Cyanobacteria, Periphyton and Aquatic Macrophytes. Evaluation of Cause – Decline in Upper Fording River Westslope Cutthroat Trout Population

Prepared for:

Teck Coal

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Date: May 10 2021

Executive Summary

Upper Fording River (UFR) periphyton includes a typical diverse and dynamic assemblage of diatoms, cyanobacteria (also known as blue-green algae), filamentous algae, and numerous bacterial families that together form the base of the UFR food web. Bryophytes (aquatic mosses) are generally distributed, while aquatic macrophytes (rooted aquatic plants) occur in slow-flowing UFR habitats. These naturally occurring primary producers are an essential component of all rivers.

The potential of cyanobacteria, periphyton and aquatic plants to apply stress directly or indirectly to Westslope Cutthroat Trout (WCT) life stages was assessed using available data, Teck Coal research projects and literature reviews. This report investigates four impact hypotheses addressing the WCT decline as either the result of a single acute or chronic stressor, or a combination of acute and/or chronic stressors. However, the absence of cyanobacteria, periphyton and macrophyte samples from the Decline Window limited this investigation.

Cyanobacteria

Hypothesis 1 examines the potential for cyanotoxicity to WCT in the UFR, through acute and chronic effects.

Like most rivers, cyanotoxins can be produced by numerous cyanobacteria taxa found in the UFR. Most benthic cyanobacteria occurring in rivers do not kill fish, but naturally occurring *Phormidium autumnale* blooms have in other river systems. *P. autumnale* grows on and in UFR biogenic calcite crusts, where dark conditions can enhance its cyanotoxin production. Chronic low-dose exposure to cyanotoxins during low flows can affect invertebrate and fish health, particularly alevin and juvenile WCT but only at sites with large cyanobacteria populations. Summer or winter cyanotoxin stress may lower overall fish health, potentially making them more susceptible to other stressors.

Cyanobacteria presence and the possibility of chronic low-dose stress to WCT is both natural and long-standing in the UFR, thus low-dose stress is unlikely to account for the WCT decline. Overall, cyanotoxins would not account for the WCT decline because early WCT life stages are more susceptible to cyanotoxins with lesser consequences to older age classes, and this is not consistent with the observed WCT decline.

Periphyton

Hypothesis 2 examines the roles of filamentous algae blooms, both positive and negative.

Proliferations of filamentous algae during late summer low flows are probable in most years since 2013 and in 2019, they exceeded observed extents in other regional rivers. Filamentous green algae blooms in Fish Pond Creek and a widespread but moderate *Didymosphenia geminata* (Didymo) bloom through the UFR mainstem were observed during stable low flows in early fall 2019. A similar bloom may have occurred in 2018 but data are limited. Sites with abundant Didymo in 2019/2020 also had some in 2015 sampling, indicating that it was not a recent introduction to these sites. Growing season conditions in the WCT Decline Window were conducive to an algae bloom due to stable low flows with low TSS. Based on fisheries research elsewhere, excessive filamentous Didymo growth is unlikely to account for the WCT decline, however its bloom phases could potentially stress juvenile WCT during their summer rearing stage. Similarly, a gradually reduced hyporheic exchange during late summer through localized substrate bioclogging is a common occurrence regionally, so is unlikely to account for or contribute to the WCT decline. Without a fall flushing flow to remove it, the unusually large amount of organic material from a filamentous algae bloom would commence decay during the winter, affecting dissolved oxygen demand, hyporheic exchange, redox conditions, all with some potential to affect overwintering WCT. Lower dissolved oxygen in slow-flowing reaches during prolonged ice cover appears to be the only mechanism by which periphyton could have contributed to the WCT decline, however data to support this mechanism are not available from the Decline Window.

Hypothesis 3 examines the roles of periphyton in the bioreactor functions of the UFR
Friable biogenic calcite (porous calcite-periphyton crusts) and periphyton biomass can gradually accumulate between strong freshets or floods. They are expected to measurably lower hyporheic exchange in low velocity areas of depositional reaches. When hyporheic exchange is restricted, periphyton and bacterial bioreactor processes that act to sequester metals and nutrients may be affected, leading to higher concentrations in solution. These water quality changes are likely attributable first to the effects of stable low flows on dilution and second on lower bioreactor functioning. A gradual and localized reduction in hyporheic exchange through bio-clogging is unlikely to account for the WCT decline or contribute to it unless a threshold or tipping point was reached. There is no evidence to suggest that periphyton-driven water chemistry changes critical to WCT health were reached within the Decline Window.

Fines trapped in the periphyton layer included organic debris, calcite and dark particulates (coal fines). These materials can increase periphyton tissue PAH and metal concentrations. Passive adsorption to periphyton surfaces may decrease metal trophic transfer while uptake into periphyton cell structures may increase it. Dry Creek results indicated that elevated Se in Didymo blooms may be concurrent with elevated Se in benthic invertebrate tissues. However, periphyton bioaccumulated Se did not measurably impact benthic invertebrate density, thus the requisite conditions for Se periphyton bioaccumulation to be the sole cause of the WCT decline were not met. Similarly, fines entrapment may reduce periphyton palatability to scraping invertebrate grazers, but fines did not result in a measurable impact on UFR benthic invertebrates. This work provides an indirect line of evidence indicating that trapped fines, PAHs and adsorbed metals in periphytonwere not the sole cause of the WCT decline.

Aquatic Macrophytes and Bryophytes

Hypothesis 4 examines the roles of aquatic macrophytes and bryophytes in the UFR.
Aquatic macrophytes and bryophytes are native to the UFR system, indicating adequate water and sediment quality to support their growth. Macrophyte stands have redeveloped in slow flowing UFR reaches following the 2013 flood. Bryophytes are more widely distributed and extensive than macrophytes in the lotic-dominated UFR watershed. Bryophytes can accumulate metals through cation exchange, but their growth was not observed to be unusual during the Decline Window. Based on the literature and studies of Elk Valley lentic sites, the highest metal concentrations are likely to occur in macrophyte root zones where bioaccumulated metals would be unavailable to benthic invertebrates or WCT. Submerged macrophyte rhizospheres host a bacterial consortium that can decompose some PAHs and attenuate metals, although selenate reduction with subsequent uptake into aquatic food chains is also known to occur. The benefits of aquatic macrophytes and bryophytes and their slow expansion following the 2013 flood provides evidence against impacts mediated by these plants as the sole cause of, or a significant contributor to the WCT decline.

Although it is uncertain at this time, stable low flows in summer and fall 2018, followed by the extremely cold winter with very low flows may have led to a cascade of winter effects. These winter effects involving deep frost, extensive ice formation, and increased oxygen demand exerted by decomposing macrophytes and periphyton may have been unusually strong in Winter 2018/2019 because a fall flush that would remove organic material from the UFR did not occur prior to this winter. The measured DO sag in Winter 2019 was part of a declining trend unique to the Decline Window, but it did not reach critical thresholds for WCT survival. If DO was depleted in late winter 2019 at lower S6, it could be an important factor in the

WCT loss, or lowered DO could contribute to overwintering WCT stress and induce them to move locations, however the strength of evidence supporting this hypothesis is weak.

Summation

The hypotheses covered in this report are all strongly affected by the UFR flow regime. Within the WCT Decline Window, stable low flows without a fall flushing flow between the summer 2018 growing season and winter 2019 would heighten potential decomposition driven impacts from cyanobacteria, periphyton and aquatic macrophytes. The accumulation of organic material and fine sediment between large freshets or floods together with the biochemical changes they induce could theoretically impact overwintering WCT in low flowing UFR reaches but only during an extreme winter such as Winter 2018/2019. Cyanobacteria, periphyton and aquatic macrophytes may have played a minor role in the WCT decline by contributing to oxygen demand when extreme winter conditions had the potential to disrupt oxygen delivery to slow flowing reaches such as lower S6. However, it is unlikely that these primary producers played a significant role in the WCT decline.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

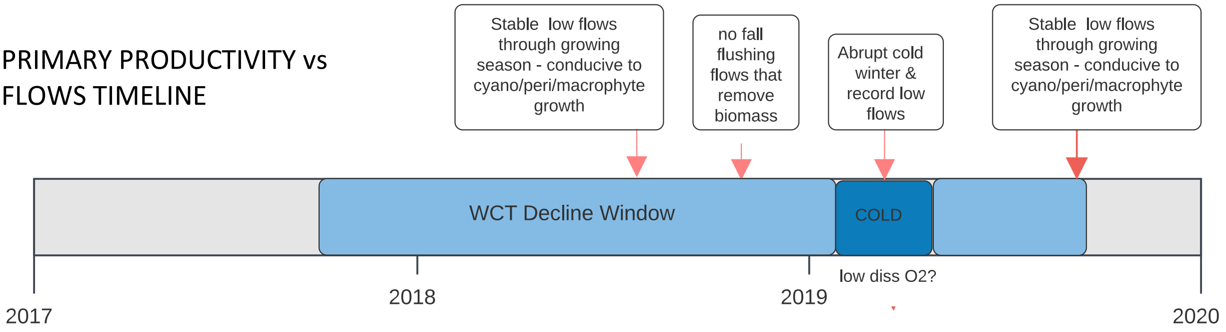
Acronyms and Definitions

Algae	A diverse group of photosynthetic aquatic plants including diatoms, filamentous green algae, cyanobacteria, chrysophyta, and flagellated forms
Anoxic	0 mg/L dissolved oxygen (DO) but available in combined forms (NO_3 SO_4 etc.); hypoxic refers to low DO 1 – 30%; anaerobic refers to cellular respiration requiring an absence of free oxygen
BOD	Biochemical oxygen demand
cBOD	Carbonaceous biochemical oxygen demand
COD	Chemical oxygen demand
Diatom	A single-celled alga that has a cell wall made of silica (glass)
DO	Dissolved oxygen
DIN	Dissolved inorganic nitrogen is the cumulative total of nitrate, nitrite and ammonia
DRP	dissolved reactive phosphorus can immediately support plant and algae growth (~ortho-P)
Hyporheic exchange	The mixing of surface and shallow subsurface water through porous sediment surrounding a river and is driven by spatial and temporal variations in channel characteristics (streambed pressure, bed mobility, alluvial volume and hydraulic conductivity)
Hydraulic conductivity	Water movement through a saturated substrate
IRB	Iron-related bacteria
PAHs	Polycyclic aromatic hydrocarbons
Periphyton	A mixture of cyanobacteria, algae and bacteria that grows on substrates in a mucilage layer
rkm	River kilometer – the distance upstream from confluence with Elk River
Spate	A sudden flood in a river, especially one caused by heavy rains or melting snow
SRB	Sulfate reducing bacteria
TSS	Total suspended solids
TTF	Trophic transfer factor
UFR	upper Fording River
WCT	Westslope Cutthroat Trout
YOY	Young of year 0+ age class, emergence to 6 – 7 cm in length = fry

RANKING of STRESSORS DEFINITIONS

Negligible	Unlikely to contribute meaningfully to the decline
Minor	A small proportion of the decline is likely attributable to the impact hypothesis
Moderate	A moderate proportion of the decline is likely attributable to the impact hypothesis
Major	This impact hypothesis is likely to explain most of the decline

PRIMARY PRODUCTIVITY vs FLOWS TIMELINE



GENERAL ACKNOWLEDGEMENTS

This report was written with extensive support from Fording River Operations staff, Teck Coal staff and their consultants, notably Mike Moore, Dan Bairos, Stefan Humphries, Patti Orr and Todd Hatfield. It was reviewed by Mariah Arnold, Marko Adzic, Cait Good, Stefan Humphries, Maggie Branton and Karsten Liber. Their contributions are gratefully acknowledged.

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Preferred Citation

Larratt, H. and J. Self 2020. Upper Fording River Evaluation of Cause: Cyanobacteria, Periphyton and Aquatic Macrophyte Impacts. Consultant's report prepared for Teck Coal Fording River Operations, Prepared by Larratt Aquatic Consulting Ltd. November 9 2020.

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TEAM QUALIFICATIONS

The Larratt Aquatic Consulting (LAC) team members involved in this project have the following relevant qualifications:

Heather Larratt 1st class Honors B.Sc. in Environmental Biology (1978) R.P. Bio. 40 years of experience

Mine reclamation	30 years research in reclaiming pit lakes and tailings ponds as simultaneous bioreactors and aquatic habitat, 25 year of work at HVC was awarded the TRCR award for mine reclamation in BC 2018
River productivity research	Productivity studies for BC Hydro on the middle and lower Columbia River, Peace River Site C | Periphyton and water quality analysis for Teck Trail Operations 2012-3, 2015 and 2018 study years
Phytoplankton periphyton studies	Algae analyst for Prov. B.C. | certified diatomist pending |
Cyanobacteria research	Extensive research into cyanotoxicity in drinking water | helped write the Interior Health Authority (IHA) cyanobacteria guidelines

Jamie Self Honors B.Sc. (2011) R.P. Bio. 10 years of experience

Statistical expertise	10 years of statistical analysis experience in professional and academic environments with focus on water quality data
Designing Programming data platforms	Created the Okanagan Regional Water Quality Database and web analytics tool hosted by Okanagan Basin Water Board
GIS, LiDAR	10 years of digital mapping and GIS experience in professional environments in conjunction with academic studies in GIS at University of Waterloo
Data analysis	Developed data analysis, visualization, and screening tools using R programming language

Innovations developed by LAC for water management clients include: the use of powdered limestone as a sediment cap for nutrient control, strategic reservoir aeration/wasting to prevent anoxic conditions, in-situ generation of copper ions for cyanobacteria control and lowering trihalomethanes through reducing cyanobacteria concentrations.

Innovations developed by LAC in mine reclamation include: developing a resilient passive sulphate reducing bacteria treatment for metal removal, identifying and correcting the link between low B vitamin concentrations and low algae production in basic pit lakes, and developing a rapid method for planting aquatic macrophytes in reclaimed tailings ponds.

READER'S NOTE

What is the Evaluation of Cause and what is its purpose?

The Evaluation of Cause is the process used to investigate, evaluate and report on the reasons the Westslope Cutthroat Trout population declined in the upper Fording River between fall 2017 and fall 2019.

Background

The Elk Valley is located in the southeast corner of British Columbia (BC), Canada. It contains the main stem of the Elk River (220 km long) and many tributaries, including the Fording River (70 km long). This report focuses on the upper Fording River, which starts 20 km upstream from its confluence with the Elk River at Josephine Falls. The Ktunaxa First Nation has occupied lands in the region for more than 10,000 years. Rivers and streams of the region provide culturally important sources of fish and plants.

The upper Fording River watershed is at a high elevation and is occupied by only one fish species, a genetically pure population of Westslope Cutthroat Trout *(Oncorhynchus clarkii lewisi)* — an iconic fish species that is highly valued in the area. This population is physically isolated because Josephine Falls is a natural barrier to fish movement. The species is protected under the federal Fisheries Act and the Species at Risk Act. In BC, the Conservation Data Center categorized Westslope Cutthroat Trout as "*imperiled or of special concern, vulnerable to extirpation or extinction."* Finally, it has been identified as a priority sport fish species by the Province of BC.

The upper Fording River watershed is influenced by various human-caused disturbances including roads, a railway, a natural gas pipeline, forest harvesting and coal mining. Teck Coal Limited (Teck Coal) operates the three surface coal mines within the upper Fording River watershed, upstream of Josephine Falls: Fording River Operations, Greenhills Operations and Line Creek Operations.

Evaluation of Cause

Following identification of the decline in the Westslope Cutthroat Trout population, Teck Coal initiated an Evaluation of Cause process. The overall results of this process are reported in a separate document (Evaluation of Cause Team, 2021) and are supported by a series of Subject Matter Expert reports.

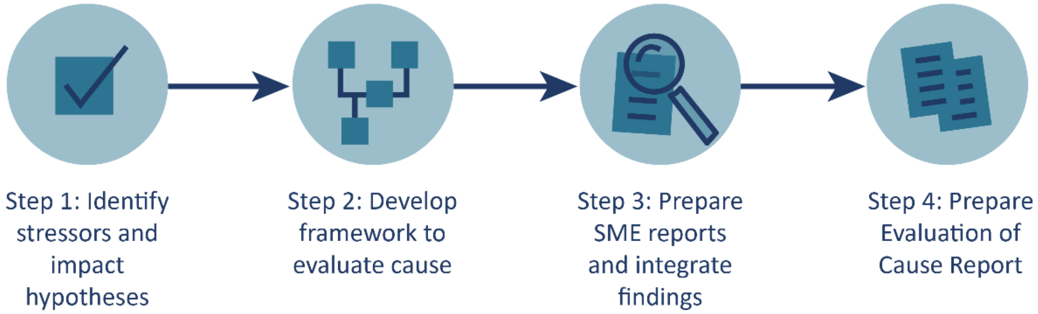
The report that follows this Reader's Note is one of those Subject Matter Expert Reports.

Monitoring conducted for Teck Coal in the fall of 2019 found that the abundance of Westslope Cutthroat Trout adults and sub-adults in the upper Fording River had declined significantly since previous sampling in fall 2017. In addition, there was evidence that juvenile fish density had decreased. Teck Coal initiated an *Evaluation of Cause* process. The overall results of this process are reported separately (Evaluation of Cause Team, 2021) and are supported by a series of Subject Matter Expert reports such as this one. The full list of SME reports follows at the end of this Reader's Note.

Building on and in addition to the Evaluation of Cause, there are ongoing efforts to support fish population recovery and implement environmental improvements in the upper Fording River.

How the Evaluation of Cause was approached

When the fish decline was identified, Teck Coal established an *Evaluation of Cause Team* (the Team), composed of *Subject Matter Experts* and coordinated by an Evaluation of Cause *Team Lead.* Further details about the Team are provided in the Evaluation of Cause report. The Team developed a systematic and objective approach (see figure below) that included developing a Framework for Subject Matter Experts to apply in their specific work. All work was subjected to rigorous peer review.



Conceptual approach to the Evaluation of Cause for the decline in the upper Fording River Westslope Cutthroat Trout population.

With input from representatives of various regulatory agencies and the Ktunaxa Nation Council, the Team initially identified potential stressors and impact hypotheses that might explain the cause(s) of the population decline. Two overarching hypotheses (essentially, questions for the Team to evaluate) were used:

- Overarching Hypothesis #1: The significant decline in the upper Fording River Westslope Cutthroat Trout population was a result of a single acute stressor[1] or a single chronic stressor[2].

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[1] Implies September 2017 to September 2019.

[2] Implies a chronic, slow change in the stressor (using 2012–2019 timeframe, data dependent).

- Overarching Hypothesis #2: The significant decline in the upper Fording River Westslope Cutthroat Trout population was a result of a combination of acute and/or chronic stressors, which individually may not account for reduced fish numbers, but cumulatively caused the decline.

The Evaluation of Cause examined numerous stressors in the UFR to determine if and to what extent those stressors and various conditions played a role in the Westslope Cutthroat Trout's decline. Given that the purpose was to evaluate the cause of the decline in abundance from 2017 to 2019[3], it was important to identify stressors or conditions that changed or were different during that period. It was equally important to identify the potential stressors or conditions that did not change during the decline window but may, nevertheless, have been important constraints on the population with respect to their ability to respond to or recover from the stressors. Finally, interactions between stressors and conditions had to be considered in an integrated fashion. Where an *impact hypothesis* depended on or may have been exacerbated by interactions among stressors or conditions, the interaction mechanisms were also considered.

The Evaluation of Cause process produced two types of deliverables:

1. **Individual Subject Matter Expert (SME) reports** (such as the one that follows this Note): These reports mostly focus on impact hypotheses under Overarching Hypothesis #1 (see list, following). A Framework was used to align SME work for all the potential stressors, and, for consistency, most SME reports have the same overall format. The format covers: (1) rationale for impact hypotheses, (2) methods, (3) analysis and (4) findings, particularly whether the requisite conditions[4] were met for the stressor(s) to be the sole cause of the fish population decline, or a contributor to it. In addition to the report, each SME provided a summary table of findings, generated according to the Framework. These summaries were used to integrate information for the Evaluation of Cause report. Note that some SME reports did not investigate specific stressors; instead, they evaluated other information considered potentially useful for supporting SME reports and the overall Evaluation of Cause, or added context (such as in the SME report that describes climate (Wright et al., 2021).
2. **The Evaluation of Cause report** (prepared by a subset of the Team, with input from SMEs): This overall report summarizes the findings of the SME reports and further considers interactions between stressors (Overarching Hypothesis #2). It describes the reasons that most likely account for the decline in the Westslope Cutthroat Trout population in the upper Fording River.

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[3] Abundance estimates for adults/sub-adults are based on surveys in September of each year, while estimates for juveniles are based on surveys in August.

[4] These are the conditions that would need to have occurred for the impact hypothesis to have resulted in the observed decline of Westslope Cutthroat Trout population in the upper Fording River.

Participation, Engagement & Transparency

To support transparency, the Team engaged frequently throughout the Evaluation of Cause process. Participants in the Evaluation of Cause process, through various committees, included:

Ktunaxa Nation Council

BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development

BC Ministry Environment & Climate Change Strategy

Ministry of Energy, Mines and Low Carbon Innovation

Environmental Assessment Office

Citation for the Evaluation of Cause Report

When citing the Evaluation of Cause Report use:

Evaluation of Cause Team, (2021). *Evaluation of Cause — Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited by Evaluation of Cause Team.

Citations for Subject Matter Expert Reports

Focus	**Citation for Subject Matter Expert Reports**
Climate, temperature, and streamflow	Wright, N., Greenacre, D., & Hatfield, T. (2021). *Subject Matter Expert Report: Climate, Water Temperature, Streamflow and Water Use Trends. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Ice	Hatfield, T., & Whelan, C. (2021). *Subject Matter Expert Report: Ice. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Ltd. Report Prepared by Ecofish Research Ltd.
Habitat availability (instream flow)	Healey, K., Little, P., & Hatfield, T. (2021). *Subject Matter Expert Report: Habitat availability. Evaluation of Cause – Decline in upper*

Focus	Citation for Subject Matter Expert Reports
	Fording River Westslope Cutthroat Trout population. Report prepared for Teck Coal Limited by Ecofish Research Ltd.
Stranding – ramping	Faulkner, S., Carter, J., Sparling, M., Hatfield, T., & Nicholl, S. (2021). *Subject Matter Expert Report: Ramping and stranding. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited by Ecofish Research Ltd.
Stranding – channel dewatering	Hatfield, T., Ammerlaan, J., Regehr, H., Carter, J., & Faulkner, S. (2021). *Subject Matter Expert Report: Channel dewatering. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited by Ecofish Research Ltd.
Stranding – mainstem dewatering	Hocking M., Ammerlaan, J., Healey, K., Akaoka, K., & Hatfield T. (2021). *Subject Matter Expert Report: Mainstem dewatering. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Ltd. by Ecofish Research Ltd. and Lotic Environmental Ltd. Zathey, N., & Robinson, M.D. (2021). *Summary of ephemeral conditions in the upper Fording River Watershed. In Hocking et al. (2021). Subject Matter Expert Report: Mainstem dewatering. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Ltd. by Ecofish Research Ltd. and Lotic Environmental Ltd.
Calcite	Hocking, M., Tamminga, A., Arnett, T., Robinson M., Larratt, H., & Hatfield, T. (2021). *Subject Matter Expert Report: Calcite. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Ltd. by Ecofish Research Ltd., Lotic Environmental Ltd., and Larratt Aquatic Consulting Ltd.
Total suspended solids	Durston, D., Greenacre, D., Ganshorn, K & Hatfield, T. (2021). *Subject Matter Expert Report: Total suspended solids. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Fish passage (habitat connectivity)	Harwood, A., Suzanne, C., Whelan, C., & Hatfield, T. (2021). *Subject Matter Expert Report: Fish passage. Evaluation of Cause – Decline*

Focus	Citation for Subject Matter Expert Reports
	in upper Fording River Westslope Cutthroat Trout population. Report prepared for Teck Coal Ltd. by Ecofish Research Ltd. Akaoka, K., & Hatfield, T. (2021). Telemetry Movement Analysis. In Harwood et al. (2021). *Subject Matter Expert Report: Fish passage. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Ltd. by Ecofish Research Ltd.
Cyanobacteria	Larratt, H., & Self, J. (2021). *Subject Matter Expert Report: Cyanobacteria, periphyton and aquatic macrophytes. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Larratt Aquatic Consulting Ltd.
Algae / macrophytes	
Water quality (all parameters except water temperature and TSS [Ecofish])	Costa, EJ., & de Bruyn, A. (2021). *Subject Matter Expert Report: Water quality. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Golder Associates Ltd. Healey, K., & Hatfield, T. (2021). *Calculator to assess Potential for cryoconcentration in upper Fording River.* In Costa, EJ., & de Bruyn, A. (2021). *Subject Matter Expert Report: Water quality. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Golder Associates Ltd.
Industrial chemicals, spills and unauthorized releases	Van Geest, J., Hart, V., Costa, EJ., & de Bruyn, A. (2021). *Subject Matter Expert Report: Industrial chemicals, spills and unauthorized releases. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Golder Associates Ltd. Branton, M., & Power, B. (2021). *Stressor Evaluation – Sewage.* In Van Geest et al. (2021). *Industrial chemicals, spills and unauthorized releases. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Golder Associates Ltd.
Wildlife predators	Dean, D. (2021). *Subject Matter Expert Report: Wildlife predation. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by VAST Resource Solutions Inc.

Focus	Citation for Subject Matter Expert Reports
Poaching	Dean, D. (2021). *Subject Matter Expert Report: Poaching. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by VAST Resource Solutions Inc.
Food availability	Orr, P., & Ings, J. (2021). *Subject Matter Expert Report: Food availability. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Minnow Environmental Inc.
Fish handling	Cope, S. (2020). *Subject Matter Expert Report: Fish handling. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Westslope Fisheries Ltd.
	Korman, J., & Branton, M. (2021). *Effects of capture and handling on Westslope Cutthroat Trout in the upper Fording River: A brief review of Cope (2020) and additional calculations.* Report prepared for Teck Coal Limited. Prepared by Ecometric Research and Azimuth Consulting Group.
Infectious disease	Bollinger, T. (2021). *Subject Matter Expert Report: Infectious disease. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by TKB Ecosystem Health Services Ltd.
Pathophysiology	Bollinger, T. (2021). *Subject Matter Expert Report: Pathophysiology of stressors on fish. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by TKB Ecosystem Health Services Ltd.
Coal dust and sediment quality	DiMauro, M., Branton, M., & Franz, E. (2021). *Subject Matter Expert Report: Coal dust and sediment quality. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Azimuth Consulting Group Inc.
Groundwater quality and quantity	Henry, C., & Humphries, S. (2021). *Subject Matter Expert Report: Hydrogeological stressors. Evaluation of Cause - Decline in upper Fording River Westslope Cutthroat Trout population.* Report Prepared for Teck Coal Limited. Prepared by SNC-Lavalin Inc.

1 INTRODUCTION

1.1 Background

1.1.1 Overall Background

This document is one of a series of Subject Matter Expert (SME) reports that support the overall Evaluation of Cause into the upper Fording River Westslope Cutthroat Trout population decline (citation for EoC report). For general information, see the preceding Reader's Note. This report is focused on the primary producers of the UFR. **Throughout this report, links to other SME reports in the UFR WCT Evaluation of Cause are in green font.**

1.1.2 Report-Specific Background

The primary producers discussed in this report form the foundation of the UFR WCT fishery food web, but they can also mediate stress to fish.

Periphyton – a mixture of algae, cyanobacteria, bacteria, heterotrophic microbes and detritus in a mucopolysaccharide matrix – occur in rivers as biofilms that coat substrates. Periphyton layers can form invisible slippery coatings on substrates or make visible mats if filamentous algae become abundant (Figure 1). Every periphyton community will reflect the dynamic balance between forces of growth: light and nutrients; and those of loss: physical disturbance and invertebrate grazing (Biggs & Kilroy 2004). Periphyton coatings are both good and bad. Periphyton provide habitat and food, absorb contaminants, serve as an early warning of environmental change, and can buffer impacts of changing biogeochemical conditions. However, their overgrowth can result in deleterious processes including bioclogging, induction of calcite precipitation, release of cyanotoxins, sequestering followed by release of metals, diurnal pH, and dissolved oxygen fluctuations. Periphyton algae are the most ubiquitous primary producers of organic carbon in flowing water, particularly in rivers without shading from riparian vegetation (Krenz et al. 2018).

Aquatic macrophytes are aquatic plants growing in or near water. Aquatic macrophytes include emergent (e.g. rushes, sedges) and submergent plants (e.g. milfoils, pondweeds) together with mosses and liverworts. These macrophytes play important roles in the structural and functional aspects of aquatic ecosystems through physical and biochemical means. Physical means include altering water movement regimes which encourages settlement of fines, providing shelter to fish and aquatic invertebrates, and serving as a food source. Biochemical means include altering water quality by regulating diel oxygen and pH balance, nutrient cycles, root zone remediation of PAHs and accumulating/attenuating metals in root zones.

Hyporheic zones are the places where stream water interacts with shallow groundwater within the streambed. This hydraulic exchange between surface water and groundwater regulates sources of oxygen, carbon and nutrients that together control trophic structure and primary productivity in the river. Hyporheic zones act as dynamic bioreactors that host crucial reactions in fluvial ecosystems (Figure 2). The microbiome of the hyporheic zones mediate numerous reactions involving carbon, nitrogen, sulphur and metals cycling/transformations.

Figure 1: Periphyton biofilm – a complex assemblage of algae, cyanobacteria, bacteria, fungi

(photo by Marek Mis)

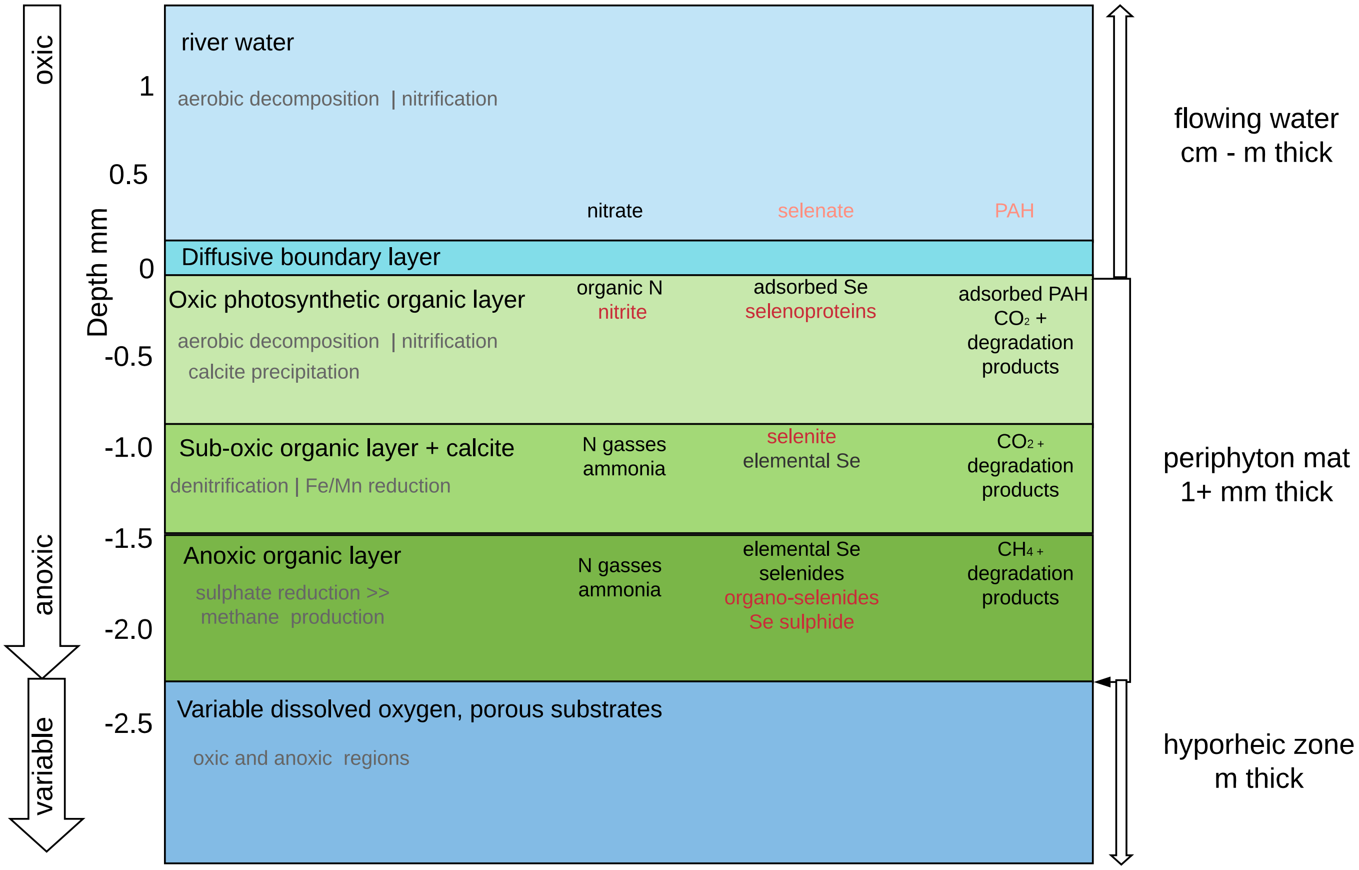

Figure 2: Hypothetical zonation of metabolic activity and redox-directed reactions in river substrates

Primary Producers in the Upper Fording River

Measurements from the 2015 Elk River watershed study that includes the UFR are compared to a range of rivers in Table 1. Most of these parameters indicate that the UFR is in the desirable "typical" category for primary productivity. This suggests that any stress exerted by UFR conditions did not result in adverse effects on periphyton growth under normal fall conditions.

Table 1: Typical ranges of Elk watershed periphyton productivity metrics from Fall 2015 study (n=240), with comparison to oligotrophic, typical and productive rivers

Metric	Oligotrophic or stressed	Typical large rivers	Eutrophic or productive	Elk River watershed (Elk reference sites in brackets)
Number of taxa (live & dead) per sample	<20 – 40	25 – 60	Variable	19-38 (11 - 39)
Chlorophyll-a ug/cm^2	<2	2 – 5	>5–10(30+)	5.1± 7.6 (3.0±3.7)
Average algae density cells/cm^2	<0.2 x10^6	1 - 4 x10^6	>10 x10^6	1.25±1.78x10^6 (0.60±0.8x10^6)
Algae biovolume cm^3/m^2	<0.5	0.5 – 5	20 - 80	10.5±54 - 11±18 (3±1.9 - 4.3±7.8)
Diatom density (frustules/cm^2)	<0.15 x10^6	1 - 2 x10^6	>10 x10^6	1.09±1.73 x10^6 (0.31±0.41x10^6)
Biomass–AFDW mg/cm^2	<0.5	0.5 – 2	>3	2.2±3.1 (1.6±1.9)
Biomass –dry wt mg/cm^2	<1	1 – 5	>10	1.22 – 60with calcite (2.3 - 112with calcite)
Organic Matter (% of dry weight)		4 - 7		0.6 - 59% (4 - 51%)
Accrual chl-a ug/cm^2/d	<0.1	0.1 - 0.6	> 0.6	Artificial substrate 0.05 - 1.4

Comparison data obtained from Flinders and Hartz 2009; Biggs 1996; Peterson and Porter 2002; Biggs and Close 1989; Durr and Thomason 2009; Romani 2010; Francoeur and Biggs 2006, Luce et al. 2010.

Flinders, C. A., & Hart, D. D. (2009). Effects of pulsed flows on nuisance periphyton growths in rivers: a mesocosm study. *River Research and Applications*, *25*(10), 1320–1330. doi:10.1002/rra.1244
Biggs, B.J.F., 1996. Algal Ecology. Academic Press, San Diego, CA

NOTE: Chl-a > 7.5 ug/cm^2 is considered high productivity in the UFR | 2015 UFR data from White and Larratt 2016 | See also Table A-16

Aquatic macrophytes have a restricted distribution in the UFR watershed as they have in adjacent mountain watersheds. They occupy 8.4 ha of the total 116 ha with a gradient ≤ 3% (Schnurr et al. 2020; Wood and Berdusco 1999). Emergent and submergent taxa are only found in riparian zones and reaches with slow-moving water: ponds and lakes. Aquatic bryophytes (mosses) are more common in the UFR, particularly in shaded reaches where their productivity can exceed that of periphyton (Worrall et al. 2016; Bowden 1999).

The distribution and intensity of hyporheic reactions fluctuate on a depth gradient and are impacted by flows (Lewandowski et al. 2019; Bencala, 1984). Figure 2 illustrates nitrogen, selenium and PAH processes in the flowing water, the periphyton mat in which oxygen gradients can be very steep, and underlying hyporheic zone with variable dissolved oxygen depending on groundwater influx and oxygen demand originating from the periphyton mat. Materials of concern are shown in red. The Elk Valley Water Quality Plan sets targets for the concentration of selenium, sulphate, nitrate, and cadmium in surface water (EMC 2019). These parameters are all affected by periphyton in the river and bacterial activities in the hyporheic zone.

The total primary productivity of the UFR may be enhanced by phosphorus from its coal-bearing watershed (Ahrens and Morrisey 2005; Davis et al. 2018), with the potential for ripple effects to its aquatic food chains and the hyporheic zones. Coal mining can increase the release of P, so that reference sites had lower P regimes than mine-influenced sites (Kruskal-Wallis Test for total phosphorus data 2012-2019, p<0.001). Phosphorus can enhance primary productivity and act to support fisheries (Wetzel 2001).
Lotic reaches support periphyton and bryophytes while deep pools support aquatic macrophytes. Expansion of these plant groups since the last flood in 2013 is indicative of stable, moderate flows (FRO flows database).

Objectives

The overarching goal of this report was to understand the interactions of cyanobacteria, periphyton and aquatic macrophytes in the UFR ecosystem, their roles, and their potential to exert stress on WCT either alone, or in combination with other stressors.

1.2 Approach and Methods

We developed four stressor-specific impact hypotheses associated with primary producers, addressing their potential to adversely impact WCT. A combination of lines of UFR evidence/data and literature research was utilized to investigate the cyanobacteria, periphyton, and aquatic macrophyte stressors. The general approach to assessing potential effects associated with cyanobacteria, periphyton, and aquatic macrophytes involved:

1. Identifying cyanobacteria/periphyton/macrophyte/bryophyte characteristics with the potential to directly affect fish or to alter other habitat parameters that affect fish using existing UFR data and published research.
2. Evaluating, where possible, how cyanobacteria/periphyton/macrophyte/bryophyte metrics varied spatially and temporally before, during and after the WCT decline window (September 2017 – September 2019). Where possible, multiple lines of evidence were used to support the evaluation of each of these hypotheses.
3. Assessing the magnitude of the cyanobacteria/periphyton/macrophyte/bryophyte-induced potential impact(s) (stressors) to WCT by relying on published literature, Teck Coal monitoring, Teck research and supportive reports, and by evaluating existing UFR data. These findings were then compared to established benchmarks, objectives, or guidelines when available.
4. Overlap of the potential stressor with WCT distributions temporally and spatially, and scale of potential impacts of the periphyton/macrophyte-driven stressor during the Decline Window.

Consistent with the approach used throughout the EoC, the requisite conditions, that is a combination of conditions that were necessary for the stressor to have caused or contributed to the decline, were examined for each hypothesis. Each hypothesis was broken down into discrete questions that address potential effects on WCT. These are presented by hypothesis in Sections 2.1 through 2.4.

We relied upon primary production data collected as part of site-specific studies and monitoring programs on the UFR. Data for most of the parameters of interest were not collected during the Decline Window however, earlier data provided information about species present, level of productivity, and periphyton community structure. Other relevant environmental data such as discharge records collected during the Decline Window were used in conjunction with the literature to assess each hypothesis. Where appropriate, our approach included statistical analyses including descriptive statistics, mixed-model averaging, correlation analysis, comparison against guidelines, data visualizations, and GIS mapping to identify and illustrate geographic distribution patterns. Statistical methods used in assessing the data are covered in full in Appendix 1.

Table 2: Pertinent data sets – lines of evidence for Hypothesis 1; cyanobacteria and cyanotoxicity

Data Set	Description
Literature	Cyanotoxin biomagnification, impacts to fish, environmental persistence, etc.
Algae data UFR	Interlab study 2013, 2013 data, 2015 UFR assessment (White and Larratt 2016), esp. for cyanobacteria and other important algae/periphyton
Algae data	Dry Creek 2018 LAEMP (Wiramanaden et al. 2019) algae grab samples collected during a Didymo bloom for taxonomy and tissue metals (Minnow 2014b)
Algae grabs	Winter 2020 periphyton samples, percentage cyanobacteria data from Teck Coal samples 2016 – present analyzed by LAC
Toxicology	sample sets in RAEMP / LAEMP reports (Minnow 2018; 2020a; Minnow and Lotic 2018, 2019, 2020) | Nautilus reports | Golder 2020b chronic toxicology report
Sediment TOC	Searched data for correlations between benthic invertebrate metrics and periphyton metrics, or surrogates (TOC) in Minnow LAEMP /RAEMP reports (Minnow 2018; 2020a; Minnow and Lotic 2018, 2019, 2020)
FRO data loggers	Winter 2020 DO/T continuous data logger results
Calcite surveys	Annual extent surveys by Lotic Environmental; Atherton 2017 report
Calcite-Metals	UGHC calcite metals project | Day and MacGregor (SRK) 2014 metals in calcite report | Goudey et al. 2009 |MacGregor and Day (SRK) 2011 | Lotic Environmental annual CI extent reports 2012 - 2019
Teck reports	Teck 2016 Measurement of Calcite Index
Observations	Photography and field observations by FRO staff and their consultants

Table 3: Pertinent data sets – lines of evidence for Hypothesis 2; filamentous algae blooms

Data Set	Description
Fall 2015 peri.	Extensive taxonomic and chl-a datasets 23 triplicate samples + duplicates from UFR
Fall 2012 peri.	Samples from 13 sites in UFR with taxonomic data
Fall 2015 2016 2019	Visual field periphyton scores (1-5) rapid assessment
Sept-Oct 2019	Photography and field observations by S. Cope
Feb 2020 peri UFR	10 samples + 2 duplicates periphyton grab samples through ice
Dry Creek 2018	2018 grab samples of bloom and invertebrates, tissue analyses Line Ck LAEMP (Minnow 2020b)
Drivepoints side channel	SNC Lavalin pH redox DO conductivity turbidity, ions, metals data from Dec 2019 to Feb 2020 in subsurface flow (SNC Lavalin SME 2020)
UFR discharge data	FRO has continuous discharge (flow) data on the UFR
In-situ DO	FRO Temp/DO loggers deployed in Feb 2020 retrieved prior to freshet
Microscope photos	Algae data 2015 - reviewed for fines entrapment
Microscope photos	Samples from February 2020 were examined and photographed for TSS/coal fines
Sediment data	(Minnow 2018; 2020a; Minnow and Lotic 2018, 2019, 2020), Golder 2020 & 2020c, SNC Lavalin 2019 sediment data for metals and PAHs

Table 4: Pertinent data sets – lines of evidence for Hypothesis 3; bioaccumulation, fines entrapment in periphyton and bioreactor functioning

Data Set	Description
AFDW 2015	Ash-free dry weight and dry weight from 2015 periphyton study
Algae data 2015	Algae data used to determine their capacity for fines entrapment
Sample site photos	Minnow 2018/2019 photos of substrates showing peri/filamentous algae growth
Microscope photos	Samples from February 2020 were examined and photographed for TSS/coal fines
Microscope examination	Samples of coal dust were side-lit and compared to photos from periphyton sample results (also compared to airborne coal dust images in (Van Staden 2012))
Sediment samples	(Minnow 2018; 2020a; Minnow and Lotic 2018, 2019, 2020), Golder 2020 & 2020c, SNC Lavalin 2019 sediment data for metals and PAHs
Didymo tissue	Minnow, 2019 Dry Ck Didymo tissue metals
Sediment PAH	Sediment PAH sample results collected from mine-influenced and reference sites in Minnow 2018, 2019
benthic Inv. metrics	Teck FRO LAEMP reports (Minnow and Lotic 2018, 2019b, 2020)
2015 periphyton data	Numerous UFR sites including those sampled downstream of the Feb 2019 wash-water spill, providing a baseline
Water quality database	Golder 2020 provided a wide range of water quality parameters in a large data base, particularly TSS. (Costa EJ., de Bruyn A. 2020c).
2020 algae grab samples	12 samples collected in Feb. 2020 along the UFR mainstem to detect residual algae from summer 2019 algae blooms
In-situ DO	FRO Temp/DO loggers deployed in winter 2020 retrieved before freshet; Field meter readings, monthly or weekly for some UFR sites
Drive point data	SNC Lavalin (2019, 2020) DO in subsurface flow adjacent to UFR
UFR discharge data	FRO has continuous UFR discharge (flow) data; also (Ecofish Wright et al. 2020)
u/w drone	Henretta Lake Feb 2020 visuals of apparent SRB sites, S6 drone videos
Seep shallow groundwater	Summary of analytical results for seep, shallow groundwater and surface water - mass balance (SNC Lavalin 2019, 2020)
T-Hg sediments	LAEMP / RAEMP sediment sampling (Minnow 2018; 2020a; Minnow and Lotic 2018, 2019, 2020)
Aqueous MeHg	Water quality database MeHg analyses and (Golder SME 2020 Water quality report)
Se in calcite	UGHC calcite project and Wright et al. 2018
Se in tissues	Dry Creek 2018-2019 Didymo bloom sampling (Minnow 2019, 2020) Line Creek LAEMP Minnow 2016 (Golder 2018a)
Teck Reports	Teck 2014 Elk Valley Water Quality Plan | Annual Water Quality Reports 2019a 2019b, 2020
UFR Se removal	Water modelling (SRK) studies by Martin et al. 2011 2018 2019
Metals in calcite	UGHC Calcite Project 2018 (Acid metals) in June 2018 (Wright et al. 2018 Ecofish)
Sediment metals	Selected samples (Minnow 2018), Golder reports, SNC Lavalin reports
Metals in water	FRO database 2017- 2019 (Golder 2019, 2020, 2020c)
Invert. tissue metals	Composite-taxa benthic invertebrate tissue chemistry, FRO LAEMP, Sept. 2018 & Sept. 2019

Table 5: Pertinent data sets – lines of evidence for Hypothesis 4; aquatic macrophytes and bryophytes

Data Set	Description
Anecdotal notes	RAEMP and LAEMP field note macrophyte and bryophyte coverage (Minnow 2018; 2020a; Minnow and Lotic 2018, 2019, 2020)
2015 periphyton	LAC noted when fragments of moss leaflets were found in 2015 periphyton samples
2012-2019	Anecdotal field observations and photographs by S. Cope (Westslope)
2012 periphyton	Lab identified moss fragment as *Jungermannia cordifolia* from CACK periphyton
In-situ loggers	In-situ temp/pH/DO logger data, 5 locations in UFR winter 2019/2020 (FRO staff)
u/w Drone ROV	ROV footage under ice Henretta Lake Feb 2020 and S6 pools open water (FRO)
Drive points	Drive point data from shallow groundwater, seeps etc. adjacent to UFR (SNC Lavalin)

2 IMPACT HYPOTHESES, ANALYSES AND FINDINGS

2.1 Impact Hypothesis 1 Cyanobacteria and cyanotoxicity impacts to WCT

HYPOTHESIS 1:
Hypothesis 1 examines the potential for cyanotoxin exposure to adversely affect WCT in the UFR.

Hypothesis 1 Questions:

A. *Are cyanotoxins in the UFR at sufficient concentrations and durations to cause adverse effects to benthic invertebrates and fish in general? Specifically, could cyanotoxins have caused mortality in juvenile and adult life stages of WCT in the UFR during the Decline Window?*
B. *Did conditions within the Decline Window permit localized calcite dissolution to release embedded cyanotoxins, particularly during winter low flows?*

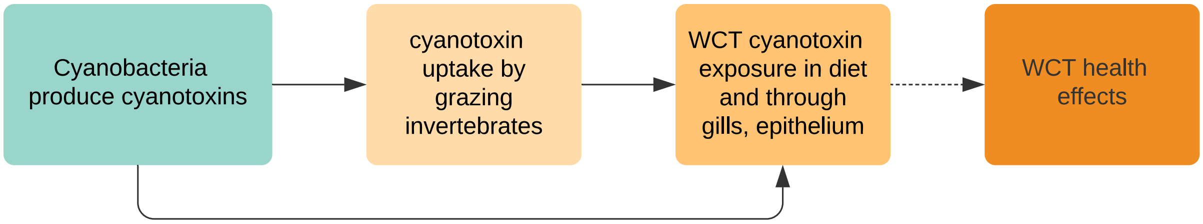

Figure 3: Conceptual interactions of cyanotoxins and WCT investigated in this report

2.1.1 Introduction to Cyanobacteria:

Cyanobacteria are naturally occurring and widespread in all types of aquatic environments. Cyanobacteria trigger concern when populations known to generate toxins rapidly expand (bloom) to exceed 2000 cells/mL for phytoplankton and 20% substrate coverage for periphyton[5]. Harmful cyanobacteria blooms are not exclusive to mine affected rivers – they are becoming more frequent and severe world-wide because of eutrophication and climate change (O'Neil et al. 2012). While most filamentous cyanobacteria species are toxin-producing, toxins are not produced at every growth phase. These toxins can induce adverse effects in benthic invertebrates and fish (Zanchett and Olivero-Filho, 2013) and they can bioaccumulate (Wiltsie et al. 2018; Zhao et al. 2020). To contribute to the WCT decline, cyanotoxins must exceed exposure concentrations that induce adverse effects in fish, and specifically adverse effects that could result in or contribute to juveniles and/or adult WCT mortality during the Decline Window.

No information on cyanobacteria densities or cyanotoxin concentrations from the Decline Window was available to support a direct evaluation of their contribution to the WCT decline. Instead, several lines of evidence were assessed that involved:

- *Assessing cyanotoxin impacts to benthic invertebrates*: there were some measurements of the invertebrate community in situ during the Decline Window, as well as toxicity tests using site media from the Decline Window. These data were used to evaluate (a) potential evidence of cyanotoxicity and (b) potential impacts to the aquatic food chain that could impact WCT food.
- *Potential for localized release of cyanobacteria during late growing season low flows but especially in winter low flows*: Available physical parameter and periphyton data collected before and after

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[5] For reference, total microcystins guideline of 0.0015 mg/L (MAC) in water is set by Health Canada for drinking water; no aquatic life guidelines have been set to date.

the Decline Window was used to assess the duration of potential cyanotoxin impact from late summer through the winter low flow season.

- The potential for a cyanobacteria bloom was assessed using data on UFR physical conditions and water chemistry that would encourage rapid cyanobacteria growth

Lotic Cyanobacteria

Cyanobacteria can be harmful to fish. Adverse impacts such as gill and organ effects are reported, while fish kills are rare. Most benthic cyanobacteria occurring in rivers do not kill fish, but *Phormidium autumnale* can and it was detected at many UFR sites. *Phormidium* can flourish under a low to moderate flow regime (flows between 0.1 and 1.0 times the median flow for several weeks ~1.0 m/s) with modest dissolved inorganic nitrogen (DIN) (>0.05-0.2 mg/L up to 0.6 mg/L as N) and low dissolved reactive phosphorus (DRP) (<0.01 mg/L as P) (Wood et al. 2017). However, water within the benthic mat is isolated from the overlying water, making it progressively independent of external nutrients.

Harmful cyanobacteria growths are most common in reaches with:

- Marked groundwater abstraction leading to stable low flows in the growing season
- Gaining reaches with high nitrate oxygenated shallow groundwater
- In reaches receiving fine sediment and riparian zones with impaired functioning (McAllister et al. 2016; Wood et al. 2020; Figure 4)

Flushing flows are used in flow-regulated rivers to remove nuisance or harmful periphyton growth (Measures and Kilroy, 2013). They were defined for the Wellington River (NZ) as three times the long-term median flow (Wood et al. 2009; Wood et al. 2017) and would be approximately 12-15 m^3/s (measured at FR_FRNTP). The alert-threshold levels for *Phormidium* are currently defined by McAllister et al. (2016) as:

1) Surveillance – up to 20% substrate coverage triggers more frequent sampling
2) Alert – 20–50% substrate coverage triggers preparation to increase flows
3) Action – greater than 50% substrate coverage triggers actions to achieve a flushing flow

Figure 4: *Phormidium autumnale* dominated periphyton in various rivers

Phormidium blooms, where substrate cover exceeds 20% are most likely to occur in summer and early autumn when water temperatures peak (Wood et al. 2017). Flows that are sufficient to enhance nutrient and gas flux, yet insufficient for shear stress that exceeds the periphyton mat tolerance, are expected to be optimal for *Phormidium* accrual (Wood et al. 2017). Detached mats that accumulate along river edges are deemed high risk and automatically raise the alert level status to action (Wood et al. 2020).

Finally, co-occurrence of toxin-producing bacteria such as the wide-spread *Bacillus cereus* in senescent periphyton biofilms can occur (Bottone 2010) and has contributed to fish kills during a cyanobacteria bloom (Landsberg et al. 2020).

Cyanotoxins

Microcystins and likely other less-studied cyanotoxin groups occur in four ways: cell-bound toxins in cyanobacterial cells, dissolved toxins in surrounding water, bioaccumulated toxins in organisms, and accumulated toxins in sediment or toxins associated with suspended substances (Song et al. 2015).

Several factors are known to determine cyanotoxin concentrations:

1. Cyanobacteria density and varieties present: particularly *Phormidium autumnale* that can continuously release cyanotoxins raising concerns about chronic anatoxin exposure (Wood et al. 2018). Refer to Table 6.
2. Cyanotoxin persistence: optimized in low light and anoxic conditions (Chorus 2012; Zanchett and Oliveira-Filho 2013) -they can last months or longer in substrates (WHO 2019), extending the period during which they can theoretically impact trout such as WCT.
3. Physical conditions: greatest concentrations at sites with stable low flows (low scour), elevated water temperature and phosphorus concentrations and low light intensity (Fetscher et al. 2015; Wiltsie et al. 2018; Gao et al. 2007; Kaebernick and Neilan 2001; Sivonen 2009 US State Dep. 2015).
4. Cyanobacteria embedded in calcite: Biogenic calcite (a soft, porous mineral) deposition is a by-product of periphyton metabolic activity and their extracellular polymeric substances (EPS) through mechanisms collectively known as microbially induced calcite precipitation (MICP). MICP involves photosynthesis, ureolysis, ammonification, denitrification, sulfate reduction, anaerobic sulfide oxidation, and methane oxidation, either by increasing pH or dissolved inorganic carbon (DIC) concentrations (Zhu and Dittrich 2016). Many taxa including *Phormidium* become embedded in biogenic calcite (Shiraishi et al. 2017). Other periphyton processes can induce the reverse process. Biogenic calcite dissolution can occur during net respiration (Carr and Whitton 1973), fermentation (Payandi-Rolland et al. 2019), Sulfide oxidizing bacteria (Leprich et al. 2021) and anoxic reactions, primarily by lowering pH, often by more than 1 pH unit. The balance between biogenic calcite deposition and dissolution processes is subject to seasonal and even diurnal fluctuations (Payandi-Rolland et al. 2019). Intense periphyton photosynthesis during summer days under moderate to low flows can encourage net biogenic calcite deposition, while dark cold winter conditions can cause net calcite dissolution (Pedley 2000; Berrendero et al. 2016; Pedley and Rogerson 2010). ***Refer to calcite chapter for information on biogenic calcite: Hocking et al. 2021 of UFR WCT Evaluation of Cause SME report.***

Overall, benthic cyanobacterial proliferations in rivers tend to form during summer when water flows are more stable (Wood et al. 2020). Global research has demonstrated that cyanotoxins can transfer through trophic levels in food webs (Flores et al. 2018). A comprehensive assessment of cyanotoxins in fresh water found evidence of a positive correlation between intracellular microcystins in the water and microcystins in fish tissues (Flores et al. 2018). However, evidence of the potential effects of cyanotoxins from laboratory studies is complex and inconsistent (Chorus and Bertram 1999). In this review, chronic cyanotoxicity is defined as the gradual development of adverse sub-lethal effects through long term or repeated exposure, while acute cyanotoxicity is defined as the rapid development of disruptive effects or death.

Invertebrates

Cyanotoxins such as microcystins can bioaccumulate in invertebrates, and particularly zooplankton, through ingestion of cyanobacterial cells (Ferrão-Filho et al. 2014; Watanabe et al. 1992; Chorus and Bertram 1999). Among invertebrates, zooplanktonic crustaceans have the highest ability to accumulate microcystins (Ferrao and Kozlowsky 2011; Thorstrup and Christoffersen 1999; Rohrlack *et al.* 2005, DeMott and Moxter 1991). Table A-17 summarizes the known impacts of cyanotoxins on benthic invertebrates and fish.

Many cyanotoxins can adversely impact the invertebrate taxa commonly used in surface water toxicology monitoring (*Ceriodaphnia*, *Chironomus*, *Daphnia, Hyalella*), causing reduced grazing, physiological disturbances, fewer progeny, and mortality (Bownik 2016; Kozlowsky-Suzuki et al. 2012; Anderson et al. 2018; Olvera-Ramirez et al. 2010). However, cladocerans can develop mechanisms to reduce their vulnerability (Ferrao-Filho and Kozlowsky-Sukuki 2011; Guo and Xie 2006, Bednarska 2006). Cyanotoxins have been reported to suppress rotifer grazing rates, population growth, and reproduction rates (Liang et al 2017), and rotifers are a trophic link between algae and some zooplankton taxa.

Fish

There are two known exposure pathways for cyanotoxins to fish – through their diet and directly through gill and/or epithelial uptake. Dietary uptake is thought to be the most important for adult fish. Uptake across the gills and epithelial layer are also important in alevins, young of year and juveniles, probably due to their thin epithelial layers relative to body size (Chorus 2012; Best and Eddy 2001). For example, in one 96 h bioassay, juvenile rainbow trout bioconcentrated anatoxin-a by a factor of 30 to 47 times and this exposure was not diet-related (Osswald et al. 2011). These early life stages may be especially susceptible to cyanotoxins (Sotton et al. 2012; Butler et al. 2009). Areas with high cyanobacteria densities and with calcite embedded with cyanobacteria could potentially affect WCT alevins and juveniles directly though uptake across the gills.

Little is known regarding accumulation and presence of cyanotoxins other than microcystins in wild fish, although co-occurrence of multiple cyanotoxins in wild fish have been detected (Flores et al. 2018). Among fish guilds, the highest cyanotoxin concentrations were found in either omnivorous (Flores et al. 2018) or zooplanktivorous fish, with mean trophic transfer factor (TTF) of 1.8 and a maximum TTF of ~2.9 for microcystins (Kozlowsky-Suzuki et al. 2012). Fish that rely on benthic invertebrates had TTF’s near 1, indicating no biomagnification and WCT would fall into this category. Higher microcystin contents were often measured in invertebrates with lower concentrations in fish tissues[6] (Larson et al. 2014).

Microcystins impact fish health at concentrations as low as a few micrograms per liter (Butler et al. 2009) and toxicity is dependent on both concentration and length of exposure. Freshwater fish are known to accumulate microcystins in their tissues, in the order of liver, gut and muscle (Zhao et al. 2020; Hardy et al. 2015; Ibelings et al. 2005; Beattie et al. 1998). Accumulation of cyanotoxins in fish livers can induce liver necrosis and it can be most severe in early life stages and juveniles (WHO, 2019; Johnson et al. 2013; Carbis et al. 1997; Beattie et al. 1998). Exposure to toxic cyanobacteria may also cause damage to kidneys,

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[6] Analytical techniques are still evolving, and tests can give different cyanotoxin concentrations when used on different tissue types (Hardy et al. 2015; Sotton et al. 2012; US EPA 2020; Flores et al. 2018).

hepatopancreas, gills, growth inhibition, reproductive impairment, abnormal behavior, and rarely fish death (Kist et al. 2011; Qiao et al. 2016). Fish can partly detoxify and excrete ingested microcystins, however chronic exposure can cause DNA damage without inducing mortality (Sotton et al. 2012), so sub-lethal effects associated with chronic exposures are far more frequently documented than fatalities associated with acute exposures (Kist et al. 2011; Qiao et al. 2016; Butler et al. 2009). Overall, the cyanotoxicity research from these studies do not align with the UFR WCT decline. This is because mortality from cyanotoxicity is rare, juveniles are expected to be more susceptible than adults, and WCT rely on benthic invertebrates, making them less susceptible to trophic transfer of cyanotoxins.

Harm to fish during growing season cyanobacteria blooms can also occur through diurnal pH and dissolved oxygen changes concurrent with exposure to cyanotoxins, particularly in ponds and pools (Butler et al. 2009). In these lentic environments, an overnight DO sag can be larger with cyanobacteria blooms compared to other algae blooms because cyanobacteria do not generate as much dissolved oxygen as other periphyton algae (Wetzel 2001).

Table 6: Cyanotoxin types that can be produced by cyanobacteria in the upper Fording River samples and their ecological effects

	Occurs in calcite in UFR	Dominant in UFR	Known toxin producer	Toxin types for dominant taxa
Anabaena sp. / Nostoc sp.			Yes	
Calothrix sp.			Yes	
Calothrix / Dichothrix sp.			Yes	
Chamaesiphon incrustans	Yes	Yes	Yes	Lip BMAA
Chamaesiphon spp.	Yes		Yes?	
Chroococcus sp.			Yes?	
Clastidium setigerum				
Heteroleibleinia kuetzingii	Yes		Yes	
Heteroleibleinia profunda		Yes	Yes	Mc Sax BMAA
Heteroleibleinia rigidula			Yes	
Heteroleibleinia sp.			Yes	
Homoeothrix janthina		Yes	Yes	BMAA
Homoeothrix varians			Yes	
Leptolyngbya lemnetica	Yes	Yes	Yes	BMAA
Lyngbya group		Yes	Yes	Lyn Apl Lip Cyl Mc Ana Sax BMAA
Merismopedia glauca				
Nostoc sp.		Yes	Yes	Apl Lip Mc Ana BMAA
Oscillatoria sp.			Yes	
Phormidium aerugineo-caeruleum	Yes		Yes?	
Phormidium autumnale	Yes	Yes	Yes	Lyn Lip Mc Ana BMAA
Phormidium sp.	Yes		Yes?	
Pseudanabaena catenata	Yes?		Yes	
Pseudanabaena sp.	Yes		Yes?	
Rivularia	Yes	Yes	Yes	Mc BMAA
Schizothrix sp.			Yes	
Spirulina sp.				
Synechococcus/ Synechocystis		Yes	Yes?	Lip Mc BMAA

Lyn =Lyngbyatoxins | Apl= Aplysiatoxins | Lip= lipopolysaccharides | Cyl=Cylindrospermopsin | Mc=Microcystins |Ana=Anatoxin-a |Sax= saxitoxin| BMAA = β-Methylamino- l-alanine

NOTE: all cyanobacteria are thought to produce BMAA neurotoxin, but this is not fully proven |
Yes? denotes detected in most but not all studies or detected in most but not all species or varieties
Many references including: Graham et al. 2008; Hoehn and Long 2002; Hudnell 2008; Sivonen and Jones 1999; Ferrão-Filho and Kozlowsky-Suzuki 2011; Borges et al. 2015; Ferrão-Filho and Kozlowsky-Suzuki 2011; Zanchett and Oliveira-Filho 2013; US EPA 2014.

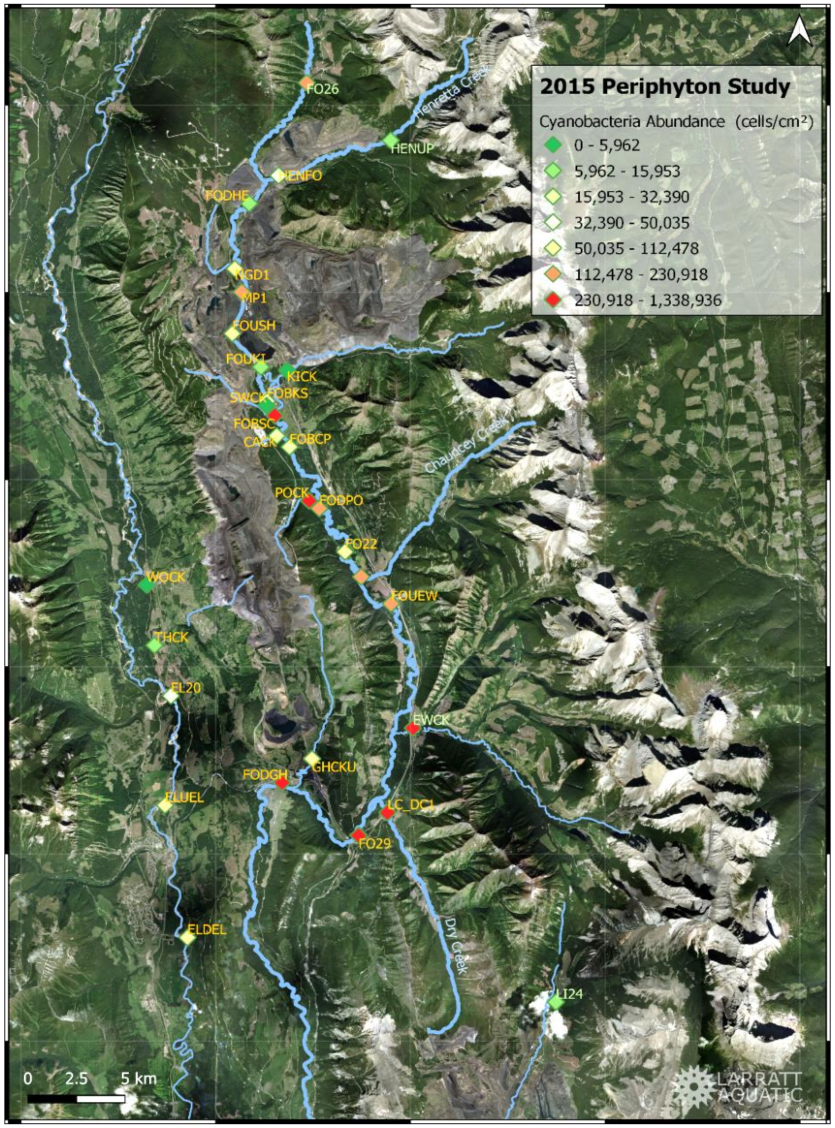

Figure 5: Cyanobacteria abundance (cells/cm2) in UFR September 2015

2.1.2 Requisite Conditions for Impact Hypothesis 1:

A low dose cyanotoxin exposure effect from late growing season through winter may occur but an acutely toxic event is very unlikely in UFR habitats. Further, an acutely toxic cyanobacteria event would be most likely in late summer which is inconsistent with the winter 2018/2019 WCT decline The following points lay out the requisite conditions that must be present before a low dose cyanotoxin impact to overall WCT health from cyanobacteria/cyanotoxins would be anticipated:

REQUISITE CONDITIONS FOR HYPOTHESIS 1:

Requisite Spatial extent	Cyanobacteria must be found generally in UFR and/or in UFR depositional habitats pools, lentic areas, WCT overwintering areas both on substrates and embedded in calcite: ***Yes*** *(based on 2013 (Minnow 2014b) , 2015,* (White and Larratt 2016); *2020 samples (this report) and field observations of biogenic calcite which are* porous calcite-periphyton crusts (Goudey et al. 2009)
Requisite Duration	More than one month of cyanobacteria bloom(s) in low flow periods during late summer through winter such as Aug 2018 - Apr 2019 that had no fall flushing flows: ***No*** *(no periphyton samples from 2017-2019 to determine cyanobacteria densities and persistence; no data loggers 2018 – 2019 to confirm diurnal DO fluctuations indicative of a bloom)*
Requisite Location	Cyanobacteria bloom would have to occur at sites that affect key WCT rearing/overwintering locations, particularly those that are difficult for WCT to leave: **Yes** *(probable based on available algae samples from WCT overwintering sites S6, RG_MP1, etc. from previous years (Minnow 2014b;* White and Larratt 2016; *this report) and on low flows, however no samples from 2017-2019 to determine cyanobacteria densities and no sampling of hyporheic zone for surrogate parameters (e.g., TOC, BOD, exchange rate)*
Requisite Timing	A cyanobacteria bloom would have to occur during the summer-fall growing season through the winter low flow period (WCT incubation-summer rearing period to overwintering), with Aug 2018-Apr 2019 low flows of particular interest: ***Yes*** *(probable based on available periphyton samples (Minnow 2014b;* White and Larratt 2016; *this report) from other years outside the decline window and low flows in summer 2018 persisting into winter 2019)*
Requisite Intensity	A cyanobacteria bloom(s) in summer followed by extensive mat decomposition and/or cyanobacterial-calcite dissolution during winter low flows with no intervening flushing flow would be required: ***No*** *(Calcite dissolution calculations showed only localized dissolution was thermodynamically possible in mainstem river flows (Hocking et al 2021) but was possible in Feb. 2019 at LC_FRDSDC (pH lowered 1 unit) and in localized areas of the periphyton mat experiencing low pH during decomposition, however, without periphyton samples to demonstrate cyanobacteria densities, this cannot be directly addressed; also no sampling of in-situ hyporheic exchange that could dilute cyanotoxins during low UFR flows)*
	Yes = requisite condition was met; No = requisite condition was not met

2.1.3 Data Gaps and Uncertainties for Impact Hypothesis 1:

The intermittent periphyton/cyanobacteria research in UFR (Sept 2012/2013, Sept 2015, Feb 2020) did not cover the WCT Decline Window (Sept 2017 – Sept 2019). A periphyton/cyanobacteria assessment during the Decline Window would have reduced uncertainty considerably. Acute cyanotoxicity to benthic invertebrates and WCT was not detected during 2015 when the most extensive paired periphyton and benthic invertebrate data was collected and is expected to be low in the Decline Window. However, it was not possible to determine if there was chronic sub-lethal stress from cyanotoxins that could have reduced individual fitness and made them more susceptible to other stressors. Natural variability in cyanobacteria densities can change dramatically over a period of days to weeks, especially during low flow events, thus 2015 data cannot be used to determine cyanotoxicity (cyanobacteria density + timing + duration/persistence) in the Decline Window.

The role of cyanobacteria in accelerating biogenic calcite formation, its morphology (strength, seasonality, metal interactions, susceptibility to erosion and dissolution) and its capacity to store and encourage cyanotoxin production by embedded cyanobacteria are not fully understood in UFR.

2.1.4 Discussion of Impact Hypothesis 1 Cyanobacteria

Hypothesis 1A: Direct potential cyanotoxin effects

In the absence of cyanobacteria data from the Decline Window, lines of evidence were followed by assessing cyanobacteria growth requirements and by inferring from densities of cyanobacteria genera in fall 2015 and in limited 2020 winter UFR samples. Because cyanobacteria blooms can change dramatically over a period of only days to weeks, especially during flow events (US State Dep. 2015), the detailed 2015 UFR data cannot be used to determine site-specific cyanobacteria conditions in the Sept. 2017 – Sept. 2019 window.

Occurrence and Prevalence

Cyanobacteria have been detected in all years with periphyton monitoring on the UFR, so are not a recent occurrence during the Decline Window (Table A-19, Table A-20). Figure 6 demonstrates that periphyton biovolume is enhanced in mine-affected areas compared to upstream reference areas for diatoms and particularly green algae, but not for cyanobacteria. This is because large populations of *Nostoc* and *Leptolyngbya* were identified at the reference site FO26 (Figure 5) which emphasises that cyanobacteria are naturally occurring in the UFR. Of the 20 cyanobacteria taxa found in UFR samples, Table 6 shows the eight cyanotoxins that can be produced by the nine dominant taxa (Table 6, Table A-18). Periphyton cyanobacteria proliferations were identified in fall 2015 samples from WCT overwintering and rearing areas. Grab samples from winter 2020 showed continued cyanobacteria growth during winter low flows (Table A-19, Table A-20). In both low flow periods a potential risk of chronic low-dose cyanotoxin exposure exists for WCT. In contrast, lentic cyanobacteria bloom taxa known to cause animal deaths (WHO 1999) were not found in any UFR 2012 - 2020 samples.

Phormidium autumnale, a bloom-forming cyanobacteria of concern due to its toxicity and prevalence on calcified substrates under low stable flows (Fetscher et al. 2015; Wiltsie et al. 2018; Gao et al. 2007; Sivonen 2009), is common at many reference and mine-influenced UFR sites (Table A-19; Figure A-29). Toxin-producing cyanobacteria inhabited all UFR reaches both reference and mine-exposed (Table 6; Table 7), notably the S6 overwintering reach and below.

Mapping of UFR *Phormidium* densities in fall 2015 and winter 2020 is provided in Figure A-29 and the entire range of cyanobacteria in Figure 5. The criteria required for harmful growths of *Phormidium* are met in some UFR reaches such as losing reaches and/or abstraction leading to stable low flows (e.g., FODPO rkm 45-50), and gaining reaches with high nitrate oxygenated shallow groundwater influx (e.g., S6 reach near Clode Ponds rkm 61.6) (S. Humphries pers. comm. 2020). *Phormidium* was common at these and adjacent sites (Figure A-29), but not at harmful densities exceeding 20% of total abundance. Total cyanobacteria densities in the fall 2015 data accounted for 13.5 ± 23.2 % of abundance and 8.9 ± 19.0 % of biovolume while in the limited winter 2020 data cyanobacteria averaged 15.4 ± 19.8 % abundance.

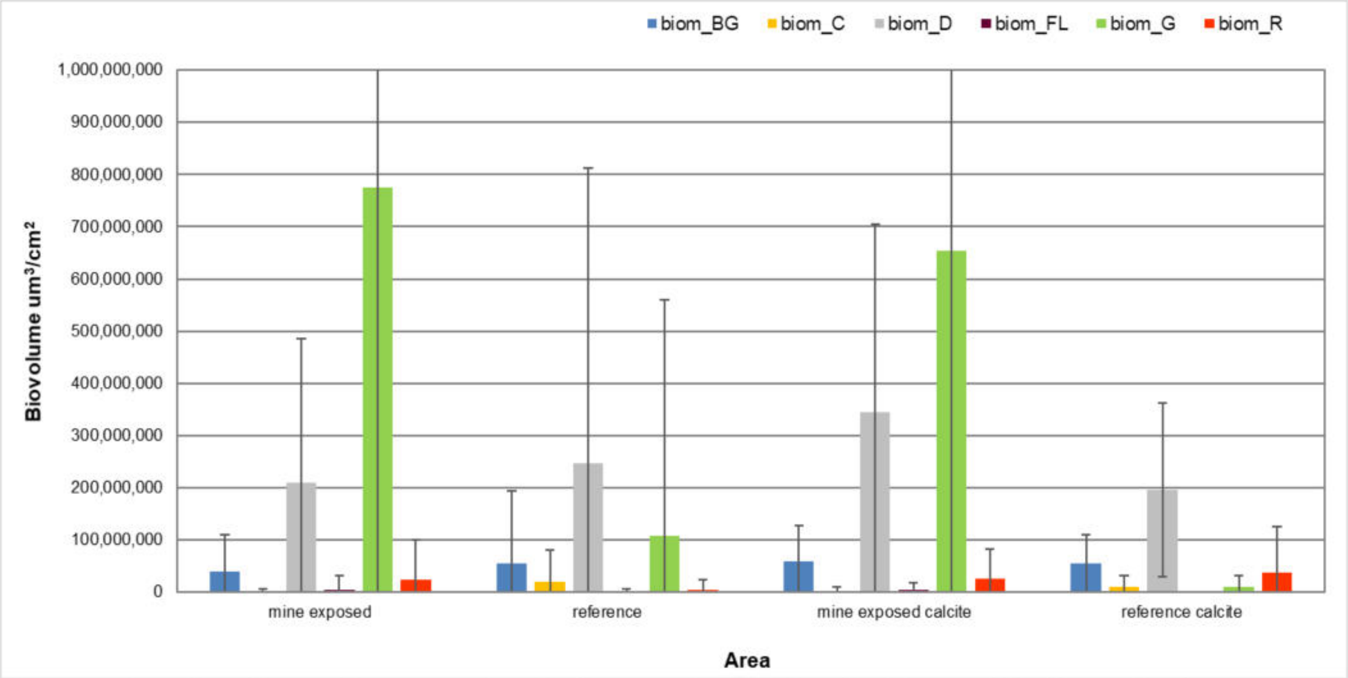

Figure 6: Algae biovolume mine-exposed and reference sites, with and without calcite (2015 data)

Note: BG = blue-green cyanobacteria C = chrysophyte D = diatom algae FL = flagellated algae G = green algae R = red algae

Table 7: Median biomass density of common cyanobacteria in Sept. 2012 and 2015 UFR samples

	2012				2015			
median biomass density	**LENTIC**		**LOTIC**		**LENTIC**		**LOTIC**	
µg/cm^2	**Ref**	**Exp**	**Ref**	**Exp**	**Ref**	**Exp**	**Ref**	**Exp**
Heteroleiblenia cf. profunda	0	21 ± NA	4	9 ± 0			1 ± 3	1 ± 3
Homeothrix cf. jantha	1 ± 2	5 ± 8	1	4 ± 3			14 ± 16	
Leptolyngbya limnetica	0	0	0	0.5 ± 2.0				
Nostoc sp.	0	0	266	0				
Phormidium autumnale	5 ± 11	15 ± 7	14	553 ± 422			31 ± 82	4 ± 60
Rivularia sp.	5 ± NA	53 ± NA	11	32 ± 35				0
	n = 25 total						n = 12	n = 60

Environmental Factors

Environmental stress such as low flows, dark conditions, elevated summer water temperature, and elevated phosphorus conditions have all been demonstrated to increase cyanotoxin production (Fetscher et al. 2015; Wiltsie et al. 2018; Gao et al. 2007; Kaebernick and Neilan 2001; Sivonen 2009).

Flushing Flows

Like all lotic environments, sites with lower flow regimes in UFR are expected to host higher densities of toxin-producing cyanobacteria than fast flowing sites. Some of these sites are important fish habitat, such as FODPO and FO22. Cyanotoxin producers that are intolerant of high flows found in the UFR include *Nostoc* and *Leptolyngbya* and these were found at both reference and mine-influenced low flow sites. These and other genera can be progressively dislodged from substrates by near-bed velocities exceeding 0.2 m/s (Guven and Howard 2006). Near-bed velocities >0.2 m/s are exceeded throughout the UFR during every freshet and in major storm flows (e.g., Dec. 19 2017). Although these will be faster than near-bed velocities, mid-column velocities at UFR mainstem sites ranged from 0.1 m/s near shore to 1.28 m/s in 48 cm depth (Minnow 2014; Figures A-37 and A-44)[7]. In mine influenced and in reference areas, stable lower flows can provide ideal environments for cyanobacterial mats and also permit the accumulation of fine sediments that can help retain cyanotoxins for weeks to months (Lahti et al. 1997; WHO 2019). Without a fall flushing flow, the potential for cyanotoxin exposure is extended from the early life stages of WCT in summer rearing, into the juvenile/adult overwintering stage. Stable low flows increase the potential for invertebrates to be exposed to cyanotoxins, thereby making them available to WCT through diet and through direct exposure.

Flows above the shear velocity for periphyton are used in flow-regulated rivers to remove nuisance or harmful periphyton/cyanobacteria growth (Measures and Kilroy, 2013). Interestingly, most rivers with *Phormidium*-dominated periphyton mats are not flow regulated by dams but have significant groundwater abstraction that stabilizes flows, and they have high nitrate concentrations like the UFR (Golder 2020a). In most years, stable low flows do not persist from summer uninterrupted through to winter. However, in Fall 2018, no flushing flow or spate intervened between summer low flows and winter low flows (FRO mean daily discharge flow data). Continued stable low flows through to Winter 2019 would retain and promote periphyton growth including cyanobacteria.

Water Temperature

In the UFR periphyton model averaging, increasing water temperature strongly encouraged cyanobacteria abundance (Figure 15, Figure 7, Appendix 1). This suggests that the important window for cyanotoxin production in UFR is during late summer when warm, low flows occur as they did in 2018 (Figure 7). Water temperatures are controlled by air temperature and influenced by low flows. Air temperatures during the Decline Window were >18 °C for either similar or fewer days compared to several preceding years (Wright et al. 2020). Mean water temperatures during the Decline Window were cooler by 0.3 – 0.8 °C in 2018 and 2019 compared to 2012 and 2013 (Ecofish 2020b; Minnow and Lotic 2020). Overall, the slight water temperature changes do not suggest a temperature-induced change in cyanobacteria densities in the Decline Window growing seasons.

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[7] Average daily discharge measured at FRNTP: Low flows ≅ <1 m^3/s | Fall flushing flows ≅ ≥2-5 m^3/s | Freshet and flood flows | ≅ ≥6-9-(16+) m^3/s

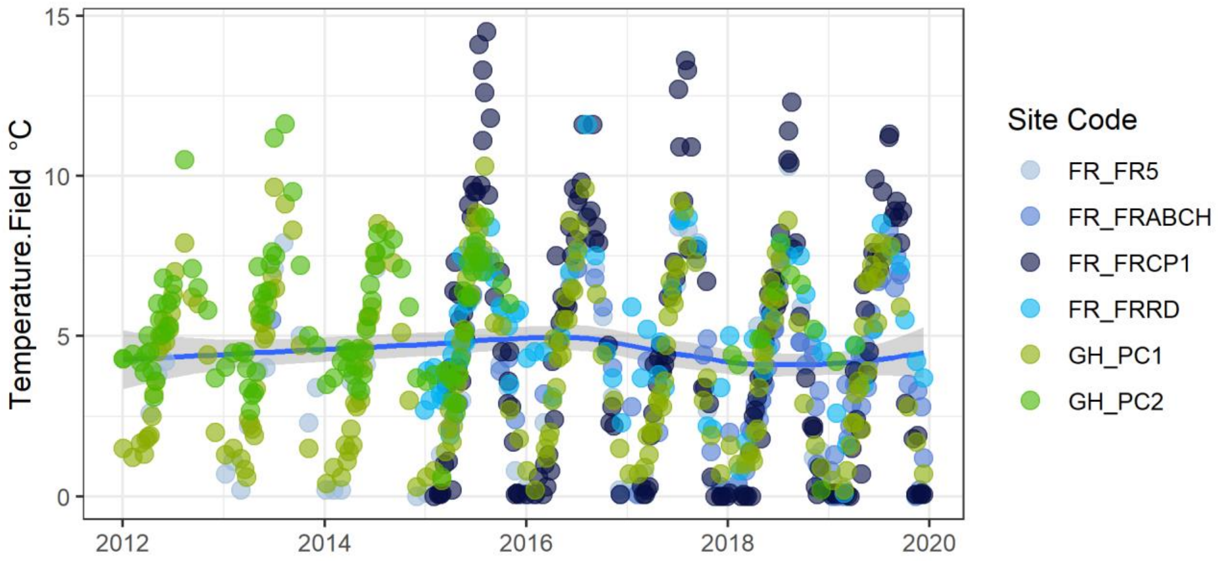

Figure 7: Temperature in UFR at sites within and adjacent to the S6 reach, 2012-2019

Nutrient Regime

Like most BC aquatic environments, the key nutrient controlling periphyton including cyanobacteria, is phosphorus. In the UFR periphyton model averaging, phosphorus was identified as being an important driver of cyanobacteria abundance (Figure 15). The UFR mainstem nutrient regime was within the range (DIN >0.05 mg/L, DRP <0.01 mg/L) reported to sustain cyanobacteria proliferations (Wood et al. 2017). Some UFR mainstem sites (notably FRABCH and FRCP1), had higher dissolved phosphorus during the Decline Window.

Cyanotoxin Production and Persistence

In the absence of analytical results for cyanotoxins, potential for cyanotoxicity were pursued using three lines of evidence – unexplained results from toxicity tests in late summer or winter, altered benthic invertebrate community metrics and a correlation between a filamentous algae bloom (Didymo), and cyanobacteria densities.

(1) Unexplained Toxicity

One means of assessing acute cyanotoxin impact potential is by reviewing UFR invertebrate and vertebrate bioassay tests to identify impacts that are not explained by water quality parameters. However, substrates and periphyton mats (reservoirs for cyanotoxins) are not included in these standardized tests. If it were to occur, cyanotoxicity should be most apparent during the summer growing season (Q3 July – Sept) and during fall low flows (Q4 Oct – Dec) when dilution is minimal. ***For more information on sediment toxicity please refer to coal dust and sediment chapter DiMauro et al. 2021 of UFR WCT Evaluation of Cause SME report.***

Bioassays with UFR water involving cladoceran zooplankton rarely demonstrated toxicity. The occurrence of toxicity without a reasonable water chemistry cause were even less frequent. Likely adverse responses were most common for algae, while most samples exhibited increased algae growth relative to the laboratory control (Golder 2020b). Toxicity to amphipods and trout occurred most often in Q4 of 2018 and of 2019 (Ings et al. 2019; Golder 2019; Minnow 2020). While we can't rule out cyanotoxicity effects, more likely, another feature of low flow water chemistry was the cause as these studies concluded. ***Refer***

to water quality chapter for more information Costa and de Bruyn 2021** of **UFR WCT Evaluation of Cause SME report.

Dry Creek stations (LC_DCDS, LC_DC1) showed infrequent adverse impacts in 7-day tests of cladoceran reproduction and survival (Golder 2020). None of the samples collected in the discharge channel (LC_SPDC) in 2017 (n=12) or in 2018 (n=5) were acutely toxic to cladocerans, with all sample results showing 100% survival (Ings et al. 2019). Similarly, chronic toxicity tests completed in 2018 showed no adverse effects on cladoceran survival from Dry Creek samples. However, an adverse effect on cladoceran reproduction was observed for the April 2018 samples. April is not usually a problematic period for cyanotoxicity in BC waters, so this adverse response is not a response to cyanotoxins. It can be concluded that Dry Creek toxicity data did not indicate cyanotoxicity.

Looking at the broader Elk Valley context, of the 80 toxicity tests conducted in the Elk Valley in 2019, 17 results were reported where no water quality constituent was associated with observed responses for invertebrates or fish in summer and early winter low flows (data from Golder 2020) (Figure 8). Most adverse aquatic vertebrate toxicological test responses could be attributed to water quality under low flow conditions. The few unexplained results occurred most often in Q3 (July – Sept) and Q4 (Oct – Dec), indicating potential cyanotoxin effects. There were no unexplained adverse response tests from UFR samples in 2019 (Appendix B-6 in Golder 2020b). Based on these results, acute cyanotoxicity appearing within the 7–8-day test run is very unlikely in the UFR, however, the potential for non-lethal chronic low dose stress from cyanotoxins cannot be determined from these studies.

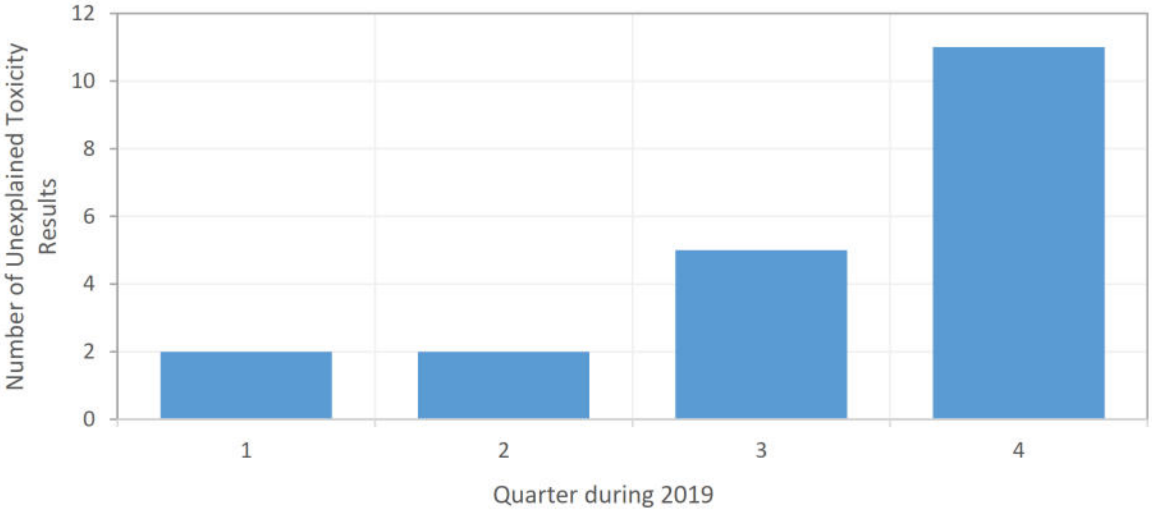

Figure 8: Toxicity results from Elk Valley 2019 samples where no water quality constiuent associated with observed responses was identified for amphipods, minnows, trout and water fleas.

Altered Benthic Invertebrate Community Metrics

Another indirect line of evidence regarding cyanotoxicity to aquatic animals involves reviewing altered benthic invertebrate distributions at sites with high cyanobacteria densities and at sites with calcite embedded with cyanobacteria (See 2.1.1. Invertebrate section and Table A-17). The detailed 2015 periphyton study was compared to benthic invertebrate abundance at UFR sites for that year (White and Larratt 2016). This analysis indicated that sites with high or very high cyanobacteria abundance upstream to downstream included:

- Very High (>180,000 cells/cm²): FO26 (reference site), FOBSC, POCK, FOUEW, EWCK, LC_DC1, FO29, FODGH
- High (60,000 – 180,000 cells/cm²): FOUSH, FODPO, FO22, CHCK (reference site), GHCKU (Figure 5)

UFR researchers determined that benthic invertebrate community metrics were within normal ranges at 11 of the 14 sites with very high/high cyanobacteria sites in the 2015 data (White and Larratt 2016). Most of them had benthic invertebrate characteristics within normal ranges (e.g., high %E and %EPT, typical overall community structure) (Minnow 2018). The three sites that had atypical invertebrate characteristics were areas that had unusual habitat characteristics or elevated levels of mine-related constituents (water quality and/or calcite; sites POCK, FODPO, and GHCKU), preventing an assessment of potential cyanotoxicity.

We can infer that the potential for acute cyanotoxicity to benthic invertebrates was negligible in the studied years, however, chronic low dose stress from cyanotoxins both to the invertebrates themselves and to the WCT that prey on them cannot be determined from these data because invertebrates can acquire cyanotoxins that can be passed onto their predators without causing measurable changes to their community metrics (Kozlowski-Suzuki et al. 2012; Ibelings and Havens 2008; Ferrao-Filho and Kozlowsky-Suzuki 2011; Anderson et al. 2018; Wood et al. 2020). The effects on WCT from chronic exposure to low dose cyanotoxins is also expected to be negligible unless a bloom occurred in the Decline Window and even if so, the sub-lethal effects are not expected to account for the observed WCT decline.

Zooplankton and rotifer habitat in the UFR is provided by lentic sites such as Fish Pond Creek and Henretta Lake, as well as in UFR reaches with slow water (<0.2 – 0.3 m/s) such as S6 and oxbows that together account for only 7% of the UFR aquatic habitat. The dietary importance of zooplankton to WCT young-of-year is not known but is likely to be important at Henretta Lake and S6 pools, as well as lotic sites that receive lentic discharge. Although it cannot be ruled out due to data constraints, bioaccumulation of cyanotoxins in zooplankton are not expected to be relevant to the WCT decline (Table A-17). Overall, the cyanotoxicity results from these studies do not align with the decline because mortality from cyanotoxicity is rare, early life stages are expected to be more susceptible than adults, and adults rely on benthic invertebrates, making them less susceptible to cyanotoxins than fish that rely on zooplankton (Table A-17).

Didymo and Cyanobacteria blooms

Finally, we searched for a correlation between the bloom-forming filamentous diatom *Didymosphenia geminata* (Didymo) and cyanobacteria dominance that could indicate the potential for an antagonistic effect between these harmful taxa. We noted increased cyanobacteria density in Didymo sample blooms in the 2015 data and postulated that a similar effect may have occurred in the Decline Window. However, the correlation between cyanobacteria and Didymo was not significant in the 2015 dataset (Pearson's correlation coefficient R=0.035) under normal, low density Didymo growth conditions. Based on work elsewhere, shading from Didymo mats is not as disadvantageous to filamentous cyanobacteria that are tolerant of low light regimes, then it is to diatom members of the periphyton biofilm that have higher light requirements (James et al. 2012; Bray 2014; Clancy et al. 2020). Further the nitrogen fixed by cyanobacteria may supply and accelerate Didymo growth (Novis et al. 2016). It is therefore possible that a Didymo bloom would exert habitat stress concurrent with cyanotoxicity.

Hypothesis 1B. calcite mediated potential cyanotoxin effects

Periphyton photosynthesis accelerates biogenic calcite precipitation principally by raising pH in the diffusive boundary layer and results in cyanobacteria embedded in calcite. In model averaging, bicarbonate ($CaCO_3$ or calcite) was the dominant driver for cyanobacteria, demonstrating that calcite is strongly associated with cyanobacteria growth (Figure 15). Conversely, decomposition processes lower pH and encourage calcite dissolution, particularly in cold water ***Refer to calcite chapter for information on biogenic calcite Hocking et al. 2021 of UFR WCT Evaluation of Cause SME report.***

Embedded cyanobacteria filaments produce more toxins under dark conditions, and these toxins can persist for months or longer. This would be most important when overwintering habitats had calcite index scores near the biological effects threshold at or above 1[8] (e.g., Fording Reach 5,6,7) and in depositional sites that accumulate calcite mud particulates in sediments (e.g., S6 pools; Figure A-30). Over-wintering WCT can be exposed to materials released during localized calcite dissolution in low flow, cold water months. Negligible cyanotoxin effects are expected with typical cyanobacteria densities, but minor effects (lowered WCT fitness) may occur if a cyanobacteria bloom of problematic taxa, particularly *Phormidium autumnale* that thrives in calcite*,* occurred during growing seasons within the WCT decline window (Ferrão-Filho and Kozlowsky-Suzuki 2011). Release of cyanotoxins from senescent cyanobacteria filaments trapped in calcite during localized dissolution or from sediments is possible, and the breakdown of those toxins under winter conditions is expected to be slow based on research elsewhere (Lahti et al. 1997; Twist and Codd, 1997; WHO 2019).

2.1.5 Concluding Summary and Strength of Evidence for Impact Hypothesis 1 Cyanobacteria and Cyanotoxins

The strength of evidence for hypothesis 1A and 1B is indeterminant due to the limited periphyton sampling in the UFR and no sampling during the WCT Decline Window. Evidence was also provided by the literature-based review which indicated that early life-stages are most susceptible to cyanotoxins, which does not align with the observed decline of WCT adults or juveniles.

HYPOTHESIS 1A *Are there cyanotoxins in the UFR at sufficient concentrations and durations to cause adverse effects to benthic invertebrates and fish in general? Specifically, could they cause mortality in juvenile and adult life stages of WCT in the UFR during the Decline Window?*

Because cyanobacteria blooms can change dramatically over a period of only days to weeks, existing UFR data cannot be used to determine site-specific cyanobacteria conditions in the Decline Window. It can be stated that cyanobacteria presence and the possibility of chronic low-dose stress to WCT is both natural and long-standing, thus chronic low-dose stress is unlikely to account for the WCT decline in the upper Fording River. Cyanotoxin stress may lower fish condition, making them more susceptible to other stressors or disease, however, cyanotoxin stress is challenging to assess without sacrificing fish and overall fish health testing had not shown a measurable effect prior to the decline (Minnow 2020a). Since cyanobacteria samples are not available from the WCT Decline Window, the presence (or absence) of a chronic low dose cyanobacteria toxin effect during that window cannot be ruled out. A cyanobacteria bloom exceeding 20% substrate coverage could have developed during summer 2018 low flows and that

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[8] CI values greater than 1 indicate that substrate concretion is occurring and is identified as an approximate effects threshold of biological impairment and increased embedded cyanobacteria densities (SME calcite report).

effect could extend through to Winter 2019 because no fall flushing flow intervened, resulting in higher WCT exposure to cyanotoxins. None the less, cyanotoxicity results from fish studies elsewhere do not align with the WCT decline because mortality from cyanotoxicity is rare, early life stages are expected to be more susceptible than adults and WCT rely on benthic invertebrates making them less susceptible to cyanotoxins than fish that rely on zooplankton.

For more information on cyanotoxin and fish health, please refer to the pathology chapter (Bolinger, 2021) of UFR WCT Evaluation of Cause SME report.

HYPOTHESIS 1B *Did conditions within the Decline Window permit localized calcite dissolution to release embedded cyanotoxins, particularly during winter low flows?*

Release of cyanotoxins from senescent cyanobacteria filaments found in periphyton mats, sediments and biogenic calcite during localized cold-water calcite dissolution is theoretically possible. The breakdown of those toxins under winter conditions is expected to be slow based on research elsewhere (Lahti et al. 1997; Twist and Codd, 1997; WHO 2019). Without flushing flows in fall 2018, cyanobacteria mats, calcite embedded with cyanobacteria and cyanotoxins stored in the sediments of depositional areas could affect overwintering invertebrates and WCT. Cyanobacteria presence and the possibility of chronic low-dose stress to WCT is both natural and long-standing, thus chronic low-dose stress is unlikely to account for or contribute to the WCT decline.

For more information on cyanotoxin and metals behavior in biogenic calcite, please refer to the calcite chapter Hocking et al. 2021 of UFR WCT Evaluation of Cause SME report.

2.2 Impact Hypothesis 2 Filamentous algae bloom impacts to WCT

Hypothesis 2 examines the positive and negative roles of filamentous algae blooms in the FRO habitat of WCT. On the positive side, filamentous algae blooms can represent habitat and grazing opportunities. On the negative side, dense mats of filamentous algae can impede water movement, restrict intra-gravel water flow and dissolved oxygen replenishment, or degradation of benthic habitat.

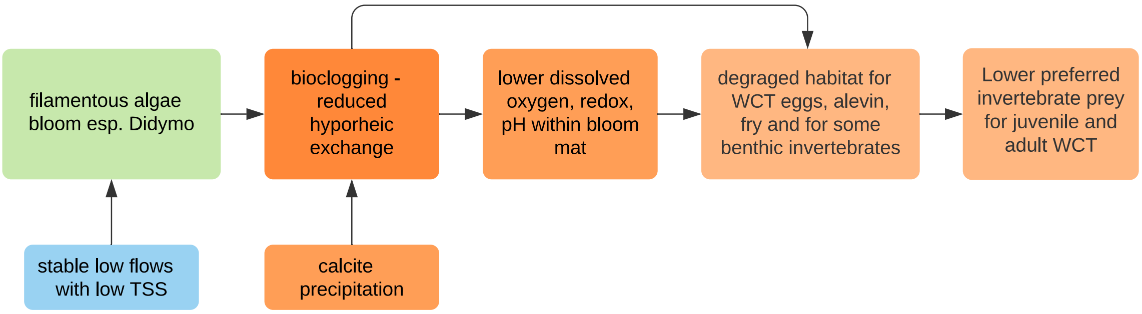

Figure 9: Conceptual effects of filamentous algae blooms on WCT investigated in this report

Hypothesis 2 Questions:

A. *Did filamentous algae blooms reduce hyporheic exchange, particularly during the decline window?*

B. *Did filamentous algae blooms, such as Didymo, provide poor forage and degrade physical habitat (Altered DO, pH, redox) for benthic invertebrates and WCT particularly in low velocity (<~1m/s) UFR reaches during the decline window?*

Q1. *What are the triggers and controls for filamentous algae blooms in the UFR*?

2.2.1 Introduction to Filamentous Algae Bloom Impacts

Filamentous algae are the largest of the periphyton taxa and their blooms make important ecological changes that affect invertebrates and early life stages of fish. Overall periphyton productivity metrics increase dramatically due to the large strand size of this algae which also provides a substrate for small diatom algae and invertebrates to attach to. Filamentous algae growth is controlled by light, flows and nutrients in all rivers. In the UFR, filamentous green taxa will proliferate as flows and phosphorus supplies allow (Stevenson and Smol 2015). Ironically, excessive stalk development in Didymo (*Didymosphenia geminata)* may be triggered by decreasing dissolved reactive phosphorus (bioavailable phosphorus), particularly below 4 µg/L (Jellyman and Harding 2016). Although filamentous algae are beneficial at low densities, heavy growth can cause strong diel pH and DO fluctuations, alter near-bed water velocities, and reduce local DO over winter as they decompose (Arnwine and Sparks 2003). For example, periphyton blooms can raise pH by >1 and supersaturate the overlying water with dissolved oxygen during the day, but lower pH by >1 and deplete DO (<4 mg/L) at night, creating conditions conducive to the release of iron-bound dissolved reactive phosphorus in sediment (Wood et al. 2020).

The current BC guideline of 100 mg/m^2 for chlorophyll-a to assist with managing stream periphyton is lower than the thresholds for nuisance levels of periphyton chlorophyll-a, estimated at 120 - 200 mg/m^2 in more recent literature (Minnow 2021a). This range corresponds to periphyton substrate coverage of about 25–30+% (McDowell et al. 2020). By comparison, the upper limits of normal ranges for chlorophyll-a collected in reference areas sampled in the Elk Valley and adjacent watersheds were 66 mg/m^2 (n=103) (Minnow 2021a).

The hypothesis questions were approached by questioning general habitat change and potential effects on the benthic invertebrates that are staple in the WCT diet.

Hypothesis 2A: Did filamentous algae blooms reduce hyporheic exchange, particularly during the decline window?

Water movement through the riverbed is primarily dependent upon substrate permeability (size, type) and thickness of the periphyton biofilm layer together with river water depth. Filamentous algae blooms form thick periphyton layers that can reduce hyporheic exchange by restricting downwelling or infiltration on a local scale. Additionally, these blooms can introduce order of magnitude changes in downwelling seepage fluxes in a matter of weeks through physical clogging in shallow areas (Newcome et al. 2016). Studies in New Zealand found that Didymo cover had no measurable effect on several hydraulic variables (flow into the substrate, hydraulic conductivity and hyporheic oxygen concentration) (Bickel et al. 2008) which agrees with a UFR tributary calcite study (Wright et al. 2018). However, there was a significant difference in the potential surface water–groundwater exchange between sites, indicating some effect of Didymo on hydraulic conditions.

Processes governing site specific infiltration include:

- scouring and sediment disturbance events (which have been shown to increase infiltration by a factor of seven (Rosenberry et al. 2010)
- Transient river flow boundaries, mechanical clogging from algal growth
- Grazer-algal food chain dynamics (Power et al. 2008; Krenz et al. 2018; Laboid et al. 2007)
- Preferential flow paths,
- River geometry
- Water temperature (Newcome et al. 2016)

Deposition of coarse sediments occurs in regions of upwelling while in regions of downwelling, fine sediments settle (Nezu and Nakagawa 1993) and both sediment types become embedded in various filamentous algae mats (Upwelling = filamentous *green, gold or red algae, cyanobacteria;* Downwelling = diatoms and Didymo) (Wyatt at al. 2008). These mats cause localized bioclogging that can reduce hyporheic exchange during low flows and therefore affect WCT habitat value. Further, Didymo blooms induce changes in the underlying hyporheic bacterial populations that in turn alter redox and dissolved organic carbon concentrations with repercussions to metal speciation and cycling (Qian et al. 2019; Briggs et al. 2015; Zarnetske et al. 2011).

The influence of algae bloom photosynthesis on pH is dramatic and well documented. Rivers show distinct and highly correlated diurnal fluctuations of DO and pH caused by photosynthesis and respiration, of ~1-2 pH units within the growing season and ~0.25 pH units in the winter (Jones and Graziano 2013). pH fluctuations have implications for river processes including biogenic calcite precipitation in which calcite precipitation occurs in the microenvironment and in the vicinity of photosynthesizing cells because the pH and the calcite saturation index are much higher than in the bulk river water (Hayashi et al. 2012). Biogenic calcite precipitation and filamentous algae biomass form a mesh that can locally increase bio-clogging.

Sites with filamentous algae blooms can impact juvenile WCT through the entire summer rearing stage by lowering hyporheic exchange rates and isolating gravels from the water column with the periphyton mat (Bickel et al. 2008; Jellyman and Harding 2016). Alevins living adjacent to a thick periphyton mat can demonstrate reduced fitness due to diel fluctuations in DO, pH and redox (Clancy et al. 2020; Furley et al. 2014). Since the effect of filamentous algae on hyporheic exchange is most important to early life stages of WCT, it is not consistent with the observed WCT decline that involved juveniles/adults.

Hypo 2B. Did filamentous algae blooms, such as Didymo, provide poor forage and degrade physical habitat (altered DO, pH, redox) for benthic invertebrates and WCT particularly in low velocity (<~1m/s) UFR reaches during the WCT Decline Window?

Several types of filamentous algae have been documented in UFR samples over the years, but blooms have been restricted to filamentous green algae and Didymo. Didymo is a stalked diatom of concern because it behaves invasively, forming thick filamentous mats that are known to cover kilometers of riverbed. Didymo has been found in most BC river systems including the Upper Fording. This stalked diatom impacts rivers with stable flows, low TSS, high light intensity, and low water temperature regimes. Didymo can proliferate under low phosphate concentrations, perhaps due to recycling of this nutrient within its filament mat (Bray 2014; James et al. 2012).

Where Didymo dominates the periphyton, nutrient cycling and ecosystem function becomes impaired (Reid and Torres 2014; Sanmiguel et al. 2016). It creates a periphyton community with low diversity, dominated by small taxa that is clearly different from communities in the same stream where there are no massive growths (Ladrera et al. 2018; Clancy et al. 2020; Kilroy et al. 2009). This effect intensifies as the Didymo mat thickens (Kilroy et al. 2009). The loss of large diatoms can reinforce the loss of larger invertebrates as illustrated in Figure 10.

In the Columbia River, Didymo filament mats correlated to lower benthic invertebrate diversity, lower EPT scores, and higher chironomid densities (Schleppe et al. 2014; Olsen-Russello et al. 2015). At high Didymo bloom coverage (20-24%), stream invertebrate assemblages originally dominated by *Ephemeroptera, Plecoptera, and Trichoptera* (EPT taxa), typically became dominated by small, opportunistic, herbivorous organisms - mainly *Chironomidae, Oligochaeta, Nematoda, or Cladocera*, that are capable of moving between filaments and are aided by the absence of large trophic competitors and predators (Kilroy et al. 2009; James and Chipps 2016; Clancy et al. 2020; Jellyman and Harding 2016). Taxa that require substrate attachment such as *Simuliidae (*blackfly) decline (Ladrera et al. 2015). Stoneflies were completely excluded by the Didymo mats in many rivers and streams. Small predators such as *Hydra* can thrive in Didymo mats, as they are favored by the increased chironomid availability and are released from their own predators (Ladrera et al. 2015).

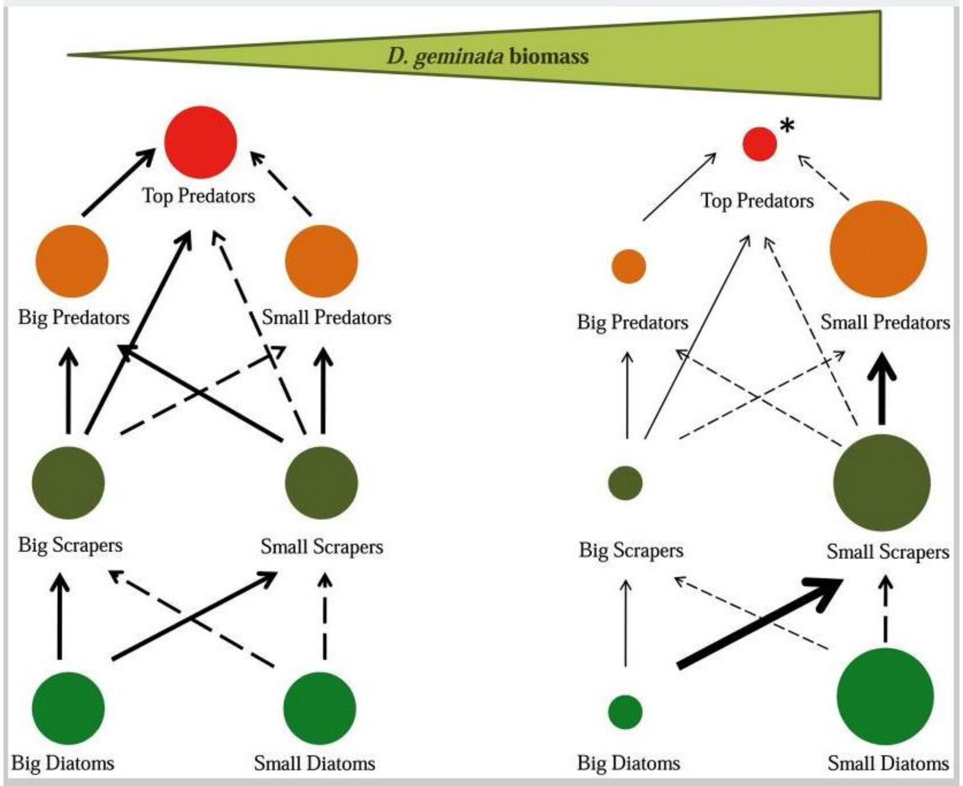

Figure 10: Trophic community alterations induced by *Didymosphenia geminata*

Diagram of the trophic interactions related to increased *D. geminata* biomass levels. Circumference size represents the density of each type of organism. The arrow thickness represents the intensity of trophic interactions, where dashed arrow represents lowest intensity. The main organisms included in each category are: Small diatoms (A. *minutissimum*, *B. neoexilis*); Big diatoms (*C. placentula*, *Gomphonema sp*); Small scrapers (Orthocladiinae); Big scrapers (Heptageniidae); Small predators (Hydra); Big predators (Perlidae); Top predator (*Salmo trutta*)* possible reduction in fish biomass with increasing Didymo biomass.

(From Ladera et al. 2018)

In a 2018 – 2019 study of the Kootenay River system, trout diets were 81% similar despite obvious differences in the composition of macroinvertebrate assemblages between the Didymo and reference streams. Further, the number of invertebrates in the drift was higher in the stream without Didymo (Clancy et al. 2020). Despite this, the overall fish condition impacts to native redband trout, westslope cutthroat trout and bull trout were small (Clancy, 2020). The Clancy study implies that the effects of a Didymo bloom were measurable but did not instigate a significant decline in resident trout.

Adverse effects of Didymo blooms have also been observed in salmonid fisheries globally (Bothwell et al. 2008, 2009; Bickel and Closs 2008; Shearer et al. 2007; Taylor 2012; Jellyman and Harding 2012; Rost et al. 2008). Altered habitat characteristics and fish feeding habits appear to drive these changes (Taylor 2012; Jellyman and Harding 2012, 2016). Many studies in New Zealand, Scandinavia, and North America have shown small Didymo impacts on trout populations and a shift to a macroinvertebrate assemblage of smaller and more abundant individuals that may favor juvenile fish (James and Chipps 2016). Other studies have found much larger impacts on adult fish such as a 50% loss of 20 cm trout and an overall 90% reduction of fish biomass (Kilroy et al. 2016). Decaying Didymo bloom masses could have consequences for trout hatching success because of the increased oxygen demand (Bickel et al. 2008).

2.2.2 Requisite Conditions for Impact Hypothesis 2:

The following points lay out the requisite conditions that must be present before an impact to WCT from filamentous algae blooms would be anticipated:

REQUISITE CONDITIONS FOR HYPOTHESIS 2:

Requisite spatial extent	Blooms would have to occur throughout the UFR, particularly in key WCT areas: ***Yes.*** *(Blooms are probable, extrapolating from available anecdotal photography, sediment TOC (DiMauro et al. 2021; Minnow 2020a, 2020b), flow data (Figure A-37), and periphyton samples (2013 (Minnow 2014b) , 2015,* (White and Larratt 2016); *2020 samples (this report))*
Requisite Duration	Increased filamentous periphyton blooms in the late growing season particularly in years between large freshets/floods would have to occur, with the refined WCT Decline Window (Feb-Mar 2019) of particular interest: ***Yes.*** *(Probable based on samples from other years showing abundant filamentous algae (2013 (Minnow 2014b) , 2015,* (White and Larratt 2016); *2020 samples (this report), but no algae samples from 2017-2019 are available to confirm, we relied on sediment TOC samples (Minnow 2020a, 2020b)*
Requisite Location	A filamentous algae bloom at key spawning/rearing/overwintering WCT locations including RG_MP1 (rkm58.5), FR_FR2 (rkm 56) through S6 to FR_FR5 (rkm 36); sites important to WCT with mine-affected fines deposition such as FOUKI (rkm 55.5) FOBKS (rkm 54) FOBSC (rkm 53.5) FRCP1 (rkm 52) would have to occur**:** ***No*** *(Bloom photography is limited and does not cover all key sites so extent of bloom is unconfirmed, and there are no periphyton samples from 2017-2019 to confirm)*
Requisite Timing	Requisite timing would involve a filamentous algae bloom in summer that persisted into winter, with no intervening fall flushing flow (≥~2-5 m^3/s average daily discharge measured at FRNTP), with the refined decline window of particular interest, thus a bloom in summer 2018 persisting into winter 2019 with no fall flushing flows)**:** ***Yes*** *(based on flow data (Figure A-37), model averaging of periphyton data prior to the Decline Window (this report* Hypo **2**$_{Q1}$*) and limited Winter 2020 periphyton samples, (this report 2.2.4)*
Requisite Intensity	Filamentous algae blooms would have to be extensive and dense enough to induce habitat degradation, leading to a significant drop in benthic invertebrate density or quality OR a bloom of *harmful filamentous algae (e.g. Didymo, Phormidium, heavy bloom of filamentous green or golden algae) extensive enough to directly affect some WCT life stages:* ***No*** *(no direct evidence based on periphyton samples (2013 (Minnow 2014b) , 2015,* (White and Larratt 2016); *2020 samples (this report), but indirect evidence based on studies showing no impact to benthic invertebrate community metrics but without algae samples from decline window, cannot be verified)*
	Yes = requisite condition was met; No = requisite condition was not met

2.2.3 Data Gaps and Uncertainties for Impact Hypothesis 2:

The biggest uncertainties from filamentous periphyton impacts arise from the lack of UFR data from the decline window, with the last systematic sampling in 2015. Natural variability is inherent in periphyton site sampling making between-years extrapolation difficult. No UFR taxonomic samples were available from the decline window. A cursory periphyton score (1-5) to support benthic invertebrate study was used in most years but not in the decline window. This was addressed in part by a review by LAC taxonomists of Minnow site photography. A rapid assessment method may be suitable to maintain data integrity between years with quantitative periphyton assessments.

2.2.4 Discussion on Impact Hypothesis 2 Filamentous Algae using Site-Specific Data

The likelihood of impacts from filamentous algae on WCT habitat in UFR was assessed using available data, field observations and by following several lines of evidence.

Flow regime conducive to filamentous algae blooms

2018 – 2019 was an unusually dry weather period with low and late snowpacks and this led to low streamflows in the UFR. Summer 2018 and 2019 had stable low summer flows and 2019 had minimum flows two months earlier than normal (Figure A-37). Very low flows occurred in Dec 2018 and in February 2019 at 15% and 20% of mean annual discharge, respectively (Wright et al. 2021). Based on UFR flow data, summer/fall storm flows that occur in most years and the annual freshet all generate the velocities needed to shear filamentous green algae and likely Didymo blooms. For example, the spate near December 19 2017, was capable of clearing out accumulated periphyton (FRO velocity data). Most years have a fall flush (exceeding ~2-5m^3/s average daily discharge at FR_ FRNTP) but 2018 did not and low flows persisted from the 2018 growing season into the 2018/2019 winter low flow period (Figure A-37). These low flows encourage filamentous algae blooms with potential consequences to winter dissolved oxygen regimes. Both the 2018 and 2019 UFR mainstem flows and flow-driven conditions such as shear velocity, TSS and water temperatures were low and conducive to a Didymo bloom. When shear velocities are not reached after a bloom has developed, the bloom material (cellulose, polysaccharides, etc.) produced in late summer/early fall will decompose in situ, creating oxygen demand through the winter months.

Changes in filamentous algae prevalence

Periphyton samples from UFR sites collected in September 2015 and a smaller sample set in February 2020 had 43 taxa of which 24 were found in both years; 8 taxa were found only in 2015 and 11 were found only in 2020 samples. Dominant taxa ranked by abundance are listed in Table A-20. Sites with Didymo in 2020 also had Didymo in 2015 (Figure 11). The 2015 samples did not involve a Didymo bloom while the winter 2020 samples likely captured the remnants of the 2019 bloom photographed by fisheries biologist S. Cope (Figure 13). In most rivers, diatoms dominate the periphyton as they did in UFR. Summary statistics for all periphyton sampling programs (2012, 2015 and 2020) showed stable diatom proportions but a possible (not statistically significant) increase in filamentous algae and cyanobacteria (Table A-19).

The proliferation of filamentous algae observed in late summer 2019 far exceeded anything documented over 2012 to present and was not seen in other regional river systems (S. Cope pers comm. 2019). Algae mats thick enough to impede nets were encountered in 2019, and Cope noted that an overall trend to increased filamentous algae production may have occurred since 1995 along the UFR. Additionally, photos taken by the Minnow team at their FRO mainstem sites in 2018 and 2019 were reviewed by LAC periphyton taxonomists. Based on those photos, 10 of 17 sites appeared to have abundant filamentous algae in 2019 and/or 2018 (Table A-28).

The 2013 flood extensively altered UFR habitat in the vicinity of FRO and appeared to “clean out” accumulated filamentous algae and macrophytes with their associated sediment build-up. More stable flows since 2013 likely encouraged the progressively enhanced algae growth witnessed in recent years. Very cold weather in February 2019 did not adversely impact these plants in the subsequent growing season. Algal proliferations in late summer/fall are likely perennial in the UFR over the past decade. A Didymo bloom like the 2019 bloom may have occurred in 2018, suggested by similar sediment organic carbon in mainstem sampling from both years (7.81% in 2018 versus 7.46% TOC in 2019 (Table A-33).

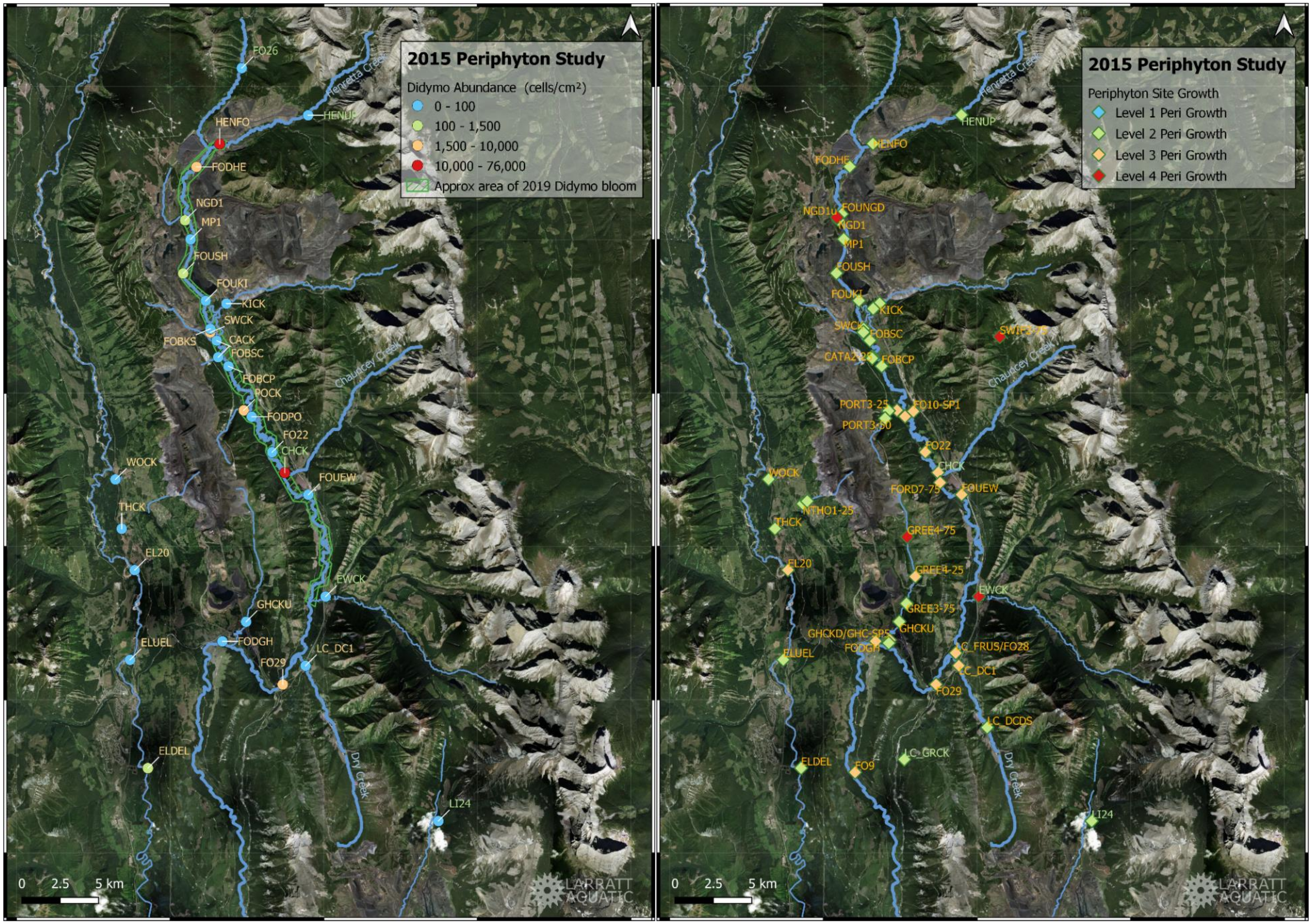

Figure 11: Didymo abundance and visual assessment levels (1 – 5) for 2015 periphyton study

Over the years, filamentous green algae were noted in riffles as dark green streamers that trapped on nets and in pools/ponds (Goudey 2009; Cope pers. comm. 2020). For example, a bright fluorescent green layer up to 60 cm thick was observed in Fish Creek ponds in summer 2019 (Cope pers. comm. 2020). Over the 50 km of Fording River surveys, more filamentous algae growth was always associated with calcite (Minnow 2018; White and Larratt 2016). In the lower areas (river segments 4/5), abundant calcite, filamentous algae, and macrophytes were noted in the 2019 snorkel survey by S. Cope.

Filamentous blooms usually occur in mid-summer through fall, thus intersecting the WCT alevin and juvenile life stages onward. The green filamentous algae bloom in Fish Pond Creek likely commenced in late August and was observed through September and October. Photographs from S. Cope illustrate the extent of the bloom in 2019 (Figure 12). Fish Pond Creek is a groundwater-fed constructed habitat that supports spawning.

The taxa responsible for the large algae bloom on the UFR mainstem in Fall 2019 was almost certainly Didymo. Extensive Didymo was still present in February 2020 samples at the same sites as in the 2015 survey (Figure 11, Figure A-32; Table A-19). This filament-forming diatom behaves invasively. The filament material will be swept downstream by flows with near-bed velocities exceeding 0.2 – 0.3 m/s (mid-column velocity >0.45 m/s) so most of this material will shear in the 2020 freshet. The sites where Didymo bloomed in 2019 (seen as residual populations in Feb. 2020 samples) had modest to low populations in 2015 (Figure A-32; Table A-28). For example, HENFO (rkm 64.4) and FODHE (rkm 63.5) had moderate Didymo abundance (0.1% of diatom frustules) in 2015. Didymo occurred in both reference and exposed lotic sites in 2012 at 6 of 13 sites. In the winter 2020 periphyton data, Didymo averaged 1.3% of the total periphyton abundance across all 2020 locations and ranged from 4% (RG_FOUKI(2), RG_FODHE) to 0.3% (RG_CLODE, RG_FOBKS). Research shows Didymo populations are controlled by TSS (Cullis et al. 2013, Figure 11) indicating that stable flows with low TSS allowed the extensive fall 2019 bloom and may have allowed a comparable Didymo bloom in 2018 as well (FRO water quality database).

Effect on hyporheic exchange

Filamentous algae blooms with the thickness shown in Figure 12 can reduce hyporheic exchange rates, although far less than fine sediments would. The bloom phase coincides with the end of the alevin incubation stage and overlaps the entire summer rearing stage.

Effect of filamentous blooms on pH, DO and redox

Samples collected by Lotic Environmental from Fish Pond Creek ponds in February 2020 through 20 cm of ice were dominated by just three diatoms, with a small amount of residual *Zygnema* filamentous algae, indicating that this was the alga blooming in summer 2019. Like most green filamentous algae, Zygnema population size is driven by available phosphorus and limited by depositing fines (Parkhill and Gulliver, 2002). *Zygnema* provides habitat and grazing opportunities as well as attachment sites for small diatoms.

During the growing season, periphyton including filamentous green algae can raise pH by >1 and supersaturate the water with DO in the sunlit hours, while bacterial and plant respiration can lower pH by >1 and DO to very low levels, even anoxia at night, that can stress fish even when criteria for DO minimum concentrations are met (Arnwine and Sparks 2003; Eiseltová and Pokorný 1994; Wood et al. 2015). These diurnal shifts are less extreme in winter. The resulting redox shifts will also affect bacterial distributions and the reactions they mediate.

These effects on pH, DO and redox are expected to be localized to the periphyton mat with the underlying hyporheic zone and their full extent may not be detectable in the overlying pond or creek water (Furley et al. 2014; Wood et al. 2015). The vast majority of a Didymo bloom biomass is composed of the mucopolysaccharide filaments that senesce during the winter. Minnow field staff noted that Didymo filaments from the 2018 Dry Creek bloom were present but breaking down to black, stringy material in early December 2018 (Wiramanaden et al. 2019) – an oxygen-consuming process.

Figure 12: Fish Pond Creek filamentous algae bloom fall 2019
Source: S. Cope

Supersaturation

Dissolved oxygen supersaturation occurs during intense photosynthesis and is most common during the growing season. Dissolved oxygen supersaturation is most common in September and occurred at many UFR mainstem sites in 2018 and 2019 (Figure A-38, Figure A-39; DO database), providing indirect evidence for algae blooms since stable, low fall flows in the UFR are ideal for algae blooms in all but the drying reaches. Oxygen supersaturation at the levels seen in the UFR is not known to harm cutthroat trout (Edsall and Smith 1990).

Didymo bloom effect on invertebrate community structure

Due to the dense, thick mats that Didymo can form within a few weeks, it has several important habitat impacts. Based on S. Cope's observations and his fall 2019 photographs, Didymo substrate coverage was significant along 50–75% of the UFR mainstem (Figure 13). Figure 13 shows the masses of Didymo dislodged by wading that became trapped on the downstream net, sufficient to cause damming. This bloom extent and intensity will alter hyporheic exchange, lower water velocities against the substrate, alter dissolved oxygen and nutrient regimes, collectively inducing a community shift away from valuable WCT food - EPT (mayflies, stoneflies, and caddisflies) to tolerant chironomids and oligochaetes.

Figure 13: Filamentous algae bloom in UFR mainstem, fall 2019
Source: S. Cope

Didymo filament masses are unlikely to be consumed by invertebrates due to their low nutrient value; however, the other algae also present in a fall 2018 Dry Creek samples were more valuable forage. Benthic invertebrate samples collected during and following the Dry Creek *Didymo/Ulothrix/Synedra* bloom at constructed, rip-rapped site LC_SPDC and at LC_DCDS showed increased *Chironomidae* and low *Simuliidae* abundance as the literature predicts, but % EPT samples were within the ranges observed in previous years (Ings et al. 2019). Microscope review of the Didymo mat samples found abundant chironomids, along with small rotifers and protozoa, none of which are preferred food for adult WCT, but may be utilized by juveniles.

In benthic invertebrate samples collected from the UFR mainstem during the Didymo bloom in September 2019, % EPT was below the normal range at RG_MP1 (rkm 58.5), and to a lesser extent at RG_FOUSH (rkm 57), unlike previous years. This decrease was driven by a decrease in % *Ephemeroptera* and % Plecoptera (Minnow 2020). Percent EPT was also lower than the normal range at RG_FOBCP (rkm 52) and likely at

RG_FOUEW (rkm 41). These samples were collected from sites that are within the estimated extent of the 2019 Didymo bloom (Figure 11, Figure A-32). We expect Didymo blooms to influence feeding habits of WCT large enough to utilize benthic invertebrates (James et al. 2012; 2016) but would not induce mortality and therefore could not account for the WCT decline.

A Didymo bloom can occupy a large portion of the stream column during low flows. Its filament mat can slow water velocity within the mat to less than the requirements for WCT age 1+ and 2+ (>1 – 22 cm/s) (Bennett 2004; CABI 2018; Cullis et al. 2013), effectively excluding WCT from habitat adjacent to the substrates.

The vast majority of a Didymo bloom biomass is composed of the mucopolysaccharide filaments. These senesce during the winter. Minnow field staff noted that Didymo filaments from the 2018 Dry Creek bloom were present but breaking down to black, stringy material in early December 2018 (Wiramanaden et al. 2019). Either the filaments were decomposing (an oxygen-consuming process), or black fines were adhering to the senescent filament masses. In both cases, Didymo mats would not enhance winter habitat values for any WCT age class. However, no reports of Didymo mats inducing WCT mortality were found.

Hypothesis 2$_{Q1}$: *What are the triggers and controls for periphyton and for filamentous algae blooms in the UFR?*

An integration of available light and flow-related factors (water velocity, shear stress, physical abrasion, and substrate characteristics) together with water temperature and chemistry is required to understand periphyton biomass and filamentous algae bloom development in all rivers including the upper Fording River. The factors controlling filamentous green algae or Didymo blooms and periphyton growth in UFR are summarized in Table 8. Their ranking by model averaging using available UFR data is discussed below:

Table 8: Overview of factors controlling the periphyton standing crop in the upper Fording River

UFR Periphyton control factor	Natural Influence	Mine-influenced
Light	Variable with riparian veg.	High due to minimal riparian vegetation
Critical flows and related factors	Precipitation-driven flows	Water use, watershed modification
TSS	Natural spates, freshet	Stormwater TSS from roads, construction
Phosphorus concentrations	Moderate influxes	Enhanced influxes
Calcite/bicarbonate/calcium	Regional geology	Influxes from spoil piles

Light: Available light is consistently ranked as a dominant driver, if not *the* dominant driver of periphyton communities in rivers (Schleppe et al. 2014; Olsen-Rusello et al. 2015). Most of the UFR recieves full sunlight so reduced light from shading is rare. Light measurement data were not available, however it is obvious that historic clearing of riparian vegetation from the mainstem UFR has maximized available light to support periphyton, and specifically, to meet the high light requirements of green filamentous algae. Light was therefore not assessed in the model averaging undertaken in this project.

Critical Flows: Periphyton biomass increases with water velocity until critical velocities are reached, after which detachment of the periphyton mat occurs. Most filamentous algae prefer near-bed water velocities around 0.05 m/s. Periphyton mat shear can be expected at velocities exceeding 0.30 m/s (Ahn et al. 2012; Biggs and Stokseth 1996). Removal rate due to elevated shear stress is influenced by the total amount of algal biomass and the condition of the periphyton mats. Thicker mats such as those produced by Didymo are predicted to be less susceptible to shear removal due to positive feedback on the near-bed hydrodynamics. Flushing flows provide an important control mechanism on periphyton growth and maintain the biodiversity of stream ecosystems (Biggs and Thomsen 1995; Jellyman and Harding 2016). Based on UFR flow data, summer/fall storms that occur in most years and the annual freshet can generate the velocities needed to shear filamentous green algae and likely Didymo blooms.

Suspended Solids and Substrate Stability: Researchers have identified that physical abrasion during periods of bed disturbance rather than simply elevated shear stress from increased flows is the primary control on accumulation and persistence of Didymo (Cullis et al. 2012, 2013). Therefore, periphyton is usually thickest on stones, cobbles, and gravel and thinnest on sand, clay, or organic material. Substrate pore sizes can shield filamentous periphyton against both hydrodynamic shear and mat tensile strength (Ahn et al. 2013). For these reasons, calcite armoring of substrates may also assist Didymo by reducing bed movement, however, the correlation between calcite index and Didymo was weak (R=0.09) because of a small sample size.

TSS: The model averaging regression technique applied to the 2015 UFR data demonstrated that TSS strongly influenced diatom abundance, including Didymo, likely through abrasion and shading from suspended solids (Figure 15, Appendix 1) (Ibisch and Borchardt 2002). The literature confirms the importance of TSS in restricting Didymo blooms (Bray 2014; CABI 2018; Cullis et al. 2012).

Nutrients**:** Of the reference nitrogen to phosphorus ratios presented in Table 9, DIN:TP best approximates the supply of N and P available to periphyton (Ptacnik et al. 2010). The UFR has high nitrogen concentrations at reference sites and especially at mine-influenced sites where DIN : TP ratios can exceed 1000:1 (Table A-21). Similarly, total phosphorus (TP) and readily bioavailable ortho-P were both in good supply at both reference and mine-influenced sites in the UFR (Table A-21). These ratios clearly show that nitrogen is in excess at both reference and mine-affected sites, leaving phosphorus as the important nutrient driver of primary productivity throughout the UFR.

Flowing water transports nutrients past cell surfaces, making nutrient guidelines or benchmarks less valuable than they are in lentic environments (Horner and Welch 1981; McDowell et al. 2009). In work elsewhere, water trapped within the mucilaginous *Phormidium* mat had on average 320-fold higher DRP concentrations than bulk river water, suggesting phosphorus release from entrapped sediment (Wood et al. 2015). Phosphorus inputs from mining have the potential to increase productivity in the UFR watershed, although phosphorus concentrations in the broader Elk River watershed are currently within the ranges observed among regional reference areas (Minnow 2020a). Nonetheless, overall primary productivity was enhanced at mine-influenced sites (Table 10; KW-Test p<0.001 for total abundance across all 2015 periphyton data).

Table 9: Generalized nutrient limitation ratios for streams compared to UFR sites, Jan 2012-Jan 2020

UFR	reference sites	mine-influenced sites	ratio descriptions (Ptacnik et al. 2010)			ratio descriptions (McDowell et al. 2009)		
			N-limited	N+P limited	P limited	N-limited	N+P limited	P limited
TN : TP	52 ± 119 : 1	907 ± 14818 : 1	<35 : 1	35 - 60	>60 : 1			
DIN : PO_4	47 ± 82 : 1	1482 ± 15620 : 1	< 16 : 1	>16 : 1	>50 : 1	< 7 : 1	7 - <15	> 15 : 1
DIN : TP	35 ± 101 : 1	866 ± 14788 : 1	<2 : 1	2 - 5	>5 : 1			

NOTE : field filtered PO_4~ SRP | 29 mine-influenced sites >300 samples | 2 reference sites >29 samples

NOTE: ratios based on median data, can be conducted site-by-site

Table 10: Selected periphyton metrics at reference and exposure UFR sites fall, 2015.

Periphyton metric	Statistic	Reference	Exposure	Significant difference
% dead:live	Mean (± SD)	0.10 ± 0.09	0.08 ± 0.1	N/A
	Median	0.09	0.04	
Chl-a mg/m^2	Mean (± SD)	19 ± 28	51 ± 76	N/A
	Median	7	13	
AFDW g/m^2	Mean (± SD)	13 ± 14	43 ± 76	No, p=0.49
	Median	9	7	
Abundance cells/cm^2	Mean (± SD)	$6.9 \times 10^5 \pm 8.6 \times 10^5$	$14.1 \times 10^5 \pm 20.3 \times 10^5$	Yes, $p<0.001$
	Median	391,000	668,000	
Biovolume µm^3/cm^2	Mean (± SD)	$4.4 \times 10^9 \pm 7.9 \times 10^8$	$5.8 \times 10^{10} \pm 5.0 \times 10^9$	Yes, p=0.005
	Median	2.0×10^8	2.9×10^8	

Notes: Sums from each 2015 replicate sample were calculated and statistics are based on the total values | these data are based upon the results from the entire 2015 periphyton study and include sites beyond the UFR | significant difference tests based on Kruskall-Wallis non-parametric tests of difference | low medians relative to means occurred because there were a few sites with large values (long-tailed histogram)

Calcite: Periphyton photosynthesis accelerates biogenic calcite precipitation by raising pH in the diffusive boundary layer, causing diatoms and cyanobacteria to become embedded in this calcite. Sites with more biogenic calcite also had higher overall periphyton productivity and frequently had higher filamentous algae productivity metrics.

Ranking UFR Periphyton drivers: Regression model averaging to capture a variable's overall effect was applied to the 2015 data set and it identified flows/TSS, calcite/bicarbonate/calcium, and phosphorus TP as the main drivers for periphyton abundance in the UFR (Figure 15). TKN, TOC, and DO also showed strong signals but because these parameters were directly affected by periphyton activity, they were not included in Figure 15. TSS was a very strong driver for diatoms (Didymo likely played a role in this by donating TOC). UFR cyanobacteria abundance was driven primarily by TP and by water temperature, and this finding agrees with their life histories and ecological preferences. Calcite was strongly involved in cyanobacteria abundance but also encouraged diatoms – both algae classes were seen embedded within calcite in 2015 UFR periphyton samples.

Timing: Finally, the timing of mine-influence and natural influences on the factors controlling algae blooms in the UFR is important to its periphyton standing crop (Table 8). For example, phosphorus is most stimulatory to the periphyton during the ice-free growing season, while high spate flows with elevated TSS will reduce the periphyton mat thickness. Dense filamentous algae mats can persist in winter, as was observed in Henretta Lake in Febuary 2020 (Figure 14).

Figure 14: Filamentous green algae in Henretta Lake under ice cover, February 2020.

Source: FRO u/w drone

Figure 15: Model averaged coefficients for periphyton classes by abundance and by biovolume

Notes: - Confidence intervals were calculated for each averaged coefficient and the results were plotted as points. The plot displays the average coefficient for each parameter fed into the model and is coloured by the type of periphyton. Points near zero and with confidence bars that cross the zero line were considered to have statistically insignificant effect on the abundance or biovolume or periphyton across the sites at FRO. Parameters with large coefficients were considered primary drivers. For example, phosphorus was flagged as a primary driver for cyanobacteria and a lesser driver for the other types of periphyton, an expected result given that many types of cyanobacteria can fix atmospheric nitrogen.

-For detailed statistical methods and how to interpret Model Average Results, see Appendix 1

2.2.5 Concluding Summary and Strength of Evidence for Impact Hypothesis 2:

The strength of evidence for Hypothesis 2_A is indeterminant and for Hypothesis 2_B is weak, due to limited periphyton sampling in the UFR and no sampling during the WCT Decline Window.

Anecdotal observations of filamentous algae blooms in late summer/early fall 2019 (outside the Decline Window) exceeded any previous observed extents in the UFR and exceeded extents in other regional rivers. Based on the fall 2019 photographs and S. Cope's observations, Didymo substrate coverage was extensive – visually estimated at 50%–75+% of the substrate surface in much of the UFR mainstem. Didymo had modest densities in 2012 and 2015 at the same sites, with the 2019 bloom likely triggered by stable flows with relatively low TSS, not nutrient concentrations. A similar bloom may have occurred in 2018, suggested by sediment organic carbon sampling and low TSS, but no periphyton monitoring occurred in that year. If a filamentous algae bloom did occur in 2018, it would have contributed to BOD/SOD oxygen demands in the 2018/2019 winter. More stable flows since 2013 likely encouraged the progressively enhanced algae growth witnessed in recent years (S. Cope pers. comm. 2020).

HYPOTHESIS $_{2\ Q1}$: *What are the triggers and controls for filamentous algae blooms in the UFR?*
Triggers and controls on filamentous algae blooms in the UFR in order of importance are: critical flows and suspended solids (TSS), calcite/bicarbonate/calcium and total phosphorus concentrations. Phosphorus is most stimulatory to the periphyton during the ice-free growing season, while high flows with elevated TSS during spates will reduce the periphyton mat thickness. The literature identifies optimal ranges of these parameters for filamentous algae blooms as: water velocity adjacent to substrates of 0.055–0.14 m/s, cobble or larger substrates, and dissolved P >30 – 50 µg/L SRP. Ironically, excessive stalk development in Didymo is triggered by decreasing dissolved reactive phosphorus, particularly below 4 µg/L (Jellyman and Harding 2016). Low bioavailable P may be a trigger for Didymo blooms but low TSS and stable low flows are a requirement. Thus, conditions in the decline window were conducive to a filamentous algae bloom.

HYPOTHESIS $_{2A}$: *Did filamentous algae blooms reduce hyporheic exchange, particularly during the decline window?*
Physical clogging of riverbed permeability due to a filamentous algae bloom can reduce downwelling seepage fluxes in slow flowing reaches by supressing hydraulic conductivity and hyporheic exchange. It is important to note that biogenically altered hyporheic exchange is a localized phenomenon that does not alter regional groundwater flows. Reduced hyporheic exchange due to bioclogging is expected to have the biggest impact during low flows (<~1 m/s) along the UFR mainstem, pools, and side channels in years between large freshets or floods, based on anecdotal observations of sediment and plant accumulations (S. Cope pers. comm.). A gradually reduced hyporheic exchange during late summer substrate bioclogging is a common occurrence regionally so is unlikely to account for or significantly contribute to the WCT decline.

HYPOTHESIS $_{2B}$: *Did filamentous algae blooms, such as Didymo, provide poor forage and degrade physical habitat (altered DO, pH, redox) for benthic invertebrates and WCT particularly in low velocity (<~1m/s) UFR reaches during the decline window?*
UFR sites with filamentous algae blooms can potentially affect alevins/young of year WCT through the summer rearing stage because these life stages live adjacent to periphyton mats. Diel oscillations in pH and dissolved oxygen during strong blooms can be harmful to fish while modest green filamentous blooms in pools such as Fish Pond Creek can benefit WCT habitat by providing forage for invertebrates.

Didymo blooms such as the UFR mainstem 2019 bloom can induce changes in the underlying hyporheic bacterial populations that in turn alter redox, DO, pH and dissolved organic carbon concentrations with repercussions to metal cycling. With the physio-chemical shifts that occur during a Didymo bloom, there is some evidence for a benthic invertebrate community shift towards chironomids in the UFR (Wiramanaden et al. 2019. A dramatic and widespread collapse of benthic invertebrate communities due to poor forage during a Didymo bloom would have to take place to directly account for the WCT decline, and this was not observed in the 2018 Dry Creek Didymo bloom nor in Didymo bloom research done elsewhere (Clancy et al. 2020).

There is some evidence for habitat degradation during a Didymo bloom that could exert stress in late summer and longer if a fall flushing flow does not occur. Therefore, if Didymo bloom occurred within the Decline Window, it could be a stressor affecting WCT health, but does not have the potential to be the sole cause the WCT decline.

2.3 Impact Hypothesis 3: Altered UFR bioreactor function impacts to WCT

Introduction to Hypothesis 3: Periphyton mats are very complex and dynamic. They are involved in promoting water quality improvement and they support the aquatic food chain as diagrammed in Figure 16. However, WCT life stages that feed on grazing benthic invertebrates could be adversely affected by periphyton entrapment of TSS, PAHs and adsorption of metals including selenium. Beneath the periphyton layer, hyporheic processes depend on a dynamic hyporheic zone driven by the exchange between oxic surface and underlying anoxic waters. Essentially a river is a linear bioreactor where water pushed through the substrate by downwelling, and upwelling can meet diverse bacteria/periphyton that mediate an array of chemical reactions. The complex array of river bioreactor processes is summarized in Figure 16 and diagrammed by season in Figure A-35.

Hypothesis 3 Questions:

A. *Did restriction of hyporheic exchange by periphyton entrapment of fines reduce habitat quality more than usual in the WCT Decline Window?*
B. *Did periphyton metal bioaccumulation and sulphate reducing bacteria (SRB)-mediated processes important to selenium or other metals cycling within the periphyton mat and the underlying hyporheic in the Upper Fording watershed have consequences to WCT during the decline window?*
C. *Did nitrogen transformations in the periphyton-influenced hyporheic zone affect UFR water quality and DO enough to have an impact on WCT in the decline window? For example, did a remnant of the Maxam wash water/ammonia spill (Feb 2019) or other mine-related nitrogen source accelerate downstream filamentous algae growth in late summer through fall low flows in 2019, particularly at S6 overwintering area?*

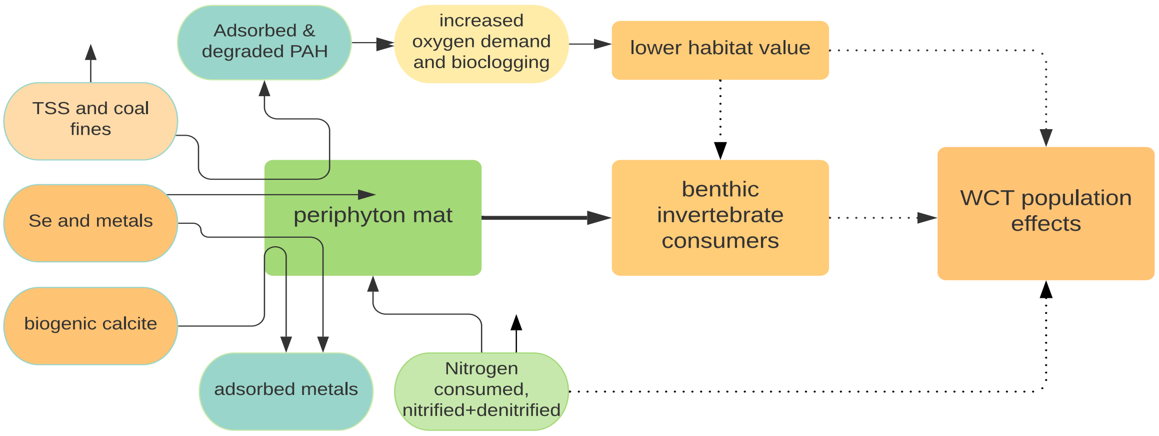

Figure 16: Conceptual effects of metals and PAH bioaccumulation and fines entrapment in periphyton and consequences to WCT investigated in this report

PAH-polycyclic aromatic hydrocarbons; Se-selenium. Arrows through periphyton mat box denote potential processing by periphyton components and associated biogenic calcite; upward arrow denotes volatilization. Thickness of arrows suggest importance of pathway.

2.3.1 Introduction to Bioreactor functioning of rivers

Like all rivers, periphyton coat wetted surfaces and support vast bacterial numbers within UFR biofilms. Beneath the periphyton layer, the interface of groundwater and stream water called the hyporheic zone also supports complex microbial biofilms that conduct a substantial portion of total river metabolic activity (Grimm and Fisher, 1984; Boulton 2007). The structure and activity of a river's microbial assemblages depends on the conditions set by oxic river water to sub-oxic groundwater exchanges within these dynamic hyporheic zones. Periphyton benefit from upwelling nutrients while hyporheic bacteria benefit from organic compounds supplied by the overlying periphyton biofilm in downwelling zones (Power et al. 2008). A river's spatially and temporally dynamic microbial population is concentrated in the upper hyporheic zone extending 10-15 cm into the bed sediments with a transition to suboxic conditions typically begins around 6 cm in uncalcified substrates (Duff and Triska 2000, 2011; Oram et al. 2010). In calcified substrates, the anoxic transition can be closer to the substrate surface (Wright et al. 2018). This redox zonation will oscillate with hyporheic flow patterns and with periphyton thickness patterns.

Periphyton biofilms and attached algae blooms such as filamentous green algae or Didymo secrete polymers (EPS) that encapsulate bed sediment grains and form filament mats that increase sediment cohesive strength (Gerbersdorf et al. 2008). Together they fill pore spaces, change hydrologic properties of bed sediments experiencing low flows, and divert pore water flow in ways that regulate microbial metabolism through redox-controlled cycling of nutrients and metals (Caruso et al. 2017). For example, friable biogenic calcite and biogenic calcite muds are expected to significantly lower hyporheic exchange in low velocity depositional reaches and will gradually accumulate between strong freshets (Boano et al. 2007; Saracho 2019).

Reduced hyporheic exchange occurs naturally through accumulation of fine material during low flow episodes; whereas flushing flows or exfiltration episodes reopen the interstices and reverse the process. Many human activities and hydrologic events cause interstitial sedimentation or disrupt surface–groundwater hydrological linkages, impacting ecological processes in the hyporheic zone. If this bioreactor function is impaired, it could induce localized DO/pH/redox/metal bioavailability close to the substrates with potential consequences to early life stages of WCT (not the life stages that are the focus of the EoC), but it could also create conditions where more metals are available in the water column. *For more information on water quality please refer to the water quality chapter Costa and DeBruyn 2021* ***of UFR WCT Evaluation of Cause SME report.*** These processes are unlikely to significantly affect UFR high volume flows but can affect shallow water in pools or slow-moving backwaters, especially during the low flow periods that frequently occur in late summer and through the winter. Parameters important to WCT mediated by hyporheic interactions include dissolved oxygen, nitrogen species, sulphate, metal species, and hyporheic exchange rates.

Polycyclic aromatic hydrocarbons (PAHs) in river sediments can undergo adsorption, volatilization, photolysis, and chemical oxidation, but transformation mediated by microorganisms is the major sink for PAHs. A wide variety of the periphyton aerobic bacterial, fungal, and algal taxa have the potential to adsorb/degrade/transform PAHs (Ghosal et al. 2016; Abdel-Shafy and Mansour 2016; Kanaly and Harayama 2000). PAHs sorbed to periphyton cells can still be bioavailable to grazers. Metabolism of PAHs occurs in some fish (e.g., carp, trout) and some benthic invertebrates (e.g., oligochaetes, chironomids, Gammaridae). Because of these processes, many researchers have confirmed that PAHs can undergo trophic dilution in aquatic food webs (Palmqvist et al. 2006).

Bacterial members of periphyton biofilms and hyporheic zones are critical to nitrogen cycling in rivers. Nitrogen is an essential nutrient but in excess, some common forms can induce toxicity as described below:

1. Nitrate can induce mortality in alevin cutthroat trout when it exceeds 2.3 – 7.6 mg/L NO_3-N (not a WCT lifestage impacted during the decline window).
2. Nitrite forms under anoxic conditions and is much more toxic to aquatic animals than nitrate, however, exposure is low due to fish exclusion from habitats where nitrite forms.
3. Ammonia occurs under anoxic conditions in two forms: the less toxic ionized ammonium ion NH_4^+, and the more toxic un-ionized ammonia NH_3. Above pH 9, NH_4^+ transforms to NH_3.
4. Nitrous oxide gas (N_2O) can form when there are adjacent oxic/anoxic conditions. As much as 25% of NH_4^+ may be converted to N_2O by nitrifying bacteria in winter conditions and can dissipate or build up under ice (Cavaliere and Baulch 2019).

The zonation and balance between nitrifying and denitrifying bacteria populations controls nitrogen cycling in the hyporheic zones of all rivers including the UFR. Nitrifying bacterial (Nitrosomonas, Nitrobacter) occupy the oxic substrates and convert ammonia through nitrite to nitrate ($NH_4+ + 2O_2 \rightarrow NO_3- + 2H+ + H_2O$). Nitrification consumes dissolved oxygen and can cause the build-up of intermediate products such as nitrite (NO_2^-) which can accumulate in fast-flowing aerobic small streams, particularly in summer (Smith et al. 1997). Despite low temperatures, nitrification rates in lentic ecosystems during winter are most strongly predicted by NH_4+ concentrations (Cavaliere and Baulch 2019). Bacterial nitrification processes consume ~4.6 kg of oxygen for every kg of ammonium ions oxidized to nitrate, far more than the 1 kg of oxygen required to oxidize 1 kg of carbonaceous organic material. Thus, nitrification can significantly impact oxygen dynamics, accounting for up to 50% of the hyporheic biological oxygen demand (Harvey et al. 2013; Cavaliere and Baulch 2019). Denitrifying bacteria capable of nitrate reduction occupy the sub-oxic hyporheic subsurface regions of rivers (Rivett et al. 2008). Denitrification ($NO_3- \rightarrow NO_2- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), is strongly linked with DOC, Mn, Fe and sulphide concentrations in substrate pore water (Kaplan and Newbold, 2000; Zarnetske et al. 2011; Broman et al. 2017; Jorgensen et al. 2008). Most bacterial denitrifying processes decline when water temperatures drop below 10°C.

The hyporheic zone acts primarily as a sink for N within NO_3-rich streams such as the UFR (Harvey et al. 2013; Danczak et al., 2016). In reaches with finer textured sediments (e.g., S6), downwelling slows and anoxic conditions can prevail, creating a nitrogen sink (Mazza et al. 2014) and the reduction of oxidized nitrogen species (Storey et al. 2004; Fischer et al. 2005). However, in coarse streambed sediment, available oxygen may not get used up by microbial respiration, allowing nitrate production and the hyporheic zone becomes a source of nitrate.

Sulphate reducing bacteria and archaea (SRB) are found in all anoxic aquatic systems where they mediate metal cycling (Martins et al. 2009) (Figure 2, Figure A-34, Figure A-35) by precipitating metals to less soluble forms (Lovley and Phillips 1994; Lloyd et al. 2001; Chardin et al. 2003). Removal of metals from solution by periphyton is consistently found to be dominated by adsorption (Almomani and Bohsale 2020) to cell wall functional groups that display electrostatic and chemical affinity for metal ions (Chen et al. 2019; Qu et al. 2019). Metals cycling in the periphyton mat and the underlying hyporheic zone affects interstitial water and to a lesser extent, the bulk river water. In most aquatic environments, SRB populations are controlled by available simple carbons and their activity by water temperature. Continuous intermittent exposure to oxygen and to nitrate and nitrite inhibits SRB activity (He et al. 2010).

Cadmium, copper, iron, lead, mercury, nickel, and zinc are some of the metals that will precipitate as metal sulfides. In addition, arsenic, antimony, and molybdenum form more complex sulfide minerals (Logan et al. 2005). Metals such as manganese, iron, nickel, copper, zinc, cadmium, mercury, and lead may also be removed to some extent by co-precipitation with other metal sulfides (Logan et al. 2005). Sulfate reduction also consumes acidity, raising pH. Increasing pH facilitates the above precipitation reactions and creates suitable conditions for precipitation of metal hydroxides (Gadd, 2004) and for calcite formation. SRB and other dissimilatory bacteria can reduce soluble selenate and selenite to nanoparticulate elemental selenium, reducing both Se toxicity and bioavailability. Thus, these bacteria have a major role in Se bioremediation. Precipitation can occur by an abiotic reaction with bacterially generated sulfide outside their cells (Hockin and Gadd 2003; Sarret et al. 2005).

Studies have demonstrated that biologically productive lentic habitats with reducing sediment conditions facilitate Se transport into those sediments, increasing exposure and enhancing uptake of Se by benthic organisms (Simmons and Wallschläger 2005; Orr et al. 2006). However, these processes are far more restricted in lotic environments such as the UFR mainstem that are important for WCT than they are in lentic sites that comprise only 7% of UFR watershed. In both environments, redox potential and bacterial activity in sediments drive the flux of Se between water and the food web.

Sulfate-reducing bacteria (SRB) and iron-related bacteria (IRB) are the important methylators of Hg in natural sediments. SRB transform Hg(II) to methyl mercury (MeHg) in hyporheic zones and can lead to accumulation of toxic MeHg in aquatic food webs. The dissolved neutral complex, HgS^0 and possibly some dissolved organic material (DOM)-complexed Hg is available for methylation while Hg bound to organic solids is not (Benoit et al. 2001; Mazrui et al. 2016).

Finally, periphyon mats provide extensive surface area for passive adsoption of metals during exposure to higher aqueous concentrations, particularly at mine-influenced sites and during low flows. Metals adsorption is universally seen in filamentous algae. Selenium often bioaccumulates from water onto particulate matter where it can then increase in macroinvertebrate tissues (Maher et al. 2010).

2.3.2 Requisite Conditions for Impact Hypothesis 3:

The following points lay out the requisite conditions that must be present before an impact to WCT from bioaccumulation and altered bioreactor functioning of the hyporheic zone would be anticipated:

REQUISITE CONDITIONS FOR HYPOTHESIS 3:

Requisite Spatial Extent	Filamentous algae or cyanobacteria blooms throughout the UFR, particularly WCT overwintering areas and possibly summer rearing areas, both in depositional and in erosional habitats would have to take place to meet requisite conditions**:** ***Yes.*** *(Based on extrapolation from extensive Didymo filamentous algae bloom on mainstem UFR observed in fall 2019 (Cope, pers. comm., 2020) and residual Didymo detected in Feb 2020 samples (this report Table A-19)*, and aqueous *Se exceedances detected at several mine-affected sites (Golder 2020a).*
Requisite Duration	Low flows would have to persist from summer through fall into winter that allowed a persistent bloom, with conditions in the 2018 growing season of greatest importance***: Yes.*** *(Based on FRO flow data (this report Figure A-37) showing no fall 2018 flushing flows, but no algae measurements or samples from 2017 – 2019 to confirm).*
Requisite Location	An algae bloom(s) would have to be persistent at key WCT locations including RG_MP1 (rkm 58.5), FR_FR2 (rkm 56) through S6 to FR_FR5 (rkm 36) to meet requisite conditions. ***No.*** *(by extrapolation from existing periphyton samples 2013 (Minnow 2014b), Sept 2015,* (White and Larratt 2016); Feb *2020 samples (this report); not all important WCT sites in UFR were likely to be affected, however, there were no samples from 2017-2019 to confirm).*
Requisite Timing	Growing season periphyton production and TSS accumulation not cleared by fall flushing flow (≥ ~2 – 5 m^3/s) would persist and decay in the winter. Winter 2018/2019 is of special concern within the Decline Window**: Yes.** (Based on extrapolating from existing periphyton and sediment data (DiMauro et al. 2021; Minnow 2020a, 2020b), and *on available hydrometric data and anecdotal observations).*
Requisite Intensity	Requisite intensity would require bioreactor-driven transformations where nitrogen (nitrate nitrite ammonia) maximum allowable concentration (MAC) guidelines were exceeded during low DO and/or DO <3 mg/L and/or significant Se or metals MAC exceedances detected in water samples**:** ***No****. (the Didymo bloom was not triggered by the Maxam spill. No unusual spikes in Se or MeHg or other metal forms arising from periphyton/hyporheic zones that could account for WCT decline were detected in water chemistry data) (UFR water quality data base).*
	Yes = requisite condition was met; No = requisite condition was not met

2.3.3 Data Gaps and Uncertainties for Impact Hypothesis 3:

Hyporheic field sampling to understand hyporheic exchange with groundwater was not available, necessitating extrapolation from shallow groundwater studies in the Greenhouse side channel conducted by SNC Lavalin (2020). Additional uncertainty also arises from infrequent periphyton sampling and infrequent tissue sampling for organic/inorganic percentages (indicates amount of algae vs sediment + calcite in sample), tissue metals and PAH associated with tissues. Similarly, the organic component of calcite and sediment samples were infrequently assessed (Table A-22), obscuring the role of periphyton in the UFR. The impact of biogenic calcite on hyporheic exchange rates during resumption of UFR low flows following freshet (minimal bio-clogging) versus hyporheic exchange rates in September low flows (maximal bio-clogging) are not known. ***For more information, refer to the calcite chapter Hocking et al. 2021 of UFR WCT Evaluation of Cause SME report.***

2.3.4 Discussion of Impact Hypothesis 3 Altered Bioreactor Functioning

A series of lines of evidence were pursued each bioreactor function hypothesis and are presented by hypothesis in the following sections.

Hypothesis 3A: Fines entrapment

All periphyton blooms present an array of surface areas for sorption and fines entrapment, allowing them to be effective nutrient, metals, and polycyclic aromatic hydrocarbons (PAH) bioaccumulators. However, periphyton palatability for grazing benthic invertebrates decreases with increased fines (Murdock et al. 2011; Parkhill and Gulliver 2002) through accidental ingestion and declines in nutritional quality as the proportion of inorganic material in the layer increases (Jones et al. 2011). Table A-25 summarizes the fines entrapment processes for common periphyton algae found in the UFR. This summary demonstrates that mechanical entrapment, adherence to the extracellular polymeric matrix, and mucopolysaccharide strands can occur in UFR periphyton mats.

Several sampling programs have been conducted in the UFR that provide information related to the contribution of periphyton to fines entrapment (Table A-25). Although these data were not collected during the Decline Window, they provide evidence that periphyton blooms together with TSS could lead to increased fines in periphyton mats relative to periods that had flushing flows. For example, ash-free dry weight (AFDW) was assessed as part of the 2015 periphyton evaluation. AFDW is elevated at sites receiving and retaining fine sediment and TSS (Figure 18). These results indicated that more fines were found in the periphyton mat from mine-affected sites than from reference sites. Most of these fines were identified as calcite by microscope assessment. Calcite ($CaCO_3$) forms on the mats induced by locally elevated pH during photosynthesis and is also trapped when upstream calcite is eroded.

Microscope observations of UFR periphyton samples collected in February 2020 demonstrated entrapment of fines that included organic detritus, calcite, and dark particulates (identified as coal fines based on color, size, light refraction), indicating a potential decrease in periphyton palatability (LAC 2020). More fines were observed in 2015 and 2020 Didymo mat samples from UFR than other UFR periphyton samples, likely due to reduced water velocity within the mat. Figure 17 shows photographs from Winter 2020 periphyton samples, with entrapped organic detritus and fines. Dark particulates were identified in many February 2020 periphyton samples. Most sites were ranked as low, but the following were ranked as moderate or moderate-high density: FOUKI, FOBKS, FOBSC, FR_CP1, FR_HC1, and FR_FC1. Sites known to receive coal fines include FOUKI rkm 55.5 through FR_CP1 rkm 52 (Minnow 2020). These dark particulates were likely coal fines based on comparison with coal dust samples - they ranged in size from 15 – 50 microns, which is within the range for coal dust (1 – 100 microns (Sapko et al. 2007) and they refracted light as coal dust does. These dark particulates were frequently associated with organic particulates and periphyton cells (Figure 17). Coal particles smaller than about 500 microns can float as a film on the water surface and would be removed from the UFR system if they do not become trapped in periphyton or sediment biofims (Ahrens and Morrissey 2005).

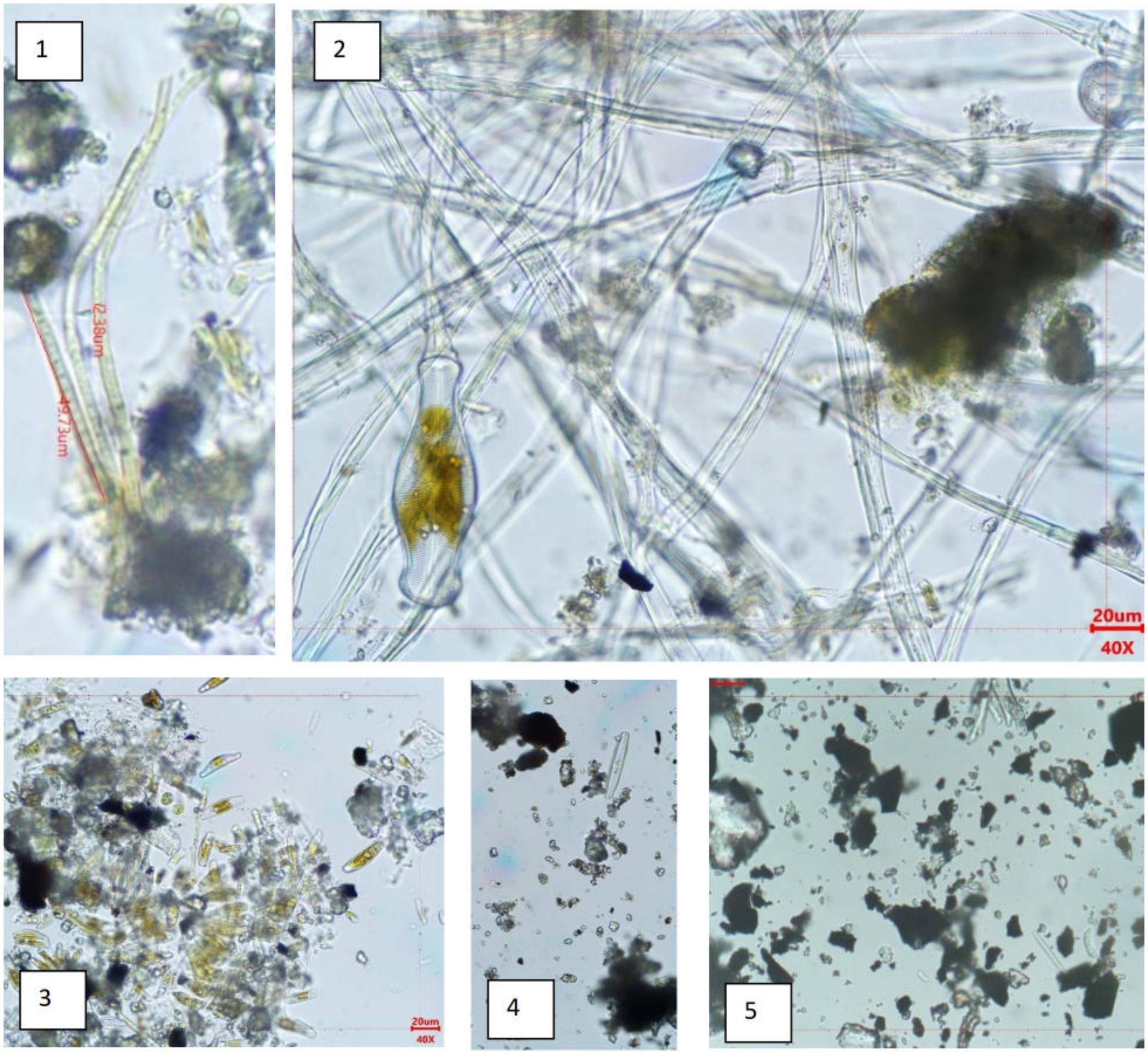

Figure 17: 1. Filamentous cyanobacteria 2. Didymo filaments 3. & 4. diatom mats showing entrapped organic debris, calcite, coal fines and fine silt in 5. Coal dust with traces of calcite or silt and diatoms

Minnow conducted benthic invertebrate community studies in 2017, 2018 and 2019. No exposure-response relationship was demonstrated in the benthic invertebrate data collected in 2019 from the sites with microscope-identified coal fines except for RG_FOBKS rkm 54, which had lower abundance metrics relative to preceding surveys (Minnow and Lotic 2020). The cause for this decline is not known. Since benthic invertebrates redistribute with drift, localized impacts from coal fines can be felt elsewhere, making impact evaluations difficult. Most effects to aquatic organisms from physical exposure to coal fines and particles in Columbia River surface water and sediments were the same as effects from other suspended sediments – scouring, abrasion, and shading due to TSS effects (Ahrens and Morrissey 2005).

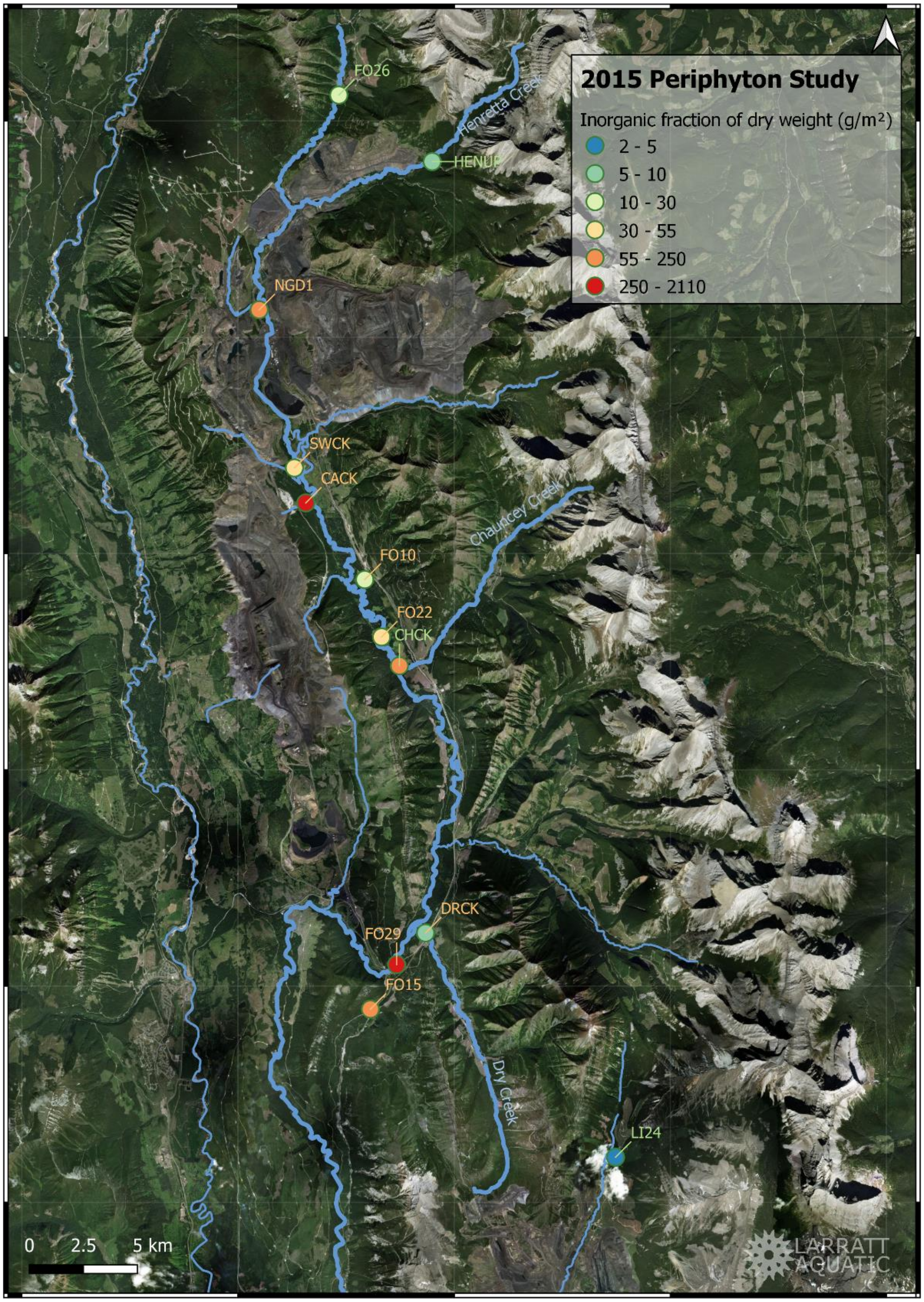

Figure 18: Inorganic fraction of periphyton dry weight (g/m²), a measure of bioclogging

Friable biogenic calcite and biogenic calcite muds are expected to lower hyporheic exchange in low velocity/flow depositional reaches (Boano et al. 2007; Saracho 2019) and can gradually deposit between strong freshets or floods. Because it is fragile, every freshet can remove this calcite and may help explain why calcite indices are stable at many UFR sites. Additionally, filamentous algae biomass can build up between large spates, potentially reducing hyporheic exchange rates (Gerbersdorf et al. 2008; Newcome et al. 2016). Both bio-clogging mechanisms can temporarily alter habitat values in discrete areas by restricting hyporheic exchange during long periods with stable low flows. In a UFR tributary study, sites with higher calcite index scores had lower dissolved oxygen in the substrate, compared to non-calcified substrates (Wright et al. 2018). Wright also confirmed that DO in the substrate decreased with higher % fines in the substrates. Finally, McCabe and Robinson (2020) concluded that UFR sites with high CI scores (>1 - 1.5) are likely to experience some reduction in hyporheic exchange. A gradually reduced hyporheic exchange through bio-clogging is unlikely to account for the WCT decline unless a water quality tipping point or threshold was reached in a parameter mediated by the UFR bioreactor functioning.

Calcite precipitates in mine-affected streams were enriched in cadmium, nickel, selenium and possibly zinc compared to calcite precipitates in reference streams (Day and MacGregor 2014). Of the six metals that exceeded lower sediment working quality guidelines in UFR calcite samples (As, Cd, Mn, Ni, Se, Zn), five of these (As, Cd, Ni, Se, Zn) were also common in UFR lotic sediment samples (Minnow 2020), in part because calcite mud (fragments/micrite/fine crystals) can accumulate in sediments through in-situ precipitation and erosion (Auerswald and Geist 2017; Turner and Jones 2005). This hypothesis is supported by very high calcium content of those sediments (Table A-18). By their nature, all depositional sediments accumulate metals, both at reference and at mine-affected sites.

In addition to using sediment guidelines, typical UFR sediment and calcite metal results can be compared to uncontaminated lake sediments from the region (Rieberger 1992). Interestingly, the range of uncontaminated lake sediments showed some exceedance of the lower working sediment quality guidelines (WSQG) for As, Cd, Cr, Cu, Pb, Mn, Ni, and Se (Rieberger 1992), indicating that this Southern Rocky Mountain Region is metal enriched (Table A-18). Cu, Fe, and Pb are among the metals that were generally *lower* in UFR lentic sediments than the range of uncontaminated regional lake sediments; As, Cr, Hg, and Ni were *within* the range of regional uncontaminated lentic sediments; and Cd, Se, and Mn *exceeded* this natural range in some mine exposed calcite and sediment samples (Table A-18). Calcite transported from the UFR by high flows can export embedded metals to downstream depositional areas where they can settle. Conditions in those environments will determine the fate of those metals and their potential to impact WCT.

PAH adsorption and decomposition

PAH concentrations were significantly higher in UFR mainstem sediments at mine-affected sites than at reference sites during 2018-2019 (KW-Test, $p<0.001$) and were strongly correlated with total organic carbon (Table A-22). In rivers withour forested riparian areas, periphyton is a critical source of TOC (Moore, 1998; USGS 2011). The absence of a fall flush in Fall 2018 may have increased the amount of periphyton TOC. Strong PAH sorption to organic carbon (periphyton) and microbial degradations of coal fines are initially high, and both mechanisms are key in limiting the bioavailability of the more toxic low molecular weight PAHs (Moermond et al. 2007). The low mwt PAHs expected to partition in algae were more than five times higher than the high mwt PAHs in the UFR mine-influenced sediment samples (Table A-22). Of the sampled sites, PAHs were highest at RG_FOUKI of the sampled sites – a site adjacent to a TSF and a rail loading yard (Schnurr et al. 2020; Table A-22) – and declined downstream, so that at RG_FRUPO (rkm 50) and RG_FO22 (rkm 44.5), the PAH concentrations were lower than those at the reference site RG_FO26 (rkm 69.5) ***Refer to section 5.5.1 from 2019 FRO LAEMP for more information.***

In general, 2018 and 2019 FRO LAEMP concluded that the highest sediment metal and PAH concentrations were not associated with locations where there were changes in benthic invertebrate communities (Minnow and Lotic 2020, Schnurr et al. 2020; Ings et al. 2018). Similarly, sites with PAHs in exceedance of sediment quality guidelines were not associated with measurable effects on benthic invertebrate communities at Fording River sample sites in 2017 and at most sample sites in 2019 (Minnow 2018, 2020). Data from sites with elevated PAHs that did not show an effect on the benthic invertebrate populations provides one indirect line of evidence indicating that organic substrate PAHs are not the sole cause of the WCT decline. ***Refer to coal dust and sediment quality chapter for more information DiMauro et al. 2021 of UFR WCT Evaluation of Cause SME report.*** Alevins and juvenile WCT using sites receiving high aqueous PAH concentrations and coal fines (e.g., FOUKI rkm 55.5) would be most affected of the WCT population. Spawning and alevin WCT could be affected by lower interstitial DO resultant from PAH break-down at a few UFR sites, but for the most part, these vulnerable life stages would not be found in substrates susceptible to DO depletion. Further, a decline among early life stages is inconsistent with the WCT decline. Overall, the oxygen demand of decomposing/degrading coal fines is expected to be small compared to biochemical oxygen demand (BOD) from decomposer flora in the periphyton biofilm and chemical oxygen demand (COD) from ammonia transformations. These PAH oxygen demands would be continuous and therefore unlikely to cause or be a significant contribution to the WCT decline.

Hypothesis 3B: UFR Bioreactor - Selenium and Nitrate

Periphyton microorganisms play a pivotal role in the selenium cycle in the environment by performing both oxidation and reduction reactions (Nancharaiah and Lens 2015). Se removal via adsorption to (not uptake into) periphyton is likely another important bioremediation process in the UFR. For example, UFR sediment metal concentrations correlated weakly with TOC despite multiple other influences (Table A-22; Pearson's R=0.631 for Se : TOC), possibly indicating adsorption to periphyton. Bioconcentration of Se in periphyton biofilms is widely observed (Cianciolo 2019; Conley et al. 2009). Below the periphyton biofilm, downwelling hyporheic exchange transports solutes into the subsurface where they can undergo further biogeochemical transformations. Microbial reduction of nitrate and selenium in suboxic zones (including deep and shallow groundwater) is strongly supported by literature (Martin et al. 2019; Dockrey et al. 2015; Hockin and Gadd 2006). Reduction in the hyporheic zone promotes sequestration of surface water Se (Oram et al. 2010) because hyporheic downwelling brings stream water into close contact with the reactive surfaces of sediment grains and periphyton (Boano et al. 2010; Winter et al. 1998). Shallow groundwater samples collected between a side channel and the main UFR channel (rkm 51) in winter 2019/2020 by SNC Lavalin showed that a significant reduction in dissolved Se occurred in samples RG_FRDP5 and RG_FRDP8 (Table 12), indicating localized biogeochemical removal. Anoxic conditions conducive to Se and nitrate removal may be more likely to occur in the meandering areas downstream of S6 (rkm 44.5 to 36).

Within the seep/shallow groundwater adjacent to the UFR that could be representative of hyporheic conditions, concentrations of lead and zinc were below levels known to inhibit SRB. Shallow groundwater at the two anoxic sites had much lower nitrate+nitrite concentrations than at upgradient, seep or side channel sites (Table 11), possibly indicating local nitrification/denitrification. However, these nitrogen concentrations may still exceed concentrations known to inhibit SRB (He et al. 2008; Korte et al. 2014). Thus, nitrate has the potential to inhibit selenate reduction in the UFR. Nitrate inhibition could be an important restriction on periphyton bacteria-mediated selenite removal in the UFR hyporheic zones (Table 11).

Table 11: Nitrate (mg/L as N) in shallow groundwater samples adjacent to UFR

Site Type	Mean	SD	Max	Min
Seep (n = 12)	43.18	6.77	50.90	34.00
Shallow anoxic groundwater (n =2)	14.61	10.64	32.20	3.44
Side Channel (n= 6)	42.30	5.76	49.70	37.40

Source: SNC Lavalin 2021

The bioreactor functions of the Fording and Elk rivers result in parallel selenium and nitrate removal via reduction in sub-oxic groundwaters, including near-surface groundwater in side-channels, hyporheic zones, and in coal piles and tailings impoundments (Martin et al. 2011; Lorax 2009; Shaw and Day 2019). The instream sinks (zones of loss) removed between 5% and 10% of the incoming selenium and nitrate mass and were active between September and April. One instance of 40% Se and NO_3 removal occurred in a year-round assessment of the sink in the Elk River upstream of the Fording River (Martin et al. 2019). These results support the hypothesis of biologically mediated removal of both selenium and nitrate in localized anoxic zones within shallow and deep groundwater.

Of the shallow groundwater samples collected by SNC Lavalin in winter 2019/2020, two samples showed field conditions that could be suitable for SRB (RG_FRDP5, RG_FRDP8). Dissolved metals of interest affected by anoxic biogeochemistry at these drive-point sites include:

- Increased Metals: D-Fe, D-Mn, D-As, D-Ni, and D-Zn
- Decreased Metals: D-Cr and D-Se

These changes in metal distributions agree with their expected anoxic chemistries (Table 12).

Table 12: Shallow groundwater, seeps and side channel drive-point samples (rkm 51 UFR)

Parameter (µg/L)	Oxic (>7 mg/L DO)		Anoxic (<0.5 mg/L DO)		Intermediate	
	Mean	SD	Mean	SD	Mean	SD
Dissolved.Arsenic	<0.10	-	0.5400	-	0.1350	0.0212
Dissolved.Cadmium	0.0580	0.0170	0.0532	-	0.1000	0.0990
Dissolved.Chromium	0.1575	0.0177	-	-	0.1400	-
Dissolved.Copper	0.2600	0.0141	-	-	0.3600	-
Dissolved.Iron	<10	-	5890	-	171	-
Dissolved.Manganese	0.2378	0.0986	2300.0	-	279.0	386.1
Dissolved.Nickel	<0.50	-	5.6600	-	2.4100	-
Dissolved.Selenium	147.7	29.0	25.0	-	94.5	38.9
Dissolved.Uranium	4.9195	0.8549	0.2800	-	2.9400	2.0365
Dissolved.Zinc	1.7500	0.3416	4.7000	-	2.0000	-

Based on field DO: Oxic = 18 samples, anoxic = 1 samples, intermediate = 2 samples : all field ORP were >300 mv : pH 7.9-8.3
Total and Dissolved Hg mercury was not detected (<0.005 µg/L) in any sample. (DATA FROM SNC LAVALIN 2021)

Green algae bloom tissue samples collected May 2016 from the Line Creek AWTF Buffer Pond were composited and showed metal concentrations within the ranges found in UFR sediment and calcite [Cd (1.1 µg/g), Ni (43.5 µg/g), Se (25.7 µg/g), As (3.0 µg/g), Cr (15.4 µg/g), Pb (2.4 µg/g)], with the exception of Mn (1960 µg/g) and Fe (80500 µg/g) (Table A-31, Table A-32). These results indicate that the lower layers of this filamentous green bloom harbored anoxic conditions despite active photosynthesis in the upper layers. We expect similar microlayers with in the periphyton mat throughout the UFR (Figures 1,2), with beneficial consequences for water quality.

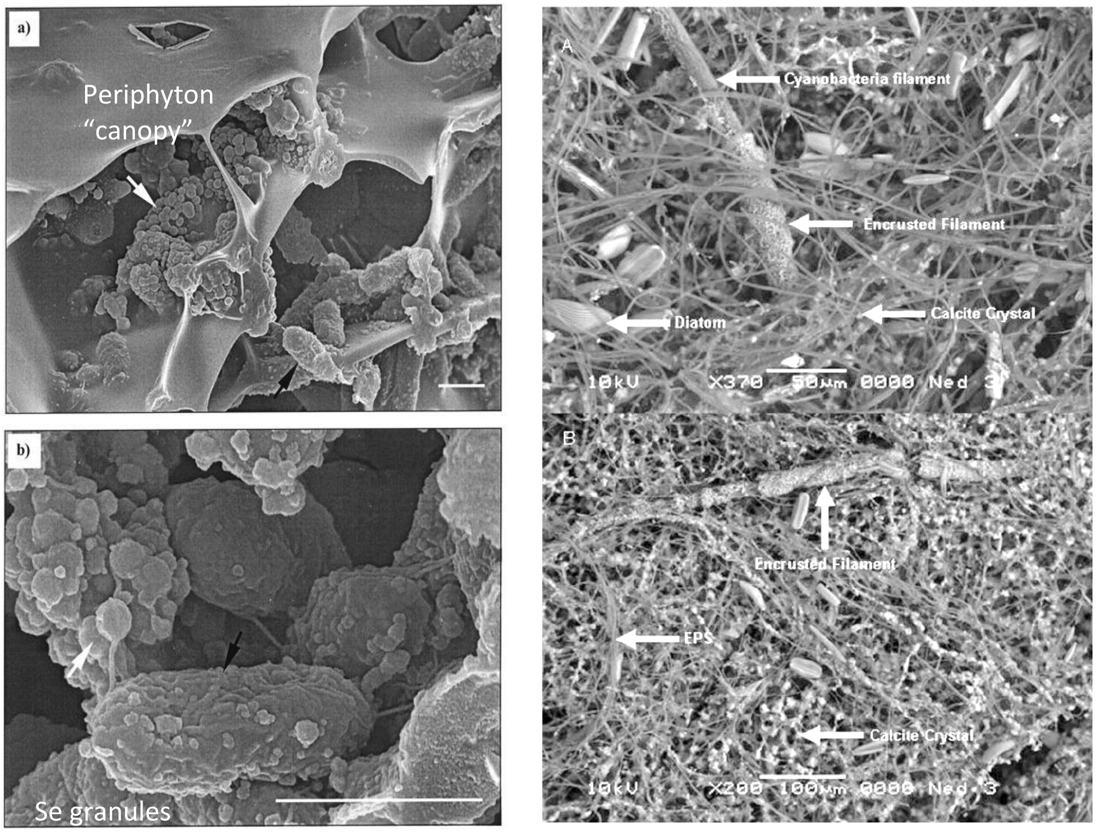

Figure 19: Occurrence of SRB and selenium granules within a periphyton canopy and calcite-embedded cyanobacteria, bacteria, diatom periphyton structure, Case Study – Dry Creek 2018
From: Hagerthey et al. 2011

A 2018 filamentous bloom at the Dry Creek tributary provided a unique opportunity to investigate selenium behavoir. Selenium often bioconcentrates from water onto particulate matter where it can then increase in macroinvertebrate tissues in mine-influenced streams (Maher et al. 2010), as it may have in Dry Creek (Minnow 2020c) and in larger studies of the Elk Valley (Orr et al. 2006; Wiramanadan et al. 2019; DeBruyn and Chapman 2007; Lemly 1993; Janz et al. 2010).

There were two significant changes that occurred in the UFR Dry Creek site in 2018. First, an increase in aqueous Se and NO_3 concentrations was observed at Dry Creek monitoring stations (Minnow 2019). The typical range for aqueous Se abruptly increased to 12-25 µg/L T-Se in 2018 compared to the typical 1-2 µg/L in earlier sampling. Second, a heavy Didymo/Ulothrix filamentous bloom occurred in Upper Dry Creek immediately below the sediment ponds (constructed in 2015) in late summer through fall 2018 and it persisted into winter 2019. This bloom was likely triggered by low flows with low TSS.

Invertebrate tissue selenium exceeded level 3 benchmarks at LC_DCDS and were above level 1 benchmarks at LC_DC1 (3 – 30 µg/g DW Se) (Table A-24). Further, selenium concentrations in Dry Creek periphyton biofilms were comparable to periphyton concentrations that adversely affected mayflies in research elsewhere (Conley et al. 2009). However, Minnow (2019) did not detect lower invertebrate density in their sampling although SPDC, DCDS, and DC2 periphyton had elevated tissue Se concentrations. Additional sampling conducted in late November 2018 and in February 2019 confirmed the September

2018 benthic invertebrate results. In this sampling, algae tissue Se samples were also collected. The periphyton T-Se range was 9 - 30 µg/g DW Se within the known Didymo/Ulothrix bloom at Upper Dry Creek (LC_SPDC LC_DCDS downstream of settlement ponds) and 6 - 12 µg/g DW Se at Lower Dry Creek sites (LC_DC4/LC_DC2a , LC_DC1) with no visible algae bloom. LC_DC1 is utilized for spawning based on observed redds but low overall WCT use (Cope et al. 2016).

When the Dry Creek Didymo bloom samples from Nov 2018 and Feb 2019 are compared, periphyton tissue Se concentrations appeared to increase over time[9]. Metal adsoprtion to bloom filaments may have caused the increased average periphyton tissue Se from 11 to 16 µg/g DW Se at LC_SPDC and from 12 to 20 µg/g DW Se at LC_DCDS (Table A-24). Alternately, these result may be anomolous (table A-34). Increased adsorption of aqueous metals to periphyton tissues during decomposition is frequently observed in aquatic environments because decomposition increases algal tissue surface area.

Trophic Transfer Factors (TTF) were calculated for the UFR Dry Creek 2018/2019 Se samples and were compared to published TTFs in Table A-23 (Presser and Luoma 2010). Average TTF for periphyton to benthic invertebrates range from 0.9 to 3.2 in high-Se streams (Table A-23), which is comparable to the TTF calculated from the 2018 Dry Creek samples from the bloom areas (LC_SPDC and LC_DCDS) of 3.57. In the Lower Dry Creek sites where a Didymo bloom was not observed, a TTF of 1.19 occurred. Despite the elevated tissue selenium found in the Dry Creek benthic invertebrate tissues and observed changes in water quality and calcite, benthic invertebrate biomass was similar in Upper versus Lower Dry Creek in 2018 (Minnow 2019) (Table A-22). The observed altered Se effect in Upper Dry Creek was localized to that small area and did not affect Lower Dry Creek so this bloom would not be expected to have impacts that extended to UFR mainstem WCT population.

The habitat changes caused by the Dry Creek Didymo bloom likely involved low flows-driven lower DO, redox and pH within the periphyton mat and these may provide a potential mechanism of increased Se uptake through altered Se cycling (Lorax 2009; Dockrey et al. 2015). For example, low DO <0.5 mg/L /low redox would favor anoxic bacteria populations such as SRB that play a pivotal role in selenium methylation. A low DO regime also supports bacteria involved in reducing selenate to selenite, which is followed by selenite sorption and reduction. These bacteria are known to inhabit hyporheic zones in the UFR, regional shallow groundwater and Elk Valley waste rock (Deen et al. 2018; Shaw and Day 2019).

The sum of these biochemical effects was either coincident with the increased Se uptake at Dry Creek, or more likely, the Didymo bloom directly or indirectly increased Se cycling. The Didymo bloom observed on the mainstem UFR in 2019 may have had a similar effect. If a UFR mainstem Didymo bloom occurred in the 2018 growing season, it could have increased Se trophic transfers within the decline window. Since Dry Creek invertebrate biomass was not significantly affected, we can assume that loss of food organisms for WCT due to an increased Se burden is not a potential contributor to the decline. Similarly, since the trophic transfer factor for WCT is 1 (Pressor and Luoma 2010), increased Se in invertebrates is not expected to biomagnify in WCT. While a potential for increased Se stress to WCT in the event of a mainstem Didymo bloom exists, this pathway does not provide a viable explanation for the observed WCT decline.

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[9] Very small sample size prevented statistical analysis of this trend; this event may have been an anomaly

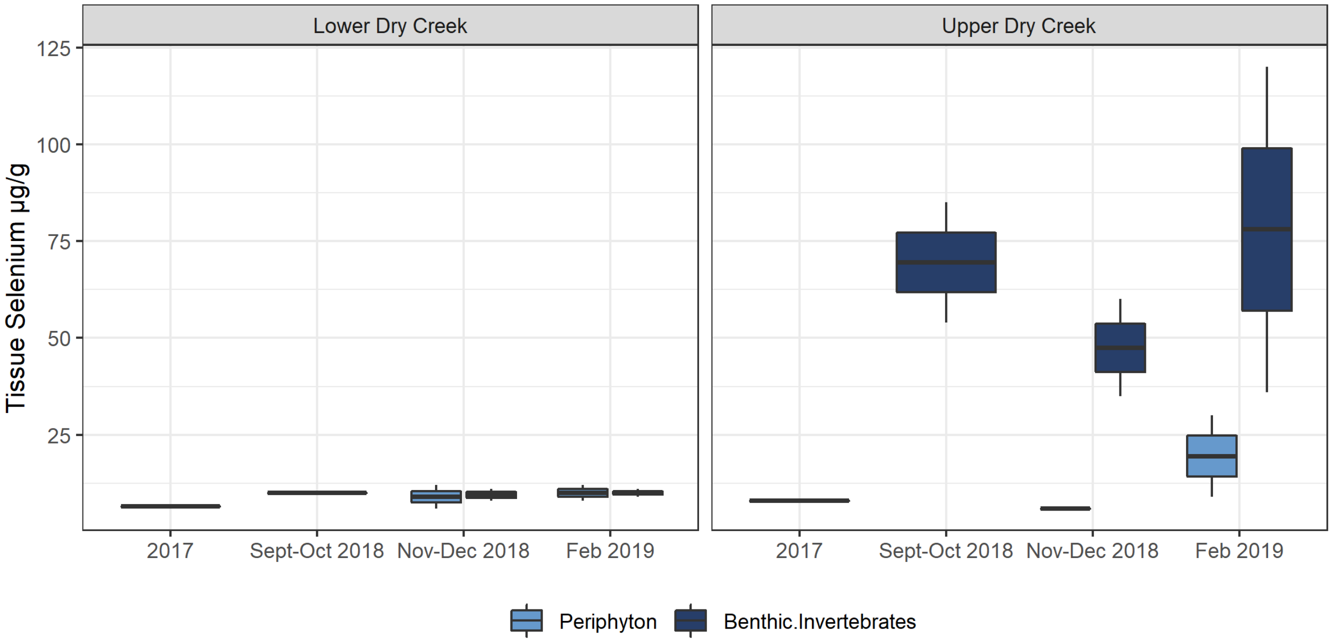


Figure 20: Periphyton and benthic invertebrate tissue selenium in Lower Dry Creek with no bloom and Upper Dry Creek with a Didymo bloom. Data period: 2017 - 2019

Note: In some cases there were few data points and boxes have minimal vertical extent, on the same time period the orientation in the legend will be the same as within the plot (i.e. periphyton is the left box and invertebrates is the right box) Source: Wiramanadan et al. 2019; Dry Ck LAEMP)

Hypothesis 3C UFR Bioreactor - SRB

Sulfate-reducing bacteria (SRB) are ubiquitous in the deep anoxic biofilm layers, but they can also thrive in anoxic microniches within oxidized periphyton layers where concentrations of dissolved selenium species are generally higher (Oremland et al. 1989; Measures and Burton 1980; Teske et al. 1998; Ramsing et al. 1996). SRB growth requires sulphate, and in the Elk Valley, sulphate loads result mainly from weathering processes in spoils. Many biofilm-forming SRB such as *Desulfomicrobium* can rapidly remove significant quantities of selenite and sulphur from solution (Hockin and Gadd 2003; Baines et al. 2004; Riedel et al. 1991; Simmons and Wallschlager 2011). Elemental selenium and elemental sulfur are precipitated outside SRB cells but within the biofilm matrix as immobile and low toxicity orange Se^0 + S granules through a reaction with bacterially generated sulfide, as opposed to enzymatic reactions within bacterial cells (Hockin and Gadd 2003). The underwater drone footage of Henretta Lake substrates showed what appear to be SRB upwellings with the typical grey-black transition metal sulphide coloring, as well as bright orange upwellings that suggest Se^0 + S granules (Figure 21; White and Gadd 1998; White and Gadd 2000). This SRB process can be an efficient method for the removal of toxic concentrations of selenite from solution (Hockin and Gadd 2003). Table A-28 shows a link between heavy Didymo growth and high bacteria (type not known) densities in the September 2015 samples. These bacteria will take advantage of the structure and organic carbon provided by the Didymo mats, and they can moderate numerous reactions relevant to water quality (Figure 19).

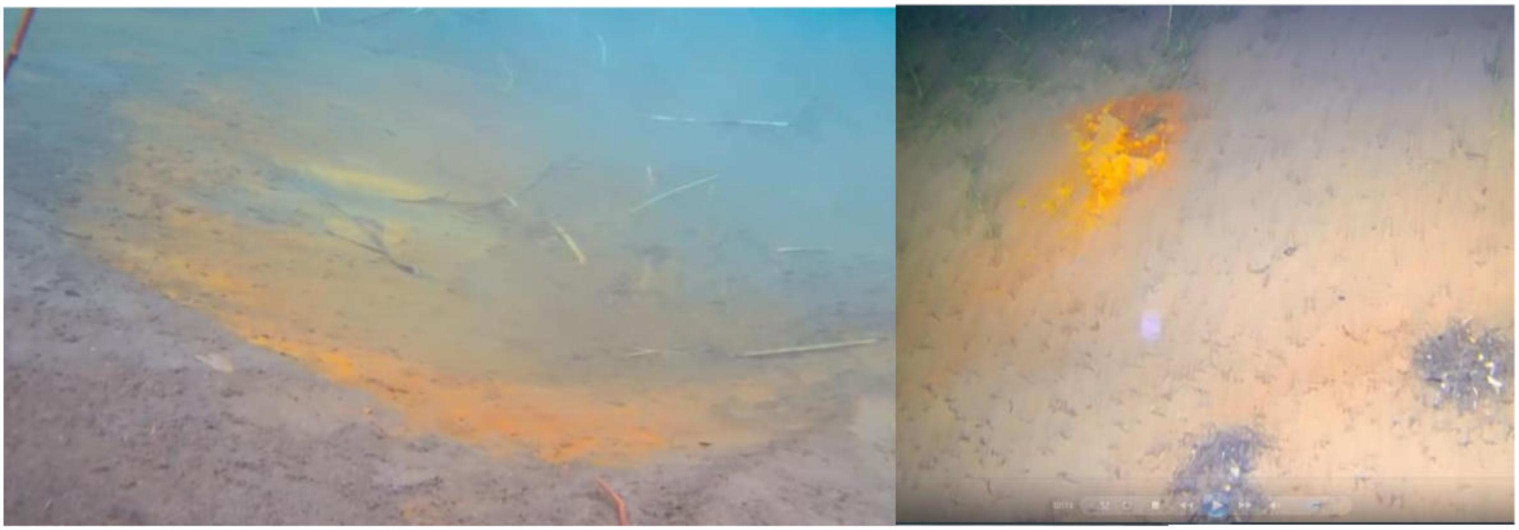


Figure 21: Possible deposits of elemental selenium in S6 pool (left) and In Henretta Lake (insert), winter 2020
Source: FRO staff underwater drone video

The UFR water quality data base also included methylated mercury (MeHg) analyses – a compound produced by SRB. Samples above the detection limit (0.00002 mg/L) were found at 17 sites in the 2017 – 2019 window (only at exposure sites). These sites showed intermittent detectable MeHg, mostly in May and all between April and July - the high freshet flow months in the UFR, and these detections were correlated to TSS. Therefore, SRB (and possibly IRB) activity is sufficient to cause detectable MeHg. Intermittent MeHg detection likely occurs because of variable upwelling from the hyporheic zone into the UFR.

The maximum concentration of MeHg in the larger 2015 – 2018 Elk Valley dataset for receiving environment locations (n= 480) was 0.00035 µg/L, which was more than an order of magnitude below the 0.004 interim CCME guideline (Hill and Turner 2019). There were only 6 of 5768 water samples at receiving environment locations from the Elk Valley (2015 – 2018) that exceeded the 0.026 ug/L T-Hg CCME interim guideline (Hill and Turner 2019). Based on these results, Hill and Turner concluded that the potential for direct toxicity to aquatic life was negligible. Shallow groundwater wells that approximate hyporheic water chemistry were analyzed for total and dissolved Hg, not MeHg (SNC Lavalin 2020). There were no patterns in total and/or dissolved Hg and most of the detections occurred in wells within the Kilmarnock area (S. Humphries pers. comm. 2020). However, no samples from shallow groundwater near the UFR mainstem exceeded the detection limit of <0.0050 µg/L D-Hg (SNC Lavalin data set. These data demonstrate that the benefits of SRB (sequestering of metals, bioremediation of PAHs) are not compromised by their adverse methylation of mercury in UFR. Thus, SRB activity cannot contribute to the WCT decline and instead, they contribute to the bioreactor functioning of the UFR.

Of the key bioreactor-mediated parameters, only SRB-mediated mercury did not deviate from typical ranges at UFR mainstem sites during the decline window (Table 13). Sulphate, selenium, nitrate and ammonia showed higher maxima and averages with increasing trends during the decline window. Notably, sulphate and selenium showed higher averages above the Level 1 benchmarks with an increasing trend. These water quality changes are likely attributable to the effects of stable low flows on dilution and to some degree on lower bioreactor functioning. These water quality shifts were likely not large enough to cause the WCT decline. *For information on metals and fish health, refer to **pathophysiology chapter** (Bollinger 2021). For more information on metals and benthic invertebrates, refer to **food availability chapter** (Orr and Ings 2021); refer to **water quality chapter for more screening information** (Costa and de Bruyn 2021); all in **UFR WCT Evaluation of Cause SME report**.*

Table 13: Selected water quality results at mainstem UFR sites in decline window (Sep 2017 – Sep 2019) compared to 2012–2019

Hyporheic exchange mediated constituent	Units	% analyses above detection in WCT decline window	Comparison to WQGs or Level 1 Benchmarks and Screening Values	Mainstem UFR Stations (% of stations)			
				No. of Stations	Higher Maxima?	Higher Average?	Increasing Trend?
Sulphate	mg/L	100%	Above Level 1	14	86%	69%	29%
Nitrate	mg-N/L	99%	Above Level 1	14	29%	15%	14%
Nitrite	mg-N/L	70%	Above Level 1	14	29%	0%	0%
Total Ammonia	mg-N/L	83%	Meets WQG	14	86%	71%	71%
Total Mercury	mg/L	34%	Meets WQG	14	0%	0%	0%
Total Selenium	mg/L	100%	Above Level 1	14	57%	79%	36%

Summarized from Costa, EJ., & de Bruyn, A. 2021 Appendix G

UFR Bioreactor - metals

In addition to concerns over Se, SO_4, NO_3, and Cd, metals of interest in UFR include Al, As, Cr, Cu, Co, Fe, Pb, Mn, Hg, Ni, U, and Zn. Of these, Al and Hg are not related to mining, while Co, Fe, and U are (EMC 2019). Periphyton in UFR can affect metal bioavailability to aquatic animals through numerous interrelated pathways involving complexing to organics, adsorption to periphyton surfaces, bacterial transformations, redox processes and chelates. We expect these processes are active within the UFR in dynamic flux driven by periphyton productivity, redox and pH, all of which are influenced by flows.

In the 2018 UFR sediment data (Table A-18; Minnow and Lotic 2019), strong correlations between TOC (periphyton, dead or alive (Krenz et al. 2018)) and metals of interest, including manganese (R=0.71), selenium (R=0.63), and cadmium (R=0.55), suggest the possible role played by periphyton in aqueous metal capture (Pearson's correlation). A similar periphyton role is likely played within calcite matrices, but since TOC was not analyzed in the UGHC Calcite project, this can only be inferred from literature studies of metal-periphyton-calcite interactions. Sediment metal concentrations were comparable between years at each area, except for selenium and zinc at RG_FOUKI, which were higher in 2018 compared to 2017 (Schnurr et al. 2020).

Periphyton biomass will drop after metal tolerances of its community members are exceeded (Mebane et al. 2020). Although metal-driven shifts in periphyton communities are possible, extensive losses of productivity have not been observed in the UFR in either anecdotal evidence or FRO/LCO Dry LAEMPs (Minnow and Lotic 2019; Minnow 2020b). Periphyton can be more sensitive to metals such as Fe Cu Zn than benthic invertebrates, resulting in invertebrate declines only after periphyton available for grazing is reduced (Cadmus et al. 2017). The absence of periphyton die-offs at UFR sites with elevated metal concentrations indicates that periphyton metal tolerances are not exceeded in the UFR.

UFR Bioreactor - nitrogen

Periphyton biofilm layers are important to nitrogen removal from N-rich streams such as UFR because they provide carbon to fuel hyporheic bacterial activity (Böhlke et al. 2009; Hubbard et al. 2010; Zarnetske et al. 2011). SNC Lavalin used drive-points to study the shallow groundwater and seeps between a side-channel and the main channel of the UFR (rkm 50–51) in winter 2019-2020. Upgradient deeper groundwater ranges from ~10 – 70 mg/L TN as N, some of which would be discharging to the UFR hyphoreic/shallow groundwater zone (S. Humphries pers. comm. 2020). Mine-influenced reaches of the UFR averaged from 0.16 mg/L TN as N upstream of mine impact to 19.0 mg/L TN as N downstream at FR_FRABCH (rkm 43), most of which was nitrate (2019-2020). Greenhouse side channel drivepoint hyporheic water averaged 42.3 ± 5.7 mg/L TN as N (Figure 22; SNC Lavalin data 2020). The most anoxic groundwater sites showed significantly less nitrate and only a small increase in ammonia, indicating bacterial nitrogen removal (yellow arrow: *refer to groundwater chapter of **UFR WCT Evaluation of Cause SME report** for isotope data (Henry and Humphries 2021).* The fact that anoxic conditions were detected in only two drive-point locations suggests that the nitrate removal is confined to a local scale (S. Humphries pers. comm. 2020). In contrast, the UFR hyporheic zone acts as a sink for nitrogen on a large scale (Martin et al. 2019). Thus, nitrogen inputs from mine-affected areas are counterbalanced to some extent by the nitrogen removal capacity of the UFR.

Nitrogen cycling in the UFR watershed is also important because it can inhibit selenium reduction, remobilization and attenuation (Dockrey et al. 2015). These bacterially-driven processes occur in sub oxic environments (Dockrey et al. 2015) such as the base of the periphyton mat and portions of the hyporheic zone (refer to Figure 2). Thus, in UFR, selenium and nitrate are biogeochemically intertwined, although the scale and magnitude of such interactions are uncertain (Dockrey et al. 2015). Most of the reactions that benefit water quality occur under anoxic conditions, while suboxic conditions where nitrate is available may promote Se oxidation and mobility (Dockrey et al. 2015; Bailey et al. 2012).

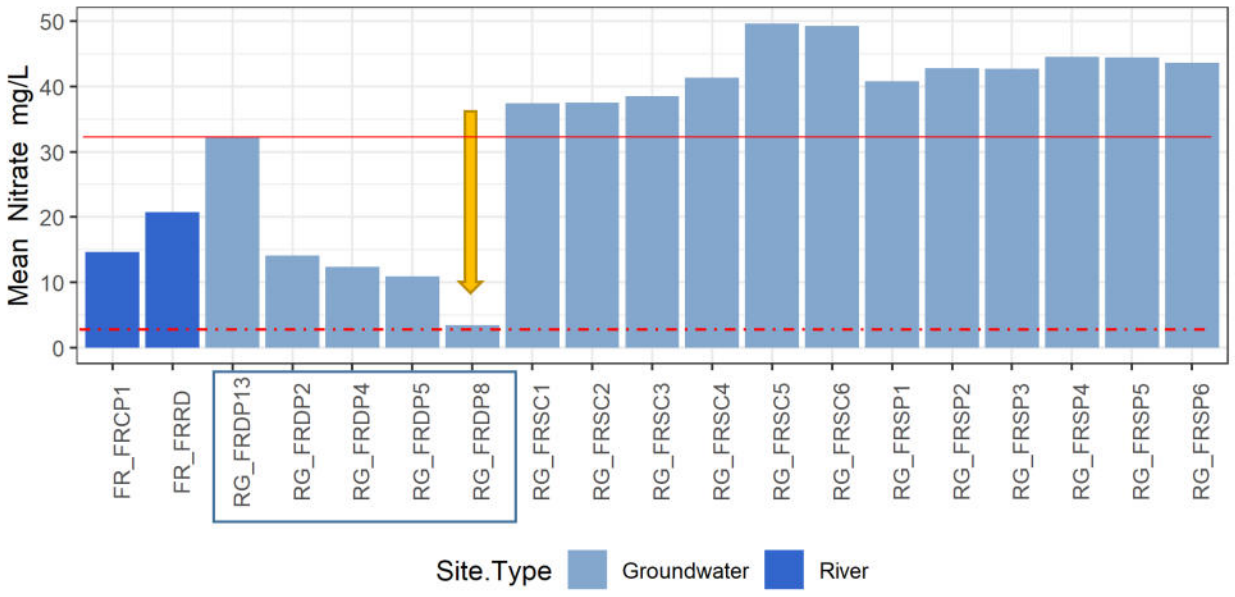


Figure 22 Mean nitrate in the UFR and in adjacent shallow groundwater and seeps (~rkm 50 - 51)

(Data from SNC Lavalin 2020) Note: solid red line = BC MAC guideline for aquatic life, dashed red line = BC 30-day guideline for aquatic life – these are provided for reference, as they do not apply to groundwater; yellow arrow denotes most anoxic groundwater site showed greatest nitrogen removal; box denotes drivepoint samples

At some of the key WCT mainstem sites, nitrate and nitrite show opposing patterns where sites with elevated nitrate had lower nitrite (Figure A-42). These results indicate adequate oxygen to support nitrification in possible combination with limited influxes of nitrite from sub-oxic hyporheic water. River water nitrate aquatic life exceedances were common while the more toxic nitrite exceedances were rare (Figure A-42) (Golder 2020a). Even in winter with ice cover, there were no ammonia exceedances in Henretta Lake/FR_HC1. An ammonia spike reached an unusually high 0.3 mg/L as N at S6 during the decline window which did not exceed aquatic life guidelines but did indicate a change in dissolved oxygen concentrations (Figure A-40). Nitrate and nitrite did not exceed their MAC's (maximum allowable concentration), however, nitrate concentrations frequently exceeded the 30-day BC guideline at several UFR sites. Figure 23 shows elevated total nitrogen on the upstream and downstream ends of the S6 overwintering area, suggestive of groundwater N influx *For more information, refer to the water chemistry (Costa and De Bruyn) of UFR WCT Evaluation of Cause SME report.*

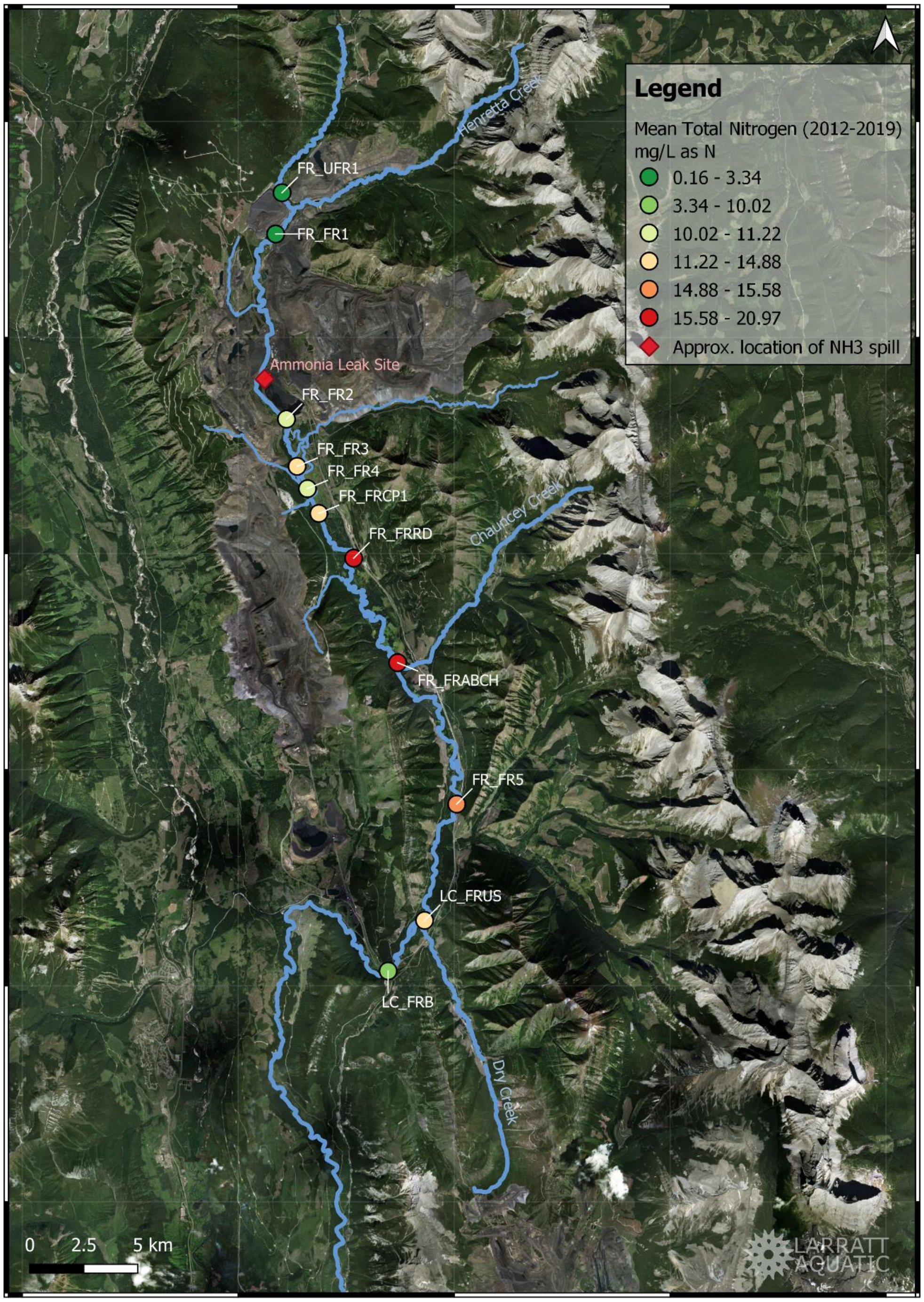


Figure 23: Mean total nitrogen at sample sites within UFR, 2012-2019

UFR Bioreactor – nutrient ratios

Most UFR mine-influenced sites had nitrogen far in excess of phosphorus concentrations and by as much three orders of magnitude (Table 14). Further increases in nitrogen will not trigger increased periphyton growth, while increases in bio-available dissolved phosphorus (PO_4, DIP) can do so (Table 14). Model Averaging on the 2015 data set identified flow-driven-TSS, calcite/bicarbonate/calcium and total phosphorus as the main drivers for periphyton abundance in the UFR, but not nitrogen (Figure 15, Table A-21). Reviewing the 2015 samples for unusual periphyton identified several mine-affected sites that were distinct from reference sites. Many of these mine-affected periphyton assemblages appeared to be reacting to calcite and not to nitrogen (Figure 15, Table A-30). If we assume that this is generally true, then the excess nitrogen is neither benefitting nor harming periphyton in the UFR. For this reason, ammonia released in the Maxam spill is not implicated in the fall 2019 Didymo bloom.

Table 14: Averaged Nitrogen to Phosphorus ratios for upper Fording River reference and mine-influenced sites

UFR	reference sites	mine-influenced	ratio descriptions (Ptacnik et al. 2010)			ratio descriptions (McDowell et al. 200		
			N-limited	N+P limited	P limited	N-limited	N+P limited	P limited
TN : TP	52 : 1	907 : 1	<35 : 1	35 - 60	>60 : 1			
DIN : PO_4	47 : 1	1482 : 1	< 16 : 1	>16 : 1	>50 : 1	< 7 : 1	7 - <15	> 15 : 1
DIN : TP	35 : 1	866 : 1	<2 : 1	2 - 5	>5 : 1			

NOTE : field filtered PO_4~ SRP | 29 mine-influenced sites >300 samples | 2 reference sites >29 samples

NOTE: ratios based on median data, can be conducted site-by-site

Shallow groundwater, seep, and side-channel samples collected by SNC Lavalin in 2019-2020 showed DIN:TP ratios far higher than UFR water, except for anoxic sites RG_FRDP5 and RG_FRDP8. These two sites had lower nitrate and therefore lower TN concentrations. Most of these shallow groundwater sites would contribute more ortho-phosphate (x=0.0037 ± 0.002 mg/L ortho-P) than the receiving Fording River water contains, indicating that upwelling sites could support greater periphyton productivity.

Low surface flow or groundwater inflow-driven spikes in ortho-phosphate can be stimulatory to periphyton and increases outside the range of historic data were observed at numerous sites during the 2017 and 2018 growing seasons within the decline window, including: FR_UFR1, FR_HC1, FR_FC1, FR_CC1, FR_LMP1, FR_FR2, FR_FRCP1, FR_FRRD, FR_FRABCH, RG_CH1, and LC_FRB (Figure 24). Sites that had ortho-P spikes in Summer 2018 included: FR_CC1, FR_FR5, FR_FRABCH,FR_LMP1, GH_FR1, and LC_FRB. Greater periphyon biomass production at those sites would persist to Winter 2019 where it would exert oxygen demand as it decomposed. This could be an important contributor to the observed DO sag in Winter 2019 (please refer to section 2.4 for potential winter DO impacts to WCT).

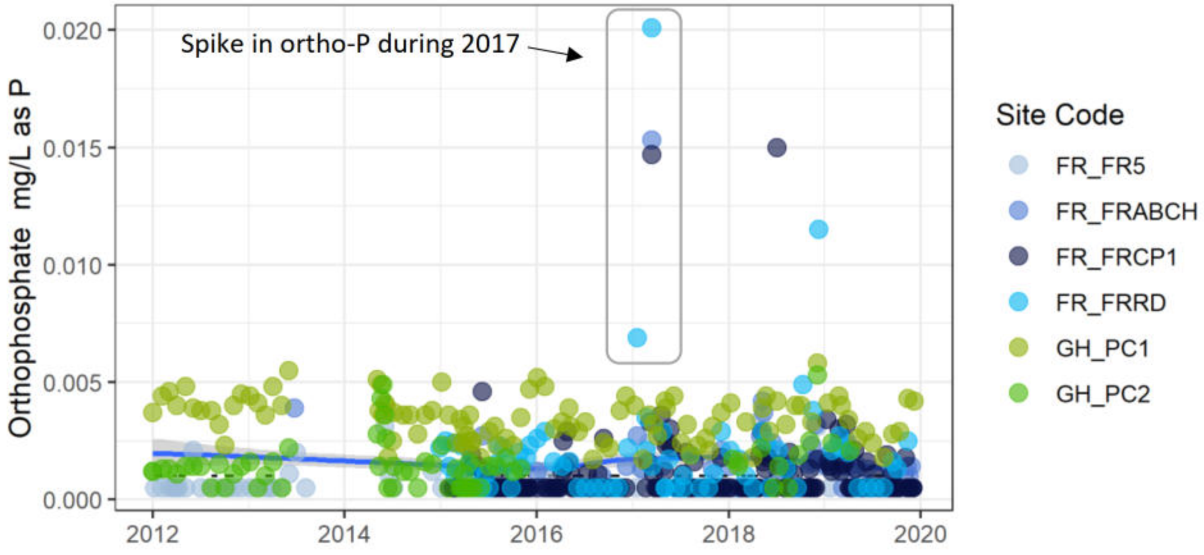


Figure 24: Orthophosphate at UFR sites within and adjacent to S6 from 2012-2019

2.3.5 Concluding Summary and Strength of Evidence for Impact Hypothesis 3:

The strength of evidence for Hypothesis 3_A, 3_B and 3_C is weak. Although UFR periphyton sampling is limited with no sampling during the Decline Window, extensive and systematic water quality analyses as well as research into UFR bioreactor functioning contributed to Hypothesis 3 analyses.

HYPOTHESIS 3_A : Did restriction of hyporheic exchange by periphyton entrapment of fines reduce habitat quality more than usual in the WCT Decline Window?

Friable biogenic calcite and periphyton biomass can gradually accumulate between strong freshets or floods. They are expected to measurably lower hyporheic exchange in low velocity/flow depositional reaches during periods with stable low flows such as the 2018 growing season, fall and winter 2018/2019. UFR ash-free dry weight data together with microscope observations from 2015 samples indicated that more fines (mostly calcite) were found in the periphyton from mine-affected sites than from reference sites, suggesting that the effect would be greater at mine-influenced UFR reaches. ***Refer to the calcite SME report (Hocking et al. 2021) for more information.*** Microscope observations demonstrated trapped fines including organic detritus, calcite and dark particulates (coal fines, 15-50 microns) in February 2020 periphyton samples, but there did not appear to be a relationship between fines and benthic invertebrate biomass or density (Ings et al. 2019). Thus, lowered periphyton palatability to invertebrate grazers cannot account for the WCT decline.

When hyporheic exchange is restricted, bioreactor processes that act to sequester metals and nutrients may not function as well, leading to higher concentrations in solution. An increase in bioreactor-mediated parameters was observed during the Decline Window. Sulphate, selenium, nitrate and ammonia showed higher maxima and averages with increasing trends during the Decline Window, while mercury did not deviate from typical ranges. These water quality changes are likely attributable to the effects of stable low flows on dilution and to lower bioreactor functioning. ***Refer to the water quality report (Costa and deBruyn 2021) for more information.***

The highest PAH sediment concentrations at mine-influenced sites were strongly correlated to total organic carbon (TOC), primarily a measure of periphyton biomass in streams without riparian forest (Moore, 1998; USGS 2011). The 2018 and 2019 FRO LAEMP concluded that the highest sediment metal and PAH concentrations were not associated with locations where there were changes in benthic invertebrate communities (Minnow 2018; Minnow 2020), providing an indirect line of evidence indicating that PAHs are not the sole cause of the WCT decline. The oxygen demand of decomposing/degrading PAH is expected to be small compared to BOD from decomposer flora in the periphyton biofilm and COD from ammonia transformations. ***Refer to the Coal dust and sediment Quality report (DiMauro, M., Branton, M., & Franz, E. 2021) for more information.***

In summary, the potential for effects of altered bioreactor function from low flow-related factors on WCT during stable low flows conditions in summer 2018 through Winter 2018/2019 would be greater than normal. However, it is unlikely to be the sole cause of or a significant contributor to the WCT decline.

HYPOTHESIS 3_B. Did periphyton metal bioaccumulation and SRB-mediated processes important to selenium or other metals cycling within the periphyton mat and the underlying hyporheic in the Upper Fording watershed have consequences to WCT during the decline window?

UFR sediment metal concentrations were strongly correlated with organic carbon (TOC), which is consistent with metal sorption to periphyton. Passive adsorption to periphyton surfaces can decrease metal trophic transfer while uptake into cell structures may increase it. Microbial SRB processes controlling metal cycling commence within the periphyton biofilm and continue into the hyporheic zone below. These processes are unlikely to affect bulk flow water chemistry, but episodes of restricted dilution can affect shallow water in pools or slow-moving backwaters, especially during low flow periods that often occur in late summer and through the winter. The bioreactor function of the Fording and Elk rivers results in measurable selenium and nitrate removal (Martin et al. 2011). The maximum concentration of SRB-generated MeHg in the large 2015 – 2018 Elk Valley water quality dataset was more than an order of magnitude below the 0.004 ug/L interim CCME guideline (Hill and Turner 2019), indicating that the potential for direct MeHg toxicity to aquatic life was negligible.

Although SRB activity apparently results in altered metal bioavailability in UFR, existing benthic invertebrate data did not show significant metal effects at sites with elevated aqueous metals (Minnow, 2019). At mine-exposed sites, significant differences in benthic invertebrate metrics between the pre-decline window and post-decline window were not evident in the data to date (Minnow 2020a). The Dry Creek 2018 Didymo bloom tissue samples indicated that elevated Se in Didymo blooms was concurrent with elevated Se in benthic invertebrate tissues. Dry Creek results suggest that Se trophic transfer from periphyton to invertebrates increased from a TTF of 1.19 with normal periphyton to a TTF of 3.57 within the Didymo bloom. From research conducted elsewhere, benthic invertebrates can accumulate the most Se (Cianciolo et al. 2020) while cutthroat trout have a TTF near 1 (Table A-23), indicating no further biomagnification. While the influence of a periphyton bloom such as Didymo on selenium cycling may merit additional study, it is unlikely to account for the WCT decline because it did not significantly impact invertebrate biomass metrics.

HYPOTHESIS 3C. Did nitrogen transformations in the periphyton-influenced hyporheic zone affect UFR water quality and DO enough to have an impact on WCT in the Decline Window? For example, did a remnant of the Maxam wash water/ammonia spill (Feb 2019) or other mine-related nitrogen source accelerate downstream filamentous algae growth in late summer through fall low flows in 2019, particularly at S6 overwintering area?

Like all lotic systems, complex interactions of periphyton-driven and bacterially mediated nitrogen transformations in the UFR hyporheic zone cause nitrogen species and DO fluctuations in both seasonally and spatially. Nitrification can significantly impact oxygen dynamics, accounting for up to 50% of the hyporheic oxygen demand. Increased nitrification oxygen demand may have contributed to the observed DO sag and elevated ammonia concentrations during the Decline Window, however these increased ammonia concentrations still met the total ammonia water quality guideline along the UFR mainstem. ***Refer to the water quality report (Costa and deBruyn 2021) for more information.*** However, the sum of these nitrogen processes could contribute to overwintering WCT DO stress particularly in winter 2019 with its unusually long period of ice cover.

Because nitrogen is in large excess of other nutrients, increases do not influence UFR periphyton communities. Consequences from periphyton stimulation by the Maxam wash water/ammonia spill or other mine-related nitrogen source cannot account for the WCT decline. The strength of evidence on this point is extensive (water quality monitoring) and compelling (established guidelines; Didymo research elsewhere).

2.4 Impact Hypothesis 4: Aquatic macrophyte and bryophyte mediated impacts to WCT

Hypothesis 4 Aquatic macrophytes and bryophytes play an important role in the structural and functional aspects of aquatic ecosystems by altering water movement regimes, serving as a food source, providing shelter to aquatic invertebrates and altering water quality by regulating oxygen balance, nutrient cycles, and interacting with metals and PAHs in root zones (Figure 25). These factors can affect WCT life stages variously. For example, fines deposition encouraged by macrophyte drag in pools and shallows can limit juvenile WCT use by filling interstitial spaces in the sediments. Further, these plants affect winter dissolved oxygen regimes in slow flowing areas and lentic habitats**.**

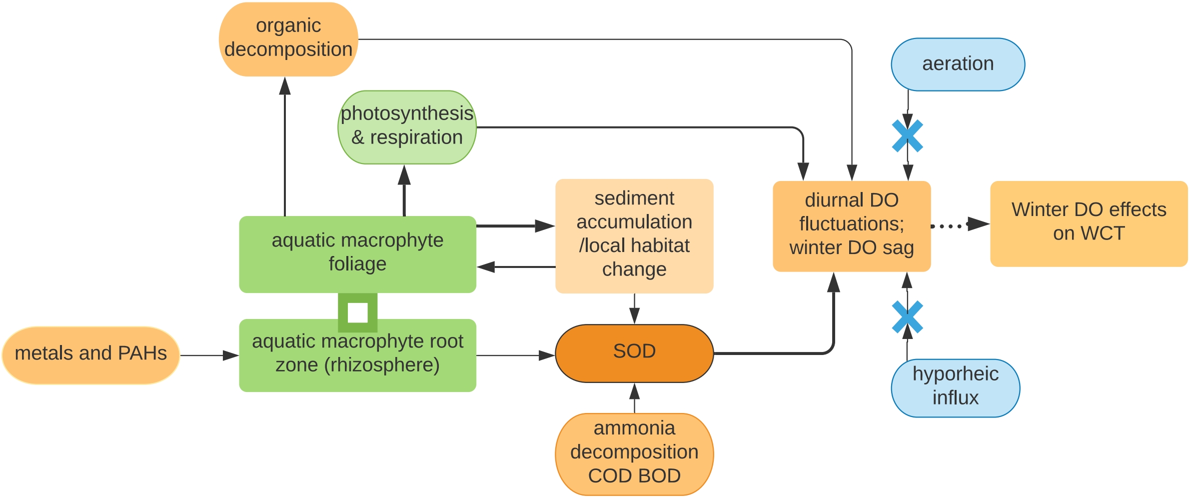


Figure 25: Conceptual macrophyte-driven winter dissolved oxygen effects on WCT in low flow UFR habitats

DO = dissolved oxygen SOD = sediment oxygen demand PAH = Polycyclic aromatic hydrocarbons "x" denotes where extreme cold winter conditions could impact oxygen influxes to UFR, particularly lower S6

Hypothesis 4 Questions:

A. *Did constituents of interest accumulate on or be taken up into macrophyte tissues and from there into grazer tissues, thus exposing WCT during the Decline Window?*
B. *Did oxygen demand from decomposing macrophytes, periphyton and from the sediments they helped accumulate, combined with ice-impeded oxygen influxes to S6 pools during the unusually cold Winter 2018/2019 cause low oxygen stress to WCT?*

2.4.1 Introduction to Macrophytes

Aquatic macrophytes include emergent (e.g., rushes, sedges) and submergent plants (e.g. milfoils, pondweeds) together with bryophytes (mosses and liverworts). Their growth will respond to increased N and P levels in both hard and soft water (Gao et al. 2019). Aquatic bryophytes can be important components of cool, headwater streams with coarse stable substrates such as the UFR, where they provide important structural habitats for macroinvertebrates (Bowden et al. 1999; Group 1999).

The interactions between macrophytes and river flows occur in three ways:

1. Flow regimes determine macrophyte stands: Stable flows are the single biggest determinant of macrophyte stand size and composition (Sand-Jensen et al. 1996; Sand-Jensen and Mebus 1999). Periods of high flow such as large freshets or storm flows can dislodge macrophyte stands.
2. Macrophytes stands alter water velocity, movement and sediment deposition: Where stands establish, macrophytes physically block the volume available for water movement and create flow resistance and sediment/detritus accumulates (Green, 2006; Bal and Meire 2009). Macrophytes can influence hyporheic exchange directly via local advective subsurface exchange and indirectly via streambed permeability factors and in-stream storage (Packman and Bencala 2000; Packman and Salehin 2003; Jones et al. 2008; White and Hendricks 2000).
3. Macrophytes alter hyporheic water chemistry: The riparian rooting zone often overlaps with the hyporheic zone, affecting both the exchange flows and biogeochemical reactions through transpiration (Larsen et al. 2014) and release of root exudates (Zhai et al. 2013; Boano et al. 2014). Vascular macrophyte roots create microzones where biochemical reactions occur affecting pH, redox, and the form/retention of nutrients (e.g., precipitation of Fe Mn compounds, co-precipitation of P, nitrification, decomposition processes, altered Se mobility).

Macrophytes are important in the UFR depositional reaches and lentic environments. These plants undergo seasonal fluctuations in biomass. In contrast, mosses and liverworts have standing crops that may represent several years growth. Organic carbon from macrophytes can be very important to local water quality (Boros et al. 2020). Macrophyte biomass fluctuations induce seasonal sediment accumulation, typically with high rates of fine sediment accumulation over the summer that can be retained through winter low flows, followed by some erosion of the accumulated sediment and plant material during the following freshet.

Bryophytes are important components of UFR reaches with coarse stable substrates. They are tolerant of calcified substrates provided their growth rates exceed the rate of calcite deposition (growing tips must be constantly above the calcite surface) (Goudey et al. 2009; Turner and Jones 2005). In shaded, turbulent streams, moss contribution to primary production can exceed that of the periphyton (Bowden et al. 1999). Bryophytes can be sheared off in floods and freshets, but they do not decompose over winter as larger macrophytes do.

Macrophytes and Metals, PAHs

Many macrophyte taxa accumulate metal ions in live (bioaccumulation) and dead (biosorption) plant tissues, and both processes are utilized in phytoremediation (Soudzilovskaia et al. 2010; Vieira et al. 2014). Where particulate organic matter or very fine sediment (e.g., clays, calcite mud) have accumulated in macrophyte beds, they can induce localized anoxic conditions that release nitrite, sulphides, ferrous iron, etc. and organic by-products. Further, the fine sediments that accumulate in macrophyte beds affect metal cycling by increasing sediment surface area available for bacterial activity.

Fine organic sediments that accumulate in macrophyte beds support biofilms that facilitate higher rates of Se biotransformation (Orr et al. 2006, Martin et al. 2011). Increased availability of sediment metals including Se leads to greater metal accumulation in emergent macrophyte roots than shoots (Martin et al. 2018; Rai 2009; Sood et al. 2012; Mishra et al. 2009) – these plants are found in UFR wetlands. Emergent macrophyte rhizosphere Se concentrations can be greater than the roots or adjacent sediments, suggesting that root exudates support bacteria that induce Se precipitation and adsorption (Martin et al. 2018). The presence or absence of emergent macrophytes presents a dominant control on sedimentary redox conditions, on the recycling and persistence of reduced Se species in lentic bottom waters and sediments, and on the uptake of metals into food chains (Martin et al. 2011).

Most metals do not biomagnify through aquatic food webs. For example, Zn and Pb frequently decrease across each trophic linkage, whereas concentrations of Cu and Cd were similar across several trophic levels. Metals with consistent bioaccumulation in lentic aquatic food chains include MeHg and Se (Rhea et al. 2013; Griboff et al. 2018). Biofilms on leaf surfaces appear to be an enriched source of organic selenium and are important food items for many benthic consumers (Franz 2012; Tse et al. 2012). In contrast, bioaccumulated metals in root zones are not available to invertebrate grazers. For example, metal uptake and retention in bryophytes is well-known (Stanković et al. 2018). Mosses have the capacity to hyperaccumulate metals including iron, lead, cadmium, copper, manganese, and zinc (Vukojevic et al. 2005; Martins et al. 2004). Dead bryophyte tissues retain polyvalent cations more effectively than live tissues (Pakarinen and Rinne 1979), likely through expanded surface area (Self and Larratt 2020).

Aquatic macrophytes can accelerate PAH removal in streams. In simulated streams, they depleted up to 31% of the mobile (fast desorbing) PAH fraction in 7 cm of sediment (Moermond 2007). The efficacy of common submerged aquatic taxa are variable but all plants performed significantly more PAH dissipation than unplanted sediments, likely related to sediment redox changes and/or microorganisms hosted by the rhizosphere (He and Chi 2016; Ghosal et al. 2016). Similarly, mosses and the microflora they host adsorb PAHs from stream water (Augusto et al. 2011; Kannangara et al. 2016). PAH-induced phytotoxic effects are rare among macrophyte taxa (Abdel-Shafy and Mansour 2016). BOD measured in periphyton mats can include BOD/COD from entrapped and degrading PAHs.

Macrophytes and Dissolved Oxygen

Aquatic macrophyte are important oxygen producers , but they also reduce re-aeration rates by modifying current velocity (Thyssen et al. 1987; Kaenal et al. 1998; Kaenel et al. 2001). Extreme diel oxygen amplitudes (8+ mg/L) with low nocturnal minima (< 1 mg/L) have been measured within dense submersed macrophyte stands in summer (Simonsen & Harremoes 1978, Dawson et al. 1982; Casey 1990; Petr 2000). Some macrophytes can continue growth at slow rates under winter ice (Boylen and Sheldon 1976), while other taxa become senescent. Submerged aquatic macrophytes can produce more DO than periphyton while bryophytes often produce less (Wetzel, 2001). Diel changes in DO are usually controlled by a combination of physical [diel water temperature, flow controls on aeration, and ice effects] and biological [photosynthesis of aquatic macrophytes and to a lesser extent, bryophytes, periphyton and cyanobacteria] (De Montety et al 2011; Slack, 1971; Ylla et al. 2007; Wetzel 2001).

The balance between autotrophic (oxygen-producing) and heterotrophic (oxygen-consuming) periphyton biofilm components is always dynamic and shifts towards oxygen consumption under dark winter conditions (Figure 25). Oxygen-consuming submerged and emergent macrophyte decomposition also varies seasonally while mosses do not (Wetzel, 2001). Macroinvertebrates are the primary shredders/consumers of macrophyte organic material in well-oxygenated riverine sites, while microbes are the primary organic material decomposers in slow-flowing backwater areas (Battle and Mihuc 2000).

Sediment Oxygen Demand (SOD) is an important variable in the oxygen balance of rivers, particularly during the winter under low flow and lengthy ice cover. Even though winter SOD (0.3 to 4.0 g/m^2/day) is typically less than half of the summer SOD in northern rivers, winter oxygen stress is more common because there is less new oxygen from inflows or photosynthesis to offset consumptive processes (Terry et al. 2017; Akomeah and Lindenscmidt 2017; Rounds and Doyle 1997). In ice-free reaches, oxygen deficits from decomposition demands are replenished through reaeration and turbulence, but ice cover prevents this gas exchange. Similarly, clear ice transmits light but snow loads reduces light penetration to a point where photosynthesis is greatly reduced. The resultant algae death and bacterial/fungal decomposition consumes oxygen from the upwelling hyporheic water that is typically well below saturation. An anoxic boundary layer adjacent to sediments is most likely to form in pools during extended ice cover with snow, and can slowly grow larger with time.

Winter sediment oxygen demand can be increased by greater growing season productivity (Terry et al. 2017). The first three months of ice-cover can have the greatest oxygen consumption due to the rapid oxidation of labile organic materials accumulated over the summer, including dead periphyton detritus (Babin and Prepas 1985; Hanson and Tenore, 1981). In winter, anchor ice can damage macrophyte foliage if plants freeze into the ice (Lind et al. 2014). After the ice melts, this material will decompose. The extent of flushing by freshet is the main factor controlling early summer SOD in streams and rivers (Rounds and Doyle 1997).

Diurnal dark conditions can lower DO around macrophyte stands and stress aquatic animals in productive pools in summer, but fish can relocate readily, however in winter, relocation can be more difficult. For example, a Bow River winter kill occurred in 2020 and in 2018, and these events are expected to be a result of a combination of low water levels together with low dissolved oxygen concentrations, where fish became isolated in a side channel of the Bow at some point in the fall or winter and were unable to move back into the main channel of the river. The fish either froze within the isolated channel or experienced decreased oxygen levels in the water. Congregated fish and aquatic vegetation continue to use oxygen from the water during the winter and this, combined with low flows can result in inadequate DO for fish (Ramsey, 2020). ***For more information on winter habitat impacts on WCT, refer to pathophysiology chapter (Bollinger 2021) of UFR WCT Evaluation of Cause SME report.***

2.4.2 Requisite Conditions for Impact Hypothesis 4:

The following points lay out the requisite conditions that must be present before an impact to WCT from aquatic macrophytes and bryophytes would be anticipated:

REQUISITE CONDITIONS FOR HYPOTHESIS 4:

Requisite Spatial extent	Macrophytes would have to occupy most slow-flowing key WCT reaches, bryophytes occupying most shaded reaches**:** ***No.*** *(Macrophyte distribution is limited but expanding in UFR pools since 2013 – they are in several key WCT rearing/overwintering habitats – Henretta Lake 12% of WCT, S6 40% of WCT but not documented in S8 20% or S1-3 14% of UFR WCT)*
Requisite Duration	Macrophyte stands would have to expand from year to year in depositional UFR sites**:** ***Yes.*** *(Stands expanding since 2013 through Decline Window) (Cope pers comm, 2020),*
Requisite Location	Macrophyte stands would have to occupy most of the substrate in key WCT habitat such as S6 (rkm 44.5 to 36), Henretta Lake etc.**:** ***No.*** *(Well-established stands are expanding in pools; but FRO ROV footage did not show macrophytes occupying >50% of videoed substrates in key WCT rearing/overwintering habitats)*
Requisite Timing	Macrophyte stands would have to expand during growing seasons leading to senescent material decaying in winter**:** ***Yes.*** *(Anecdotal evidence suggests gradual macrophyte stand expansion since the 2013 flood)*
Requisite Intensity	Dense, senescent macrophyte stands would be required in slow flowing reaches to have a measurable effect**:** ***No.*** *(Although stands are expanding, FRO underwater drone showed patchy stands of viable plants in winter 2020)*
	Yes = requisite condition was met; No = requisite condition was not met

2.4.3 Data Gaps and Uncertainties for Impact Hypothesis 4:

A formal macrophyte survey is needed with macrophyte identifications and samples to create mapping and an herbarium for future reference. Macrophytes may be more prevalent than anecdotal observations suggest, however macrophytes are unlikely to ever achieve problematic density in UFR. A systematic tissue metals sampling of macrophyte and bryophytes was not available to this analysis.

Although BOD from macrophyte decomposition could be important in depositional UFR sites with persistent ice cover (as in Winter 2019), a data gap exists involving temp / DO /pH /redox under those conditions. Should another intensely cold winter period like Winter 2019 occur, assessing reduced oxygen production + increased consumption with ice/snow shading and comparing those results to winters with open water would clarify effects of long-term ice cover at UFR depositional sites. Further, measurements of sediment oxygen demand would benefit assessments of WCT overwintering habitats from a DO and a water chemistry perspective.

2.4.4 Discussion of Impact Hypothesis Aquatic Macrophytes

Aquatic Macrophyte Populations in UFR

Aquatic macrophytes have a restricted distribution in the UFR watershed due to topography, with potential habitat limited to 7% or about 8.4 ha of the total 116 ha with a gradient ≤ 3% (Minnow, 2020; Wood and Berdusco 1999). Along the mainstem, submergent taxa were found in UFR riparian zones with lentic character and in reaches with slow-moving water.

General field observations indicate low emergent macrophyte abundance in the UFR (Figure A-31). Submergent pondweeds and milfoils may occur in several deeper ponds or wetlands but were seldom noted. The submerged macrophytes seen in the UFR ROV videos may be from the milfoil group (*Myriophyllum*) as well as the narrow-leafed pondweed group (*Potamogeton*) (Figure 27). Both filamentous algae and macrophytes retained silt/fines and contributed to a soft sediment accumulation that exceeded 50 cm thick in some pools. Cope (pers. comm. 2019) noted that macrophytes are rarely seen in other regional river systems but were seen in most depositional sites along the Fording system.

Aquatic mosses (bryophytes) are widely distributed in the study area, most frequently near the wetted edge of the mainstem and tributaries. They were more common than submerged macrophytes in the UFR, particularly in shaded mainstem reaches where their productivity in shallows can exceed that of periphyton (Worrall et al. 2016; Bowden 1999). UFR moss distribution was determined by combining observations of moss when visibly abundant at benthic invertebrate sample sites, with moss occurrence in periphyton samples, although the target periphyton sample depth was deeper than optimal moss habitat (White and Larratt 2016)(Figure A-31). Two identifications have been offered in reports to Teck thus far: *Jungermannia cordifolia* - a moist shoreline moss – and *Fontinalis antipyretica* – a submerged aquatic moss (PlanktonRUs 2012; Goudey et al. 2009). *F. antipyretica* grows in large clumps and mats and provides refuge for benthic invertebrates and WCT fry.

In the Upper Fording watershed, slow moving reaches conducive to submerged aquatic macrophyte growth are rare. Macrophytes have only been noted by Minnow field staff in three locations, and all in 2019 (Minnow, 2020) (Figure 27). These locations are RG_FC1, RG_CLODE, and RG_MP1. Additionally, the February 2020 video showed viable macrophytes in Henretta Lake and in the lower S6 pools (Figure 28). Of these, MP1, S6 and Henretta Lake have higher use as WCT overwintering areas and the other sites may be valuable as either overwintering or rearing areas (M. Robinson pers. comm. 2020). Senescence or decay of the summer aquatic macrophyte crop persisting and decaying through winter low flows is common. This can lower dissolved oxygen measurements. However, the underwater drone footage of the S6 pools showed viable macrophytes and bryophytes during Winter 2020 with shoreline ice and open water (Figure 28). Their coverage appeared to be <50% of available pool surface area.

In the 2015 periphyton sampling, LAC noted moss fragments in the following eight samples: RG_SWCK, RG_CATCK, RG_POCK, RG_FOUKI, RG_FODPO, RG_FO22, RG_FO29, and LC_FRUS (Figure A-31). There may have been more shaded sites with moss stands but the samplers were encouraged to avoid such areas due to the interference of moss leaflets in chlorophyll-a analyses performed on periphyton samples. Of these eight sites, the section of FODPO downstream past FO22 to the confluence of Chauncey Creek (S6) is important fish rearing/overwintering (M. Robinson, pers. comm. 2020). In contrast to the macrophyte seasonal decline, moss standing stock can represent several years' growth, provided the plants were not plucked by freshet flows. Decomposition of older leaflets is continuous as they become shaded by new growth overhead. This oxygen-consuming process is gradual and not restricted to winter. Where filamentous green algae occurred along the mainstem in the 2015 samples, it was invariably embedded in the moss, making it impossible to separate the effects of bryophytes and filamentous algae.

Aquatic macrophyte growth in 2019 was the largest extent since the 2013 flood – a flood which altered UFR habitat on mine site areas and removed accumulated filamentous algae and macrophytes with their associated sediment build-up (Cope pers. comm. 2019). More stable flows since 2013 may have encouraged the observed expansion of macrophyte growth. Very cold weather in Winter 2019 did not adversely impact these plants, although it may have impacted aquatic animals. Both filamentous algae and macrophytes are retaining silt and coal fines, contributing to a soft sediment accumulation that is >50 cm thick in some UFR pools. For example, the low gradient conditions at S6, characterized as low gradient, highly sinuous meandering channel dominated by deep glide habitat and fine sediment, have implications for metal cycling. No macrophyte identifications or estimates of stand size were available at the time of writing, beyond the underwater drone footage from Winter 2020.

Hypothesis 4A: PAHs and metals

The effect of PAHs and metals upon macrophytes and the potential for phytoremediation of these constituents by macrophytes has not been studied specifically within the UFR. Nonetheless, their volunteering at numerous sites (Figure 27) indicates that water and sediment chemistry is suitable for their growth. Similarly, bryophytes were established at eight 2015 periphyton sample sites, and are likely more widespread in the UFR watershed beyond these sites. Both plant types are utilized extensively for phytoremediation of PAHs and heavy metals, suggesting that they may be performing those functions in the UFR.

Aquatic macrophyte and algal responses usually have significant agreement, and algae are often more sensitive to organics and metals (Lewis and Thursby 2018; Paixão et al. 2008). Toxicity tests conducted on the floating macrophyte *Lemna minor* (duckweed) can be used to infer macrophyte tolerance of UFR conditions. Dry Creek chronic toxicity tests completed in 2018 showed no adverse effects on cell yield of the green alga, *Pseudokirchneriella subcapitata*. However, adverse effects on the macrophyte duckweed (*Lemna minor*) dry weight were observed for the April samples, based on IC_{25} values of 67.2% and 24.1% for LC_DCDS and LC_DC1, respectively, with no cause identified (Golder 2020). Related adverse effects on duckweed frond production were observed in the April sample collected at LC_DC1 (IC25 of 79.2%), and in the October sample collected at LC_DCDS (IC_{25} of 5.8%) (Nautilus 2019; Minnow 2019). In Dry Creek toxicity results, phytotoxicity demonstrated by duckweed but not by the alga indicates that continued monitoring is warranted but toxicity is unlikely to affect aquatic macrophytes in UFR.

The impact of the wide-spread aquatic moss in the UFR is largely beneficial through increased benthic invertebrate populations, high metal accumulation (Stanković et al. 2018), and increased overall primary productivity (Bowden 1999). The aquatic moss in the UFR is expected to be *Fontinalis antipyretica* based on the Goudey et al. (2009) report and its preference for calcareous streams.

Emergent macrophytes are distinct from submergent types by their transport of oxygen to their roots and they occupy lentic--type habitats. Regional UFR lentic organic sediments serve as pronounced sinks for Se (Martin et al. 2011; 2018). Martin et al. (2018) concluded that the presence or absence of emergent macrophyte vegetation presents a dominant control on sedimentary redox conditions as well as on the recycling and persistence of reduced Se species in bottom waters and sediments. These plants were not documented along the UFR mainstem, and their range in the UFR is very limited (Minnow 2020).

Based on the literature, the highest tissue metal concentrations are likely to occur in submerged aquatic macrophyte root zones through rhizo-filtration (root-microbe interactions) as opposed to macrophyte foliage. Metals in root tissues are largely unavailable to benthic invertebrates. The rhizosphere should host a bacterial community that decomposes some PAHs, although this has not been tested in the UFR. In addition, periphyton coatings on macrophyte leaves and the leaf tissues can bioaccumulate metals through adsorption as can bryophytes through cation exchange. No UFR macrophyte tissue samples were available at the time of writing, however, no impacts on benthic invertebrates were detected at UFR monitoring sites hosting bryophytes (example sites: Figure A-31; Minnow 2019 2020).

Hypothesis 4B: Dissolved oxygen

LENTIC In lentic areas such as Henretta Lake, some macrophytes can remain physiologically active while others experience up to 95% attenuation under winter ice cover (Wetzel 2001). Maximum winter photosynthetic activity is typically 10-20% of summer rates among plants that remain active. The macrophytes shown in the Winter 2020 Henretta Lake drone image may be *Potamogeton pectinatus* and the macroalgae *Chara* (Figure 19*).* Their winter viability means that instead of decaying and consuming oxygen, they are still producing some oxygen during daylight hours, even with ice cover. Macrophyte, periphyton and phytoplankton photosynthesis will be greatest when the snow cover on the lake ice is thin (Hrycik and Stockwell 2020). The large water volume of Henretta Lake represents a large DO reservoir. Thus, no demonstration of macrophyte decomposition that could adversely impact WCT survival in Henretta Lake through DO stress was detected in 2020, even during winter dark hours with months of ice cover. Additionally, the cold inflow from Henretta Creek should keep an ice lead open for most of the winter and travel under the ice in extreme cold weather, both of which will create a DO refuge.

LOTIC In lotic areas along the UFR mainstem, the hyporheic zone provides some oxygen to the river despite its chemical oxygen demand and restricted access to atmospheric oxygen. SNC Lavalin used drive-points to study the shallow groundwater and seeps between a side-channel and the main channel of the UFR (rkm 50–51) in winter 2019-2020. The aquifer is unconfined in this area, so it has higher DO than the anoxic conditions common in confined aquifers (S. Humphries pers. comm. 2020). DO ranged from 0.43 – 3.2 mg/L in the two drive-point sites with anoxic behavior but measured 7.67 - 8.67 mg/L at the seep locations (SNC Lavalin 2020); DO at all those sites was lower than the open side channel water at 9.15 – 11 mg/L. Full saturation would occur at ~12.5 mg/L at the 4 – 6°C range. In this data, seep drive points showed 61 – 72% saturation, while side-channel drive-points showed 70 – 86% saturation. This work indicated that shallow groundwater hyporheic exchange can deliver water to the UFR within the DO range tolerated by WCT. The winter 2020 DO logger data indicated 1 mg/L diel swings, with DO concentrations maintained well above WCT tolerances under open water conditions (Figure 26). For reference, hypoxia tolerance of many trout species occurs around 3 mg/L DO.

Sediments accumulate in the UFR during low flows, particularly following channel over-widening during floods. This accumulation of fine sediments in the UFR pools since the last major flush in 2013 is supporting macrophytes which also accelerate sediment deposition. Accumulated sediment is expected to exert increasing BOD/COD and lower dissolved oxygen transfer (Wright et al. 2018). This may be occurring at RG_FC1, RG_CLODE, and RG_MP1, based on snorkel surveys and ROV observations. In this sediment, nitrification of ammonia can impact oxygen dynamics, accounting for up to 50% of the hyporheic biochemical oxygen demand (Harvey et al. 2013; Cavaliere and Baulch 2019). *Refer to Section 2.3.4 for more information*.

Minimum monthly DO concentrations (2012 – 2019) below the 3 - 6 mg/L screening values were very rare in the UFR and invariably occurred during low flows almost exclusively in winter at: FR_UFR1, rkm 65, FR_HC3 rkm 64.5, FR_FRCP1 rkm 52, LC_FRUS rkm 31.5, FR_FR1 (rkm 66), FR_FRABCH (rkm 43), GH_FR1 (rkm 25), and Dry Creek (Golder 2019a). Low DO at these sites may relate to a combination of periphyton/ organics decomposition, restricted DO upwelling, ice cover, and chemical oxygen demand, but they are not all sites with known macrophytes stands (Figure A-31). Therefore, macrophyte decomposition is an unlikely contributor to these observed low DO measurements. Further, FRO underwater drone footage from the S6 pools from winter 2020 with open water showed viable macrophytes and bryophytes (Figure A-31, Figure 27).

Set-up conditions unique to Summer 2018 through Winter 2019

An unusually dry weather period occurred in 2018 – 2019, with low and late snowpacks and this led to low streamflows in the UFR. At FR_FRNTP mean flows in February 2019 were 20% of mean annual discharge - the lowest of any month from September 2016 to September 2019 (Wright et al. 2021). Flows were low from the 2018 growing season through winter 2018/2019 (0.36 m^3/s discharge), indicating that accumulated organic material could have persisted and exerted BOD. Added to this, both winter 2017/2018 and winter 2018/2019 were unusually cool. A warm January was followed by an abrupt air temperature drop of more than 20 °C over two days in February 2019 and persisted into the beginning of March (Wright et al. 2021). These winter weather conditions set up unusual ice conditions. ***For more information on ice in Feb. 2019, refer to Ice report (Hatfield, T., & Whelan, C. 2021) of UFR WCT Evaluation of Cause SME report.***

Decomposition of organic materials residual from the previous summer will exert more BOD when flows remain low between late summer and the winter low flow period on the UFR. For example, a brief flow spike to ~6m^3/s average daily discharge at FR_FRNTP occurred in December 2017 and twice in February 2020, but not in fall 2018 or fall 2019 (FRO flow data). Without a flushing flow, the oxygen demand from the summer productivity will be higher, especially during the first few winter months. For the same reason, winter sediment oxygen demand can be elevated following greater growing season productivity (Terry et al. 2017). It is therefore possible that BOD + SOD from the decomposition of the summer 2018 periphyton crop exerted a greater than normal oxygen demand during the exceptionally cold February 2019, when ice cover was more extensive and persistent than normal.

Winter sediment oxygen demand (SOD) in northern rivers ranges from 0.1-4.0 $g/m^2/day$ with the 0.3-2 $g/m^2/day$ range reported frequently (Terry et al. 2017; Akomeah and Lindenscmidt 2017). Even though winter SOD is typically less than half of the summer SOD in northern rivers, winter oxygen stress is more common because there is less oxygen influx from aeration, inflows, or photosynthesis to offset consumptive processes (Terry et al. 2017; Akomeah and Lindenscmidt 2017; Rounds and Doyle 1997). An anoxic boundary layer adjacent to sediments is most likely to form in pools during extended ice cover and can slowly expand during low flows. If this boundary layer became anoxic during Winter 2019, it would dramatically alter metal cycling in addition to a restriction on WCT overwintering habitat.

Available field meter data (monthly and some weekly readings from the S6 area) indicate a DO sag during the cold February/early March 2019 period (Figure A-38, Figure A-39) Daytime readings as low as 55% (7 mg/L) were detected at FRABCH. This period had anomalously low DO at FRABCH and upstream FR_FRCP1, suggesting that there could be low DO in the slowest flowing areas of S6 at night, particularly if temporary ice dams or other ice features formed. This sag did not reach critical DO levels for WCT and confirms the findings of the DO screening work. A declining DO trend at S6 over the two-year Decline Window was detected (Mann-Kendall DO% p=0.003; DO mg/L p=0.018) but not in the 2012-2019 window, indicating it was a short-term pattern (Figure A-4).

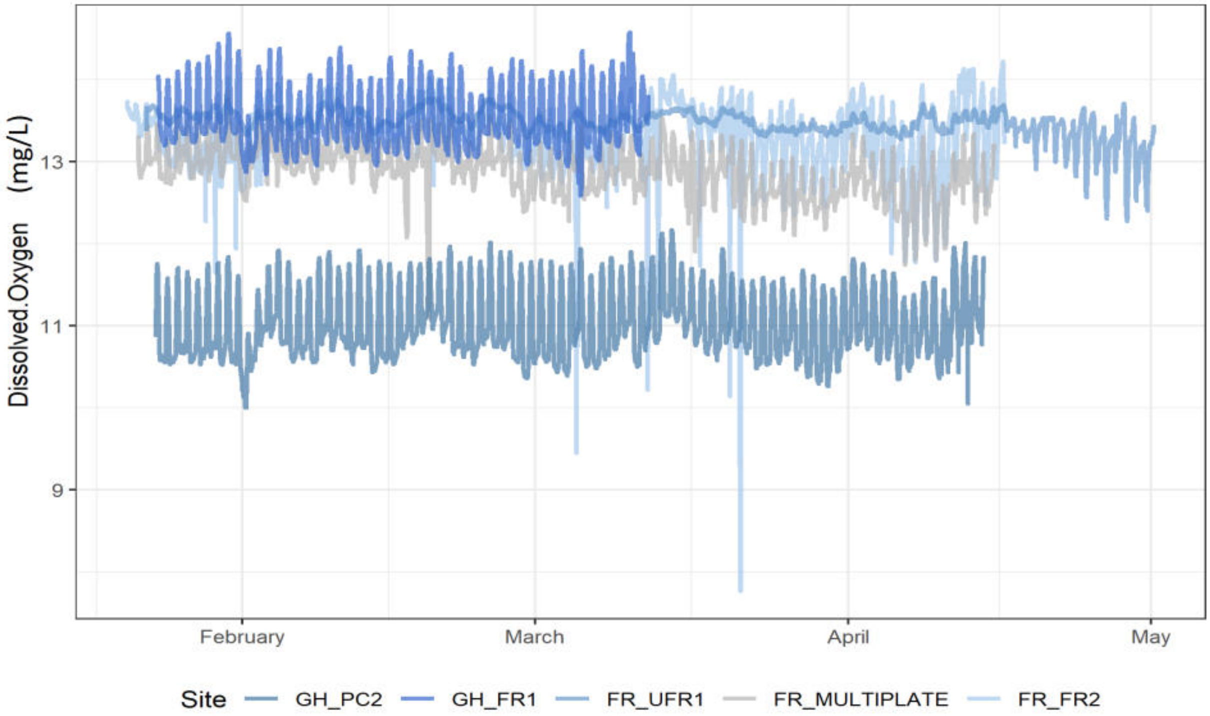


Figure 26: Dissolved oxygen data at sites along UFR during January to May 2020 with open water
Note: dissolved oxygen threshold of concern for WCT is below the scale of this graph (see Golder SME report for screening values)

Although it is uncertain at this time, the sustained cold February to early March 2019 weather may have resulted in frost penetrating the hyporheic zone, causing unusually extensive anchor ice. If this occurred, it could have impeded groundwater influx through the hyporheic zone (Nafziger et al. 2017). With impeded dissolved oxygen delivery from shallow groundwater, and with solid ice cover preventing atmospheric gas exchange, dissolved oxygen concentrations could decline in the lower half of the S6 pools during this time. However, this effect is not anticipated to occur in the 'regional groundwater discharge zone in the upper half of the S6 region (i.e., Greenhouse Side Channel and approximately 1.5 km downstream) due to significant upwelling of relatively warmer groundwater and due to coarse substrates. If these restricted DO influx conditions occurred, high BOD from the decomposition of an unusually large summer 2018 macrophyte and periphyton crop may have instigated the observed DO sag (Figure A-39). Additionally, a 50% reduction in light penetration occurs under ice cover relative to open water (Hrycik and Stockwell 2020); thus at S6, lower photosynthesis by macrophytes is expected during this period. DO showed depletion to 55% of saturation (7 mg/L) in February 2019 at S6 in daytime meter readings and would be approximately 1 mg/L less at night based on 2020 light logger data. Indirect evidence for the scale of SOD at S6 is provided by declines in DO, ORP, pH, and increased decomposition products (ammonia, TKN & organic nitrogen, DOC, MeHg), where:

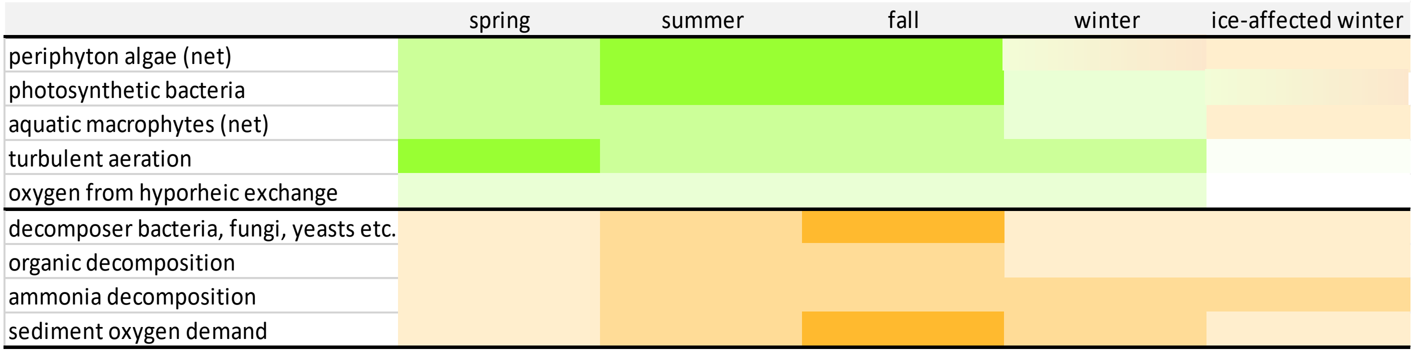
- Fall/Winter 2019 had lower pH, an unusual ammonia spike (Figure A-40), elevated TKN (Figure A-43) and higher dissolved organic carbon, indicating organic decomposition.
- FRABCH redox fluctuated seasonally between 0 and 300mV whereas the UFR mainstem averaged 200 – 550 mV
- Detectable MeHg at FRABCH (met all guidelines) indicated anoxic SRB activity

These parameters align with elevated SOD from the fine sediments at FRABCH in the Decline Window.

If ice blockage caused stagnant conditions in pools, full DO depletion of the S6 volume would take between 30 days to as little as 2 days using available estimates of SOD. If water continued to flow at the winter flow rate of 0.36 m^3/sec., water could become depleted of oxygen within S6 under a high SOD scenario. These calculations only serve to indicate a potential for a dissolved oxygen deficit at lower S6 during the unusually cold five weeks in Winter 2019 ***For more information, refer to the dissolved oxygen Appendix E of UFR WCT Evaluation of Cause SME report.***

In other Rocky Mountain research, work on the Kananaskis River determined that anchor ice alters hyporheic exchange by altering flow paths between surface and groundwater (Nafziger et al. 2017). Work on the Bow River 2018 and 2020 winterkills concluded that oxygen consumption may have played a role in winter fish kills (Ramsey 2020). These examples suggest that under extreme cold with low flow conditions, ice effects could impede oxygenated inflows to UFR mainstem sites like the S6 overwintering, making them vulnerable to oxygen depletion through biochemical oxygen consumption. ***For more information, refer to pathophysiology chapter (Bollinger 2021) and the ice chapter (Hatfield and Whelan 2021 of UFR WCT Evaluation of Cause SME report.*** If DO was depleted in late winter 2019 at lower S6, it could be an important factor in the WCT loss, or lowered DO could contribute to overwintering WCT stress and induce them to move locations, however the strength of evidence supporting this hypothesis is weak. Table 15 shows the theoretical difference in oxygen budgets between a typical winter and an ice-affected winter such as the record-cold February 2019.

Table 15: Theoretical seasonal oxygen budget contributions for UFR mainstem pools during winter low flows



	spring	summer	fall	winter	ice-affected winter
periphyton algae (net)	▒	█	█	░	◔
photosynthetic bacteria	▒	█	█	░	◔
aquatic macrophytes (net)	▒	▒	▒	░	◔
turbulent aeration	█	▒	▒	▒	░
oxygen from hyporheic exchange	░	░	░	░	
decomposer bacteria, fungi, yeasts etc.	◔	◑	●	◔	◔
organic decomposition	◔	◑	◑	◔	◔
ammonia decomposition	◔	◑	◑	◑	◑
sediment oxygen demand	◔	◑	●	◑	◔

░	low oxygen provider
▒	moderate oxygen provider
█	high oxygen provider
◔	low oxygen consumer
◑	moderate oxygen consumer
●	high oxygen consumer

The composite of winter 2019 conditions may include:

- No fall flushing flows
- Biochemical oxygen demand/sediment oxygen demand from decomposition of a large periphyton crop due to stable low flows in the 2018 growing season resulting in elevated winter sediment oxygen demand (BOD COD)
- Lengthy surface ice cover
- Frazil and anchor ice impacts locally impede hyporheic inflows
- Potential interruption of suboxic subsurface drainage by deep frost

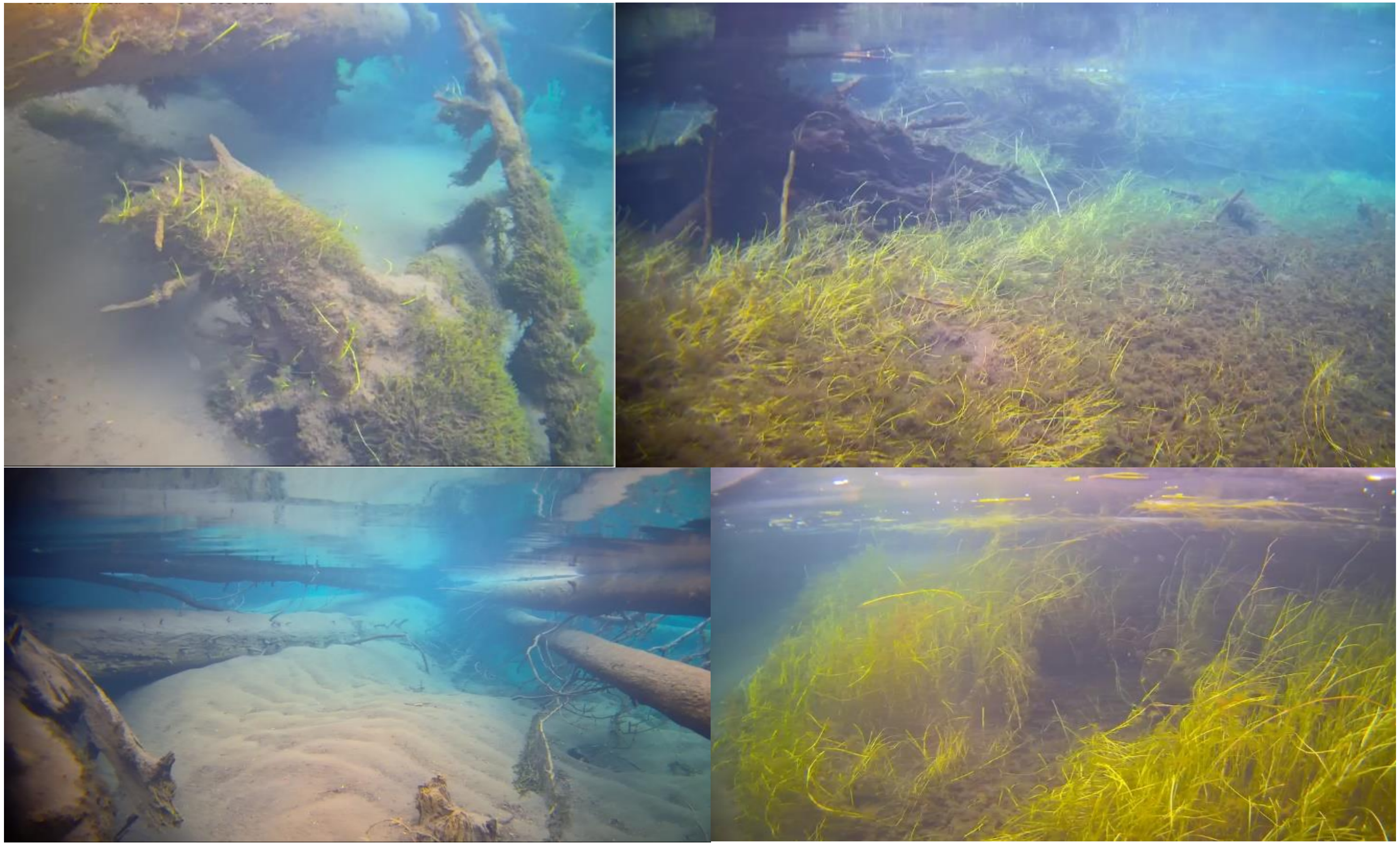
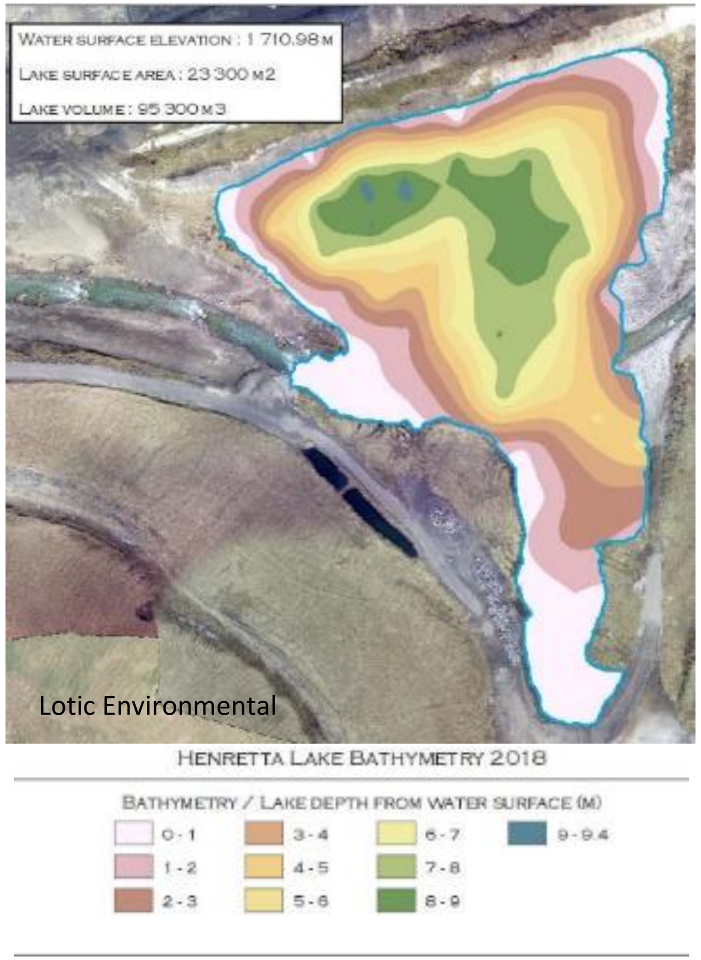


Figure 27: Underwater drone image of aquatic macrophytes in S6 pools, February 2020 - FRO staff

Note: Site 1 S6 pools



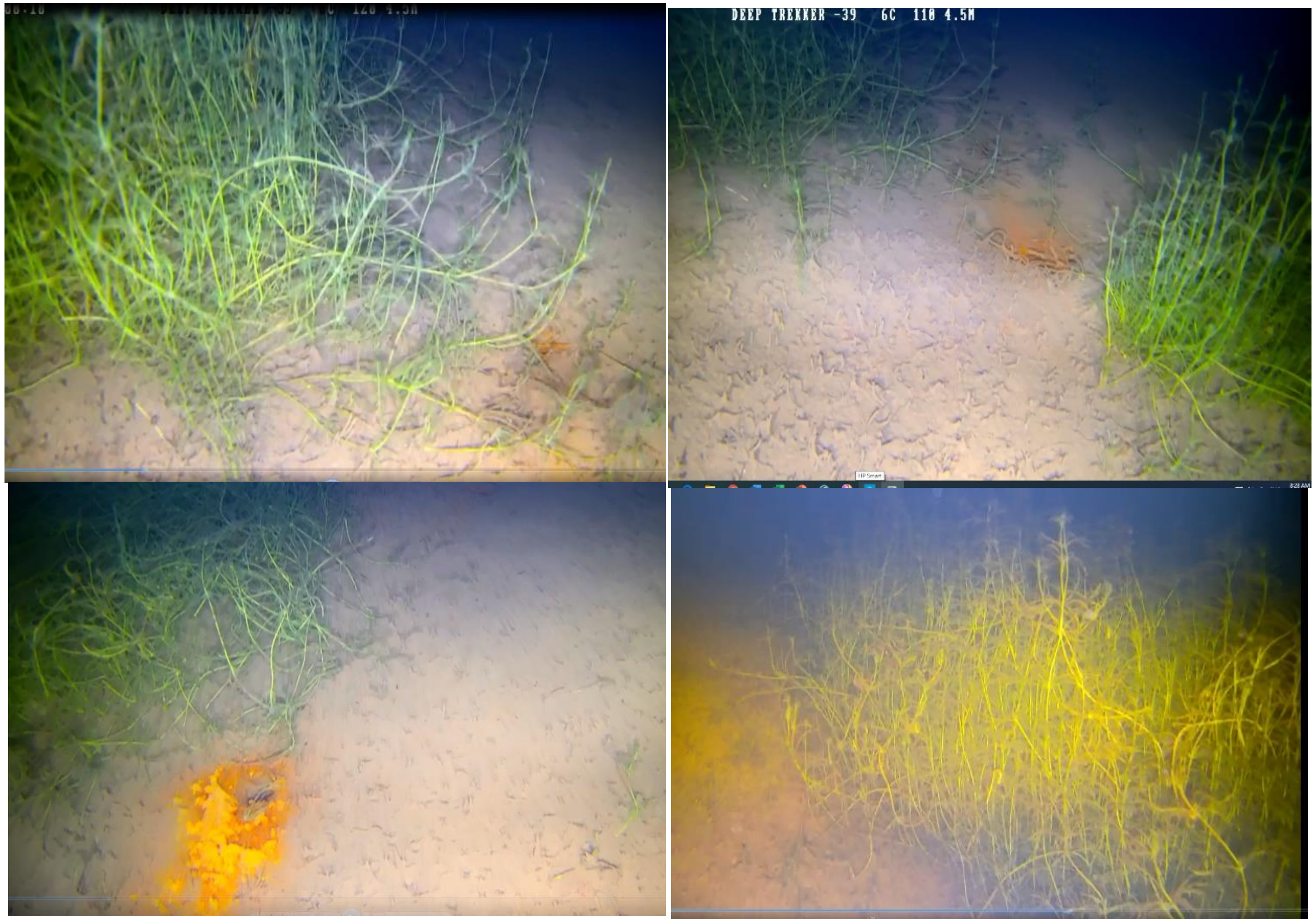


Figure 28: Bathymetry and underwater drone image of aquatic macrophytes in Henretta Lake 3-4.5m depth, January 2020 - FRO staff

2.4.5 Concluding Summary and Strength of Evidence for Impact Hypothesis 4:

The strength of evidence for Hypothesis 4_A and 4_B is indeterminant due to a lack of macrophyte/bryophyte surveys in the mainstem UFR and limited temperature/DO/pH/redox sampling during the Decline Window. There are no systematic aquatic plant surveys or macrophyte tissue samples available, but the observed gradual population expansion since the 2013 flood means that unusual macrophyte growth within the WCT Decline Window is very improbable. Macrophyte populations remain small (S. Cope pers. comm. 2020; ROV footage 2020) and are limited by UFR topography.

HYPOTHESIS 4_A Did constituents of interest accumulate on or be taken up into macrophyte tissues and from there into grazer tissues, thus exposing WCT during the Decline Window?

Based on the literature and studies of Elk Valley lentic sites (Martin 2011; 2018), the highest metal concentrations are likely to occur in macrophyte root zones where bioaccumulated metals would be unavailable to benthic invertebrates or WCT. The macrophyte rhizospheres should host bacterial consortia that decompose some PAHs and attenuate metals, although selenate reduction with subsequent uptake into aquatic food chains can also occur. In depositional reaches with finer textured sediments (e.g., S6 pools), with slowed hyporheic exchange, anoxic sediment conditions can occur within sediment layers, possibly allowing localized nitrogen build-up at the same time as dissolved oxygen production is slowed by winter conditions.

Bryophyte stands are more extensive in the UFR than macrophytes, and these mosses provide important structural habitats for macroinvertebrates in shaded, turbulent reaches. Bryophytes perform cation exchange so present a metal accumulation risk, but their growth was not novel during the Decline Window (Minnow 2018). Gradual expansion of aquatic macrophyte and bryophyte stands to their current small extent since the 2013 flood and their benefits prevent bioaccumulation by these plants from being the sole cause of the WCT decline.

HYPOTHESIS 4_B Did oxygen demand from decomposing macrophytes, periphyton and from the sediments they helped accumulate, combined with ice-impeded oxygen influxes to S6 pools during the unusually cold Winter 2018/2019 cause low oxygen stress to WCT?

Bryophyte decomposition is continuous, not seasonal, so they do not contribute to over-winter BOD to the same extent as aquatic macrophytes. Their gradual expansion since the last large flush in 2013 and their benefits prevent bryophytes from contributing to the WCT decline.

The large DO volume of Henretta Lake, its stream inflow, subsurface inflow, and detection of viable macrophytes and filamentous algae in Winter 2020 all act to limit DO depletion despite its annual ice cover. Winter DO consumption in the pit lake based on a low winter SOD with no DO inputs from creek or groundwater inflows or photosynthesis is estimated at 140 days (5 months) to full water column anoxia given the volume to surface area of Henretta pit lake[10] . A bottom water anoxic zone may form but refugia such as open water leads from Henretta Creek are expected. Late winter DO profiles from Henretta Lake to confirm this expected scenario were not available at the time of writing.

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[10] Using a low DO consumption estimate of 0.3 g/m²/day with a surface area of 23000 m² and volume of 95000 m³ yields an estimate consumption time of 138 days assuming static conditions. Given that continuous inflows of high DO water exist, it is not expected that DO depletion would occur in Henretta Lake.

Oxygen demand from decomposing macrophytes and periphyton in UFR depositional reaches on the mainstem during winters with extreme ice cover such as Winter 2018/2019 could lower dissolved oxygen concentrations at discrete depositional pools, particularly under dark conditions. Available field meter data (monthly and some weekly daytime readings from the S6 area) indicated a DO sag during the cold Feb/early March 2019 period. This period had anomalously low DO at FRABCH and upstream FR_FRCP1, suggesting that there could be low DO in the slowest flowing areas of S6 at night, particularly if temporary ice dams or other ice features formed. The measured DO sag in Winter 2019 was part of a declining DO trend unique to the Decline Window. The detected sag did not reach critical thresholds for WCT survival. However, the DO sag detected the top (FR_FRABCH) and bottom (FR_FR5) of S6 did not reach the <3 mg/L DO threshold known to be lethal to WCT, even if 1 mg/L is subtracted to account for diurnal fluctuations measured by UFR DO sensors.

Under extreme winter ice conditions DO concentrations may have dropped below the tolerance of overwintering WCT in low flow segments of lower S6 during February through early March 2019. If all DO sources were cut off by a combination of surface ice, anchor ice, and deep frost, a simplistic calculation of SOD alone predicts depletion the S6 volume in 30 days to as little as 2 days. If water continued to flow at the winter flow rate of 0.36 m^3/sec., it could become oxygen-depleted during travel through S6 under a high SOD scenario. These calculations only serve to indicate a potential for a dissolved oxygen deficit at lower S6 during the unusually cold weeks in Winter 2019. Please refer to Appendix E for full explanation. SOD data are not available for this period to test this scenario. If DO was depleted in late winter 2019, it could have caused the loss of WCT from lower S6 or more likely, lowered DO would contribute to overwintering WCT stress and induce them to move locations.

In contrast, the continuous Winter 2020 field probe data shows excellent oxygen concentrations under open water conditions, further confirming that low DO from macrophyte decomposition in a typical winter does not occur at these important WCT overwintering sites.

2.5 Preliminary Evaluation of Cause

In all cyanobacteria and periphyton hypotheses, the strength of evidence is indeterminant or weak due to limited periphyton sampling and no periphyton sampling during the WCT Decline Window from UFR mainstem sites. Similarly, the strength of evidence for aquatic macrophyte and bryophyte mediated impacts is indeterminant due to the lack of systematic macrophyte and bryophyte study in the upper Fording River. When it was possible, other lines of evidence were considered where the strength of evidence was stronger. For example, the extensive water quality database and reports were reviewed for parameters that are affected by these primary producers. Based on the available data, Teck Coal research projects, a review of the literature and professional judgement, the following conclusions were reached:

HYPOTHESIS$_1$ Cyanobacteria and Cyanotoxicity: Because cyanobacteria blooms can change dramatically over a period of only days to weeks, existing UFR data cannot be used to determine site-specific cyanobacteria conditions in the Decline Window. It can be stated that cyanobacteria presence and the possibility of chronic low-dose stress to WCT in the UFR is both natural and long-standing. *Phormidium autumnale* occurs naturally in UFR calcite crusts, where dark conditions can enhance its cyanotoxin production. Low flows extending through the growing season increase the potential for cyanotoxin exposure of invertebrates and early life stages of WCT. Fall flushing flows capable of removing cyanobacteria mats and calcite may not have occurred in 2018. There may be some potential for juvenile/adult WCT to be exposed to cyanotoxins in winter 2019 low flows due to low dilution and to localized calcite dissolution concurrent with periphyton mat decomposition. None the less, cyanotoxicity results from fish studies elsewhere do not align with the WCT decline because mortality from cyanotoxicity is rare, early life stages are expected to be more susceptible than adults and WCT rely on benthic invertebrates making them less susceptible to cyanotoxins than fish that rely on zooplankton. In conclusion, cyanotoxins may have potential to lower fish health in low flow conditions but would not account for the WCT decline.

Hypothesis$_1$ LINKAGE TO OTHER POTENTIAL STRESSORS – cyanobacteria to biogenic calcite formation

HYPOTHESIS$_2$ Filamentous algae blooms: Proliferations of filamentous algae during late summer low flows are probable in most years since the 2013 flood and in 2019, they exceeded observed extents in other regional rivers. Controls on filamentous periphyton blooms in the UFR were determined to be critical flows and suspended solids (TSS), as well as calcite/bicarbonate/calcium and total phosphorus concentrations. Nitrogen was not stimulatory to filamentous algae growth. Filamentous green algae blooms in Fish Pond Creek and a widespread but moderate density Didymosphenia geminata (Didymo) bloom through the UFR mainstem were observed during stable low flows in early fall 2019, however taxonomic periphyton data from the Decline Window were not available. Sites with abundant Didymo in 2019/2020 also had some Didymo in 2015 sampling, indicating that this taxon was not recently introduced. Based on fisheries research elsewhere, excessive filamentous Didymo growth is unlikely to account for the WCT decline, however its bloom phases could potentially stress juvenile WCT during their summer rearing stage. Similarly, a gradually reduced hyporheic exchange during late summer through localized substrate bioclogging is a common occurrence regionally during low flows so is unlikely to account for or contribute to the WCT decline. Without a significant 2018 fall flushing flow to remove it, the unusually large amount of organic material from a filamentous algae bloom would decay in winter 2018/2019, increasing dissolved oxygen demand. Lower dissolved oxygen concentrations in slow flowing reaches under weeks of ice cover appears to be the only way periphyton could potentially contribute to the WCT decline, however data to support or refute this mechanism are not available.

Hypothesis$_2$ LINKAGE TO OTHER POTENTIAL STRESSORS – low stable flows for more than three weeks and filamentous algae blooms

HYPOTHESIS3 Bioaccumulation, fines entrapment and bioreactor function of UFR:
Friable biogenic calcite and periphyton biomass can gradually accumulate between strong freshets or floods, and they are expected to measurably lower hyporheic exchange in low velocity areas of depositional reaches. When hyporheic exchange is restricted, bioreactor processes that act to sequester metals and nutrients may not function as well, leading to higher concentrations in solution. In the nitrogen rich UFR flows, bacterially mediated N transformations in the hyporheic zone cause nitrogen species and dissolved oxygen fluctuations both seasonally and spatially, but because nitrogen is in large excess of other nutrients, increases do not influence UFR periphyton communities. The benefits of SRB (sequestering of metals, bioremediation of PAHs) were not countered by mercury methylation in UFR samples. Sulphate, selenium, nitrate, and ammonia showed higher maxima and averages with increasing trends during the Decline Window, while mercury did not deviate from typical ranges. These water quality changes are likely attributable mainly to the effects of stable low flows on dilution and secondarily on lower bioreactor functioning. A gradual and localized reduction in hyporheic exchange through bio-clogging is unlikely to account for the WCT decline or contribute to it unless a threshold or tipping point was reached. There is no evidence to suggest that periphyton driven water chemistry changes critical to WCT health were reached within the Decline Window.

Fines trapped in the periphyton layer included organic debris, calcite and coal fines, and these materials can increase periphyton tissue PAH and metal concentrations. PAH concentrations were strongly correlated to organic carbon which is consistent with periphyton biomass - the material that helps adsorb and degrade toxic low molecular weight PAHs. Passive adsorption to periphyton surfaces may decrease metal trophic transfer while uptake into periphyton cell structures may increase it. While the influence of a periphyton bloom such as Didymo on selenium cycling may merit additional study, it is unlikely to account for the WCT decline because it did not significantly impact invertebrate biomass metrics. Similarly, fines entrapment may have reduced periphyton palatability to invertebrate grazers but did not result in measurable impacts. These findings provide evidence that periphyton mediated fines entrapment and PAH/metal's adsorption cannot account for the WCT decline.

Hypothesis3 LINKAGE TO OTHER POTENTIAL STRESSORS - low stable flows to filamentous algae blooms, metal bioaccumulation and to altered bioreactor function

HYPOTHESIS4 Aquatic macrophytes and bryophytes: Aquatic macrophytes and bryophytes are native to the UFR system, indicating adequate water and sediment quality to support their growth. Macrophyte stands have redeveloped in slow flowing reaches following the 2013 flood. Bryophytes are more widely distributed and extensive than other macrophytes in the lotic-dominated UFR watershed. Bryophytes can accumulate metals through cation exchange, but their growth was not observed to be unusual during the decline window. Based on the literature and studies of Elk Valley lentic sites (Martin 2011; 2018), the highest metal concentrations are likely to occur in macrophyte root zones where bioaccumulated metals would be unavailable to benthic invertebrates and WCT. Submerged macrophyte rhizospheres host a bacterial consortium that can decompose some PAHs and attenuate metals, although selenate reduction with subsequent uptake into aquatic food chains is also known to occur. The benefits of aquatic macrophytes and bryophytes and their slow expansion following the 2013 flood provides evidence against bioaccumulation by these plants as the sole cause of, or a major contributor to, the WCT decline.

Aquatic macrophyte decomposition could only affect DO regimes when stable low flows through summer and fall are followed by an extremely cold winter that interrupts oxygen influxes. The composite of extreme cold winter 2019 conditions may include:

- No fall flushing flows
- Biochemical oxygen demand from decomposition of a large periphyton crop due to stable low flows in the 2018 growing season, resulting in elevated winter sediment oxygen demand (BOD, COD)
- Lengthy surface ice cover

- Frazil and anchor ice that locally impede hyporheic inflows
- Potential interruption of suboxic subsurface drainage by deep frost

These winter conditions may have progressed to the point that this composite of conditions reduced dissolved oxygen (DO) below the tolerance of overwintering WCT in low flow segments of lower S6 during February through early March 2019. If DO was depleted in late winter 2019 at lower S6, it could be the sole cause of the WCT loss or lowered DO could contribute to overwintering WCT stress and induce them to move locations. However, the strength of evidence for this hypothesis is weak. The measured DO sag in Winter 2019 was part of a declining DO trend unique to the Decline Window, but it did not reach critical thresholds for WCT survival. At Henretta Lake, its large DO content, creek inflow and viable macrophyte beds in winter limits a similar issue despite its annual ice cover.

Hypothesis$_4$ LINKAGE TO OTHER POTENTIAL STRESSORS – macrophyte and bryophyte stand impacts to sediment accumulation and to lower hyporheic exchange

2.6 Other Relevant Observations & Findings

All four hypotheses covered in this report are strongly affected by the UFR flow regime. The accumulation of organic material and fine sediment between large freshets or floods can have a profound effect on the UFR ecosystem. More importantly, stable low flows that persist from the growing season through to winter without interruption by a fall flushing flow can:

- In the positive: stable low flows allow more periphyton growth that can adsorb metals, generate DO in daylight hours, limit sediment transport and lower TSS
- In the negative: stable low flows prevent the clearing of periphyton biomass prior to winter low flows, encourages Didymo and cyanobacteria blooms, allows accelerated photosynthesis-driven calcite-periphyton crust formation and allows the retention of macrophyte biomass with accrued sediment

Therefore, if stable low flows persist through the growing season without fall flushing flows, increased organic material from cyanobacteria, periphyton and aquatic macrophytes together with sediment accumulated in and around them can affect the following winter’s dissolved oxygen, hyporheic exchange, and bioreactor function in low flowing UFR reaches. However, it is unlikely that these primary producers played a significant role in the WCT decline.

3 Literature Cited - Cyanobacteria, Periphyton and Macrophytes

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Humphries, S. 2020 SNC Lavalin email and phone conversations to Heather Larratt (Larratt Aquatic Consulting Ltd.) March 4 to Sept 19 2020)

Robinson, M. Lotic Environmental email and phone calls to Heather Larratt (Larratt Aquatic Consulting Ltd.) February 4 2020 to August 22 2020)

FIGURES

LIST OF AFTER-TEXT FIGURES

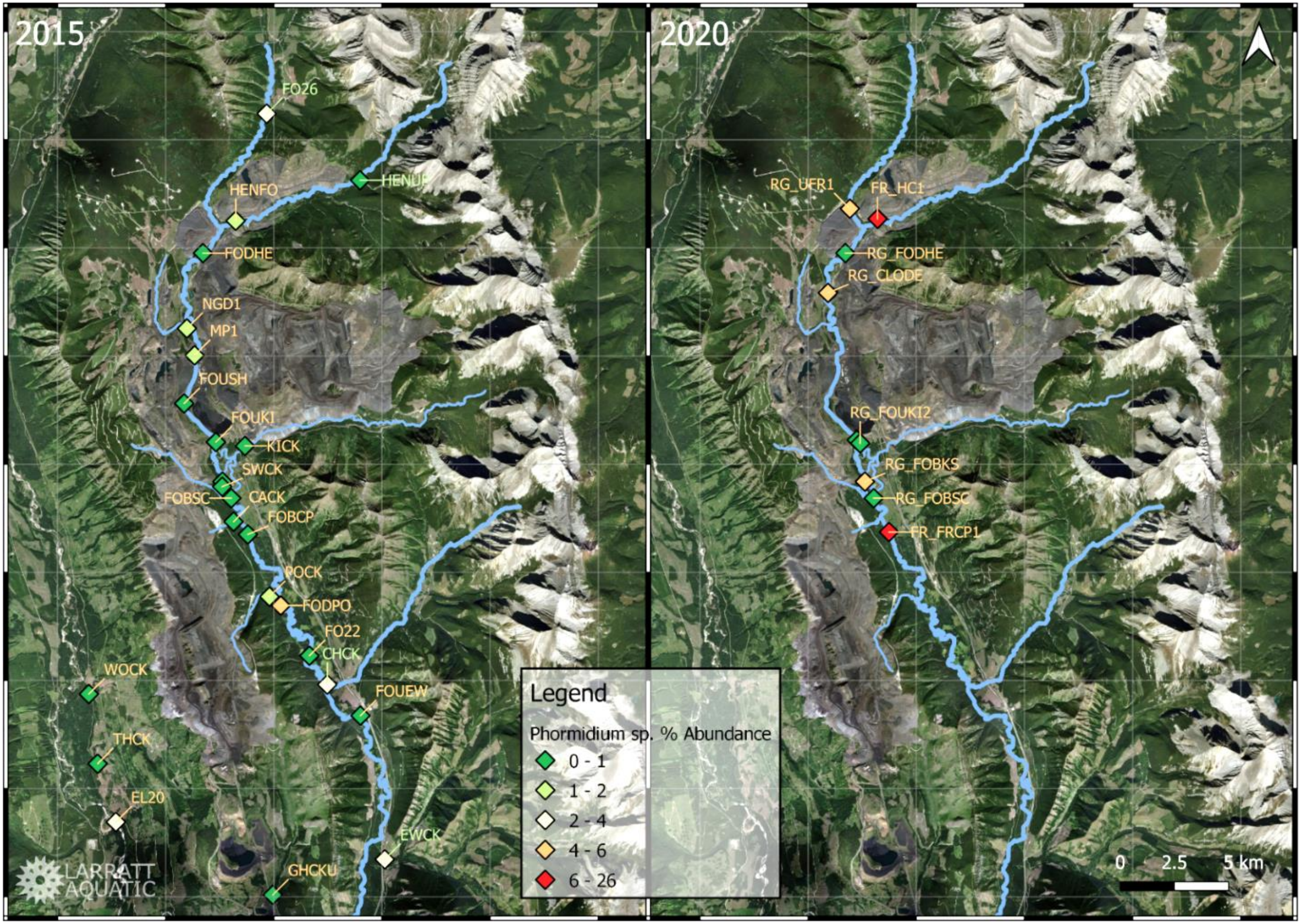


Figure A-29: Percent abundance for Phormidium sp. comparing September 2015 and February 2020 results

Note: Orange site labels = exposed site, green site labels = reference site

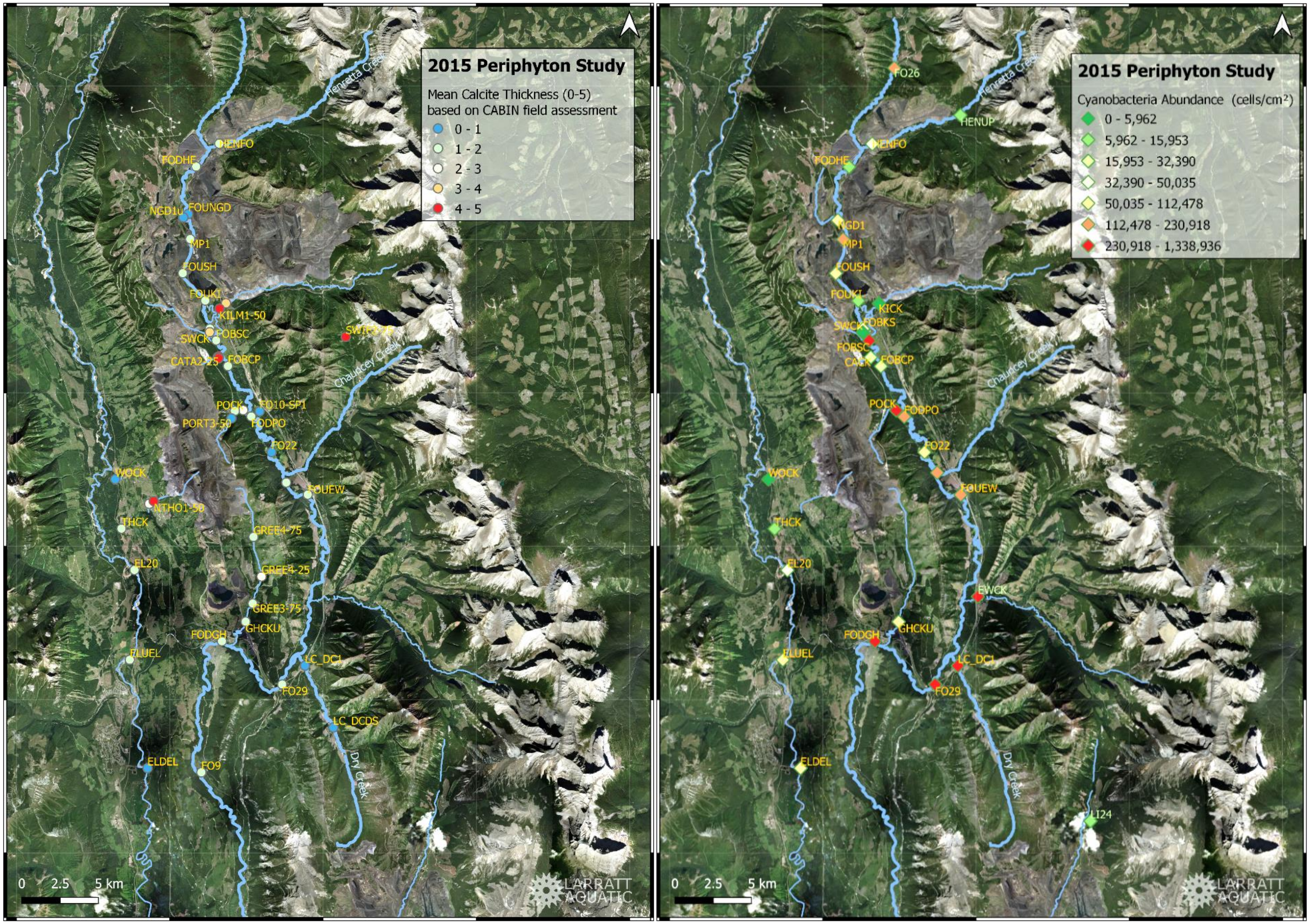


Figure A-30: Maps of field-observed calcite from CABIN assessment (1 – 5) and cyanobacteria abundance (cells/cm2)

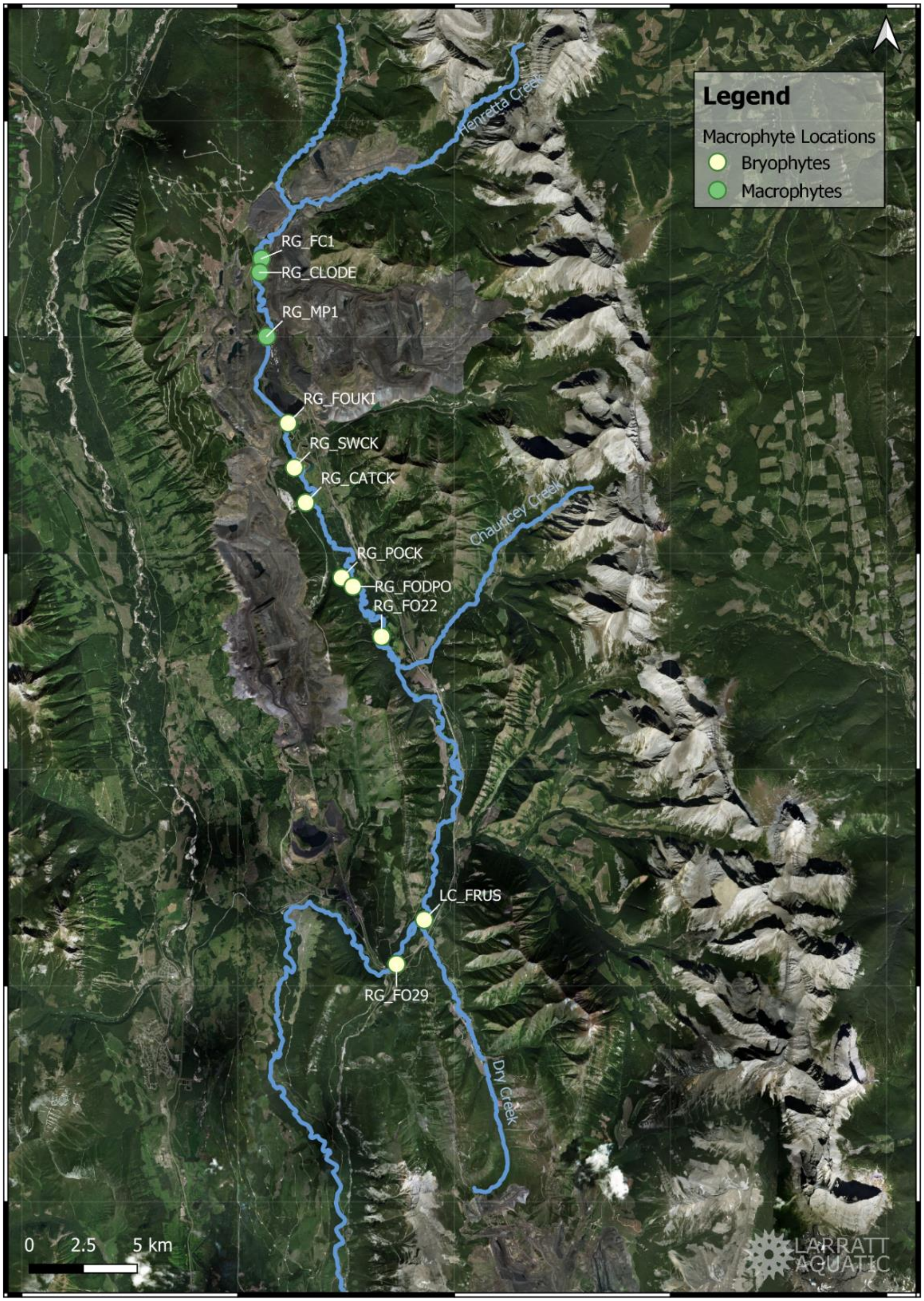


Figure A-31: Monitoring sites with aquatic macrophytes or bryophytes noted

Source: S. Cope, Minnow, Lotic, FRO staff

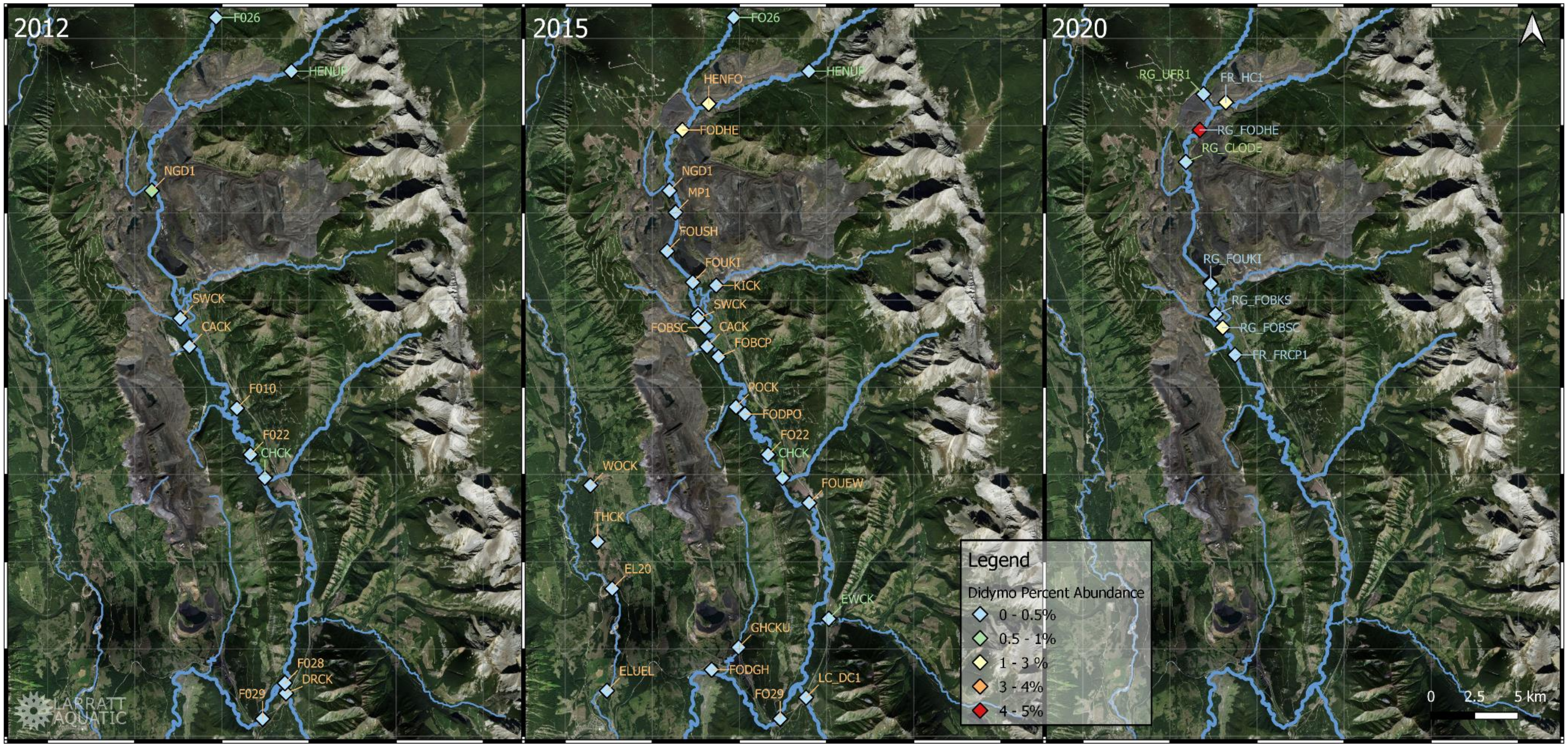


Figure A-32: Didymo percent abundance for Fall 2012, 2015 and Winter 2020 UFR periphyton samples

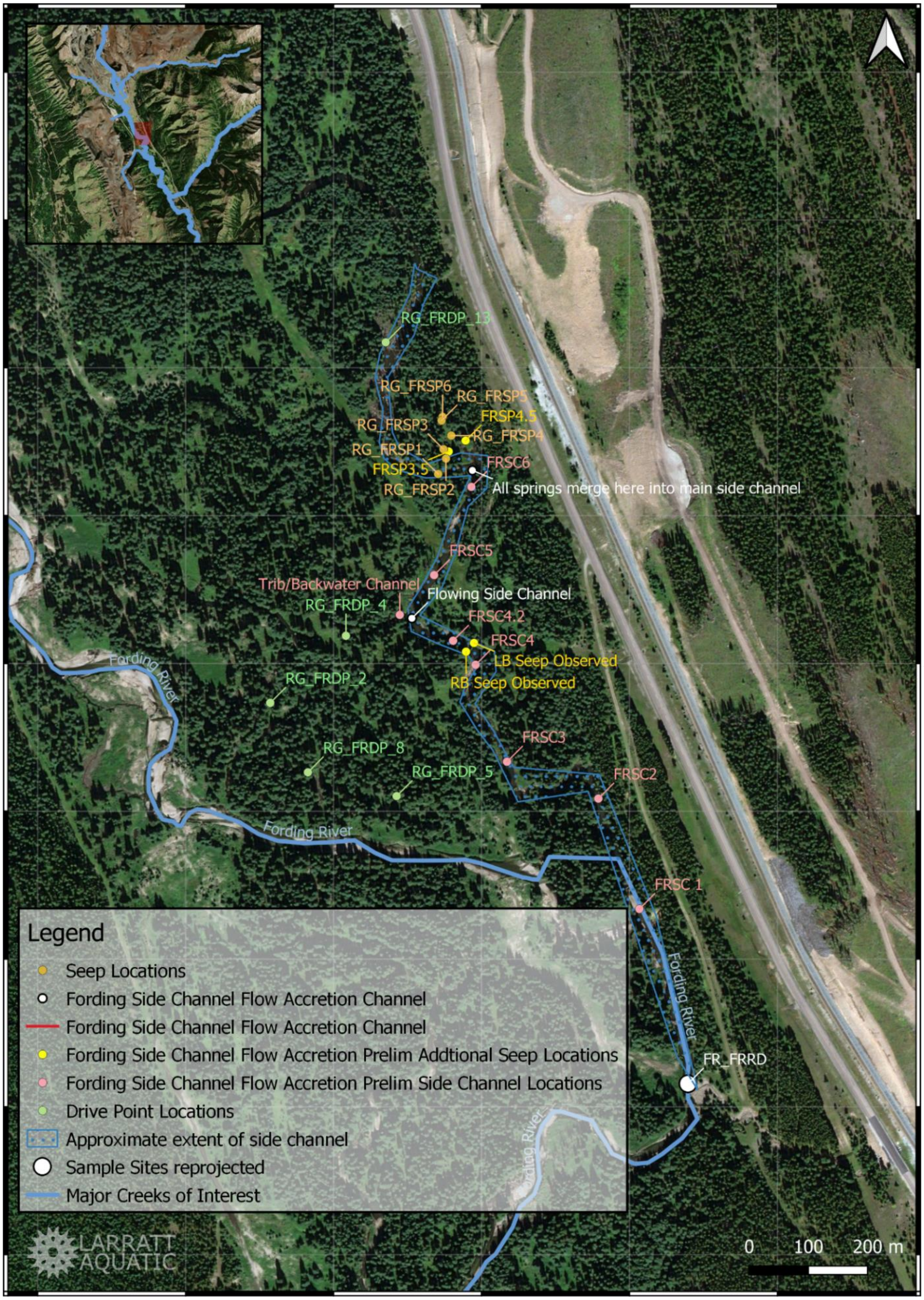


Figure A-33: UFR side channel sample locations from SNC Lavalin study

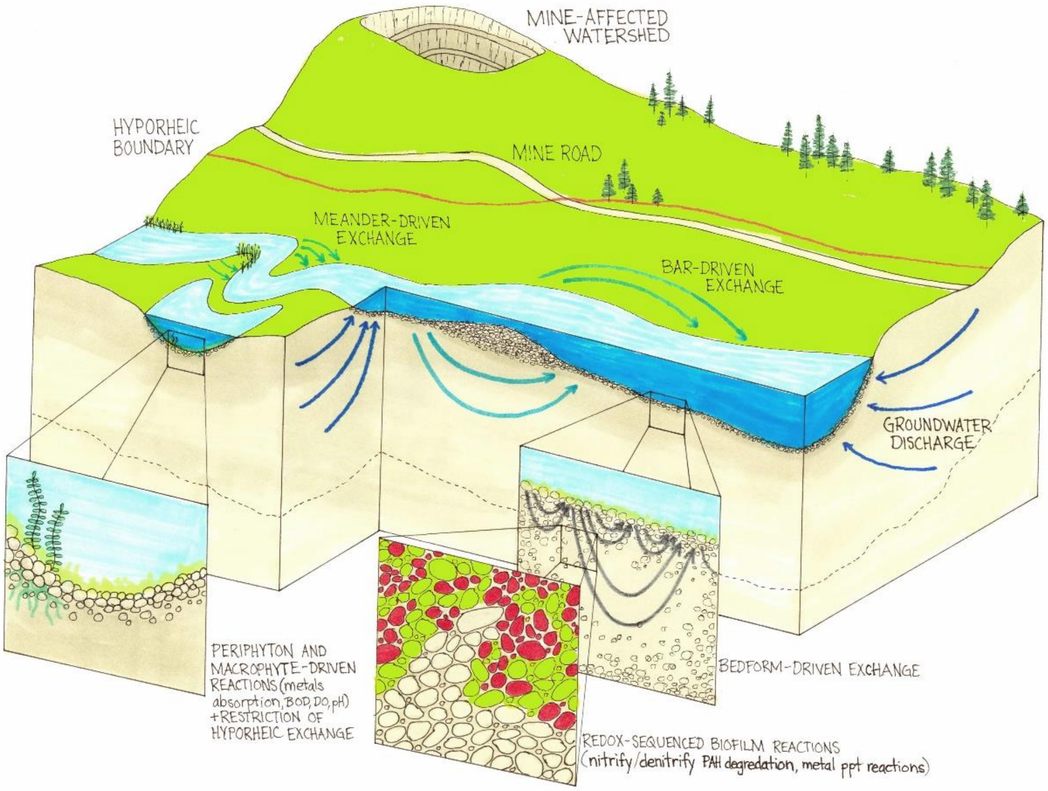


Figure A-34: Generalized schematic of hyporheic biogeochemistry showing a localized scale
(after Stonedahl et al. 2009).

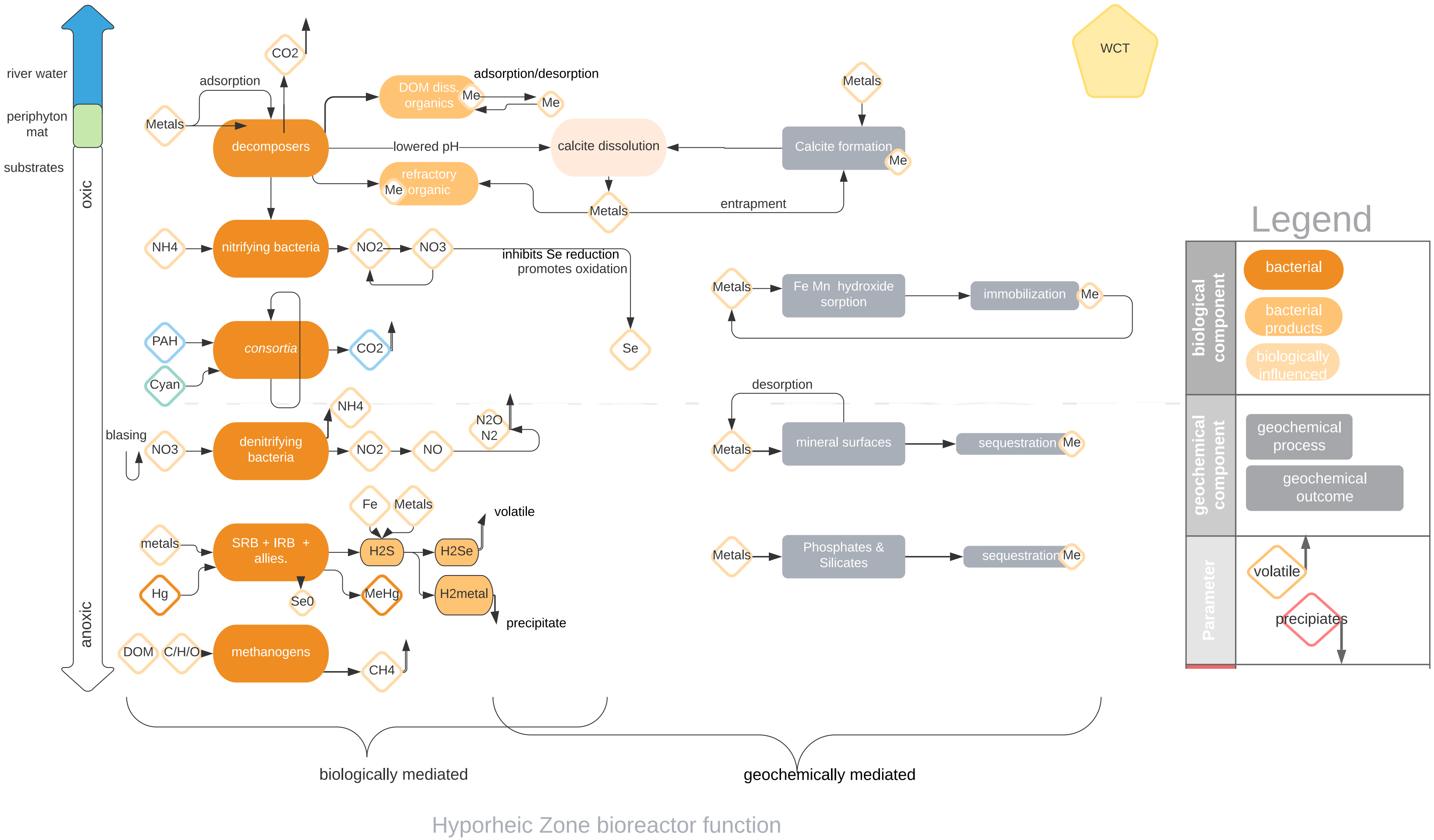


Figure A-35: Hyporheic zone bioreactor function showing process pathways and end products

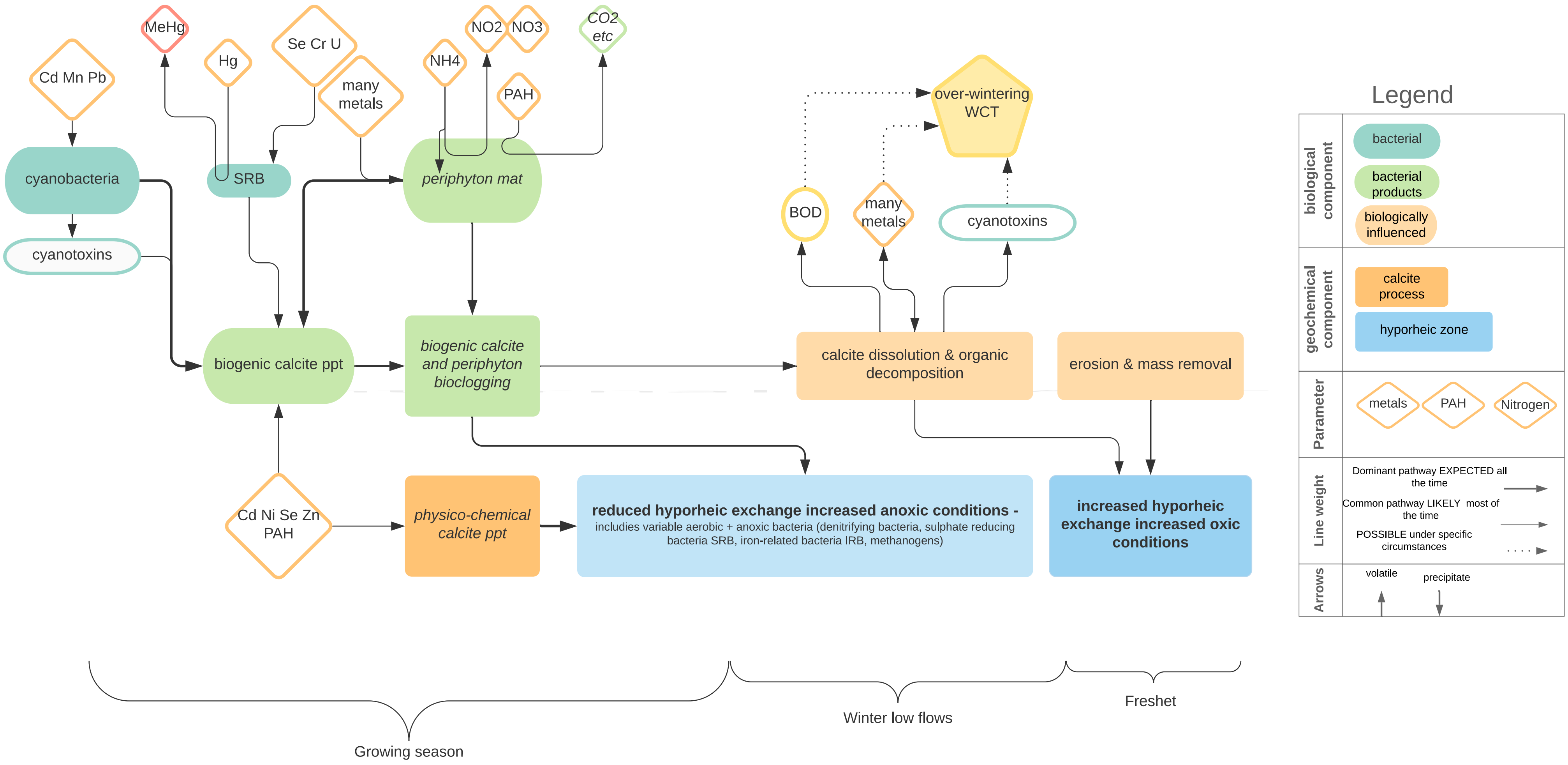
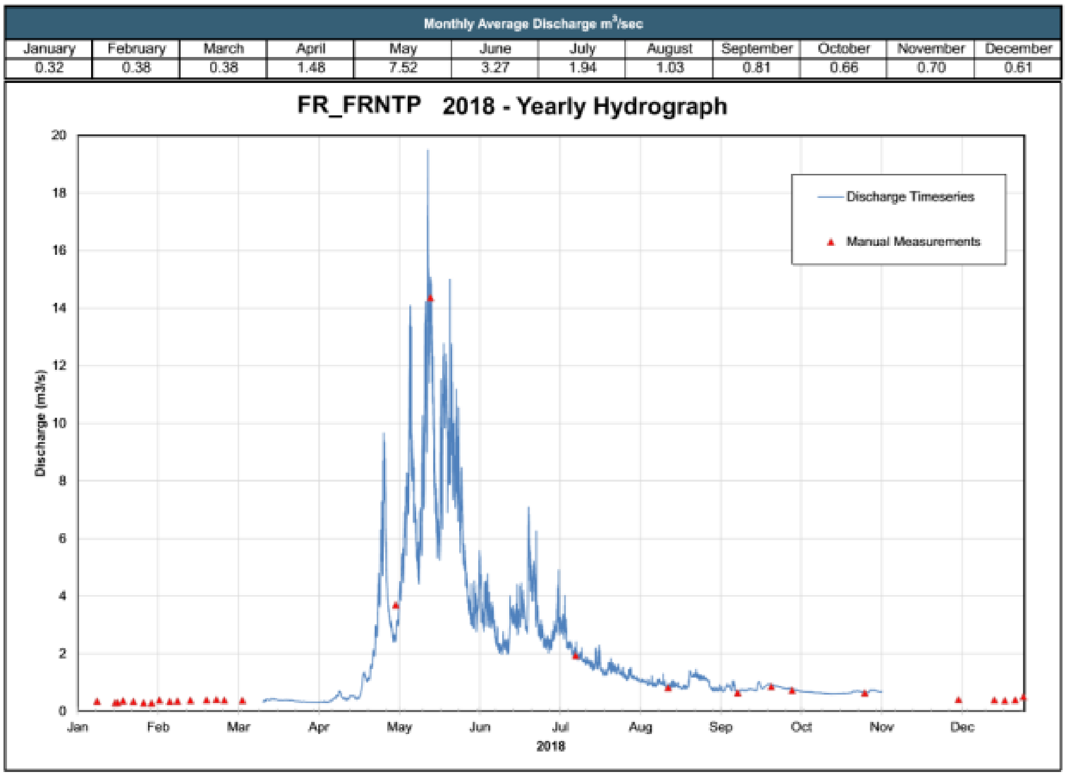


Figure A-36: Seasonal Periphyton mat bioreactor functions investigated in this report



Monthly Average Discharge m^3/sec

January	February	March	April	May	June	July	August	September	October	November	December
0.32	0.38	0.38	1.48	7.52	3.27	1.94	1.03	0.81	0.66	0.70	0.61

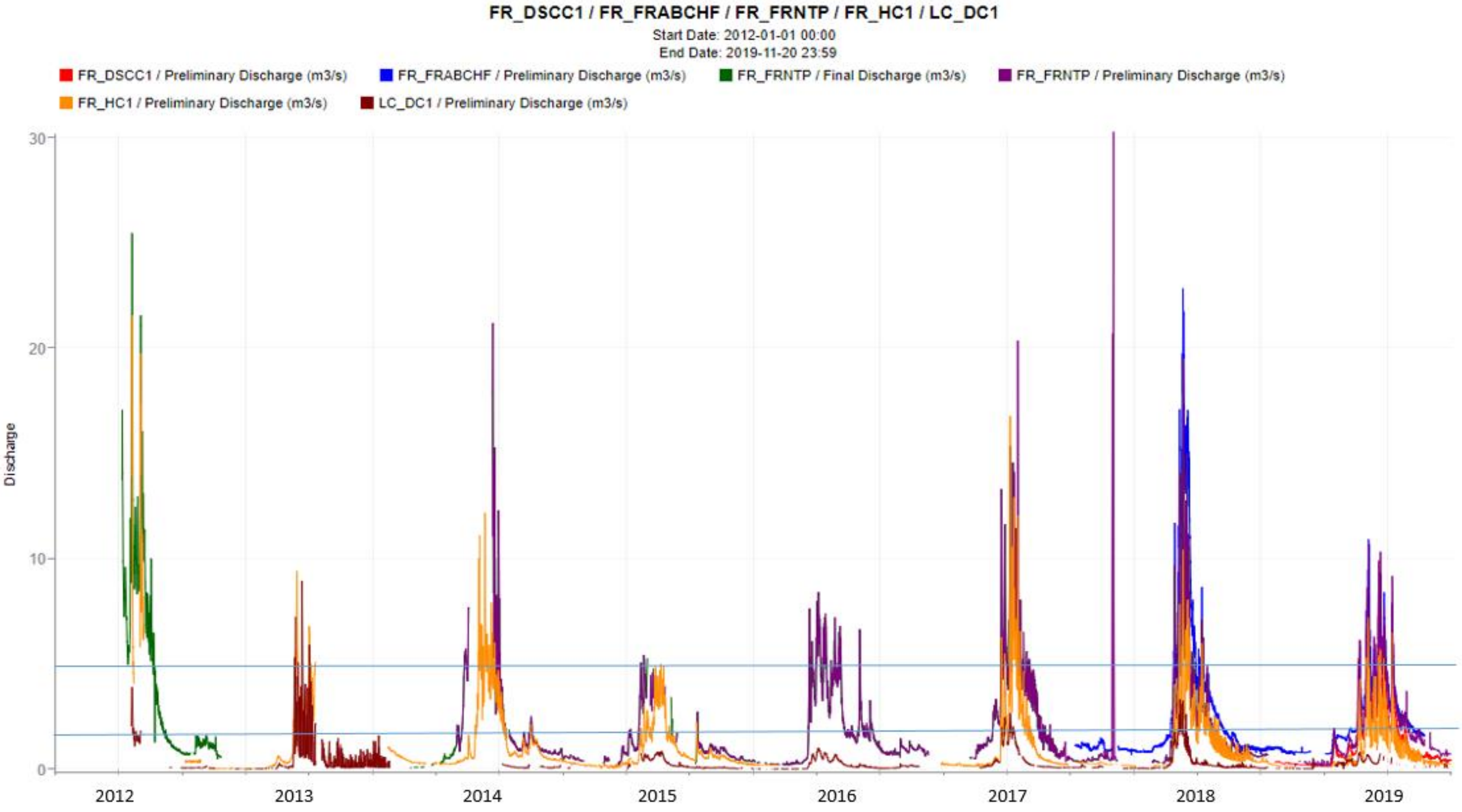


Figure A-37: Mean Annual Discharge for selected UFR mainstem sites 2012 - 2019

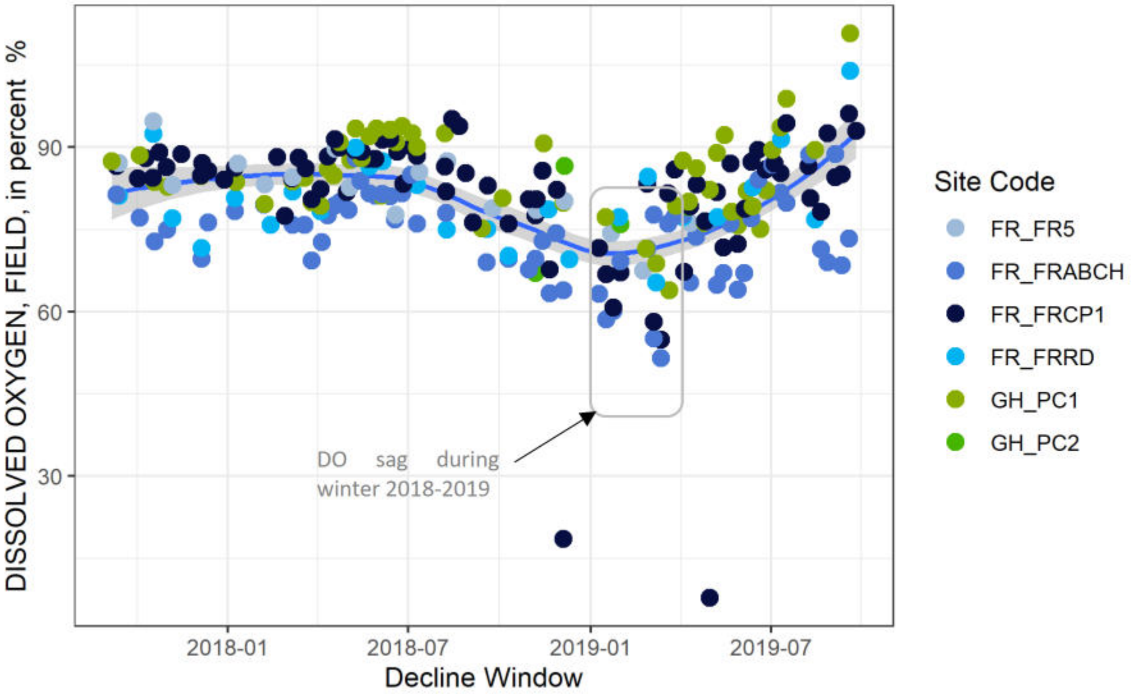


Figure A-38: Dissolved oxygen (% saturation) at UFR sites within or adjacent to S6 during the Decline Window

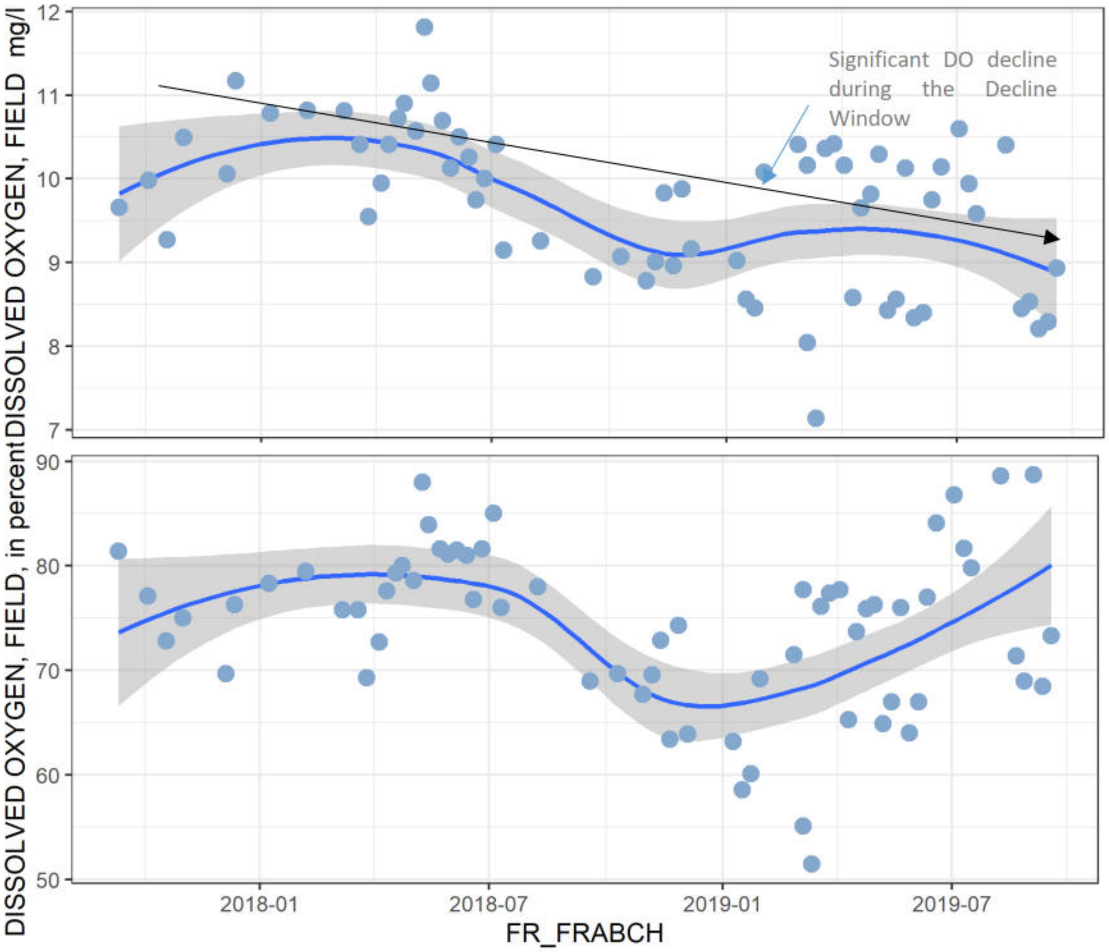


Figure A-39: Dissolved oxygen sag at FR_FRABCH during the WCT Decline Window

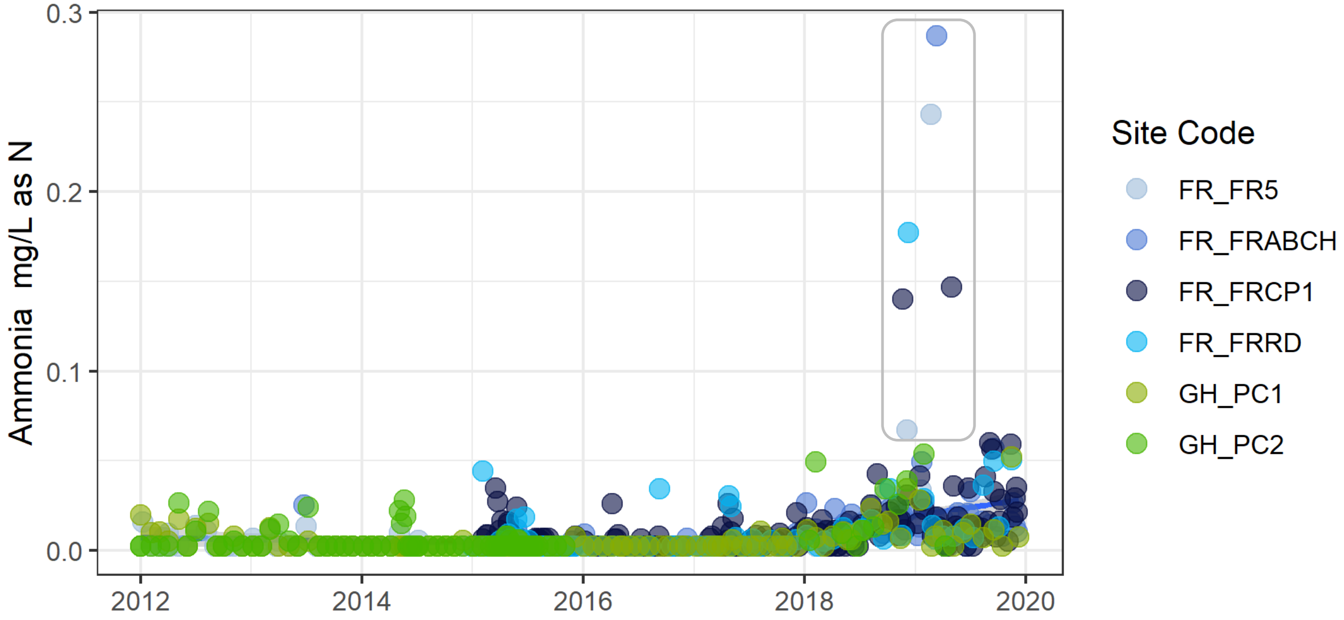


Figure A-40: Ammonia spike during Winter 2019 at UFR S6 region

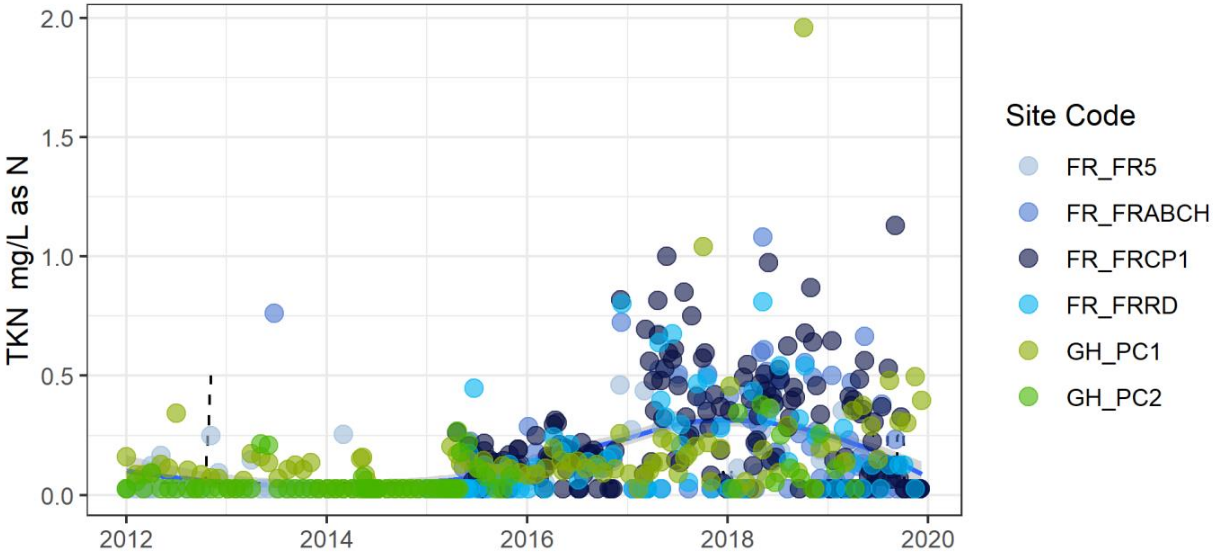


Figure A-41: TKN in sites near S6 reach from 2012-2020

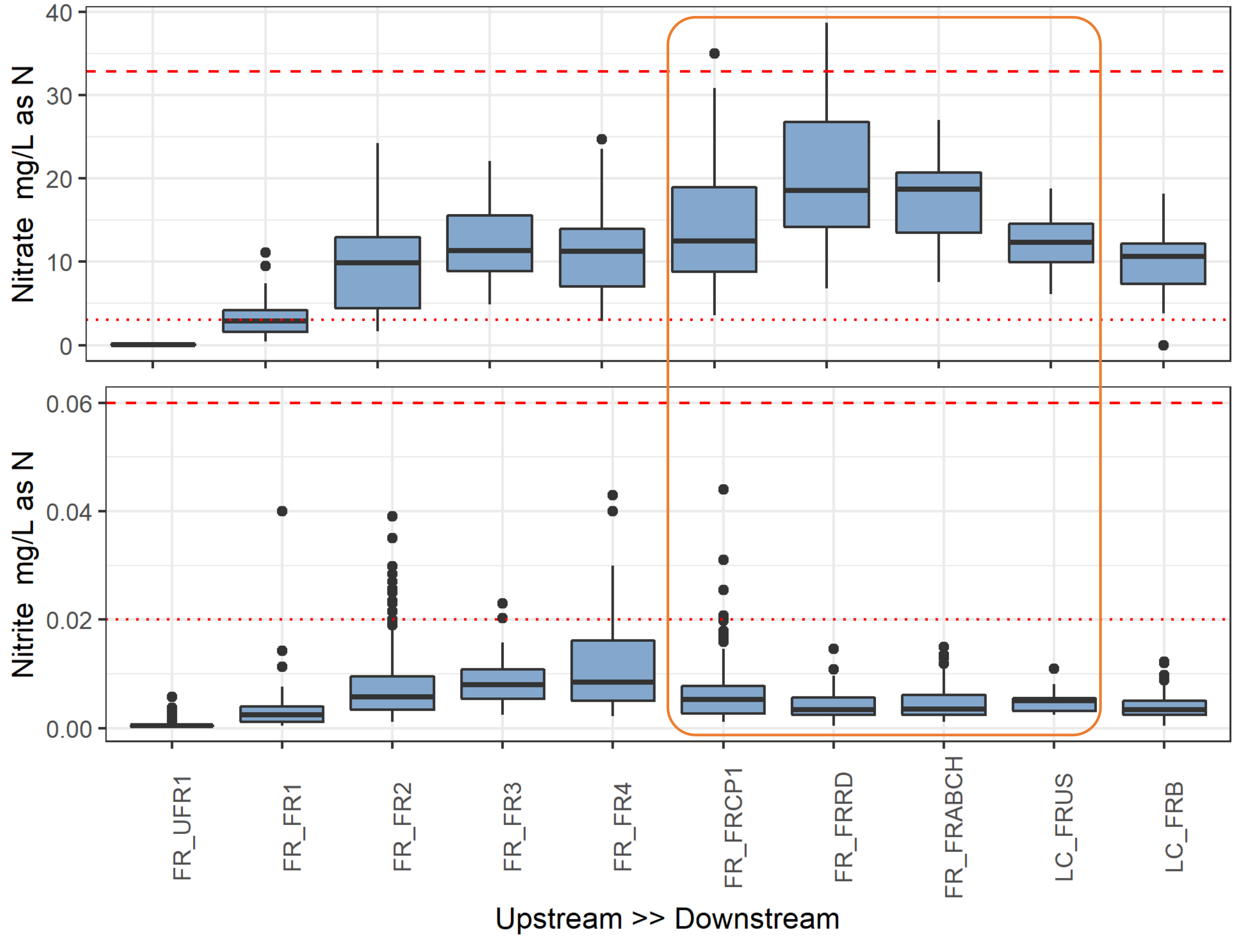


Figure A-42: Nitrate and Nitrite at upper Fording River mainstem sites in 2013 to 2019

Note: red dashed line = BC Aquatic Life MAC, red dotted line = BC Aquatic Life 30 day; orange box = area of high habitat value

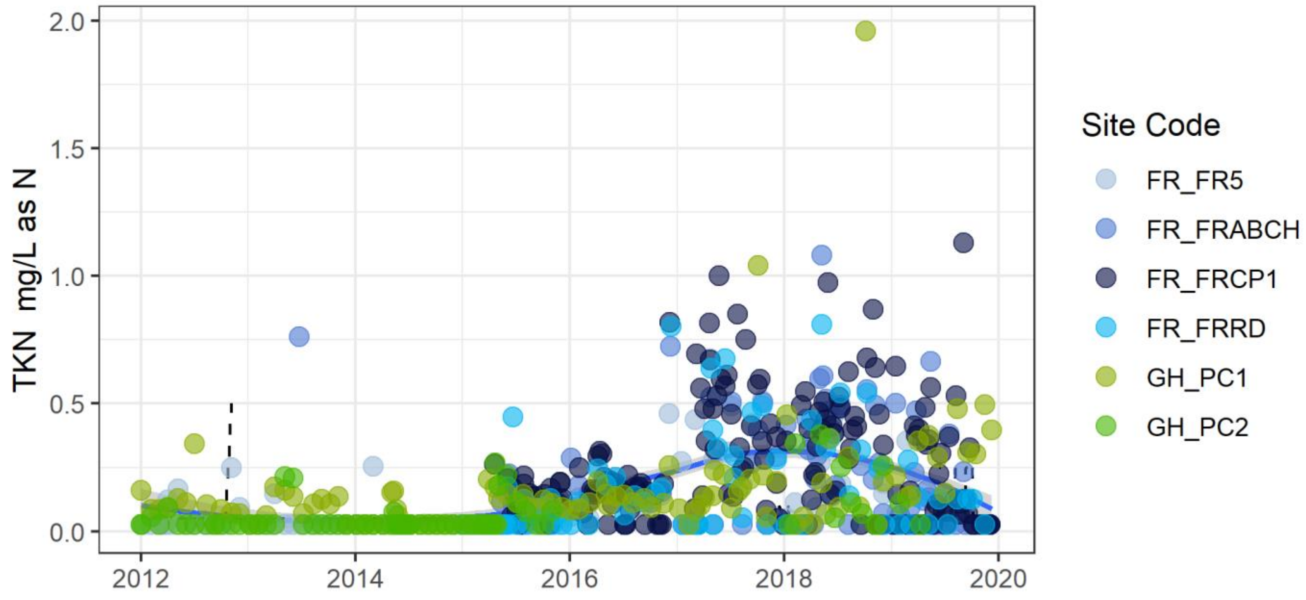


Figure A-43: TKN at upper Fording River S6 region in 2012 through 2019

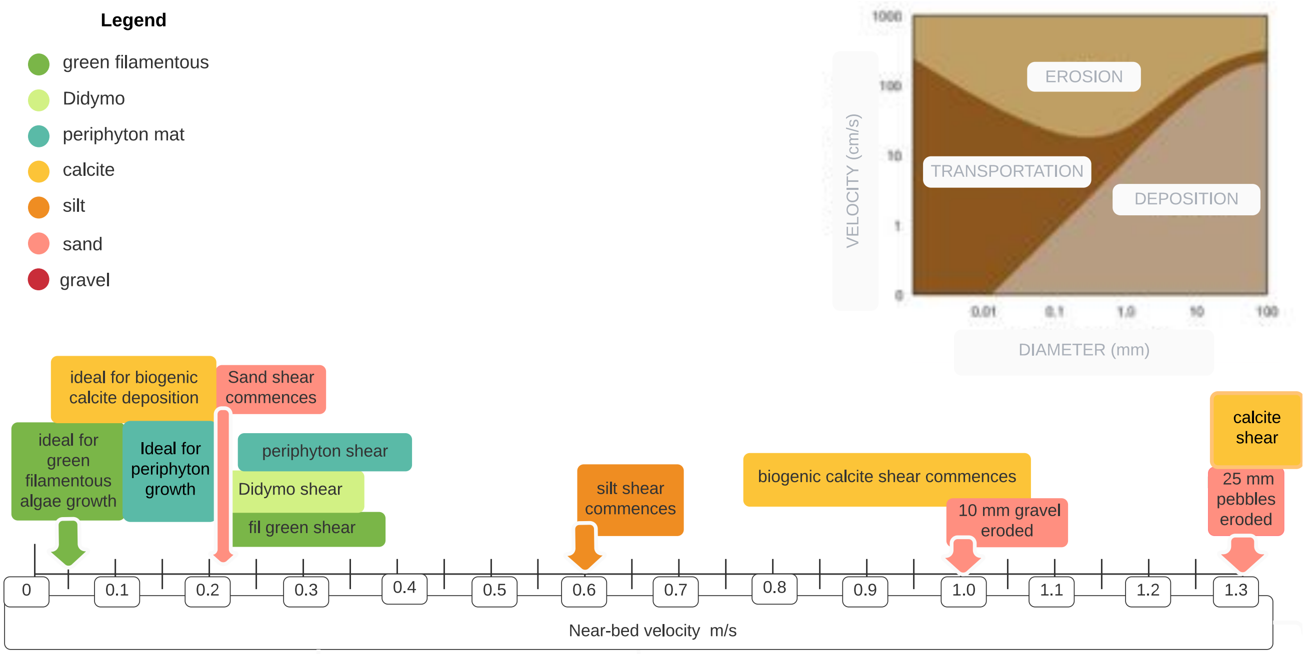


Figure A-44: Approximate near-bed velocities (m/s) at which growth occurs or shear commences for stream substrates

TABLES

LIST OF AFTER-TEXT TABLES

Table A-16: Trophic status table

	Lotic trophic status classifications and thresholds for phosphorus (TP ug/L)						
	Ultra-oligotrophic	Oligotrophic	Mesotrophic	Meso-eutrophic	Eutrophic	Hyper-eutrophic	Jusidiction/Reference
Trophic status	<4	4 - 10	10 - 20	20 - 35	35 - 100	>100	CCME 2004, 2016
	---	1 - 10	10 - 30	---	>30	---	BC WQG Nordin 1985 (lentic)
	---	4 - 10	10 - 30	---	30 - 100	---	Quebec in Galvanez-Cloutier and Sanchez 2007
Total P Thresholds					50		Alberta - aquatic life (AENV 1999)
			18				Alberta Bow River (Sosiak 2002)
	2						Alberta Montaine (Chambers and Guy 2004)
		5 avg 10 max					BC Vancouver Island (Nordin 2009; BCMOE 2012)
				25	50		Manitoba (Man. Wat. Steward. 2011)
			12				New Brunswick (Chambers et al 2008)
			24 - 30				Ontario (MOEE 1994)
			24 - 30				Quebec (MDE 2009)
			24				PEI (Van Den Heuvel 2009)

NOTE: Many of the TP thresholds are aligned with other primary guidelines (TN, chl-a, periphyton biomass)

Table A-17: Impacts of cyanotoxins on invertebrates and fish

Invertebrate Group	Exposure route	Impact of cyanotoxins	Tissue cyanotoxin concentrations
Zooplankton - cladocerans	Food	Highest cyanotoxin bioaccumulation. | Failure to thrive and reproduce| mortality |Some evidence of gene expression to detoxify cyanotoxins	Microcystin avg 383 ug/g range 0.01 – 1226 ug/g Nodularins ND – 6.2 Saxitoxins 0.07 – 0.38 ug/g
Zooplankton – copepods	Food	Moderate cyanotoxin bioaccumulation | Can avoid ingestion of toxic cyanobacteria, but that selectivity depends upon food availability and on degree of starvation	Microcystin avg 0.2 – 0.7 ug/g
Zooplankton - rotifers	Food	Reduced grazing rate, growth, and reproduction	
Invertebrates - chironomids	Food	Reproductive failure and mortality | High cyanotoxin bioaccumulation	
Invertebrates - Gammarus	Food	Freshwater shrimp accumulate microcystins in various organs, mostly in the hepatopancreas and gonads	Microcystin avg 0.09 – 0.7 ug/g Nodularin ND – 0.002 ug/g
Gastropods	Food and gills	Can expel living toxic cyanobacteria into pseudofeces and activate efficient depuration of tissues from microcystins	Microcystin up to 436 ug/g
Mollusks	Food and dissolved	Can expel living toxic cyanobacteria into pseudofeces and activate efficient depuration of tissues from microcystins (variable among taxa)	Microcystin avg 57 ug/g (96 - 195 ug/g) Nodularins/microcystins ND - 140 ug/g saxitoxinx 0.8 – 6.2 ug/g BMAA 251 – 305 ug/g
Decapods	Food and dissolved	Accumulate microcystins in various organs, mostly in the hepatopancreas and gonads	Microcystins avg 4.2 ug/g BMAA ND - 6976
Planktivorous fish	Food and dissolved	Bioaccumulation up to 2.9 times and at least partial detoxification of some cyanotoxins has been documented	Microcystins 49 – 137 ug/g Nodularins 0.15-0.80 ug/g
Omnivorous fish	Food and dissolved		Microcystins ND – 678 ug/g Nodularins ND – 0.9 ug/g
Carnivorous fish (e.g. Trout)	Food and dissolved	Biodilution of microcystins is possible, while accumulation of other cyanotoxins has been determined. Early life stages are usually found to be more susceptible than adults.	Microcystins 0.04 – 0.79 ug/g Nodularins 0.003 – 1.1 ug/g

Fadness 2018 | Bownik 2013 |Guo and Xie 2006 | Bednarska 2006 | Gustafsson and Hanson 2004 | Rohrlack et al 2005 | Demott and Moxter 1991 |Ferrao-Filho and Kozlowsky-Suzuki 2011

Cyanotoxin LD50 (ip) ug/kg mouse assay: 50-1000 microcystins | 50 nodularin | 8-10 saxitoxins | 20-250 anatoxins | 299-2100 Cylindrospermopsins (Zanchett and Oliveira-Filho 2013)

Table A-18: Working Sediment Quality Guidelines (BC WSQGs) and mean uncontaminated lentic ranges (minimum-maximum) from the upper Fording River

sediment				Calcite		Lotic Sediment		Lentic Sediment MU1	
Metal (mg/kg DW)	Lower WSQG	Upper WSQG	Mean Lentic Sediment - S. Rockies	reference (2 sites)	mine influenced (3 sites)	reference (2 sites)	mine influenced (6 sites)	reference (RG_UPGHC)	mine influenced (7 sites)
Arsenic (As)	5.9	17	15.48 ± 10.35	4 - 10	<2 - 4	1.15 - 5.86	2.32 - 7.28	0.90 - 1.68	2.48 - 7.63
Cadmium (Cd)	0.6	3.5	1.22 ± 0.64	0.66 - 0.95	1.68 - 21.3	0.289 - 1.39	0.828 - 2.39	1.28 - 1.58	0.59 - 4.75
Calcium (Ca)			77500 ± 79460			38600 - 269000	35700 - 124000	16700 - 22800	16500 - 341000
Chromium (Cr)	37.3	90	252.2 ± 166.8			5.15 - 21.3	5.07 - 28.8	6.61 - 15.0	4.16 - 35.0
Copper (Cu)	35.7	197	178.9 ± 84.46			1.33 - 18.1	7.32 - 16.1	12 - 18	3.40 - 40
Iron (Fe)	21,200	43,766	23400 ± 15970			2350 - 20100	6990 - 34500	2670 - 6540	2270 - 14100
Lead (Pb)	35	91	37.89 ± 19.69			1.70 - 10.2	4.77 - 9.94	8.35 - 11.0	1.25 - 20.0
Manganese (Mn)	460	1,100	738.2 ± 1485	25 - 41	49 - 309	94.5 - 672	250 - 1260	43 - 170	38.0 - 7330
Mercury (Hg)	0.17	0.486	0.09 ± 0.04			0.007 - 0.067	0.027 - 0.055	0.058 - 0.113	0.011 - 0.124
Nickel (Ni)	16	75	27.26 ± 25.68	4.4 - 9.1	16.3 - 36.1	6.74 - 24.6	21.2 - 70.4	4.64 - 7.57	14 - 42
Selenium (Se)	2	2	14.33 ± 5.67	2.9 - 3.8	1.4 - 25.3	0.28 - 1.88	1.13 - 8.91	1.33 -3.16	0.83 - 21.0
Zinc (Zn)	123	315	106.2 ±100.8	30 - 182	74 - 550	40.4 - 115	88.6 - 169	64 - 132	32 - 183
Phosphorus (P)	--	--	888.7 ± 458.3	190 -1390*	420 - 630	360 - 1380	700 - 1980	1320 - 1940	348 - 1650
Kjeldahl N	--	--	7510 ± 5760						
Organic carbon %	--	--	7.78 ± 5.97			5.1 - 11.7	4.68 - 13.8	25 - 30	6.58 - 23
			Reiberger 1992	SRK 2014 *GHO		Minnow 2017 - 2019		Minnow 2018	
			n = 27	n = 2	n = 3	n = 2	n = 16	n = 1	n = 7

From :Rieberger 1992

Table A-19: Summary statistics of 2012, 2015, and 2020 periphyton samples in UFR

Periphyton Data	Sep 2012	Sep 2015	Feb 2020
	Quantitative samples	Quantitative samples	Grab samples
Average species richness /site	17	23	19
Range of species richness	7 - 44	11 - 32	14 - 27
Average abundance (cells/cm^2 x 10^5)	9.9	51.6	--
Range of abundance (cells/cm^2 x 10^5)	1.1 - 55.6	2.4 - 274.8	--
% rhodophyta (red algae)	0.2	3.9	0
% chlorophyta (green algae)	1.8	3.3	4.4
% cyanophyta (blue-green algae)	10.6	11.0	15.5
% chrysophyta (golden algae)	15.8	13.5	7.4
% diatoms	71.3	67.1	72.3
Phormidium autumnale abundance (cells/cm^2 x 10^5)	5.18	0.75	(0.80%)
Didymo abundance (cells/cm^2 x 10^5)	0.34	0.047	(1.30%)
Filamentous green algae (cells/cm^2 x 10^5)		0.016	(4.40%)
number of sites sampled	19	24	11
number of samples (n) including duplicates	27	72	12

NOTE: 2020 winter samples were grab samples due to field sampling conditions

Data from LAC work on UFR (White and Larratt 2016; this report)

Table A-20: Dominant taxa by abundance in periphyton samples, fall 2015 vs winter 2020

	2015		2020
	Exposed	**Reference**	**Exposed**
1	*Achnanthidium minutissimum*	*Hydrurus foetidus*	*Achnanthidium minutissima*
2	*Homeothrix sp.*	*Achnanthidium minutissimum*	*Achnanthidium linearis*
3	*Achnanthidium minutissimum*	*Cymbella minuta*	*Phormidium sp.*
4	*Diatoma vulgare*	*Homeothrix sp.*	*Leptolyngbya*
5	*Hydrurus foetidus*	*Navicula spp.*	*Flagellates lrg.*
6	*Diatoma elongatum*	*Phormidium autumnale*	*Diatoma vulgaris*
7	*Cladophora sp.*	*Encyonema silesiacum*	*Gomphonema sp.*
8	*Audouinella sp.*	*Heteroleibleinia sp.*	*Oscillatoria sp*
9	*Cymbella minuta*	*Cymbella excisiformis*	*Cymbella exisiformis*
10	*Navicula capitatoradiata*	*Gomphonema minutum*	*Synedra sp.*

NOTE: There were no reference sites samples in winter 2020
Dominants selected by ranking all taxa abundance and selecting the highest 10 taxa
Data from LAC work on UFR (White and Larratt 2016; this report)

Table A-21: Nutrient limitation ratios for streams compared to results from UFR sites

UFR	reference sites	mine-influenced sites	ratio descriptions (Ptacnik et al. 2010)			ratio descriptions (McDowell et al. 2009)		
			N-limited	N+P limited	P limited	N-limited	N+P limited	P limited
TN : TP	52 : 1	907 : 1	<35 : 1	35 - 60	>60 : 1			
DIN : PO_4	47 : 1	1482 : 1	< 16 : 1	>16 : 1	>50 : 1	< 7 : 1	7 - <15	> 15 : 1
DIN : TP	35 : 1	866 : 1	<2 : 1	2 - 5	>5 : 1			

NOTE : field filtered PO_4~ SRP | 29 mine-influenced sites >300 samples | 2 reference sites >29 samples

NOTE: ratios based on median data, can be conducted site-by-site

Data from Minnow 2020a 2020b

Table A-22: Sediment TOC to PAH Correlations and Low vs High molecular weight PAH, UFR 2018 and 2019

Correlations between 2018-2019 UFR Sediment Total Organic Carbon (Periphyton) and PAHs, Metals of Interest

PAH	Correlation
Acenaphthene	not detected
Acenaphthylene*	0.798
Benz(a)anthracene*	0.916
Benzo(a)pyrene	0.847
Chrysene	0.858
Dibenz(a,h)anthracene	not detected
Fluorene	0.859
2-Methylnaphthalene	0.851
Naphthalene	0.844
Phenanthrene	0.855
Pyrene	0.861
Metal of Interest	**Correlation**
Arsenic..As.	-0.283
Cadmium..Cd.	0.553
Chromium..Cr.	-0.245
Iron..Fe.	-0.138
Manganese..Mn.	0.711
Nickel..Ni.	0.388
Phosphorus..P.	-0.341
Selenium..Se.	0.631
Zinc..Zn.	0.428

*> 70% of samples below RDL (See Appendix 1 for Non-detect treatment) Pearson's Correlation coefficient (r) In low vs high m.wt. calculations, ND data was handled as ½ of RDL

2018	TOC %	low m wt PAHs (mg/kg)	high m wt PAHs (mg/kg)
RG_HENUP (ref)	5.2	0.01	0.01
RG_FO26 (ref)	5.88	0.09	0.01
RG_FOUKI	13.6	0.67	0.11
RG_FOBKS	12.6	0.37	0.05
RG_FOBCP	5.6	0.10	0.02
RG_FRUPO	4.4	0.04	0.01
RG_FO22	4.75	0.05	0.01
RG_SCOUT			
2019			
RG_HENUP (ref)	5.6	0.02	0.01
RG_FO26 (ref)	8.39	0.15	0.02
RG_FOUKI	9.37	0.32	0.03
RG_FOBKS	8.9	0.24	0.04
RG_FOBCP	6.6	0.24	0.03
RG_FRUPO	5.64	0.11	0.02
RG_FO22	5.87	0.06	0.01
RG_SCOUT	7.03	0.21	0.02

NOTES: Low molecular wt PAH: Fluorene | 2Methylnapthalene | Napthalene | Phenanthrene | Acenaphthalene | Acenapththylene
High molecular wt PAH: Pyrene | Benz(a)anthracene | Benzo(a)pyrene | Chrysene | Dibenz(a,h)anthracene
(Data from Minnow 2020a 2020b)

Table A-23: Summary of selected selenium TFFs for invertebrates and fish

Invertebrate	TTF
Amphipod (freshwater) (*Hyalella azteca, Gammarus fasciatus, Corophium spp.)*	0.9
Zooplankton (freshwater composite)	1.5
Daphnia (*Daphnia magna*)	1.9
Stonefly (*Perlodidae/Perlidae, Chloroperlidae*)	2.6
Damselfly (*Coenagrionidae*)	2.6
Mayfly (*Baetidae, Heptageniidae, Ephemerellidae*)	2.7
Chironomid (*Chironomus sp.*)	2.7
Caddisfly (*Rhyacophilidae, Hydropsychidae*)	3.2
Aquatic insect (average)	2.8
Aquatic insect composite	3.2
Fish (whole-body or muscle)	**TTF**
Rainbow trout (*Oncorhynchus mykiss*)	0.98
Fathead minnow (larval and adult) (*Pimephales promelas*)	1
Cutthroat trout (*Oncorhynchus clarkii*)*	1
Brown trout (*Salmo trutta*)	1.3
Mottled sculpin (*Cottus bairdi*)	1.4
Longnose dace (*Rhinichthys cataractae*)	1.5

Dry Creek TTF for benthic invertebrates 3.57 at sites with Didymo bloom
Dry Creek TTF for benthic invertebrates 1.19 at sites without Didymo bloom
Source: (Presser and Luoma 2010; Cianciolo et al 2019) (see also Orr et al. 2006) *
May be similar to WCT

Table A-24: Dry Creek tissue selenium concentrations 2018-2019

PERIPHYTON Se ug/g dry wt

	Upper Dry Creek		Lower Dry Creek	
Timing	Site			
	LC_SPDC	LC_DCDS	LC_DC4 / L	LC_DC1
Nov/Dec	11	12	-	5.9
	10	9.6	-	5.9
	11	10	-	6.2
Feb-19	9.1	30	12	
	14	16	12	
	26	14	8.2	

BENTHIC INVERTEBRATE Se ug/g DW

	Upper Dry Creek		Lower Dry Creek	
Timing	Site			
	LC_SPDC	LC_DCDS	DC4 / LC_D	LC_DC1
Nov/Dec-18	35	40	10	10
	48	46	10	10
	44	52	10	10
	32	60	7.8	11
	60	54	7.9	10
Feb-19	61	120	11	
	48	49	11	
	40	47	10	
	36	55	9.3	
		50	11	

(Data from Minnow 2020a 2020b)

Table A-25: Fines entrapment potential of dominant UFR periphyton taxa by abundance, Fall 2015

	Taxa	Mechanical entrapment	Extracellular polymers	Muco-polysaccharide strands
DIATOMS	Achnanthidium minutissimum		✓	
	Cymbella minuta (E. minutum)		✓	
	Diatoma vulgaris		✓	
	Gomphoneis minuta			✓
	Gomphonema minutum			✓
	Navicula cryptocephala		✓	
	Synedra ulna		✓	
	Didymosphenia geminata	✓	✓	✓
CYANOBACTERIA				
	Heteroleibleinia sp. (profunda?)	✓	✓	
	Homeothrix sp. (janthina?)	✓		
	Nostoc sp.	✓	✓	
	Phormidium autumnale	✓	✓	
GOLD	Hydrurus foetidus or sp.	✓	✓	
RED	Audouinella sp.	✓	✓	
GREEN				
	Cladophora sp. (glomerata?)	✓	✓	
	Stigeoclonium spp.	✓	✓	

Data from LAC periphyton analyses (White and Larratt 2016)

Table A-26: Instream and Streamside Vegetation in the upper Fording River, September 2015, 2016, 2019

Station ID		2015				2016			2019		
		Canopy Coverage (%)	Macrophyte Coverage (%)	Periphyton Coverage Score (1-5)	Moss ID'd in Peri samples	Canopy Coverage (%)	Macrophyte Coverage (%)	Periphyton Coverage Score (1-5)	Canopy Coverage (%)	Macrophyte Coverage (%)	Periphyton Coverage Score (1-5)
Reference	RG_HENUP	1	0	2		1 - 25	-	-	1 - 25	0	1
	RG_CHCK	0	0	3		-	-	-	-	-	-
	RG_EWCK	5	0	4		-	-	-	-	-	-
	RG_DCEF	-	-	-		-	-	-	26 - 50	0	3
	RG_FO26	12	0	2/3		1 - 25	0	-	1 - 25	0	1
Mine-exposed Tributaries	RG_HENFO	1	0	2		-	-	-	-	-	-
	RG_FC1	-	-	-		-	-	-	1 - 25	1 - 25	3
	RG_CLODE	-	-	-		-	-	-	0	1 - 25	2
	RG_KICK	0	0	2		-	-	-	0	0	2
	RG_SWCK	99	0	2	M	-	-	-	-	-	-
	RG_CATCK	2	0	2	M	-	-	-	-	-	-
	RG_POCK	76 - 100	0	3	M	-	-	-	-	-	-
	LC_DC3	-	-	-		-	-	-	1 - 25	0	3
	LC_SPDC	-	-	-		-	-	-	0	0	4
	LC_DCDS	31	0	2		26 - 50	0	3	51 - 75	0	3
	LC_DC2	-	-	-		-	-	-	1 - 25	0	3
	LC_DC4	-	-	-		-	-	-	1 - 25	0	3
	LC_DC1	33	0	3		28	0	2	1 - 25	0	3
	RG_GHCKU	76 - 100	0	2		-	-	-	-	-	-
	RG_GHCKD	44	0	3					1 - 25	0	1
Mine-exposed Main Stem Areas	RG_FODHE	0	0	2		0	0	-	0	0	4
	RG_FOUCL		-	-		-	-	-	1 - 25	0	3
	RG_FOUNGD	26	0	2		-	-	-	0	0	3
	RG_FODNGD	40	0	2		-	-	-	1 - 25	0	2
	RG_MP1	0	0	2		-	-	-	0	26 - 50	2
	RG_FOUSH	5	0	2		-	-	-	0	0	2
	RG_FOUKI	2	0	2	M	0	0	2	0	0	2
	RG_FOBKS	26	0	2		1 - 25	0	1	1 - 25	0	1
	RG_SCOUTDS		-	-		-	-	-	0	0	1
	RG_FOBSC	2	0	2		1 - 25	0	1	0	0	2
	RG_FOBCP	10	0	2		1 - 25	0	1	1 - 25	0	2
	RG_FRCP1SW		-	-		-	-	-	0	0	3
	RG_FRUPO		-	-		-	-	-	1 - 25	0	2
	RG_FODPO	51 - 75	0	3	M	1 - 25	0	1	-	-	-
	RG_FO22	7	0	3	M	-	-	-	1 - 25	0	-
	RG_FO29	-	-	-	M	-	-	-	-	-	-
	RG_FOUEW	22	0	3		1 - 25	0	1	1 - 25	0	2
	LC_FRUS	47	0	3	M	26 - 50	0	3	1 - 25	0	2
	LC_FRB	18	0	3		12	0	3	1 - 25	0	3
	RG_FODGH	76 - 100	0	3		1 - 25	0		1 - 25	0	2

Notes: Periphyton coverage scores are as follows:
1 - Rocks not slippery, no obvious colour (<0.5mm thick)
2 - Rocks slightly slippery, yellow-brown to light green in colour (0.5-1mm thick)
3 - Rocks have noticeble slippery feel, patches of thicker green to brown algae (1-5mm thick)
4 - Rocks are very slippery, numerous clumps (5-20mm thick)
5 - Rocks mostly obscurred by algae mat, may have long strands (>20mm thick)
"-" indicates no data recorded.

Data combined from FRO LAEMP Reports 2016-2019 (Minnow reports)

Table A-27: LAC Visual assessment of substrate and periphyton photography from Minnow Environmental

	field score	September Periphyton						Winter Periphyton		Winter substrate illumination		
Site	Minnow Sept 2019	Periphyton cover Sep 2018	Periphyton cover Sep 2019	Filamentous green? 2018	Filamentous green? 2019	Possible Didymo? 2018	Possible Didymo? 2019	Periphyton cover Dec 2018	Periphyton cover Feb 2019	Snow crust Feb 2019	Deep ice Feb 2019	Silt?
HENUP	1	low	moderate	no	no	no	no	n/a	n/a	n/a	n/a	
FO26	1	low	low	yes	yes	no	no	n/a	n/a	n/a	n/a	
FODHE	4	moderate	mod-high	no	no	yes	yes	--	--	yes	yes	
FOUNDG	3	low	mod	no	no	no	no	low	high	no	no	some
MP1	2	high	mod-high	no	no	yes	?	--	--	yes	yes	
FOUSH	2	low	low	?	no	no	no	--	--	yes	yes	
FOUKI	2	--	--	no	no	--	?	low	high	no	no	
FOBKS	1	low	low	no	no	no	no	--	--	yes	yes	
FOBSC	2	--	moderate	no	?	no	?	low	--	yes	yes	
FOBCP	2	low	n/a	no	no	no	no	low	low	yes	yes	some
FRUPO	2	--	--	--	--	--	--	high	mod	no	no	
FODPO	-	?	low	no	no	?	no	high	low	no	no	
FO22	-	moderate	low	no	no	?	no	mod	low	no	no	yes
FOUEW	2	mod-high	high	no	no	yes	yes	high	low	no	no	some
FO20 FRUS	2	moderate	mod-low	no	no	?	?	n/a	n/a	n/a	n/a	
FO29 FRB	3	moderate	mod-low	some	no	some	no	n/a	n/a	n/a	n/a	some
FODGH	2	high	--	no	--	yes	n/a	n/a	n/a	n/a	n/a	

n/a	no photo
--	can't tell
?	possible

Table A-28: Periphyton metrics from February 20, 2020 grab sampling

	February 19-21 2020 Winter Periphyton	FR_HC1 R. km 64.5	RG_UFR1 r.km 65	RG_CLODE R.km 61.5	FR_FODHE R. km 63.5	(FR_FCWP1) Fish Pond R. km 62	FR_CC1H Clode Pond R.km 61.5	FR_FC1 Fish Pond Ck R.km 62	RG_FOUKI (1) R. km 55.5	RG_FOUKI (2) R. km 55.5	RG_FOBKS R. km 54	RG_FOBSC R.km 53.5	FR_CP1 R. km 52	Average
Sample Results 2020	Percent cyanobacteria abundance	40.1%	52.4%	0.0%	2.5%	0.0%	1.2%	13.2%	41.6%	0.0%	4.4%	0.0%	30.1%	15.5%
	Percent diatoms abundance	57.1%	18.5%	83.6%	90.0%	82.1%	86.6%	81.5%	48.7%	76.6%	84.0%	87.7%	53.2%	70.8%
	Percent Didymo abundance	2.0%	1.2%	0.3%	4.2%	0.0%	0.0%	0.0%	1.3%	4.3%	0.3%	1.4%	0.5%	1.3%
	Percent filamentous green abund.	1.2%	12.5%	3.1%	5.0%	4.5%	0.0%	2.0%	0.0%	12.8%	1.2%	6.8%	4.2%	4.4%
	Percent flagellates abundance	1.7%	9.5%	13.3%	2.5%	13.4%	12.2%	0.0%	8.7%	0.0%	10.4%	5.5%	11.6%	7.4%
	Species Richness	14	22	22	19	27	14	20	19	21	13	22	16	19
	Diatom Species Richness	23	9	16	19	11	20	15	16	12	18	11	14	15
	Cyanobacteria species richness	2	2	0	1	0	1	2	3	0	1	0	2	1
	Density rating (total # cells counted)	347	168	293	120	179	82	303	577	47	338	73	216	
	Diversity (Shannon-Weaver)	2.47	2.07	2.37	2.55	1.85	2.62	2.50	2.06	2.32	2.07	2.31	2.14	
2015 samples	Percent cyanobacteria abundance 2015	5.9%			6.9%				14.8%	14.8%	22.5%	32.8%	6.6%	14.9%
	Percent diatoms abundance 2015	89.4%			85.8%				58.5%	58.5%	58.6%	48.0%	91.2%	70.0%
	Percent greens abundance 2015	1.9%			0.0%				6.3%	6.3%	0.0%	1.4%	0.0%	2.3%
Comments on samples	Bacteria	M	M	VL	M-H	VH	VH	M	L	L	H	VL	L-M	
	Didymo filaments	M	L	l	H	N	N	L	L	L	L	VL	L	
	Organic debris	M	M	L	M	L	H	L	M-H	M	M	L	M	
	silt	M-H	M	VL	L	M	H	M-H	L	L-M	L-M	VL	L-M	
	possible coal fines	M	L	L	L	L	L	L-M	M	M-H	L-M	M	M	
	fungal filaments					P	P						P	
	moss leaflet fragments						1	3				1		
	Benthic Invertebrates 2019 (Minnow)										↓ biomass,density			
Site	substrate in sample	cobble	cobble	cobble	cobble	silt	cobble	cobble	cobble	cobble	cobble	cobble	cobble	
	open water Feb 2020?	yes	no	yes		no	no	yes	yes	yes	yes	no	no	
	water depth (cm)	20	15	15		0.15	50	25	25	25	25	15	20	
	ice thickness (cm)		15	--		20	20	--	--	--	--	25	50	

NOTE:

P = Present: encountered in 1 or 2 microscope field out of 50
V low = encountered occasionally (3-5 microscope fields out of 50)
Low = encountered regularly but accounts for less than 5% of sample material
Moderate = encountered in most fields and accounts for 6 – 49% of sample material
High = encountered in all fields and accounts for >50% of sample material

Cobble Grab Samples collected by Lotic Environmental; Periphyton was removed at the LAC lab using brushed and a water jet; samples were analyzed on phase-contrast inverted microscope

Table A-29: Groundwater field parameters from Upper Fording River

Sample Name	Date	Time	Temperature (°C)	DO (mg/L)	SPC (µS/cm)	pH	ORP (mV)	Turbidity (NTU)
RG_FRSP1_WG_2019_12_03_NP	12-03-2019	13:15	4.4	8.52	1268	7.48	206.7	0.49
RG_FRSP2_WG_2019_12_03_NP	12-03-2019	12:30	5.7	7.67	1294	7.2	228.6	0.28
RG_FRSP3_WG_2019_12_03_NP	12-03-2019	14:15	5.3	8.06	1291	7.45	174.3	3.17
RG_FRSP4_WG_2019_12_03_NP	12-03-2019	14:40	5.9	8.27	1324	7.45	173	1.47
RG_FRSP5_WG_2019_12_03_NP	12-03-2019	15:05	5.9	8.67	1320	7.47	174	2.87
RG_FRSP6_WG_2019_12_03_NP	12-03-2019	15:20	5.9	8.07	1311	7.42	179	0.84
RG_FRDP_2_WG_2019_12_04_NP*	12-04-2019	10:30	2.7	9.77	1025	7.17	236.3	2.91
RG_FRDP_4_WG_2019_12_03_NP*	12-04-2019	11:45	6.4	6.92	955	7.44	196.7	1.68
RG_FRDP_5_WG_2019_12_04_NP	12-04-2019	13:00	3.6	0.43	948	6.99	5.9	3.91
RG_FRDP_8_WG_2019_12_04_NP	12-04-2019	14:15	3.9	3.22	950	7.09	-89.6	9.84
RG_FRDP_13_WG_2019_12_04_NP	12-04-2019	15:35	4.1	3.05	1249	7.32	50	27

Data from SNC Lavalin

Table A-30: UFR periphyton samples with distinctive characteristics (2015 project)

2015 Selected Upper Fording River Periphyton Sample Sites	**River Km**	Filamentous green	GOLD *Hydrurus* dominated	RED *Audouinella* dominated	FLAGELLATES common	Didymo filaments low	Calcite heavy	Calcite moderate	Moss or leaf noted in sample	Moss noted on-site, not in sample	bacterial density	organic debris	silt	High Achnanthidium density cells/cm2 (> 2 St Dev from reference sites)	High Fragilariaceae density cells/cm2 (>2 St Dev from reference sites)	High Green Density cells/cm2 (>2 St Dev from reference sites)
FO9	**17.5**							**X**			C-D	L-C	D	7E+05		
FO10					**X**			**X**			L	C	L	1E+06	2E+05	
FO22	**44.5**					**X**			**X**		DD	D	VL-L			
FO23								**X**			D	D	L-C			
FO26 (ref)	**69.5**		**X**					**X**			C-D	C-D	VL-C			
FO29	**29**						**(X)**			**X**	L	C-D	C	3E+06		
FOBCP	**52**						**(X)**				L-C	C	L-C		3E+05	
FOBKS	**54**						**X**				L	C	L			
FOBSC	**53.5**		**X**			**X**		**(X)**			D	**L-C**	**C**			
FODGH	**25.5**							**X**			L	C	C-D	6E+06		
FODHE	**25.5**					**X**	**(X)**				L-D	L-C	L-C			
FODNGD				**X**							C-D	L-C	L			
FODPO	**48**								**X**		C	D	L-C	2E+06		27072
FOUEW	**41**	**X**					**X**				L-C	C	L		8E+06	6E+05
FOUKI	**55.5**						**(X)**		**X**		C	C	C			
FOUL											L-C	C	C-D			

Data from White and Larratt 2016

Table A-31: Metals in regional calcite samples

Calcite analyses - Acid Metals	metals of interest in calcite							Major calcite constituents				
Sample ID	**Cd**	**Cu**	**Cr**	**Mn**	**Ni**	**Se**	**Zn**	**Ca**	**Mg**	**Fe**	**P**	**S**
Units	**ppm**	**ppm**	**ppm**	**ppm**	**ppm**	**ppm**	**ppm**	**%**	**%**	**%**	**%**	**%**
GH-GCI (UPSTREAM)	0.7	16.8	48	637	31.1	<0.5	105	3.70	0.59	2.90	0.139	0.12
GH-CTF (UPSTREAM)	2.9	5.2	7	232	58.4	1.3	253	26.8	0.44	0.45	0.019	0.47
GH-HWGH-BRB (UPSTREAM)	1.1	6.5	13	186	22.9	2.9	62	27.9	0.47	0.39	0.041	0.44
GH-GCI (DOWNSTREAM)	0.6	4.3	11	294	10.7	<0.5	32	27.9	0.44	0.60	0.044	0.48
GH-CTF (DOWNSTREAM)	7.8	12.9	9	337	92.9	4.8	600	27.0	0.45	0.45	0.042	0.45
GH-HWGH-BRB (DOWNSTREAM)	1.6	10.8	21	290	44.3	4.6	114	21.6	0.51	0.73	0.063	0.34

Data from: SNC Lavalin 2020

Table A-32: UFR calcite metal content

Site	elemental content of UFR calcite					
mg/kg	As	Cd	Mn	Ni	Se	Zn
KC-0-FRO ref	10	0.95	25	9.1	3.8	182
KC-3-FRO mine-a	4	21.3	54	36.1	1.4	550
UFR1-FRO ref	4	0.66	41	4.4	2.9	30
SP2-FRO mine-a	<2	5.03	49	16.3	1.5	151
LM-2-FRO	8	1.68	309	17.9	25.3	74

Data from: SRK 2014

Table A-33: UFR mainstem sediment quality - median values for selected parameters

2018	**Units**	**Lower WSQG**	**Upper WSQG**	**RG_HENUP ref**	**RG_FO26**	**RG_FOUKI**	**RG_FOBKS**	**RG_FOBCP**	**RG_FRUPO**	**RG_FO22**
				06-Sep-18	07-Sep-18	07-Sep-18	08-Sep-18	09-Sep-18	09-Sep-18	08-Sep-18
Total Organic Carbon	%	-	-	5.20	5.88	13.6	12.6	5.60	4.44	4.75
Arsenic (As)	mg/kg	5.9	17	1.85	6.57	4.24	4.64	4.41	5.10	5.53
Cadmium (Cd)	mg/kg	0.6	3.5	0.317	0.943	1.62	2.07	1.53	1.28	1.09
Chromium (Cr)	mg/kg	37.3	90	12.6	19.0	12.5	11.6	13.8	13.4	14.2
Iron (Fe)	mg/kg	21,200	43,766	4,010	17,600	14,000	13,700	13,700	14,700	14,900
Manganese (Mn)	mg/kg	460	1100	141	667	854	984	493	462	390
Nickel (Ni)	mg/kg	16	75	12.8	27.1	41.3	47.4	47.0	32.4	25.3
Phosphorus (P)	mg/kg	-	-	479	1,630	1,160	1,280	1,230	1,610	1,650
Selenium (Se)	mg/kg	2		0.430	1.35	6.36	3.26	2.10	1.62	1.69
Zinc (Zn)	mg/kg	123	315	53.5	115	147	142	120	109	113

2019	**Units**	**Lower WSQG**	**Upper WSQG**	**RG_HENUP Ref**	**RG_FO26**	**RG_FOUKI**	**RG_FOBKS**	**RG_FOBCP**	**RG_FRUPO**	**RG_FO22**	**RG_SCOUTDS**
				11-Sep-19	10-Sep-19	05-Sep-19	09-Sep-19	06-Sep-18	07-Sep-19	16-Sep-18	05-Sep-19
Total Organic Carbon	%	-	-	5.60	8.39	9.37	8.91	6.61	5.64	5.87	7.03
Arsenic (As)	mg/kg	5.9	17	1.21	5.29	3.19	3.70	4.20	4.81	5.99	5.54
Cadmium (Cd)	mg/kg	0.6	3.5	0.296	1.06	1.16	1.32	1.52	1.16	1.31	1.47
Iron (Fe)	mg/kg	21,200	43,766	2,630	14,900	10,500	11,500	12,700	13,500	17,200	16,900
Manganese (Mn)	mg/kg	460	1100	95.4	551	576	619	588	518	470	741
Nickel (Ni)	mg/kg	16	75	6.97	22.9	32.0	36.7	42.5	33.2	30.5	42.7
Phosphorus (P)	mg/kg	-	-	360	1,310	878	1,040	1,270	1,400	1,930	1,530
Selenium (Se)	mg/kg	2	2	0.330	1.57	2.75	1.98	2.34	1.99	2.84	1.94
Zinc (Zn)	mg/kg	123	315	42.6	111	90.3	99.2	125	102	117	127

░ " one or more subsamples exceeded lower WSQG

▒ " median exceeded lower WSQG

▓ " median exceeded upper WSQG

Data from Minnow 2019 and 2020a 2020b

Table A-34: Comparison of selenium concentrations in water vs selenium concentrations in benthic invertebrate tissues in UFR, 2012-2019

		Selenium in Water		Selenium in Tissues	
	Month	**Mean**	**SD**	**Mean**	**SD**
Exp	2	66.97	26.15	6.16	2.00
	3	72.69	24.78	5.59	1.03
	6	21.18	16.00	6.56	6.94
	9	55.54	36.08	7.68	1.94
	12	89.11	102.27	5.86	4.00
Ref	2	1.020	0.000	4.633	0.737
	6	0.600	0.000	3.467	0.115
	9	0.819	0.243	4.027	0.841
	12	0.775	0.049	4.200	0.352

Data from Minnow 2020a 2020b

APPENDICES

APPENDIX 1:
STATISTICAL METHODS

Model Averaging

Mixed model averaging was used to compare periphyton growth data (abundance and biovolume) against several likely drivers (Table B-1). Mixed model averaging is a process where linear models are computed for every variable combination and then averaged using the algorithms included in the R package “MuMIn”. This process involves combining the different parameters into a data frame where each record has a value for each parameter to be used in the model. The data was standardized by subtracting the mean from each value and dividing by 2x the standard deviation. Standardizing the data makes the different variables comparable by scaling their variation. Data was used from sites that had periphyton data, water chemistry data, and calcite data. The models were automatically ranked based on their ability to predict the variation in the modeled parameter and the 1000 most accurate models were averaged. Averaging the models takes the linear equation coefficients and averages them based on the weights assigned by the modeling algorithm.

Table B-1: Parameters used in mixed model average calculations

Parameters modeled for	Parameters modeled from
Cyanobacteria abundance	Total phosphorus
Cyanobacteria biovolume	Total nitrogen
Diatom abundance	Nitrate
Diatom biovolume	DIN:TP ratio
Green algae abundance	TSS
Green algae biovolume	TDS
Total abundance	Total Selenium
Total biovolume	Dissolved Selenium
	pH
	Temperature
	Total calcium
	Calcite thickness
	Bicarbonate as $CaCO_3$

Confidence intervals were calculated for each averaged coefficient and the results were plotted (Figure B-1). The plot displays the average coefficient for each parameter fed into the model and is coloured by the type of periphyton. Points near zero and with confidence bars that cross the zero line were considered to have statistically insignificant effect on the abundance or biovolume or periphyton across the sites at FRO. Parameters with large coefficients were considered primary drivers. For example, phosphorus was flagged as a primary driver for cyanobacteria and a lesser driver for the other types of periphyton, an expected result given that many types of cyanobacteria can fix atmospheric nitrogen.

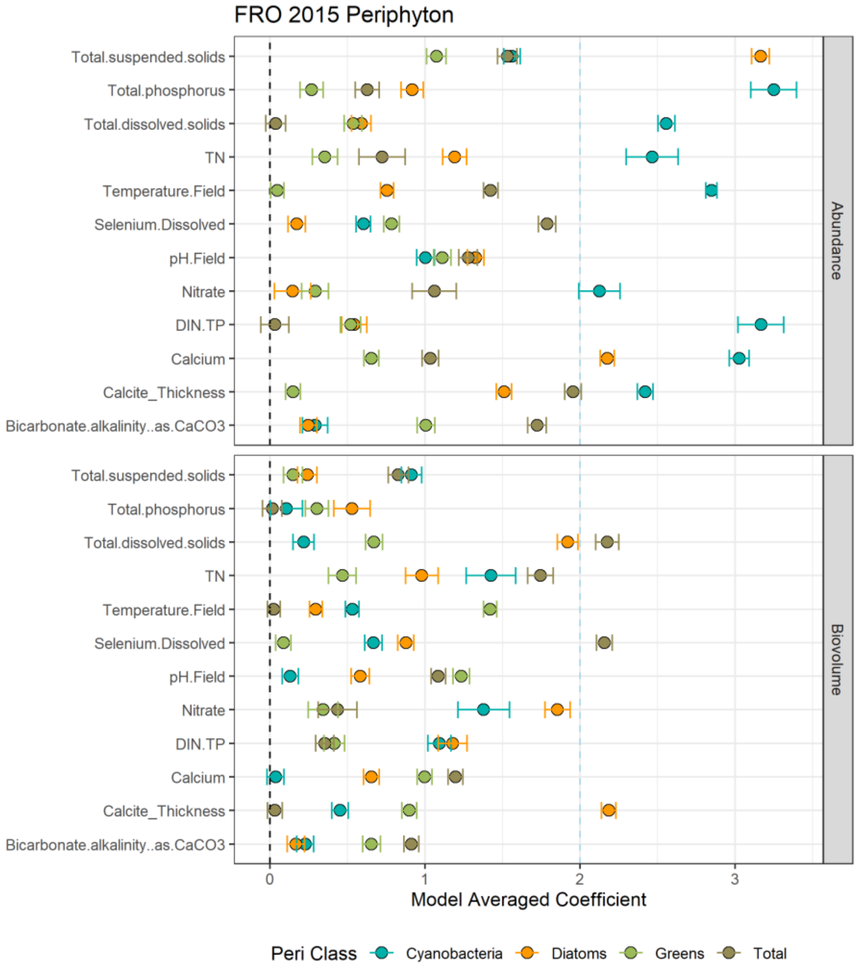


Figure B-1: Example plot from mixed model averaging

Correlations

Correlations were frequently conducted between variables of interest. Throughout this report, all correlations were conducted using the Pearson's correlation coefficient using complete pairs. Where referenced in the text, the corresponding R value is cited for the strength of the correlation.

Biovolume calculations (2015, 2020 data)

Periphyton samples were collected during February 2020 and their results are included in several places throughout the LAC section for comparison against 2012 and 2015 data, and as a residual from the 2019 algae blooms in the UFR. Using measurements of each taxa found within the UFR samples, cell volumes were calculated for each taxa using standard taxon formulae (sphere, cylinder, cymbelloid etc). The geometric mean of the measurements (length, width, height, etc.) were used for calculating biovolume metrics.

General water quality statistics

Descriptive statistics were computed for the 2012, 2015, and 2020 periphyton data in the UFR as well as upon other parameters used in the LAC section such as calcite, water chemistry, sediment chemistry.

Water quality plotting

Several types of plots were frequently used in the LAC section including scatterplot (Figure B-2), boxplots (Figure B-3), and bar plots (Figure B-4).

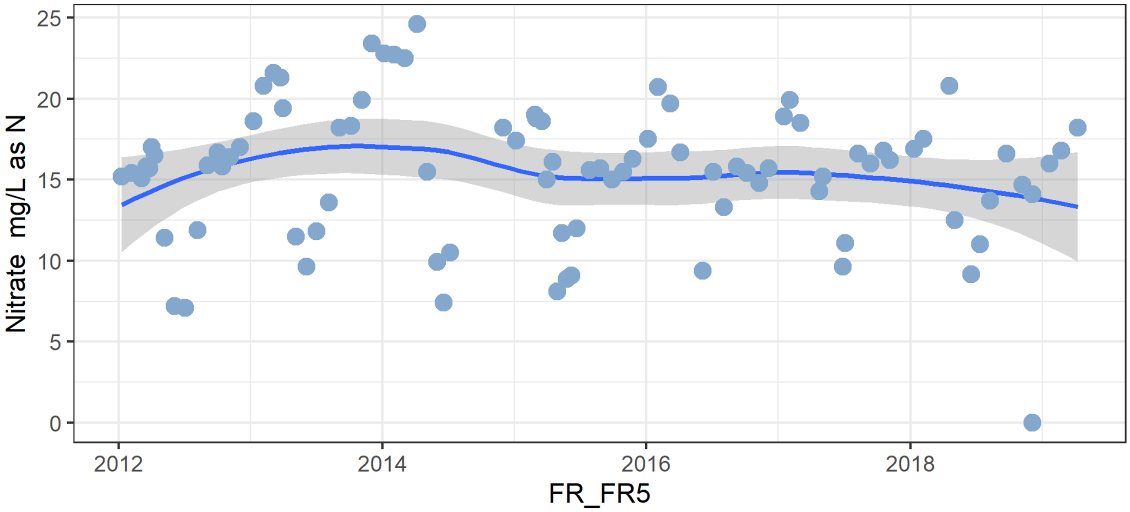


Figure B-3: Example of scatterplot with LOESS trend line

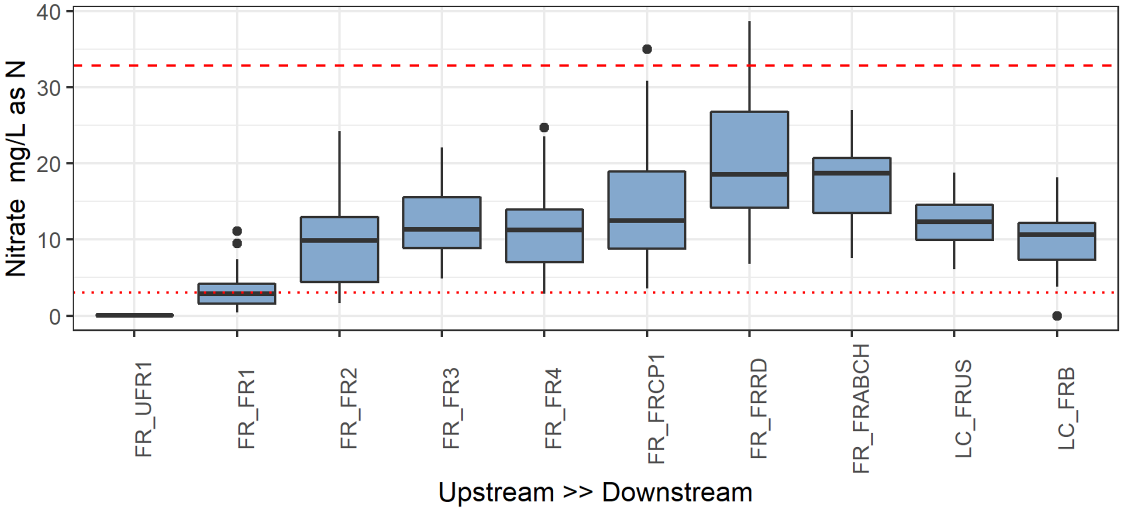


Figure B-4 Example boxplot with BC aquatic life 30-day guideline (dotted line) and BC aquatic life MAC (dashed line)

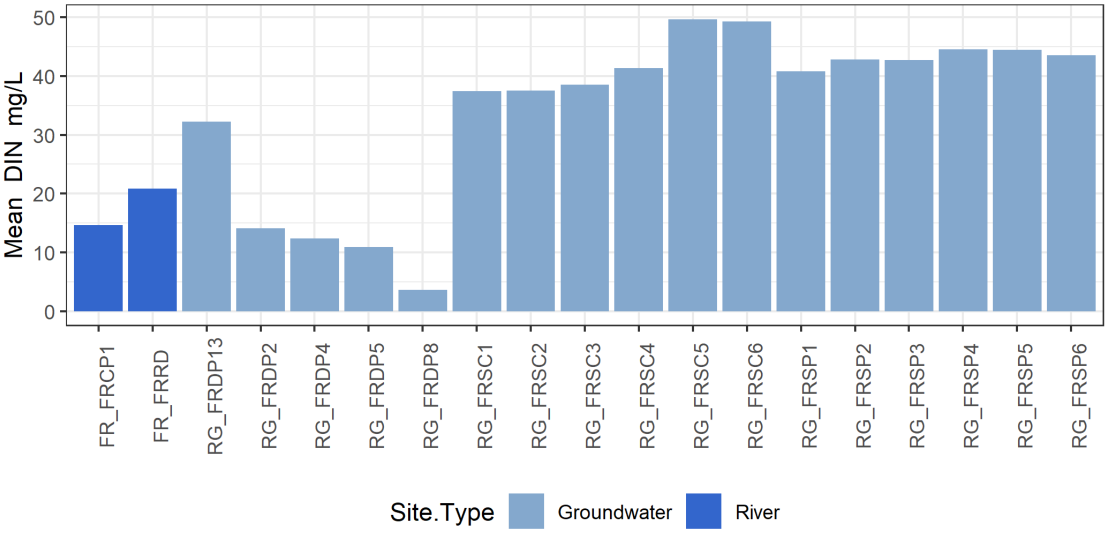


Figure B-5: Example of bar plot coloured by site type

Exceedance Calculations

For nitrate and ammonia, water chemistry data was compared against relevant BC aquatic life guidelines for maximum allowable concentration and 30-day chronic exposure concentration.

GIS

GIS software was frequently employed during this project and numerous data-based maps are included in the report. Plotting data on a map allows for easier visual interpretation of geographic trends and provides more context to the data with so many sites on numerous sub-watersheds. The main types of data plotted this way were periphyton results (abundance, biovolume) and calcite data. General maps illustrating sites locations and important events such as the 2019 algae bloom were also included.

For calculation of the model averaging, it was important to locate sites that had multiple types of data available. This was conducted in GIS by nearest neighbor analysis. The results were then hand tailored to ensure accurate links between sites and data types.

Periphyton data from 2015 and 2020 were already available to LAC because it was the taxonomic lab for those projects. 2012 data was obtained from the FRO OneDrive and the inter-lab periphyton review by Minnow (2013). Base layers including sample locations, stream polylines, and lake polygons were obtained from the FRO OneDrive. Calcite data locations were sent to LAC from Lotic Consultants directly.

Calcite

Calcite data was used in two forms. The primary source of data were the field observations from the 2015 Periphyton Study conducted by Minnow Environmental and LAC. These results could be directly compared against periphyton results and were very useful. Calcite data was also obtained from the Lotic Consultants studies including calcite metals, thicknesses, etc. These data were informative to the general understanding but could not be compared directly to periphyton results.

Sediment data

Sediment metals results were analyzed between 2018 and 2019 for metals and PAHs with the specific focus on determining if a change had occurred between 2018 and 2019. This was tested using the Kruskall-Wallis non-parametric test of difference. The test compared each parameter at each site between 2018 and 2019 and assigned a significance value (p). If $p \leq 0.05$, a significant difference between years was identified.

Treatment of ND values

Non-detect values are common in water and sediment chemistry samples. Throughout the LAC section, all non-detect values were assigned as ½ the detection limit. Detection limits were included in plots as dashed black lines for parameters where at least one sample was below detection. For parameters where all samples were >RDL, a dashed line does not appear on the scatterplot.

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