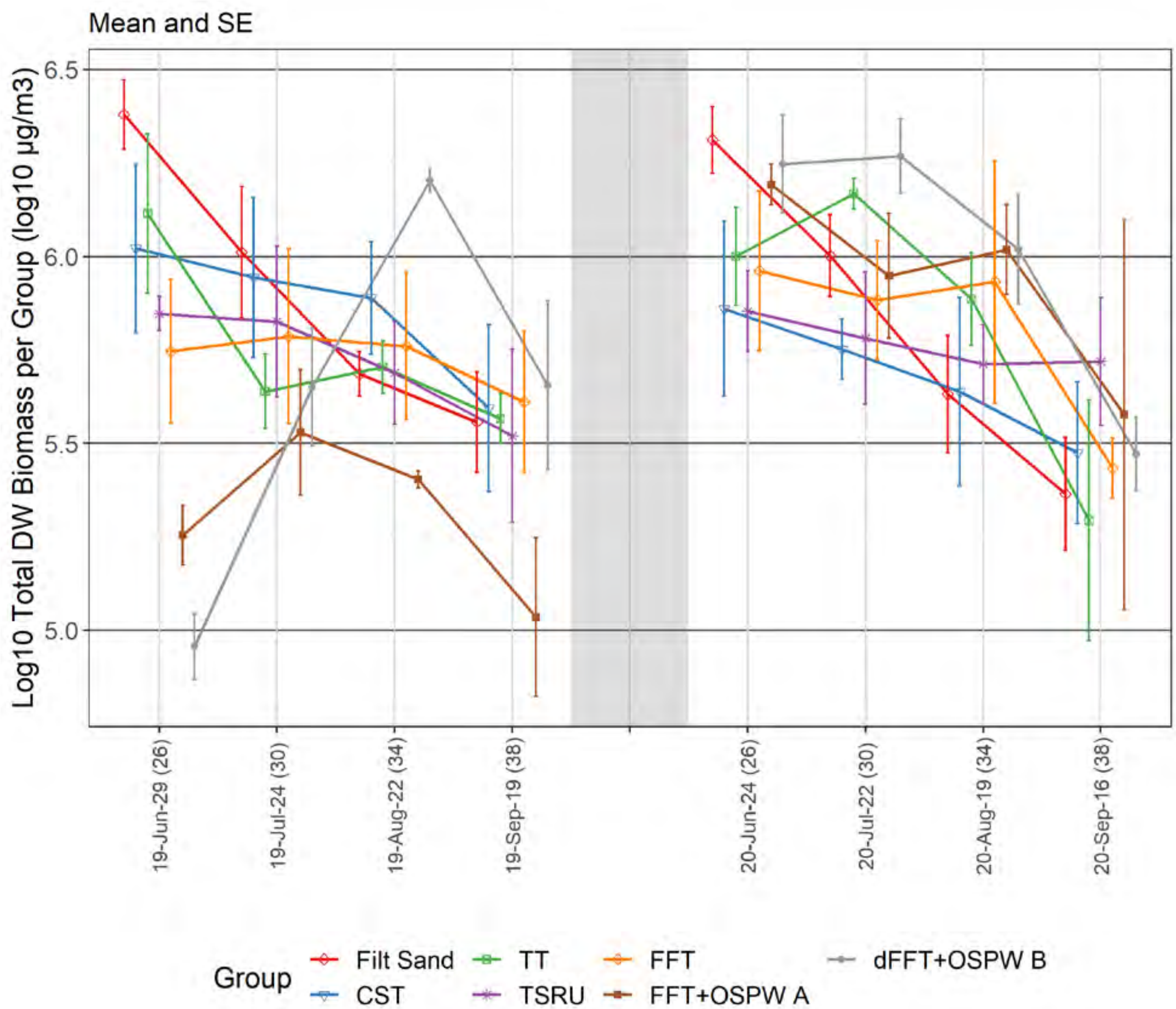

Figure 39. Zooplankton biomass in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.5.1 Effect of treatment material presence (CTL vs. each TRT)

3.5.1.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

In 2019, there were no significant differences in zooplankton species richness between tailings-only treatments (TRTs 1, 2, 3, and 4) and CTL. However, the zooplankton abundance data showed significantly reduced values in the presence of TT (TRT 2), TSRU (TRT 3) and FFT (TRT 4) compared to CTL during the beginning of 2019 (on Week 26). Unlike richness and abundance, the zooplankton diversity of TRTs 2, 3, and 4 was significantly higher than the CTL mesocosms. However, a significant reduction in diversity was observed in TRT 1 (CST) compared to CTL from Weeks 30 to 38, 2019.

Similar zooplankton families were found in CTL and TRTs 1, 2, 3, and 4 mesocosms. The exception to this was except Diptera (Figure 40), which were absent from TRT 2 (Week 34, 2019), TRT 3 (Week 34, 2019), and TRT 4 (Week 30, 2019) mesocosms. In terms of total biomass, there were no significant effects associated with the presence of tailings only treatments in 2019. The only exception was significantly lower biomass values in TRT 4 (FFT) at the beginning of 2019. In 2020, TRT 4 (FFT) had significantly lower richness and abundance at Week 30 and lower abundance during Week 30 compared to CTL.

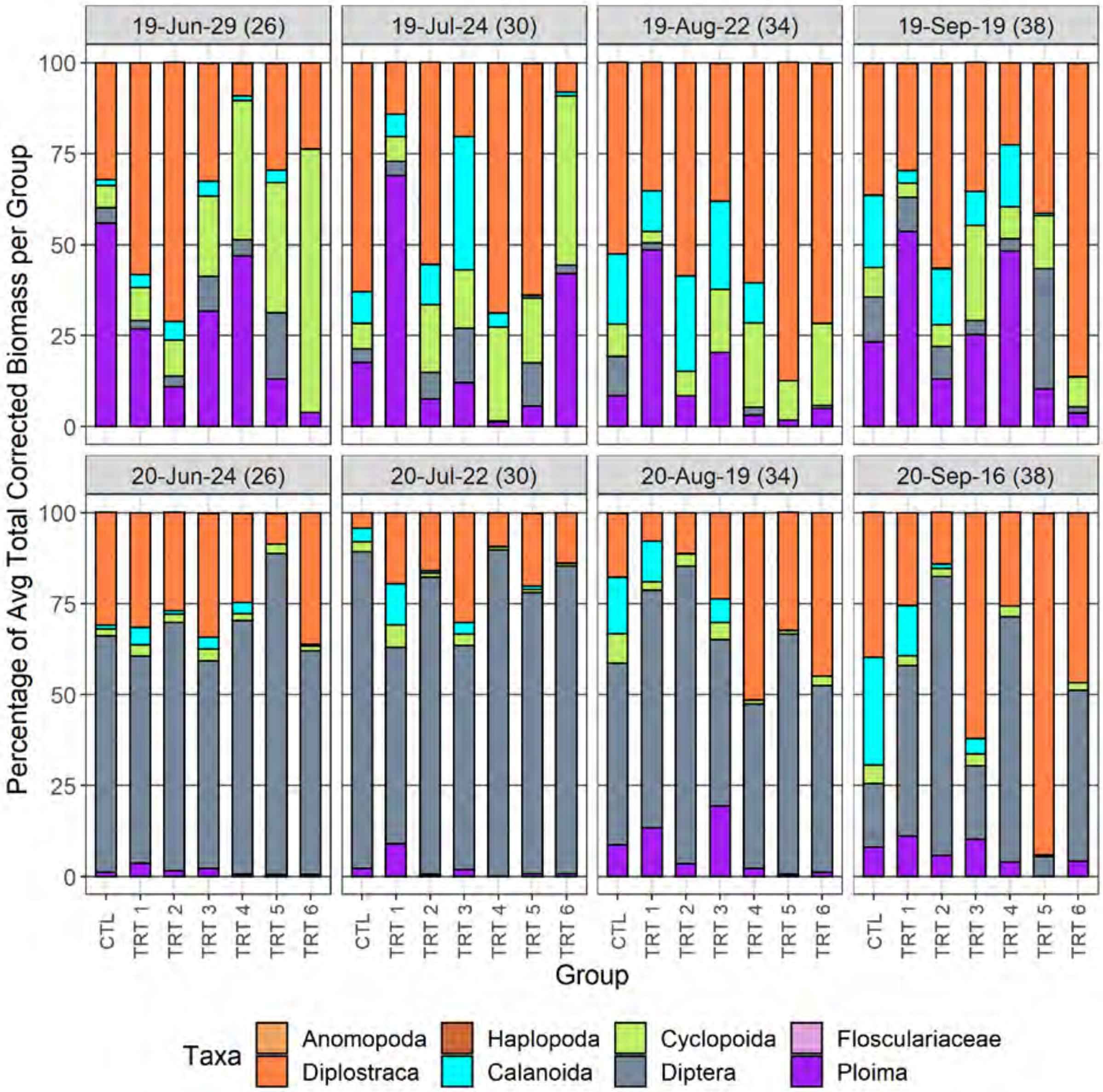

Figure 40. Zooplankton percentage of average total biomass per group taxa across groups in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW + FFT; TRT 6: 50% Suncor OSPW +dFFT.

3.5.1.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

TRT 5 mesocosms had significantly lower zooplankton species richness than CTL in Weeks 34 and 38 of 2019 and abundance was significantly lower in Weeks 26, 34, and 38 of 2019. Interestingly, TRT 5 demonstrated significantly higher diversity than CTL during Week 26 of 2019. However, diversity values dropped significantly in TRT 5 compared to CTL during Weeks 30 and 34. It was also noted that Diptera was not present in Week 34 (Figure 40). There was a significant effect in total zooplankton biomass associated with the materials in TRT 5 at the beginning and end of 2019.

TRT 5 had significantly lower richness and abundance, compared to CTL in Week 26 of 2020 and Weeks 26 and 34 of 2020, respectively. Zooplankton diversity and biomass were not significantly different between TRT 5 and CTL.

3.5.1.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

TRT 6 mesocosms had significantly lower zooplankton species richness, abundance, diversity, and total biomass compared to CTL at least once in 2019. These significant reductions might be linked to the fact that TRT 6 lacked Diptera in 2019 (Figure 40). Interestingly, TRT 6 demonstrated a significantly higher abundance and total biomass than CTL in Week 34 of 2019 and diversity values were significantly higher in TRT 6 compared to CTL during Week 26 of 2019.

In 2020, there were no significant effects on zooplankton species richness, abundance, diversity and total biomass associated with the presence of 50% Suncor OSPW with dFFT. The only exception noticed was that the abundance of zooplankton in TRT 6 was significantly lower compared to CTL in Week 34.

3.5.2 Effect of treatment of tailings (TRT 2 vs. TRT 4)

Overall, there were no significant effects associated with different tailings materials on zooplankton richness, abundance, diversity, and biomass in 2019 and 2020. The exceptions noticed were that the richness of TRT 4 (FFT) was significantly lower than TRT 2 (TT) during Week 30 of 2019, Weeks 30 and 34 of 2020. In addition, diversity was significantly reduced in TRT 4 compared to TRT 2 at the end of 2020.

3.5.3 Effect of 100% Imperial OSPW in the Presence of FFT (TRT 4 vs. TRT 5)

There were no significant effects on zooplankton species richness, abundance, diversity, and biomass associated with 100% Imperial OSPW in the presence of FFT, with a few exceptions. TRT 4 (FFT) produced significantly higher richness, abundance, and diversity than TRT 5 (FFT+100% Imperial OSPW) twice over the study. However, diversity in TRT 4 was significantly lower than TRT 5 at the end of 2020. Zooplankton biomass results per group showed minimal Calanoida in TRT 5 than TRT 4 at the end of 2019 (Figure 40). The absence of Diptera was noticed in TRT 4 and TRT 5 during Weeks 30 and 34 of 2019, respectively (Figure 40).

3.5.4 Effect of Time

For zooplankton species richness, a statistically significant mid-study increase was found in CTL, TRT 1 (CST), TRT 4 (FFT), and TRT 6 (dFFT+50% Suncor OSPW) mesocosms. No trend or fluctuation in abundance was found overtime in TRT 1 (CST) and TRT 4 (FFT). Zooplankton abundance in CTL, TRT 2 (TT), TRT 3 (TSRU) and TRT 5 (FFT + 100% Imperial OSPW) mesocosms showed a significant mid-study decrease, while a significant mid-study increase was found in TRT 6 mesocosms. A significant mid-study increase in zooplankton diversity was observed for CTL, TRT 3 and 4 mesocosms.

There were no clear zooplankton biomass trends for any zooplankton family, except Diptera were less abundant in 2019 than in 2020 (Figure 40). However, zooplankton biomass in TRT 1 mesocosms decreased significantly over the study, while TRT 6 mesocosms had a significant mid-study increase in biomass over time.

3.6 Macroinvertebrates

Figures and tables detailing macroinvertebrate statistical analyses can be found in Section A.11 of Appendix A. Macroinvertebrate communities were compared according to species richness, abundance, diversity (measured by Shannon Weiner H), taxa tolerance (expressed by Hilsenhoff Biotic Index), and Functional Groups.

3.6.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

3.6.1.1 Activity traps

In 2019, only TRT 4 (FFT) demonstrated significantly lower taxa richness than CTL in Weeks 30 and 34 (Figure 41). No significant differences in abundance and diversity were seen between experimental groups and CTL. The only exception was that TRT 4 (FFT) had significantly less diversity at the beginning of 2019. The presence of tailings did not produce significantly lower Hilsenoff Biotic tolerance values in 2019. In examining the functional groups, the reduction in taxa richness and diversity in TRT 4 (FFT) mesocosms might be associated with the loss of Collector-Filter at the beginning of 2019. In 2020, no significant differences were seen between CTL and TRTs 1, 2, 3, and 4 in any of the measured ecological parameters.

3.6.1.2 Hester-Dendy samplers

TRT 4 (FFT) demonstrated significantly lower Hilsenhoff Biotic values than CTL in Week 40 of 2019. The reduction of HBI values at the end of 2019 might be associated with a higher percentage of Predators. No other significant differences were seen between experimental groups and CTL for richness, abundance, diversity, and HBI in 2019. Exceptions only applied to elevate abundance in TRT 2 (TT) at the beginning of 2019. In examining the functional groups, the elevation in abundance in TRT 2 mesocosms might be associated with the more Collector-Gathers at the beginning of 2019.

In 2020, TRT 2 (TT) demonstrated significantly higher richness than CTL in Weeks 32 and 40 (Figure 42). TRT 4 (FFT) demonstrated significantly higher abundance values than CTL in Week 36 of 2020. No other significant differences were detected between CTL and TRTs 1, 2, 3, and 4 in any of the measured ecological parameters.

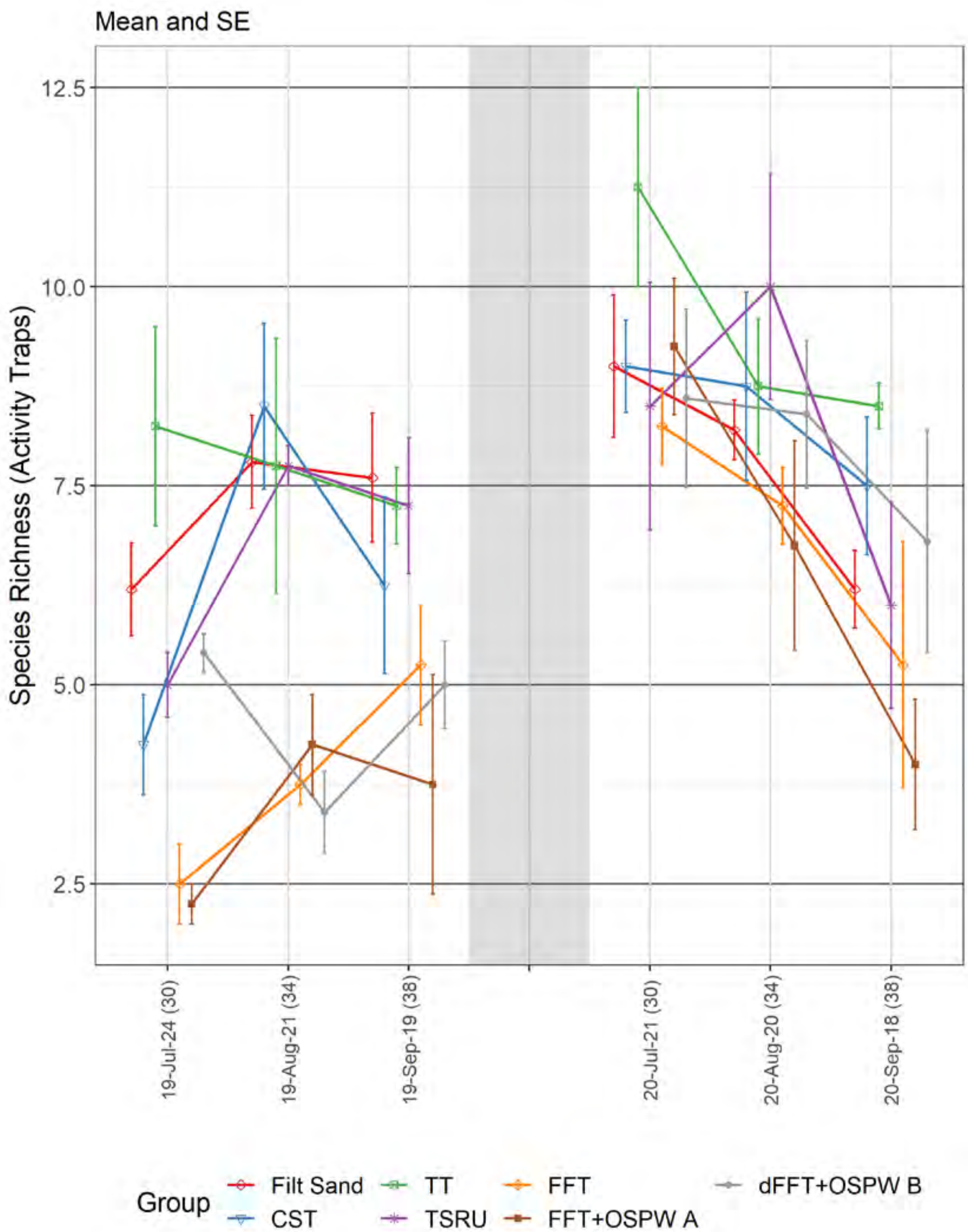

Figure 41. Macroinvertebrate species richness in samples collected by activity traps in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

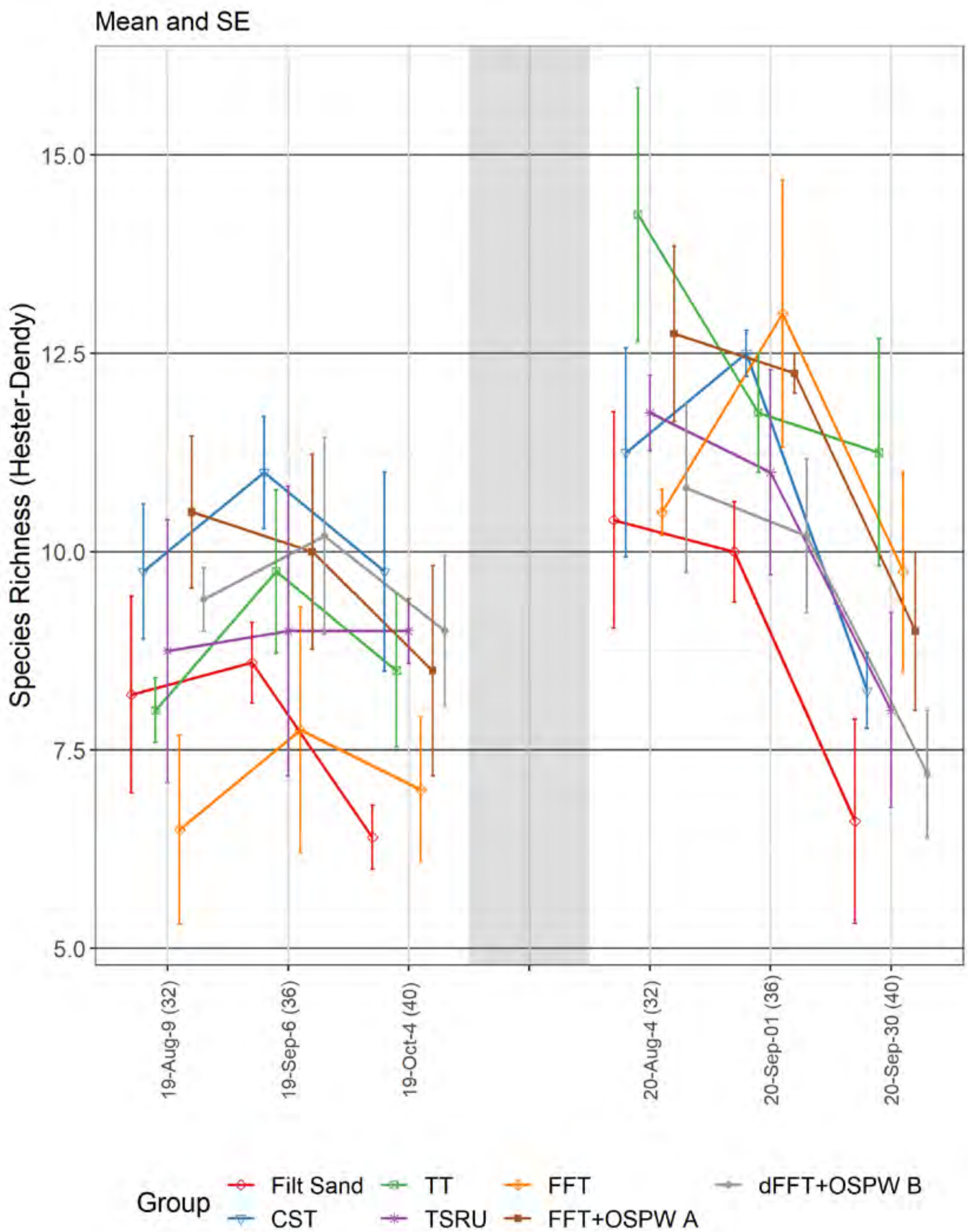

Figure 42. Macroinvertebrate species richness in samples collected by Hester-Dendy (H-D) samplers in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.6.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

3.6.2.1 Activity traps

A significant reduction in macroinvertebrate richness, abundance, and diversity was observed in TRT 5 compared to CTL in at least one sampling time point in 2019 (Figure 43). However, there were no significant differences in HBI in samples collected by activity traps. The reduction in richness, abundance and diversity in TRT 5 might be related to the Shredder-Herbivores group's presence at the beginning of 2019 and the Collector-Filter group's presence end of 2019. In 2020, no significant differences were observed between CTL and TRT 5 for any of these parameters.

3.6.2.2 Hester-Dendy samplers

In 2019, there were no significant effects on macroinvertebrate species richness and diversity associated with 100% Imperial OSPW with FFT. Interestingly, TRT 5 produced significantly higher macroinvertebrate abundance than CTL during Weeks 36 and 40 (Figure 44). Also, HBI values in TRT 5 dropped significantly in Weeks 32 and 40.

In Week 26 of 2020, macroinvertebrate abundance in TRT 5 was significantly higher than CTL. However, diversity HBI values dropped significantly at least once in TRT 5 compared to CTL. The lack of Scrapers functional group in TRT 5 might account for the differences observed.

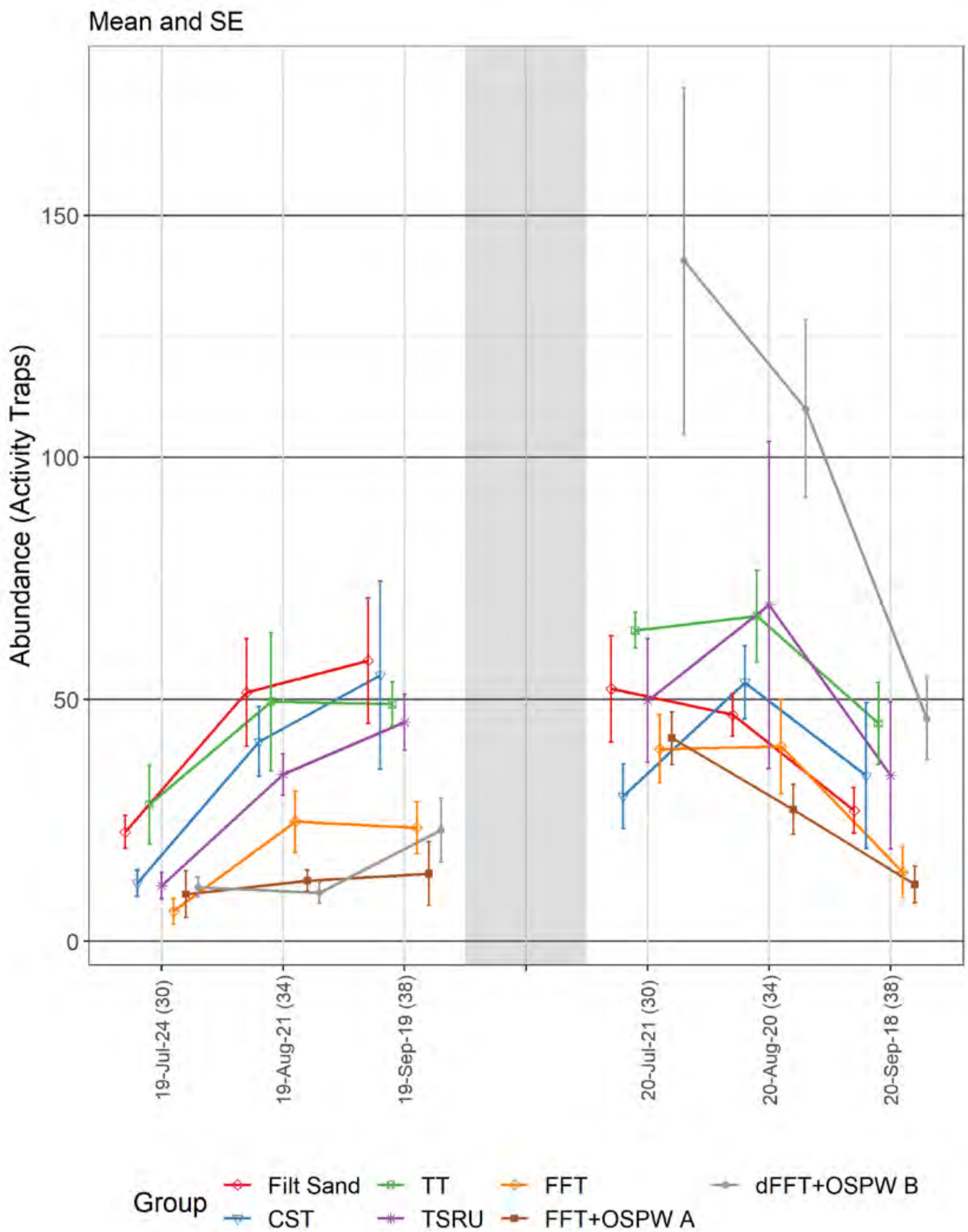

Figure 43. Macroinvertebrate abundance in samples collected by activity traps in 2019 and 2020. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

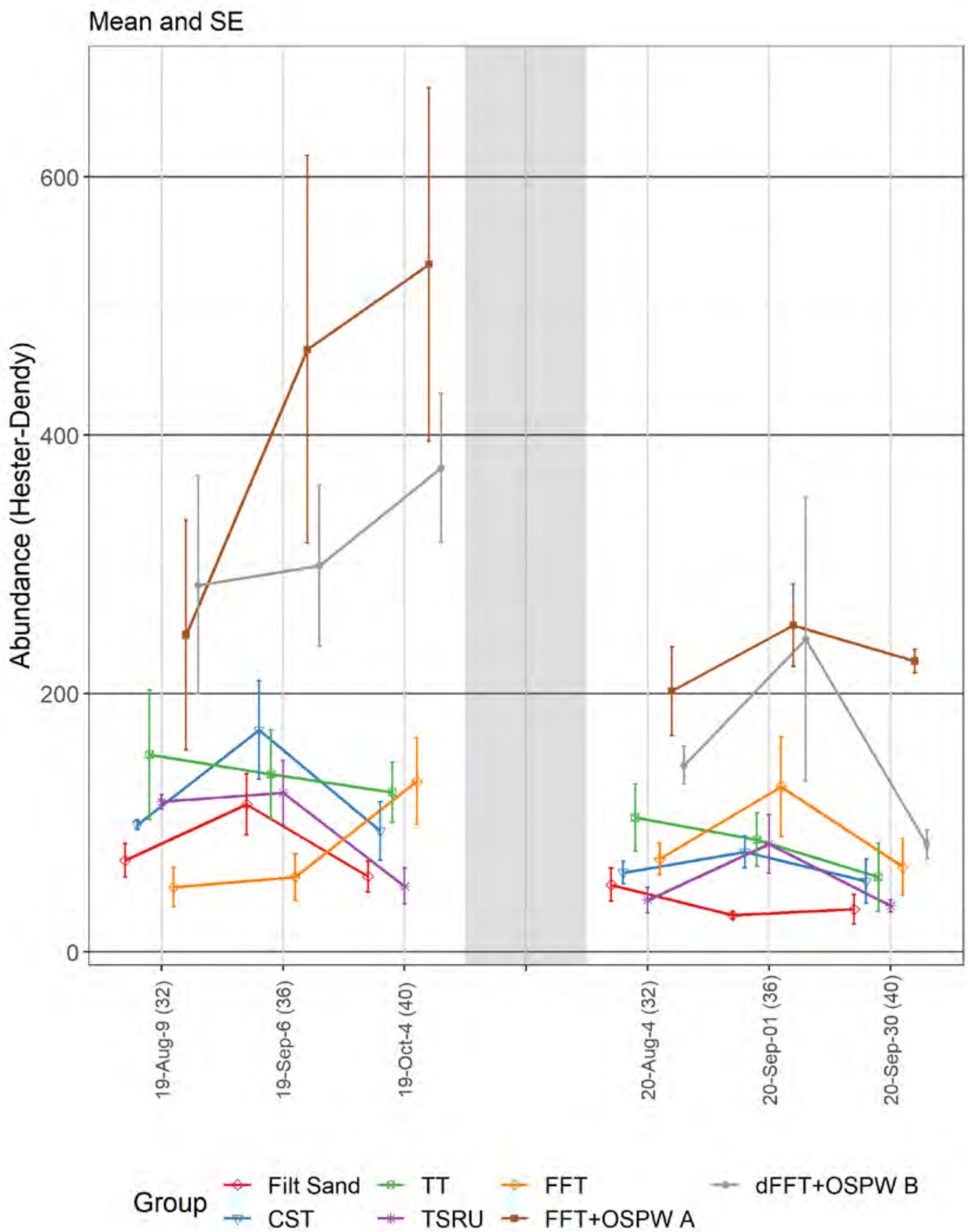

Figure 44. Macroinvertebrate abundance in samples collected by Hester-Dendy (H-D) samplers in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.6.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

3.6.3.1 Activity traps

TRT 6 had significantly lower macroinvertebrate species richness and abundance at least once in 2019. This could be attributed to differences in abundances of functional groups; there was a lower proportion of Scrapers and a higher proportion of Collector-Gathers group in early 2019.

There were no significant effects on diversity and HBI in the presence of 50% Suncor OSPW with dFFT in 2019. However, significant diversity reductions were noted at the beginning of 2020 (Figure 45). Surprisingly, TRT 6 demonstrated significantly higher abundance compared to CTL during Weeks 30 and 34 of 2020.

3.6.3.2 Hester-Dendy samplers

For the samples collected in 2019 by Hester-Dendy samplers, there were no significant effects in macroinvertebrate richness and diversity in the presence of 50% Suncor OSPW with dFFT. Similar to what was observed in TRT 5, macroinvertebrate abundance was significantly higher, whereas HBI was significantly reduced compared to CTL, on at least two occasions in 2019. In 2020, TRT 6 had significantly higher abundance at Week 36 than did in CTL. In addition, TRT 6 demonstrated significantly lower diversity values compared to CTL at Weeks 32 and 36 of 2020 (Figure 46).

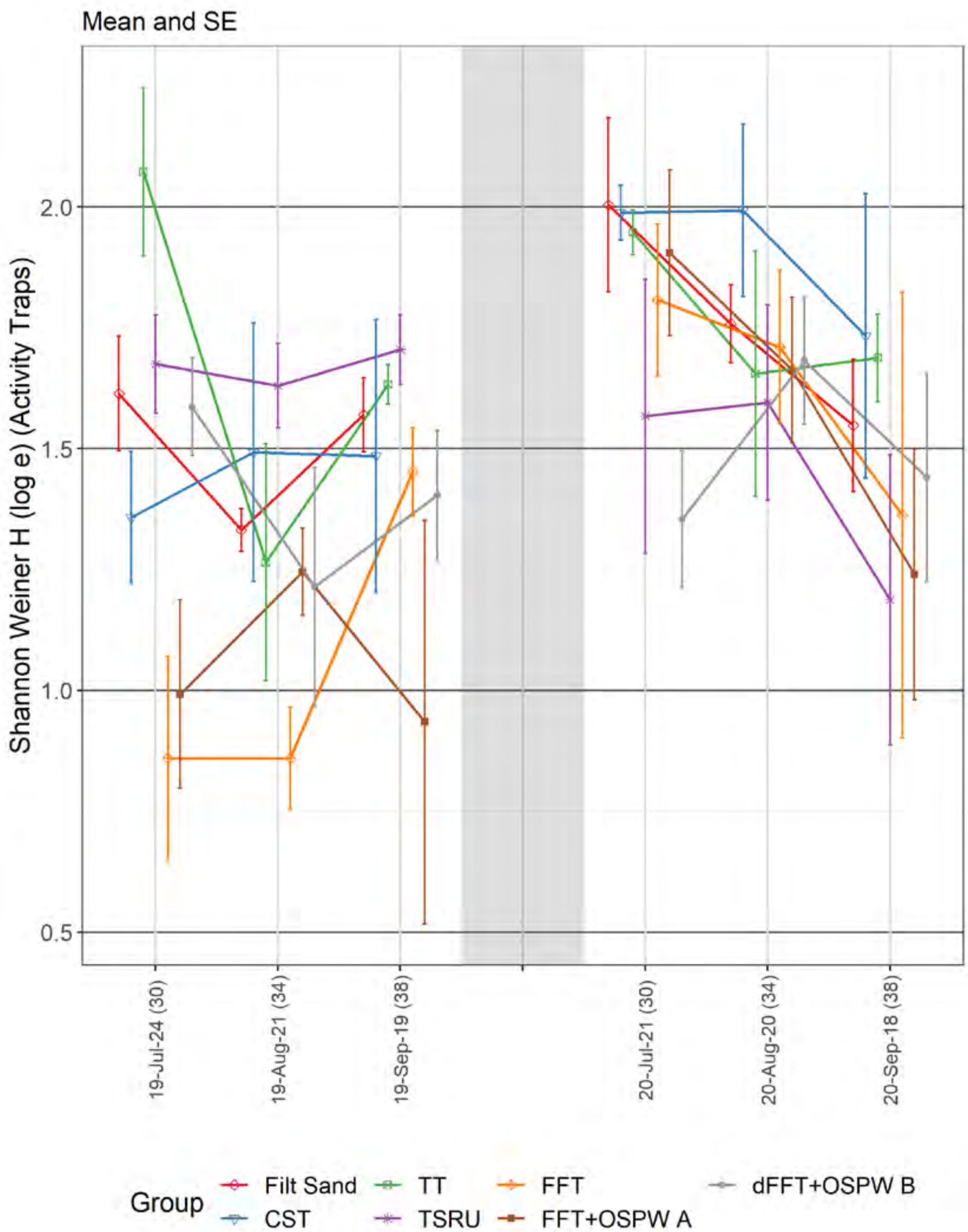

Figure 45. Macroinvertebrate diversity in samples collected by activity traps in 2019 and 2020. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

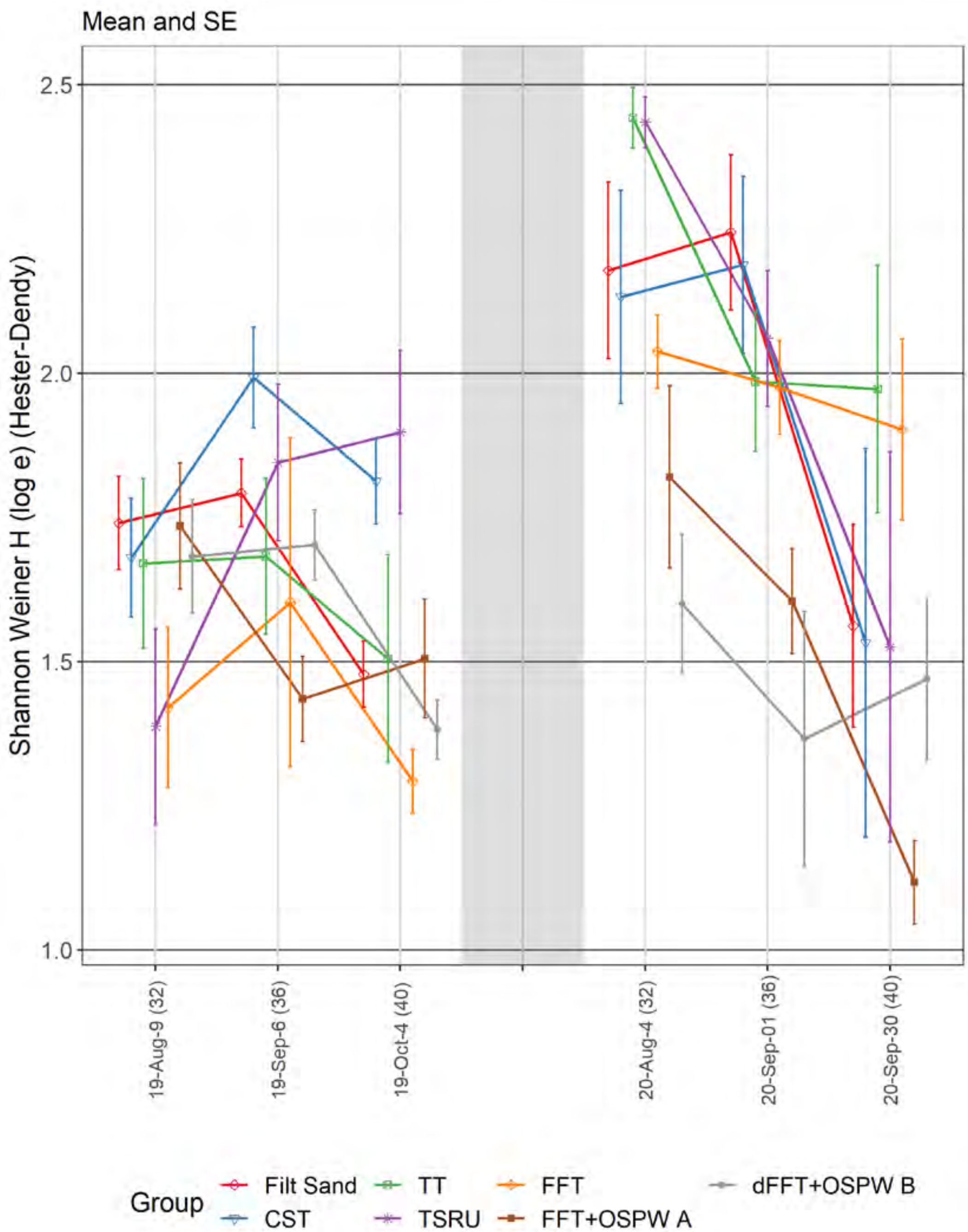

Figure 46. Macroinvertebrate diversity in samples collected by H-D samplers in 2019 and 2020. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.6.4 Effect of treatment of tailings (TRT 2 vs. TRT 4)

3.6.4.1 Activity traps

By Week 34 of 2019, TRT 4 (FFT) had a significant reduction in macroinvertebrate species richness compared to TRT 2 (TT), but this difference was not significant until the end of 2019. Overall, there were no significant differences in abundance, diversity and HBI associated with the tailing treatments. The only exception was significantly lower diversity values were observed in TRT 4 during the beginning of 2019. This reduction could be associated with the loss of the Collector-Filter group (Figure 47). In 2020, TRT 4 had significantly higher richness values at Weeks 30 and 38. No other significant differences were detected between TRTs 2 and 4.

3.6.4.2 Hester-Dendy samplers

By Week 36 of 2019, TRT 4 (FFT) demonstrated significantly lower macroinvertebrate abundance values, compared to TRT 2 (TT). TRT 4 also had a significant reduction in macroinvertebrate species richness compared to TRT 2 at Week 32, 2020. This difference might be associated with a lower portion of Scrapers and a higher percentage of Predators group in TRT 2 in early 2020 (Figure 48). No significant differences for any other measured parameters were detected between TRTs 2 and 4 in 2019 and 2020.

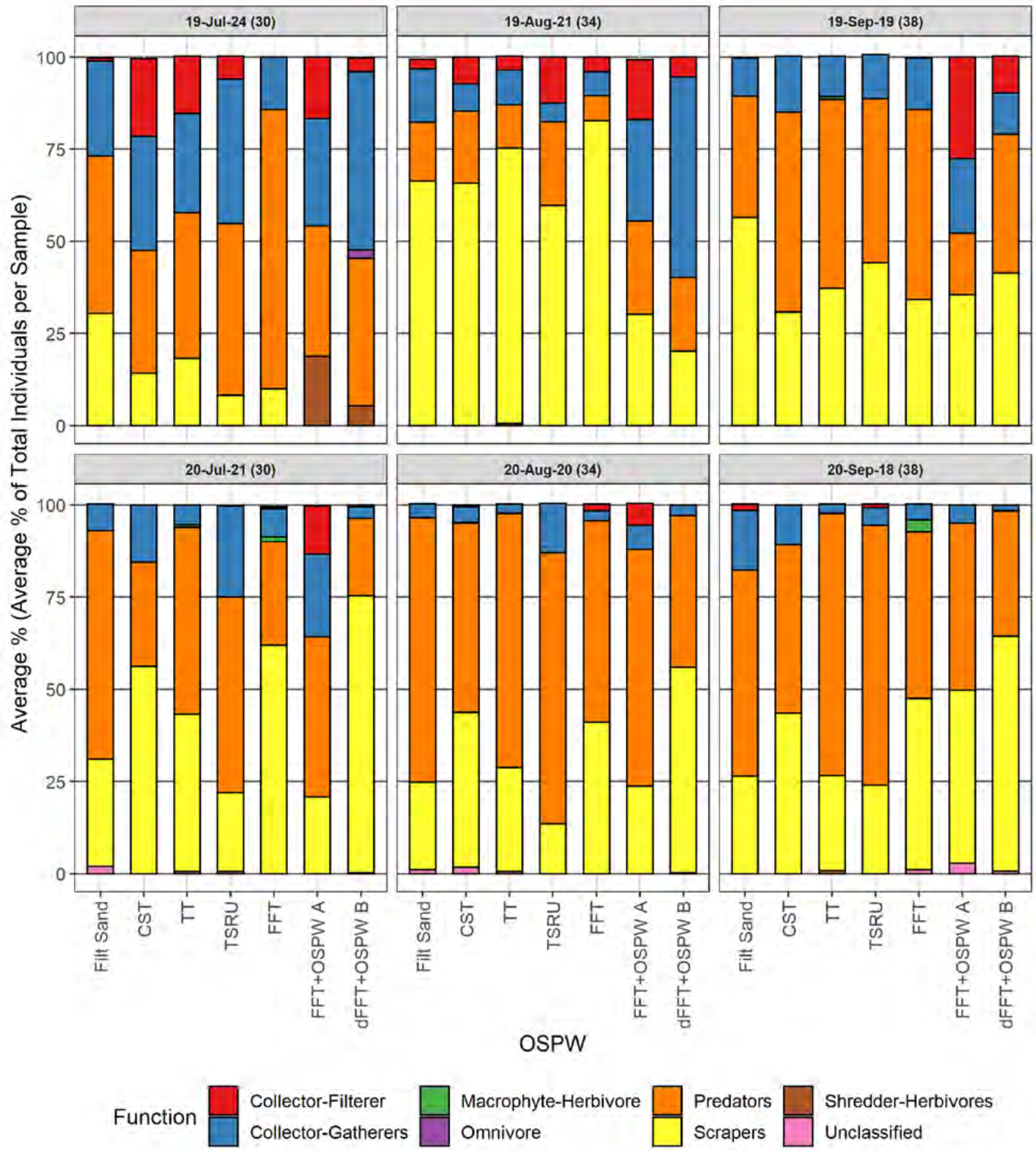

Figure 47. Macroinvertebrate functional groups in samples collected by activity traps in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

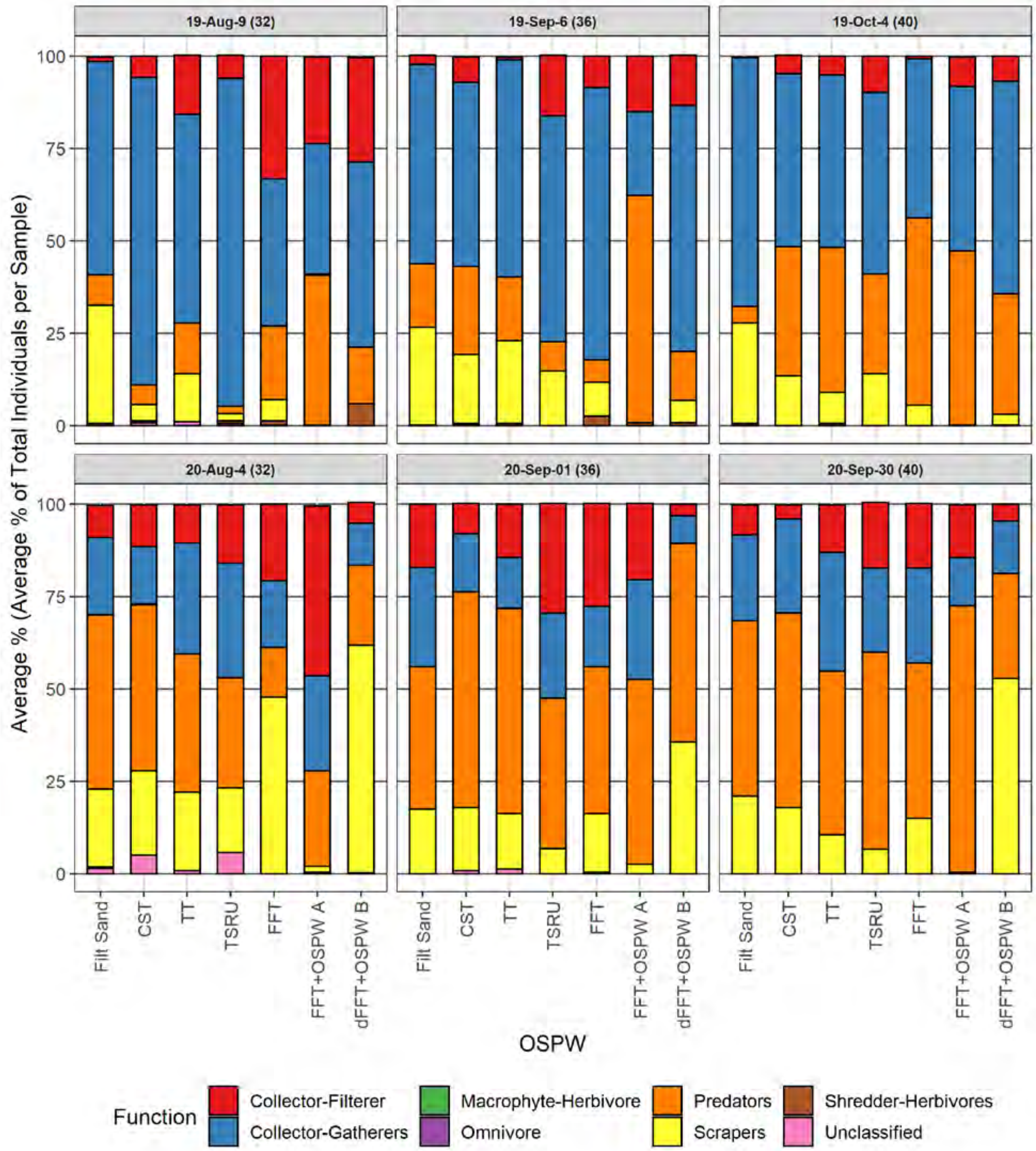

Figure 48. Macroinvertebrate functional groups in samples collected by H-D samplers in 2019 and 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.6.5 Effect of 100% Imperial OSPW in the Presence of FFT (TRT 4 vs. TRT 5)

3.6.5.1 Activity traps

During the study, there were no significant effects on macroinvertebrates (as collected by activity traps) associated with mesocosms with 100% Imperial OSPW and FFT, compared to mesocosms with FFT only.

3.6.5.2 Hester-Dendy samplers

It is noteworthy that TRT 4 (FFT) had significantly lower macroinvertebrate richness and abundance along with significantly higher HBI than TRT 5 (FFT+100% Imperial OSPW) during at least one sampling week in 2019. The diminished richness and abundance in TRT 4 might be related to less Predators group. In 2020, TRT 4 demonstrated a significantly higher diversity than TRT 5 at Week 40. No other significant differences were seen between TRTs 4 and 5.

3.6.6 Effect of Time

3.6.6.1 Activity traps

No change or fluctuation in macroinvertebrate species richness was observed between TRT 2 (TT) and TRT 6 (dFFT+50% Suncor OSPW). However, a significant mid-study increase in species richness was noticed for CTL, TRT 1 (CST), TRT 3 (TSRU), TRT 4 (FFT), and TRT 5 (FFT+100% Imperial OSPW). In general, species richness values were the highest at Week 30 of 2020. There were no clear overall trends in abundance for TRTs 1, 2, 4, and 5. However, a significantly mid-study increase in abundance was noticed for CTL, and TRTs 3 and 6.

A significant increase or mid-study increase in macroinvertebrate diversity was noted for TRT 1 (CST), TRT 4 (FFT), and TRT 5 (FFT+100% Imperial OSPW). Only one significant increased case was found in TRT 6 (dFFT+50% OSPW) for tolerance values (HBI). In examining the functional groups, noticeable abundance of Shredder-Herbivores and lack of Scrapers were observed in OSPW associated groups (TRT 5 and TRT 6) in early 2019, while limited Collector-Filterers were found in CTL and TRTs 1, 2, 3, and 4 (tailings-only treatments) in late 2019. In 2020, a limited number of Macrophyte-Herbivores was found in FFT only treatment (TRT 4).

3.6.6.2 Hester-Dendy (H-D) samplers

No trends or fluctuations in macroinvertebrate species richness was detected for most samples collected by Hester-Dendy samplers over time. However, a significant increase was found in TRT 2 (TT) and TRT 4 (FFT). Interestingly, macroinvertebrate abundance showed a significant mid-study increase for TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT+50% Suncor OSPW), and these values were the highest at Week 40, 2019 over the study period.

In terms of macroinvertebrate diversity, no trends or fluctuations was seen over time, except for a significant increase or mid-study increase in tailings-alone mesocosms (TRTs 1, 2, 3, and 4). The tolerance values (HBI) trend was similar to activity traps, except for a significant decrease in CTL and a significant increase in TRT 6 (dFFT+50% OSPW, Figure 50). It is noteworthy that Scrapers appeared over time in TRT 6 but rarely in TRT 5 (FFT+100% Imperial OSPW).

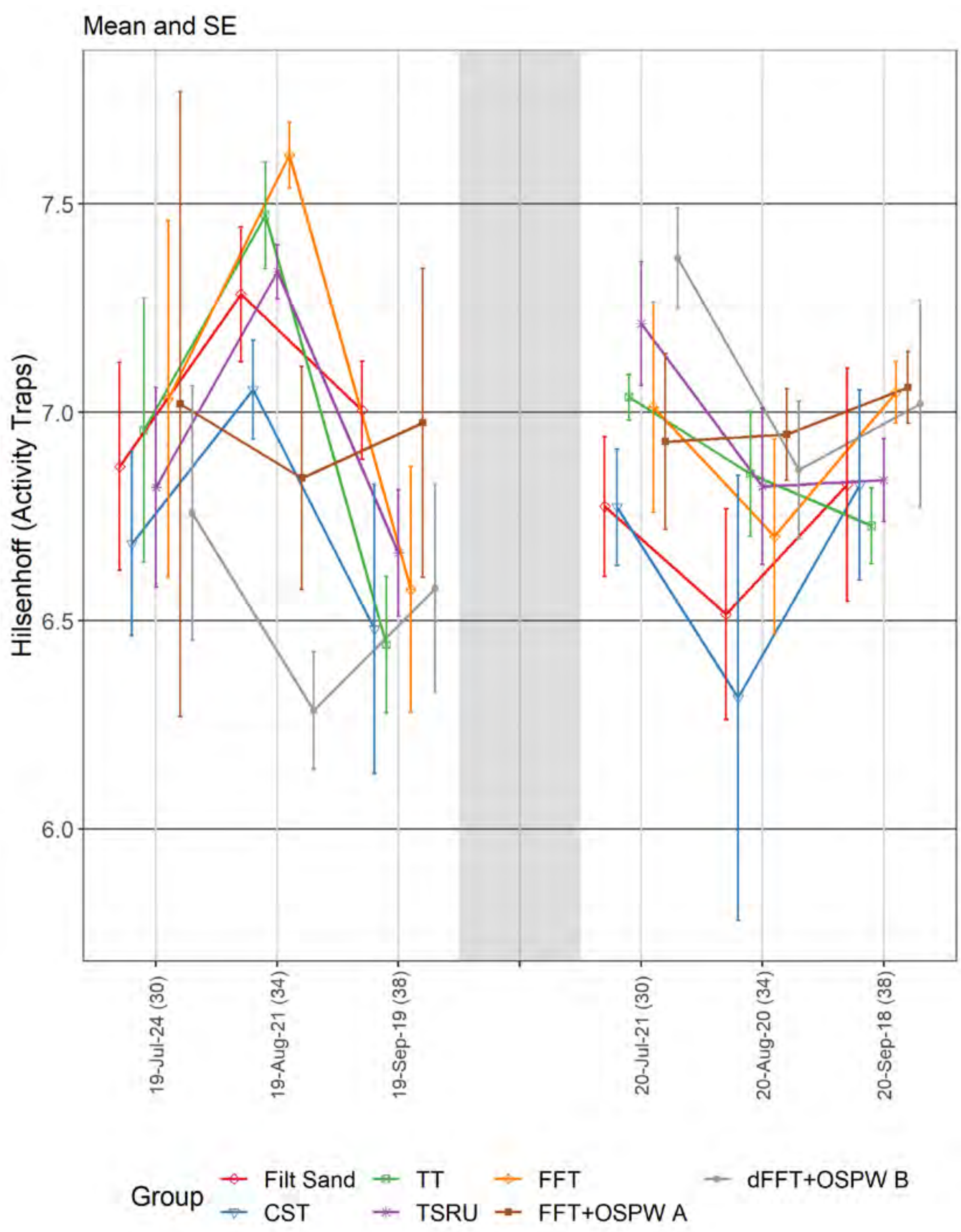

Figure 49. Macroinvertebrate HBI in samples collected by activity traps in 2019 and 2020. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

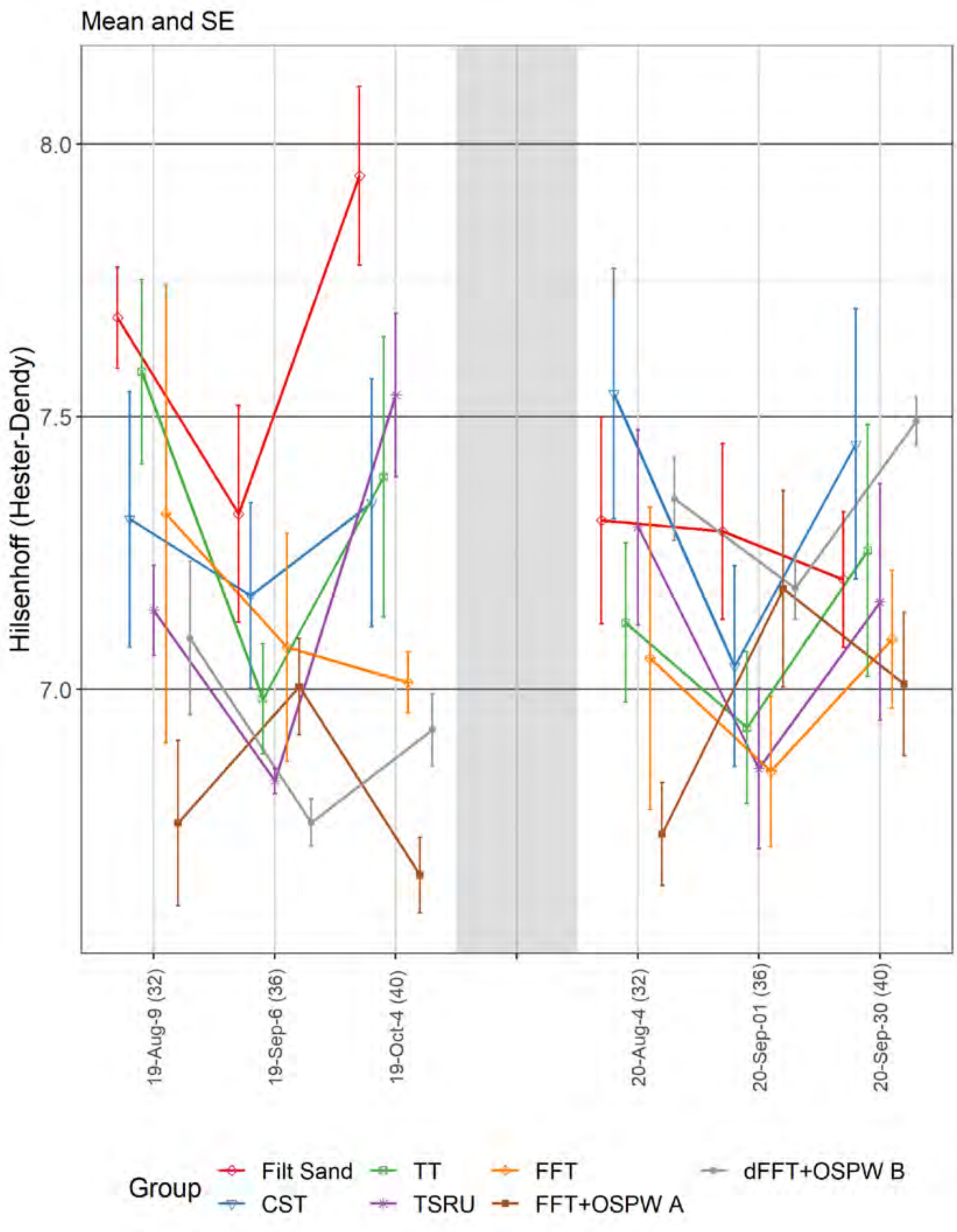

Figure 50. Macroinvertebrate HBI in samples collected by H-D samplers in 2019 and 2020. CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.6.7 Effect of Sampling Methods

Macroinvertebrate taxa caught by each method were compared by species richness, abundance, diversity and HBI. It was perceived that activity traps produced relatively lower values for richness and abundance compared to H-D samplers, which was expected due to longer deployment period of H-D samplers. The abundance level from activity traps was generally much lower (an order of magnitude for some cases) than that for H-D samplers. However, the similarity of diversity and HBI was perceived overall. The level of variability observed was large and perceived as similar between the two methods. As expected, ecological functional groups shifted between two different methods. Most notably, more Scrapers were found in samples collected by activity traps, while more Collector-Gathers from H-D samplers. The lack of relationship in the indices of activity traps and H-D samplers indicates that both sampling methods should be used to maximize the success of capturing macroinvertebrates communities.

3.7 Visual Estimation of Phytoplankton

As introduced in Section 2.13.8, the term phytoplankton is used here to describe what visually appears to be algal material with no specific form but is free-floating in the water column. However, as the taxonomic analysis is beyond this study's scope, the coverage estimation was most likely confounded with the presence of metaphyton. More details about the visual assessment of phytoplankton can be found in Section A.7 of the Statistical Analysis. Phytoplankton surface area coverage is summarized below for 2020 only (Figure 51), because phytoplankton/metaphyton was not observed in 2019. The comparative analyses focus on relative differences between mesocosms rather than absolute value quantification. Hence, when estimating phytoplankton coverage, the total surface area of the mesocosm was used; the area covered by the floating rafts was not accounted for.

3.7.1 Effect of treatment material presence (CTL vs. each TRT)

3.7.1.1 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

A significant difference phytoplankton coverage between CTL and TRTs 1, 2, 3, and 4 was observed from Weeks 26 to Week 41, 2020.

3.7.1.2 Effect of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

The overall coverage of phytoplankton in CTL was significantly higher than in TRT 5 (100% Imperial OSPW with FFT) from Weeks 26 to Week 41, 2020.

3.7.1.3 Effect of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

As TRT 5, a significant reduction compared to CTL was observed in TRT 6 (50% Suncor OSPW with dFFT) from Weeks 26 to 41, 2020.

3.7.2 Effect of treatment of tailings (TRT 2 vs. TRT 4)

There were no significant differences in phytoplankton coverage between TRT 2 (TT) and TRT 4 (FFT).

3.7.3 Effect of 100% Imperial OSPW in the Presence of FFT (TRT 4 vs. TRT 5)

At Week 24 of 2020, TRT 4 (FFT) mesocosms produced significantly higher phytoplankton coverage than TRT 5 (FFT+100% Imperial OSPW). However, in Week 26 of 2020, phytoplankton coverage in TRT 4 reduced significantly, compared to TRT 5 (FFT+100% Imperial OSPW). No significant differences between TRTs 4 and 5 could be found at any other time point.

3.7.4 Effect of Time

Phytoplankton (metaphyton) surface area coverage followed a mid-study increase in CTL and TRT 3 (TSRU). There were no clear trends for the other treatment mesocosms.

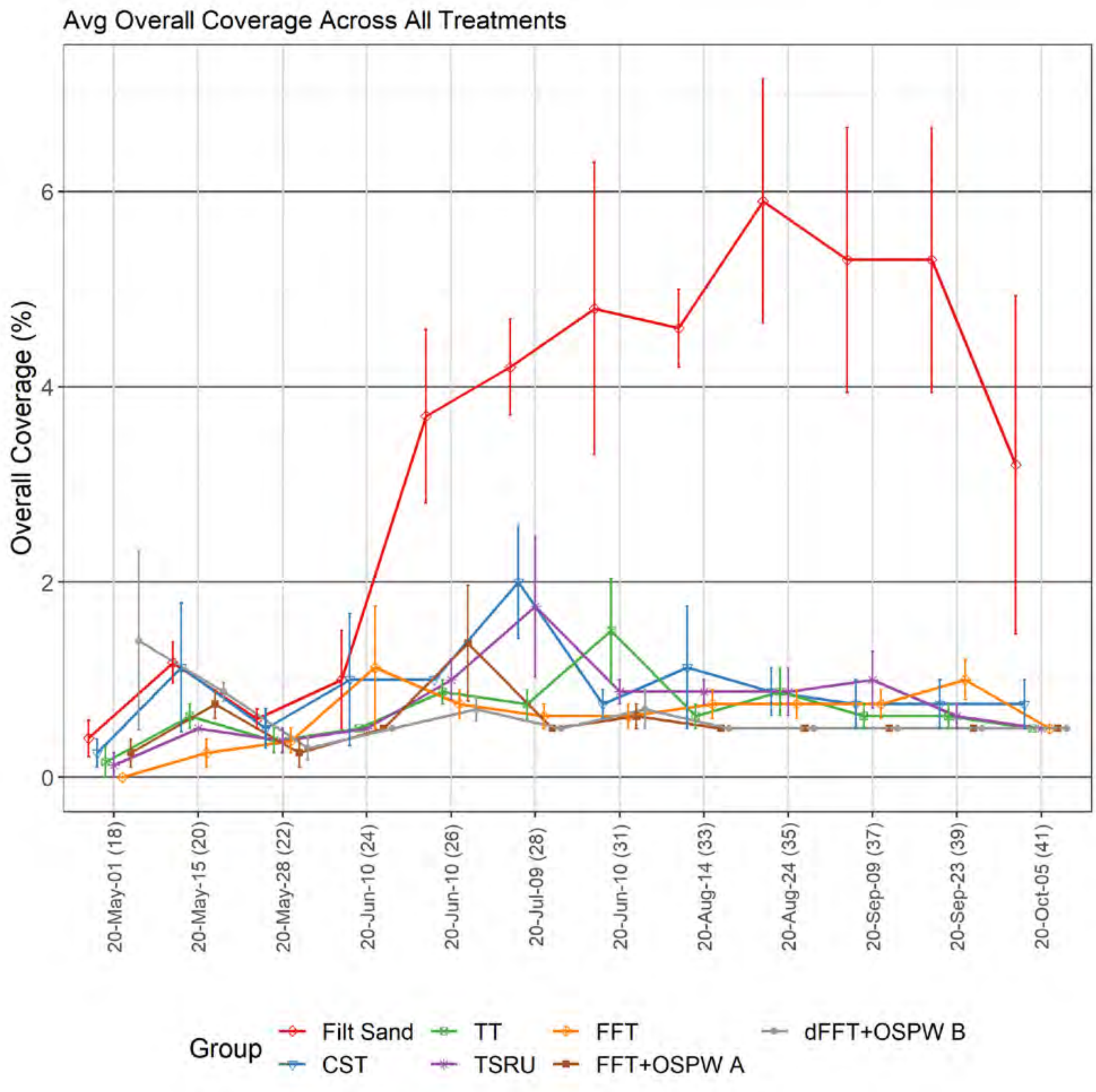

Figure 51. Phytoplankton surface area coverage in mesocosms in 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.8 Data Loggers

Detailed figures recorded by the data loggers, including dissolved oxygen and conductivity, can be found in Section A.4 of Appendix A. Note that data loggers were placed on the soil trough floor (around 100 cm depth), while data sonde was measurements were taken below the surface of the water and at a depth of 125 cm. Since only one logger was deployed at each experimental group, no statistical comparisons were conducted. Because water temperature was used to calculate specific conductivity by the loggers, the loggers' temperature data was not reported. In 2019, some loggers failed where no data was shown, hence the results from 2020 mainly reported in the following sections.

3.8.1 Dissolved Oxygen

In general, diel fluctuations in DO did not vary substantially between CTL and TRTs 1, 2, and 3 (CST/TT/TSRU) in 2020. However, FFT and OSPW associated mesocosms (TRTs 4, 5, and 6) appear to have produced qualitatively smaller swings in DO compared to CTL in 2020. The CTL mesocosms produced qualitatively decreasing DO concentrations in 2020, especially at the beginning of the year (Week 19 to Week 29). No similar trend was observed in other treatment groups in 2020 (Figure 52). Interestingly, it is noted that TRT 6 (dFFT + 50% Suncor OSPW) had an increasing DO trend over the first half of the 2020 study and relatively lower DO levels compared to other experimental groups at the beginning (Weeks 19 to 29) and end of 2020 (Weeks 35 to 41).

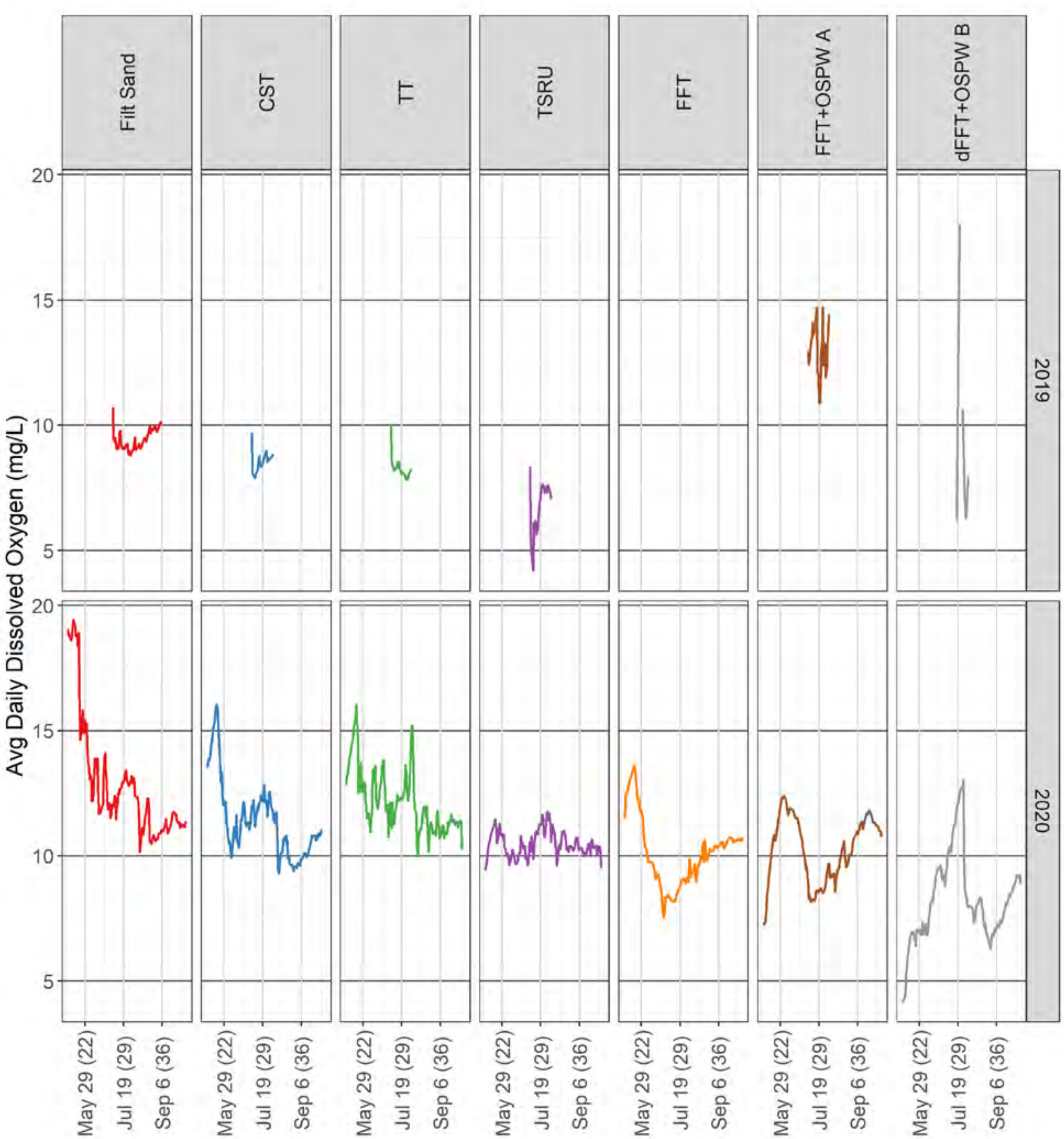

Figure 52. Dissolved oxygen (DO) recorded in mesocosms.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.8.2 Specific Conductivity

All mesocosm treatments had decreasing conductivity trends over time in 2019 and at the beginning of 2020. In 2020, this gradual decline disappeared around Week 22 for CTL and TRTs 1, 2, 3, and 4 (tailings-only) (Figure 53). In 2020, the initial decrease in conductivity in TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW) lasted longer (i.e., l Week 27 and Week 29, respectively), and was followed by a slight increase. The diel swings did not vary visibly between experimental groups in 2020.

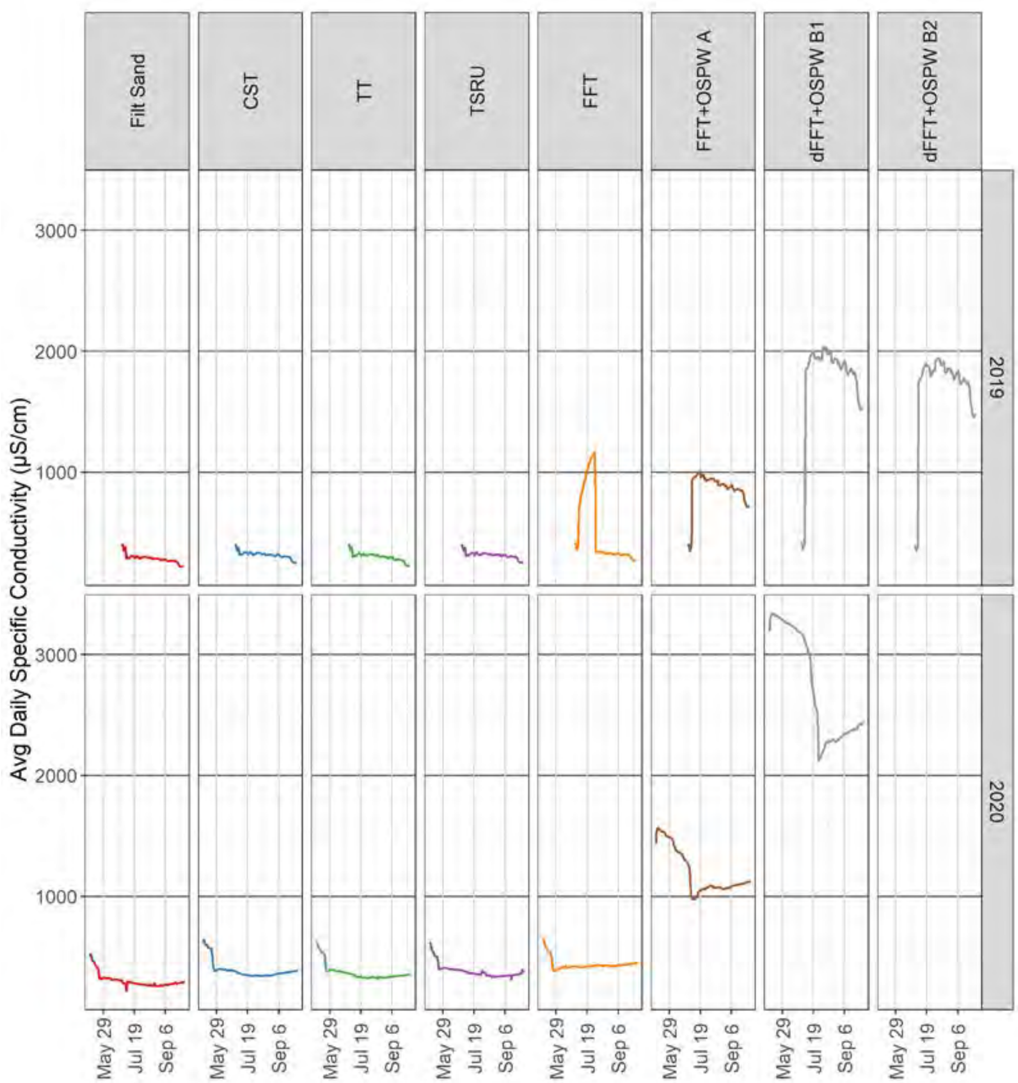

Figure 53. Specific conductivity recorded in mesocosms.
Note that two conductivity probes were used for TRT 6 in 2019 and only one in 2020.
CTL: 100%ARW+FS; TRT 1: 100%ARW+CST; TRT 2: 100%ARW+TT; TRT 3: 100%ARW+TSRU; TRT 4: 100%ARW+FFT; TRT 5: 100% Imperial OSPW A+ FFT; TRT 6: 50% Suncor OSPW B+dFFT.

3.9 Anecdotal Observations

In 2020, several anecdotal observations were made and are qualitatively reported below with accompanying representative photos.

3.9.1 Adventitious Vegetation

Natural colonization of adventitious vegetation was noticeable in CTL and TRTs 1 (CST), TRT 2 (TT), and TRT 3 (TSRU) mesocosms, especially in the soil troughs (Figure 54). It was outside of this study's scope to do complete detailed vegetation assessments for these species. These plants were preliminarily identified based on morphology, and included *Callitriche palustris* (vernal water starwort), *Potamogeton pusillus* (slender pondweed), *Ranunculus aquatilus var eradicus* (white water crowfoot), *Marsilea vestita* (hairy water clover) and *Chara spp.* (stonewort). Note that the last species is a green alga and not a vascular plant. However, few aquatic plants were found in the FFT and OSPW associated mesocosms (TRTs 4, 5, and 6, Figure 55).

CTL, 100%ARW+FS   TRT 1, 100%ARW+CST

TRT 2, 100%ARW+TT   TRT 3, 100%ARW+TSRU

Figure 54. Extensive adventitious vegetation colonization in CTL and TRTs 1, TRT 2, and TRT 3 mesocosms.
Pictures were taken on October 5, 2020.

Data loggers in CTL and TRTs 1, 2, and 3 mesocosms were covered over by massive adventitious plants/algae (Figure 56).

TRT 4, 100%ARW+FFT TRT 5, 100%OSPW-I+ FFT TRT 6, 50%OSPW-S+dFFT

Figure 55. Low adventitious vegetation colonization in TRT 4, TRT 5, and TRT 6 mesocosms. Pictures were taken on October 5, 2020.

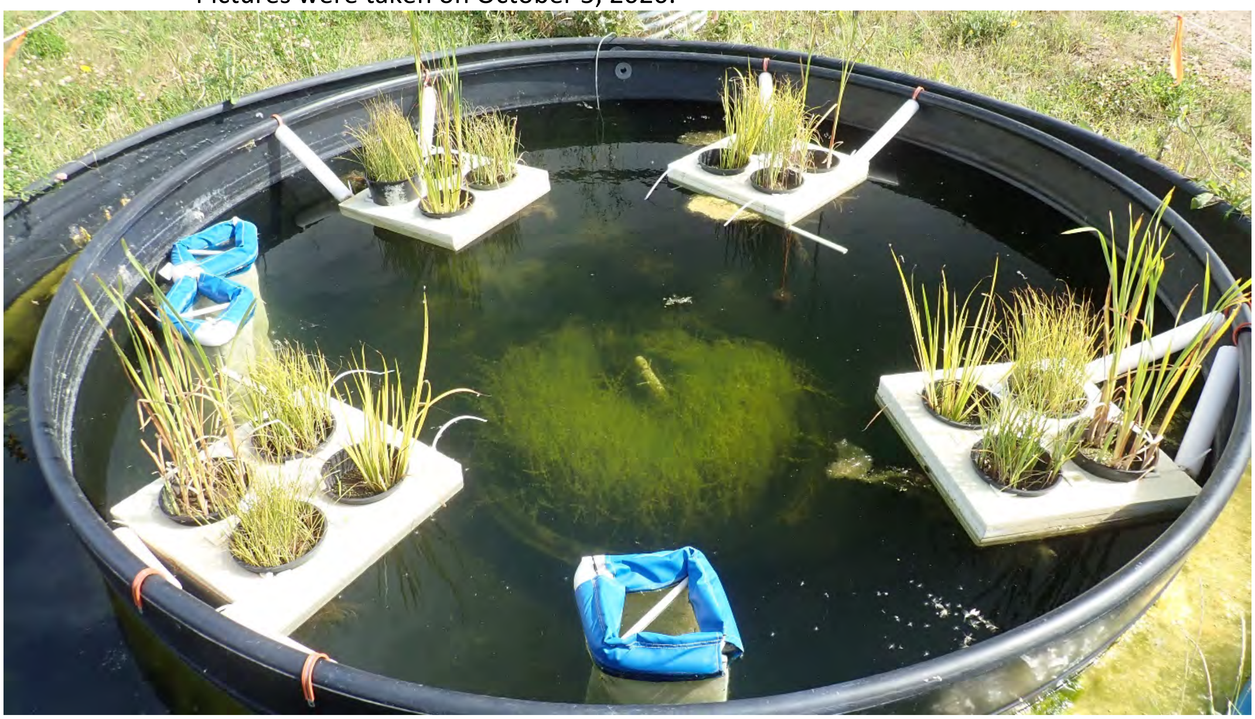

Figure 56. Adventitious vegetation-covered data loggers in TRT 3 (TSRU) mesocosms (D3). Pictures were taken on August 24, 2020.

3.9.2 Algal Mats

Algal mats were observed in the spring of 2020 in TRT 6 (dFFT + 50% Suncor OSPW) mesocosms (Figure 57), though very little was present during the remainder of 2020. It is suspected that some benthic algal mats may have floated to the surface at the beginning of 2020 and then disappeared due to frequent precipitation events.

Figure 57. Algal mats were observed in TRT 6 (50%OSPW-S+dFFT) mesocosms. Pictures were taken on May 15 (left) and May 25, 2020 (right).

3.9.3 Mesh Sock Coloration

A noticeable difference in the mesh socks' color was observed at the end of the 2020 study for TRT 5 (Figure 58). In general, the socks from mesocosms containing 100% Imperial OSPW and FFT appeared darker in color than others.

CTL, 100%ARW+FS

TRT 1, 100%ARW+CST

TRT 2, 100%ARW+TT

TRT 3, 100%ARW+TSRU

TRT 4, 100%ARW+FFT

TRT 5, 100%OSPW-I+FFT

TRT 6, 50%OSPW-S+dFFT

Figure 58. Mesh socks on September 21, 2020.

3.9.4 Submergent Plants

In the summer of 2020, *Potamogeton richardsonii* was observed in most CTL and TRT 1, 2, 3, and 4 (tailings alone) mesocosms, but rarely in TRTs 5 and 6 (Figure 59). *Myriophyllum sibiricum*, another submergent plant species, survived in all the treatment groups (Figure 60). As shown below, this species seemed to be more shaded by relatively higher levels of turbidity in TRT 4 (FFT) and TRT 5 (FFT + 100% Imperial OSPW) (Figure 60).

CTL, 100%ARW+FS TRT 1, 100%ARW+CST TRT 2, 100%ARW+TT

TRT 3, 100%ARW+TSRU TRT 4, 100%ARW+FFT TRT 5, 100%OSPW-I+FFT TRT 6, 50%OSPW-S+dFFT

Figure 59. *Potamogeton richardsonii* observed on July 6, 2020.

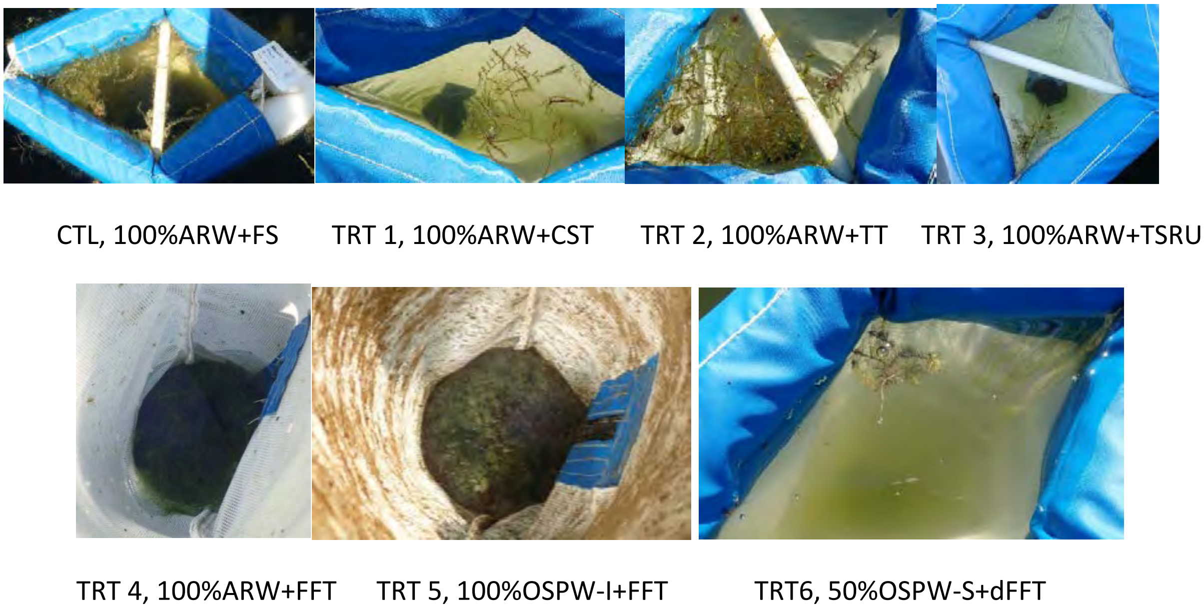

Figure 60. *Myriophyllum sibiricum* was observed on July 6, 2020.

3.9.5 Floating Plants

Ceratophyllum demersum successfully overwintered, as evidenced by their presence in the mesocosms during the summer of 2020 (Figure 61). However, these plants species did not appear healthy in treatments with OSPW (TRTs 5 and 6); plant biomass appeared to be shrinking/ compressed and was always surrounded by slimy texture which was hard to remove by physical contact (Figure 61).

Figure 61. *Ceratophyllum demersum* observations on June 11 and 12, 2020.

3.9.6 Periphyton on the Mesocosm Walls and Socks

The term periphyton is used here to denote the flora/debris (without taxonomical identification) associated with the mesocosm walls. Colonization of periphyton was visible at the end of 2020 and was cataloged as high, medium, low, and not observed and is shown in Figure 62 and summarized in Table 18 and Table 19. Periphyton relative abundance was not a response to the treatment material. There was no apparent inhibition of periphyton growth by experimental materials. It should be noted that a lack of periphyton was not necessarily indicative of growth inhibitive conditions, because grazers (e.g., snails) may have consumed the periphyton (Section 3.9.9).

Figure 62. Periphyton observations on the mesocosm walls on October 15, 2020. The observation was cataloged as High, Medium, Low, and not observed (from left to right).

Table 18. Periphyton relative abundance on the mesocosm walls at the end of 2020.

Group	CTL	TRT 1	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6
H	1		1	1		3	2
M				1	3		1
L	4	4	3	1	1	1	2
NO				1			

Note: H: High, M: Medium, L: Low, N: Not Observed

Table 19. Periphyton relative abundance on the socks at the end of 2020.

Group	CTL	TRT 1	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6
H	1					3	
M			1	1	1		3
L		1	3	2	3	1	1
NO	4	3		1			1

Note: H: High, M: Medium, L: Low, N: Not Observed

3.9.7 Bitumen Adhesion on Rafts

At the end of 2020, bitumen spots were noticed on the floating rafts. Bitumen spots were qualitatively described as high, medium, low, and not observed and are shown in Figure 63, and summarized in Table 20. Bitumen spots were more associated with OSPW (TRT 5 and TRT 6) treatments in general, because all OSPW associated mesocosms were described as high or medium.

Figure 63. Bitumen observations on the rafts on October 15, 2020.
Observations were arbitrarily cataloged as High, Medium, Low (from left to right).

Table 20. Bitumen observations on the rafts at the end of 2020.

Group	CTL	TRT 1	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6
H						3	
M					1	1	5
L		2	4	3	3		
NO	5	2		1			

Note: H: High, M: Medium, L: Low, NO: Not Observed

3.9.8 Metaphyton

Observations of floating masses of filamentous algae are described as metaphyton in the present study (Figure 64). Metaphyton was mainly found in the CTL mesocosms, often not extensive and was observed in proximity to emergent vegetation, mesh socks, and vegetation in the soil troughs (Figure 64). Some small amounts were observed in some of the TRT mesocosms by the end of 2020; their abundance was not comparable to those found in the CTL mesocosms.

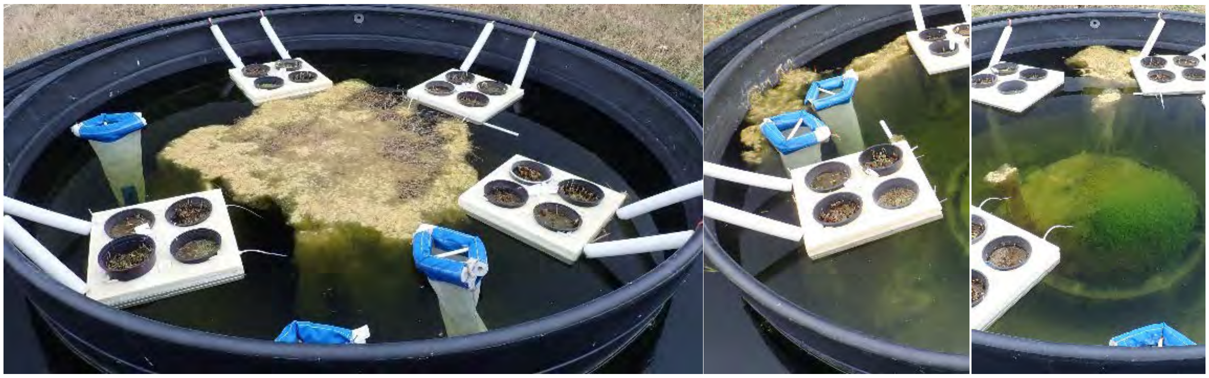

Figure 64. Metaphyton mass observations in CTL mesocosms on October 5, 2020.

3.9.9 Snails

The relative abundance of snails was qualitatively assessed as high, medium, low, and not observed and is summarized in Table 21 and Table 22. Snails were found on the mesh socks and mesocosm walls in all the experimental groups at the end of 2020 (Figure 65).

Table 21. Relative abundance of snails on the mesocosm walls at the end of 2020.

Group	CTL	TRT 1	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6
H			1				
M	4	4	1		1		1
L	1		2	4	3	2	4
NO						2	

Note: H: High, M: Medium, L: Low, NO: Not Observed

Table 22. Relative abundance of snails on the socks at the end of 2020.

Group	CTL	TRT 1	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6
H							
M	3	3		1	1		3
L	2	1	4	3	3	3	2
NO						1	

Note: H: High, M: Medium, L: Low, NO: Not Observed

Figure 65. Snails' observations on the socks and mesocosm walls in CTL, TRT 1, TRT 2, and TRT 6 (from left to right).

3.9.10 Density of Tailings

The relative increase in effort required to push the depth gauge through tailings/sands could indicate compaction over time. This relative effort was qualitatively assessed as easy, hard, and extra hard, summarized in Table 23. In October 2020, CST and TSRU became quite dense and very difficult to push the depth gauge through. However, the depth gauge was easily pushed through FFT (TRTs 4 and 5) and dFFT (TRT 6) tailings materials; these tailings materials had a density similar to toothpaste.

Table 23. Relative effort required to push the depth gauge through treatment materials at the end of 2020.

Group	CTL	TRT 1	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6
E					X	X	X
H	X		X				
EH		X		X			

Note: E: easy, H: hard, and EH: extra hard

4.0 DISCUSSION

Caution should be taken when examining results against over-extrapolation, considering the static model feature of the mesocosm study, which is differentiated from “real world” ecosystem. Aquatic mesocosms are outdoor artificial systems, and often, comparing trends over time and pattern differences across groups should be paid more attention than absolute values.

4.1 The Effect of Overwintering

This aquatic mesocosm study was conducted over two years in ambient outdoor conditions, established in the spring of 2019 and decommissioned in the fall of 2021. The results presented are for the 2019/2020 study, and the 2021 study will be reported as an addendum to this report. As such, the mesocosms froze over the winter period for approximately six months. The effect of ice cover, known as brine rejection, has been discussed in the 2018 mesocosms report (Melnichuk, 2020). Briefly, when the surface water was frozen in winter, the ice excluded a large proportion of the dissolved salts in water, and the salt excluded was mixed in the water column under the ice (Pieters and Lawrence, 2014). In the spring, salt levels at the surface were diluted by the ice melting. This suppression is more likely to occur when the salinity is high (Pieters and Lawrence, 2009). The influence of brine rejection is supported by the conductivity measurements taken by data sonde, which showed conductivity gradients in the water column (see Section 3.1.4). This is further supported by logger measurements taken in the spring of 2020, which showed a decrease in conductivity (see Section 3.8.2). Due to the mixing in the water column with time, the concentration gradient was gradually attenuated until Week 23 for TRT 1, 2, 3, and 4, Week 27 for TRT 5 (FFT+100% Imperial OSPW), and Week 31 for TRT 6 (dFFT + 50% Suncor OSPW), respectively.

In addition to salt, laboratory experiments suggested that freezing could also reject organic matter, such as synthetic NAs in ice samples (Reynolds, 2013). This agrees with what has been observed for some laboratory water quality measurements collected for the first set of samples in the 2020 season. For example, at Week 26, 2020, the NAs and DOC levels appeared low relative to samples taken later in the season, especially in TRT 5 (FFT+100% Imperial OSPW) and TRT 6 (dFFT+50% Suncor OSPW), where conductivity is relatively high. In the following sections, where specific analytes are discussed in detail, the ice thaw effect could account for change in concentrations of analytes that do not degrade (e.g., metals) or volatile (e.g., NAs) in the spring of 2020. In contrast to a highly controlled laboratory study, in mesocosm studies, the effects of seasonal variation may often be more pronounced than the influence of treatment materials (Melnichuk, 2020).

4.2 The Effect of Depth

The only data collected at different depths were the physicochemical measurements described under Water Quality – Field Measurement (Section 3.1).

4.2.1 Temperature

The temperature within any given mesocosm was generally higher at the surface than at the bottom. This pattern was expected as vertical concentration gradients reflected the soil and jacket water surrounding the mesocosm. The thermal gradient was not noticeable in the fall of both years when ambient air temperatures were relatively lower.

However, it was noted that the temperature differences between depths were more evident and lasted longer in 2019 in TRT 4 (FFT), TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW), compared to CTL and other treatment groups. This effect could be explained by the turbidity associated with FFT, dFFT, and/or OSPW. Relatively higher turbidity was detected in TRT 4 (FFT), TRT 5 (FFT+100% Imperial OSPW) and TRT 6 (dFFT+50% Suncor OSPW) in 2019, resulting in shading at the bottom, and therefore keeping the bottom cooler than the surface. As the turbidity decreased over time, so did the shading effect. As a result, the temperatures at the surface and the bottom became similar.

A previous study by Schwerdtfecer (1963) demonstrated that high salinity in water could depress the freezing point, and Jackett et al. (2006) reported a linear relationship between freezing temperature and salinity. However, in the spring of 2020, the influence of brine rejection was not apparent for temperature in high salinity groups (TRT 5 and TRT 6) compared to CTL. Generally, bottom temperatures were significantly cooler than that of the surface for all experimental groups until Week 31 (with a few exceptions). Because this thermal stratification was observed both for CTL and TRTs, it is likely a result of the weather and natural freeze-thaw cycle.

4.2.2 pH

Generally, in the groups that contained FFT and dFFT (TRT 4, TRT 5, and TRT 6), pH tended to be lower at the bottom at the start of the open water season each year. This pattern is more evident for TRT 6 containing dFFT than TRT 4 and TRT 5, which contained FFT. It is unlikely to be explained by anaerobic conditions considering the thickness of the tailing layer and the DO levels at the bottom of the water column. A plausible explanation could be that the source of substrates (FFT/dFFT) might release low pH constituents or contribute to the production of CO_2, resulting in the reduction of pH. The release of constituents or CO_2 production diminished over time so that the pH values at the surface and bottom converged. In the spring of 2020, the gradients observed in pH were less noticeable compared to conductivity. This indicates that pH in the mesocosms might be primarily influenced by the degree of biological factors (Wait et al., 2006), such as lack of adventitious plants in TRT 4, TRT 5, and TRT 6.

Similar trends in reduced pH with depth in dFFT mesocosms was observed in the previous mesocosm experiment (Melnichuk, 2020). Air bubbles were also observed in the activity traps at the first several weeks of each year but not at the end (Appendix E). However, it is unknown whether the air bubbles were CO_2 or methane (CH_4), because the air sample analyses were outside of this Project's scope. The gas composition could be analyzed in future studies. In addition, stable isotopes can be used to determine where the gases came from (e.g., generated by bacteria vs. a chemical reaction); microbes preferentially use the lighter isotope resulting in an enrichment of light isotopes in the metabolic products and heavy isotopes in the remaining reactants.

4.2.3 Turbidity

Overall, turbidity within each experimental group did not vary significantly between the surface and the bottom of the water column. Occasionally, the bottom turbidity values were significantly higher than the surface. It is possible that water quality measurements by way of the data sonde may have resulted in increased turbidity in cases where the data sonde contacted the tailings layer, subsequently raising a plume of suspended solids. It should be noted that higher turbidity levels at the bottom were detected at the first time point of 2019 in most groups, except CTL and TRT 1 (CST). The high turbidity levels at the bottom occurred until Week 29, 2019. The installations' artificial effect can explain this, since the installation of water column suspended tailings even all reasonable effort was made to minimize this effect, and this effect diminished after tailings settled through the water column with time. Differences in the longevity of turbidity difference between depths might also indicate the sedimentation rate (solid particles setting out of a liquid by gravity) of different materials (Mkpenie et al., 2007).

4.2.4 Specific Conductivity

In 2019, there were almost no significant differences in conductivity between water depths in all mesocosms. The few exceptions could be due to data sonde contact with the sediment layer or material installation, as described above in Section 4.2.3. However, early in 2020, specific conductivity was significantly higher at the bottom than the surface in all mesocosms. As described in Section 4.1, in the winter of 2019, the surface water froze, and brine rejection likely concentrated salt into the deeper water column. Differences in length of duration of conductivity differences between depth could be related to the salinity.

4.2.5 Dissolved oxygen

In 2019, there were no significant differences between DO bottom and surface water depths in the CTL group. However, in the beginning of 2019, reduced DO levels were measured at the bottom of the treatment mesocosms. These lower DO levels were likely a result of less photosynthetic activity at the bottom. As outlined in Section 4.2.3, it is expected that turbidity had an initial shading effect and as this turbidity gradient diminished, the DO gradient resolved.

In 2020, however, depth did not have a uniform effect on DO. In CTL, TRT 1 (CST), TRT 2 (TT), and TRT 4 (FFT), DO was higher at the bottom for the first few measurements. One plausible explanation could also be photosynthetic activity at the bottom. To some degree, this explanation could be supported by observed adventitious vegetation in CTL and TRT 1, 2, and 3 (Section 3.9.1), although few aquatic plants/algae were found in TRT 4 (FFT). Also, at the beginning of 2020, significantly higher chlorophyll levels at the bottom were detected in TRT 1 (CST) and TRT 2 (TT). Chlorophylls are pigments of photosynthesis, and their degradation in aqueous environments is complicated and unclear (Scheer, 2012).

For initial measurements in 2020, reduced DO at the bottom was detected in TRT 3 (TSRU), TRT 5 (FFT+100% Imperial OSPW), and TRT 6 (dFFT+50% Suncor OSPW). The most plausible explanation is that some decomposition was taking place at the bottom. The decomposition likely occurred at a rate that did not consume the oxygen faster than it was replenished. This hypothesis could partially be supported observation of algal mats in the spring of 2020 in TRT 6 (dFFT+50% Suncor OSPW) mesocosm. The DO concentrations between depths were then conveyed later in 2020 due to the diffusion of oxygen from the atmosphere.

4.2.6 Total Algae (Chlorophyll and Blue-green Algae)

In all mesocosms, there was at least one sampling event where chlorophyll and BGA concentrations were significantly higher at the bottom than at the surface. One plausible explanation could be more feeding activities of herbivorous protists at the surface than phototrophic protists (Kashiyama et al., 2012), and the growth rates of herbivorous are known to exceed that of phototrophic protists above certain temperature (Rose and Caron, 2007).

As per the manufacturer's manual, chlorophyll readings should exhibit low interference from dissolved organics and turbidity (YSI Incorporated, 2014). However, the YSI sensor's precision could become low and incur higher variability under certain field conditions (Hodges et al., 2018), which is suspected to be the case, especially for the bottom measurements. A few parameters are likely to influence the probe's measurement of cyanobacteria, such as turbidity, boundary, and phytoplankton composition (Brient et al., 2008). Therefore, the data sonde-derived total algae values were considered qualitative by the authors to some degree.

4.3 The Effects of time

4.3.1 Water Quality – Field Measurement

The water temperatures in the mesocosms increased mid-season in both years. The water temperature rose with the seasonal rise in ambient air temperature and fell as the ambient temperature decreased in the fall.

An overall increase for two years in pH over time was observed, although the increase was not significant in TRT 5 (FFT+100% Imperial OSPW). The increase in pH was likely an interaction of multiple factors, such as CO_2 depletion from adventitious plants and algae during photosynthesis (Shiraiwa et al., 1993).Lower pH (3.5 - 6) was reported to improve water clarity of OSPW, although this could be affected by the water ionic strength (Brandon, 2016). However, the pH in all treatment groups (including CTL) are in the alkaline range (7.5-9.5).

Turbidity levels decreased significantly over time (except for CTL), with the most marked decrease observed in 2019 and minimal change in 2020. This observed trend was similar to what Brandon (2016) observed in OSPW and FFT exposure over three to four months. As per the *Environmental Quality Guidelines for Alberta Surface Waters* (Government of Alberta, 2018), Table 3, the threshold for recreational users is less than 50 NTU. The turbidity levels in the mesocosm only exceeded this threshold at the first measurement in 2019 for TRT 4. Given that the high turbidity levels after installation resolved over time in these mescosms, it is possible that with time, turbidity levels in end pit lakes could fall under the threshold. However, due to limitations of the mesocosm study design and length of trial, it is not possible to accurately predict turbidity patterns at a field trial or end pit lake scale.

In 2019, conductivity was unchanged in most cases, and the concern of precipitation and evaporation seems minimal for any influence on conductivity change over time. In 2020, the effect of time on specific conductivity was significant. The effect is most likely due to the brine rejection, as discussed in Section 4.1 and 4.2.3.

Dissolved oxygen increased significantly over two years in general, except for TRT 5 (FFT + 100% Imperial OSPW) at the surface. It also showed convergence between most groups at both depths. DO was likely influenced by several factors through time, including adventitious vegetation/algae abundance and the decomposition of detritus.

Although this study's purpose was not to differentiate between major types of phytoplankton, the trend of BGA values was also analyzed. Chlorophyll and BGA values did not change significantly with exception of fluctuations in the CTL and tailings only mesocosms (TRTs 1, 2, 3, and 4). Exceptions included BGA readings in FFT mesocosms at the bottom. Chlorophyll and BGA measurements at the bottom showed a significant decrease in OSPW mesocosms (TRT 5 and TRT 6) and FFT mesocosms. The decrease in chlorophyll and BGA could have been due to decreased phytoplankton, the degradation of residual extracellular chlorophyll from detrital material, or a decrease in chlorophyll's cellular concentration. Given the static feature of the mesocosm experiment, a decrease in phytoplankton is possible due to the ageing of the water exposed.

4.3.2 Water Quality – Laboratory Measurement

Time-related trends in laboratory water quality results varied according to the analyte and experimental group, which is difficult to generalize. Since a broad range of water chemistry analytes was included in the current study, the depth of analyses was performed in balancing brevity and comprehensibility and to serve the study purpose. One objective of this study was to evaluate tailings streams' chemical properties, reporting on general patterns. Another objective was to examine how different tailings streams affect the water column in chemical, biological, and toxicological properties. Unexpected or uncommon patterns would also be selected and analytes, which were expected to affect aquatic biota considerably.

As a general pattern discussed above (Section 4.1), brine rejection substantially affected the water quality parameters analyzed. Over two years, the water chemistry analytes often showed a significant mid-study decrease, mainly due to the lowest analytes' concentration in the spring of 2020. This effect was relatively pronounced in TRT 5 (FFT+100% Imperial OSPW) and TRT 6 (dFFT+50% Suncor OSPW), where TDS was high. For example, molybdenum showed a significant mid-study decrease in TRT 5 and TRT 6, while no change was observed in CTL and TRT 1, 2, 3, and 4 mesocosms. It is noteworthy that the brine rejection appeared more obvious in TRT 6 than TRT 5. For example, the lithium (total) concentration was approximately 73% and 56% in TRT 5 and TRT 6, respectively, when comparing the value at Week 26 of 2020 to Week 38 of 2019. Since water samples collected for laboratory analysis were only collected at the surface, the significant mid-study decrease might not mainly be due to reducing the analyte itself in the mesocosm since water samples at depth was not captured. It is recommended to take an integrated depth (or composite) sample in future studies. Depending on the specific research questions and budget, discrete samples from multiple depths could also be considered. An alternative suggestion could be to collect water samples after the conductivity readings are similar between the surface and bottom. However, it might introduce uncertainty on the sampling schedule and incur potential conflict with other sampling activities.

Total NAs decreased significantly over two years in the groups which contained both tailings and OSPW (TRT 5 and TRT 6). The original OSPW contained 24.9 mg/L and 39.6 mg/L NAs for Imperial and Suncor, respectively, resulting in expected concentrations of approximately 25 mg/L and 20 mg/L NAs for TRT 5 and TRT 6 (50% OSPW), respectively, neglecting the potential release or adsorption from tailings. The initial measurement in 2019 showed 33.2±2.7 mg/L and 22.0±2.3 mg/L for TRT 5 and TRT 6, respectively. The addition of FFT/dFFT and mixing with ARW (for TRT 6) were suspected to contribute to the discrepancy. The initial NAs concentration in TRT 4 (FFT) was 2.5 mg/L. It indicated that the NAs presented in water did not suffer excessive adsorptive losses to the mesocosm structures rapidly. In general, NAs are not considered to bioaccumulate (Scott et al., 2020), although recent research suggested that some vascular plants can uptake NAs into tissue (Alberts et al., 2021). The accumulation in soils/tailings might partially explain decreased NAs levels over two years (Headley and McMartin, 2004), although NA in soils/tailings after installation was not analyzed. Note that the topsoil in all mesocosms was "washed" with potable water before installation, potentially removing a large amount of organic content. Janfada et al. (2006) reported sorption of NAs on soil, and sorption levels were associated with soil's organic content levels. However, Stewart, (2013) suggested limited adsorption of NAs on sediment. Consequently, we recommend that NAs in the soil and tailings should be analyzed in the future. Toor et al., (2013) showed that in laboratory microcosms used to minimc wetland environments, natural degradation of total NAs in OSPW was observed after 52 weeks. The indigenous microbial community was proved to contribute to the biodegradation of NAs (Herman et al., 1994), and in turn, NAs would influence the composition of the microbial community (Hadwin et al., 2006). Therefore, microbial characterization is recommended for the 2021 mesocosm program.

For most tailings-only groups (TRT 1, 2, 3, and 4), the proportion (%) of NA families of Z-2 to Z-6 significantly decreased, whereas and the proportion of Z-10 to Z14 groups increased over time. To some degree, these findings are consistent with a laboratory evaluation conducted by Armstrong et al., (2009), which showed a decrease in abundance of two- and three-ring (Z-4 and Z-6), accompanied by an increase of one- and six-ring NAs (Z-2 and Z-12), observed over 30 days under a laboratory evaluation. A microcosm study also observed by Rio (2004) the significant degradation of Z-2 and Z-4 NA surrogates although process-derived NAs appeared to be recalcitrant over 2 years (Rio et al., 2006). For groups with both tailings and OSPW (and most tailings only groups as well), the most complex proportion of NAs (Z-16 and Z- 18) showed a significant decrease, along with an increase in Z-4 to Z-12. It is assumed that the most complex NA molecules had been degraded into simpler ones over time. However, changes in NAs profile over time cannot be fully confirmed in this study, because of the single source/method used. In the future, results may be obtained from Dr. Gamal's lab at the University of Alberta, so that HPLC-Orbitrap-MS analysis could be consulted with analysis by FTIR and TOFMS to confirm.

Some other analytes also showed decreases over time, such as PAHs in all treatments and some metals in certain mesosms. Some PAH compounds are slightly soluble and highly volatile (e.g., naphthalene), while some are relatively non-volatile (e.g., dibenzo(ah)anthracene) (Douben, 2003). Cancelli and Gobas showed that a treatment wetland could substantially reduce concentrations of PAHs in OSPW (Cancelli and Gobas, 2020). Over time, the decrease of PAHs could be explained by volatilization, degradation or sedimentation (Douben, 2003). A review on the environmental impact of PAHs suggested that some of PAHs could be incorporated into sediments by sorption, thereby increasing the mobility and bioavailability of PAHs (Abdel-Shafy and Mansour, 2016), especially for higher molecular weight compounds (4 to 7 fused benzene rings) (Collier et al., 2014). Overall, PAHs levels were low in all treatment groups (< 0.8 μg/L) and most compounds were undetectable with current analytical methods. The level of cobalt in TRT 5 (FFT + 100 % Imperial OSPW) only exceeded metal guidelines (chronic, 1.1 or 1.2 μg/L depending on the hardness) for the protection of aquatic life at the first two sampling points of 2019 (Government of Alberta, 2018), which was 2.7 and 1.5 μg/L, respectively although the cobalt level is lower than guideline level for the protection of agricultural water uses (50 μg/L). In 2020, cobalt concentration was less than 0.4 μg/L in all treatment groups.

Presumably, the decrease of metals could be associated with adsorption to mesocosm structures (e.g., soil) or particulate matter or by biological uptake. For example, the decrease in potassium concentration tends to be an essential nutrient for plants and algae (Talling, 2010), while sodium is not the case. An investigation conducted in Ells River, Alberta, indicated that both PAHs and metals have a strong affinity for sediment particles (Droppo et al., 2015). Sedimentary PAHs might have a different composition than in water (Wang et al., 2020). Consequently, we recommend that PAHs and metals should be chosen as targets for soil chemistry analysis. The transformation ability of metals from one oxidation state to another should also been investigated, since it could potentially affect metals' bridgeable property and their toxicity (Ore and Adeola, 2021).

Some observations were notable for increase or mid-study increase trends, usually more evident in tailings only groups, such as time-related increases of boron in TRT 4 (FFT). The simplest explanation is the efflux of these analytes from the tailings, as some analytes (e.g., metals) are likely to mobilize from the pore water in the tailings.

Some analytes did not demonstrate any uniform trends across all mesocosms. For example, no change of total Sr was observed in TRT 4 (FFT), and a significant mid-study increase showed in TRT 3 (TRSU). However, a significant decrease of Sr was detected in TRT 1 (CST). The simplest explanation could be adsorption and desorption since elemental metals do not undergo degradation. For instance, strong strontium adsorption has been shown for wetland sediments, while desorption of strontium from cattails was also observed (Boyer et al., 2018).

It should be cautioned that some artifacts can appear in the data, which near or drop below the reporting limit in most mesocosms. For example, a significant increase of dissolved chromium was detected by contrast analysis in TRT 3 (TSRU), mainly due to the last measurement in 2020. However, the dissolved chromium concentration measured at the end of 2020 was 0.41 ± 0.26 µg/L, while the reporting limit (Section 2.16.6) is 0.3 µg/L. Mercury (total) levels (up to 0.09 µg/L in TRT 5 and 0.21 µg/L in TRT 6) exceed acute guideline values for the protection of freshwater aquatic life (0.013 µg/L)(Government of Alberta, 2018), especially in the presence of OSPW (TRT 5 and TRT 6) in 2019. The mercury levels in mesocosms were not consistent with the values observed for material characterization of OSPW (i.e., total mercury was 0.05 µg/L for Imperial OSPW and 0.27 µg/L for Suncor OSPW). The initial contribution of FFT (0.026 µg/g) and dFFT (0.039 µg/g), which is higher compared to FS (0.005 µg/g), may explain this discrepancy. At the end of the 2020 sampling season, all mercury concentrations were below 0.05 µg/L. However, the reporting limit of mercury (total) is 0.02 µg/L. Future studies could include more sensitive methods for determining mercury (total and methyl) concentrations.

It was also interesting to note the difference between sulfur and sulfate concentrations in early 2020 compared to the last time point of 2019 (Section 3.2.12), which might be associated with the abundance of sulfate/sulfur reducing bacteria (Warren et al., 2016). Anaerobic conditions are unlikely to be achieved expect for TRT 6 (Section 3.8.1), although loggers were not deployed over winter. In the future, microbiological analysis and sediment characterization might reveal a possible explanation at the end of the study.

4.3.3 Installed Vegetation

The emergent plants growth followed a normal trend; plants grew taller in all mesocosms before peaking at maturity height and then reached a plateau or slightly decreased in height and senesced at the end of the season. Only *A. americanus* flowered in 2019; however, flowering was observed in all four species in 2020.

However, a different trend for *C. demersum* was observed. Initially, there was an increase in wet weight in CTL and tailing only mesocosms (TRT 1 to TRT 4), followed by an overall decline towards the end of 2020 (including CTL). A plausible explanation for the decline could be a change in water chemistry or low nutrient load in the water. *C. demersum* also appeared fragile (tend to fall apart when handled) during the early season of 2020. The manipulation of *C. demersum* during the sampling process, (i.e., removal from the water and the attempt to separate metaphyton), may have also caused additional stress to the plants. In the groups with OSPW (TRT 5 and TRT 6), biomass dropped throughout the study until little or no viable biomass persisted. This finding was not unexpected. *C. demersum* is considered a robust indicator species and was shown to be absent in industrial wetlands associated with reclaimed oil sands wetlands (Trites and Bayley, 2009). For future studies, less frequency is suggested for repeated plant growth measurements or even conduct measurements only at installation and harvest to minimize additional stress to this species during sampling process.

4.3.4 Toxicity

Fathead minnows (*Pimephales promelas*) are native to most northeastern Alberta wetlands (Cruz-Martinez and Smits, 2012). In general, both the trout and fathead minnow assays detected low toxicity levels in the presence of tailings alone. However, decreased toxicity to trout (mortality) occurred in OSPW-containing groups (TRT 5 and TRT 6) throughout 2019, while fathead minnow mortality results stayed mainly unchanged in both groups. Toor et al. (2013) found a reduction in rainbow trout toxicity to OSPW with time in simulated wetlands. Plants, such as sedges, are known to detoxify OSPW (Simair et al., 2021). The observed toxicity of trout could be associated with NA levels and Rio (2004) showed that it can be reduced by microbial degradation. Based on Ahad et al., (2018)'s study, we could expect that the natural microbial population might break down some of the NAs. However, Marentette et al., (2015) showed no decrease in NA fraction components' toxicity from aged sources of OSPW to fathead minnow embryos. This agrees with this current study that the mortality of fathead minnow was relatively low and mainly unchanged overtime. PCA analysis also found little correlation between the Fathead Minnow LC_{50} and NA toxicity (Appendix F).

Trends in fathead minnow biomass were variable across treatments. For example, the biomass of fathead minnow in CTL mesocosms slightly but statistically significant increased from 0.380 ± 0.013 mg in 2019 to 0.421 ± 0.009 mg in 2020. Although decreased hardness (from 124 to 84 mg/L $CaCO_3$) in CTL mesocosms is suspected to be partially responsible for increased biomass (C.Blanksma et al., 2009), comparing between years must be done with some degree of caution. It is possible that larval fish of different batches used for the assays may have slightly different sensitivity. Reference toxicant test resulted in IC_{50}s (95% CL, g NaCl/L) for biomass are 5.7 (5.4, 6.0) and 4.4 (4.1, 4.7) in 2019, while the values were 5.7 (5.4, 6.1) and 5.9 (5.6, 6.2) in 2020. The different trends produced by each group suggest that different mechanisms or substances may have affected fathead minnow growth. Identification and exploration of toxicity mechanisms are out of the current study's scope. However, the overall toxicity of the biomass endpoint is low and has not increased over time.

4.3.5 Zooplankton

A mid-study significant increase in zooplankton richness and diversity was observed in some mesocosms, while a significant mid-study decrease in zooplankton abundance was observed. These results are not surprising, given that the systems were new in 2019. Olmo et al. (2012) showed that zooplankton communities might be re-established over time after winter, and any decreases in abundance may be related to the age of the ecological communities housed in the mesocosm tanks.

There is some indication that time influenced the zooplankton communities' structure. In general, the highest richness and biomass values were found at the beginning of 2020. This result could be attributed to the brine rejection effect, because zooplankton traps were deployed near the surface (Section 2.13.5) and zooplankton has the potential to avoid hydrocarbon-contained sources by swimming away (Seuront, 2010). However, if brine rejection were responsible for increased zooplankton richness and biomass, mid-study increases would be expected for indices and biomass in the TRT groups, especially TRT 5 (FFT+100% Imperial OSPW) and TRT 6 (dFFT+50% Suncor OSPW). Such a pattern was not always observed. An alternative explanation for changes in the zooplankton community might change seasonally associated with diet, such as phytoplankton and bacteria (Taipale et al., 2009).

4.3.6 Macroinvertebrates

Macroinvertebrate species richness appeared to increase or have little change in 2019 and decreased in 2020. In general, the highest species richness in each treatment group was observed in Week 32, 2020. The similarity in showing the highest species richness in the spring of 2020 across all experimental groups would suggest the influence of an environmental factor common to all mesocosms (e.g., overwintering and time) and not a specific treatment effect. The decrease of species richness in 2020 might be related to limiting resource availability.

For the abundance of macroinvertebrates, a significant mid-study increase was detected for CTL, TRT 3 (TSRU), and TRT 6 (dFFT+50% OSPW) from samples collected by activity traps, while there was a significant mid-study increase for TRT 5 (FFT+100% Imperial OSPW) and TRT 6 (dFFT+50% OSPW) by H-D samplers. Our results indicate the bias of each sampling method. Activity traps catch mainly mobile macroinvertebrates, while H-D samplers primarily target colonized organisms (Macanowicz et al., 2013). In this study, the combined use of activity traps and H-D samplers represented more complete macroinvertebrate communities and is still recommended in future studies to obtain a good representation of the macroinvertebrate community.

The diversity of macroinvertebrates was similar to what was observed for richness and abundance, with no trend/fluctuation or mid-study increase. A deeper analysis of community structure is beyond the scope of this report. Additional information about macroinvertebrate diversity changes over time and mechanisms driving change could be obtained by examining detailed community composition in future studies.

HBI, as the best-known index for aquatic biomonitoring, was used to summarize the overall pollution tolerance of macroinvertebrates, especially as an indicator of organic pollution (Alain Armellin, Donald Baird and Nancy Glozier, Adam Martens, 2019). This index traditionally classified the quality of surface water into excellent (0.00 – 3.75), very good (3.76-4.25), good (4.25-5.00), fair (5.01-5.75), fairly poor (5.76-6.50), poor (6.51-7.25), and very poor (7.26-10.00) (Klemm et al., 1990). In the current study, no trend or fluctuation was found in most experimental groups. However, caution should be taken when examining the HBI values, considering the static feature of the mesocosm study.

The effect of oil sands materials on macroinvertebrates is expected to diminish over time as the water continues to age due to organic fraction contribution (White and Liber, 2020). For samples collected by activity traps, Macrophyte-herbivores was an addition to 2020 in TRT 4 (FFT), and Shredder-herbivores were only detected in TRT 5 (FFT+100% Imperial OSPW) and TRT 6 (dFFT+50% Suncor OSPW) at the beginning of 2019. For samples collected by H-D samplers, a substantial increase in the proportion of Scrapers in TRT 6 (dFFT+50% Suncor OSPW) was observed in 2020. These results suggest the structure of the mesocosm community evolved over time.

4.3.7 Phytoplankton coverage

Phytoplankton (filamentous metaphyton) coverage was only observed in 2020 and increased mainly in the CTL. Since phytoplankton was estimated by visual observation for cover near the water surface, the trend observed in CTL is suspected to be associated with the competition between phytoplankton and adventitious plants/algae from soil trough for light absorption (Lassen et al., 1997). Also, water quality parameters, such as pH, DO, and conductivity, could be considered as integrative indicators for phytoplankton's photosynthetic activity (Knauer and Hommen, 2012). Future studies should consider completing more detailed investigations of phytoplankton communities within the mesocosms to aid in the interpretation of water quality data.

4.3.8 Data Loggers

Dissolved oxygen loggers demonstrated that the bulk waters were not anoxic, although the loggers were installed a few days after ice-off in 2020. More continuous monitoring over winter may help in addressing this concern in future studies (if practical).

Over the first few weeks of 2020, dissolved oxygen increased dramatically in TRT 6 (dFFT + 50% Suncor OSPW) but decreased in CTL. In 2020, specific conductivity appeared to creep downwards for several weeks then plateaued. The period preceding the plateau is associated with the levels of conductivity of each treatment. These are the same patterns described in Section 3.1.4.7 and 3.1.5.7. Diel fluctuations were also observed in the data but more evident for DO than for conductivity. These findings are in partial agreement with the literature; for example, monitoring of a stormwater pond observed diurnal variations in water temperature and DO, but not in conductivity (He et al., 2015).

It was expected that CTL and TRT 1 – 3 would demonstrate different diel cycles compared to TRT 4 - 6 due to the photosynthesis and respiration of adventitious plants/algae. While diel cycles in DO and conductivity were observed, their amplitude did not appear to differ substantially between the groups with and without adventitious plants/algae. Loggers in CTL and TRT 1 -3 mesocosms were covered by massive adventitious plants/algae (Section 3.9.1), and the monthly clean process of loggers could have interrupted the growth of submerged adventitious plants in the soil trough. Future studies might benefit from installing smaller containers of soil and including loggers away from soil containers.

4.4 Effect of the presence of tailings (CTL vs. TRT 1, CTL vs. TRT 2, CTL vs. TRT 3, CTL vs. TRT 4)

4.4.1 Water Quality – Field Measurement

While tailings treatments were associated with temporary or intermittent effects on pH, turbidity, temperature (bottom only), dissolved oxygen, chlorophyll, and BGA, it was only specific conductivity measurements at the bottom that responded consistently (higher) to the presence of FFT (TRT 4). The elevation in specific conductivity in TRT 1 (CST), TRT 2 (TT), and TRT 3 (TSRU) in 2020 reflected an efflux of ions from the tailing layer into the overlying water. The elevation in conductivity is not unexpected, given the possibility of releasing solute-rich pore-water from tailings. The mixing of water influenced by the initial gradient due to brine rejection made the tailing associated elevation in specific conductivity more detectable in 2020.

4.4.2 Water Quality – Laboratory Measurement

As discussed in the preceding section, primary effect of the tailings treatments on water quality was related to the release of solutes into the overlying water. This effect was most noticeable in the treatments with river water (TRT 1, 2, 3, and 4). Tailing alone is generally associated with an increase in some analytes, similar to OSPW, but the magnitude of the increase was small relative to the concentrations found in the OSPW-treated mesocosms (TRT5 and TRT6) in the current study. Average water quality constituent concentrations were summarized in Appendix F with their CCME water quality guidelines. No values exceed CCME guidelines was observed for the long-term and short-term freshwater quality guidelines for the Protection of Aquatic Life.

Arsenic, copper, and uranium were the most noteworthy analytes which were observed to reduce in the presence of tailings. Metals might be transported from water to the soils/tailings, or biota or vice versa (Sheoran and Sheoran, 2006). Plant and microbial activities alter the distribution of metals in wetland sediments (Weis and Weis, 2004). In future studies, it is recommended to measure metal levels and microbial characterization in soils and tailings.

4.4.3 Installed Vegetation

In general, tailings alone did not affect the growth of the emergent plants most of the time. Interestingly, TR3 (TSRU) had the most flowers of *T.latifolia* in late 2020. Literature suggested that *Typha* can serve as standard test species for ecological risk assessments (Sesin et al., 2021), and it is recommended to use this species to represent emergent macrophytes in future experiments. Conversely, significant differences in *C. demersum* wet weights were observed between CTL and TRT 1, 2, 3, and 4 in 2019. In 2020, only FFT affected the wet weight of the floating plant. *C. demersum* is known as a hyperaccumulator for arsenic (Weis and Weis, 2004). It might also contribute to lower arsenic level in TRT 3.

Compared to FS (CTL), an interesting effect of FFT (TRT 4) was the absence of adventitious vegetation. This observation is consistent with the literature (Roy et al., 2016) and suggests that FFT alone is sufficient to impede vegetation colonization. Once established in CTL, TRT 1, TRT 2, and TRT 3, aquatic plants can impact their environment's physical, chemical, and biological characteristics with time, e.g., increase oxygen concentrations. Additional studies of identification of adventitious vegetation (plants or algae) would be required to confirm the species colonized.

4.4.4 Toxicity

Trout and minnow survival appeared unaffected by the presence of tailings alone. Much of the research regarding toxicity has focused on OSPW, and only limited information about the toxicity of tailings alone is currently available. It should be noted that water used for current toxicity tests was collected from the surface of the mesocosms. Recently, laboratory exposure of early life stages of fish to tailings pond sediment resulted in decreased survival with increasing sediment concentration for walleye (Raine et al., 2017), northern pike (Raine et al., 2018) and fathead minnow (Parrott et al., 2019). Based on these results, sediment toxicity tests should be considered in the future.

4.4.5 Zooplankton

Occasional reduced zooplankton richness, abundance, diversity, and biomass in TRT 1, 2, 3, and 4 relatives to CTL suggest that tailings alone can negatively affect the zooplankton community, although the negative effects tends to be short-termed (mainly in 2019). Significantly higher diversity values were observed in TRT 2, 3, and 4 than CTL at the beginning of 2019. Various environmental conditions could affect the development of zooplankton eggs, such as light, salinity, temperature, and oxygen concentration (Dodson et al., 2010). Non-metric multidimensional scaling (NMDS) analysis of four years (2017 - 2020) zooplankton data suggested zooplankton richness tended to increase with dissolved oxygen content (Appendix F). The temperature was reported to contribute to the generation of freshwater zooplankton in seasonal dynamics (Gillooly, 2000). Our observation at the beginning of 2019 could support this hypothesis. For example, significant lower temperature in TRT 3 (TSRU, 12.99 ˚C) and TRT 4 (FFT, 13.12 ˚C) than CTL (14.89 ˚C) was observed at the bottom at Week 25, 2019. Significantly higher turbidity (shading effect) in TRT 2 (4.34 FNU), TRT 3 (TSRU, 14.61 FNU), TRT 4 (45.19 FNU) than CTL (0.45 FNU) was also found at the surface at the first sampling point of 2019.

An alternative explanation could be fewer zooplankton eggs in filter sand in the CTL mesocosms. As per the provider's statement, the filter sands are NSF 61 certified and meet or exceed American Water Works Association (AWWA) B100 Standards. "NSF/ANSI 61 was developed to establish minimum requirements for the control of potential adverse human health effects from products that contact drinking water"(NSF International, 2016). Significantly higher diversity values in TRT 5 (FFT + 100% Imperial OSPW) and TRT 6 (dFFT + 50% Suncor OSPW) in early 2019 could partially support this hypothesis. The high diversity in TRT 2, 3, and 4 than CTL could also be attributed to the slight suspension of TT/TSRU/FFT compared to FS during installation. However, no direct evidence showed zooplankton eggs in tailings, although zooplankton can be expected from wastewater treatment ponds (Hogg et al., 2017).

To our knowledge, literature focusing on the interaction of individual tailings alone with zooplankton is limited. Diptera seems more sensitive to some tailing types. The Insecta Diptera within the samples consisted of a single-family in 2019, which is Chaoboridae. Chaoboridae is considered both planktonic and associated with the benthos (Personal communication, Sarah Steinerstauch at Biologica Environmental Services, Ltd., June 7, 2021). Additional and more focused investigation of individual taxa would be beneficial to explore further this area of research but beyond the scope of the current study plan.

4.4.6 Macroinvertebrates

FFT may simplify macroinvertebrate communities during the first few weeks of exposure as evidenced by TRT 4 (FFT) which demonstrated lower taxa richness and diversity than CTL. TT (TRT 2) appeared to have a positive influence on macroinvertebrate richness and abundance. Such an effect might be expected from the presence (TT) or absence (FFT) of adventitious plants/algae since macroinvertebrates richness and diversity were associated with macrophyte communities (Waters and San Giovanni, 2002).

It is difficult to conclude whether for CST (TRT 1) and TSRU (TRT 3) that the effect is genuinely absent or present because the differences were not statistically significant. Literature suggests that Diptera Chironomidae has been successfully used as indicators to assess water quality (Liu, 2016). Ecosystems with high productivity might be functionally redundant, and effects on species may not be evident (Graney, 1994). Further analysis of specific species and metrics is suggested. For example, a list of metrics and calculations was proposed by US EPA for macroinvertebrate indices, such as percent dominance and Crustacea/Mollusca (United States Environmental Protection Agency, 2003). However, such is beyond the scope of this study.

4.4.7 Phytoplankton coverage

Phytoplankton (filamentous metaphyton) coverage appeared to be inhibited in the presence of tailings alone, although this statement cannot be supported by the laboratory measurement of Chlorophyll a. Chlorophyll a is commonly used to surrogate total phytoplankton biomass (Gregor and Maršálek, 2004). However, there is no relationship between phytoplankton coverage and lab-measured Chlorophyll a. The presence of chlorophyll in both water chemistry samples and as detected by the data sonde would indicate that phytoplankton was present in the mesocosms containing tailings only. Literature suggested that zooplankton grazing might be more effective for a particular group of phytoplankton (Florencia et al., 2021). Phytoplankton species composition, which is out of the scope of the current study, could be considered in the future to interpret better.

4.5 The Effects of 100% Imperial OSPW with FFT (CTL vs. TRT 5)

4.5.1 Water Quality – Field Measurement

Conductivity and turbidity levels were higher in 100% Imperial OSPW with FFT (TRT 5) (more evident in 2019 at the surface), while temperature (mainly in 2019 at the bottom) and DO (mainly in 2020) were reduced. These are not unexpected findings. As discussed above, the shading effect of higher turbidity can explain the reduced temperature for the first few weeks of 2019, while lack of adventitious plants from soil trough can attribute to reduced DO in TRT 5.

pH was significantly higher for the OSPW with FFT treatments in the first half of 2019. The initial elevation in pH was expected because the measured pH of Imperial OSPW and ARW were substantially different according to the source material characterizations (8.26 and 7.61, respectively). However, the pH in TRT 5 in 2020 was significantly lower than CTL. The variation in pH in different years might be due to biological factors. For example, wetland plants can affect the pH of the medium, especially surrounding the roots (Yang and Ye, 2009). The TRT 5 mesocosms contained significantly more total algae than CTL for the first few weeks of 2019. The differences in the original material (ARW and OSPW) could explain it. The original chlorophyll-a values of Imperial OSPW and ARW were 4.9 µg/L and 1.0 µg/L, respectively.

4.5.2 Water Quality – Laboratory Measurement

The addition of OSPW with FFT to the mesocosms was associated with a marked elevation in the vast majority of analytes' concentration.

However, this was not the case for BOD, manganese (dissolved), and silver (dissolved), which showed no significant change at any sampling point. BOD is often used to estimate the health of the aquatic ecosystem, and lower values generally indicate cleaner water (Afzal et al., 2019). It was noted that the BOD values in TRT 5 (FFT + 100% Imperial OSPW) were within the range of typically measured in natural freshwater between 0.2 to 2.9 mg/L (Albakistani, 2018), and no significant differences in BOD values were detected between CTL and TRT 5 mesocosms. In the presence of OSPW with FFT, few analytes appeared to be reduced, including aluminum (dissolved), copper, iron (dissolved), and thallium. Reduced dissolved Al is suspected to be related to the coagulant behavior, which might settle with FFT particles (Wei et al., 2021). No average values exceed CCME guidelines was observed for the long-term and short-term freshwater quality guidelines for the Protection of Aquatic Life (Appendix F). For example, the level of total nickel (4.91 – 7.55 µg/L) was significantly higher than that in CTL (1.22 – 2.48 µg/L). However, this level of nickel is well below the long-term (chronic) guidelines for the for the Protection of Aquatic Life (52 µg/L, calculated by the lowest hardness observed) (Government of Alberta, 2018).

Hardness (calculated) and calcium concentration (i.e., a component of hardness) trends, were not uniform. For example, in 2019, the calcium concentration was significantly less in TRT 5 (FFT+100% Imperial OSPW) compared to CTL. However, by the end of 2020, this the trend reversed. For reference, the dissolved calcium in ARW was 34.9 mg/L, slightly higher than in Imperial OSPW (31.3 mg/L); and the total calcium in filter sand (19.7 mg/g) was considerably higher than FFT (6.46 mg/g), which could explain the higher calcium concentration in CTL initially. Correlation plot of analytes for metals (dissolved and total) suggested that calcium is not highly correlated with other metals (loadings between -0.5 and 0.5). Since calcium is a nutrient for aquatic vegetation and invertebrates, it is possible that the adventitious vegetation/zooplankton/macroinvertebrates in CTL could have consumed more calcium in water over time, compared to TRT 5. This explanation is supported by minimal adventitious vegetation cover in TRT 5 (FFT+100% Imperial OSPW) by the end of 2020, as well as lower zooplankton abundance compared to CTL. Also, scrapers (including gastropods) were not observed in TRT 5, but in CTL in 2019. Relative less abundance of snails (requiring calcium to produce shells) in TRT 5 was observed compared to CTL. In addition, the differences in pH between TRT 5 and CTL could further contribute to the uniformity, since calcium ions react at higher pH to precipitate.

Previous literature reported that the NAs from the OSPW were predominated by Z-4 and Z-6 (Grewer et al., 2010), which agrees with the current study. Though fluoride (dissolved) concentration in TRT 5 was significantly higher than CTL, these concentrations (~ 4 mg/L) fall in the typical fluoride range for natural surface waters (0.1 – 10 mg/L)(Edmunds and Smedley, 2013). Note that the fluoride (dissolved) concentration was 5.09 mg/L in the original Imperial OSPW. It is suspected that soils might contribute to difluoride concentrations in water (Kumar et al., 2019).

4.5.3 Installed Vegetation

The only significant differences in emergent plant growth patterns observed were related to the flowering rate of *T. latifolia*, where fewer flowers were observed in TRT 5, which contained both FFT and OSPW. Restricted growth rates of *T. latifolia* has been reported in the oil sands wetlands with lower aboveground biomass (Mollard et al., 2013), which was not observed in the current study. A greenhouse experiment found that *Carex* species were not impacted by OSPW, which contained up to 569 mg/L Na and 54 mg/L NAs (Pouliot et al., 2012). However, over time, *C. demersum* disappeared and adventitious colonizers were rarely observed in soil troughs. These findings align with AESRD *Criteria and Indicators Framework for Oil Sands Mine Reclamation Certification* (2013), which references a decrease in relative cover of *C. demersum* as an indicator of stress for open water marsh zones in reclaimed oil sands mining landscapes.

For future study design, fewer pots of each emergent plant species might be sufficient to address differences between experimental groups, since only *T. latifolia* showed significant treatment effects

4.5.4 Toxicity

Several studies have reported LC_{50} values for rainbow trout exposed to raw OSPW , ranging from 3.2% to 92% (Mahaffey and Dubé, 2016). 96-hour trout LC_{50} values for TRT 5 mesocosms were within the range of values reported at Week 26, 2019. Conversely, the survival LC_{50} and biomass IC_{25} to fathead minnow were higher than 100% (% v/v) most of the time in the fall of 2019 and 2020. Given the complexity of the exposure material, the exact causative analytes could not be determined. Literature suggested that NAs and PAHs might be correlated with toxicity, while higher salinity or metals seem unlikely (McNeill et al., 2012). Although toxic unit analysis indicated concentrations of NA toxicity were lower in control mesocosms than in the treatment mesocosms (Appendix F), joint toxicity effects of compounds and elements beyond those specified in the Study Plan.

4.5.5 Zooplankton

The indices and biomass of the zooplankton community in the presence of 100% Imperial OSPW with FFT were not always significantly lower than CTL, especially in 2020. Recently, a mesocosm experiment reported that higher Mg could alter freshwater zooplankton structures (Mooney et al., 2020). However, it is unlikely to explain the zooplankton community changes by the effect of metals.

The presence of 100% Imperial OSPW and FFT is associated with the loss of organisms/taxa. In viewing the taxonomic breakdown of the data, it is tempting to assume that the Calanoida might be sensitive to the oil sands materials in TRT 5. The Calanoida group (Crustacea Copepoda) within the samples consisted of one distinct family (Diaptomidae) and an unknown number of genera represented by juveniles that had not matured sufficiently for their genus to be determined. A majority of the Calanoida biomass in our samples belonged to taxon *Leptodiaptomus sp.*, *Skistodiaptomus sp*., and Calanoida copepodite. Literature indicated that Calanoid copepods might be sensitive to temperature fluctuations (Keenan, 2020). Understanding the relative sensitivity of each genus identified in this study is outside the scope of this study but could be assessed in future studies if desired.

4.5.6 Macroinvertebrates

A previous study showed that the composition, abundance, and diversity of macroinvertebrates in an OSPW-affected wetland was influenced by water chemistry, such as pH and salinity, but not directly influence by sediment (Barr, 2009). In 2019, the macroinvertebrate communities of TRT 5 (FFT+100% Imperial OSPW) collected by activity traps had lower richness, abundance, and diversity compared to CTL. Macroinvertebrate diversity has shown to be lower in OSPW associated wetlands (Kennedy, 2012). The literature has also indicated that macroinvertebrate taxa richness might be affected by NAs concentrations, and the development of macrophytes may initially correlate with macroinvertebrate abundance (Leonhardt, 2003). These explanations agree with the results in the current study; for example, higher NAs concentration and minimal adventitious plants were observed in TRT 5 mesocosms, compared to CTL.

At the beginning of 2019, the Scrapers functional group was absent in TRT 5. Lab research using a limited catalogue of NAs indicated that freshwater gastropod could be progressively affected by 10 mg/L NAs (Johnston, 2015), although the severity of NA toxicity could be related to NA structure (Marasco, 2017). Previous mesocosm studies also reported that the relative abundance of snails was lower in the OSPW-containing mesocosms than the control mesocosms without OSPW during the first year of exposure (Davies, 2018).

However, in 2020, differences in richness, abundance, diversity and HBI for macroinvertebrates collected by activity traps were not statistically significant. As discussed in Section 4.3.6, over time, it is possible that the macroinvertebrate community might recover from the effect of treatment materials. These results align with a previous study that reported an insignificant reduction of macroinvertebrate richness and abundance in OSPW-affected wetlands (Whelly, 2000).

However, in 2020, for samples collected by H-D samplers, there was a significant elevation in abundance in both years and reduced diversity and HBI. As discussed in Section 4.3.6, H-D samplers collected mostly epifaunal (surface-dwelling) invertebrates, while activity traps primarily collected free-swimming macroinvertebrates. For example, a relatively larger abundance of Naididae (may be referred to as detritus worms) appeared in TRT 5 samples collected by H-D samplers, and an ecological study assessing aquatic invertebrates of the AOSR reported that more of this family appeared on the oiled bricks than the controls (Barton and Wallace, 1980). A more detailed species-by-species examination of the data may yield additional information on the relative sensitivity of different macroinvertebrate taxa to OSPW and FFT.

4.5.7 Phytoplankton coverage

Phytoplankton (filamentous metaphyton) coverage was reduced in the second half of 2020, in TRT 5 (100% Imperial OSPW and FFT) compared to CTL. The literature suggested that phytoplankton biomass was not systematically related to NA or major ion levels associated with oil sands development (Leung et al., 2003). However, NA concentrations resulted in significant ecological effects on phytoplankton (Leung et al., 2001). Multivariate analysis indicated several potential stressors contributed to variations in biological responses in general, such as specific conductivity, metals, chloride and NAs. Assessment of the influence of covariate factors, such as salinity and metals, may require further investigation.

4.6 The Effects of 50% Suncor OSPW with dFFT (CTL vs. TRT 6)

4.6.1 Water Quality – Field Measurement

No significant differences were found in water temperature (at the surface) and chlorophyll (2020) between TRT 6 and CTL treatments. Turbidity was higher in TRT 6 (50% Suncor OSPW with dFFT) mesocosms, but differences were only significant for surface samples taken at the first 4 sample measurement dates in 2019. The higher turbidity levels were likely associated with installation.

50% Suncor OSPW with dFFT had significantly higher conductivity compared to CTL. This was expected, because the source material characterization (Appendix A.5.3) showed Suncor OSPW contained a far higher conductivity (3,940 µS/cm, 25°C) than the ARW (345 µS/cm, 25°C).

50% Suncor OSPW with dFFT mesocosm had significantly reduced DO levels, compared to CTL, especially during the first several weeks of each year. Dissolved oxygen level did not meet acute guideline for surface water in Alberta (5 mg/L) until Week 27 at the surface and Week 29 at the bottom in 2019 (Shaw, 1997). The reduction in DO in early 2019 was associated with high conductivity and suspected because lower initial DO in Suncor OSPW than ARW. Although DO level was not measured during material characterization, Chlorophyll a level of Suncor OSPW (0.15 µg/L) was lower than that in ARW (1 µg/L), indicating less photosynthetic activity in Suncor OSPW. Algal mats were only observed at the surface in TRT 6 in early 2020 (Section 3.9.2) and could partially explain lower DO values by differential photosynthetic activity, as well as inhibited mixing in TRT 6.

4.6.2 Water Quality – Laboratory Measurement

Similar to 100% Imperial OSPW with FFT (TRT 5), water quality samples taken in 50% Suncor OSPW with dFFT showed higher levels for most analytes. As expected, NA concentrations were higher in the presence of 50% Suncor OSPW with dFFT. For this study, the NA fractions of more than 20% were Z-4 DBE3 and Z-6 DBE4 in TRT 6. NAs with DBE of 3 and 4 were found to be the dominant NA fractions in tailings ponds of AOSR (Vander Meulen et al., 2021), which to some degree is in agreement with the current study. For the current study, TRT 6 mesocosms only produced higher PAH concentrations than CTL in 2019, but not in 2020. In the 2017 mesocosm study, PAHs were associated with dFFT at the beginning of the exposure. However, PAH compounds were almost non-detectable after several weeks (Davies, 2018), which is consistent with what was observed in this study.

Average water quality constituent concentrations were summarized in Appendix F with their CCME water quality guidelines. Analytes exceed CCME guidelines included chloride, boron, molybdenum, and selenium. The levels of chloride (total) in TRT 6 ranged from 250 to 447 mg/L which is between chronic (120 mg/L) and acute (640 mg/L) guideline levels as per the surface water for the protection of freshwater aquatic life (Government of Alberta, 2018). It should be noted that the establishment of guideline levels for chloride might be in debate (Kindzierski and Bari, 2018). Boron (total) concentrations measured in TRT 6 ranged from 1.5 to 2.2 mg/L which is slightly higher than the long-term (chronic) threshold level 1.5 mg/L as per the surface water quality guidelines to protect freshwater aquatic life (Government of Alberta, 2018). However, the boron (total) level in TRT 6 is lower than the upper limit of water quality guidelines for the protection of agricultural water uses (6 mg/L) (Government of Alberta, 2018). Molybdenum (total, 88 – 173 µg/L) modestly exceeded their CCME water quality guideline for the protection of freshwater aquatic life (73 µg/L) (Government of Alberta, 2018) . Selenium levels were in excess of the CCME guideline of 1 µg/L (Appendix F). Since selenate is generally more toxic than selenite while selenite could be more bioavailable than selenate (Donner et al., 2018), there would be value in measuring selenium species for more accurately estimating potential risks to aquatic organisms in future studies.

Besides metals and metalloids mentioned above,, vanadium (V) is a metal that is receiving increasing attention due to high V content in oil sands in Alberta and its possible toxicity (Gustafsson, 2019). For example, V concentration in AOSR plants was reported greater compared to those in reference locations (Stachiw, 2019). However, to the authors' best knowledge, risk-based guidelines for V remain uncertain and there is no CCME water quality guideline for the protection of aquatic life (https://ccme.ca/en/summary-table). The total concentration of V in TRT 6 is well under 100 µg/L, which is the threshold level cited in irrigation guidelines to protect agricultural water uses (Government of Alberta, 2018). The maximum permissible concentration in freshwater was proposed to be 1.2 µg/L dissolved V for Dutch surface waters, which is only three times higher than local background values (Smit, 2012).

The addition of 50% Suncor OSPW with dFFT was associated with lower concentration of a few analytes, including calcium, magnesium, hardness (calc), copper, and thallium, compared to CTL. The lower concentrations of calcium, magnesium and hardness (calc) might be attributed to the softening of water before or during bitumen extraction. Material characterization (Appendix A.5.3) demonstrates calcium (extractable) in Suncor OSPW (9.91 mg/L) and dFFT (2.42 mg/L) was lower than ARW (35.1 mg/L) and filter sand (11.8 mg/L).

4.6.3 Installed Vegetation

The effect of 50% Suncor OSPW with dFFT on installed vegetation was varied. The presence of 50% Suncor OSPW with dFFT appeared to increase growth and flowering rates of emergent vegetation in general. PCA analysis revealed that biological parameters were highly correlated along with boron, molybdenum, and NAs (Appendix F). This growth stimulation could have resulted from multiple factors, such as the higher concentration of essential elements for plant growth in the bulk water, such as Boron (Woods, 1994). A previous study by Wort (1976) demonstrated that potassium naphthenate can stimulate plant growth, however the results are not directly comparable, because the NAs in OSPW have a different structure. The increased growth rate may have also resulted from an adaptive stress response called hormesis (Agathokleous and Calabrese, 2019). Hormesis was defined as “a dose-response relationship where low doses enhance and high doses are inhibitory”, and has been documented in plants exposed to various chemicals (Agathokleous et al., 2019).

A previous study reported *C. aquatilis* plants had lower heights and shorter leaves in oil sands wetlands compared to natural wetlands (Mollard et al., 2012). The relative frequency of occurrence of *C. aquatilis* was reported to have a negative relationship with salinity (Cowan, 2017). Furthermore, moderate salinity levels have been reported to influence the growth of *A. americanus* plants (Calvo-Polanco et al., 2014). However, this contrasts with what was observed in TRT 6, where no growth inhibition was observed in terms. *C. demersum* generally dominates wetlands where the water nutrients and TSS are high and Ca/Mg levels are low (Rooney, 2011). However, hornwort (*C. demersum*) diminished in the presence of 50% Suncor OSPW with dFFT. Understanding the drivers of these plant responses was beyond the scope of this study. However, if desired, future studies could be designed in a way test the influence of multiple variables, specifically on *C. demersum* and *A. Americanus*.

4.6.4 Toxicity

High NA concentrations (25 mg/L) and conductivities (2 000 µS/cm) were reported to induce an adverse effect for the reproduction of fathead minnow, without significant larval mortality (Kavanagh et al., 2011). Based on the results from Kavanagh et al., (2011), we expected that water from TRT 6 mesocosms would not incur apparent mortality but show growth inhibition. It is in agreement to the observation in the current study. While an attempt to correlate toxicity results with specific compounds or elements is beyond the scope of this study, it is interesting to note that the NA concentration decreased from > 20 mg/L to < 10 mg/L, and conductivity remained >2 000 µS/cm at both sampling years. Any synergistic, additive, or antagonistic effect of OSPW and dFFT might contribute to what was observed. A recent study found that the toxicity of aged OSPW to aquatic species might depend on the different polarities of organic fractions (Bauer et al., 2019). In addition, hardness (calcium), which is significantly lower in TRT 6 than CTL in 2019, is known as a toxicity modifying factor and several guidelines for the protection of aquatic life varies with hardness (Government of Alberta, 2018). Further investigation could consider estimating a series of hydrocarbon fractions in water and applying the Biotic Ligand Model (BLM), which is a new way of developing and expressing metal guidelines for the toxicity of metals (Government of Alberta, 2018).

4.6.5 Zooplankton

Many zooplankton species are sensitive to salinity (Dodson et al., 2010). Previous research has shown richness and diversity of zooplankton are lower with higher salinity (Gutierrez et al., 2018). For example, salinity higher than 1 000 mg/L (~1 470 µS/cm) was reported to cause a reduction in zooplankton richness and abundance (Nielsen et al., 2003). For the sake of simplicity, the conversion for total soluble salts (mg/L) = 0.68 x electrical conductivity (µS/cm) (Hart et al., 1991). However, the presence of 50% Suncor OSPW with dFFT (> 2 000 µS/cm; ~1360 mg/L initially) did not always significantly affect zooplankton outside of the significant reduction in richness, abundance, diversity, and biomass. The majority of effects, if detected, were occurred in 2019. It should be noted that the initial zooplankton communities in the ARW/filter sand and Suncor OSPW/dFFT were expected to be different. It is possible that lower zooplankton taxa richness in TRT 6 in 2019 was a result of a dilutional effect (the zooplankton community in ARW was "diluted" by OSPW to 50%). Additionally, the initial zooplankton community differences could explain the occasional higher abundance, diversity, and biomass in TRT 6 than CTL in early 2019. It is likely that over time, the zooplankton introduced by the inoculation from the local wetland (Section 2.3.3) started to propagate after installation. In the end of 2020, there were no significant effects associated with the presence of 50% Suncor OSPW with dFFT.

4.6.6 Macroinvertebrates

Macroinvertebrate community structure has been considered indictive of stress imposed by oil sands effluent (Bendell-young et al., 2000). Similar to TRT 5, reduced macroinvertebrate richness, abundance, and diversity were found occasionally but at least once in TRT 6 (50% Suncor OSPW with dFFT) compared to CTL over two years. Since adventitious vegetation in TRT 6 was minimal, it is possible that these effects might be correlated with the cover and detritus of adventitious vegetation (Batzer, 2013). Previous research has shown lower macroinvertebrate diversity in OSPW wetlands and was speculated to be related to salinity levels, in addition to the cover of aquatic plants (Kovalenko et al., 2013). In addition, the toxicity of OSPW to invertebrates has been primarily attributed to NAs (Bartlett et al., 2017). Commercial NAs at 7 mg/L was reported to significantly reduce macroinvertebrate abundance in an outdoor mesocosm study (Howland, 2019). Apart from NAs and metals, PAH concentrations have been linked with adverse effects on the macroinvertebrates community in the AOSR by a field monitoring study (Gerner et al., 2017). By assessing the biomass attributed to each Order, it is also perceived that the portion of Diptera in TRT 6 was less than the CTL. Diptera is known to be sensitive to salinity in general (up to 1,000 mg/L) (Hart et al., 1991), which agrees with the highest conductivity observed in TRT 6 (~1,500 mg/L initially). Some Insecta may "choose" mesocosms to colonize, while flightless macroinvertebrates (e.g., Gastropoda) may have to adapt to whatever conditions developed (Batzer et al., 2004). Interestingly, Gastropoda was not found in any sampling method at the beginning of 2019 in TRT 6 but was abundant in CTL. The literature also suggested that Gastropoda can potentially be bioindicators of toxicity for OSPW (Chen et al., 2021).

Interestingly, in both sampling methods, macroinvertebrate abundance was higher in TRT 6 compared to CTL mesocosms. It is suspected to be related to differences in water chemistry. The literature indicates dissolved oxygen could be inversely correlated with macroinvertebrate abundance (Arimoro and Ikomi, 2008). As outlined in Section 4.6.1, dissolved oxygen levels were lower in TRT 6 compared to CTL mesocosms. By assessing detailed community composition, it is perceived that the portion of Oligochaeta may attribute to the higher abundance, which is primarily found in untreated sewage from literature (Arimoro and Ikomi, 2008). It would be beneficial to explore detailed species composition further, in order to understand the relative sensitivity of different macroinvertebrate taxa to OSPW and dFFT.

4.6.7 Phytoplankton coverage

Phytoplankton has been used as an indicator of water quality for decades (Parmar et al., 2016). Although not considered in phytoplankton coverage estimation, algal mats were observed in the spring of 2020 in TRT 6 mesocosms (Section 3.9.2). It is suspected that the algal mats were microbenthic algal communities that were resuspended from the bottom to the surface due to brine rejection mixing or gas generated from the bottom. However, no quantification or taxonomic characterization of the algal mats was completed. In future studies, taxonomic analysis is recommended to determine the response of this foundational component of the food web to oil sands materials.

4.7 Effect of Tailings Treatments (TRT 2 vs. TRT 4)

4.7.1 Water Quality – Field Measurement

Higher conductivity levels in TRT 4 (FFT, 98 – 642 µm/cm) compared to TRT 2 (TT, 97 – 582 µm/cm) is likely mediated by the dissolution of ions, albeit to a lesser degree from TT than FFT and of differing composition, although the conductivity levels of both treatments are not substantially higher compared to natural wetlands in Canada (Trites and Bayley, 2009). In 2019, turbidity measurements in FFT mesocosms were higher than TT mesocosms. The “shading” effect of turbidity could also explain the lower temperature at the bottom of the TRT 4 (FFT) mesocosms than TRT 2 (TT) mesocosms during the first few sampling weeks in 2019. The turbidity likely resulted from disturbing the tailings during installation and physical properties of different tailing types. This hypothesis is supported by observation (Section 3.9.10) that TT seemed more solid while FFT was similar to toothpaste or even watery. Physical properties of tailings, such as water content/solids content and particle size, could also be considered in the future.

pH measurements were significantly lower in the presence of FFT than TT, especially in 2020. Respiration and metabolic byproducts of adventitious vegetation observed in TT but not FFT mesocosms probably played a role in the pH difference.

4.7.2 Water Quality – Laboratory Measurement

Compared to TT, FFT was associated with higher or no difference in most water chemistry analytes, such as NAs, DOC, and hardness. A few analytes were lower in FFT, compared to TT, at least once, including chlorophyll a, BOD, aluminum (dissolved), arsenic, chromium (dissolved), cobalt (dissolved), copper, iron (dissolved), magnesium (dissolved), zinc (dissolved), phosphorus (dissolved). While an extensive assessment of the fate of any particular element is beyond the scope of the current study, it was reported that some blue-green algae species play an essential role in arsenic cycling in aquatic environments (Yin et al., 2012). The difference in BOD could be due to the biological respiration associated with bacteria initiated from the TT/FFT tailing-water interface (Chen et al., 2013). If a microbial characterization study is conducted in the future, sampling is recommended to include tailings and perhaps different depths within the water column.

For some analytes above, dissolved concentrations significantly differed, whereas differences in total concentration were not significant. It is suspected that these analytes had a substantial undissolved fraction that was captured in the total but not the dissolved measurement. Dissolved metals in the current study were those present in the sample after passing through a 0.45 µm filter. Although there is some debate on how to define the dissolved fraction (Chen et al., 2012), for this study we used a widely accepted SOP (Section 2.13.2). Metal speciation testing was not part of the scope of this study but could be considered in future studies.

4.7.3 Installed Vegetation

TRT 2 (TT) had more adventitious submerged vegetation than TRT 4 (FFT), which suggests that TT had less influence on aquatic plant growth than FFT. However, emergent plants' response to TT (TRT 2) and FFT (TRT 4) are identical, in terms of height, flowering rate, or dry biomass. In contrast, *C. demersum* wet weight was significantly lower in TRT 4 (FFT) than TRT 2 (TT) from Week 27 to Week 33, 2020.

4.7.4 Toxicity

Trout survival appeared unaffected by the presence of TT or FFT. There was also a lack of any observable effect from the results of fathead minnow tests. While there is evidence that the FFT did release more different elements or compounds (e.g., boron and lithium) to the water column than TT (Section 3.2.4.4), the release was below any threshold that would elicit any toxicological effect according to the toxicity tests conducted in the current study.

4.7.5 Zooplankton

Anecdotal observations (Section 3.9.1) indicate a visible presence of adventitious plants/algae colonized the soil troughs in TRT 2 (TT) but not in TRT 4 (FFT). The presence of aquatic plant debris at the bottom of the mesocosms could favor zooplankton diversity (Eskinazi-Sant'anna and Pace, 2018). Compared to unplanted systems, plants reduced the acute toxicity of NAs on freshwater zooplankton *Daphnia magna* (Armstrong, 2008). However, *Daphnia sp*. was observed in both treatment mesocosms at all sampling points, and no considerable difference was perceived for abundance and biomass by examining detailed zooplankton species data. This effect seems not substantial by the presence of tailings alone in terms of abundance and biomass.

4.7.6 Macroinvertebrates

The macroinvertebrate community has higher richness, abundance, and diversity in TRT 2 (TT) than TRT 4 (FFT) at least once, it could because almost no coverage of adventitious plants/algae in FFT contained mesocosms (TRT 4), which was expected to create habitats and act as food for aquatic macroinvertebrates.

4.7.7 Phytoplankton

There were no significant differences in relative phytoplankton abundance between the different tailing types (TRT 2 vs. TRT 4), however these results were likely confounded by the low percent cover. A higher precision method for quantifying phytoplankton species abundance could be considered if this response variable is of interest in future studies.

4.8 Effect of 100% Imperial OSPW in the Presence of FFT (TRT 4 vs. TRT 5)

4.8.1 Water Quality – Field Measurement

In the presence of FFT and 100 % Imperial OSPW, surface water conductivity levels measured in the field were higher in both years, while the higher turbidity, pH, DO, chlorophyll and BGA levels only lasted for a few weeks after installation in 2019. Slight reductions in turbidity and DO levels were observed occasionally in late 2019 or 2020 season. Theoretically, dissolved oxygen content in water decreases as salinity increase (Shaw, 1997). However, the non-uniformity pattern in DO levels could be a result of photosynthesis (Dings-Avery, 2019). In the future, it could be considered to investigate more details of phytoplankton communities by identifying taxa presented, if interested.

4.8.2 Water Quality – Laboratory Measurement

Water samples taken for TRT 5 (FFT + 100 % Imperial OSPW) showed higher concentrations of most analytes, compared to TRT 4 (FFT + ARW). However, this was not true for lead (total), magnesium (dissolved), silver (dissolved), thallium, thorium (dissolved), and titanium (total), which showed almost no change between the two treatments. Calcium, aluminum (dissolved), and iron (dissolved) concentrations were lower on occasion for TRT 5, compared to TRT 4. Possible mechanisms for these reductions are discussed in Section 4.5.2.

4.8.3 Installed Vegetation

The effect of 100% Imperial OSPW in the presence of FFT on installed vegetation was varied. The increased growth (height and dry biomass) in TRT 5 (FFT + 100% Imperial OSPW) was evident occasionally for some emergent plants, while fewer flowers of *T.latifolia* were observed during the second half of 2020. These results suggest that there may be a stimulation effect of 100% Imperial OSPW in the presence of FFT for certain macrophyte species. As expected, hornwort (*C. demersum*) wet weight was reduced in the presence of OSPW due to its sensitivity to changing water quality, as described in Section 4.5.3.

4.8.4 Toxicity

NAs are generally believed to be the origin of most of the toxicity associated with OPSW (Li et al., 2017). Overall, low levels of trout toxicity were observed at the end of 2020. Biomimetic extraction via solid-phase microextraction (BE-SPME) is believed to be a valuable tool to predict the toxicity of complex mixtures of OSPW (Redman et al., 2018). NMDS analysis found some indices of zooplankton and macroinvertebrate communities (e.g., richness) tended to decrease with increases in BE-SPME. More details were discussed in Appendix F.

4.8.5 Zooplankton

Occasionally, there was reduced zooplankton species richness, abundance, and diversity occasionally in TRT 5 compared to TRT 4. These differences could be related to differences in pH, temperature, phosphorus, and DOC concentration (Shurin et al., 2010). OSPW has been previously reported to affect the feeding of *D. magna* (Lari et al., 2017). However, the source of OSPW in the former study is expected to be different than that used in our experiment and would have different chemical with potentially different impacts on zooplankton populations. The relative sensitivity of each genus identified in different treatments may be worth further analysis.

4.8.6 Macroinvertebrates

The macroinvertebrate communities collected by H-D sampler experienced occasional differences between TRT 4 and TRT 5. An outdoor mesocosm study found that macroinvertebrate communities were sensitive to 10% OSPW, although commercial mixtures were used (Howland et al., 2019). However, monitoring lower Athabasca River exposed to oil sand mining activities revealed that the macroinvertebrates appeared more affected by municipal sewage effluent than mining activities (Culp et al., 2020). For samples collected from activity traps, TRT 5 (100% Imperial OSPW + FFT) had no significant effect in richness, abundance, diversity, and HBI, compared to TRT 4 (FFT + ARW).

For samples collected by H-D samplers, the macroinvertebrate communities of TRT 5 showed reduced diversity at the end of 2020. Moreover, the functional group Scrapers was absent in TRT 5. Conversely, macroinvertebrate samples taken in TRT 4 (FFT + ARW) had lower richness and abundance in 2019. These results exemplify the difficulty in separating the effects of OSPW on macroinvertebrate communities. The combined effect of OSPW and FFT on macroinvertebrate community composition requires future investigation by a more detailed species-by-species examination of the data obtained.

4.8.7 Phytoplankton

There were rarely any significant differences in phytoplankton relative abundance between TRT 4 and 5. It is likely that FFT in the mesocosms did not amplify the effect of OPSW, although the effects of FFT and OSPW on phytoplankton communities could not be fully determined. In future studies, taxonomic analysis is recommended to determine the response of this foundational component of the food web to oil sands materials.

5.0 CONCLUSION AND RECOMMENDATIONS

The first goal of the 2019 – 2021 aquatic mesocosm research project was to evaluate the physical and chemical properties of tailing types and the resulting pore water, as well as OSPW. Learnings mainly related to the first objective include:

- The pH in all treatment groups (including CTL) was in the neutral to alkaline range (7.5 – 9.5). The pH was usually lowered by treatment materials, but typically no more than 1 pH unit from the CTL mesocosms.

- DO levels were initially reduced following the introduction of tailings and OSPW. DO levels did not meet the acute guideline for surface water in Alberta (5 mg/L) for the first few weeks in each open water season in mesocosms with 50% OSPW and dFFT. However, these conditions did not persist, and DO concentrations in all treatment groups (including CTL) converged later in the growing season in each year.

- Overall, the treatments with tailings alone had relatively similar conductivity to the CTL mesocosms. However, OSPW groups exhibited higher specific conductivity than CTL units. Experimental groups rarely exhibited any depth-related differences within treatments and weeks in 2019. Early in 2020, specific conductivity was significantly higher at the bottom than the surface in all mesocosms. Differences in the length of time these depth-related conductivity differences persisted could be related to degree of salinity of the test materials i.e., differences between surface and bottom water lasted longer for treatments containing OSPW, as they were more saline.

- After installation, the high turbidity levels decreased over time and remained low in all treatment groups.

- Treatments with both OSPW and tailings (TRT 5 and TRT 6) were associated with higher concentrations of most elements and compounds.

- The concentrations of some analytes exhibited a subtle increase over the two study years, with this increase usually being more evident in tailings-only groups (e.g., time-related increases in boron concentration in TRT 4 (FFT)).

- Mesocosms containing both OSPW and tailings (TRT 5 and 6) showed significantly higher levels of NAs relative to CTL mesocosms. However, there was a significant decrease in NAs for both TRT 5 and 6 mesocosms over two years. In spring of 2020, a chemocline artificially lowered relative NAs concentrations at the surface, but not absolute concentrations across the entire water column.

- Brine rejection substantially affected the water quality parameters analyzed, and this effect was relatively pronounced in TRT 5 and TRT 6. For example, molybdenum showed a significant mid-study decrease in TRT 5 and TRT 6, while no change was observed in CTL and tailings-alone mesocosms. Since water samples for laboratory analysis were only collected at the surface, some of the significant mid-study decreases observed may be an artefact of the heterogenous vertical distribution of analytes following the overwintering period, since water samples were not collected at depth.

- Concentrations of hydrocarbons associated with OSPW and tailings treatments, such as BTEX, PAHs and phenols, were generally low, and most of these compounds were undetectable with current commercially available analytical methods in the second year of the experiment. The addition of tailings alone to the mesocosms was not associated with exceedance of CCME guidelines for the long-term and short-term freshwater protection of aquatic life based on the mean concentrations of most compounds; the only exception was for fluoride (TRTs 1, 2, 3, and 4). Though fluoride concentration exceeded the long term CCME guideline (120 µg/L) - mostly in treatments with tailings alone - fluoride concentrations were only statistically significantly higher in the presence of FFT (TRT 4) compared to CTL at the beginning of 2019. In 2020, the concentration of fluoride was statistically significantly higher in the presence of TT (TRT 2), TSRU (TRT 3), and FFT (TRT 4) when compared to CTL.

- Besides fluoride, selenium concentrations were higher than the CCME water quality guideline (1 µg/L) during the last two sampling points in TRT 5 in 2020. Mercury levels exceeded long term CCME guideline values for the protection of freshwater aquatic life (0.026 µg/L) mainly in 2019. However, the reporting limit of mercury in this study is 0.02 µg/L.

- In TRT6 (50% Suncor OSPW with dFFT), chloride and molybdenum exceeded the CCME chronic guidelines in 2019 and 2020. Arsenic and boron also slightly exceeded the chronic guidelines (Table S3). Several guidelines for the protection of aquatic life vary with hardness, and TRT 6 exhibited lower hardness (62 - 99 mg/L $CaCO_3$) compared to CTL (84 – 140 mg/L $CaCO_3$).

Recommendations:

- In future studies, there would be value in measuring selenium species in water and bioaccumulation in organisms at different trophic levels for more accurately estimating potential risks to aquatic organisms.

- Future studies could include more sensitive methods for determining mercury (total and methyl) concentrations.

- Brine rejection effect is expected to affect water quality parameters for any overwintered studies when surface samples are collected. It is recommended to take an integrated depth (or composite) sample in future studies to reflect the chemistry of the entire water column and minimize the artifacts in trend analysis.

- We recommend that NAs, PAHs and metals should be measured in the sediments (e.g., soil and tailings) in addition to the water column in future studies, as hydrocarbons and metals are expected to mobilize between sediment and water in the mesocosm structures and the characteristics of sediments are likely to change with time.

The second goal was to determine how different tailing types alter the water column in terms of biological and toxicological properties. Learnings mainly related to the second objective include:

- From the mid- to end-point of 2020 sampling season, phytoplankton coverage in CTL mesocosms was significantly higher than that observed in TRT mesocosms. Algal mats were only observed in TRT 6 mesocosms in the spring of 2020.

- In general, tailings alone and OSPW with FFT did not affect the growth of the emergent plants (ratroot, water sedge, awned sedge, and cattail) most of the time over two years of exposure with few exceptions, suggesting that these plants were tolerant of the various constituents associated with these materials. However, TRT 6 had a significant stimulatory effect on the growth of all four emergent species. Emergent plants growing in Suncor OSPW (50%) with dFFT were significantly taller. The aboveground biomass of all four emergent species was significantly greater in TRT 6 than CLT mesocosms in both years.

- Tailings alone groups negatively affected hornwort in 2019, but this effect only persisted into 2020 for FFT mesocosms. However, tailings with OSPW negatively impacted hornwort in both years, which did not survive in mesocosms containing OSPW with tailings treatments in 2020. These findings align with the AESRD *Criteria and Indicators Framework for Oil Sands Mine Reclamation Certification*, which references a decrease in relative cover of hornwort as an indicator of stress for open water marsh zones in reclaimed oil sands mining landscapes.

- There were no statistically significant differences between TRTs3, 5, 6 and CTL in terms of uptake of PAHs. Compared to CTL mesocosms, statistically significant accumulation of trace metals was rarely documented in the above-ground tissues of all four species of emergent plants by the end of the first sampling season, although concentrations of metals in tissues could be influenced by different OSPW and tailings treatments. Overall, differences in metals uptake by emergent plants were more evident in the presence of both OSPW and tailings (TRTs 5 and 6) than tailings alone (TRT3).

- The presence of treatment materials occasionally affected zooplankton and macroinvertebrate communities. Occasional reduced zooplankton and macroinvertebrate indices, including richness, abundance, and diversity, in TRTs relative to CTL suggest that even tailings alone can negatively affect the zooplankton community, although the negative effects tend to be short-lived (mainly in 2019).

- The acute 96-hour LC_{50} for rainbow trout, and the chronic 7-day LC_{50} and IC_{25} for fathead minnow, tests were included for estimating toxicity of the treatment materials. In general, both the trout and fathead minnow assays detected "non-lethal" in the presence of tailings alone. TRT 5 (FFT + 100% Imperial OSPW), and one TRT 6 mesocosm (dFFT + 50% Suncor OSPW) had rainbow trout LC_{50} values less than 100% (~55% and 82%, respectively) at the beginning of the 2019 sampling season. In 2020, some level of mortality was also observed in TRT 5 mesocosms. Limited biomass effects were observed early in 2019, for one mesocosm in each of the TRT 4, TRT 5, and TRT 6 mesocosms.

Recommendations:

- Taxonomic analysis is recommended to further determine the response of phytoplankton communities to oil sands materials.
- The toxicity in this study was quantified using standard test organisms. Non-standard test species, including those native to the Athabasca oil sands region, should be considered in the future.
- Microbial communities play a pivotal role in maintaining overall ecosystem functioning. Microbial analysis is recommended to obtain taxonomic data to understand how different treatment materials alter different microbial community assemblages.

The third goal was to provide information on early biological establishment rates and water quality trajectories. Learnings mainly related to the third objective include:

- By the end of two years of exposure, vascular plants had colonized installed submerged soil in CTL, and TRT 1, 2, 3 mesocosms, but colonization was limited in mesocosms containing FFT and OSPW (TRT 4, 5, and 6).
- There was rarely any indication of growth inhibition of installed emergent plants over the two years of the study. For TRT 6, the growth and flowering rates of emergent plants were higher than in other treatments or the controls. No statistically significant effect on emergent plant growth was observed in TRT 5 (which also contained OSPW and FFT) compared to CTL mesocosms.
- Trout and minnow survival was unaffected by the presence of tailings alone. Overall, toxicity to trout declined over time in OSPW-containing groups (TRT 5 and TRT 6) throughout 2019, while fathead minnow mortality results stayed mainly unchanged in both of these treatments. Some biomass reductions were observed in TRTs 4, 5, and 6 in 2019.
- There is some indication that time influenced the structure of the zooplankton community in both CTL and TRTs mesocosms. In general, the highest richness and biomass values were documented at the beginning of 2020. Zooplankton richness was low initially during establishment stage, and the communities might be re-established over time, and any decreases in abundance could be age-related.

- All mesocosms were found to support populations of macroinvertebrates in both years. Macroinvertebrate species richness appeared to increase or exhibit little change in 2019 but decreased during 2020. The similarity in the pattern of species richness across all experimental groups suggests the influence of an environmental factor common to all mesocosms (e.g., overwintering and time) and not a specific treatment effect.

- Some level of risk to zooplankton and aquatic macroinvertebrates related to the experimental materials was indicated. The presence of OSPW and tailings together significantly impacted the zooplankton and macroinvertebrate communities during some sampling periods; the majority of these effects were detected in 2019. For example, TRT5 and 6 mesocosms had significantly lower zooplankton/macroinvertebrate species richness and abundance compared to CTL at least once in 2019. Our results suggest the overall ecological structure of the mesocosm community changed over time, and it is possible that the zooplankton and macroinvertebrate community might recover from the effect of treatment materials over time.

- The Spearman Rank correlations (Appendix F) indicated some measures of zooplankton and macroinvertebrate species richness and/or abundance exhibited a negative relationship with increased concentrations of metals (i.e., boron, molybdenum, nickel, vanadium), metalloids (i.e., selenium), naphthenic acids, specific conductivity, and chloride, and with decreased dissolved oxygen levels.

Recommendations:

- Additional studies of identification of adventitious vegetation (plants or algae) would be required to confirm the species colonized and provide additional information related to the changes in vegetation establishment in the mesocosms.

- It should be noted that water used for current toxicity tests was collected from the surface of the mesocosms. Sediment toxicity tests are not conducted and are recommended to consider in the future.

- A more detailed species-by-species examination of the macroinvertebrate/zooplankton data may yield additional information on the relative sensitivity of different taxa to different tailings and OSPW.

- A Principal Component Analysis (PCA) for water quality data indicated that total and dissolved fractions of metals were highly correlated and seemed to correlate with similar biological effects. Therefore, dissolved metals measurement may be redundant for future studies.

6.0 GLOSSARY OF TERMS AND ACRONYMS

6.1 Terms

OSPW (Oil Sands Process (affected) Water)

Water used in the bitumen extraction process.

Commissioning

The installation of internal structures and materials, including shelving/rafts, mesh socks, installed plants, troughs, filter sand, and conditioned/unconditioned topsoil.

Establishment

A short period during which simple food webs stabilize and biotic and abiotic elements of the mesocosm start to interact.

Exposure period

The period during which the mesocosms are exposed to tailings and/or OSPW.

Mesocosm decommissioning

The process whereby the last, often destructive, samples are collected, and the tanks are refurbished for a new study.

LC_{50}

LC_{50} is the Median lethal concentration, i.e., the concentration of substance or material in the water that is estimated to be lethal to 50% of the test organisms.

IC_{25}

IC_{25} is the concentration estimated to cause a 25% reduction in growth (including that measured as biomass) of larval fish relative to the control.

6.2 Acronyms

ARW	Athabasca River Water
AOSR	Athabasca Oil Sands Region
BE–SPME	Biomimetic Extraction via Solid Phase-micro Extraction
BGA	Blue-Green Algae
BLM	Biotic Ligand Model
BML	Base Mine Lake
BTEX	Benzene, Toluene, Ethylbenzene, Xylenes
BOD	Biological Oxygen Demand
cfMFT	coagulated-flocculated Mature Fine Tails
COD	Chemical Oxygen Demand

COSIA	Canada's Oil Sands Innovation Alliance
CST	Coarse Sand Tailings
DBE	Double Bond Equivalents
dFFT	Densified Fluid Fine Tailings
DPL	Demonstration Pit Lake project
DOC	Dissolved Organic Carbon
EAS	Environmental Analytical Services
FFT	Fluid Fine Tailings
FS	Filter Sand
FTIR	Fourier Transform Infrared Spectroscopy
HBI	Hilsenhoff Biotic Index
H-D	Hester-Dendy
HDPE	High-density Polyethylene
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
JIP	Joint Industry Partnership
MAIS	Monitoring, Analytics & Informatics Services
NAs	Naphthenic Acids
OSPW	Oil Sands Process (affected) Water
PAHs	Polycyclic Aromatic Hydrocarbons
PCA	Principal Component Analysis
PL	Pit Lakes
PVC	Polyvinyl Chloride
SOPs	Standard Operating Procedures
TOFMS	Time-of-Flight Mass Spectrometry
TPW	Tailings Pond Water
TSRU	Tailings Solvent Recovery Unit Tails
TT	Thickened Tailings
WEPA	Water Environmental Priority Area

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