Subject Matter Expert Report:

CALCITE. Decline in Upper Fording River Westslope Cutthroat Trout Population

Prepared for:

Teck Coal Limited
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Sparwood, BC, V0B 2G0

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EXECUTIVE SUMMARY

Abundances of both juvenile and adult life stages of Westslope Cutthroat Trout (WCT) in the upper Fording River were substantively lower in 2019 relative to 2017. Teck Coal Ltd. initiated the "Evaluation of Cause" (EoC) to assess factors responsible for the population decline. The EoC evaluates numerous impact hypotheses to determine whether and to what extent various stressors and conditions played a role in the decline of WCT. Ecofish Research Ltd., Lotic Environmental Ltd., and Larratt Aquatic Consulting Ltd. were asked to provide support as Subject Matter Experts to the EoC. The EoC project team is investigating two "Over-arching" Hypotheses related to multiple potential stressors:

- **Over-arching Hypothesis #1:** The significant decline in the UFR WCT population was a result of a single acute stressor or a single chronic stressor.
- **Over-arching Hypothesis #2:** The significant decline in the UFR WCT population was a result of a combination of acute and/or chronic stressors, which individually may not account for reduced WCT numbers, but cumulatively caused the decline.

Stressors are defined as any biological, physical, or chemical factor that causes adverse responses in the environment.

This report investigates the potential for the precipitation of calcite on the streambed downstream of Teck mines[1] to cause or contribute to the observed decline of WCT in the UFR. The investigation draws on existing reports and new analyses of calcite data from the UFR to determine if there were changes in calcite conditions during or before the WCT Decline Window (between September 2017 and September 2019) that may have caused or contributed to the WCT population decline. Results of the calcite analyses are meant to support the evaluation of Over-arching Hypothesis 1 and Over-arching Hypothesis 2. The report first provides an overview of spatial and temporal trends in calcite throughout the UFR, and then examines five effect pathways through which calcite can impact fish populations: 1) effects to spawning suitability, 2) effects to rearing – invertebrate prey availability, 3) effects to rearing – biogenic calcite precipitation and dissolution, 4) effects to incubation conditions, and 5) effects to rearing – overwintering habitat. Overall, this report examines the following impact hypothesis related to calcite:

> Impact hypothesis**:** Did accumulations of calcite in WCT habitat cause or contribute to the observed decline in WCT abundance through decreased spawning, rearing or overwintering success?

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[1] Teck mines include Fording River Operations (FRO), Greenhills Operations (GHO) and Line Creek Operations (LCO).

Spatial and temporal trends in calcite

Data to assess the spatial and temporal trends in calcite were gathered through the Regional Calcite Monitoring Programs from 2013-2019 (e.g., Robinson *et al.* 2013; Robinson and MacDonald 2014; Robinson *et al.* 2016; Robinson and Atherton 2016; McCabe and Robinson 2020). These programs sampled calcite throughout the UFR in areas downstream of Fording River Operations (FRO), Greenhills Operations (GHO), Line Creek Operations (LCO), Elkview Operations (EVO), and Coal Mountain Operations (CMO). As part of the Regional Calcite Monitoring Program, the UFR watershed is monitored for calcite annually over eight mainstem reaches (Fording River reaches 5-10), and 28 tributary reaches, using a stream reach or segment geographic breakdown as the spatial unit within which calcite is monitored and reported. Results were summarised in terms of calcite index (CI), calcite presence, and calcite concretion for four stream categories: tributaries (reference), tributaries (mine-exposed), Fording mainstem (mine-exposed), and Elk mainstem (mine-exposed). This level of assessment allowed for a detailed examination of temporal and spatial trends throughout the UFR. For direct comparison with WCT population data from Cope (2020), calcite concretion data were also binned into four additional geographic strata: mainstem headwaters, middle mainstem, lower mainstem, and lower tributary reaches.

Increasing trends in CI but not calcite concretion were observed in both reference and exposed reaches across the UFR. For example, a gradual increase in CI was observed in the Fording River mainstem from 2013-2019, with current CI values averaging 0.80. In contrast, calcite concretion values did not change across the pre-window versus Decline Window periods. CI represents a composite index of calcite presence and concretion and therefore increases in CI in the UFR largely reflect increases in calcite presence. CI values greater than 1 are observed when concretion of the substrate is occurring. Reach mean CI values greater than 1 only exist in several mine-exposed tributaries, and largely do not occur within fish habitat assessed by Cope (2020).

Within the fish habitat area surveyed by Cope (2020), calcite concretion values were low (mean = 0.06) and showed little change in the pre-window to Decline Window periods. A recent increase was observed in the middle mainstem (which is key WCT habitat), but the increase was relatively minor and driven by an isolated increase in one stream reach (FORD9).

The trends in calcite in the UFR were assessed in context with available dose-response relationships for the five effect pathways to evaluate the potential for calcite to have caused or contributed to the WCT decline.

Effects to spawning suitability

The effect of calcite on spawning suitability was assessed by drawing on results from on-going studies in the Elk watershed of the effects of calcite to spawning habitat (Hocking *et al.* 2019, 2020). The draft spawning suitability curve for mean redd counts developed in Hocking *et al.* (2020) was applied to spatial and temporal trends in calcite concretion data corresponding to fish-bearing reaches and strata monitored by Cope (2020), which was used to evaluate changes in spawning suitability in pre-window versus Decline Window periods. Trends in calcite concretion were used instead of CI because the concretion of spawning substrate is hypothesized to be the main mechanism that calcite could affect spawning suitability.

Calcite concretion levels in the UFR were similar between pre-window and Decline Window periods and were also not high enough to lead to a substantial decline in mean redd count. There is uncertainty inherent in the modeled response curve and its application, but the general shape and slope of the response curve provides a tool for assessing the general magnitude of effects—concretion levels did not reach intensities needed to satisfy requisite conditions for this pathway to be considered causal. While increases in calcite concretion were observed in the middle mainstem strata in 2018, these were limited in geographic extent to a single reach, and any decreases in fry through decreased spawning success would not immediately translate into a decline in other life stages, and thus do not solely explain the WCT decline. It is possible, however, that this pathway could contribute to the WCT juvenile decline.

Effects to rearing – invertebrate prey availability

This pathway was assessed based on characterization of calcite effects to benthic invertebrates and periphyton by Barrett *et al.* (2016), who completed biological sampling in 2014 and 2015 in the UFR watershed. The study aimed to characterize relationships between 1) calcite and benthic invertebrate production and community characteristics, and 2) calcite and periphyton productivity endpoints to determine the level of calcite at which biological effects occur. The study showed some increases in periphyton productivity with calcite, but conditions did not deviate from the expected normal range at any calcite level. Benthic invertebrate communities were more strongly affected by calcite above CI value of 1; key findings included a decrease in Ephemeroptera, Plecoptera and Tricoptera (EPT) and Ephemeroptera proportion with increased calcite, an increase in Chironomidae with increased calcite, an increase in Diptera with increased calcite, and no significant change in total invertebrate abundance with increasing calcite. Higher CI values were also found to be correlated with changes in water quality, and therefore Barrett *et al.* (2016) noted the uncertainty as to whether the effect of CI on invertebrate communities was driven by CI or by water quality.

Although EPT are a preferred prey for drift-feeding salmonids, it is unclear whether a change in invertebrate species composition with no change in total invertebrate biomass would translate into effects on WCT growth and abundance. Deviation from normal benthic community composition as a result of changes in calcite was also only observed at CI values >1. Calcite levels in the UFR mainstem and lower tributaries remain below CI = 1 for all reaches surveyed by Cope (2020) except for Clode Creek. Further, no distinct changes in calcite occurred pre-window versus decline window, other than an isolated increase in reach FORD9. The effect of calcite on food production is therefore unlikely to be a sole or contributing cause of WCT declines for adults or juveniles.

Effects to rearing – biogenic calcite precipitation and dissolution

Biogenic or bio-mediated calcite is a soft, porous mineral formed in river water when carbonate is precipitated in association with periphyton biofilms. The presence of a microbial biofilm strongly influences carbonate precipitation and dissolution (Pedley *et al.* 2009; Roche *et al.* 2019; Payandi-Rolland *et al.* 2019). Effects of biogenic calcite precipitation and subsequent dissolution were assessed through a collation of available periphyton and cyanobacteria data, UFR monitoring parameters that predict calcite precipitation and dissolution, and information available in the literature on periphyton growth and release of cyanotoxins during calcite dissolution.

While periphyton likely accelerates and enhances calcite deposition, changes in calcite in the UFR have been gradual with no distinct shift in calcite index or concretion during the Decline Window that could clearly account for the WCT decline. Many of the harmful cyanobacteria impacting rivers globally do not occur in the UFR, however, naturally occurring *Phormidium* is of concern because its growth is associated with biogenic calcite. For all fish life stages, the main mechanism of cyanotoxin harm appears to be accumulation in liver tissue inducing liver necrosis and research elsewhere indicates it may be most acute in early life stages and juveniles.

Biogenic calcite impacts from cyanotoxicity are predicted to exert the greatest influence when a fall spate (flushing flow) does not occur. No fall flush occurred in 2018 (FRO FRNTP data), indicating that biogenic calcite and associated cyanobacteria could have persisted from summer 2018 into winter 2018/2019. Theoretically, a calcite dissolution event could release cyanotoxins built up in embedded cyanobacteria filaments. Review of UFR mainstem dissolution data indicates that the potential for a significant calcite dissolution event is low and localized to depositional sites with organic decomposition or sites receiving hyporheic upwelling, resulting in limited potential for release of embedded cyanotoxins or metals to an intensity that could account for the WCT decline or be a significant contributor to it. However, two episodes were observed in the Decline Window during winter 2018 and winter 2019 at one site in the lower Fording River mainstem below LCO Dry Creek where river water quality indicated that calcite dissolution was thermodynamically possible. This site overlaps with the lower watershed residents accounting for roughly 10% of the UFR WCT population. Therefore, release of cyanotoxins during calcite dissolution as a sole cause or a significant contributor is unlikely. However, it remains possible that cyanotoxin stress contributed to the WCT decline of juveniles through an interaction of flow conditions and the extreme cold conditions in winter 2018/2019 in the lower mainstem area. Overall, there is high uncertainty associated with this pathway, stemming from the unavailability of cyanobacteria and cyanotoxin data within the Decline Window.

Effects to incubation conditions

Studies by Wright *et al.* (2017, 2018) were used to evaluate the effects pathway for incubation conditions on WCT in the UFR. These studies examined dissolved oxygen and hyporheic flow in relation to calcite in the UFR and found that while calcite index had no effect on hyporheic flow, it was an important predictor of dissolved oxygen in the substrate. However, the effect on dissolved oxygen was found at depths in the substrate greater than the average excavation depth for a WCT redd. Declines in interstitial dissolved oxygen was also associated with moderate to high calcite levels

(CI >1), which are not present in most of the fish-bearing reaches of the UFR. Overall, the requisite conditions (in particular, high levels of calcite intensity) are not met and effects to incubation conditions are unlikely to be a sole or contributing cause of WCT declines for adults or juveniles.

Effects to rearing – overwintering habitat

Increases in calcite concretion could affect WCT overwintering habitat via a decrease in interstitial spaces. This pathway was evaluated by drawing on results from Cope *et al.* (2016), Cope (2019) and Hatfield and Whelan (2021), as well as a literature review of the importance of interstitial spaces for overwintering WCT. Cope (2019) highlighted the importance of interstitial space as refuge for overwintering small fish and noted that winter mortality can affect fish through predation mortality and ice effects, while Hatfield and Whelan (2021) outlined the potential for winter habitat to be a limiting factor that can be affected by other stressors. Although the relationship between calcite and the use of substrate refuge was not examined explicitly, a defined, widespread increase in calcite concretion during the Decline Window was not observed. Similarly, because overwintering effects of calcite concretion would be expected to affect young (i.e., small) fish more strongly, this pathway does not meet the requisite conditions to explain the decline in all age classes of WCT, and thus is unlikely to be a sole or contributing cause of WCT adult declines. However, it is possible that this pathway could have contributed to cumulative impacts from other pathways for WCT juveniles.

Conclusion

Calcite in the UFR watershed has the potential to impact WCT through several pathways. These pathways were evaluated in the context of spatial and temporal trends in calcite throughout the watershed and interpreted in terms of requisite conditions for each pathway to cause or contribute to the WCT decline. The main trend in calcite conditions documented was a general increase in calcite index in the UFR in both exposed and reference reaches. In particular, a gradual increase in calcite index from 2013 to 2019 has been observed in the Fording River mainstem. However, levels of calcite concretion have remained low and relatively stable across years, and there were no sharp increases in calcite index or concretion during or immediately prior to the Decline Window. The requisite conditions for all pathways examined here required moderate to high levels of calcite index (CI>1) and/or concretion and timing of calcite increases to be associated with the Decline Window. The calcite pathways were also generally unable to explain the observed decline in WCT across all age classes during the Decline Window. Calcite as a sole cause of the decline is therefore unlikely for both WCT adults and juveniles.

Although calcite is unlikely to be a sole cause of decline, the pathways examined could contribute cumulatively to WCT population abundance. Like all aquatic ecosystems, the potential fish stressors at play in the UFR co-occur and interact. Such interactions could have been important in winter 2019 when flows were low and ice was prevalent. Calcite as a contributor to the decline of WCT juveniles is therefore assessed as possible, but with high uncertainty given the lack of explicit dose-response relationships for many pathways and limited data on interactions among pathways.

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ACRONYMNS AND ABBREVIATIONS

AFDM	Ash-Free Dry Mass
AIC$_c$	Akaike Information Criterion
CABIN	Canadian Aquatic Biomonitoring Network
CI	Calcite Index
CI$_c$	Calcite Concretion
CI$_p$	Calcite Presence
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EoC	Evaluation of Cause
FRO	Fording River Operations
SME	Subject Matter Expert
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
TKN	Total Kjeldahl Nitrogen
TSS	Total Suspended Solids
UFR	Upper Fording River
WCT	Westslope Cutthroat Trout

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

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.

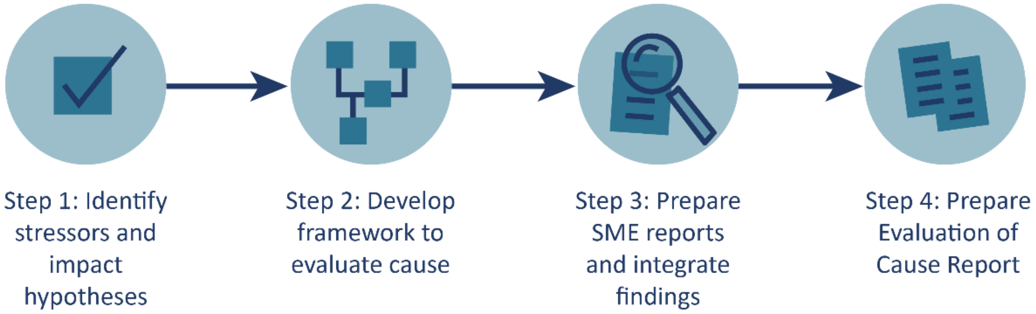
watershed, upstream of Josephine Falls: Fording River Operations, Greenhills Operations and Line Creek Operations.

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[2] or a single chronic stressor[3].
- 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[4], 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

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[2] Implies September 2017 to September 2019.

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

[4] 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.

whether the requisite conditions[5] 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.

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

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[5] 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.

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 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.

Focus	Citation for Subject Matter Expert Reports
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 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*

Focus	Citation for Subject Matter Expert Reports
	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.

Focus	Citation for Subject Matter Expert Reports
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

Abundances of adult and juvenile life stages of Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*) (WCT) in the upper Fording River (UFR) have been estimated since 2012 using high-effort snorkel and electrofishing surveys, supported by radio-telemetry and redd surveys (Cope *et al.* 2016). Annual snorkel and electrofishing surveys were conducted in the autumns of 2012-2014, 2017, and 2019. Abundances of both juvenile and adult life stages were substantively lower in 2019 than 2017, indicating a large decline during the two-year period between September 2017 to September 2019 (Westslope Cutthroat Trout Population Decline Window; hereafter referred to as the Decline Window; Cope 2020). The magnitude of the decline as well as refinements in the timing of decline are reviewed in detail by Cope (2020) and the Evaluation of Cause Team (2021).

Teck Coal Limited (Teck Coal) initiated the "Evaluation of Cause" (EoC) to assess factors responsible for the population decline. The EoC evaluates numerous impact hypotheses to determine whether and to what extent various stressors and conditions played a role in the decline of WCT. Given that the primary objective is to evaluate the cause of the sudden decline over a short time period from September 2017 to September 2019, it is important to identify stressors or conditions that changed or were different during the Decline Window. However, it is equally important to identify all potential stressors or conditions that did not change during the Decline Window but nevertheless may be important constraints on the population. Finally, interactions between stressors are also considered in the Evaluation of Cause. Where an impact hypothesis depends on interactions among stressors or conditions, or may be exacerbated by particular interactions, the mechanisms of interaction are considered as specific impact hypotheses.

A project team is evaluating the cause of decline and is investigating two "Over-arching" Hypotheses:

- Over-arching Hypothesis #1: The significant decline in the UFR WCT population was a result of a single acute stressor[6] or a single chronic stressor[7].
- Over-arching Hypothesis #2: The significant decline in the UFR WCT population was a result of a combination of acute and/or chronic stressors, which individually may not account for reduced WCT numbers, but cumulatively caused the decline.

Ecofish Research Ltd., Lotic Environmental Ltd., and Larratt Aquatic Consulting Ltd. were asked to provide support as subject matter experts (SMEs) to evaluate some of the stressors. This report investigates potential calcite-related stressors on WCT in the UFR.

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[6] Implies September 2017 to September 2019.

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

1.1. Background

1.1.1. Overall Background

This document is one of a series of SME reports that supports the overall EoC of the UFR WCT population decline (EoC Team 2021). For general information, see the preceding Reader's Note.

1.1.2. Report-specific Background

Calcite is a calcium carbonate deposit that occurs on organic and inorganic substrate in freshwater streams. Although naturally occurring, the magnitude and extent of calcite formation can increase because of open pit mine runoff (Teck 2017). Calcite formation can lead to consolidation of substrate, which alters streambeds by cementing rocks together (concretion), thereby affecting sediment transport and habitat structure. Concretion can adversely influence fish spawning and benthic invertebrate communities and may also affect WCT through other pathways such as alteration of incubation habitat and overwintering success (Robinson 2010; Barrett *et al.* 2016; Wright *et al.* 2018; Hocking *et al.* 2020). Calcite can also potentially impact fish populations through a biogenic precipitation pathway, where periphyton can accelerate calcite deposition and mediate release of metals and cyanotoxins at concentrations that can be detrimental. For more information, also see the Cyanobacteria, Periphyton and Aquatic Macrophyte Impacts Evaluation of Cause (Larratt and Self 2021).

In the Elk River watershed, there are wide ranges in the spatial extent and magnitude of calcite cover. Calcite cover ranges from areas with minimal calcite to areas in certain streams where calcite completely covers portions of the stream bed, making the gravels largely immovable (Smithson *et al.* 2018). There are concerns that moderate to high calcite levels have adverse effects on WCT and other biota. Since 2016, Ecofish has been involved in studies in the Elk River watershed to quantify the relationships between calcite and fish spawning and incubation success (Wright *et al.* 2017; 2018; Hocking *et al.* 2019; 2020). The basic premise for these studies is that calcite accumulation on a streambed reduces the suitability of spawning habitat and incubation conditions, and thereby the carrying capacity of fish habitat. These studies complement ongoing studies by Lotic Environmental Ltd., Minnow Environmental Inc., and Larratt Aquatic Consulting Ltd. The effects of calcite on fish are described in the following causal effect pathways linking calcite to fish production (also summarised in Figure 1):

1) Calcite reduces spawning habitat suitability, which decreases quantity and quality of spawning habitat, which in turn limits spawning success, population recruitment, resulting in lower fish abundance.

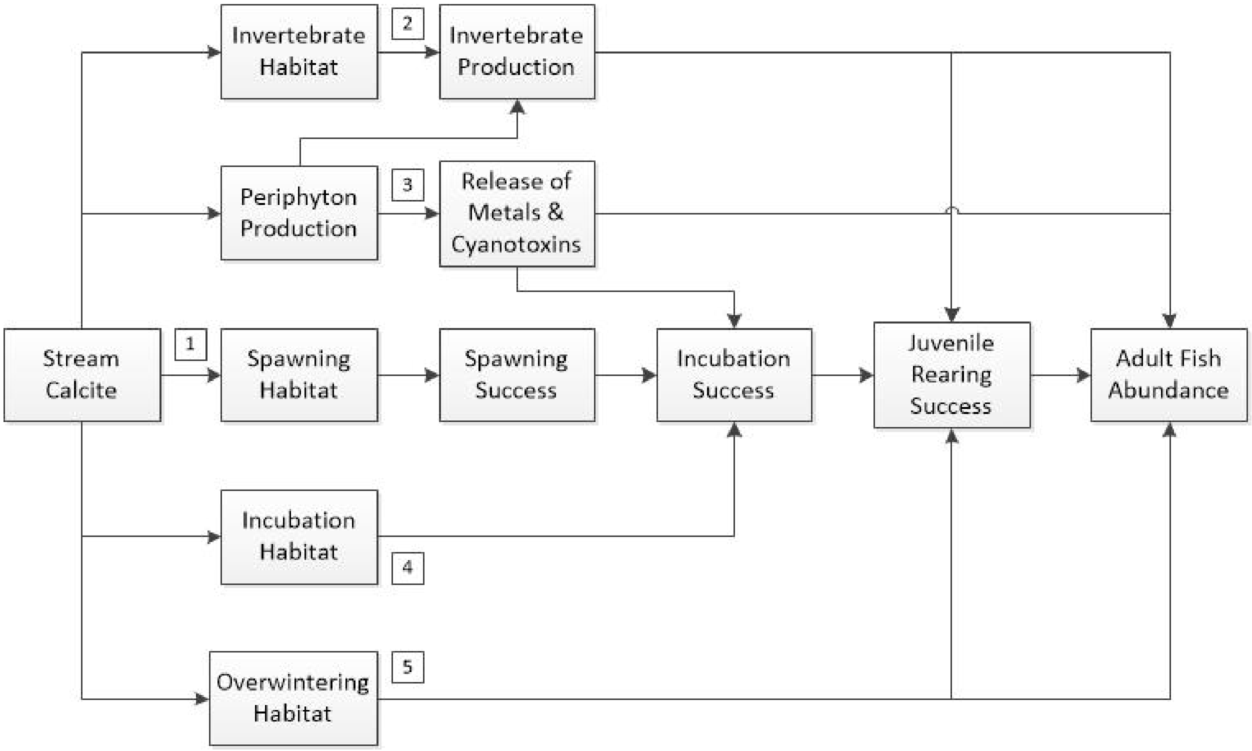
2) Calcite reduces invertebrate habitat suitability, which decreases invertebrate prey availability for WCT, limits juvenile and adult rearing success, resulting in lower fish abundance.

3) Calcite increases periphyton productivity, which accelerates calcite precipitation and causes release of cyanotoxins and metals during calcite dissolution, resulting in lower fish abundance.

4) Calcite reduces hyporheic flow and dissolved oxygen during incubation, which decreases incubation success and limits population recruitment, resulting in lower fish abundance.

5) Calcite reduces overwintering habitat suitability, which increases overwintering mortality, resulting in lower fish abundance.

Figure 1. Effect pathway diagram linking calcite on the streambed to fish production.



1.1.3. Author Qualifications

Todd Hatfield, Ph.D., R.P.Bio.

This project is being led by Todd Hatfield, Ph.D., a registered Professional Biologist and Principal at Ecofish Research Ltd. Todd has been a practising biological consultant since 1996 and he has focused his professional career on three core areas: environmental impact assessment of aquatic resources, environmental assessment of flow regime changes in regulated rivers, and conservation biology of freshwater fishes. Since 2012, Todd has provided expertise to a wide array of projects for Teck Coal: third party review of reports and studies, instream flow studies, environmental flow needs assessments, aquatic technical input to structured decision making processes and other decision support, environmental impact assessments, water licensing support, fish community baseline studies, calcite effects studies, habitat offsetting review and prioritizations, aquatic habitat management plans, streamflow ramping assessments, development of effectiveness and biological response monitoring programs, population modelling, and environmental incident investigations.

Todd has facilitated technical committees as part of multi-stakeholder structured decision making processes for water allocation in the Lower Athabasca, Campbell, Quinsam, Salmon, Peace, Capilano, Seymour and Fording rivers; he has been involved in detailed studies and evaluation of environmental flows needs and effects of river regulation for Lois River, China Creek, Tamihi Creek, Fording River, Duck Creek, Chemainus River, Sooke River, Nicola valley streams, Okanagan valley streams, and Dry Creek. Todd was the lead author or co-author on guidelines related to water diversion and allocation for the BC provincial government and industry, particularly as related to the determination of instream flow for the protection of valued ecosystem components in BC. He has worked on numerous projects related to water management, fisheries conservation, and impact assessments, and developed management plans and guidelines for industry and government related to many different development types. Todd is currently in his third 4-year term with COSEWIC (Committee on the Status of Endangered Wildlife in Canada) on the Freshwater Fishes Subcommittee.

Morgan Hocking, Ph.D., R.P.Bio.

Morgan is Senior Environmental Scientist with Ecofish with over 20 years of experience conducting salmonid conservation and watershed resource management projects in British Columbia. For much of his career, he has studied how spawning Pacific salmon affect terrestrial biodiversity, and how this information can be used in ecosystem-based management. He uses a combination of field studies, experiments, watershed spatial data, quantitative modelling, and novel tools in ecology such as stable isotopes and environmental DNA to assess watershed status and the relationships between watershed developments and biodiversity, and has published 23 peer-reviewed articles on his work. Morgan has extensive experience in designing and implementing large-scale monitoring programs and has over 15 years of experience working with First Nations, primarily related to fisheries management in the Great Bear Rainforest.

With Ecofish, Morgan works on technical project management, community engagement, experimental design, data analysis, reporting and senior technical review on a diversity of projects such as the Cumulative Effects Monitoring Program in the Skeena watershed (Environmental Stewardship Initiative), the Fish and Wildlife Compensation Program (FWCP) Action Plan Update (FWCP Coastal and FWCP Peace), the Site C Tributary Mitigation Program (BC Hydro) and the Ecofish environmental DNA program. Morgan is also the technical lead of the Calcite Biological Effects Program with Teck and the Teck Kilmarnock eDNA study. Morgan also holds a position as an Adjunct Professor in the School of Environmental Studies at the University of Victoria.

Heather Larratt, H.B.Sc., R.P.Bio.

The Larratt Aquatic Consulting (LAC) team contributed to this project, led by Heather Larratt, a registered Professional Biologist with 40 years of experience. Heather was the first recipient of a 1st class Honors B.Sc. in Environmental Biology (1978) U of Calgary. Her core research revolves around lake and reservoir management to optimize water quality. In 1985, she also became involved in the reclamation of mine tailings ponds as wetlands and pit lakes as bioreactors using microflora. In 2009, she began collaborating on river productivity studies for BC Hydro on the Columbia and Peace rivers.

These projects involved identification of periphyton and phytoplankton, including potential impacts of these microflora and what their present indicates. She has conducted research on cyanotoxicity in drinking water and helped write the Interior Health Authority (IHA) cyanobacteria guidelines. 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 trihalomethane production 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.

Awards include:

- Professional Canadian Mineral Analysts, Best Paper 1995;
- Major's Environmental Award for Best Professional Volunteer Organization (EAC) 2001;
- BC Water Supply Association MSC Award for Outstanding Contribution to the Water Supply Industry 2014; and
- TRCR award for 20 years of research and implementation on the use of tailings ponds and pit lakes as aquatic habitat and passive water treatment facilities 2017.

Mike Robinson, M.Sc., R.P.Bio.

Mike has been working in the field of aquatic sciences for over 19 years. During this time he has become recognized in two key fields: the ecology of Westslope Cutthroat Trout and Aquatic Ecosystem Restoration. His work with Westslope Cutthroat Trout has covered most of their range in British Columbia and Alberta. Mike completed his MSc degree where his thesis investigated, for the first time, the ecological consequences of hybridization between native Westslope Cutthroat Trout and introduced Rainbow Trout. This study in part, considered the effects of human-impacts, such as habitat degradation, on the fish community and the potential spread of introduced species. This work was completed in the Oldman River watershed. Later on, Mike completed genetic inventory and mapping work for the Government of Alberta, mapping the remnant Westslope Cutthroat Trout population in Alberta in support their SARA listing.

As a consultant, Mike's experience includes: fish population monitoring, effects assessments, effectiveness monitoring, fish habitat assessments; however with particular focus on Ecological Restoration and Environmental Assessments. Regarding Ecological Restoration, Mike has experience designing and implementing habitat restoration prescriptions and Fish Habitat Offsetting Plans. He is currently leading a multidisciplinary team in identifying and designing habitat offsetting projects throughout the Elk River watershed; acting as the Project Manager and lead Biologist for Teck Coal's ongoing fish habitat offsetting strategy, since 2007. In regards to Environmental Assessment Mike has been involved in multiple projects, acting as the Fish and Fish Habitat discipline lead. He has been an author on gap analysis reports, baseline assessments, and effects assessments. Mike has also completed environmental effects studies, including assessing the effects of substrate manipulation on benthic macroinvertebrate communities. Mike has lead projects investigating fish distributions in British Columbia and Alberta, to assist with Federal Species At Risk recovery plans. Mike has a strong understanding of scientific design and statistical analysis, and provides a balance of research, education and experience.

1.2. Objective

This report presents a summary of calcite studies relevant to the observed decline in WCT over the 2017-2019 period, providing new analyses of calcite data and interpretation of existing studies within an evaluation of cause framework to examine the following general impact hypothesis:

> **Impact hypothesis:** Did accumulations of calcite in WCT habitat cause or contribute to the observed decline in WCT abundance through decreased spawning, rearing or overwintering success?

1.3. Approach

The overall approach used in the evaluation of cause for calcite and assessment of requisite conditions included two main parts: 1) an assessment of the spatial and temporal trends of calcite in the UFR, and 2) an assessment of the biological effects related to calcite for each of the five impact pathways in reference to the calcite trends found in #1. For the spatial and temporal assessment of calcite, the calcite data from the Regional Calcite Monitoring Program (Robinson *et al.* 2013) was compared over time including in the pre-window versus Decline Window periods. Calcite data was also isolated for direct comparison to the fish habitat reaches in the UFR surveyed by Cope (2020). The calcite data provides key context for all requisite conditions including spatial extent, duration, location, timing, and intensity for all five impact pathways.

The second section on assessment of biological effects is divided into subsections for each impact pathway and generally consists of a literature review to document any known dose-response relationships between calcite and fish spawning, rearing or overwintering conditions or habitat. These dose-response relationships are described in the methods (since they are results of other studies not this one) and applied to the trends in calcite in the UFR in the results section. The dose-response relationships provide important context regarding the requisite condition for the intensity of calcite needed to elicit a WCT response, as well as the spatial extent and location conditions that overlap with critical WCT habitat. Not all impact pathways have dose-response relationships available and therefore these pathways are evaluated more qualitatively and therefore have greater uncertainty.

2. METHODS

2.1. Spatial and Temporal Trends in Calcite

Teck has been documenting the occurrence of calcite in streams downstream of its Elk Valley operations since 2008 (Berdusco 2009), with a formal Regional Calcite Monitoring Program (the Program) implemented in 2013 (Robinson *et al.* 2013). The Program was conducted using a stream reach-based sampling design in 2013-2015. A revised Program was implemented in 2016-2018 to sample stream segments[8] (Robinson and Atherton 2016). Following the 2016-2018 sampling period, the Program was again modified based on recommendations from the 2016-2018 reports (e.g., Robinson *et al.* 2016). Currently, sampling uses a hybrid approach of the full "reach-by-reach" Program (2013-2015) and the stream segment/indicator reach approach (2016-2018) to estimate spatial distribution of calcite relative to each of the mines. This approach was developed to allow for higher-resolution monitoring in key areas of interest and surveillance monitoring in areas with lower potential of calcite deposition.

Since 2013, sample locations have been visited annually in areas downstream of Fording River Operations (FRO), Greenhills Operations (GHO), Line Creek Operations (LCO), Elkview Operations (EVO), and Coal Mountain Operations[9] (CMO). The study area extends to near Fernie, BC, the downstream limit of the Elk River Reach 8.

Specific to the EoC, the UFR watershed is monitored annually over eight mainstem reaches (Fording River reaches 5-10), and 28 tributary reaches (Figure 2). Thirty-three reaches are classified as mine-exposed and three are reference. Fording River – Reach 4 covers a higher gradient, confined reach that is bisected by Josephine Falls. It therefore technically has approximately half of the reach in the UFR. However, it was omitted from the UFR in this assessment, mainly because it does not overlap with core WCT habitat.

Calcite at a location is described using the calcite index (CI), which is the sum of the amount of calcite present (calcite presence = CI_p) and the degree to which calcite is binding individual streambed particles (calcite concretion = CI_c).

The regional calcite program uses stream reach or segments as the spatial unit within which calcite is monitored and reported. Stream reach and segment are described here to aid understanding when reviewing and interpreting results. Stream reaches were first delineated over the entire Elk Valley study area in 2013 (Robinson *et al.* 2013). A stream reach is a stream network subdivision that represents a spatial scale finer than a valley segment (or catchment), but larger than a mesohabitat unit (Bisson *et al.* 2006). Several benefits of using the stream reach as a sampling unit were noted, but key to this was the expectation that a reach, representing a relatively homogeneous section of stream in

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[8] Segment = one or more reaches grouped based on historical calcite survey results and similar exposure to mining from a water quality perspective.

[9] Coal Mountain Operations is no longer operating and is in a Care and Maintenance status.

terms of channel morphology, riparian cover and flow (RISC 2001), would be expected to exhibit low spatial variability of calcite deposition.

In the 2016 - 2017 programs, effort was redistributed from areas where CI variability was low to areas where CI variability was higher. This was accomplished by sampling of stream segments. A stream segment was defined as one or more contiguous reaches with: (1) similar CI values observed during current monitoring; and, (2) a similar exposure to mining activity. Segments were monitored at an indicator reach, which was selected based on a combination of past monitoring results and logistical field sampling criteria. The most downstream reach was selected as the indicator reach to represent most stream segments as it would represent all upstream inputs in terms of water quality and land-use affecting calcite values.

Replication was required to accurately describe reach-based calcite observations. Since within-reach calcite variability was relatively unknown in 2013, triplicate sampling was recommended using systematic-stratified sampling set at 25%, 50% and 75% of the total reach length. With an improved understanding that within-reach variability was a function of the amount of calcite in a reach, the number of replicates (i.e., sites) was set as a function of the degree of calcification (Table 1).

Figure 2. Upper Fording River watershed study area map, including calcite index data by stream reach in 2019.

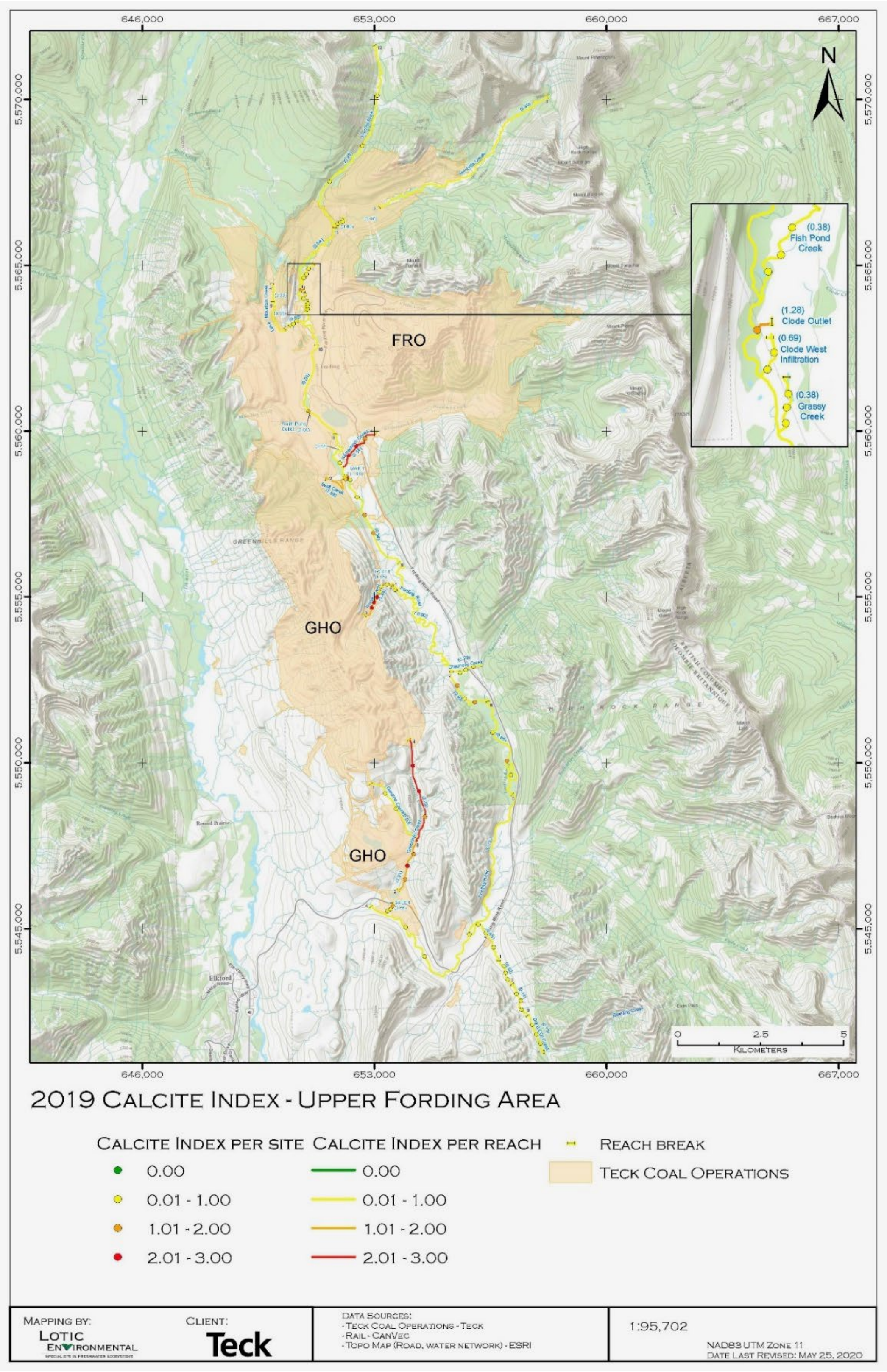


Table 1. **Number of sample sites per stream reach by CI bin (modified from Robinson and Atherton 2016).**

CI Bin	N
0.00-0.25	3
0.25-1.00	3
1.00-1.50	6
1.50-2.00	6
2.00-2.50	3
2.50-3.00	3

Calcite was measured at each site using a modified Wolman pebble count (Wolman 1954). Beginning at the downstream end of a site, a sampler entered the stream and haphazardly selected an individual pebble and then attempted to remove the rock with the index finger. This was repeated for 100 observations per site, with the following observations recorded for each particle:

a) Calcite concretion (CI_c):

- Was the particle removed without calcite-induced resistance (0)?
- Was the particle removed with any noticeable amount of force to overcome calcite-induced resistance (1)?
- Was the particle non-movable or fully concreted by calcite (2)?

b) Calcite presence/absence (CI_p) – Did the individual particle have calcite deposition? 0= No, 1= Yes.

Concretion score and calcite presence score were calculated and summed to form the CI according to:

$$CI_p = Calcite\ Presence\ Score\ = \frac{Sum\ of\ pebbles\ with\ calcite}{Number\ of\ pebbles\ counted}$$

$$CI_c = Calcite\ Concretion\ Score = \frac{Sum\ of\ pebble\ concretion\ scores}{Number\ of\ pebbles\ counted}$$

$$CI = Calcite\ Index = CI_p + CI_c$$

Annual reports use CI ranges or "bins" to describe the general spatial distribution of CI by stream length (stream kilometer). Six bins of 0.5 CI intervals are used to divide the range of potential CI scores (0.00 – 3.00). Results are then summarized for four stream categories:

- Fording and Elk mainstems (reference);
- Tributaries (reference);
- Fording and Elk mainstems (mine-exposed); and
- Tributaries (mine-exposed).

In the Elk River and UFR alike, temporal change in calcite deposition was assessed in annual reports from 2013–2016 using linear regression of CI versus time (year) (e.g., Robinson and MacDonald 2014). For 2017 and onward, a non-parametric Mann-Kendall trend test was used in place of linear regressions. Similarly, Mann-Kendall was used to determine if there were trends (positive or negative) in the calcite data over time (2013-2019). An α-value of 0.10 was selected for both linear regression and Mann-Kendall results to account for the data from a shorter time period (i.e., it was more difficult to accurately detect trends with shorter time periods). Having this larger alpha value allowed for a conservative interpretation of significance while not overlooking potential trends at an early stage in monitoring.

Regional monitoring reports have demonstrated the relationship of the calcite index and its sub-components. The relationships of CI to calcite presence (CI_p) and calcite concretion (CI_c) were first reported in 2013 (Robinson and MacDonald 2014) and remained consistent when reassessed in 2018. The assessments show that CI scores below 1.00 are largely driven by CI_p (Figure 3). As the maximum CI_p is 1.00, any increase in CI beyond that is primarily driven by increased CI_c (Figure 4). From this it is generally understood that concretion is indicative of a more advanced state of calcite deposition, occurring more substantially as CI approaches 1.00.

Spatial patterns were presented within the UFR by mapping reaches with a colour coding system based on reach-mean CI (Figure 2). A table was prepared to show CI and CI_c by reach for 2013-2019 with results of the linear trend analyses completed in each annual report since 2015. Cells in the table have been shaded to indicate significant linear trends for all years prior to that cell. The 2019 column shows the assessment of the entire 2013-2019 dataset (from McCabe and Robinson 2020). CI trends from 2013-2019 were assessed within the UFR through linear regression binned by exposed and reference reaches. CI was subsequently assessed at a reach scale with linear regression. Results show significant increasing and decreasing trends for both α-value of 0.10 and 0.05.

Because concretion indicates an advanced state of calcite deposition, concretion in particular was selected as a key factor that could drive WCT declines for several effect pathways in the UFR. The importance of concretion was also highlighted by Hocking *et al.* (2020), who found that calcite concretion had the strongest influence (as opposed to calcite index or calcite presence) on WCT spawning suitability throughout the Elk River valley. Spatial and temporal trends in CI_c were therefore also examined by calcite monitoring reach as described above and binned by reference versus mine-exposed reaches and mainstem versus tributary reaches in the UFR. Regression analysis was run to assess trends within each spatial category but keeping exposed and reference reaches separate.

In addition to above analyses, calcite data was further binned into strata for direct comparison to the available fish population data and strata and river segments surveyed in Cope (2020). For example, not all tributaries with calcite data in UFR support fish access due to the presence of barriers. Fish monitoring also typically occurs in the lowest reaches of each tributary where there is the majority of fish habitat associated with the Fording River mainstem. The strata used by Cope (2020) divided the watershed into sections representing the mainstem headwaters, mid-mainstem, lower mainstem, lower tributary and upper tributary reaches. A summary of the geographic locations and sample years for the strata used in Cope (2020) is shown in Table 2. The calcite data reaches from McCabe and Robinson (2020) were aligned with strata from Cope (2020) as shown in Table 3. This geographic binning allowed for a direct comparison of trends in calcite in WCT productivity in relation to spatial and temporal changes in calcite and in the context of fish declines.

Figure 3. Calcite index versus calcite presence scores from 2018 calcite regional monitoring data (McCabe and Robinson 2020).

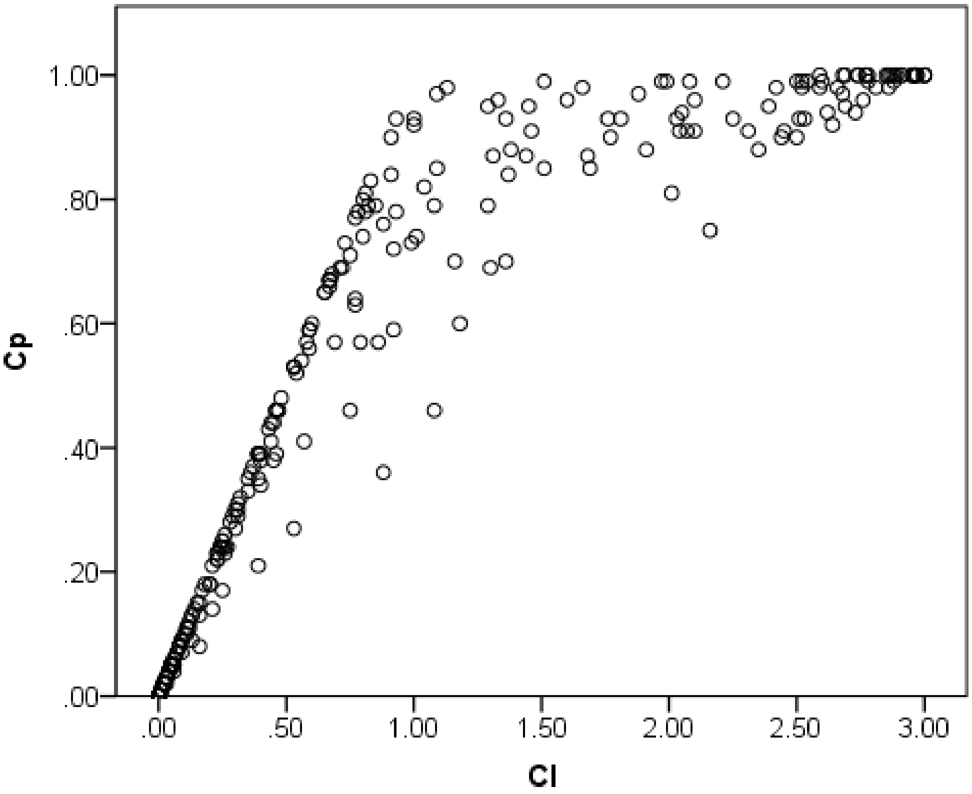


Figure 4. Calcite index versus calcite concretion scores from 2018 calcite regional monitoring data (McCabe and Robinson 2020).

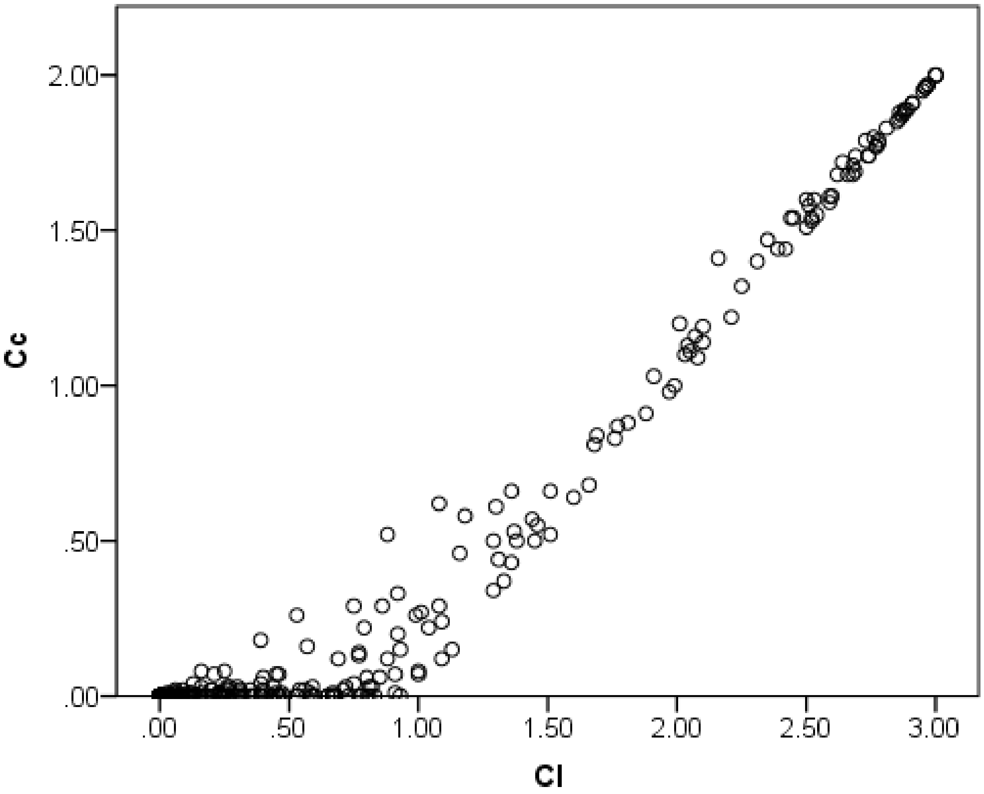
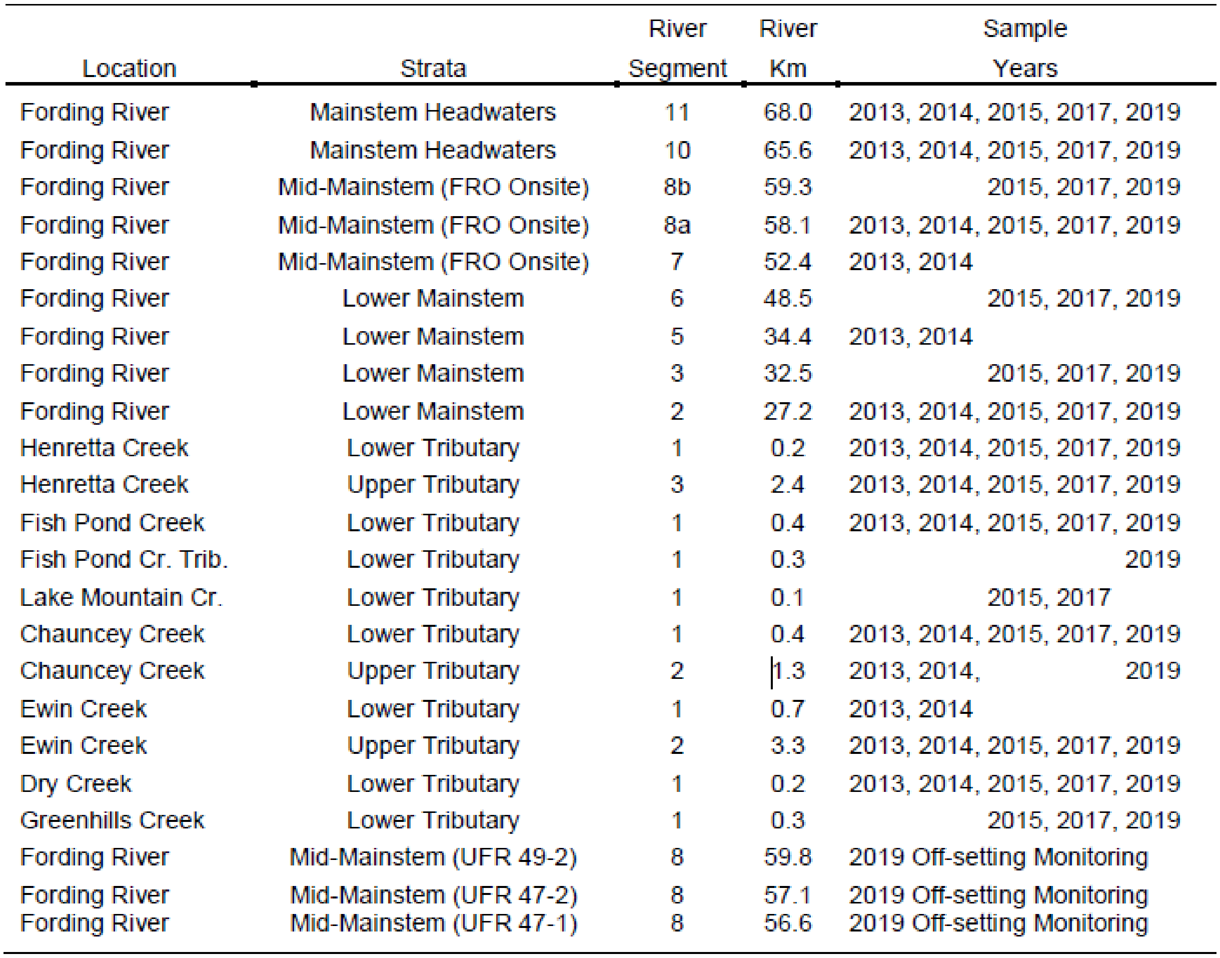


Table 2. Summary of UFR strata relative to WCT sample locations for 2013, 2014, 2015, 2017 and 2019 (from Cope 2020).



Location	Strata	River Segment	River Km	Sample Years
Fording River	Mainstem Headwaters	11	68.0	2013, 2014, 2015, 2017, 2019
Fording River	Mainstem Headwaters	10	65.6	2013, 2014, 2015, 2017, 2019
Fording River	Mid-Mainstem (FRO Onsite)	8b	59.3	2015, 2017, 2019
Fording River	Mid-Mainstem (FRO Onsite)	8a	58.1	2013, 2014, 2015, 2017, 2019
Fording River	Mid-Mainstem (FRO Onsite)	7	52.4	2013, 2014
Fording River	Lower Mainstem	6	48.5	2015, 2017, 2019
Fording River	Lower Mainstem	5	34.4	2013, 2014
Fording River	Lower Mainstem	3	32.5	2015, 2017, 2019
Fording River	Lower Mainstem	2	27.2	2013, 2014, 2015, 2017, 2019
Henretta Creek	Lower Tributary	1	0.2	2013, 2014, 2015, 2017, 2019
Henretta Creek	Upper Tributary	3	2.4	2013, 2014, 2015, 2017, 2019
Fish Pond Creek	Lower Tributary	1	0.4	2013, 2014, 2015, 2017, 2019
Fish Pond Cr. Trib.	Lower Tributary	1	0.3	2019
Lake Mountain Cr.	Lower Tributary	1	0.1	2015, 2017
Chauncey Creek	Lower Tributary	1	0.4	2013, 2014, 2015, 2017, 2019
Chauncey Creek	Upper Tributary	2	1.3	2013, 2014, 2019
Ewin Creek	Lower Tributary	1	0.7	2013, 2014
Ewin Creek	Upper Tributary	2	3.3	2013, 2014, 2015, 2017, 2019
Dry Creek	Lower Tributary	1	0.2	2013, 2014, 2015, 2017, 2019
Greenhills Creek	Lower Tributary	1	0.3	2015, 2017, 2019
Fording River	Mid-Mainstem (UFR 49-2)	8	59.8	2019 Off-setting Monitoring
Fording River	Mid-Mainstem (UFR 47-2)	8	57.1	2019 Off-setting Monitoring
Fording River	Mid-Mainstem (UFR 47-1)	8	56.6	2019 Off-setting Monitoring

Table 3. Strata designations in the UFR watershed to align stream reach calcite data (McCabe and Robinson 2020) and WCT population data (Cope 2020).

Location	**Strata**	**River Reach (Calcite data)**	**River Segment (WCT data)**
Fording River	Mainstem Headwaters	12	10, 11
Fording River	Middle Mainstem (FRO onsite)	9, 10, 11	7, 8a, 8b, 9
Fording River	Lower Mainstem	5, 6, 7, 8	1, 2, 3, 5, 4, 6
Chauncey Creek	Lower Tributary	1	1
LCO Dry Creek	Lower Tributary	1	1
Fish Pond Creek	Lower Tributary	1	1
Greenhills Creek	Lower Tributary	1	1
Henretta Creek	Lower Tributary	1,2	1
Clode Creek[1]	Lower Tributary	1	-
Lake Mountain Creek	Lower Tributary	1	1

[1] No WCT data available for Clode Creek

2.2. Assessment of Biological Effects

2.2.1. Effects to Spawning Suitability

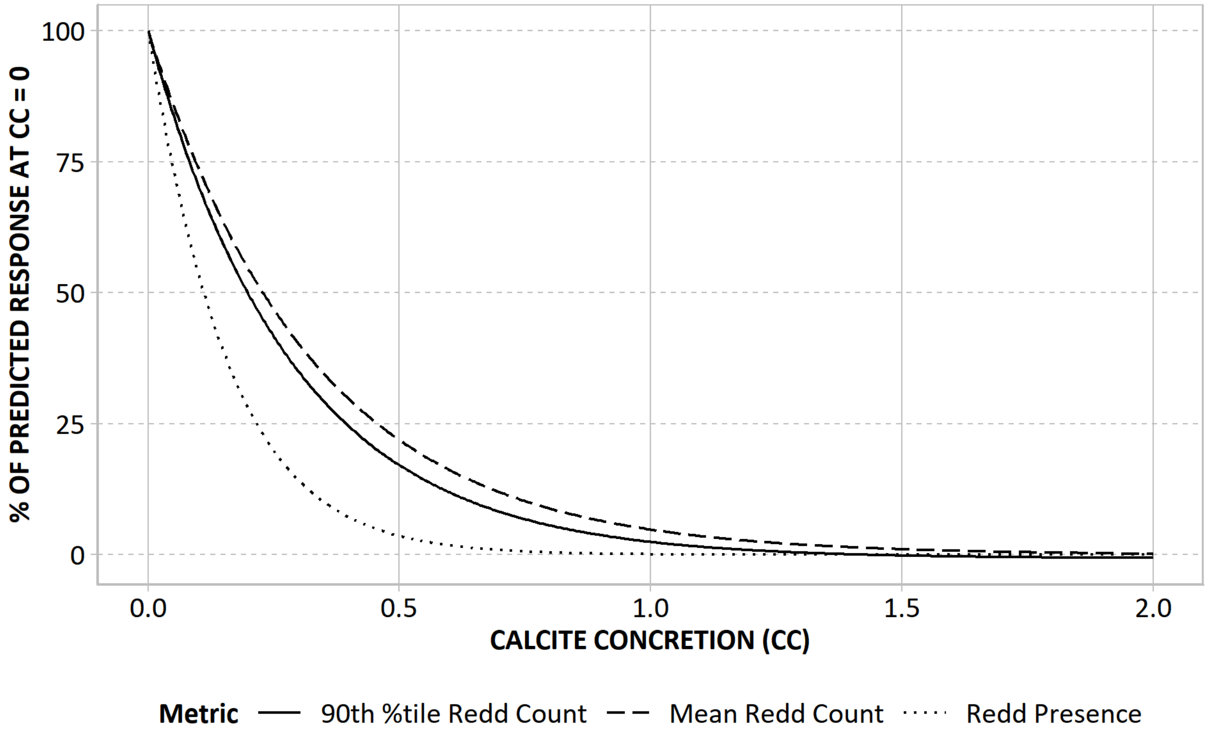
This pathway was assessed by applying the results from on-going studies in the Elk Valley that are investigating the effects of calcite to WCT spawning habitat (Hocking *et al.* 2019, 2020). The studies were conducted over a range of sites with varying levels of calcite and results were used for the EoC to predict effects of calcite prior to and during the Decline Window.

To develop a predictive spawning suitability curve, Hocking *et al.* (2020) sampled 5 streams in 2018 and 17 streams in 2019 across the Elk River valley. At each stream, the presence and abundance of WCT redds, calcite, and other fish habitat data were measured at the mesohabitat scale. A mesohabitat is a defined hydromorphological unit within a stream reach such as a pool, riffle or glide habitat. The relationship between spawning habitat suitability and calcite conditions was assessed with two measures: presence of redds, and number of redds per mesohabitat unit a stream. To provide a characterization of the relationship between calcite and the number of redds in a stream, effects on redd counts were assessed using two main approaches: the effect on the mean number of redds and the effect on the 90th quantile of redd counts (i.e., calcite effect on the probability of having high counts of redds).

Relationships of redd presence and redd counts versus explanatory variables including calcite and other fish habitat variables such as spawning substrate, depth, velocity, and water quality were investigated using a model selection approach where alternate models with different combinations of explanatory variables were competed against one another and ranked using Akaike Information Criterion (AIC_c) scores. It was determined that calcite concretion (CI_c) was the most important explanatory variable for describing variance in redd presence, mean redd count, and the 90th quantile of redd counts. Modeled suitability curves are shown in Figure 5. Overall, redds were observed only in habitats with low concretion; no redds were observed at moderate to high concretion or high calcite index. In all cases, the influence of calcite concretion on the response variables was negative, and the three spawning habitat suitability models for WCT decreased exponentially with increasing levels of calcite concretion. In particular, the mean likelihood of redd presence decreased steeply from ~0.15 at a calcite concretion score of 0 to a probability close to zero at 0.5 calcite concretion. Redd counts in a stream also decreased exponentially with increasing calcite concretion, although at a slightly slower rate than redd presence.

The suitability curve developed by Hocking *et al.* (2020) for mean redd count was applied to the calcite concretion data from the UFR watershed (McCabe and Robinson 2020) to support the evaluation of whether calcite can explain WCT population declines. This pathway assesses the potential effect of calcite on WCT through recruitment reduction or failure from reduced spawning success. The spawning suitability curve was applied to the annual trends in calcite concretion data for the overall UFR watershed by year and by fish strata used in Cope (2020) (Table 3). This allowed an evaluation of predicted trends in spawning suitability for the major fish-bearing reaches and strata for the UFR as driven by spatial and temporal changes in calcite conditions. However, one uncertainty of applying the spawning suitability curves in this way is that they were based on calcite data collected at the mesohabitat scale in Hocking *et al.* (2020), whereas data collected by McCabe and Robinson (2020) occur at a larger stream segment or reach scale. We therefore focus on applying the spawning suitability curve to describe potential trends in suitability, and in particular comparing spawning suitability within the Decline Window to suitability prior to the Decline Window, rather than predicting absolute spawning suitability across the UFR.

Figure 5. **Draft WCT spawning suitability response curves for calcite concretion based on data collected in 2018 and 2019 from 17 tributary streams of the Elk River, B.C. Curves are model averaged predictions of the effects of calcite concretion on redd presence and redd counts (mean and 90th quantile) (from Hocking *et al.* 2020).**



2.2.2. Effects to Rearing – Invertebrate Prey Availability

Calcite can potentially affect WCT rearing success by impacting invertebrate habitat availability and quality, which in turn can reduce invertebrate production and food availability for WCT juveniles and adults. The investigation into potential reductions in food availability more broadly is presented in the SME Food Availability Report by Orr and Ings (2021). This pathway related to potential effects of calcite to invertebrate production was assessed here primarily based on characterization of calcite effects to benthic invertebrates by Barrett *et al.* (2016), who completed biological sampling in 2014 and 2015 in the UFR watershed. The study aimed to characterize relationships between 1) calcite deposition and benthic invertebrate community characteristics, and 2) calcite deposition and periphyton productivity endpoints to determine the level of calcite at which biological effects occur. Thirty-one areas (24 mine-exposed and seven reference) were sampled in 2014, and 114 areas were sampled in 2015, with an additional 15 mine-exposed areas added to cover a broader range of calcite conditions.

At each sampling area, *in situ* water quality measurements were taken to assess temperature, pH, dissolved oxygen, and specific conductivity. Water samples were also collected for laboratory analysis of total organic carbon (TOC), dissolved organic carbon (DOC), total suspended solids (TSS), total dissolved solids (TDS), turbidity, total alkalinity, bicarbonate alkalinity, total and dissolved metals/metalloids, anions (nitrate, nitrite, sulphate, chloride, fluoride, bromide), ammonia, total Kjeldahl nitrogen (TKN), and total phosphorus. Benthic invertebrate communities were sampled following the 3-minute kick sampling method of the Canadian Aquatic Biomonitoring Network (CABIN) for sampling wadeable streams (Environment Canada 2012). Samples were sorted and identified to lowest practical taxonomic level.

Barrett *et al.* (2016) found significant positive correlations ($p < 0.001$) between calcite index and periphyton productivity in 2014 and 2015 (Figure 6). They attributed this to the possibility that calcite deposits may provide a surface favourable to periphyton growth, that periphyton growth alters water quality near the periphyton surface in a manner that favours calcite formation, and/or that bioavailable nutrient concentrations may be elevated in areas with more calcite. However, no specific calcite index value was determined above which periphyton productivity would be expected to deviate from the normal ranges (Barrett *et al.* 2016, White and Larratt 2016). The normal range is defined as 2.5th and 97.5th percentiles of the reference area data collected (Barrett *et al.* 2016).

The Barrett *et al.* (2016) results for invertebrate prey availability are shown in Figure 7. All plots shown in Figure 7 indicate significant positive or negative relationships with increasing calcite. These results suggest that seven selected benthic invertebrate community endpoints correspond directly with relative calcite exposure. Key findings include a decrease in Ephemeroptera with calcite, an increase in Chironomidae with calcite, and an increase in Diptera with increasing calcite. In contrast, total invertebrate abundance, which is a measure of total invertebrate production, was not correlated to increasing calcite index ($p = 0.71$; Barrett *et al.* 2016). Although these relationships cannot be solely ascribed to the effects of calcite due to the confounding effects of water quality on benthic invertebrates, the data indicate that benthic invertebrate community structure (and especially the proportion of Ephemeroptera) deviates from the normal range when calcite index is greater than 1.

The calcite index value of >1 was thus used as a reference point for evaluation of this pathway relative to calcite conditions observed prior to and during the Decline Window.

Figure 6. **Scatterplot of chlorophyll-a and AFDM in relation to calcite index for all reference and mine-exposed areas sampled in Elk Valley in 2014 and 2015 (Barrett *et al.* 2016). Grey shade represents the normal range for each periphyton endpoint, defined as values between the 2.5th and 97.5th percentiles for reference area data collected in 2015. Values below the detection limited are plotted as open symbols at the detection limit. The lower limit of the normal range for AFDM is <0.5.**

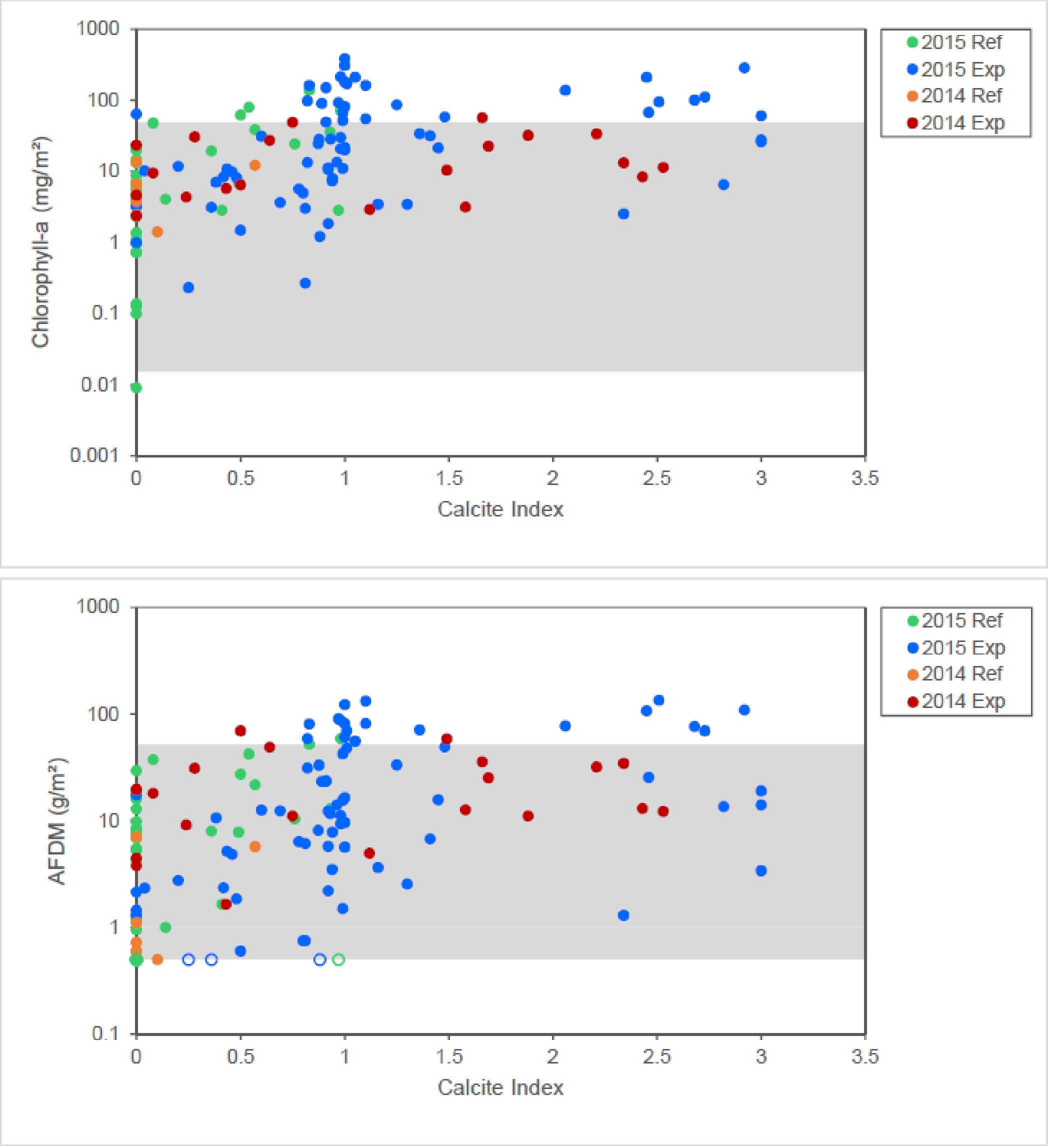
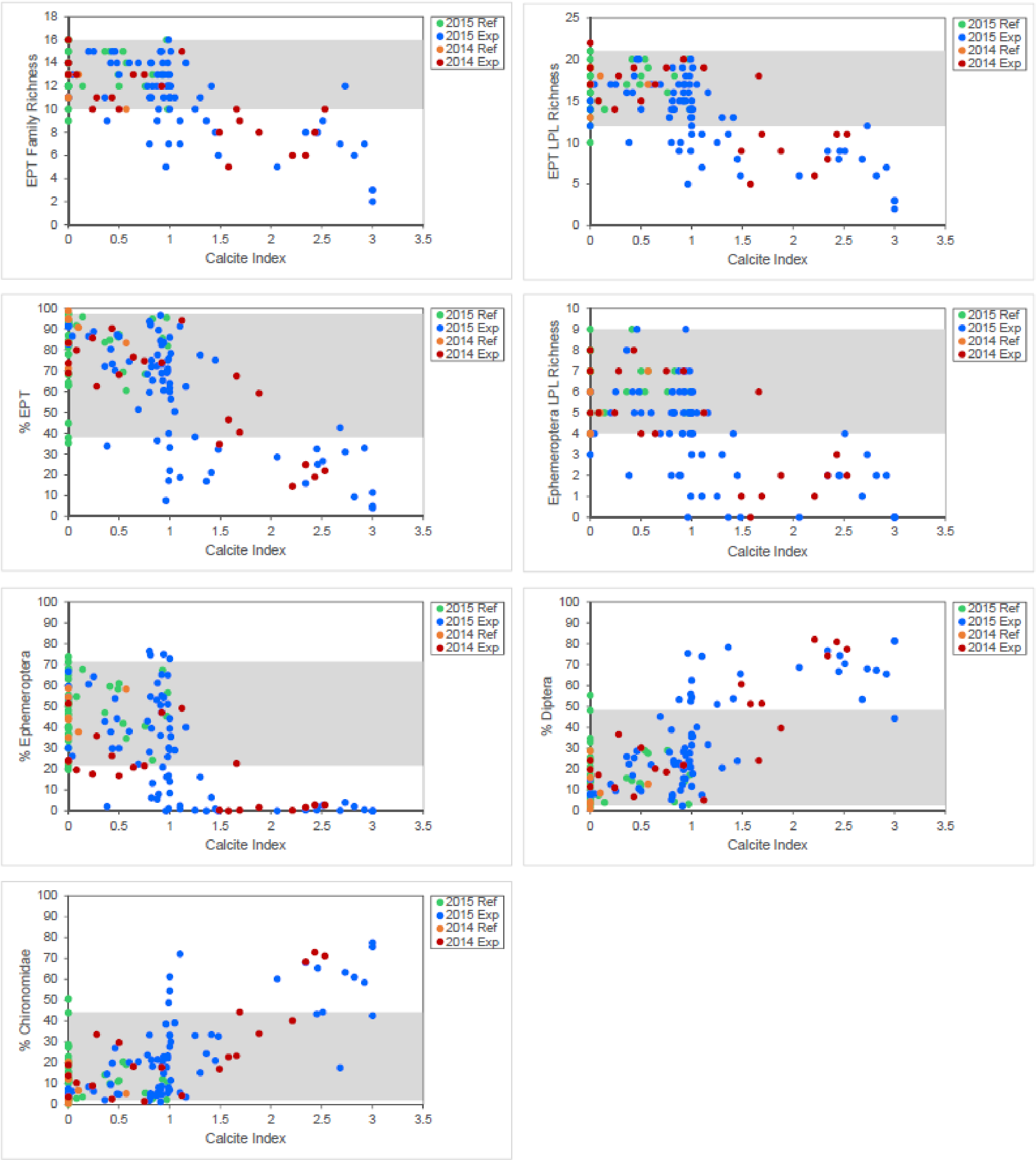


Figure 7. **Scatterplot of selected benthic invertebrate endpoints in relation to calcite index for all reference and mine-exposed areas sampled in Elk Valley in 2014 and 2015 (Barrett *et al.* 2016). Gray shade represents the normal range for each benthic endpoint, defined as the 2.5th and 97.5th percentiles for reference data collected in 2015.**



2.2.3. Effects to Rearing – Biogenic Calcite Precipitation and Dissolution

Calcite can potentially affect WCT incubation success, juvenile rearing success and adult productivity via a biogenic precipitation and dissolution pathway, where periphyton can accelerate calcite deposition and mediate the release of cyanotoxins and metals during localized calcite dissolution. Potential effects are hypothesized to be most influential for young of year and rearing juveniles. There is significant uncertainty regarding the prevalence and magnitude of this pathway. Evidence for this pathway was compiled from literature and monitoring data in the UFR and Elk Valley more broadly and is evaluated qualitatively in the results section due to the unavailability of dose-response relationships. For more information, also see the Cyanobacteria, Periphyton and Aquatic Macrophyte Impacts Evaluation of Cause (Larratt and Self 2021).

There are two known routes for cyanotoxin impacts 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, while uptake across the gills and epithelial layer are also important in juveniles (Ferrão-Filho and Kozlowsky-Suzuki 2011). Juvenile WCT (alevins/fry) are more likely to utilize zooplankton than older life-stages (Luecke 1986). For all life stages, the main mechanism of harm appears to be accumulation in liver tissue inducing liver necrosis and it may be most acute in juveniles (Carbis *et al.* 1997; Beattie *et al.* 1998; Johnson *et al.* 2013; WHO 2019). It is possible that cyanotoxins produced in UFR could biomagnify in WCT food including benthic invertebrates, and particularly in zooplankton, both reducing food quality for fish and increasing the cyanobacteria dose through trophic transfer. These effects are well documented elsewhere and may occur in the UFR (Chorus and Bertram 1999; Watanabe *et al.* 1992; Ferrão-Filho and Kozlowsky-Suzuki 2011).

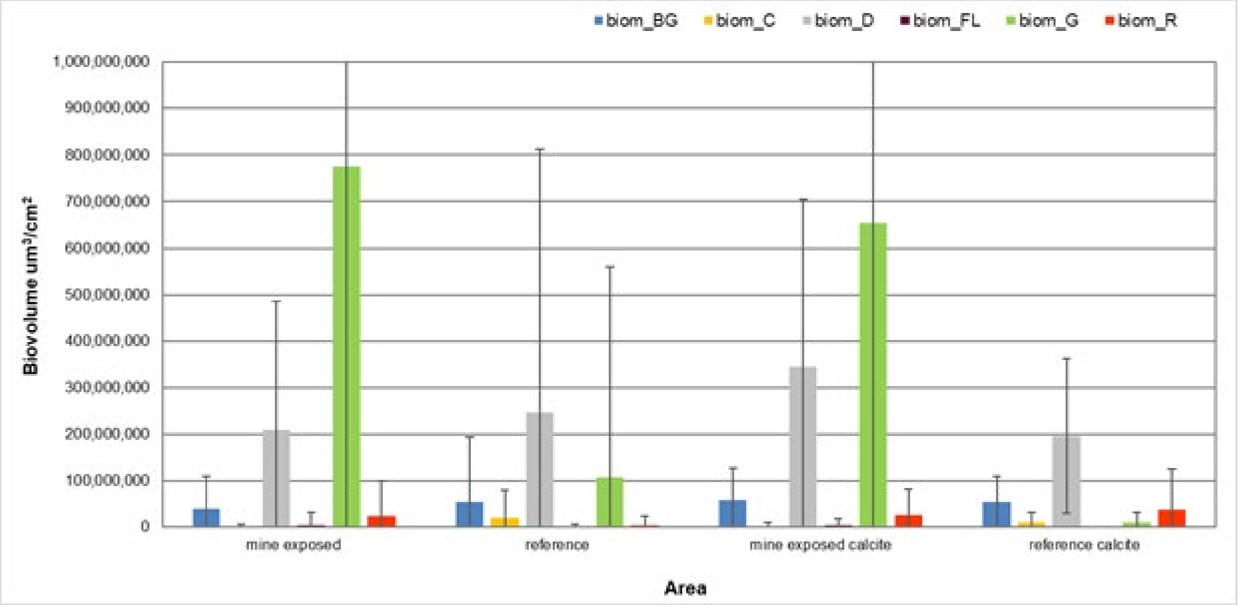
During the research phase of this project, Larratt Aquatic conducted a literature review and a review of other research conducted at FRO (Table 4). The data assessment phase involved a comparison of calcite data collected for FRO and periphyton data. The 2015 periphyton community study data and results were referred to extensively as this was the most exhaustive dataset from UFR for this question. Correlations between chl-a and AFDM (a metric of calcite) were conducted as part of 2015 periphyton community study.

Table 4. **Pertinent data sets – lines of evidence for cyanobacteria and cyanotoxicity.**

Data Set	**Description**
Literature	Cyanotoxin biomagnification, impacts to fish, environmental persistence, biogenic calcite characteristics
Algae data UFR	Interlab study 2013, 2013 data, 2015 UFR assessment (White and Larratt 2016), esp. for cyanobacteria and other important algae/periphyton
Algae grabs	Winter 2020 periphyton samples, percentage cyanobacteria data
Toxicology	sample sets in RAEMP / LAEMP reports (Minnow) | Nautilus reports
Sediment TOC	Searched data for correlations between benthic invertebrate metrics and periphyton metrics, or surrogates (TOC) in Minnow LAEMP /RAEMP
Calcite surveys	Annual extent surveys by Lotic; Atherton 2017 report | biogeochemical modelling of calcite precipitation (Goudey *et al.* 2009)
Calcite-Metals	UGHC calcite metals project | Day and MacGregor (SRK) 2014 metals in calcite report
Water Quality	Monthly sampling at UFR sites for calcite dissolution predictions from FRO staff| shallow groundwater data from SNC-Lavalin (S. Humphries).
Flow Data	FR-FRNTP flow data for fall spates
Teck reports	Cause reports: Goudey *et al.* 2009 | MacGregor and Day (SRK) 2011 | Lotic annual CI extent reports 2012 - 2019
Observations	Photography and field observations by FRO staff and their consultants

Significant positive correlations ($p < 0.001$) have been observed between CI and periphyton metrics (chlorophyll-a, AFDM) measured at Elk Valley sites in 2014 and 2015 (Barrett *et al.* 2016; Worrall *et al.* 2016), indicating that increases in calcite can correspond to increases in periphyton production (see Figure 6). In the extensive 2015 periphyton productivity program, samples from sites with moderate CI scores of 1.0 – 1.5 usually had tightly felted mats of cyanobacteria with diatoms on and in the calcite because these algae both encouraged deposition and became trapped within the calcite layer. While heavy calcification and associated concretion (CI>2.0) reduced periphyton diversity, sites with moderate CI scores (1.0 – 1.5) had high periphyton biovolume and diversity, both at mine-exposed and at reference sites (Figure 8) (White and Larratt 2016). Assessment of the organic component of calcite in the UFR showed a 5.2 - 7% loss on ignition (MacGregor and Day 2011; Solenis 2018), indicating presence of periphyton trapped within the calcite matrix.

Figure 8. **Algae biovolume in mine-exposed and reference sites, with and without calcite, throughout the Elk Valley in 2015.**



Periphyton, especially cyanobacteria, can enhance biogenic calcite precipitation through photosynthesis, exudates, and cell surface interactions with stream water. A shift in the calcite precipitation mechanism in streams takes place from a dominantly physico-chemical precipitation below a resurgence point to a predominantly biogenic precipitation further downstream (Merz and Zankle 1991; Ford and Pedley 1996). Thus, physico-chemical processes (from de-gassing CO_2) are expected to predominate downstream of rock drain emergence sites, while calcite deposition at most sites in the UFR is expected to also demonstrate biogenic control (Goudey *et al.* 2009). UFR geochemical modelling supported the hypothesis that carbon dioxide consumed by photosynthesis of algae, cyanobacteria, and aquatic plants can increase pH and enhance biogenic calcite precipitation (Goudey *et al.* 2009).

Calcite crusts in UFR have different morphologies, strengths and colors depending on the deposition process and periphyton taxa involved (Goudey *et al.* 2009). Physico-chemically precipitated calcite has a stronger, compact crystalline structure, while biogenic calcite is more porous and weaker but with a notably high capacity for metal removal (Zhou *et al.* 2017).

Cyanobacteria enhance biogenic calcite production and they can produce cyanotoxins. Many of the harmful cyanobacteria impacting rivers globally do not occur in the UFR. The taxa associated with calcite in the UFR include many potential toxin producers, of which *Phormidium autumnale* is of increasing concern world-wide because it has been involved in lotic fish kills. Physical conditions known to increase cyanotoxin production include low flows, low light, elevated phosphorus and water temperature >10°C (Fetscher *et al.* 2015; Wiltsie *et al.* 2018; Sivonen 2009). These toxins can persist in substrates for weeks to months, even years under anoxic conditions (Lahti *et al.* 1997; WHO 2019;

Klitzke *et al.* 2012; Henao *et al.* 2019. The storage of cyanotoxins in calcite and in fine sediments extends the potential for cyanotoxin harm from the UFR summer rearing life stage and into the overwintering stage. Entrapped cyanobacteria and their toxins can be delivered to the stream during winter low flows by calcite dissolution combined with the slow break-down of those toxins under cold water temperatures (WHO 2019; Lahti *et al.* 1997), creating a potential for cyanotoxin exposure and biomagnification during winter low flows as well as in summer low flows.

In the absence of analytical toxicity tests from the Decline Window, potential for cyanotoxicity were inferred from the biovolumes and types of cyanobacteria known to produce toxins in the UFR and from the conditions under which toxins are generated and persistent. These cyanobacterial types were compiled from surveys in the UFR and compared to those that have caused documented fish impacts elsewhere.

Calcite dissolution can cause release of cyanotoxins and it demonstrates seasonality in response to flows, water temperature, solution strength, and through localized microbially-driven pH/CO_2 and decomposition conditions (Atherton 2017). Biogenic calcite accumulations are likely to be highest in the fall after a period of warm temperatures, low summer flows, and high periphyton productivity. High flows during the fall can break up calcite and accelerate dissolution, which subsequently reduces the likelihood for cyanotoxicity in winter during winter low flows. Three processes are involved:

1) Mechanical erosion occurs when flows exceed the shear stress of the substrate, and above that threshold, mechanical erosion continues to increase with discharge (Mulec and Prelovost 2015; Covington *et al.* 2015).
2) Chemical dissolution is driven by pH and carbon dioxide dynamics as well as by discharge-driven dilution with plateaus above a certain discharge (Covington *et al.* 2015).
3) Biochemical dissolution is driven by periphyton biofilm decomposition processes and researchers elsewhere have found environmental parameters including discharge and light can be used in estimating calcite dissolution rates enhanced by several microbial activities within periphyton mats (Mulec and Prelovsek 2015).

Flows are a critical driver of these three processes. UFR flow data was examined (FR-FRNTP) prior to and during the Decline Window for spates exceeding ~2-5 m^3/s that would be strong enough to dislodge periphyton and biogenic calcite. In particular, flows during the fall of 2013 to 2019 were examined. This flow range was determined using available flow velocity data at a given discharge, field observations and a review of periphyton/calcite removal research.

Additional work was undertaken by Teck to develop predictions of when calcite will form and dissolve based on solution strength, pH and thermodynamics in the UFR mainstem. Three sites were investigated for the period prior to and during the Decline Window from January 2013 to December 2019, including FR_FRCP1 (Fording River downstream of Greenhills Creek), LC_FRDSDC (Fording River downstream of LCO Dry Creek) and GH_FR1 (Fording River downstream of Swift/Cataract Creek). Shallow groundwater from the Greenhouse Side-Channel was also investigated for 2019 to represent hyporheic water that could affect the calcite/periphyton layer.

For these three sites, the Langelier saturation index (LSI) that approximates the base 10 logarithm of the calcite saturation level and a Stability Factor that determines the ratio of the calcium/bicarbonate concentration product to a "calcite-stable effluent" were determined. A negative LSI indicates dissolution should proceed, while a positive LSI indicates calcite precipitation should occur. Waters with a stability factor less than 1 are considered "calcite stable" and will not precipitate calcite. In both these indices, pH is the master driver. Biologically driven pH fluctuations in the periphyton mat will seasonally encourage calcite precipitation during photosynthesis (high pH) and encourage calcite dissolution during decomposition (low pH).

The precipitation, dissolution and erosion of calcite crusts can also affect metal mobilization. The literature demonstrates that calcite dissolution and recrystallization allowed sequestration of metals including Zn, Cd, Pb, and Cu in solid solution within calcium carbonate (Schlosseler *et al.* 1999; Mugwar and Harbottle 2016). This involves sorption to crystal and cell surfaces, and incorporation into re-forming calcium carbonate lattices metals (Mugwar and Harbottle 2016).

Calcite in 34 mine-affected samples from the Elk Valley were observed to be enriched in cadmium, nickel, selenium, and zinc, compared to calcite from 5 reference streams (Day and MacGregor 2014). The five UFR samples from Day and MacGregor (2014) are presented in the Results section. The UFR sediment and calcite metal results were screened against BC sediment guidelines and compared to uncontaminated lake sediments from the Southern Rocky Mountain Region (DiMauro *et al.* 2021; Rieberger 1992).

2.2.4. Effects to Incubation Conditions

Calcite accumulations can potentially influence incubation habitat for buried WCT embryos/alevins by interfering with exchange of surface water and hyporheic water that causes a reduction in flow and dissolved oxygen in the interstitial spaces of spawning gravel. Studies by Wright *et al.* (2017, 2018) investigated dose-response relationships for this pathway through field studies of calcite index, hyporheic conditions (i.e., DO concentration at depth and hyporheic flow), as well as other naturally varying potential covariates (i.e., key fish habitat variables, hyporheic water quality, substrate composition, and surface hydrology). Study sites in 2016 for the Wright *et al.* (2017, 2018) were selected within the upper Fording River watershed to represent both mainstem and tributary spawning habitat used by WCT in the upper Fording River and to represent the full range of calcite conditions based on previous calcite monitoring. Spawning was visually confirmed (i.e., redds, spawning fish) at the sites selected in the upper Fording River, Clode Creek, and lower Greenhills Creek. These sites were supplemented with sampling at LCO Dry Creek and Henretta Creek in 2017. Two methods were employed to measure hyporheic flow: a hydraulic head method using piezometers (30 cm and 50 cm substrate depth readings) and a temperature method employing Tidbit temperature loggers installed in a vertical array at substrate depths of 10 cm to 40 cm. Results were used by Wright *et al.* (2017, 2018) to model relationships between hyporheic conditions and CI taking into consideration site characteristics and covariates (e.g., % fines), using linear mixed-effects models. Model outputs were then compared to BC Water Quality Guidelines for buried embryos/alevins and assessed in the context of WCT red digging behaviour.

Overall, Wright *et al.* (2017, 2018) found that calcite index was an important predictor of dissolved oxygen in the substrate (calcite index reduced dissolved oxygen), and that this effect increased with depth in the substrate and was also related to other covariates, such as substrate % fines. Calcite index was not found to be related to hyporheic flow. The model for dissolved oxygen predicted that at a maximum calcite index score of 3, the average instantaneous dissolved oxygen is ~7.5 mg/L at a depth of 30 cm and ~6 mg/L at a depth of 50 cm, both of which are at or above the instantaneous minimum BC Water Quality Guidelines for buried embryos/alevins. For reference, the average redd depth for Westslope Cutthroat Trout is between 10 and 30 cm (DeVries 1997, Magee and McMahon 1996). Therefore, although DO concentrations observed in the studies were at times below the minimum guidelines for the protection of buried life stages, the most significant effects on incubation conditions were predicted at sites with CI scores higher than ~1.25, relatively high % fines, and at depths deeper than typical redd depths. This suggests that at depths less than 30 cm, increases in calcite may not be an important factor in determining incubation success.

2.2.5. Effects to Rearing – Overwintering Habitat

Calcite may affect WCT by altering habitat required for overwintering, particularly for juveniles. This pathway was evaluated qualitatively via a literature review as there have been no studies explicitly examining the link between calcite, overwintering conditions and impacts to salmonids. Generally, fish in the UFR migrate to habitat that protects them from the harshest winter conditions (Cope *et al.* 2016), reflecting a preference for areas that favour reduced energy use (Cunjak 1996). Salmonids, including WCT, tend to move to habitat that provides cover and lower water velocities (Cunjak 1996, Hiscock *et al.* 2002, Huusko *et al.* 2007, Brown *et al.* 2011). Slow velocity areas used for overwintering may include pools, backwater areas, off-channel ponds, logjams, swamps, side channels, beaver ponds, and tributaries, and the amount of available cover influences the number of fish that overwinter in an area (Tschaplinski and Hartman 1983, Bustard 1986, Swales *et al.* 1986, Meyer and Griffith 1997). Areas with these types of habitats are often limited in streams and rivers, so it is common for fish to be found in large groups or aggregations within optimal habitats (Huusko *et al.* 2007). Small salmonids seek cover in interstitial spaces in the stream substrate, whereas large-bodied individuals may have to move into slow velocity areas to find suitable shelters from ice and predators (McMahon and Hartman 1989; Lindstrom and Hubert 2004). This difference in shelter preference between juveniles and adult trout was also confirmed by Jakober *et al.* (1998), who found that adult WCT in Montana tended to use pools dominated with large wood accumulations, while small trout concealed in substrate interstices.

The highest utilized areas for WCT for overwintering sub-adults and adults (>200 mm) in the UFR are the Fording River S6 oxbows and Henretta Pitt Lake (Cope *et al.* 2016). Both of these areas and other overwintering habitats in the UFR provide deep water cover for protection from ice and predators. Less is known about movements of juveniles to overwintering areas in the UFR but it is generally thought that they overwinter in areas close to their summer rearing habitats and utilize interstitial areas for overwintering cover (Cope *et al*. 2016; Cope 2019).

Because overwintering habitat can be limiting and substrate shelter provides important habitat for rearing WCT, this pathway is based on the premise that calcite concretion can affect the availability of and access to interstitial areas that small fish use for refuge during winter. To further evaluate this pathway, including the potential for interactions with other stressors such as winter conditions, results from Cope (2019) and Hatfield and Whelan (2021) were examined. The Cope (2019) study assessed WCT habitats and populations in Harmer and Grave Creek using removal depletion electrofishing population assessments, radio telemetry, radio tags, spawning studies, habitat characterization to the mesohabitat level, and monitoring of water temperature, discharge, and ice conditions. The study extended through the winter season and documented changes in population and age classes over time. Cope (2019) did not evaluate potential effects of calcite on overwintering mortality but did find that predation from predators such as mink and river otters can contribute to direct WCT mortality during the winter. Further, Cope (2019) found that increased mortality during ice exposure can occur. Hatfield and Whelan (2021) evaluated the potential for overwintering conditions and ice formation to cause or contribute to the decline of WCT in the UFR, drawing on a range meteorological and hydrological data. The authors concluded that if the usual overwintering locations that feature deep water pools or groundwater influence were accessible then it is unlikely that overwintering conditions resulted in WCT decline. However, if WCT could not access their usual overwintering habitat due to issues with stream connectivity and migrations barriers, then they likely would have experienced severe winter conditions could lead to population level effects. Because calcite concretion has the potential to limit overwintering refuge for WCT, in particular juveniles, this pathway was evaluated in the context of calcite trends and intensity in key overwintering areas, and in relation to the timing of WCT declines.

2.3. Requisite Conditions

Requisite conditions are factors that must have been met for calcite to have resulted in the observed decline of WCT in the UFR. Each calcite pathway was evaluated in terms of a specific set of requisite conditions (addressing *spatial extent*, *duration*, *location*, *timing*, and *intensity*), and summarized in Table 5. The requisite conditions are focused on Over-arching Hypothesis #1 related to the circumstances required for calcite as a single stressor to have caused the WCT decline. The requisite conditions for Over-arching Hypothesis #2, related to calcite as a cumulative contributing stressor, would be similar except that not all requisite conditions would have to be fully met. For example, calcite could cumulatively contribute to WCT decline when there is no change pre-Window versus Decline Window (*timing* requisite condition) but other requisite conditions are met such as high calcite *intensity* throughout WCT habitat (*spatial extent* and *location*).

Table 5. Summary of requisite conditions for each pathway.

Pathway	Requisite Conditions
Effects to Spawning Suitability	**Spatial extent:** Widespread calcite in mainstem and tributary areas of the UFR that support WCT **Duration:** Calcite reduces spawning habitat suitability during WCT spawning period and during the Decline Window **Location:** Widespread calcite in UFR mainstem and tributary WCT spawning habitat **Timing:** Calcite index and concretion would have to change between the pre-window and Decline Window to explain the observed decline **Intensity:** Moderate to high calcite index and/or concretion scores would be needed
Effects to Rearing - Invertebrate Production	**Spatial extent:** Widespread calcite in mainstem and tributary areas of the UFR that support WCT **Duration:** Calcite reduces rearing habitat productivity during the Decline Window **Location:** Widespread calcite in UFR mainstem and tributary WCT rearing habitat **Timing:** Calcite index and concretion would have to change between the pre-window and Decline Window to explain the observed decline **Intensity:** Moderate to high calcite index and/or concretion scores would be needed
Effects to Rearing - Biogenic Calcite Precipitation and Dissolution	**Spatial extent:** Widespread biogenic calcite dissolution in mainstem and tributary areas of the UFR **Duration:** Biogenic calcite dissolution occurs from calcite accumulations during the Decline Window **Location:** Widespread calcite in and upstream of UFR mainstem and tributary WCT habitat **Timing:** Biogenic calcite dissolution would have to change between the pre-window and Decline Window to explain the observed decline **Intensity:** Moderate to high calcite index and/or concretion scores would be needed
Effects to Incubation Conditions	**Spatial extent:** Widespread calcite in mainstem and tributary areas of the UFR that support WCT **Duration:** Calcite reduces incubation habitat suitability during WCT spawning and incubation periods and during the Decline Window **Location:** Widespread calcite in UFR mainstem and tributary WCT spawning habitat **Timing:** Calcite index and concretion would have to change between the pre-window and Decline Window to explain the observed decline **Intensity:** Moderate to high calcite index and/or concretion scores would be needed
Effects to Rearing - Overwintering	**Spatial extent:** Widespread calcite in mainstem and tributary areas of the UFR that support WCT **Duration:** Calcite reduces overwintering habitat quality during the Decline Window **Location:** Widespread calcite in UFR mainstem and tributary WCT rearing habitat **Timing:** Calcite index and concretion would have to change between the pre-window and Decline Window to explain the observed decline **Intensity:** Moderate to high calcite index and/or concretion scores would be needed

3. RESULTS

3.1. Spatial and Temporal Trends in Calcite

The spatial and temporal trends in calcite collected during the Regional Calcite Monitoring Program (Robinson *et al.* 2013) provides key context for all requisite conditions including spatial extent, duration, location, timing, and intensity for all five impact pathways presented in Table 5. The sections below evaluate the calcite data in different spatial and temporal contrasts relevant to the EoC for WCT declines.

3.1.1. Calcite Index in the Elk River Watershed

Over the full Elk River watershed study area, the distribution of mine-exposed stream kilometers among CI bins has been similar from 2013-2019, with the majority of mainstem and tributary kilometers having CI scores within the 0.00-0.50 bin (McCabe and Robinson 2020). However, spatial and temporal trends have been noted suggesting an increase in CI over the watershed. A decreasing trend in total stream kilometers of both mainstem ($p<0.001$; df=6) and tributaries ($p=0.03$; df=6) in the 0.00-0.50 bin was found to be highly significant through linear regression. As well, the subsequent increase in mainstem kilometers categorized into the 0.51-1.00 bin was also found to be highly significant ($p < 0.001$; df=6).

In 2019, most of the reference mainstem stream kilometers were categorized into the 0.00 - 0.50 CI bin. 2019 marked the first year where a portion (8.1 km) of the reference tributary stream kilometers were categorized in a higher bin (CI range 0.51-1.00). Alexander Creek – Reach 3 has been sampled as a reference for this Program since 2013 and typically reports the highest calcite values for reference streams. In 2019, the 8.1 km represented by Alexander Creek – Reach 3 had an average CI value of 0.86 and was the only reference tributary reach with a CI score higher than the lowest (0.00-0.50) bin.

3.1.2. Calcite Index in the Upper Fording River

Similar observations to the full regional dataset are reported when looking at just the upper Fording River watershed. CI values in both exposed ($p=0.10$; df=212) and reference ($p<0.001$; df=13) reaches have significantly increased on average from 2013-2019 (Figure 9). Assessment by reach type (i.e., exposed versus reference) also shows that while both types have increased over the period of record, CI values are consistently elevated at exposed reaches relative to references. Exposed reaches averaged a CI score of 0.85 and reference reaches averaged 0.10. This difference was highly significant ($p=0.004$; df=216).

At a reach-scale, significant increases in CI were first reported for the upper Fording River watershed in 2017 with three reaches having significant trends (one tributary and two mainstem, one of which was reference). By 2019, 13 reaches showed significant increasing trends (Table 6). For mainstem reaches, this included Fording River reaches 5, 9 and the reference Reach 12. All others were reference. In all three cases, these trends were significant using an α-value of 0.05 by 2019.

Ten tributary reaches were found to have significant increases from 2013-2019, with six tributaries observed to have significant increases in CI solely in 2019 (Table 6). Dry Creek (LCO) alone contained three reaches with significant increases in calcite observed. Another tributary with a significant increasing trend is the reference Chauncey Creek – Reach 1. Three reaches, Porter Creek reaches 1 and 3, and Eagle Pond Outlet resulted in significant decreases in mean CI.

Figure 9. Mean CI versus Year (2013-2019) for exposed and references streams in the upper Fording River. Error bars represent one standard error.

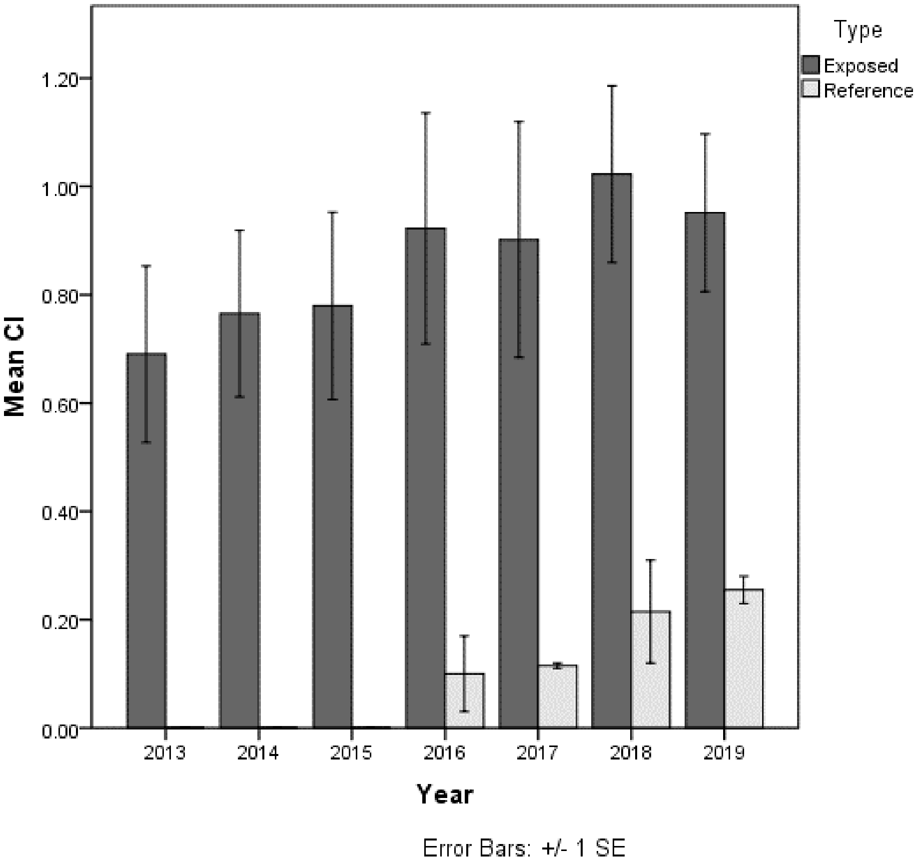


Table 6. Mean calcite index score for the upper Fording River watershed for all years sampled showing significant increases and decreases in CI over time. Shading shows individual linear trend analysis results summarized from annual reports.

Type	Stream and Reach	Mean Calcite Index						
		2013	2014	2015	2016	2017	2018	2019
Mainstem	Fording River - Reach 5	0.32	0.35	0.53	0.58	0.73	0.70	0.80
	Fording River - Reach 6	0.74	0.43	1.53	0.64	0.68	0.79	0.98
	Fording River - Reach 7	0.43	0.97	0.55	0.63	-	0.89	0.90
	Fording River - Reach 8	0.31	0.49	0.48	-	-	0.61	-
	Fording River - Reach 9	0.00	0.00	0.00	0.00	0.32	0.73	0.53
	Fording River - Reach 10	0.00	0.00	0.00	-	-	0.63	-
	Fording River - Reach 11	0.00	0.00	0.00	-	-	0.27	-
	Fording River - Reach 12 (reference)	0.00	0.00	0.00	0.03	0.11	0.31	0.28
Tributary	Cataract Creek - Reach 1	3.00	3.00	3.00	3.00	3.00	2.96	-
	Cataract Creek - Reach 3	3.00	2.64	2.56	-	-	2.89	-
	Chauncey Creek - Reach 1 (reference)	0.00	0.00	0.00	0.17	0.12	0.12	0.23
	Clode Pond Outlet	0.00	1.01	1.03	1.21	0.29	1.46	1.28
	Clode West Infiltration	-	0.18	0.00	0.50	0.21	0.67	0.69
	Dry Creek (LCO) - Reach 1	0.00	0.00	0.00	0.00	0.02	0.57	0.65
	Dry Creek (LCO) - Reach 2	0.00	0.00	0.00	0.00	0.00	0.24	0.52
	Dry Creek (LCO) - Reach 3	0.00	0.00	0.00	0.00	0.00	0.06	0.16
	Dry Creek (LCO) - Reach 4	0.00	-	0.00	0.00	0.00	0.32	0.15
	Dry Creek (LCO) - Reach 5	0.00	-	-	-	-	-	-
	Dry Creek (LCO) - Reach 6	0.00	-	-	-	-	-	-
	Eagle Pond Outlet	1.90	1.31	0.58	0.20	0.25	0.21	-
	East Dry Creek - Reach 1	-	-	-	-	-	-	0.01
	Fish Pond Creek - Reach 1	0.00	0.03	0.00	0.08	0.20	0.17	0.38
	Gardine Creek - Reach 1	0.29	0.70	0.32	0.14	0.60	0.64	0.50
	Grassy Creek - Reach 1	0.00	0.09	0.00	0.04	0.29	0.25	0.38
	Greenhills Creek - Reach 1	0.35	1.06	0.45	0.86	1.07	0.64	0.66
	Greenhills Creek - Reach 3	1.30	2.22	2.46	2.18	-	2.49	1.91
	Greenhills Creek - Reach 4	1.62	2.78	2.80	2.61	2.68	2.74	2.32
	Henretta Creek - Reach 1	0.00	0.00	0.00	0.00	0.04	0.32	0.40
	Henretta Creek - Reach 3 (reference)	0.00	0.00	0.00	-	-	0.00	-
	Kilmarnock Creek - Reach 1	2.16	1.64	1.97	2.59	2.77	2.30	2.56
	Lake Mountain Creek - Reach 1	0.00	0.33	0.00	0.15	0.18	0.39	0.88
	Porter Creek - Reach 1	0.92	0.84	0.85	0.75	0.74	0.85	0.85
	Porter Creek - Reach 3	2.78	1.94	1.94	1.46	1.62	1.65	1.44
	Smith Pond Outlet	2.61	2.24	2.24	3.00	2.60	2.45	2.00
	Swift Creek - Reach 1	2.58	2.18	2.39	2.43	2.45	1.69	1.88
	Swift Creek - Reach 2	0.00	1.04	0.82	-	-	1.12	-

Statistical increasing change over all prior years	$p<0.10$	$p<0.05$
Statistical decreasing change over all prior years	$p<0.10$	$p<0.05$

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3.1.3. Concretion in the Upper Fording River

Calcite concretion may be the most relevant performance measure to monitor when considering potential ecological effects of calcite to WCT. Studies suggest impairment to benthic communities and spawning habitat occurs near a CI of 1.00, where concretion begins (Robinson 2010; Barrett *et al.* 2016; Hocking *et al.* 2020). Similar to CI, concretion was found to be significantly elevated in exposed reaches relative to references reaches from 2013-2019 (p=0.02; df=216). However, the trend for both reference and exposed reaches has not changed significantly over time (exposed: p=0.928, df=212, reference: p=0.187; df=13) (Figure 10).

Table 7 shows reach mean CI_c scores calculated for the upper Fording River watershed. The same shading scheme used for CI was used here. This presentation demonstrates that concretion shows few trends, with significant Mann-Kendall results returned for only 6 of 36 reaches in the UFR. Three of these significant reaches show decreasing CI_c from 2013-2019 and three show increases. Of the three increasing, FORD9 is the most relevant to the UFR evaluation of cause as it has seasonal use by WCT observed to have exhibited the decline, including summer rearing and spawning. Chauncey Creek is a reference stream that showed a significant increase of CI_c from 2013-2017 and 2013-2018, but not from 2013-2019. This is likely an artefact of the data being zero and then showing some values slightly above zero and not a meaningful change in CI_c. In other words, the sampling methods are such that changes of this scale would be outside of the sensitivity of the method.

Figure 10. **Mean CI_c versus Year (2013-2019) for exposed and references streams in the upper Fording River. Error bars represent one standard error. Mean CI_c has not changed significantly over time in exposed or reference reaches ($p > 0.18$).**

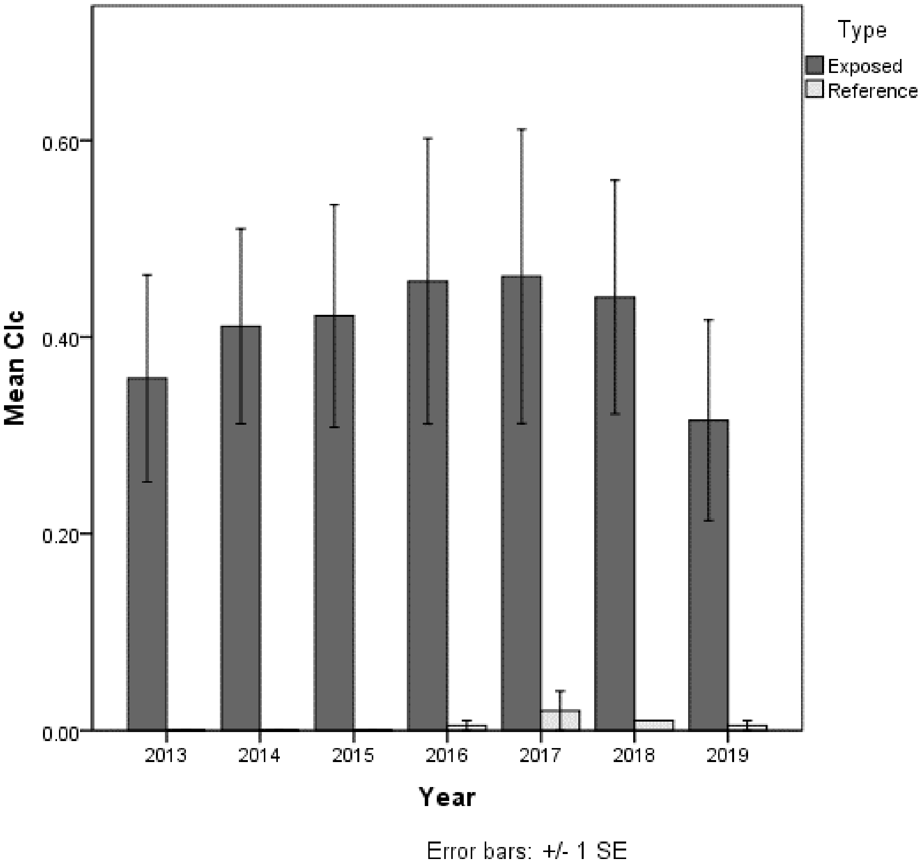


Table 7. Mean calcite concretion scores for the upper Fording River watershed from all years sampled. Shading shows individual linear trend analysis results summarized from annual reports.

Type	Stream and Reach	Mean Concretion Score						
		2013	2014	2015	2016	2017	2018	2019
Mainstem	Fording River - Reach 5	0.00	0.07	0.00	0.00	0.00	0.01	0.00
	Fording River - Reach 6	0.06	0.10	0.70	0.02	0.05	0.14	0.06
	Fording River - Reach 7	0.03	0.37	0.00	0.01	-	0.07	0.08
	Fording River - Reach 8	0.01	0.05	0.01	-	-	0.00	-
	Fording River - Reach 9	0.00	0.00	0.00	0.00	0.09	0.18	0.13
	Fording River - Reach 10	0.00	0.00	0.00	-	-	0.03	-
	Fording River - Reach 11	0.00	0.00	0.00	-	-	0.00	-
	Fording River - Reach 12 (reference)	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Tributary	Cataract Creek - Reach 1	2.00	2.00	2.00	2.00	2.00	1.96	-
	Cataract Creek - Reach 3	2.00	1.76	1.58	-	-	1.89	-
	Chauncey Creek - Reach 1 (reference)	0.00	0.00	0.00	0.01	0.04	0.01	0.01
	Clode Pond Outlet	0.00	0.36	0.16	0.25	0.05	0.55	0.38
	Clode West Infiltration	-	0.00	0.00	0.00	0.00	0.01	0.00
	Dry Creek (LCO) - Reach 1	0.00	0.00	0.00	0.00	0.00	0.00	0.03
	Dry Creek (LCO) - Reach 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Dry Creek (LCO) - Reach 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Dry Creek (LCO) - Reach 4	0.00	-	0.00	0.00	0.00	0.00	0.00
	Dry Creek (LCO) - Reach 5	0.00	-	-	-	-	-	-
	Dry Creek (LCO) - Reach 6	0.00	-	-	-	-	-	-
	Eagle Pond Outlet	0.90	0.73	0.32	0.06	0.04	0.00	-
	East Dry Creek - Reach 1	-	-	-	-	-	-	0.00
	Fish Pond Creek - Reach 1	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	Gardine Creek - Reach 1	0.00	0.40	0.06	0.02	0.28	0.29	0.01
	Grassy Creek - Reach 1	0.00	0.01	0.00	0.01	0.11	0.09	0.12
	Greenhills Creek - Reach 1	0.01	0.41	0.04	0.27	0.42	0.20	0.09
	Greenhills Creek - Reach 3	0.47	1.31	1.52	1.23	-	1.51	0.92
	Greenhills Creek - Reach 4	0.83	1.80	1.84	1.64	1.69	1.75	1.32
	Henretta Creek - Reach 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Henretta Creek - Reach 3	0.00	0.00	0.00	-	-	0.00	-
	Kilmarnock Creek - Reach 1	1.33	1.05	1.28	1.64	1.81	1.40	1.65
	Lake Mountain Creek - Reach 1	0.00	0.06	0.00	0.00	0.00	0.00	0.00
	Porter Creek - Reach 1	0.14	0.06	0.23	0.00	0.00	0.06	0.00
	Porter Creek - Reach 3	1.80	1.02	1.16	0.79	0.95	0.88	0.78
	Smith Pond Outlet	1.72	1.39	1.39	2.00	1.66	1.54	1.09
	Swift Creek - Reach 1	1.71	1.27	1.53	1.48	1.47	0.84	0.91
	Swift Creek - Reach 2	0.00	0.58	0.51	-	-	0.25	-

Statistical increasing change over all prior years	p<0.10	p<0.05
Statistical decreasing change over all prior years	p<0.10	p<0.05

3.1.4. Trends in UFR Mainstem Versus Tributaries

Calcite index values have increased significantly from 2013-2019 in mainstem-exposed (p=0.002; df=38), mainstem-reference (p=0.007; df=6), and tributary-reference (p=0.011; df=6) grouped reaches, but not tributary-exposed reaches (p=0.242; df=173) (Figure 11). Across all years, mean CI was higher in tributary-exposed (0.93 +/-0.07) reaches than mainstem-exposed (0.48+/-0.15) reaches (p=0.007; df=213), although this difference has decreased in recent years. CI has increased in the UFR mainstem-exposed reaches from an average of 0.26 in 2013 to 0.80 in 2019. Across all years, mainstem and tributary reaches did not differ significantly in reference areas (p=0.840; df=14).

In comparison to CI, no significant annual trends have been observed for CI_c across mainstem-exposed (p=819; df=38), mainstem-reference (p=0.363; df=6), tributary-exposed (p=0.952; df=173), and tributary-reference grouped reaches (p=0.263; df=6) from 2013-2019 (Figure 12). Similar to CI, concretion has been significantly higher in tributary-exposed (0.49 +/-0.05) compared to mainstem-exposed (0.06 +/-0.10) reaches across all years. CI_c also did not differ significantly between mainstem and tributaries within reference reaches (p=0.147; df=14).

Overall, it appears that CI has increased gradually over time in the UFR mainstem alongside and downstream of Fording River Operations. However, this does not appear to have translated into significant increases in CI_c during the decline window in the UFR mainstem.

Figure 11. Mean CI score from 2013-2019 for pooled reaches within the UFR mainstem and tributaries in both exposed and reference reach types.

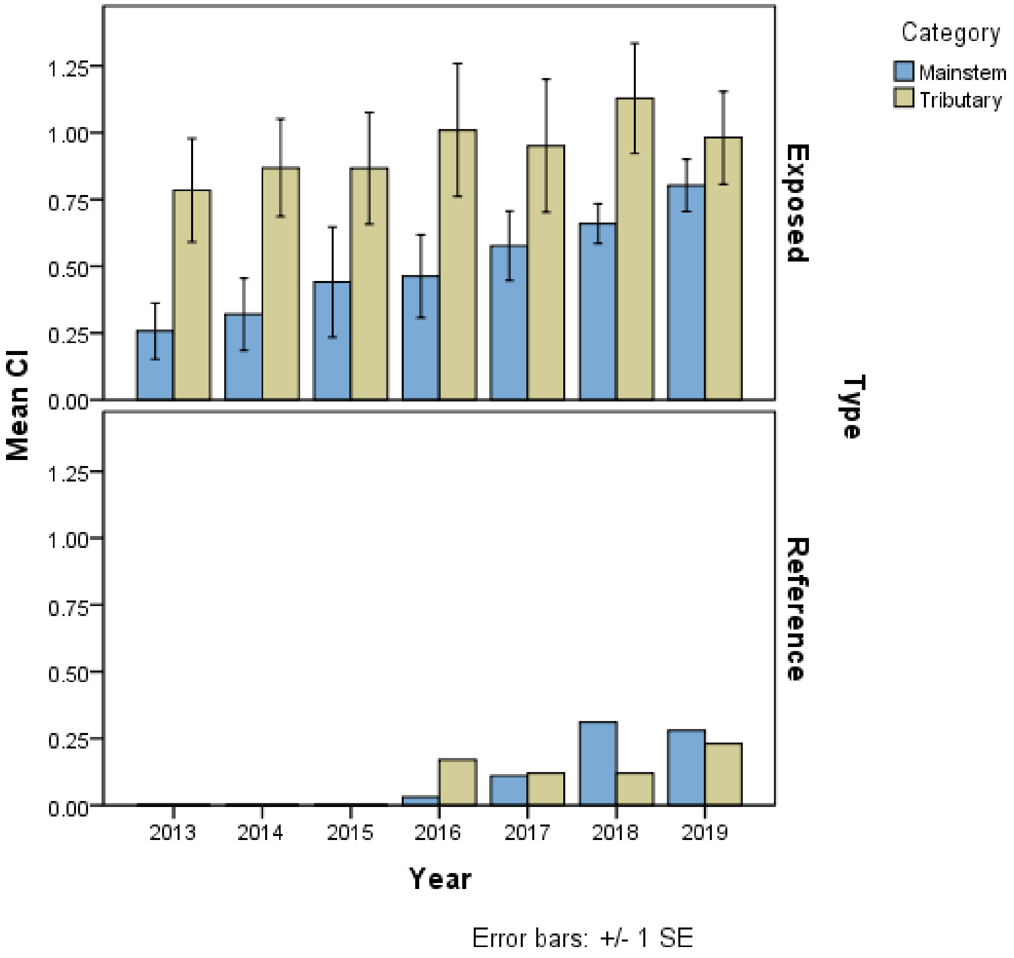
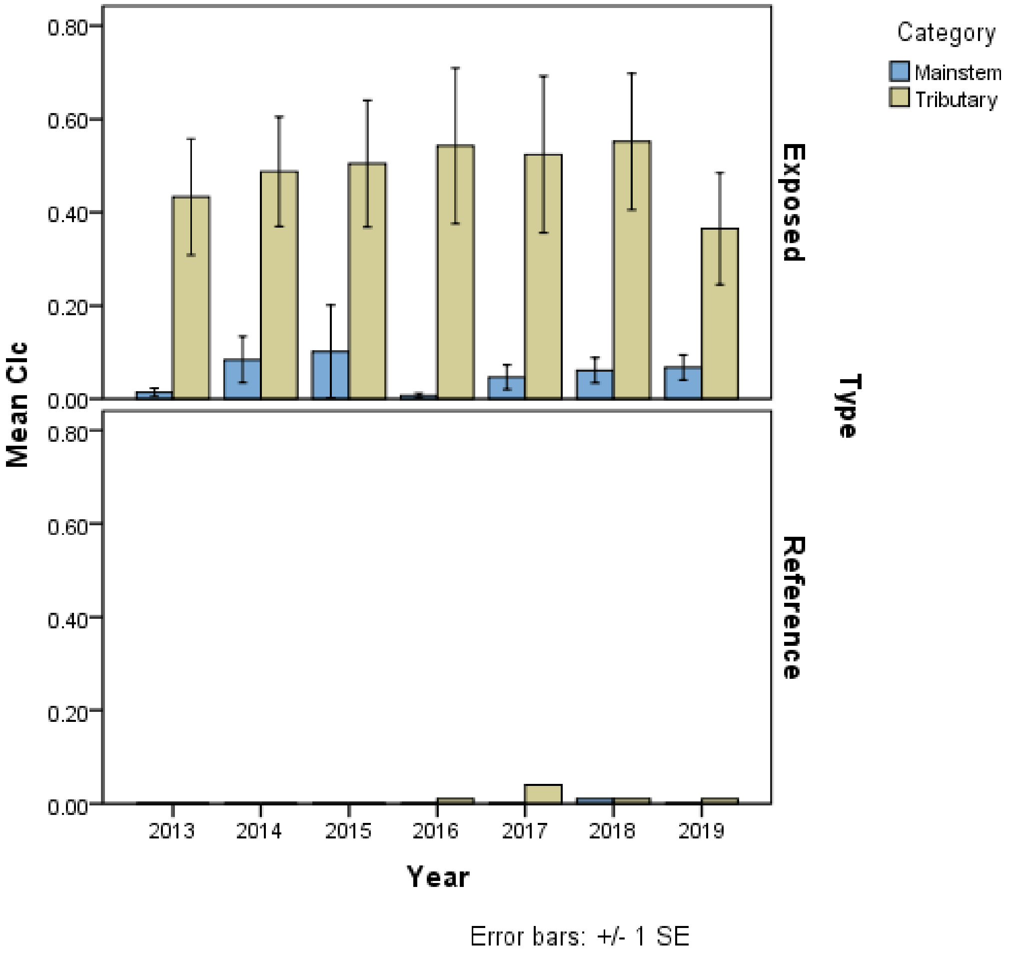


Figure 12. **Mean CI_c score from 2013-2019 for pooled reaches within the UFR mainstem and tributaries in both exposed and reference reach types.**



3.1.5. Calcite Concretion in Fish-Bearing Strata

To align calcite data with WCT data, the trends in calcite concretion were summarized by fish-bearing strata used in Cope (2020) as shown in Table 2 and Table 3. Results from this analysis are shown in Figure 13 and Figure 14. Figure 13 confirms that calcite concretion in exposed reaches in UFR was consistently higher than calcite concretion in reference reaches but did not change significantly in the pre-window versus the decline window in UFR. Concretion in exposed reaches was lowest in 2013 (~0.03), rose in 2014 (~0.11), followed by a decrease from 2014-2016 to 0.04 and a stabilization at a concretion value of about 0.07 between 2017-2019 (Figure 13, Table 7). Concretion in reference reaches was zero from 2013-2015; low levels of concretion appeared in 2016 and were highest in 2017 (<0.03), decreasing again slightly into 2019.

In terms of the sampled UFR strata in Cope (2020), the lower mainstem (exposed) and lower tributary (all tributaries except Chauncey Creek are exposed) strata had the highest concretion scores in

2013-2016; no concretion was documented in mainstem headwaters or middle mainstem strata during these years (Figure 14). In 2017, concretion was measured in the middle mainstem strata at levels similar to lower mainstem and lower tributary strata. The middle mainstem concretion was highest in 2019, but average values for middle mainstem are based on limited data for this year and largely driven by the FORD9 reach (Table 7). No concretion was documented in the mainstem headwaters aside from a low level (<0.02) in 2018.

Figure 13. Trends in calcite concretion for the UFR watershed 2013-2019 in exposed versus reference fish-bearing reaches surveyed by Cope (2020) (see Table 3). Error bars represent one standard error.

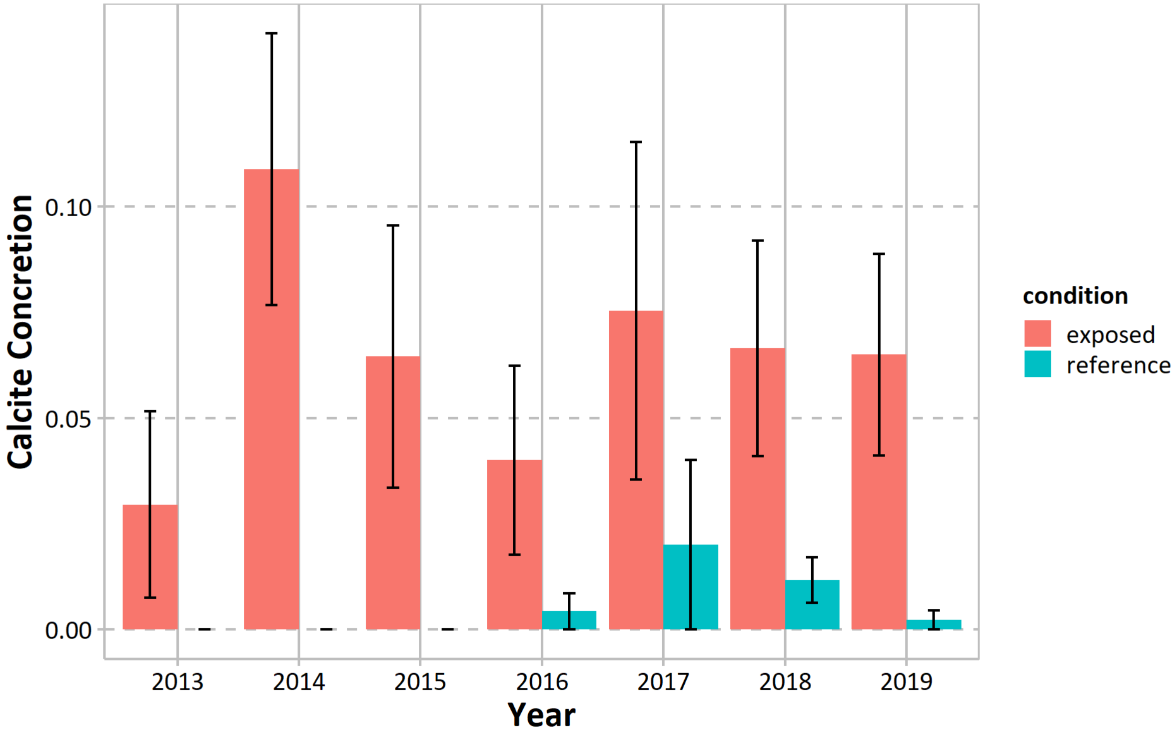
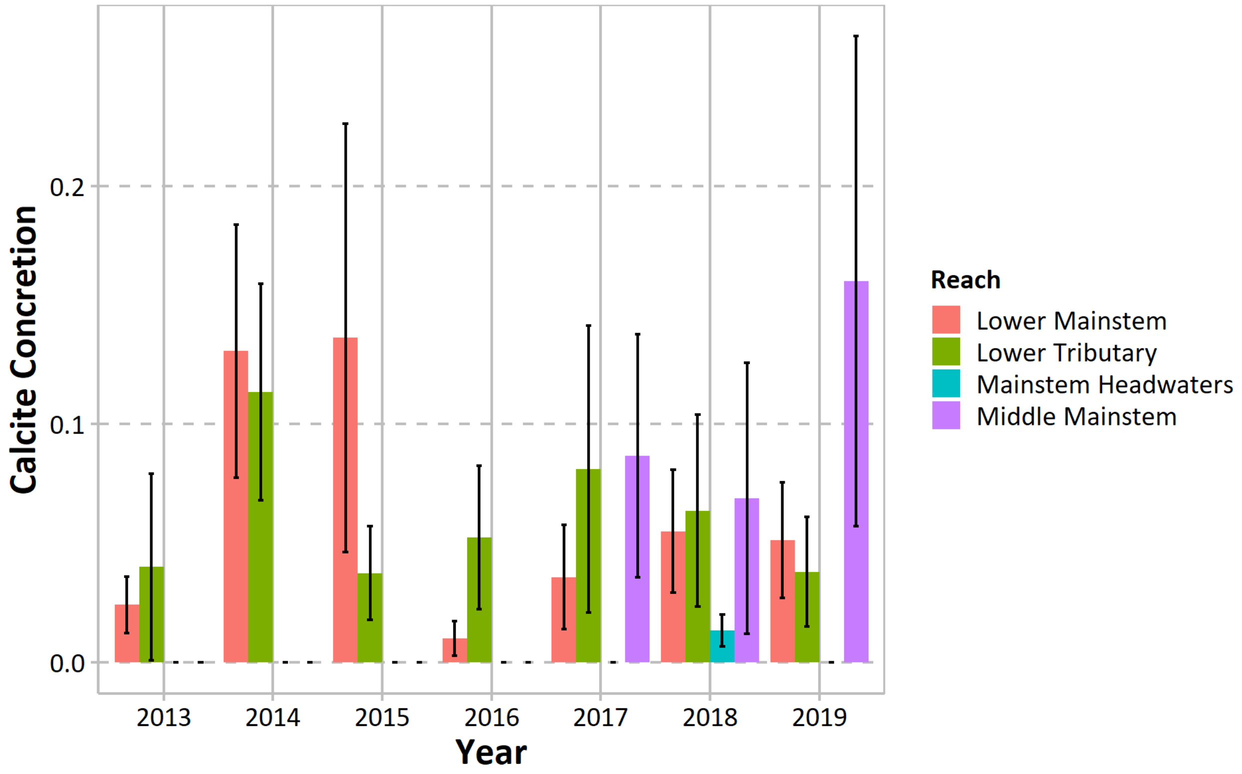


Figure 14. Trends in calcite concretion for the UFR watershed 2013-2019 in fish-bearing strata surveyed by Cope (2020) (see Table 3). Error bars represent one standard error.



3.2. Assessment of Biological Effects

The spatial and temporal trends in calcite data reported above are used along with dose-response relationships to evaluate each biological effects pathway to determine whether the intensity of calcite in the UFR is sufficient to elicit a WCT response and whether calcite explains, wholly or in part, the observed WCT decline.

3.2.1. Effects to Spawning Suitability

The draft spawning suitability curve for mean redd count developed by Hocking *et al.* (2020) and shown in Figure 5 was used to predict estimated spawning suitability based on the annual trends in calcite in fish-bearing strata of the UFR shown in Figure 13 and Figure 14. Predicted responses of spawning suitability to calcite concretion are shown in Figure 15. Results are presented as the percent decline in spawning suitability relative to a concretion level of zero, based on the spawning suitability curve for mean redd count per mesohabitat unit. The predicted declines in spawning suitability mirror the observed trends in calcite concretion by strata shown in Figure 14.

Overall, spawning suitability is estimated to be high and stable across most fish-bearing reaches of the UFR watershed for the years 2013-2019. From 2013-2016, a maximum estimated loss of spawning suitability of ~25% was predicted in the lower mainstem, with most effects in the pre-Decline Window period seen in the lower mainstem and lower tributary strata. Between 2017 and 2019, calcite concretion levels remained at similar levels to previous years in the lower mainstem, lower tributary and mainstem headwaters strata with no major shifts in calcite concretion pre-Decline Window compared to the WCT Decline Window in any strata. An increase in calcite concretion was observed in the middle mainstem strata between 2018 and 2019 to an average of approximately 0.16 CI_c, which corresponds to a predicted decline in spawning suitability of approximately 27% in the middle mainstem strata. The middle mainstem area overlaps with FRO onsite and is considered one of several core spawning areas for WCT (Cope *et al.* 2016). This middle mainstem strata has been estimated to support ~13% of migratory WCT spawners and ~52% of upper watershed resident spawners, which sums to roughly 19% of spawners within the UFR (Cope *et al.* 2016).

The predicted decline in spawning suitability in the middle mainstem strata is indicative of a potential trend in the middle mainstem region of UFR but is also uncertain for several key reasons. Figure 16 shows the changes in calcite concretion within stream reaches for the middle mainstem over the 2013-2019 period. This figure shows that an increase in calcite concretion occurred in the FORD9 unit of the middle mainstem in 2018. No similar increase in calcite concretion was observed for the other two units (FORD10 and FORD11) of the middle mainstem for 2018, and no data are available for these units in 2019 (Table 7). The predicted declines in spawning suitability in the middle mainstem strata are therefore driven by an increase in calcite concretion in one reach only (FORD9) and thus the predicted decline in spawning suitability of 27% may not be reflective of the full middle mainstem of UFR. Although an increase in calcite concretion (and therefore decreased predicted spawning suitability) was observed over this timeframe, there is high uncertainty in the result due to the limited geographic extent and small dataset. Additional data are needed to resolve this uncertainty.

Figure 15. Predicted spawning suitability based on calcite concretion. Error bars represent one standard error.

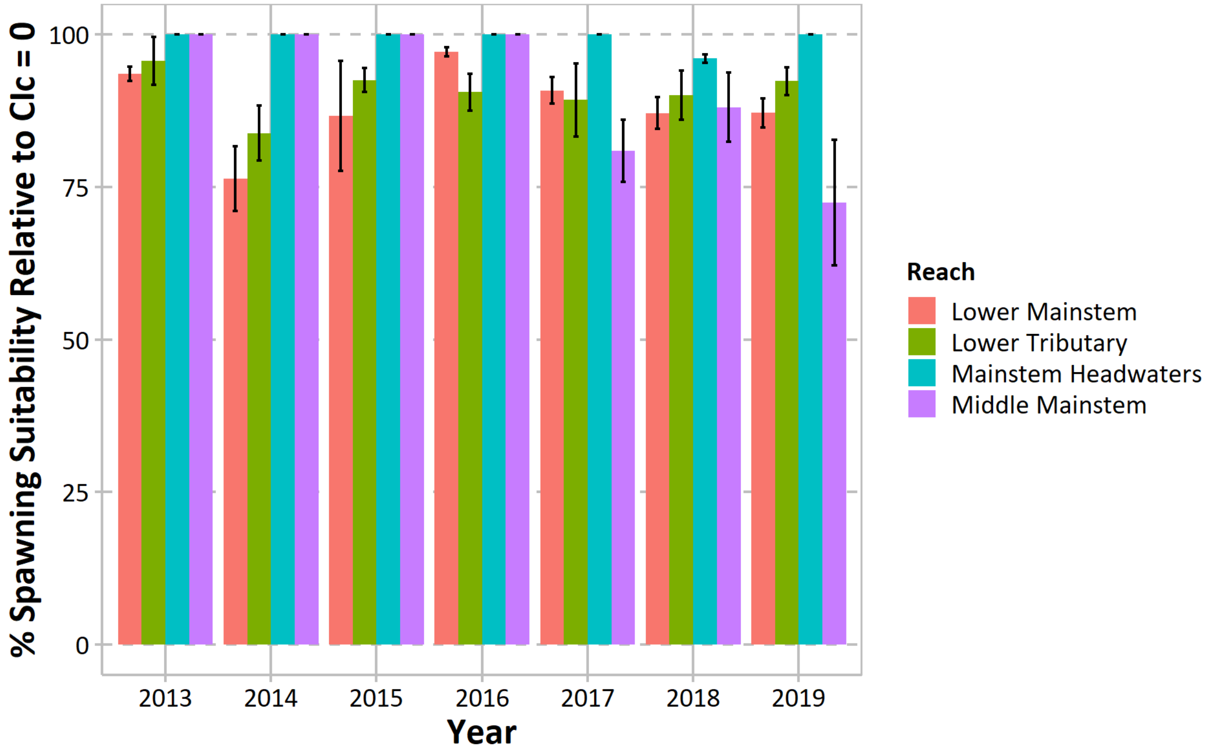
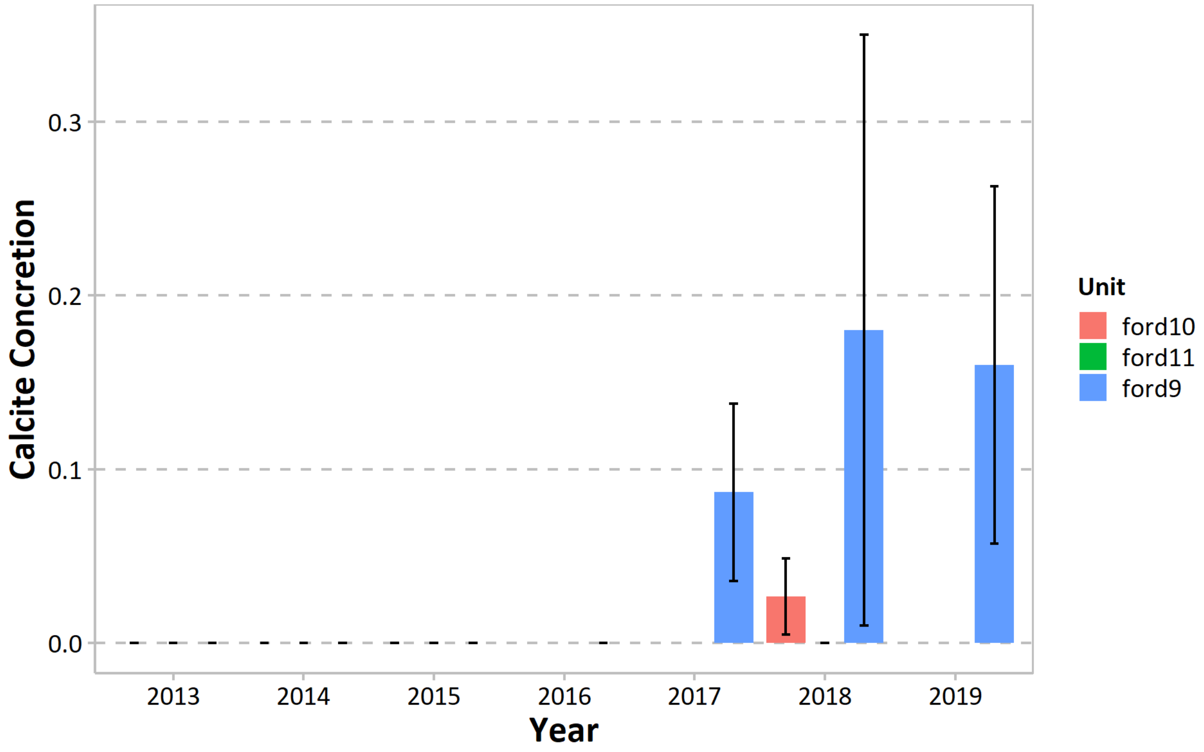


Figure 16. **Calcite concretion trends for each stream reach in the middle mainstem strata. Error bars represent one standard error. Individual reach data is also shown in Table 7. Zero concretion was observed in 2013-2016.**



Interpretation of the spawning suitability pathway was based on the requisite conditions needed to be met for the pathway to be considered explanatory for declines in WCT in UFR during the 2017-2019 Decline Window. The spatial extent/location of calcite concretion partially meets the condition of being present throughout the UFR as calcite and calcite concretion are present in most reaches of UFR where WCT spawning occurs. There was a modest increase in calcite concretion in the middle mainstem between 2017 and 2019 (Figure 14). However, the documented increase in calcite concretion was driven by a single stream reach within the middle mainstem strata; calcite concretion values were relatively low (<0.2) throughout the majority of the UFR watershed and did not change substantially between the pre-Window and the Decline Window periods. Applying the spawning suitability model to these calcite levels to evaluate requite conditions for intensity shows predicted declines in spawning suitability of 27% or less for all years, reaches and strata, with similar predicted declines in spawning suitability in the pre-Window and Decline Window periods. Further, although a decrease in spawning suitability could lead to a decrease in WCT fry, any decrease in fry through recruitment declines or failure would not immediately translate into declines in older WCT age classes. In the Cope (2020) report, the observations of juvenile density included widespread declines in juveniles in the Decline Window throughout the UFR (exception lower mainstem strata) and the highest juvenile densities in 2017 across all strata. Therefore, any declines in spawning suitability from calcite prior to or during the Decline Window are not consistent with the observed juvenile density data in Cope (2020).

Overall, calcite concretion scores do not meet requisite levels to cause large declines in WCT abundance across age classes throughout the UFR watershed through the spawning suitability pathway. Calcite concretion levels remained relatively stable and low (<0.5) throughout the pre-window and Decline Window periods. Declines in spawning suitability in the middle mainstem are predicted to be relatively minor compared to the WCT declines observed. This causal pathway would be also expected to only directly impact the fry age class, and therefore cannot explain the observed rapid decline in WCT across all age classes. In particular, it does not explain the substantial decline in adult abundance throughout the UFR. Therefore, the evidence to support this causal pathway as the sole cause is weak. This pathway could, however, be a contributing factor to the WCT juvenile decline.

3.2.2. Effects to Rearing – Invertebrate Prey Availability

The available data provides an initial dose-response relationship between calcite and invertebrate prey availability, which can be used to qualitatively assess whether the requisite conditions for a causal or contributory mechanism are met. Results from Barrett *et al.* (2016) showed that calcite can increase periphyton productivity (Figure 6), but a specific calcite value above which periphyton productivity would be expected to deviate outside the normal range was not identified. Benthic community structure was more strongly affected by calcite; shifts in benthic invertebrate community endpoints were observed above calcite index scores of ~1, including a noticeable decrease in Ephemeroptera proportion (Figure 7). However, total invertebrate abundance did not change across the range in CI measured and some groups such as Diptera increased with increasing CI. Barrett *et al.* (2016) also indicated some uncertainty as to whether changes in invertebrate composition were related to CI or water quality, which is correlated to CI. Ephemeroptera are a preferred and common prey for drift-feeding salmonids, including for WCT in the Elk Valley (Minnow 2004, EVS 2005), but it is unclear if changes in invertebrate species composition would translate into effects on fish growth and abundance when total invertebrate abundance does not differ. Cutthroat Trout are known to feed on a variety of invertebrate prey, including terrestrial invertebrates and chironomids (McDonald and Strosher 1998; Romero *et al.* 2005; Fisheries and Oceans Canada 2016), so a shift in benthic species composition may not result in a change in fish growth or condition.

In terms of requisite conditions, the spatial extent and location requirements are met in that calcite is present throughout the area; the requisite condition for timing of calcite changes is not met, aside from an isolated increase in the Ford9 unit of the middle mainstem strata. Any calcite effects to invertebrate prey availability for WCT would be relevant for all free feeding life stages and therefore may be consistent with effects observed across all age classes; however, the intensity of calcite levels is likely not sufficient to explain the observed rapid WCT decline because predicted effects to benthic invertebrates are only expected when CI >1. Changes in invertebrate communities were also not observed by Orr and Ings (2021) when comparing the pre-Window to the Decline Window in the

UFR. The evidence that calcite effects on food production can be the sole cause of declines is therefore weak. Although possible, it is unlikely that this causal pathway could be a contributing factor to the WCT decline.

3.2.3. Effects to Rearing – Biogenic Calcite Precipitation and Dissolution

Given the lack of data to develop an explicit dose versus response relationship between calcite accumulation, biogenic precipitation, and dissolution, and WCT population response, this pathway can only be examined qualitatively to assess whether the requisite conditions for a causal or contributory mechanism are met. Data presented include examination of cyanotoxin types produced from cyanobacteria, flow data, Teck calcite dissolution data and calcite metal data in the UFR.

Biogenic calcite can create formations in the Upper Fording watershed, specifically in tributaries. For example, the process of moss encrustation with continual growth along the surface together with filamentous algae on the lee edge can cause terracing. This biogenic calcite terracing has been observed at several locations such as Cataract Creek (Goudey *et al*. 2009). Biogenic calcite is fragile compared to physico-chemically precipitated calcite and may help explain why calcite indices are not increasing year over year in the UFR.

There are 10 cyanobacteria taxa that have been frequently documented from periphyton and calcite samples in the UFR, and eight known cyanotoxins documented from dominant cyanobacteria taxa (Table 8). Cyanotoxins include Lyngbyatoxins, Aplysiatoxins, Lipopolysaccharides, Cylindrospermopsin, Microcystins, Anatoxin-a, Saxitoxin, and Beta-methylamino-L-alanine. Cyanotoxicity causing fish kills in rivers is increasing globally (McAllister *et al*. 2016), and of the harmful taxa, naturally occurring *Phormidium* is of special concern in the UFR because the growth of this cyanobacteria is also associated with calcite. There are no data on the cyanotoxicity concentrations present from these cyanobacteria taxa in the UFR and during the Decline Window. In addition, the dose required to elicit a WCT population response is also not known. The potential for cause was thus evaluated by assessing the flow and water quality conditions during the Decline Window that could be correlated to a toxicity event. A fuller discussion of the cyanotoxicity literature can be found in the Cyanobacteria, Periphyton and Aquatic Macrophyte Impacts Evaluation of Cause (Larratt and Self 2021).

The important window for cyanotoxin production is during late summer low flows with maximal water temperatures. These toxins can persist in fine substrates for weeks to months or even 100's of years under anoxic conditions (Klitzke *et al*. 2012; Henao *et al*. 2019), thus long-term storage in calcite matrices due to factors including restricted DO and shielding from sunlight UV, is probably analogous to storage observed in fine substrates (WHO 2019). The storage of cyanotoxins in calcite and in fine sediments extends the potential for cyanotoxin harm from the WCT summer rearing life stage and into the overwintering stage in the UFR. Entrapped cyanobacteria and their toxins can be delivered to the stream during winter low flows by calcite dissolution combined with the slow break-down of those toxins under cold water temperatures (WHO 2019; Lahti *et al*. 1997), creating a potential for greater cyanotoxin exposure and biomagnification during winter low flows.

The retention of late summer/early fall cyanotoxins is expected when flows remain below the threshold where accumulated periphyton mats and biogenic calcite are dislodged. Biogenic calcite impacts from cyanotoxicity are therefore predicted to exert the greatest influence when no fall flush of greater than ~2-5 m^3/s measured at FR_FRNTP occurs in between the summer biogenic calcite production and winter low flow conditions When late summer to early winter flow data are examined: Fall 2015 had a flow peak or flush of 2 m^3/s, July 2016 had a peak near 5 m^3/s with flows maintained near 2 m^3/s through that summer, Fall 2017 had a >5 m^3/s in December, but in 2018, average daily discharge was below 2 m^3/s from the end of June onwards (no flushing of accumulated periphyton mat prior to winter low flows) (Figure 17, Figure 18). Therefore, no fall spate (flush) occurred in 2018 (FRO average daily discharge data at FR_FRNTP), indicating that biogenic calcite and associated cyanobacteria could have persisted into winter 2018/2019. Biogenic calcite impacts are also expected to gradually increase between years with a large freshet or flood, with the last major flood occurring in 2013.

Table 8. Cyanotoxin types that can be produced by cyanobacteria occurring in the upper Fording River samples and their ecological effects.

Cyanobacteria	Occurs in UFR calcite	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	
Homeothrix janthina		Yes	Yes	BMAA
Homeothrix varians			Yes	
Leptolyngbya limnetica	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 sp.	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 = beta-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.
Blank cells indicate that the cyanobacteria taxa has not been detected to date.

Cyanobacteria references include: 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 17. Daily streamflow (m^3/s) at FR_FRNTP from 2010-2019 (from Wright *et al.* 2021).

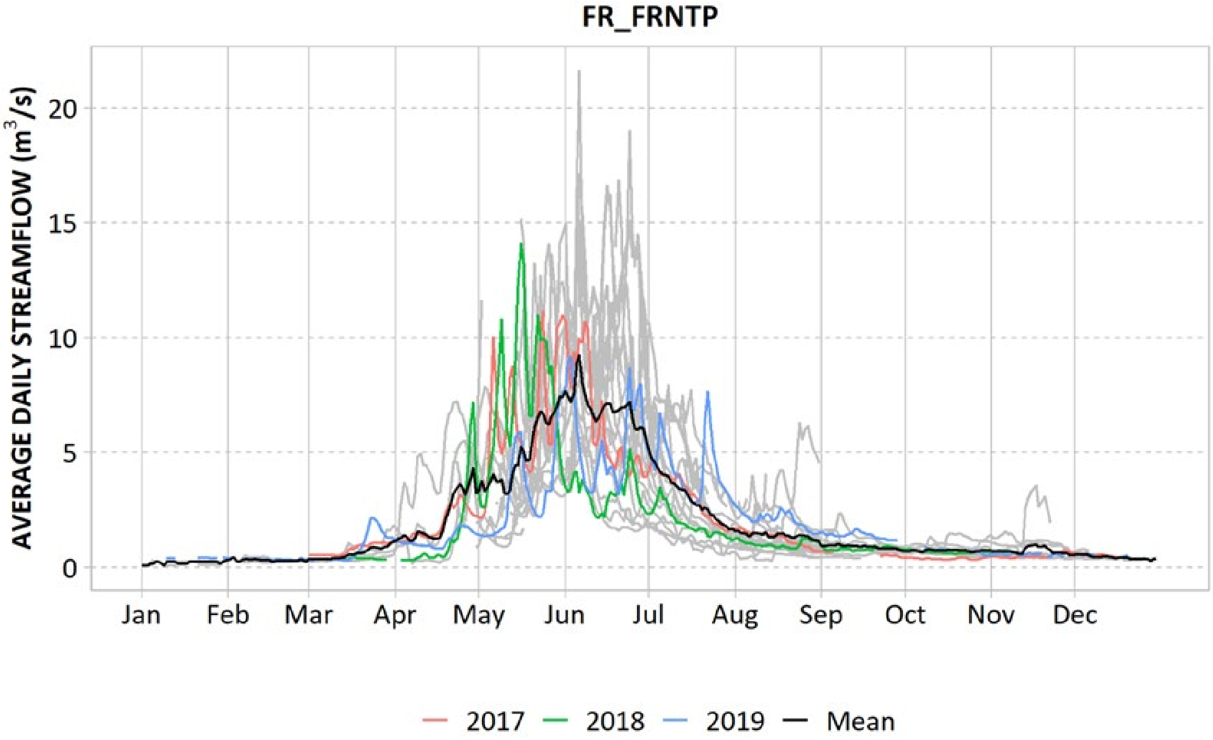
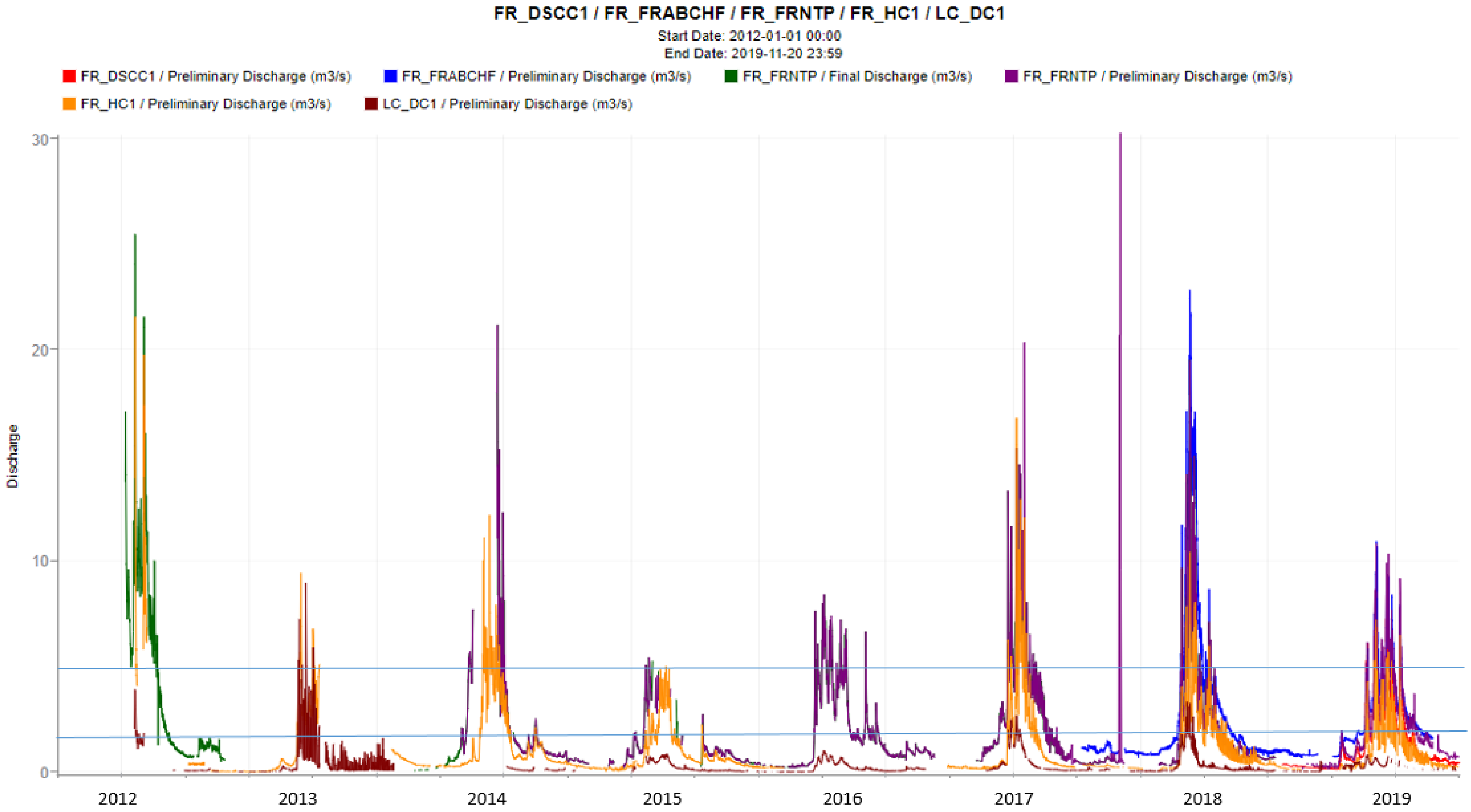


Figure 18. Discharge hydrographs at UFR mainstem sites FR_DSCC1, FR_FRABCHF, FR_FRNTP, FR_HC1, and LC_DC1 from 2012 – 2019.



Monthly WQ sampling at all three sites from 2013 to 2019 indicates that the main flows of the UFR rarely enter a regime where calcite dissolution would be thermodynamically possible (Figure 19, F**igure** 20). Only rare instances of calcite dissolution are predicted by indices calculated for the three UFR mainstem sites. Of the three sites investigated by SPO staff for the period from January 2013 to December 2019, the most dilute site and therefore the most prone to calcite dissolution was LC_FRDSDC. This site occurs in the Fording River downstream of the LCO Dry Creek within the Lower Mainstem strata, which overlaps with the Lower Watershed resident WCT population area (Cope *et al.* 2016). Two episodes in which dissolution was thermodynamically possible were observed in the time series at LC_FRDSDC, one in March 2018 (LSI = -0.42) and another in February 2019 (LSI = -0.68).

Specifically, the LSI = -0.68 calculated for January 2019 at LC_FRDSDC does indicate potential for calcite dissolution in the bulk UFR water due to a low pH of 7.1 compared to the average 8.2±0.3 (Figure 20). Within the periphyton mat where calcite dissolution is expected, other researchers have found pH supressed by more than 1 pH unit during respiration or decomposition, demonstrating the potential for localized biogenic calcite dissolution (Wood *et al.* 2015; Hayashi *et al.* 2012; Dodds 2013). This effect could be reinforced in some areas by hyporheic exchange with lower pH shallow groundwater. Shallow groundwater locations RG_FRDP2, DP4, DP5 and DP8 in Dec 2019 were under-saturated in calcite, making calcite dissolution by these hyporheic upwelling waters thermodynamically possible in 2018 samples. This was not the case at the same locations in Feb 2020, nor at seep locations (except for RG_FRSP2 in Dec 2019).

In comparison, the sites GH_FR1 (Fording River downstream of Greenhills Creek) and FR_FRCP1 (Fording River downstream of Swift/Cataract creeks) were less prone to calcite dissolution and showed higher propensity for calcite precipitation than LC_FRDSC (Figure 19,F**igure** 20). Water with high calcite forming potential enter the UFR from Greenhills, Swift and Cataract creeks just upstream of these locations. Calculated typical values of the LSI ranged from 0.3 to 1.1 at FR_FRCP1 and 0.1 to 0.7 at GH_FR1, while LSI values were as low as 0.06 to -0.13, respectively. The FR_FRCP1 site occurs just upstream of one of the most important overwintering areas for WCT in the S6 oxbows. Available data thus suggest that the WCT overwintering in this core area did not experience a calcite dissolution event during the Decline Window.

Figure 19. Trends in Langlier Saturation Index (LSI) from 2013 to 2018 at GH_FR1, FR_FRCP1 and LC_FRDSDC.

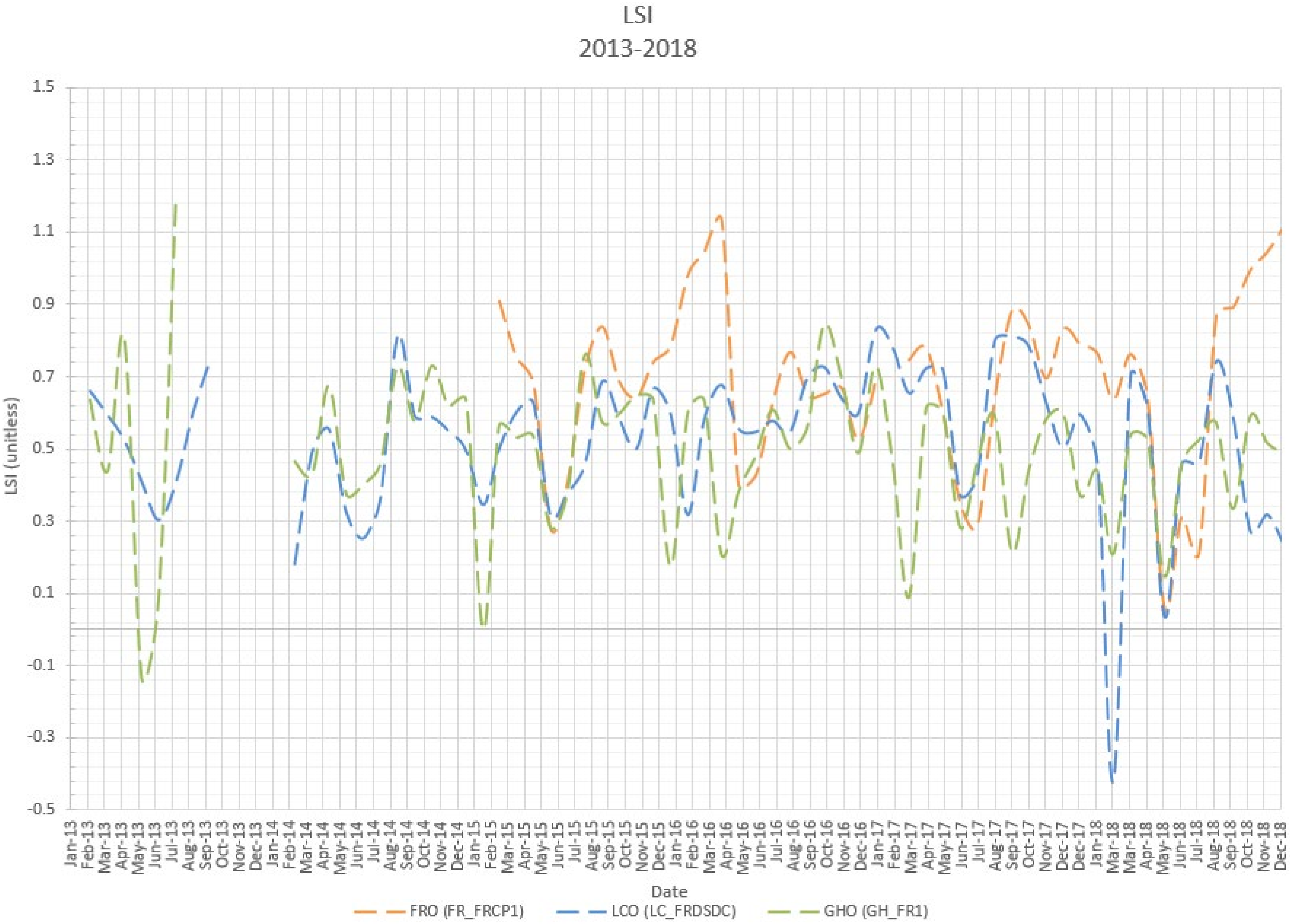
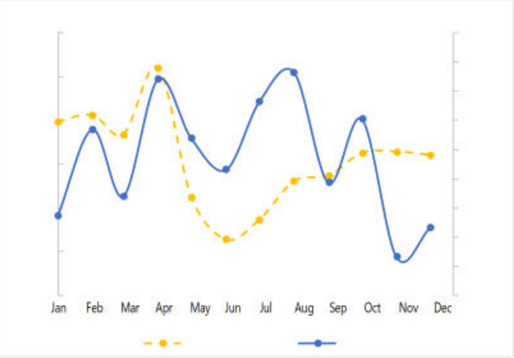


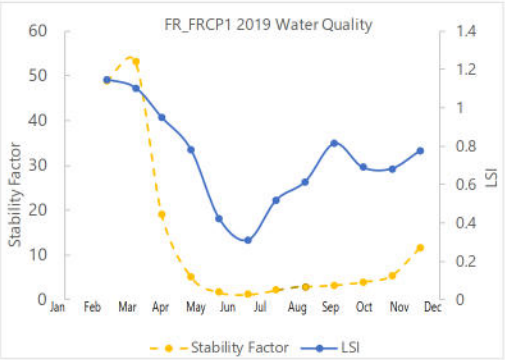
Figure 20. UFR mainstem calcite stability at GH_FR1, FR_FRCP1 and LC_FRDSDC for 2019.

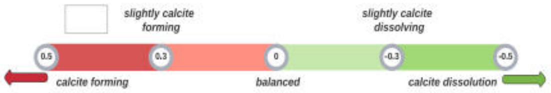
Date	GH_FR1 2019 Water Quality			
	Average Total Alkalinity, as CaCO3 (mg/L)	LSI	Stability Factor	Dissolved CO2 (mg/L)
Jan-19	212	0.27	4.0	4.77
Feb-19	214	0.57	4.1	2.47
Mar-19	208	0.34	3.7	3.83
Apr-19	244	0.74	5.2	2.43
May-19	180	0.54	2.2	1.97
Jun-19	154	0.43	1.3	1.53
Jul-19	174	0.67	1.7	1.23
Aug-19	196	0.77	2.6	1.59
Sep-19	198	0.39	2.7	3.81
Oct-19	203	0.61	3.3	1.86
Nov-19	204	0.13	3.3	5.68
Dec-19	204	0.23	3.2	4.28
MAXIMUM	243.60	0.77	5.20	5.68
MINIMUM	153.75	0.13	1.27	1.23
Average	199.1	0.5	3.1	3.0
StdS	22.4	0.2	1.1	1.5
2*StdS	44.8	0.4	2.2	2.9

Date	FR_FRCP1 2019 Water Quality			
	Average Total Alkalinity, as CaCO3 (mg/L)	LSI	Stability Factor	Dissolved CO2 (mg/L)
Jan-19	397	1.15	48.8	6.61
Feb-19	403	1.10	53.2	7.98
Mar-19	309	0.95	19.1	4.39
Apr-19	227	0.78	5.2	2.13
May-19	167	0.42	1.7	1.86
Jun-19	154	0.31	1.2	2.01
Jul-19	190	0.52	2.1	2.21
Aug-19	202	0.61	2.9	2.62
Sep-19	200	0.81	3.3	1.70
Oct-19	207	0.69	4.0	2.08
Nov-19	227	0.68	5.4	2.54
Dec-19	274	0.77	11.6	4.10
MAXIMUM	403.00	1.15	53.18	7.98
MINIMUM	154.25	0.31	1.17	1.70
Average	246.4	0.7	13.2	3.4
StdS	83.3	0.3	18.4	2.0
2*StdS	166.6	0.5	36.8	4.1

Date	LC_FRDSDC 2019 Water Quality			
	Average Total Alkalinity, as CaCO3	LSI	Stability Factor	Dissolved CO2 (mg/L)
Jan-19	202	0.38	3.5	3.32
Feb-19	202	-0.68	3.2	35.97
Mar-19	203	0.46	3.5	2.90
Apr-19	247	0.71	5.3	2.65
May-19	174	0.41	1.9	2.14
Jun-19	176	0.16	1.5	3.29
Jul-19	172	0.24	1.6	2.95
Aug-19	191	0.04	2.3	6.91
Sep-19	206	0.50	3.1	2.88
Oct-19	202	0.38	3.2	3.56
Nov-19	196	0.44	3.0	2.49
Dec-19	203	0.22	3.0	4.21
MAXIMUM	247.20	0.71	5.31	35.97
MINIMUM	172.33	-0.68	1.54	2.14
Average	197.8	0.3	2.9	6.1
StdS	19.9	0.3	1.0	9.5
2*StdS	39.9	0.7	2.1	19.0







Note that LSI remains above zero at all sites throughout the year except for January 2019 at LC_FRDSDC. Langelier saturation index (LSI) approximates the base 10 logarithm of the calcite saturation level | Stability Factor determines the ratio of the calcium/bicarbonate concentration product to a "calcite-stable effluent"

Calcite interacts with metals as it precipitates. Metal ions can occur throughout the porous organic-inorganic hybrid structure of biogenic calcite. Calcite in 34 UFR mine-affected samples from the Elk Valley were observed to be enriched in cadmium, nickel, selenium, and zinc compared to calcite from five reference streams (Day and MacGregor 2014). The five UFR calcite samples from this project are presented in Table 9, and they also demonstrate metal enrichment in mine-affected samples, although selenium was elevated in the UFR1-FRO reference calcite sample. Most metals that are elevated in the UFR sediment samples – Cd, Mn, Ni, Se and Zn, are also elevated in calcite – Cd Ni Se and Zn (MacGregor and Day 2011; Ings *et al.* 2019). In part, this is because calcite fragments/mud can accumulate in sediments, but it is also a reflection of UFR water chemistry. The range of uncontaminated lake sediments in the South Rockies Region showed some exceedance of the lower BC WSQG for As, Cd, Cr, Cu, Pb, Mn, Ni, and Se, indicating that this entire region is metal enriched (Table 9).

Calcite transported by high flows can export embedded metals to depositional locations where they may settle. Conditions in those environments will determine the fate of those metals. During calcite dissolution, sorption to crystal and cell surfaces and incorporation into re-forming calcium carbonate lattices can recapture released metals (Mugwar and Harbottle 2016). This means biogenic calcite is unlikely to release metal ions during dissolution, thus retaining its role as a metal sink that makes metals less bioavailable.

In terms of requisite conditions, the spatial extent and location conditions are met in that calcite is present throughout the UFR, but the duration and timing of calcite changes are not significant despite an isolated increase in the FORD9 unit of the middle mainstem strata (Figure 16). Although this biogenic precipitation pathway may be consistent with effects across all age classes, greatest effects are predicted in juveniles compared to adults. Additionally, the intensity of calcite levels and their rate of change pre-window versus Decline Window are likely not high enough to explain the observed rapid WCT decline. Further evaluation of the potential for calcite dissolution, which could release cyanotoxins and metals found that potential dissolution events were rare in the UFR mainstem with only two found at site LC_FRCP1 during the Decline Window. This site occurs within the Lower watershed resident WCT population area, which contains an estimated 10% of the overall UFR WCT population (Cope *et al.* 2016). Therefore, biogenic precipitation as a sole cause is unlikely, both for WCT adults and juveniles. However, it remains possible that cyanotoxin stress may have contributed to the WCT decline, particularly for juveniles residing in the Lower Mainstem area, via an interaction with a lack of a fall flush in 2018 and winter conditions in 2018/2019. For example, calcite dissolution may have occurred in January 2019 concurrent with ice cover that may restrict dissolved oxygen concentrations and while aqueous concentrations of cyanotoxins experience the least dilution. The impact of cyanotoxin release during calcite dissolution in UFR winter low flows cannot be determined at this time but is possibly a contributing factor to WCT juvenile declines due to their smaller body size, proximity to the substrate, and mechanism of uptake across the gills and epithelial layer. Overall, there is high uncertainty associated with this pathway due to the unavailability of a dose-response relationship.

Table 9. Metal content in UFR calcite and sediment samples, compared to the BC WSQG guidelines.

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

NOTE: the BC WSQG sediment guidelines are screening tools for evaluating potential for toxicological risk. The lower WSQG aligns with the CCME ISQG (interim sediment quality guideline, while the upper WSQG aligns with the CCME PEL (probable effects level) were not based on dose-response curves but instead concentrations in samples that showed toxicity.

3.2.4. Effects to Incubation Conditions

As with other calcite pathways to WCT, the effects of calcite on incubation conditions have not been formalized into explicit dose-response curves given the small available datasets and lack of corresponding hatch success information. However, results from Wright *et al.* (2017, 2018) provided general conclusions with which to test the requisite conditions for an incubation pathway. Overall, Wright *et al.* (2017, 2018) found that calcite index was not an important factor in determining incubation success, as measured by DO and flow in the substrate. Because the calcite index averaged 0.85 across the UFR ($CI < 1$) and calcite concretion averaged 0.07, and that any effects of this pathway during the Decline Window would be observed in juveniles and not adults, these combined findings do not meet the requisite conditions for this pathway to be considered causal, either as a sole or contributing cause.

3.2.5. Effects to Rearing – Overwintering Habitat

Data with which to examine relationships between calcite and overwintering effects are minimal, making it difficult to provide an estimate of population-level effects of this pathway. Inferences based on literature and previous studies can be used to estimate the importance of this pathway in the context of requisite conditions, albeit with some uncertainty. The highest utilized areas for overwintering for adult WCT are the S6 oxbows in the Fording mainstem just above Chauncey Creek and Henretta Pit Lake (Cope *et al.* 2016). No calcite data is available for these specific reaches although conditions are expected to be similar to adjacent reaches with $CI < 1$ and $CI_c < 0.1$. WCT juveniles are expected to overwinter in similar habitat to summer rearing areas and therefore calcite conditions in fish-bearing strata can be used to evaluate requisite conditions.

The importance of interstitial refuge for juvenile trout was highlighted by Cunjak (1996), Jakober (1995), and Jakober *et al.* (1998). Cope *et al.* (2016) and (2019) noted predation and ice as potential mortality mechanisms for juvenile overwintering fish that burrow into interstitial space for cover. If calcite concretion covers substrates and reduces available interstitial space, it could exacerbate winter stressors such as increased metabolic requirements from lack of shelter or exposure to direct mortality. However, requisite conditions for this pathway assume that moderate to high levels of calcite concretion would be necessary for this pathway to be considered explanatory. Overwintering mortality can potentially affect fish of all ages (fry, parr, adults), but it is expected that small fish (fry and parr) would be more susceptible to this causal pathway, as large-bodied individuals tend to make use of deep pools and rely less on interstices.

Given the relatively low calcite levels, the lack of an intense, widespread increase in calcite coincident with the WCT decline, and the expectation that effects would be possible for juveniles but not adults, evidence for the overwintering pathway to be considered causal on its own is weak. Adults are expected to overwinter in deep-water habitats in the UFR and therefore the requisite conditions for adults are not met as either a sole or contributing cause. For juveniles, calcite concretion may compound overwintering stressors described in Hatfield and Whelan (2021) and reduce juvenile WCT carrying capacity through effects on winter habitat, meaning that it is possible that this causal pathway could have contributed to impacts from other pathways for WCT juvenile declines.

4. DISCUSSION

4.1. Spatial and Temporal Trends in Calcite

A general trend of increasing CI is reported throughout much of the UFR, and the larger Elk River watershed. The increasing trends are occurring in both exposed and reference reaches. From this finding, McCabe and Robinson (2020) proposed two hypotheses, which are likely to be happening concurrently. The first hypothesis is that the large flood of 2013 resulted in extensive bedload movement and bank erosion, which introduced new material to the streams and reduced the observed amount of calcite in exposed and reference streams throughout the watershed. Coincidently, the regional calcite monitoring program was initiated in 2013. Under this hypothesis, the increasing trends may be in part a result of calcite deposition returning to pre-flood levels as newly deposited streambed substrates become subjected to calcite deposition. The relatively gradual nature of these trends (i.e., low rate of change) are consistent with this explanation. The second hypothesis is that increasing trends are the result of increasing mine activity. Portions of the UFR (i.e., Dry Creek-LCO) have provided an opportunity to study the process of calcite accumulation in response to mining activity. However, exposure to mining alone does not explain significant trends in reference areas. McCabe and Robinson (2020) concluded that both hydrology and mine activity are likely contributing to the observed trends.

Increasing trends in calcite index but not calcite concretion was observed in both reference and exposed reaches across the UFR. For example, a gradual increase in calcite index was observed in the Fording River mainstem from 2013-2019, with current CI values averaging 0.80. In contrast, calcite concretion values did not change across the pre-window versus Decline Window periods. Further, reach mean CI values greater than 1 only exist in mine-exposed tributaries, and largely do not occur within fish habitat assessed by Cope (2020), which supports the critical habitat and largest part of the WCT population area. CI values greater than 1 is the value identified as an approximate effects threshold by Robinson (2010) and Barrett *et al.* (2016) in independent invertebrate effects assessments. Important in these effects studies is the observation that biological impairment appears to be more closely related to advanced stages of calcite development where concretion begins to occur (Robinson 2010; Barrett *et al.* 2016; Hocking *et al.* 2020). The predictive spawning curve developed in Hocking *et al.* (2020) also shows a steep decline in spawning suitability with initiation of calcite concretion.

Assessment of trends in calcite concretion specific to strata sampled by Cope (2020) that support WCT showed that the highest concretion scores occur in upper tributaries and areas currently not accessible to WCT. However, a moderate increase in calcite concretion was observed in the middle mainstem strata between 2018 and 2019 to an average of approximately 0.16 CI_c. This stratum includes high use spawning and rearing habitat near Clode Creek. However, an important caution is that this increase in calcite concretion is driven primarily by the FORD9 unit of the middle mainstem. No similar magnitude increase in calcite concretion was observed for the other two units (FORD10 and FORD11) of the middle mainstem for 2018, and no data are available for these two units in 2019.

The requisite conditions for all five biological pathways depend on the spatial extent, duration, location, timing, and intensity of calcite in the UFR. Overall, while calcite levels are generally increasing in the UFR, the current data do not support the requisite conditions for sole cause related to duration and timing (no distinct increase in calcite during Decline Window) and intensity (CI < 1 in fish habitat) for all biological pathways. The requisite conditions are discussed for each biological pathway further below, including the potential of each biological pathway to cumulatively contribute to cause for both WCT adults and juveniles.

4.2. Assessment of Biological Effects

4.2.1. Effects to Spawning Suitability

The spawning suitability curve for mean redd counts developed in Hocking *et al.* (2020) was applied to spatial and temporal trends calcite concretion data corresponding to fish-bearing reaches and strata monitored by Cope (2020) to predict changes in spawning suitability in pre-window versus Decline Window periods. The spawning suitability curves in Hocking *et al.* (2020) provide a draft dose-response relationship between calcite concretion and spawning success. However, there remains uncertainty in applying the spawning suitability curves in this way and we therefore focus on applying the spawning suitability curve to describe potential trends in spawning habitat suitability, and in particular comparing spawning suitability within the Decline Window to suitability prior to the decline window, rather than predicting absolute spawning suitability across the UFR.

Calcite concretion levels throughout the UFR were generally similar between pre-window and Decline Window periods and were also not high enough to lead to a substantial decline in mean redd count. There is uncertainty inherent in the modeled response curve and its application, but the general shape and slope of the response curve provides a tool for assessing the magnitude of effects; concretion levels do not reach intensities needed to satisfy requisite conditions for this pathway to be considered causal. While increases in calcite concretion were observed in the middle mainstem strata in 2018, these were limited in geographic extent to a single reach, with a very localized potential impact. Further, any decreases in fry through decreased spawning success would not immediately translate into a decline in other life stages, and thus do not explain the WCT decline, particularly for adult WCT. The effect of calcite on spawning suitability is therefore not expected to be a sole explanation of the declines, though it is a possible cumulative contributing factor to juvenile WCT declines.

4.2.2. Effects to Rearing – Invertebrate Prey Availability

Results from Barrett *et al.* (2016) provide insight into relationships between periphyton and invertebrate production and potential effects on rearing WCT. The study showed some increases in periphyton productivity with calcite, but conditions did not deviate from the expected normal range at any calcite level. Benthic invertebrate communities were more strongly affected by calcite than periphyton; proportions of EPT and Ephemeroptera decreased with increasing calcite. Although EPT are a preferred prey for drift-feeding salmonids, it is unclear whether a change in invertebrate species composition with similar total abundance would translate into effects on WCT growth and abundance. Deviation from normal benthic community composition was also only observed at CI values >1, which did not occur in most rearing habitat. Calcite levels in the UFR mainstem and lower tributaries remain below CI = 1 for all reaches surveyed by Cope (2020) except for Clode Creek. Further, no distinct changes in calcite occurred pre-window versus Decline Window. The effect of calcite on food production is therefore unlikely to be a sole or contributing cause of WCT declines for either WCT adults or juveniles.

4.2.3. Effects to Rearing – Biogenic Calcite Precipitation and Dissolution

Evaluation of cause for biogenic calcite impacts to WCT resulted in several conclusions, including that the magnitude of this pathway could not be determined due to the lack of UFR cyanobacteria and cyanotoxin data to build a dose-response relationship. While periphyton likely accelerates and enhances calcite deposition, which can be harmful to WCT at CI exceeding 1, changes in calcite throughout the UFR have been gradual with no distinct shift in calcite index or concretion during the Decline Window that could clearly account for the WCT decline.

Metals were a component of this pathway that were examined, and it was determined that metals associated with UFR calcite could theoretically be released during winter calcite dissolution; however, rapid resorption to crystal and cell surfaces in re-forming calcium carbonate lattices can recapture released metals, making it unlikely that metals released from calcite contributed to the WCT decline.

The final component of this pathway that was examined was the release of cyanotoxins during calcite dissolution. Biogenic calcite impacts from cyanotoxicity are predicted to exert the greatest influence when no fall flush (>~2-5 m^3/s measured at FR_FRNTP) occurs in between the summer biogenic calcite production and winter low flow conditions. No fall spate (flush) occurred in 2018, indicating that biogenic calcite and associated cyanobacteria could have persisted into winter 2018/2019. Biogenic calcite impacts are also expected to gradually increase between years with a large freshet or flood, however, because it is fragile, every freshet will remove some of this calcite and may help explain why calcite indices are stable at many UFR sites.

The main flows of the UFR rarely approach calcite dissolving conditions despite frequent oscillations. Instead, dissolution is predicted to occur in localized zones of hyporheic upwelling and in microsites at the base of the periphyton where decomposition suppresses pH, particularly in late fall and winter. The calculated calcite dissolution indices (LSI) indicate that the scale of biogenic calcite dissolution and therefore a related cyanotoxin release event will be localized and infrequent. However, the LSI = -0.68 calculated for January 2019 at LC_FRDSDC does indicate potential for calcite dissolution in the bulk river water due to a low pH of 7.1 compared to the average 8.2 ± 0.3. This site overlaps with the Lower Mainstem strata and the Lower watershed resident WCT population, which contains an estimated 10% of the UFR WCT population.

Therefore, although cyanotoxicity as a sole cause is determined to be unlikely, chronic low dose cyanotoxin stress may have contributed to the WCT decline via an interaction with fall and winter conditions in 2018/2019, particularly for WCT juveniles within the lower watershed. However, there is high uncertainty associated with this pathway, stemming from a lack of cyanobacteria and cyanotoxin data within the Decline Window.

4.2.4. Effects to Incubation Conditions

Results from Wright *et al.* (2017, 2018) based in tributaries of the UFR were used to evaluate the effects pathway for incubation conditions. These studies examined dissolved oxygen and hyporheic flow in relation to calcite and found that while calcite index had no effect on hyporheic flow, it was an important predictor of dissolved oxygen in the substrate. However, the effect on dissolved oxygen was most pronounced at depths greater than the average excavation depth for a WCT redd. Declines in interstitial DO was also associated with moderate to high calcite levels (CI >1), which are not present in most of the fish-bearing reaches of the UFR. This pathway would also only have the potential to affect WCT juveniles during the Decline Window and not adults. Overall, the requisite conditions (in particular, high levels of calcite intensity) are not met and effects to incubation conditions are unlikely to be a sole or contributing cause of WCT declines for both WCT adults or juveniles.

4.2.5. Effects to Rearing – Overwintering Habitat

Inferences from Cope *et al.* (2016), Cope (2019), and Hatfield and Whelan (2021) give insight into overwintering mortality and the relationships between calcite and overwintering conditions for WCT. Cope (2019) highlighted the importance of interstitial space as refuge for overwintering small fish and noted that winter mortality can affect fish through predation mortality and ice effects, and Hatfield and Whelan (2021) outlined the potential for winter habitat to be a limiting factor that can be affected by other stressors. Although the relationship between calcite and the use of substrate refuge was not examined explicitly, a defined, widespread increase in calcite during the Decline Window was not observed. Similarly, because overwintering effects would be expected to affect young (i.e., small) fish more strongly, this pathway does not meet the requisite conditions to explain the decline in adult WCT. It remains possible that calcite accumulations contribute cumulatively to juvenile WCT declines, although there is uncertainty, based on the lack of a dose-response relationship for this pathway.

4.3. Discussion of Uncertainty

Each of the pathways presented here has uncertainties associated with either original study implementations or interpretations of findings in an evaluation of cause framework. A primary limitation for some of the pathways (effects to rearing – invertebrate prey availability, effects to rearing – overwintering, effects to biogenic precipitation) is the lack of a quantified relationship between calcite dose and mechanisms of WCT population response. For these pathways, evaluation of cause is limited to qualitative (albeit informed) interpretations of ecological relationships between the factors examined and WCT population response. Although the absence of quantitative dose-response relationships introduces uncertainty to each pathway, evaluation of the requisite conditions is still feasible based on the WCT life history stages that would be affected and a comparison of the requisite conditions for calcite that overlap with key WCT spawning, rearing or overwintering habitat.

In contrast, the effects to spawning suitability pathway does have a defined response curve between calcite and spawning suitability, based on modelling results from Hocking *et al.* (2020). While this facilitates direct assessment of how calcite can influence recruitment through reduced spawning success, such a modelling approach also has uncertainties. The spawning suitability curves are currently in draft form and further sampling and analysis are planned for 2020 to increase statistical confidence of the curves, particularly at low to moderate calcite concretion levels. There is also uncertainty in the degree to which the spawning suitability curves derived from mesohabitat scale data can be expanded to reach-level calcite and fish population data. For example, patchiness in calcite within a reach may allow fish to select favourable locations in the reach, particularly if spawning habitat is not limiting. If spawning habitat is not limiting and WCT are able to avoid areas with high calcite levels to spawn successfully in other areas, the population level response to calcite effects on spawning would not be as steep as predicted by the modeled suitability curve. Overall, we therefore focus on applying the spawning suitability curve to better assess spatial and temporal trends in suitability, rather than predicting absolute spawning suitability across the UFR.

Another key uncertainty is how these pathways interact and how calcite accumulations in UFR interact with other (non-calcite related) pathways of effect. For example, interactions between calcite effects to overwintering and biogenic calcite precipitation with stream flow and temperature conditions in winter are possible but also highly uncertain due to a lack of studies.

4.4. Summary Evaluation of Requisite Conditions to Cause or Contribute to the Decline

Calcite in the UFR watershed has the potential to impact WCT through several pathways. These pathways were evaluated in the context of spatial and temporal trends in calcite throughout the watershed and interpreted in terms of requisite conditions for each pathway to cause or contribute to the WCT decline. The main trend in calcite conditions documented was a significant increase in calcite index in the UFR in both exposed and reference reaches. In particular, a gradual increase in calcite index from 2013 to 2019 has been observed in the Fording River mainstem. However, levels of calcite concretion have remained low and relatively stable across years, and there were no sharp increases in calcite index or concretion during or immediately prior to the Decline Window. The requisite conditions for all pathways examined here required moderate to high levels of calcite index (CI>1) and/or concretion and timing of calcite increases to be associated with the Decline Window. The biological pathways were also generally unable to explain the observed decline in WCT across all age classes during the decline window, particularly for adults. Calcite as a sole cause of the decline is therefore unlikely.

Although calcite is unlikely to be a sole cause of decline, the pathways examined could contribute cumulatively to WCT population abundance. Like all aquatic ecosystems, the potential fish stressors at play in the UFR co-occur and interact. Such interactions could have been important in winter 2019 when flows were low and ice was prevalent. Calcite as a contributor to the decline of WCT juveniles is therefore assessed as possible, but with high uncertainty given the lack of explicit dose-response relationships for many pathways and limited data on interactions among pathways.

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