Subject Matter Expert Report: Selenium

Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population

Submitted to:

Teck Coal Limited

Submitted by:

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Background

The Elk Valley (Qukin ʔamaʔkis) is located in the southeast corner of British Columbia (BC), Canada. “Ktunaxa people have occupied Qukin ʔamaʔkis for over 10,000 years. . . . The value and significance of ʔa·kxamis ʼqapi qapsin (All Living Things) to the Ktunaxa Nation and in Qukin ʔamaʔkis must not be understated” (text provided by the Ktunaxa Nation Council [KNC]).

The Elk Valley contains the main stem of the Elk River, and one of the tributaries to the Elk River is Grave Creek. Grave Creek has tributaries of its own, including Harmer Creek. Harmer and Grave Creeks are upstream of a waterfall on Grave Creek, and they are home to isolated, genetically pure Westslope Cutthroat Trout (WCT; Oncorhynchus clarkii lewisi). This fish species is iconic, highly valued in the area and of special concern under federal and provincial legislation and policy.

In the Grave Creek watershed[1], the disturbance from logging, roads and other development is limited. The mine property belonging to Teck Coal Limited’s Elkview Operations includes an area in the southwest of the Harmer Creek subwatershed. These operations influence Harmer Creek through its tributary Dry Creek, and they influence Grave Creek below its confluence with Harmer Creek (Harmer Creek Evaluation of Cause, 2023)[2]. Westslope Cutthroat Trout populations in both Harmer and Grave Creeks are part of Teck Coal’s monitoring program.

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[1] Including Grave and Harmer Creeks and their tributaries.

[2] Harmer Creek Evaluation of Cause Team. (2023*). Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited.

The Evaluation of Cause Process

The Process Was Initiated

Teck Coal undertakes aquatic monitoring programs in the Elk Valley, including fish population monitoring. Using data collected as part of Teck Coal's monitoring program, Cope & Cope (2020) reported low abundance of juvenile WCT in 2019, which appeared to be due to recruitment failure in Harmer Creek. Teck Coal initiated an Evaluation of Cause — a process to evaluate and report on what may have contributed to the apparent recruitment failure. Data were analyzed from annual monitoring programs in the Harmer and Grave Creek population areas[3] from 2017 to 2021 (Thorley et al. 2022; Chapter 4, Evaluation of Cause), and several patterns related to recruitment[4] were identified:

- *Reduced Recruitment*[5] occurred during the 2017, 2018 and 2019 spawn years[6] in the Harmer Creek population and in the 2018 spawn year in the Grave Creek population.
- The magnitude of Reduced Recruitment in the Harmer Creek population in the 2018 spawn year was significant enough to constitute *Recruitment Failure*[7].
- Recruitment was *Above Replacement*[8] for the 2020 spawn year in both the Harmer and Grave Creek populations.

The recruitment patterns from 2017, 2018 and 2019 in Harmer Creek are collectively referred to as Reduced Recruitment in this report. To the extent that there are specific nuances within 2017-2019 recruitment patterns that correlate with individual years, such as the 2018 Recruitment Failure, these are referenced as appropriate.

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[3] Grave Creek population area" includes Grave Creek upstream of the waterfall at river kilometer (rkm) 2.1 and Harmer Creek below Harmer Sedimentation Pond. "Harmer Creek population area" includes Harmer Creek and its tributaries (including Dry Creek) from Harmer Sedimentation Pond and upstream.

[4] Recruitment refers to the addition of new individuals to a population through reproduction.

[5] For the purposes of the Evaluation of Cause, Reduced Recruitment is defined as a probability of > 50% that annual recruitment is <100% of that required for population replacement (See Chapter 4, Evaluation of Cause, Harmer Creek Evaluation of Cause Team 2023).

[6] The spawn year is the year a fish egg was deposited, and fry emerged.

[7] For the purposes of the Evaluation of Cause, Recruitment Failure is defined as a probability of > 50% that annual recruitment is <10% of that required for population replacement (See Chapter 4, Evaluation of Cause, Harmer Creek Evaluation of Cause Team 2023).

[8] For the purposes of the Evaluation of Cause, Above Replacement is defined as a probability of > 50% that annual recruitment is >100% of that required for population replacement (See Chapter 4, Evaluation of Cause, Harmer Creek Evaluation of Cause Team 2023).

How the Evaluation of Cause Was Approached

When the Evaluation of Cause was initiated, an *Evaluation of Cause Team* (the Team) was established. It was composed of *Subject Matter Experts* (SMEs) who evaluated stressors with the potential to impact the WCT population. Further details about the Team are provided in the Evaluation of Cause report (Harmer Creek Evaluation of Cause Team, 2023).

During the Evaluation of Cause process, the Team had regularly scheduled meetings with representatives of the KNC and various agencies (the participants). These meetings included discussions about the overarching question that would be evaluated and about technical issues, such as identifying potential stressors, natural and anthropogenic, which had the potential to impact recruitment in the Harmer Creek WCT population. This was an iterative process driven largely by the Team's evolving understanding of key parameters of the WCT population, such as abundance, density, size, condition and patterns of recruitment over time. Once the approach was finalized and the data were compiled, SMEs presented methods and draft results for informal input from participants. Subject Matter Experts then revised their work to address feedback and, subsequently, participants reviewed and commented on the reports. Finally, results of the analysis of the population monitoring data and potential stressor assessments were integrated to determine the relative contribution of each potential stressor to the Reduced Recruitment in the Harmer Creek population.

The Overarching Question the Team Investigated

The Team investigated the overarching question identified for the Evaluation of Cause, which was:

> **What potential stressors can explain changes in the Harmer Creek Westslope Cutthroat Trout population over time, specifically with respect to Reduced Recruitment?**

The Team developed a systematic and objective approach to investigate the potential stressors that could have contributed to the Reduced Recruitment in the Harmer Creek population. This approach is illustrated in the figure that follows the list of deliverables, below. The approach included evaluating patterns and trends, over time, in data from fish monitoring and potential stressors within the Harmer Creek population area and comparing them with patterns and trends in the nearby Grave Creek population area, which was used as a reference. The SMEs used currently available data to investigate causal effect pathways for the stressors and to determine if the stressors were present at a magnitude and for a duration sufficient to have adversely impacted the WCT. The results of this investigation are provided in two types of deliverables:

1. Individual Subject Matter Expert reports (such as the one that follows this Note). Potential stressors were evaluated by SMEs and their co-authors using the available data. These evaluations were documented in a series of reports that describe spatial and temporal patterns associated with the potential stressors, and they focus on the period of Reduced Recruitment, including the Recruitment Failure of the 2018 spawn year where appropriate. The reports describe if and to what extent potential stressors may explain the Reduced Recruitment.

   The full list of Subject Matter Expert reports follows at the end of this Reader's Note.

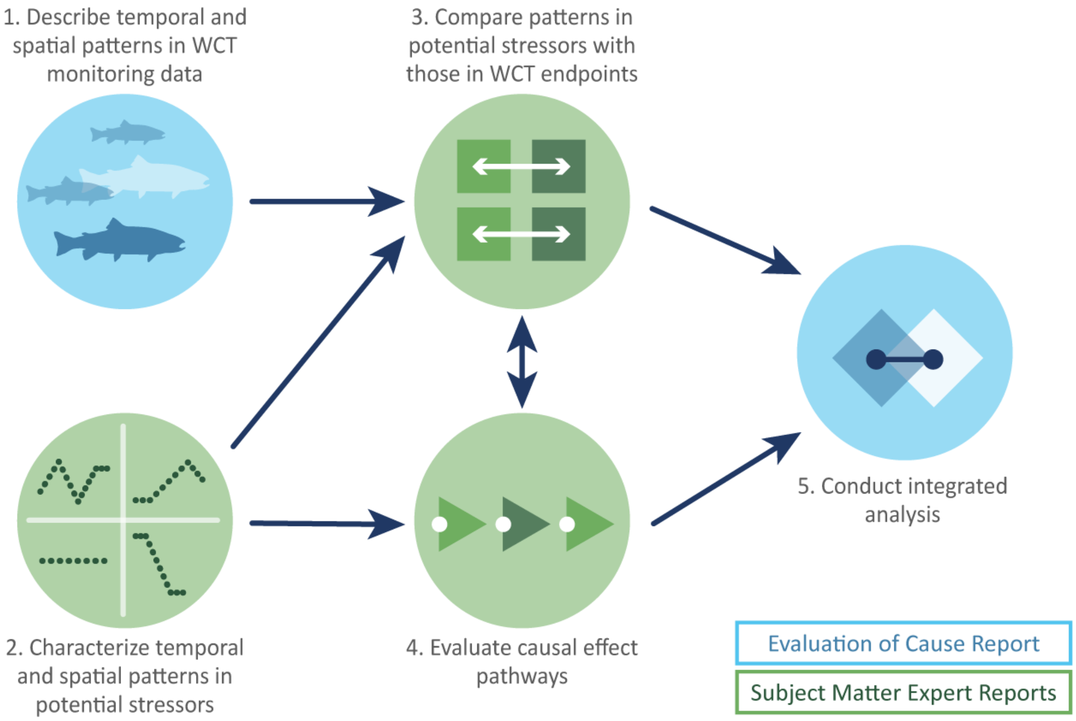
2. The Evaluation of Cause report. The SME reports provided the foundation for the Evaluation of Cause report, which was prepared by a subset of the Team and included input from SMEs.

   The Evaluation of Cause report:

   a. Provides readers with context for the SME reports and describes Harmer and Grave Creeks, the Grave Creek watershed, the history of development in the area and the natural history of WCT in these creeks

   b. Presents fish monitoring data, which characterize the Harmer Creek and Grave Creek populations over time

   c. Uses an integrated approach to assess the role of each potential stressor in contributing to Reduced Recruitment in the Harmer Creek population area.



Conceptual approach to the Evaluation of Cause for the Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout population.

Participation, Engagement & Transparency

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

- Ktunaxa Nation Council
- BC Ministry of Forests,
- BC Ministry of Land, Water and Resource Stewardship
- BC Ministry Environment & Climate Change Strategy
- Ministry of Energy, Mines and Low Carbon Innovation
- Environmental Assessment Office

Citations for Evaluation of Cause Team Reports

Focus	Citation
Harmer Creek Evaluation of Cause report	Harmer Creek Evaluation of Cause Team. (2023). *Evaluation of Cause - Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited.
Calcite	Hocking, M. A., Cloutier, R. N., Braga, J., & Hatfield, T. (2022). *Subject Matter Expert Report: Calcite. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Dissolved oxygen	Abell, J., Yu, X., Braga, J., & Hatfield, T. (2022). *Subject Matter Expert Report: Dissolved Oxygen. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Energetic Status	Thorley, J.L. & Branton, M.A. (2023) *Subject Matter Expert Report: Energetic Status at the Onset of Winter Based on Fork Length and Wet Weight. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population*. Report prepared for Teck Coal Limited. Prepared by Poisson Consulting Ltd and Branton Environmental Consulting.
Food availability	Wiebe, A., Orr, P., & Ings, J. (2022). *Subject Matter Expert Report: Food Availability. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Minnow Environmental Inc.
Groundwater	Canham, E., & Humphries, S. (2022). *Evaluation of Groundwater as a Potential Stressor to Westslope Cutthroat Trout in the Harmer and Grave Creek Watersheds.* Memo prepared for Teck Coal Limited. Prepared by SNC-Lavalin Inc.

Focus	Citation
Habitat availability (instream flow)	Wright, N., Little, P., & Hatfield, T. (2022). *Subject Matter Expert Report: Streamflow and Inferred Habitat Availability. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Sediment quality	Wiebe, A., Orr, P., & Ings, J. (2022). Subject Matter Expert Report: Sediment Quality. *Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population*. Report prepared for Teck Coal Limited. Prepared by Minnow Environmental Inc.
Selenium	de Bruyn, A., Bollinger, T., & Luoma, S. (2022). *Subject Matter Expert Report: Selenium. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by ADEPT Environmental Sciences Ltd, TKB Ecosystem Health Services, and SNL PhD, LLC.
Small population size	Thorley, J. L., Hussein, N., Amish, S. J. (2022). *Subject Matter Expert Report: Small Population Size. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Poisson Consulting and Conservation Genomics Consulting, LLC.
Telemetry analysis	Akaoka, K., & Hatfield, T. (2022). *Harmer and Grave Creeks Telemetry Movement Analysis.* Memo prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Total suspended solids	Durston, D., & Hatfield, T. (2022). *Subject Matter Expert Report: Total Suspended Solids. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Water quality	Warner, K., & Lancaster, S. (2022). *Subject Matter Expert Report: Surface Water Quality. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by WSP-Golder.

Focus	Citation
Water temperature and ice	Hocking, M., Whelan, C. & Hatfield, T. (2022). *Subject Matter Expert Report: Water Temperature and Ice. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.

1.0 INTRODUCTION

1.1 General Introduction

Teck Coal undertakes aquatic monitoring programs in the Elk Valley, including fish population monitoring. Using data collected from 2017 to 2019 in Harmer and Grave Creeks, Cope & Cope (2020) reported low abundance of juvenile Westslope Cutthroat Trout (WCT; *Oncorhynchus clarkii lewisi*), which indicated apparent recruitment failure in Harmer Creek. Teck Coal initiated an Evaluation of Cause — a process to evaluate and report on what may have contributed to the apparent recruitment failure. Data were analyzed from annual monitoring programs in the Harmer and Grave Creek population areas[9] from 2017 to 2021 (Thorley et al. 2022; Chapter 4, Evaluation of Cause), and several patterns related to recruitment[10] were identified:

- *Reduced recruitment*[11] occurred during the 2017, 2018 and 2019 spawn years[12] in the Harmer Creek population and in the 2018 spawn year in the Grave Creek population.
- The magnitude of Reduced Recruitment in the Harmer Creek population in the 2018 spawn year was significant enough to constitute *recruitment failure*[13].
- Recruitment was *above replacement*[14] for the 2020 spawn year in both the Harmer and Grave Creek populations.

The recruitment patterns from 2017, 2018, and 2019 in Harmer Creek are collectively referred to as reduced recruitment in this report. To the extent that there are specific nuances within 2017-2019 recruitment patterns that correlate with individual years, such as the 2018 recruitment failure, these are referenced as appropriate.

The Evaluation of Cause Project Team investigated one overarching question: **What potential stressors can explain changes in the Harmer Creek Westslope Cutthroat Trout population over time, specifically with respect to patterns of Reduced Recruitment?** To investigate this question, the Team evaluated trends in WCT population parameters, including size, condition, and recruitment, and in the potential stressors[15] that could impact these parameters. They evaluated the trends in WCT population parameters based on monitoring data collected from 2017 to 2021 (reported in Thorley et al., 2022 and Chapter 4, Harmer Creek Evaluation of Cause Team, 2023). The Grave Creek population area was used as a reference area for this evaluation.

The approach for analyzing potential stressors for the Evaluation of Cause included, as appropriate to the potential stressor in question, (1) characterizing trends in the Harmer and Grave Creek population areas, (2) comparing the trends between the two population areas, (3) identifying any changes in Harmer Creek during

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[9] "Grave Creek population area" includes Grave Creek upstream of the waterfall and Harmer Creek below Harmer Sedimentation Pond. "Harmer Creek population area" includes Harmer Creek and its tributaries (including Dry Creek) from Harmer Sedimentation Pond and upstream.

[10] Recruitment refers to the addition of new individuals to a population through reproduction.

[11] For the purposes of the Evaluation of Cause, reduced recruitment is defined as a probability of > 50% that annual recruitment was < 100% of that required for population replacement (See Chapter 4, Evaluation of Cause, Harmer Creek Evaluation of Cause Team, 2023).

[12] The spawn year is the year a fish egg was deposited, and fry emerged.

[13] For the purposes of the Evaluation of Cause, recruitment failure is defined as a probability of > 50% that annual recruitment is < 10% of that required for population replacement (See Chapter 4 Evaluation of Cause, Harmer Creek Evaluation of Cause Team, 2023).

[14] For the purposes of the Evaluation of Cause, recruitment above replacement is defined as a probability of > 50% that annual recruitment is > 100% of that required for population replacement (See Chapter 4 Evaluation of Cause, Harmer Creek Evaluation of Cause Team, 2023)

[15] The Evaluation of Cause process was initiated early in 2021 with currently available data. Although the process continued through mid-2022, data collected in 2021 were not included in the Evaluation of Cause because most stressor reports were already complete. Exceptions were made for the 2021 fish monitoring data and selenium data because (1) the selenium report was not complete and substantive new datasets were available and (2) water temperature data for 2021 in the temperature report because a new sampling location was added in upper Grave Creek that contributed to our understanding of the Grave Creek population area.

the period of reduced recruitment, including the recruitment failure of the 2018 spawn year where appropriate, and (4) evaluating how the stressor trended relative to the fish population parameters. The Team identified mechanisms by which the potential stressors could impact WCT and determined if the stressors were present at a sufficient magnitude and duration to have an adverse effect on WCT during the period of reduced recruitment. Together, these analyses were used in the Evaluation of Cause report to support conclusions about the relative contribution of each potential stressor to the reduced recruitment observed in the Harmer Creek population area.

This document is one of a series of Subject Matter Expert (SME) reports that supports the integrated Harmer Creek Westslope Cutthroat Trout Evaluation of Cause (Harmer Creek Evaluation of Cause Team, 2023). For more information, see the preceding Reader's Note.

1.2 Background for the Selenium Assessment

The SME reports on Surface Water Quality (Warner and and Lancaster 2022) and Sediment Quality (Wiebe and Orr 2022) prepared in support of the EoC Process concluded that selenium warranted further evaluation as a potential contributor to reduced recruitment. Selenium concentrations in the Harmer Creek watershed relate to waste rock deposited by Teck Coal’s Elkview Operations (EVO) in the upper portions of Dry Creek, which is a tributary of Harmer Creek. Selenium is distinct from the other water quality constituents considered in the SME Report on Surface Water Quality because it is widely understood to be a bioaccumulative substance, in that it is disproportionately accumulated in biota relative to concentrations in water (discussed further in Section 2.1). Selenium can cause effects to sensitive species and life stages of fish when bioaccumulated concentrations exceed tissue-based thresholds for effect (discussed further in Section 2.2). The exposure routes and modes of effects of selenium are distinct from most other water quality constituents, for which potential effects are evaluated by considering direct exposure to aqueous concentrations.

1.3 Overview of Approach to Selenium Assessment

The approach to selenium assessment herein follows the scientific community’s current understanding of selenium fate and toxicology, as summarized by an international workshop of selenium scientists (Chapman et al. 2010), British Columbia Ministry of Environment and Climate Change Strategy (BC MOE 2014), United States Environmental Protection Agency (US EPA 2021), and Environment and Climate Change Canada (ECCC 2017). These summaries align on an understanding of pathways of potential exposure to selenium in the aquatic environment, sensitive receptor taxa, and relevant effects endpoints.

Key principles of selenium risk relevant to the present assessment are discussed in Section 2.0. The assessment begins by summarizing what is known about selenium bioaccumulation (Section 2.1) and toxicology (Section 2.2). This information is then used to identify plausible impact hypotheses describing different ways that selenium could have contributed to reduced recruitment of WCT in Harmer Creek (Section 2.3). A summary is then provided of available site-specific information (Section 3.0) and this information is used to test the strength of evidence for each impact hypothesis (Section 4.0). Site-specific information considered in the assessment includes spatial gradients of aqueous selenium concentrations in the watershed, evidence for how selenium bioaccumulation varies across the watershed, evidence for fish feeding in (and thus being exposed to bioaccumulated selenium) and moving between (and thus integrating this exposure across areas) different portions of the watershed, and consideration of how selenium could interact with other stressors to cause cumulative stress.

1.4 Authorship

The following report was prepared as a collaboration of selenium SMEs from ADEPT Environmental Sciences Ltd. (Dr. Adrian de Bruyn), Samuel N Luoma PhD LLC (Dr. Sam Luoma), and TKB Ecosystem Health Services (Dr. Trent Bollinger).

2.0 PRINCIPLES OF SELENIUM RISK

The objective of this section is to review what is known about selenium fate and toxicity to generate hypotheses for how selenium could have contributed to reduced recruitment in the Harmer Creek WCT population. The review presented herein focuses on processes and conditions that are relevant to selenium exposure in the Harmer Creek population area and that plausibly could contribute to reduced recruitment in a fish population.

2.1 Bioaccumulation

Selenium bioaccumulation is of interest to the Harmer Creek EoC because bioaccumulation is a quantitative indicator of selenium exposure. Bioaccumulated selenium concentrations are quantitatively related to toxicological effects, reducing uncertainties in linking selenium exposure and effects. Understanding the processes that govern selenium bioaccumulation can help quantify pathways of exposure (e.g., sediments, forms of selenium in water, food) when direct analyses of tissues (e.g., benthic invertebrates, fish) are incomplete. This allows asking questions relevant to the Harmer Creek EoC, such as: What is the source of selenium in Harmer Creek? Do reaches of the creek differ in their exposure to aqueous or sedimentary selenium? Is there evidence of selenium exposure sufficient to cause adverse effects in Harmer Creek compared to Grave Creek? Did exposures in 2018 exceed exposures in other years that did not show the same evidence of reduced recruitment?

Important drivers of bioaccumulation include aqueous selenium concentrations, selenium speciation, transformation of selenium from dissolved to particulate forms, and the species-specific attributes of bioaccumulation into food webs. Understanding selenium exposures and assessing how selenium may have contributed to population scale changes in recruitment requires a full consideration of these factors.

Aqueous Concentrations of Selenium

Bioaccumulation, and thus selenium exposure, is concentration dependent. For example, selenium concentrations in benthic invertebrates correlate significantly with aqueous selenium concentrations across lotic environments in the Elk Valley (Teck Coal 2014; Golder 2020). Drivers of selenium bioaccumulation like speciation and food webs do not differ greatly among such environments. In such circumstances, aqueous selenium concentrations can be a useful first line of evidence about selenium exposures. For example, if selenium concentrations are higher in one location or increase through time, a change in selenium exposure of the food web is likely. The degree of that change depends upon other factors, but aqueous selenium concentration is the first consideration.

Selenium Speciation

Aqueous selenium concentrations alone are not sufficient to quantify selenium exposure, especially if biogeochemical conditions change. It is now well established that it is overly simplistic to assume a direct linkage between aqueous selenium and either bioaccumulated selenium in the food web or selenium toxicity (Stewart et al. 2010). Geochemical speciation of selenium is perhaps the most important factor contributing to the weak link between aqueous selenium and risk to the food web. Geochemical speciation is defined by the distribution of aqueous selenium among different geochemical forms or oxidation states. Aqueous selenium can occur in four oxidation states: elemental selenium (Se0), selenate (SeVI), selenite (SeIV), and organic selenides (Se -II, which we will abbreviate as org-Se).

The biogeochemical cycle of selenium is more complex than that of most trace elements. The speciation of selenium in natural waters does not correspond to predictions based upon chemical thermodynamics alone, like many metals do. This is because transformation reactions and kinetics are mostly driven by biological processes that are not predictable from geochemical thermodynamics (Cutter and Bruland 1984). On the other hand, selenium is one of the few elements for which different forms can be directly analyzed at environmental concentrations (Cutter and Cutter 2004). Thus, enough empirical data are available to explain important speciation processes.

Selenate is the predominant form of selenium released to waterways by geologic processes and thus the predominant form of selenium in most mine-affected waters. Selenate is not reactive with particle surfaces and it can be taken up only slowly by plants, algae, and microbes (Cutter and Bruland 1984). These organisms then transform the selenium to organo-selenides, although some types of bacteria produce elemental selenium as well (Oremland et al. 1990).

The primary forms of selenium within plant, algal, or microbial tissues are seleno-amino acids (selenomethionines and selenocysteines), no matter what the form bioaccumulated. These seleno-amino acids are eventually broken down into organic metabolites and selenite (SeIV) and released into the environment via excretion of metabolic by-products, cell lysis when they die, or when grazed upon by consumer organisms (Wrench 1978). The reduced forms of selenium are taken up much faster than selenate by the organisms at the base of the food web, accelerating the further generation of reduced selenides. Re-released selenium is not reconverted to selenate because the back reaction from either selenite or organo-selenium to selenate has a half time of hundreds of years (Cutter and Bruland 1984). Conceptually, the result could be a build-up of organo-selenides and selenite as streams proceed from their headwaters through their hydrologic units to their ultimate repository in the sea (Lemly 1999).

The degree of transformation, and thus the proportion of different selenium forms, differs widely among watersheds. The exact proportion of the different forms and phases depends upon the opportunities for biological transformation and accumulation of by-products. The degree to which this build-up of reduced selenium occurs is affected by the original uptake rate of selenate at the base of the food web. Recycling is increased when the residence time of the water body increases, i.e., when there is more time for contact between sediment, plants, and microbes and more time for accumulation of by-products in the water column or sediments. Sulphate concentrations are also important because sulphate competes with selenate for uptake. High sulphate concentrations reduce selenate uptake. Microbe and plant productivity are also important because more abundant life at the base of the food web means more uptake and opportunities for recycling.

In contrast, in lotic waters like the Fording and Elk Rivers with high sulphate concentrations and relatively low productivity, the rate of selenate uptake can be slow compared to other forms of selenium. The low residence time of flowing waters limits opportunities for reduced selenium to grow in concentration via recycling. Based upon current understanding, selenium is released from mine works as near 100% selenate. Selenate remains ≥99% of total selenium, selenite is ≤1%, and organic selenides are usually below detection in the mainstem Fording River and Elk River (Minnow 2018). In contrast, the higher residence times and greater productivity of some wetlands and ponds are examples of environments that can generate organ-selenides and selenite. For example, selenite was 1 – 7% of the total selenium and the geomean of concentrations of organo-selenides was 0.05 ± 0.02 µg/L in waters from Goddard Marsh on the Elk River, in 2019. Metabolites of organic selenides appear to be lost rapidly downstream from where they are generated (Golder 2021).

Selenate can also be sequestered into sediments via a microbial biotransformation reaction called extracellular dissimilatory reduction. The transformation is enhanced by prolonged contact with sediment, providing another pathway for generation of selenite and organo-Se and accumulation of reduced selenium as residence times increase. Oremland et al. (1990) showed that nitrate-reducing microbes living in sediment take up selenate in a process that is not affected by sulphate. These bacteria reduce selenate or selenite to elemental selenium, Se(0), and some organo-Se, which they deposit as particles into the sediment. The reaction occurs just below the interface between the water column and the sediments. Transformation appears to be enhanced by prolonged contact between a parcel of water and the sediment. A sharp gradient between oxidized water and reduced sediment is also conducive to the occurrence of the microbes that conduct the reaction. Rapid rates of selenate uptake are observed where ponded water remains in contact with sediments (Tokunaga et al. 1998).

Once sequestered into sediments, selenium is not necessarily retained in its original form. While wetland sediments retain some Se(0) (Tokunaga et al. 1996), it can also be oxidized to Se(IV), which can adsorb to iron oxides in oxidized sediments or form selenium sulfide in anoxic sediments. Microbial uptake and further generation of organic metabolites can also occur. In surface sediments from natural waters, combinations of Se(0), adsorbed selenium, and particulate Se(-II) are often found (Velinsky and Cutter 1991; Zhang and Moore 1996). Sequestration by processes including dissimilatory reduction, microbial uptake and deposition of plant detritus can result in accumulation of a large mass of selenium in sediments and provide a pathway whereby organisms living within the sediment are exposed to selenium.

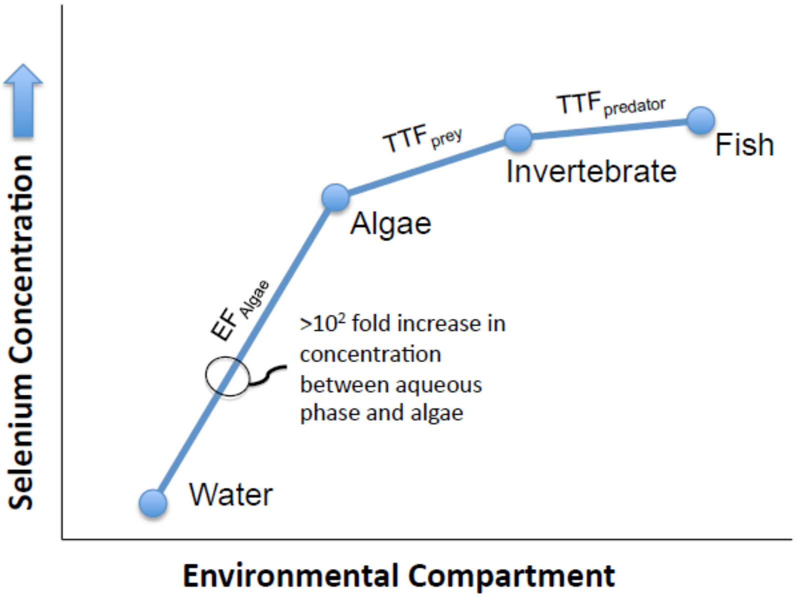
Speciation is relevant to the Harmer Creek EoC. The Harmer Creek population area of interest to the EoC is largely flowing lotic waters with high sulphate concentrations. The likelihood that reduced forms of selenium would be generated in those waters are low. However, the source of selenium is Dry Creek. Generation of organic selenides appears to be occurring in the Dry Creek beaver ponds and/or sedimentation pond. The highest concentrations of metabolites of selenoamino-acids are detected in Dry Creek downstream of the sedimentation pond (EV_DCOUT, EV_DC1) and at the outlet of the Harmer Creek Sedimentation Pond (EV_HC1, RG_HACKDS). Lower concentrations of metabolites are detected in Harmer Creek between Dry Creek and the Harmer Creek Sedimentation Pond (RG_HACKUS). Harmer Creek Sedimentation Pond also appears to be a source of reduced selenium, but the detection of org-Se upstream of the pond at times suggests at least some proportion of the organic selenium generated in the Dry Creek Sedimentation Pond is transported downstream. Thus, exposure of the Harmer Creek food web to reduced forms of selenium generated in the Dry Creek Sedimentation Pond could make linkages between aqueous selenium and bioaccumulated selenium complex.

Transformation at the Base of the Food Web

By far the largest step in bioconcentration of selenium is its bioaccumulation and transformation by the organisms that serve as the food web base (plants, algae, and microbes) (Stewart et al. 2010; Figure 1). As noted above, as inorganic selenium is taken into plants, algae, or microbes, it is transformed into organo-selenium compounds. These transformations not only drive recycling but also drive transfer of selenium up the food chain because the plants, microbes, and detritus containing the transformed selenium provide the food for consumer organisms at the next step in the food web.

When measured in the same units, selenium concentrations at the base of the food web can exceed aqueous concentrations by 100,000× in some cases (e.g., pure algal cultures; Baines and Fisher 2001) or less than 100× in other cases (e.g., selenate-dominated, high sulphate lotic systems; de Bruyn and Luoma, 2021). The variability of this ratio decouples the correlation of aqueous selenium with bioaccumulated selenium and toxicity throughout the food web. The concentration at the base of the food web, not the concentration in water, determines how much selenium is taken up by animals at the lower trophic levels. Transfer to the lower trophic levels determines exposure of higher trophic level animals such as fish and birds. The degree of internal exposure in these organisms determines whether toxicity is manifested in individuals. The degree of growth, survival and reproductive effects determines whether populations are adversely affected.

Figure 1: Conceptual schematic of food web bioaccumulation of selenium (after Stewart et al. 2010).



Stewart et al. (2010) recommended the term “enrichment function” to describe the ratio of bioaccumulated selenium at the base of the food web to aqueous selenium. Others have equated this ratio to a traditional equilibrium partitioning coefficient (K_d; Presser and Luoma 2010). The principles that govern traditional partitioning coefficients do not apply to selenium (thermodynamic equilibrium; similar forms in each media; Stewart et al. 2010), but operationally the formula for both is the same. The K_d term is most often used in the literature, but should be recognized as an operational observation of the biogeochemically-driven distribution between water and the base of the food web, not a thermodynamic equilibrium constant.

Geochemical speciation of selenium, as described above, influences selenium uptake by plants and microbes and the rate of transformation of selenium from dissolved form to the base of the food web. It was long observed in nature that selenium concentrations in algae, microbes, sediments, or suspended particulates are 100 – 500 times higher than dissolved concentrations in lotic environments (K_d on the order of 100 to 500 in streams and rivers), whereas in lentic environments bioaccumulation throughout the food web can be much higher (K_d up to 10,000) (van der Veer and Canton 1997). An important difference between lotic and lentic environments is the difference in speciation of selenium. The most bioavailable reduced forms of selenium increase in lentic areas. Where residence times of water are longer and productivity is higher (e.g., many lentic environments), reduced forms of selenium and bioaccumulation both increase (see above), suggesting higher bioavailability of the reduced forms of the element.

Experimental studies have quantified the differences in bioaccumulation among the dominant geochemical species of aqueous selenium—selenite, selenate, and organic selenides. Reduced forms are accumulated at significantly different rates by algae and microorganisms. An overall K_d can vary widely among watersheds and among environments within a watershed (Presser and Luoma 2010) if speciation differs. Quantifying the K_d for each form of selenium is one way to better understand selenium bioaccumulation in food webs with differing speciation (de Bruyn and Luoma 2021).

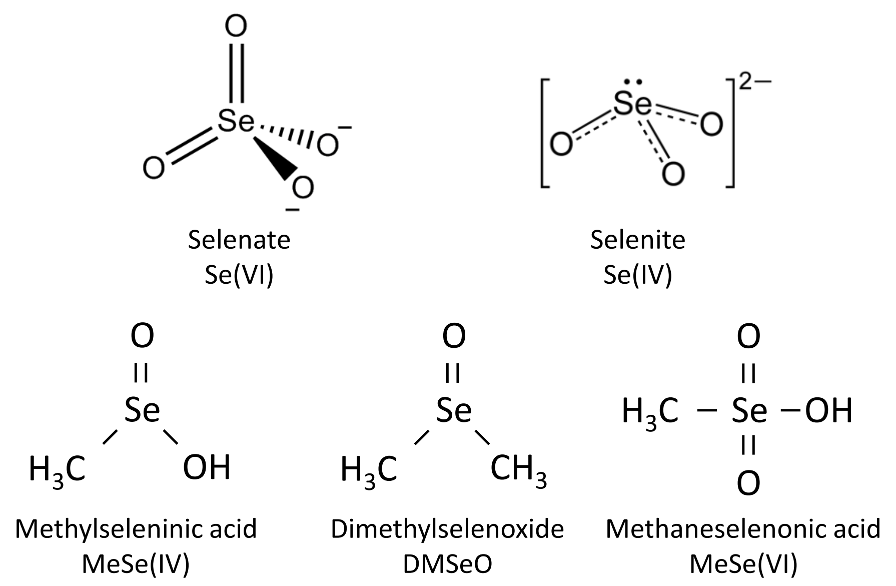
In most studies, uptake rates of aqueous selenate by algae and plants are considerably slower than uptake of the reduced forms of selenium, especially in the presence of sulphate. This has been attributed to uptake of selenate into algal cells and other organisms at the base of the food web via the same pathway as sulphate (Bailey et al. 1995). In many environments, sulphate concentrations are orders of magnitude higher than selenate concentrations, inhibiting uptake of the latter and reducing K_d. For example, in the Elk Valley watershed, geochemical speciation is dominated by selenate (as high as 1 µM) accompanied by an abundance of sulphate (average 1 mM). Selenium concentrations that are high by the standards of most environments are of low bioavailability because of that combination.

The K_d of selenate for algal cells also decreases as selenate concentrations increase; it is concentration dependent (Lo 2014; Van Geest et al. 2016). In a watershed like the Elk Valley, estimating a K_d for selenate becomes complex because sulphate concentrations correlate with selenium concentrations and K_d declines as selenate concentration increases. In a model that took into account both concentration dependence and sulphate competition, de Bruyn and Luoma (2021) calculated K_d's for selenate ranging from ~2,000 where selenate concentrations were low (1 µg/L Se(VI) and 25 mg/L SO_4) to ~20 at 100 µg/L Se(VI) and 500 mg/L sulphate. These values encompass the range of K_d's typically ascribed to selenate-dominated systems (e.g., 140 to 493 [Presser and Luoma 2010]).

In general, reduced forms of selenium have higher rates of uptake than selenate. Selenite is typically the most abundant reduced form of selenium. Laboratory experiments have estimated different K_d's for selenite depending upon selenite concentration, phosphate concentration, and the type of primary producer (algae, periphyton). In general, K_d's ranging from ~700 to ~4,000 roughly characterize selenite bioaccumulation (Conley et al. 2011, 2013; de Bruyn and Luoma 2021). Thus, as the proportion of selenite compared to selenate increases in a water body, food web bioaccumulation will increase.

Uptake rates of organo-selenides can greatly exceed uptake rates for inorganic forms of selenium. Mechanistically, seleno amino acids are taken up via same pathway as the amino acid itself (methionine or cysteine; Stewart et al. 2010). The affinity of organic selenides for the pathways is very high. The biogeochemistry of the organo-selenides is also complex. Thus, when seleno amino acids or their metabolites are added to experimental media they can be rapidly depleted by a combination of rapid metabolism to different forms, volatilization, or rapid bioaccumulation by both experimental organisms and incidental microbes (Zhang and Moore 1997). The resulting K_d's are difficult to quantify. In general, different estimates suggest bioavailability of organic selenides can be 5 to 10-fold or more greater than the bioavailability of selenite. For example, de Bruyn and Luoma (2021) estimated a K_d of 28,000 for dimethylselenoxide and methylseleninic acid, two metabolites of seleno-methionine sometimes found in the Elk Valley watershed (Figure 2). This estimate was derived using a statistical approach with field data and is consistent with experimental studies (Besser et al. 1993).

Figure 2: Selenium species included in the de Bruyn and Luoma (2021) bioaccumulation tool



Selenate
Se(VI)

Selenite
Se(IV)

Methylseleninic acid
MeSe(IV)

Dimethylselenoxide
DMSeO

Methaneselenonic acid
MeSe(VI)

Uptake of selenium at the base of the food web can be estimated from measurements in nature. However, directly analyzing the exact food of one or a community of consumer organisms is rarely feasible. Presser and Luoma (2010) suggested that consistent analysis of one surrogate can aid understanding of site-specific bioavailability to the food web. The more closely the surrogate represents the food of their consumers (e.g., benthic invertebrates in Harmer Creek represent the food of fish), the less uncertainty about consumer exposure. Plants, plant/microbial complexes like periphyton, suspended fine particulates, suspended detritus, and sediments are, in that order, potential surrogates to indicate selenium exposure at the base of the food web in stream environments.

Bioaccumulation can differ among taxa at the base of the food web under similar conditions. No one primary producer can directly represent all the food of a variety of consumer taxa. Algal species in the water column are usually a small proportion of the total suspended material and cannot be separated from inorganic and detrital particles. Also, the bioavailability of selenium in these components can vary. Thus, reliably predicting selenium concentrations in the food web from suspended material can be challenging. Access to sufficient material for analysis can also be a challenge. Sediments are the least reliable of the surrogates because both grain size and a complex mixture of materials affect selenium bioavailability. In the Elk Valley watershed, periphyton analyses are a useful surrogate because of the availability of material for analysis and similarity to food for the dominant primary consumer taxa, aquatic insect larvae. But a small dynamic range of concentrations, variable growth of plant materials from different locations, and contamination with suspended particulate material can distort analyses. Direct determination of selenium concentrations in benthic invertebrate tissues accounts for these complexities and therefore is the most straightforward indicator of selenium exposure to lower trophic levels in a food web.

The measures of selenium accumulation and transformation at the base of the food web available to the Harmer Creek EoC are selenium concentrations in sediment (an indirect and complex measure of the base of the food web) and benthic invertebrates (which reflect feeding on the concentrations at the base of the food web). K_d's calculated from available sediment data range from ~250 to ~750. Using these as a direct measure of selenium in taxa at the base of the food web would over-estimate selenium bioaccumulation in benthic invertebrates from the same location by about 3 – 10× (see next section; methods from de Bruyn and Luoma 2021). Determining bioavailability from sediments is always a challenge, but the degree of over-estimation using total sedimentary selenium as a food source in the de Bruyn and Luoma (2021) bioaccumulation model suggests a bioavailability from the sediments of 10% – 30%.

Trophic transfer: Predators

Bioaccumulation of selenium at the second step of food webs (consumer organisms) is not a simple function of aqueous selenium concentrations. This is because: a) dietary bioaccumulation, not direct uptake of aqueous selenium, dominates uptake by consumers; and b) bioconcentration into the base of the food web, the source of food for consumers, varies widely with differences in speciation and taxa.

Aqueous concentrations and geochemical speciation determine how much selenium enters the food web at its base (as discussed in the previous subsection), but direct uptake of dissolved selenium contributes little to bioaccumulation by animals. Decades of literature support the concept that selenium bioaccumulation by animals is dominated by uptake from diet. When quantified at concentrations typical of nature, dissolved selenium uptake cannot explain the level of bioaccumulation in nature or in studies experimentally dosing with food and water together (e.g., Luoma et al. 1992; Wang et al. 1996). Besser et al. (1993) concluded that diet was the primary source of inorganic selenium bioaccumulation in a water>daphnid>bluegill food chain. Lemly (1996) showed that the principal source of selenium to fish was their food in an episode of toxicity in Belews Lake, North Carolina, USA. Xu and Wang (2002) showed that dietary uptake always dominates selenium accumulation in the predatory mangrove snapper (*Lutjanus argentimaculatus*). Multiple chapters in a selenium expert workshop (Chapman et al. 2010) drew a similar conclusion: virtually all the selenium found in the tissues of animals originates from dietary bioaccumulation.

Stewart et al. (2010) did note that there could be exceptional circumstances where dissolved selenium might contribute to overall selenium bioaccumulation. For example, dissolved selenium could be important if, because of physiology or functional ecology, the invertebrate has a low assimilation efficiency of selenium from its food or selenium concentrations in food are low relative to bioavailable selenium in water. Algal taxa differ widely in the efficiency with which they take up selenium. If an invertebrate feeds exclusively on algal taxa that themselves do not take up selenium efficiently, then dissolved selenium might be a more important pathway of exposure in the invertebrate relative to uptake from food. For example, zebra mussels (*Dreissena polymorpha*) have a high filtration rate, rapidly passing water over their gills. Selenium in their food is absorbed into tissues (assimilated) with less than 50% efficiency. Stewart et al. (2010) estimated a contribution from water of 24 – 61% of uptake for the zebra mussel. But the perfect storm of factors that lower uptake from food and increase uptake from water is rare. Typically, from 60% to 90% of the of the selenium in algal cultures is assimilated into the tissues of zooplankton (Stewart et al. 2010) and nearly all bioaccumulation is from diet. If selenate or selenite are the dominant dissolved forms of selenium, as they are in the Elk Valley, it is unlikely that a significant proportion of uptake could come from dissolved forms under any circumstances (Besser et al. 1993; Stewart et al. 2010).

Even if highly bioavailable organic selenides are present in the water column, their efficient uptake at the base of the food web will generate a highly bioavailable dietary source for consumers in food that will outstrip dissolved uptake. More importantly, transformation of selenium to organic selenides in organisms at the base of the food web presents consumers with a concentrated form of selenium that is efficiently taken up in the gut. As a result, most consumers also bioaccumulate selenium to levels that exceed the concentration in their food. For example, Conley et al. (2009, 2011, 2013) showed that biomagnification of selenium by aquatic insect larvae (mayflies) typical of the benthos in Harmer Creek, can lead to two- to three-fold higher concentrations than occur in their food. Once the organic selenides in plants and microbes are bioaccumulated, they are retained as an organic form in the consumer and passed on efficiently to predators.

Differences in the physiological processes driving bioaccumulation and differences in food sources cause bioaccumulation to differ among consumer taxa. As noted above, aquatic insect larvae bioaccumulate selenium to concentrations 2 – 3× higher than the periphyton they ingest (Conley et al. 2011). Bivalves can bioaccumulate selenium up to 10× higher than the suspended material they ingest (Luoma et al. 1992; Lee et al. 2006). Annelids like aquatic oligochaetes appear to bioaccumulate selenium somewhere between aquatic insect larvae and bivalves (Dubois and Hare 2009). Amphipods are an exception to this rule. Selenium concentrations in amphipods do not appear to be greater than those in their food. These differences are caused by differences among taxa in digestive (assimilation) efficiency, feeding rate and choice of food (which can affect selenium concentrations in diet).

Dominance by dietary bioaccumulation has important implications for site-specific assessments of the effects of selenium, as in the Harmer Creek EoC. Firstly, guidelines established by traditional dissolved selenium toxicity tests do not reflect toxicity in nature. The dissolved toxicity testing literature is not a useful guide for predicting selenium effects in natural waters. Thus, traditional aquatic toxicity-based guidelines (e.g., USEPA's criteria up until 2016) are not useful for determining reproductive or growth risks as a function of aqueous selenium concentrations. Universal BCFs or BAFs (ratio of $[\mathrm{Se}]_{\mathrm{consumer}}/[\mathrm{Se}]_{\mathrm{water}}$) are also of questionable value for estimating bioaccumulation. Any choice of one value for a BAF will be highly uncertain if speciation or aqueous selenium concentration changes within a stream or over time.

It is possible to quantify bioaccumulation into consumer organisms by quantifying the mechanisms that drive bioaccumulation and solving a few simple equations (e.g., Schlekat et al. 2004). In the simplest terms bioaccumulation of selenium at steady state is defined by

$$C_{ss} = C_{ss,water} + C_{ss,food} \qquad (1)$$

Where C_{ss} is the steady state concentration of selenium in the consumer organism, $C_{ss,water}$ is steady state concentration originating directly from dissolved selenium and $C_{ss,food}$ is steady state concentration derived from food. Numerous studies show that uptake from dietary and dissolved forms of selenium are additive (e.g., Luoma et al. 1992; Besser et al. 1993), as are uptake of different forms of dissolved selenium (Maier et al. 1993). Therefore, if uptake from each pathway can be quantified, then the relative contribution of each can be determined. First, steady state for water and food must be defined:

$$C_{ss} = I / (k_e + k_g) \qquad (2)$$

I is the influx rate from each source at concentrations typical of nature in µg g^{-1} d^{-1}, k_e is the rate constant of loss of selenium (d^{-1}) and k_g is rate constant of growth (d^{-1}). This equation simply says bioaccumulation is a balance between influx rate of selenium into tissues and efflux rate out of tissues. Efflux increases exponentially over time as concentrations in the tissue increase if influx rates are constant. When influx and efflux rates balance one another, concentrations of selenium in tissues are stabilized (steady state concentration). Growth (k_g) is important when it is comparable in magnitude to k_e. When organisms grow rapidly, measured selenium in tissues can be diluted by the addition of tissue. For example, age 0 trout might biodilute selenium in the most rapid phase of their growth. However, rapid growth is rarely sustained in organisms over a lifetime. Again, as trout mature their growth rate constant (in % body weight per day) slows. In the trout example, selenium concentrations in tissues at the same dietary exposure would be lower in rapidly growing age 0 fish compared to slower-growing, older fish. The difference in the rate constant of growth (k_g) would determine the difference in selenium concentrations in the fish.

Experimental studies can quantify uptake from food and water by determining uptake rate constants from different geochemical forms in water, uptake rate constants from different diets, and the rate constants of loss. Quantifying these terms consistently shows that $C_{ss,water}$ can be ignored in almost all circumstances (see citations above).

Uptake via the dominant dietary route of exposure is defined by quantifying the dominant processes described above (Presser and Luoma 2010):

$$C_{ss,food} = C_w \times K_d \times TTF \qquad (3)$$

where $C_{ss,food}$ is the steady state uptake concentration by the consumer, C_w is the concentration of selenium in water, K_d is the transformation coefficient for the organism at the base of the food web (i.e., ratio of selenium in periphyton to water under relevant geochemical speciation conditions) and TTF is the trophic transfer factor from food (e.g., periphyton) to the consumer (e.g., aquatic insect larvae). This equation shows that the relationship between consumer bioaccumulation and aqueous selenium (C_w) is dependent upon the factor (K_d) that drives transformation to particulate selenium, which in turn is driven by geochemical speciation of selenium.

If geochemical speciation is known, experimental or field-based transformation constants specific to each form of selenium can be used predict bioaccumulation via an expanded version of equations 1- 3 (de Bruyn and Luoma 2021). This also requires uptake rate constants for the relevant consumer species (e.g., aquatic insect larvae for Harmer Creek). These models can also be used to address relevant questions such as: how would bioaccumulated selenium concentrations change if speciation or aqueous selenium concentrations changed?

While it is useful to understand the processes driving selenium bioaccumulation in consumer organisms, direct determination of bioaccumulation by consumer organisms in nature is also important. As noted above, there are many consumer taxa and bioaccumulation can differ among them. One advantage of directly determining tissue concentrations is that when exposures to selenium increase, bioaccumulated selenium will increase in all exposed organisms, although the degree of increase will differ among taxa (Cain et al. 1992). Therefore, use of a surrogate species to represent whether or not food web exposure has changed (in time or space) is feasible. Consistent analysis of one surrogate (either composite BI or individual taxa) can aid understanding of site-specific bioavailability to the food web. The more closely the surrogate represents the food of predators of interest (e.g., WCT in Harmer Creek), the less uncertainty about exposure of the predator. Luoma and Rainbow (2008) defined criteria for choosing suitable taxa to act as surrogates.

Aquatic insect larvae typical of cobble bottom streams are widely used as surrogates for metal and metalloid exposure in streams (Cain et al. 1992). These consumers are usually dominated by EPT taxa: a mixture of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). Samples are collected by kick nets, therefore other potential food items are occasionally present, including annelids and dipterans. Teck takes pictures of each sample and notes the presence of annelids, in particular. Teck Coal has studied bioaccumulated selenium concentrations in multi-taxa composite samples of benthic invertebrates in cobble bottom streams in the Elk Valley watershed over the last decade (e.g., Minnow 2018). The vast majority of such samples are composed of EPT taxa. Best sampling protocols, requirements for replication, and comparisons of single taxa vs composites of multiple taxa are well defined in these studies.

Single composite samples can be used to explore different environments but replicates are necessary to reduce uncertainties about exposure (Minnow 2018). Minnow (2018) showed that although bioaccumulation differs among EPT taxa, those differences are small. Thus, EPT composite samples from cobble bottom streams can define selenium exposure as effectively as samples of single taxa, and are much more practical to collect over a wide range of habitats.

Less is known about selenium exposure in upper trophic level taxa or life stages that feed specifically on taxa like aquatic annelids (e.g., aquatic oligochaetes), or use of such taxa as indicators of exposure. Annelids are found in about 5% of composite samples from lotic habitats in the Elk Valley (Golder 2021), but the Minnow (2018) protocols that were employed in the Harmer Creek EoC to estimate consumer exposures to selenium did not identify if composite samples include annelids. Annelids are more common where stream bottoms are composed of finer grained sediments rather than cobble. It appears that selenium can bioaccumulate to higher concentrations in annelids than in EPT taxa from the same location, although the bioavailability of this bioaccumulated selenium to higher trophic levels may be lower from annelids compared to other taxa (Teck, in progress). Selenium exposure of life stages or taxa that feed specifically on these invertebrate taxa seems to be rare where data are available. A specific study of predator exposure from these taxa is underway.

The purpose of determining selenium bioaccumulation in consumer taxa in the field is to directly assess bioavailability of selenium when data on aqueous exposure, speciation, and transformation in the base of the food web are sparse, unavailable, or of questionable reliability. Any analysis of cause, however, will be strengthened if data on all of the above are used as multiple lines of evidence to define selenium exposure.

Predator Bioaccumulation

Ultimately, selenium exposure of WCT is the metric of most interest to the Harmer Creek EoC. Selenium is always efficiently transferred from one trophic level to the next. Biomagnification, or at least maintenance of selenium concentrations from lower to higher trophic levels is more the rule than the exception (Luoma and Rainbow 2008). Because of the difference in the amount of selenium bioaccumulated by different prey, some types of predators are exposed to much more selenium than others. This is one of several factors that cause the poor correlation between bioaccumulation in predators and dissolved selenium concentrations. Assessments of risks from selenium must consider these complexities of trophic transfer.

As in the case of invertebrates, dietary bioaccumulation (trophic transfer) is unquestionably the pathway of selenium exposure in fish. If feeding relationships are simple or accurately identified, concentrations in predator and prey are related. Such relationships are relatively strong across habitats, suggesting that selenium concentrations in prey are good indicators of selenium exposure to predators. One result is that differences in selenium bioaccumulation among predators in the same habitat can reflect differences in bioaccumulation among prey species.

When exact feeding relationships are identified and each prey is compared to its specific predator, different exposures among prey can be observed. For example, Stewart et al. (2004) compared selenium concentrations among a broad array of predator and prey fauna in San Francisco Bay. Trophic level was determined by stable isotope analysis; ^{15}N is accumulated preferentially to other N isotopes when one organism eats another, so it proportionately increases (higher $\delta^{15}N$) up food chains. The correlation between $\delta^{15}N$ and selenium concentrations was not strong among all data in the Bay. But when food chains were carefully separated, biomagnification was greater in a predator from a bivalve-based (clam) food web (sturgeon); than in predators from a zooplankton-based (crustacean) food web (e.g., striped bass). Bivalves bioaccumulated much more selenium than the zooplankton. As a result, much higher selenium concentrations were found in sturgeon (whose stomachs were full of bivalves) than in striped bass (which ate zooplankton), although both individual food webs biomagnified selenium. The difference between the food webs occurred because initial differences in prey selenium concentrations were propagated up each food web to create two correlational relationships.

Speciation, transformation, physiological dynamics, and food choice thus combine to determine selenium exposure of predators. As we saw earlier, phase transformation and prey bioaccumulation are driven by biogeochemistry and physiology. That influence is propagated up the food web.

The complexities that influence trophic transfer of selenium have important management implications. First and most importantly, aqueous selenium concentrations are unlikely to be effective predictors of risk if speciation changes. Exposure may also change if the same predator eats different prey in different habitats. Production of organic selenides in the Dry Creek and Harmer Creek sedimentation ponds or other lentic areas could add complications to understanding exposure of WCT, especially if organic selenide concentrations change over time. Exposure of WCT could also differ in different reaches if trout eat annelids near the ponds (where annelids may be more abundant) than in Harmer Creek (where annelids are largely absent). Supporting evidence like concentrations of selenium in prey can facilitate prediction of predator exposure better than concentrations in water. It follows that use of benthic invertebrates is an excellent surrogate for estimation of exposure of WCT.

Fish also lose selenium (k_e in equation 2) at relatively slow rates, with whole body k_e's typically less than 0.1 d^{-1} (Presser and Luoma 2010), whereas efflux rate constants in mayflies are typically ~0.2 d^{-1}. One example is the mangrove snapper *Lutjanus argentimaculatus* (Xu and Wang 2002) with a k_e of 0.03 d^{-1}. Half the body burden of selenium in this species is lost about every 20 to 40 days. As a result, biomagnification of selenium into fish is likely. Presser and Luoma (2010) found a trophic transfer factor of ~1 – 2 among a wide variety of fish species. Minnow (2018) reported the ratio of selenium in the eggs of WCT to be almost 2× higher than selenium concentrations in composite benthic invertebrate taxa from the same reach of the Elk Valley watershed.

Fish also move and that can affect their exposure to selenium. Palace et al. (2007) used selenium concentrations in the otoliths of rainbow trout to show that individuals caught in a selenium-enriched stream were primarily residents of an unenriched tributary.

2.2 Toxicology

Selenium is an essential nutrient for all species but at high concentrations can be toxic. The toxicity of selenium to fish and other vertebrate species has been reviewed by Janz et al. (2010), US EPA (2021), and others. The precise mechanism of selenium toxicity at the cellular level is still not fully understood. The current understanding was briefly summarized in Bollinger (2021) and is repeated here.

Mechanisms of Toxicity

At least three mechanisms have been proposed to explain the toxicity to fish and observed lesions resulting from exposure to sufficiently high concentrations of bioaccumulated selenium. The first is that selenium is substituted for sulfur in specific amino acids and the resulting enzymes and proteins are altered in their function. The second is that high selenium levels results in increased production of reactive oxygen species (ROS), which overwhelms antioxidant capacities of the cell resulting in oxidative injury to tissues. The third is that selenium alters the metabolism of glycogen and triglycerides in fish (Knight et al. 2016). Most recently, a study showed that exposure of fish embryos to seleno-L-methionine alters expression of key genes involved in cartilage differentiation and bone formation (Wang et al. 2020), providing a mechanistic explanation for the teratogenic abnormalities observed.

The production of ROS is thought to play an important role in selenium toxicity. ROS can be lethal to cells due to their ability to damage cellular membranes, denature proteins, and degrade nucleic acids. Antioxidant processes within the cell remove ROS, but if production of ROS exceeds antioxidant removal, oxidative stress occurs. Selenite reacts with reduced glutathione (GSH) to produce hydrogen selenide (H_2Se) and oxidized glutathione (GSSG). Hydrogen selenide then reacts with oxygen to produce ROS. Selenomethionine is reportedly metabolized into methylselenol and subsequently superoxide radicals (Palace et al. 2004). Superoxide dismutase and glutathione peroxidase are key antioxidant enzymes. The conversion of reduced GSH to GSSG is an important pathway for removal of ROS. A reduced ratio of GSH to GSSG is indicative of oxidative stress. Excess selenium and the production of ROS can deplete antioxidant defenses leading to oxidative stress and tissue damage (Birnie-Gauvin et al. 2017).

Toxicity via Maternal Transfer

Reproduction in fish can be divided into various stages (Figure 3) and selenium can potentially affect reproduction at all levels. A review by Janz et al. (2010) concluded that high selenium exposure does not appear to affect fertilization rates in fish, except at very high levels (including in the two studies of WCT reviewed therein: Kennedy et al. 2000; Rudolph et al. 2008), but instead effects observed are embryo-larval mortality, teratogenesis, and larval edema (Nautilus and Interior Reforestation 2011; Covington et al. 2018; Table 1). Similarly, there appears to be no relationship between selenium concentration in eggs and time to hatch (Kennedy et al. 2000). To date, nothing appears to have significantly changed that conclusion; however, there is a study showing that chronic dietary selenomethionine exposure in female rainbow trout can alter steroid hormone production and vitellogenesis (Wiseman et al. 2011). In that study, selenomethionine fed to rainbow trout at concentrations of 4.54 mg/kg wet weight for 126 days resulted in significantly increased plasma sex steroid hormone concentrations and, among other changes noted, there was increased production of vitellogen and zona-radiata protein RNA transcripts in the liver. Further research is needed to understand the effects of these alterations on reproduction. As well, a recent publication showed that life-cycle exposure of zebrafish to waterborne selenite (Na_2SeO_3) significantly delays ovarian maturation and reduces fertility of female zebrafish (Mo et al. 2020). These effects were observed at chronic exposures to selenite of 25 µg Se/L and above.

The effect of selenium in larval fish is related to transfer of selenium from females to eggs during oocyte development. Reportedly, the primary mechanism of transfer of selenium in fish is via vitellogenin, a main precursor of yolk protein. Vitellogenin is a phospholipoglycoprotein synthesized in the liver and transported via the blood to the developing ovarian follicle where it is taken up by receptor-mediated endocytosis. Selenium is thought to nonspecifically replace sulfur during vitellogenin synthesis. In the ovarian follicle, vitellogenin is enzymatically cleaved into the yolk proteins lipovitellin and phosvitin. These and other yolk proteins are utilized by embryos during development. These vitellogenins provide protein, carbohydrate, and lipid nutrition to developing embryos and yolk-sac larvae at specific time points during development (Reading et al. 2018) and are critical to proper growth. Other pathways of maternal selenium transfer have been described in other vertebrate taxa (Unrine et al. 2006) but their significance in fish have not been investigated.

Although selenium is readily incorporated into the egg during development, the amount of maternal selenium transferred to the eggs varies among fish species and can vary depending on spawning strategy. In a review of studies examining relationships between maternal tissue selenium and egg concentrations, there was considerable difference among species, with rainbow trout having among the highest ratios of egg to adult muscle selenium concentrations and brook trout the least (de Bruyn et al. 2008). In WCT, there was a linear relationship between concentrations of selenium in female tissues and eggs with the mean egg to muscle ratio being 1.6 (Nautilus and Interior Reforestation 2011).

Maternal transfer of selenium is affected by levels of selenium in diet, environmental exposure, and tissue concentration at the time of vitellogenesis. Reproductive strategies vary greatly among fish species, but salmonids typically undergo vitellogenesis over several months prior to spawning and tissue stores likely play a greater role in maternal transfer of selenium than does diet prior to spawning (Janz et al. 2010). There has been very little research on this aspect of selenium metabolism.

Since virtually all constituents (proteins, carbohydrates, lipids, and minerals) for larval development are contained within the egg, the concentration of selenium in larvae on a dry weight basis is roughly equivalent to the initial concentration in the egg, until such time as selenium excretion pathways become functional. Although poorly studied, it is reasonable to assume that depuration is minimal until the swim-up period when fry begin feeding. Under this assumption, fry from eggs with selenium concentrations of ~16 µg/g dw, corresponding to adult female muscle concentrations of 10 µg/g dw, would have tissue concentrations that have been reported to alter physiological processes in some species (Knight et al. 2016, Berntssen et al. 2017, Attaran et al. 2020, Thomas et al. 2013). Whether these altered physiological processes affect growth and survival is not known and the outcome is affected by additional factors such as selenium concentration in feed and water, environmental factors, and so on. For example, survival, frequency of deformities, length, weight, and condition factor at 28-days post swim-up of WCT under experimental conditions was unaffected by egg selenium concentration over the range studied (3.9 to 24.8 µg/g dw; Nautilus and Interior Reforestation 2011). Growth rates of post swim-up fry from eggs collected at sites with high selenium concentrations were reported to be higher than those from the reference sites but data were not presented; however, larval weights and condition factors at the end of the 28 day-day post swim-up were still less than fry reared from reference sites. This was attributed to the higher initial weight of eggs from the reference site. Fry were fed trout ration and kept at 11°C with a 16:8 hour light:dark photoperiod. Unfortunately, selenium concentrations of fry at the beginning and end of the growth period were not analyzed.

Egg or post swim-up fry weight has not been identified as a sensitive endpoint for selenium toxicity in fish (Janz et al. 2010), although it may be important in settings where hatch is late relative to winter and freeze-up as juvenile size and body condition is important in over-winter survival (Post and Parkinson 2001). Unfortunately, egg weight and larval weight comparisons between selenium exposed and reference lakes in studies of reproductive toxicity in WCT have not consistently been reported (Rudolph et al. 2008; Kennedy et al. 2000).

Selenium depuration in fish is affected by several factors including species, size, sex, age, and by selenium concentration and form in the diet and in the environment during depuration. Deng et al. (2007), in reviewing the literature, reported a range in half-lives for selenium in juvenile fish of between 19 and 30 days; half-lives were reported to be longer in adult fish.

Teratogenesis is a well described effect of high concentrations of selenium in eggs. Deformities include craniofacial deformities, deviation of the vertebral column, and pericardial edema. Effect concentrations are similar to, or higher than, those reported for larval survival (Table 1) and deformities may be the cause of larval mortality if severe enough (Rudolph et al. 2008). Oxidative stress has been suggested as a cause of these abnormalities and recent studies are beginning to unravel the cellular mechanisms by which they occur. Oxidative stress, unfolded protein response, and endoplasmic reticulum stress, as well as apoptosis (programmed cell death) were implicated in the development of deformities in Japanese medaka embryos exposed to high levels of selenomethionine during a specific stage of embryogenesis (Kupsco and Schlenk 2016). Wang et al. (2020), using a similar exposure model, demonstrated that selenomethionine in embryos modulated expression of genes involved in cartilage differentiation and bone formation during development. The pathogenesis of selenium toxicity in embryogenesis and larval development is complex (Figure 3).

Figure 3: Summary of processes and stages of oocyte growth through to late embryogenesis (modified from Reading et al. 2018)

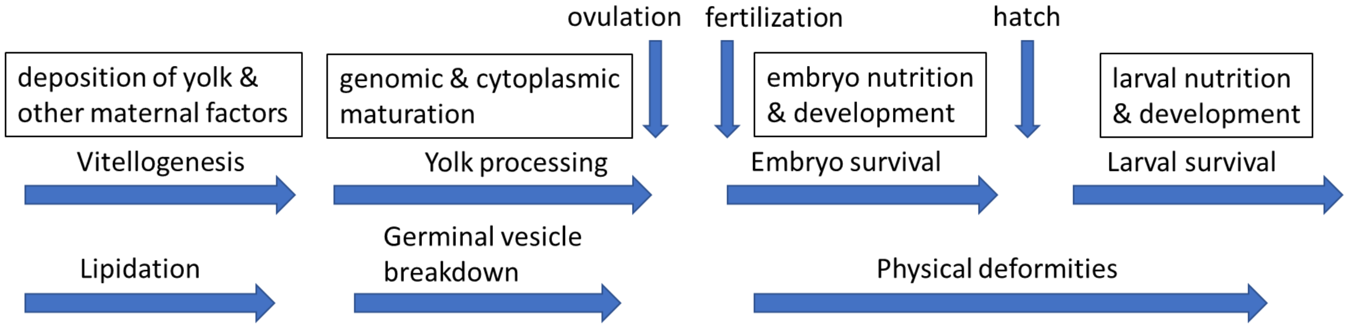
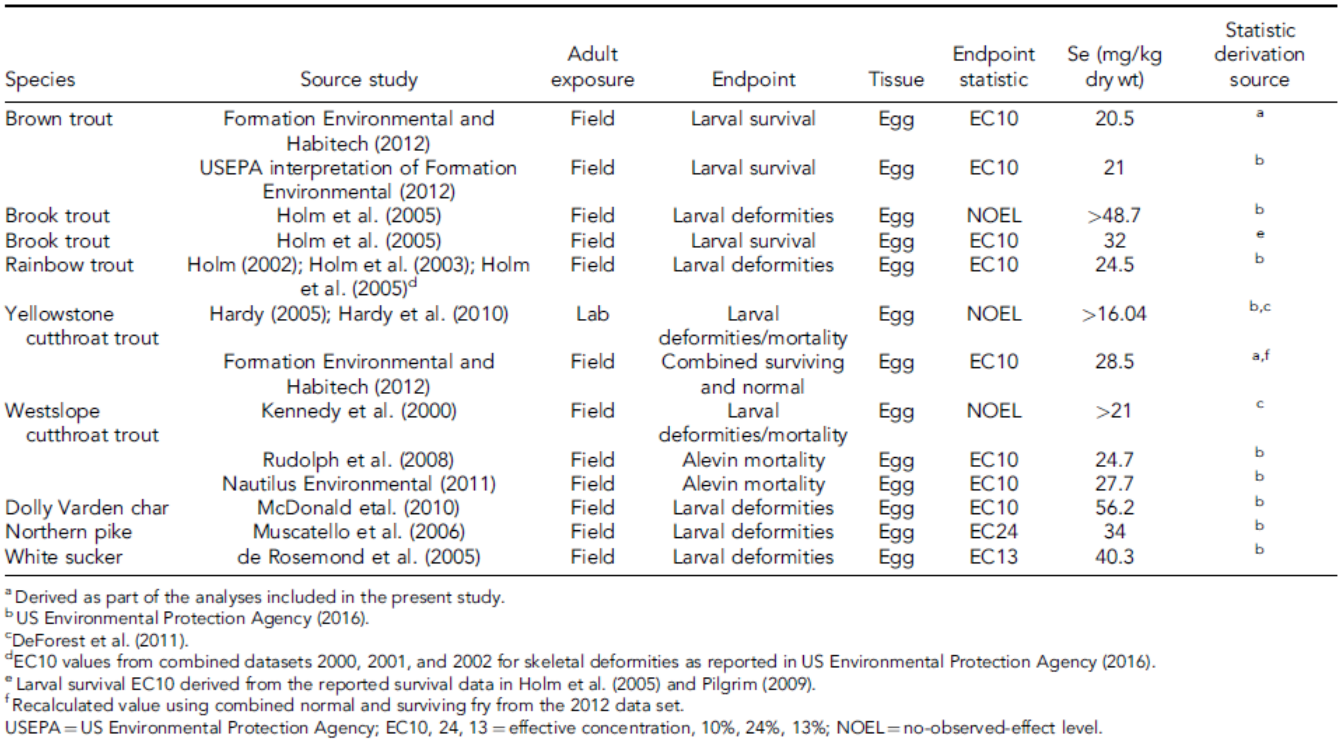


Table 1: Effects endpoints from maternal transfer studies for cold-water species (from Covington et al. 2018)



Species	Source study	Adult exposure	Endpoint	Tissue	Endpoint statistic	Se (mg/kg dry wt)	Statistic derivation source
Brown trout	Formation Environmental and Habitech (2012)	Field	Larval survival	Egg	EC10	20.5	a
	USEPA interpretation of Formation Environmental (2012)	Field	Larval survival	Egg	EC10	21	b
Brook trout	Holm et al. (2005)	Field	Larval deformities	Egg	NOEL	>48.7	b
Brook trout	Holm et al. (2005)	Field	Larval survival	Egg	EC10	32	e
Rainbow trout	Holm (2002); Holm et al. (2003); Holm et al. (2005)[d]	Field	Larval deformities	Egg	EC10	24.5	b
Yellowstone cutthroat trout	Hardy (2005); Hardy et al. (2010)	Lab	Larval deformities/mortality	Egg	NOEL	>16.04	b,c
	Formation Environmental and Habitech (2012)	Field	Combined surviving and normal	Egg	EC10	28.5	a,f
Westslope cutthroat trout	Kennedy et al. (2000)	Field	Larval deformities/mortality	Egg	NOEL	>21	c
	Rudolph et al. (2008)	Field	Alevin mortality	Egg	EC10	24.7	b
	Nautilus Environmental (2011)	Field	Alevin mortality	Egg	EC10	27.7	b
Dolly Varden char	McDonald etal. (2010)	Field	Larval deformities	Egg	EC10	56.2	b
Northern pike	Muscatello et al. (2006)	Field	Larval deformities	Egg	EC24	34	b
White sucker	de Rosemond et al. (2005)	Field	Larval deformities	Egg	EC13	40.3	b

[a] Derived as part of the analyses included in the present study.
[b] US Environmental Protection Agency (2016).
[c] DeForest et al. (2011).
[d] EC10 values from combined datasets 2000, 2001, and 2002 for skeletal deformities as reported in US Environmental Protection Agency (2016).
[e] Larval survival EC10 derived from the reported survival data in Holm et al. (2005) and Pilgrim (2009).
[f] Recalculated value using combined normal and surviving fry from the 2012 data set.
USEPA = US Environmental Protection Agency; EC10, 24, 13 = effective concentration, 10%, 24%, 13%; NOEL = no-observed-effect level.

Dietary Toxicity

Once yolk sacs are depleted, fry rely on ingested food for growth and development. Selenium in food is an essential micronutrient, and sufficient levels of selenium are required for normal growth, but if in excess it can reduce growth and survival. At sublethal levels, the reported effect of selenium on condition factor, growth, and weight gain in fish is highly variable (Table 2). Larval (24-day-old) rainbow trout exposed to dosages of selenomethionine of 4.6, 12 and 18 µg/g dw in their diet for 90 days showed significant decrease in body weight and fork-length in the 4.6 and 12 µg/g treatment groups at 90 days (Vidal 2005). At 30 and 60 days, the other time periods when measurements were taken, there were no significant differences. Body weight and fork length were lower but not significantly different from controls in the 18 µg/g dw high dose group. Whole body total selenium concentrations were significantly higher at 90 days in the two highest treatment groups compared to controls. Fish at 90 days had lower whole body selenium concentrations than at 60 days, which was suggested to be the result of increased relative body mass associated with transition from larval to juvenile stages which occurred over this time. Biomarkers of oxidative damage in the liver did not differ from controls. A summary of other papers reporting effects of selenium exposure on growth and body condition is provided in Table 2.

Table 2: Summary of literature on effects of selenium exposure on growth, mortality, and condition of fish

Exposure	Species	Age	Condition Factor	Mortality	Effect on energy stores	Tissue Se	Reference
Se-Met dietary (1.3, 6.4, 15.8, 47.8 µg/g dw) 60 days, 12˚C	Rainbow trout	Juv.	No difference among groups	No difference among treatments	Liver: elevated triglyceride and glycogen stores at 15.8 and 47.8 dosages Skeletal muscle: no difference Heart: no difference	Muscle (56 days): ~ 40 mg/kg at 15.8 dosage; ~80 mg/kg at 47.8 dosage. All in dw	Pettem et al. 2018
Diet: Selenite (1-2 or 15 mg/g ww; Se-Met (1-2 or 15 mg/g ww); 90 days, 9˚C	Atlantic salmon	18 mon.	High selenite group sig. different final weight	No mortalities	Whole body: lipid content reduced in high dose group but not significant	Whole body: ~8 mg/kg DW at 15 mg/g dosage. Muscle: ~12 mg/kg dw at 15 mg/g Se-Met	Berntssen et al. 2017
Diet: Se-Met: 1.3, 7.1, 10.7, 19.5, 31.8 mg/kg dw: 60 days, 11˚C	Rainbow trout	Juv.	Dose groups lower weights	No difference among groups	Liver: triglycerides in 2 high dose groups lower than controls	Whole body: 1, 4.4, 6, 10.4 & 15 mg/kg dw respectively	Knight et al. 2016
Diet: Se-Met: 1.1, 10.3, 28.8 µg/g dw 90 days, 28˚C	Zebrafish	Adult	No difference among groups	Similar among groups	Muscle glycogen: 1.8, 4.4 & 5.3 mg/g DW respectively dosed grps sig. higher Triglycerides: 2.2, 2.2, 2.6 mg/g dw respectively	Whole body: 0.7, 3.3, 9.2 µg/g dw respectively	Pettem et al. 2017
Diet: Se-Met: 3.7, 9.6, 26.6 µg/g dw, 60 days, 28˚C	Zebrafish	Adult	No difference among groups: tx groups higher BW and total length; except 9.6 µg/g group	Mortalities were significantly higher in 26.6 µg/g group: % mortality: control =10.7, tx= 21, 23, 39, respectively	Exposed fish had greater whole-body triglycerides and glycogen levels than controls	Whole body Male, female 1.25, 1.92; 8.19, 6.13; 11.42, 13.43; 15.28, 21.93 µg/g dw, respectively	Thomas and Janz 2011

dw – dry weight; ww – wet weight; BW – body weight; tx – treatment; Juv. – juvenile; Mon. - months

Although selenium has several potential mechanisms of toxicity, there are currently two main hypotheses to explain the mechanism by which elevated selenium may affect growth in fish. The first is that oxidative stress and the resultant damage caused by ROS affects growth, and the second is selenium disrupts metabolic pathways of triglyceride and glycogen storage, which affects growth. These mechanisms are not necessarily mutually exclusive and are part of integrated metabolic pathways that may have compensatory pathways, which may explain inconsistent findings. Unravelling the role of selenium in these complex integrated pathways is challenging and an area of current research.

Research by Knight et al. (2016) examined the relative roles of oxidative stress and altered triglyceride metabolic pathways in the adverse effects of elevated selenium in rainbow trout fry. They fed juvenile rainbow trout a nominal dose of 5, 10, 20, or 40 mg/kg dw and found those fed the two highest dosages had lower body weight and shorter lengths than controls but no differences in body condition (Table 2). They found that markers of an oxidative stress response, such as total glutathione, 8-isoprostane levels, and levels of mRNA for glutathione peroxidase isoforms, did not differ from control groups. They did, however, find in the highest treatment group lower triglyceride levels in the liver and a corresponding increase in molecules associated with long-chain fatty acid transport, lipid transport, and low-density lipid peroxidation. As well, they detected up-regulated gene networks for epidermal growth factor and Notch signaling, which is involved in cellular communication and gene transcription regulation.

Similarly, Berntssen et al. (2017) evaluated mechanisms of selenium toxicity in Atlantic salmon and found both oxidative stress and altered lipid metabolism were associated with reduced growth and other apparent adverse effects. Effects were observed in the highest selenium exposure groups of 15 mg/kg selenite but not 15 mg/kg SeMet-yeast, highlighting how differences in forms of selenium and routes of exposure can affect outcomes. Impaired lipid synthesis could potentially affect the build-up of energy reserves required for over-winter survival.

Interactions with Temperature

The term "winter stress syndrome" was coined by Lemly (1993) to explain the increase in oxygen consumption, decreased body condition, and increased mortality he observed in bluegill sunfish exposed to low water temperatures and selenium for 180 days under experimental conditions. He indicated three conditions needed to be present concurrently for this syndrome to occur: 1) the fish need to be at temperatures <10°C; 2) the cold temperatures must result in reduced activity and feeding in fish; and 3) a metabolic stressor such as a contaminant or parasite needed to be present. Lemly (1993) reported that these stressors in combination created a metabolic deficit resulting in severe lipid depletion and mortality (Table 3). McIntyre et al. (2008) also evaluated the effects of reduced water temperature on fish exposed to high levels of selenium in food and water. They established three different experimental exposures using juvenile bluegill sunfish (Table 3). All three experiments began with a 30-day period at 20°C followed by a weekly decline of 2°C/week until reaching 4°C in exposure systems one (ES1) and two (ES2) and until reaching 9°C in the third (ES3). The temperature regime was identical in all three exposure systems until about day 63, at which time ES3 was held at 9°C and the other two were lowered to 4°C over the remaining weeks. In ES1 and ES3, fish were exposed to six nominal concentrations of selenium in water and in their diet via worms that had been fed selenized yeast. ES2 was designed to replicate the Lemly (1993) study and although not identical, it was similar enough for comparison. The study ran for 182 days.

Although Lemly reported 40% mortality after 180 days, McIntyre reported "no meaningful mortality" in ES2 after 182 days. The difference in results is even more apparent given that tissue selenium concentrations in fish at the end of the Lemly study were 5.85 to 7.91 ug/g dw, whereas there was no effect on survival in fish with up to 10 µg/g dw in the McIntyre et al. (2008) study. However, in the other experimental exposures, as whole-body selenium concentrations exceeded 11 µg/g dw, survival of bluegill sunfish declined rapidly. These concentrations were only reached in the two highest exposure treatment groups of ES1 and ES3 and were reached faster in the highest exposure group. At the highest treatment in ES1 and ES3, mortality began at about day 45 at temperatures of 14°C, and although conditions were nominally equivalent until about day 60, fractional survival was ~50% in ES1 and ~83% in ES3 at this time. This difference in mortality under identical conditions up to that point may explain the reported conclusion that fish were more sensitive to selenium at the 4-5°C temperature regime (EC_{10} = 9.56 µg/g dw) compared to the 9°C regime (EC_{10} = 13.29 µg/g dw). As well, in the highest treatment group (ES1), there were 11 mortalities on day 65, which is incongruent with daily mortalities in all other treatments. Without replicates of the treatments, this individual variability in mortality can have a significant influence on results. Fish in the 20° declining to 9°C groups accumulated more selenium than those at the lower temperature. Unfortunately, there were no treatment groups where temperature was held at 20°C to determine the role temperature played in the mortality. There was no decrease in body condition or lipid content of fish in the three experimental treatments, suggesting a different mechanism of mortality in this study compared to that proposed by Lemly (1993). The toxicity of selenium to bluegill sunfish was 1.9 times less in the McIntyre et al. (2008) study. Although some reviews state the findings in the McIntyre and Lemly studies are similar (Janz et al. 2010), there are significant differences, and other reviewers have concluded that the McIntyre study did not corroborate Lemly's findings (DeForest and Adams 2011).

The high mortality rates in bluegill sunfish at whole body selenium concentrations greater than 11 µg/g dw beginning at temperatures of 14°C at day 45 in the McIntyre study contrast with no significant difference in mortality of rainbow trout among treatment groups with similar dietary exposures for 60 days at 11°C with whole body selenium concentrations up to 15 mg/kg dw (Knight et al. 2016) and up to ~80 mg/kg dw in skeletal muscle in rainbow trout at 12°C for 60 days (Pettem et al. 2018) (Table 1). There appear to be significant differences among fish species in response to selenium and temperature.

Table 3: Literature on effect of cold on selenium toxicity in bluegill sunfish

Exposure	Species	Age	Condition Factor	Mortality	Effect on energy stores	Tissue Se	Reference
Water: 5 µg/L selenate& selenite Diet: Se-Met: 5 µg/g: 180 days: 20°C & 20 to 4°C	Bluegill	Juv.	Significant decline in cold and Se treated group	Significantly higher mortality in cold + Se group	Whole body: "depletion of 50-80 % of body lipid"	Whole body: cold water 7-8 µg/g DW; warm water 5-6 µg/g DW	Lemly 1993
ES2: Water: 5 µg/L selenate&selenite Diet: Se-Met: 5 µg/g: 182 days: 20°C to 4°C	Bluegill	Juv.	No decline in condition score	No significant mortality	No decline in lipid content	Whole body: 9.41 and 10.61 µg/g DW	McIntyre et al. 2008
ES1: 6 treatments: Water: 1.25, 2.5. 5, 10, 20, 40 µg/L; 1:1 selenate:selenite Diet: Se-Met: 1.25, 2.5, 5, 10, 20, 40 µg/g dw: 182 days: 20°C to 4°C	Bluegill	Juv.	No decline in condition score	High mortality in 2 highest treatment groups	No decline in lipid content	Whole- body in 2 highest: treatment groups: 9.21 and 12.66 µg/g DW	McIntyre et al. 2008
ES3: 6 treatments: Water: 1.25, 2.5. 5, 10, 20, 40 µg/L ;1:1 selenate:selenite Diet: Se-Met: 1.25, 2.5, 5, 10, 20, 40 µg/g dw: 182 days: 20°C to 9°C	Bluegill	Juv.	No decline in condition score	High mortality in 2 highest treatment groups	No decline in lipid content	Whole- body in 2 highest: treatment groups: 15.14 and 17.24 µg/g DW	McIntyre et al. 2008

Timing of mortality was also different between the Lemly (1993) and McIntyre et al. (2008) studies. In the Lemly study "most of the mortality occurred 60 days after the water temperature reached its low point of 4°C which occurred between days 50 and 60", whereas in the McIntyre et al. (2008) study, mortalities only occurred in the high treatment groups and began before temperatures reached 4°C and 9°C. In ES2, meant to replicate the Lemly (1993) study, no significant mortality occurred. Mortality did occur in the treatment groups receiving the two highest dosages of selenium, but in the highest treatment group it began at day 45- 50 when temperatures were approximately 14°C and by approximately day 85 to 90 survival was ~10%. At this time, temperatures had been 4°C for less than a week in ES1, and had been at 9°C for only a few weeks in ES3. In the second highest treatment group in both ES1 and ES3, mortality began at ~85 days and by 182 days mortality was approximately 40 to 50%. The mortality rates are consistent with estimated mortality for these dosages in larval warm water fish reported by DeForest et al. (1999) without cold stress. They reviewed existing literature and estimated an EC_{50} for larval mortality in warm water fish of 19 mg/kg dw dietary selenium and estimated 100% mortality at 40 mg/kg dw dietary selenium. This study also estimated the EC_{10} for larval mortality at 10 mg/kg dw selenomethionine in the diet. In the McIntyre et al. (2008) study the mortality for this concentration of dietary exposure was reported to be very low (3%) over the 182 days, despite the additional stress of fish being exposed to 4°C (ES1) and 9°C (ES3). For comparison, the Lemly "winter stress" study and the replicate by McIntyre et al. exposed fish to 5 µg/g dw dietary selenomethionine.

Unfortunately, there have been only two experimental trials addressing the effects of cold on selenium toxicity and their results differ in spite of using the same fish species and very similar experimental designs. Lemly (1993) showed lipid depletion followed by mortality in fish fed a diet high in selenomethionine at cold temperatures, McIntyre et al. (2008) had "no meaningful" mortality in the experiment meant to replicate the Lemly study and there was no evidence of lipid depletion in that experiment or any of the other experiments examining selenium and cold exposure. Both studies measured feed consumption and both reported minimal feeding activity in fish at 5°C. McIntyre discusses the differences between their study and that of Lemly and points out that in the Lemly study fish were removed from each treatment group for oxygen consumption measurements and then returned to the exposure tanks. They suggest the possibility that the additional stress of handling may have contributed to the higher mortality but suggest the number of fish used for oxygen measurements does not explain all the mortality; however, their explanation does not take into consideration the stress experienced by fish within the exposure tanks during the capture and re-release activities. Lemly (1993) demonstrated increased oxygen consumption in selenium exposed fish which is consistent with findings by Thomas et al. (2013) in adult zebrafish. The effect of dietary selenomethionine on oxygen consumption appears to be dependent on species as McPhee and Janz (2014), using the same techniques as Thomas et al. (2013), did not detect an effect of selenium on oxygen consumption in juvenile fathead minnows or in juvenile rainbow trout (McPhee 2014).

Attempts to demonstrate "winter stress syndrome" under field conditions, within the broad context proposed by Lemly of water pollutants and winter conditions (cold temperature and reduced photoperiod) causing increased rates of lipid depletion, have been unsuccessful. These field studies are potentially confounded by other factors within aquatic systems that can affect behaviour, metabolism, energy depletion, and survival, but they do address the general applicability of the hypothesis that fish exposed to contaminants in winter will deplete lipid stores at a greater rate than fish that are not exposed to effluent. Driedger et al. (2009) evaluated overwinter survival potential in juvenile fathead minnows, creek chub, and white suckers from a creek receiving metal mining and municipal wastewater through measurement of growth and energy stores immediately before and after winter ice, and compared these to non-impacted sites. Energy stores were not depleted at exposure sites as compared to controls and therefore they concluded their findings did not support the winter stress syndrome hypothesis. Whole body selenium concentrations in fathead minnows and white suckers at the exposure site ranged from 11-42 µg/g dw. Bennett and Janz (2007) similarly showed that overwintering northern pike and burbot from lakes in northern Saskatchewan receiving metal mine effluent did not have reduced energy reserves compared to non-impacted reference lakes, again failing to support the winter stress hypothesis. The two exposure lakes were classified as having "low" (Se 1.0 µg/L) and "high" (Se 3.0 µg/L) concentrations of selenium in the water; however, exposure levels were below those in Lemly's experiments described above. Selenium content of food was also not estimated and there were no estimates of selenium tissue concentrations in fish from these lakes, which limits the interpretation of these findings in relation to experimental exposures. Changes in other water characteristics, such as nutrient inputs, may also have impacted the results.

In summary, winter can induce physiological stresses in fish via several mechanisms, but the effect of cold on selenium toxicity is unclear. Cold temperatures altering metabolic rates and cellular processes at a time when fish are reliant on endogenous energy stores is a likely primary mechanism. Research described above that demonstrated elevated selenium can alter pathways involved in glycogen and lipid metabolism (Knight et al. 2016, Berntssen et al. 2017) identifies potential pathways by which selenium could contribute to the physiological stress of cold and winter. However, the concept that detoxification of selenium induces a metabolic cost to fish not feeding during cold temperatures, as hypothesized by Lemly and termed "winter stress syndrome", has not been supported by subsequent studies.

2.3 Impact Hypotheses

The impact hypotheses outlined in Table 4 describe different ways that selenium could plausibly have contributed to reduced recruitment, and recruitment failure in the 2018 spawn year, considering the types of exposure that could have occurred in the Harmer Creek population area (Section 2.1) and the types of effects that selenium can cause in fish (Section 2.2). The impact hypotheses are organized by the life stages potentially affected by different exposure pathways and mechanisms of toxicity, progressing from embryos to alevin to fry. As discussed in the EoC Report (Harmer Creek EoC Team 2022), the nature of reduced recruitment implicates effects on early life stages; the available evidence does not indicate that effects occurred to adults.

Table 4: Impact hypotheses for a role of selenium in reduced recruitment of Harmer Creek WCT

Life Stage(s) Affected	Exposure Pathway	Mechanism of Toxicity	Impact Hypothesis
Embryos and alevin	Maternal transfer of dietary selenium to yolk, mobilization during embryonic development	Oxidative stress and/or gene regulation effects causing embryo mortality and/or larval deformity	Adult female WCT accumulated sufficient dietary selenium to result in egg selenium concentrations exceeding a threshold for embryo-larval toxicity. Resulting embryo-larval deformity and/or mortality was severe enough to cause or contribute to reduced recruitment.
Alevin and/or fry	Direct exposure to reactive species of aqueous selenium	Oxidative stress, in combination with other metabolic stressors and/or exacerbated by low temperatures, causing alevin and/or fry mortality	Alevin and/or fry were exposed to aqueous concentrations of reactive selenium species exceeding a threshold for direct toxicity. Resulting alevin and/or fry mortality was severe enough to cause or contribute to reduced recruitment.
Fry	Dietary selenium intake	Oxidative stress, in combination with other metabolic stressors and/or exacerbated by low temperatures, causing fry mortality	Fry were exposed to dietary selenium concentrations, potentially in combination with residual egg selenium, exceeding a threshold for lethality. Resulting fry mortality was severe enough to cause or contribute to reduced recruitment.
Fry	Dietary selenium intake	Metabolic cost of sublethal selenium toxicity and/or gene regulation effects, leading to impaired growth and contributing to failure of fry to reach minimum size to survive winter	Fry were exposed to dietary selenium concentrations, potentially in combination with residual egg selenium and/or oxidative stress from direct exposure to reactive species of aqueous selenium, exceeding a threshold for growth effects, leading to impaired growth and contributing to a failure of fry to reach minimum size to survive winter. Resulting overwinter mortality of fry was severe enough to cause or contribute to reduced recruitment.

3.0 SUMMARY OF AVAILABLE MONITORING DATA

3.1 Strengths and Limitations of Monitoring Data

In evaluating patterns of selenium exposure across the Harmer Creek population area, this assessment integrates selenium data from water, benthic invertebrates, and WCT tissue. Sediment selenium data are summarized herein and evaluated in detail in Wiebe and Orr (2022). Each of these data types has particular strengths and limitations in terms of the evidence they bring to the assessment.

Aqueous selenium concentrations have been measured in several areas of the watershed, and a few key locations have been monitored monthly over many years. These data are supplemented in some areas by sediment selenium concentrations that provide a more time-integrated measure of environmental concentrations. However, selenium concentrations in water and sediment are not always reliable indicators of the magnitude of exposure for fish because patterns of bioaccumulation can be strongly affected by local biogeochemical conditions (Section 2.1), for example in areas downstream of sedimentation ponds (Golder 2021).

Selenium speciation analysis can help evaluate such local changes in bioaccumulation (de Bruyn and Luoma 2021), and the limited speciation data available from the Harmer Creek population area are used for that purpose herein. More reliable indicators of selenium exposure are measured concentrations in biota. Benthic invertebrate selenium concentrations, which are a measure of potential dietary exposure for juvenile and adult WCT, have been monitored at several locations in the Harmer Creek population area. These data are supplemented by measurements of selenium concentrations in WCT. Although available for fewer locations and in fewer years compared to other data types, WCT tissue selenium concentrations provide the most direct measure of potential effects on WCT by integrating the locations and prey types that actually contribute to WCT exposure.

None of these data types alone is sufficient to characterize selenium exposure conditions throughout the Harmer Creek population area during the period of interest, but taken together (Figure 4) they provide a reasonable basis for evaluating where and to what extent selenium may have contributed to reduced recruitment.

3.2 Aqueous Selenium Data

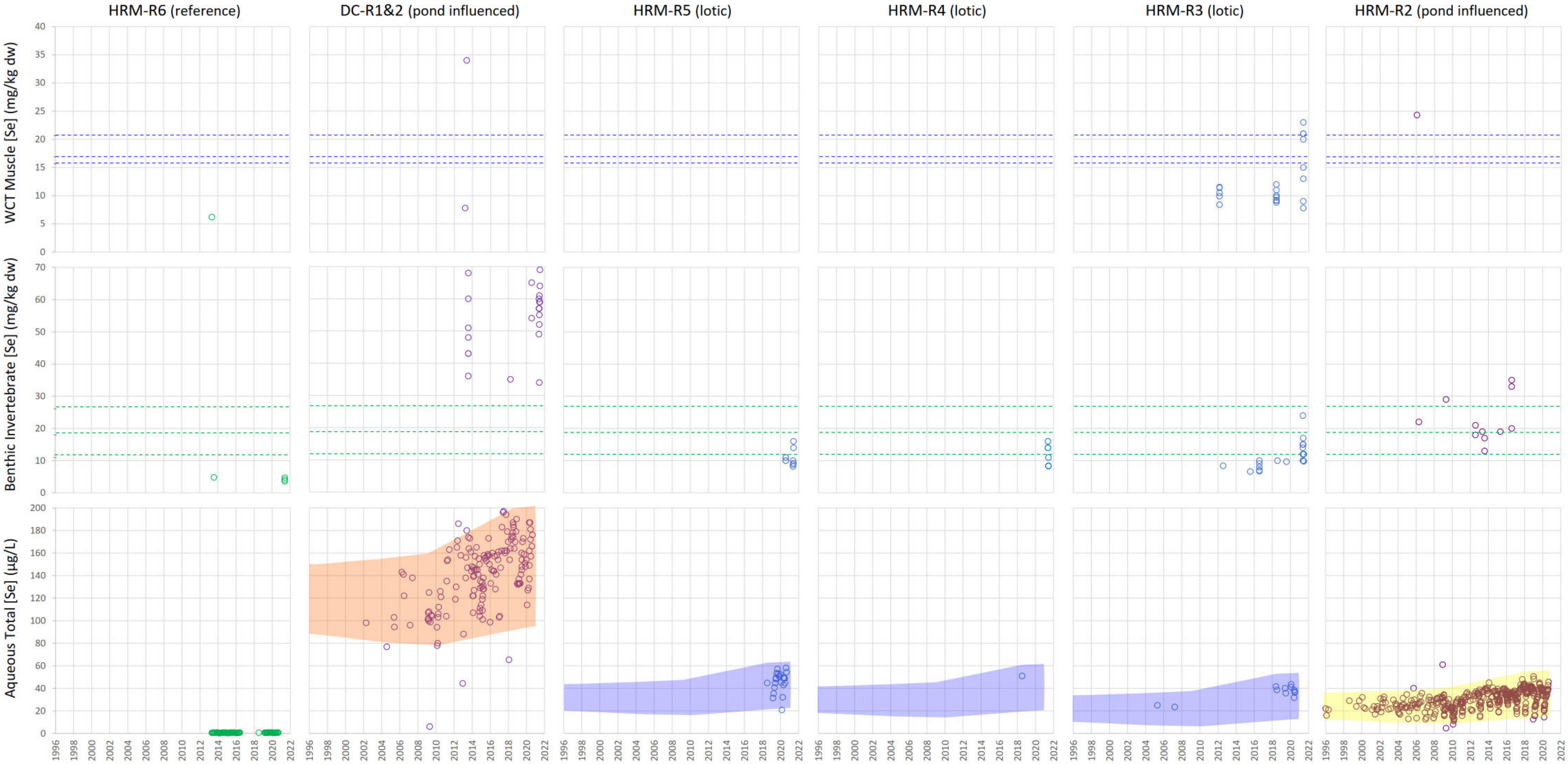
Aqueous selenium monitoring data from the Harmer Creek population area are plotted in the bottom panels of Figure 4. The longest and most consistent records of water quality monitoring are at the points of discharge from the Dry Creek Sedimentation Pond (reflecting conditions in the pond [DC-R2] and the reach downstream [DCR1] since 2002) and the Harmer Creek Sedimentation Pond (reflecting conditions in the pond [HRM-R2] and the reach downstream [HRM-R1] since 1996). Water quality monitoring has also been conducted since 2013 in reference (i.e., without mining influence) reaches of Harmer Creek (HRM-R6) and Grave Creek (GRV-R3) and at the mouth of Grave Creek (GRV-R1) (Warner and Lancaster 2022). More limited monitoring data are available for 2019, 2020, and 2021 in Harmer Creek downstream of Dry Creek (HRM-R5 and -R4) and upstream of Harmer Creek Sedimentation Pond (HRM-R3), and in Grave Creek downstream of the Harmer Creek confluence (GRV-R2).

As discussed in Section 1.2.2 of Warner and Lancaster (2022), the available water quality monitoring data provide a reasonable characterization of conditions in the Harmer Creek population area. Although there are reaches and years with few or no monitoring data, conditions in these reaches and years can reasonably be inferred from monitored conditions in other reaches and years. Such inference can be supported because Dry Creek (which has a long monitoring record) is the only source of mine-influenced water to Harmer Creek. Specifically:

- The highest aqueous concentrations of selenium in the Grave Creek watershed occur in Dry Creek, which has a period of record that includes the years of reduced recruitment and prior years. There is no plausible mechanism by which higher aqueous concentrations of selenium could occur in Harmer Creek compared to Dry Creek.
- Consistent spatial patterns of water quality are evident in the available monitoring data (Figure 4), indicating that reaches of Harmer Creek downstream of Dry Creek exhibit consistent and progressive improvement of water quality with inputs of non-mine-affected water from upstream Harmer Creek (HRMR6), reference tributaries, and groundwater accretion (Lorax 2019). These spatial patterns are evident in recent years of monitoring data at HRM-R5 and HRM-R3 and are also reflected in the long monitoring record at the decant of the Harmer Creek Sedimentation Pond (HRM-R2). Therefore, water quality in Harmer Creek reaches HRM-R3 and HRM-R4 can reasonably be inferred to be intermediate between that in HRM-R5 (downstream of Dry Creek) and HRM-R2 (Harmer Creek Sedimentation Pond). It is possible that there are localized areas of Harmer Creek in which water quality is affected by upwelling groundwater from the Dry Creek catchment (Canham and Humphries 2022). To the extent that such groundwater inputs might affect surface water quality, they would be reflected in the overall spatial pattern of monitoring data across reaches.
- Trends over time in water quality in Dry Creek are expected to translate into similar trends, albeit at lower concentrations, in reaches of Harmer Creek downstream of Dry Creek. This expectation is supported by the similarity of trends between the long-term monitoring locations on Dry Creek (EV_DC1 in DC-R2) and Harmer Creek (EV_HC1 in HRM-R2) evident in Figure 4, indicating about a 20% increase in aqueous selenium concentrations between 2010 and 2020 at both monitoring locations. Therefore, temporal trends in concentrations of selenium in Dry Creek can be used to infer how concentrations have changed over time at locations throughout Harmer Creek. Inferred trends over time are illustrated in Figure 4 as shaded areas.

Observed temporal trends in long-term monitoring data at the Dry Creek Sedimentation Pond (DC-R1 and R2) and the Harmer Creek Sedimentation Pond (HRM-R2) are illustrated in Figure 4 by orange and yellow shaded areas (shading corresponds to the categories of organoselenium concentration in Table 5, discussed further in Section 3.3). Inferred temporal trends in reaches with fewer monitoring data (HRM-R3, -R4, and -R5) are illustrated by blue shaded areas. For reasons discussed in the bullets above, there is low uncertainty in these inferred temporal trends and the ranges of selenium concentrations that occur in each reach.

Figure 4: Selenium monitoring data from study reaches in the Harmer Creek population area



Notes: Blue dashed lines in top panels are muscle equivalent level 1, 2, and 3 benchmarks for reproductive effects in Westslope Cutthroat Trout. Green dashed lines in middle panels are dietary level 1, 2, and 3 benchmarks for juvenile growth effects in sensitive fish species. Not shown (off scale) is a benthic invertebrate concentration of 98 mg/kg dw reported downstream of Dry Creek sedimentation pond (EV_DCOUT) in 2020. Shaded areas illustrate general temporal trends of aqueous selenium concentrations in reaches with organoselenium concentrations corresponding to the categories in Table 5 (no shading <0.025 µg/L; blue 0.025-0.05 µg/L; yellow 0.05-0.1 µg/L; orange >0.1 µg/L).

3.3 Selenium Speciation Data

Teck Coal began quarterly monitoring of selenium speciation in 2017 at all compliance locations listed in Permit 107517, including EV_HC1 at the spillway of the Harmer Creek Sedimentation Pond. Non-routine samples have also been collected at several locations in Dry Creek, Harmer Creek, and Grave Creek. Available speciation data (Table 5) were used as inputs to the de Bruyn and Luoma (2021) bioaccumulation model to calculate expected concentrations in benthic invertebrates (right-most column of Table 5).

Table 5: Selenium speciation and modelled BI Se from the Harmer Creek and Grave Creek population areas.

Reach	Location	Sample Date	[DMSeO]	[MeSe(IV)]	Total OrganoSe	[Se(IV)]	[Se(VI)]	Modelled BI [Se]
DC-R4	EV_DC3	22-Sep-20	0.021	0.022	0.043	0.846	147	8.7
	EV_DCSP_UP	28-Aug-21	<0.010	0.019	0.019	0.916	124	11.1
DC-R1	EV_DC1	05-Jul-18	0.031	0.142	0.173	1.05	130	21.6
	EV_DCOUT	22-Sep-20	0.010	0.094	0.104	1.19	146	16.3
	EV_DC1	17-Feb-21	<0.010	0.013	0.013	0.286	99.7	6.7
	EV_DC1	11-Mar-21	<0.010	0.034	0.034	0.310	131	8.8
	EV_DC1	06-Apr-21	<0.010	0.023	0.023	0.455	103	8.2
	EV_DC1	03-May-21	<0.010	0.041	0.041	0.525	84.7	10.6
	EV_DC1	19-May-21	<0.010	0.013	0.013	0.299	50.6	7.7
	EV_DC1	02-Jun-21	<0.010	0.029	0.029	0.531	75.5	9.3
	EV_DC1	17-Jun-21	0.013	0.033	0.046	0.820	137	10.7
	EV_DC1	12-Aug-21	0.017	0.215	0.232	2.24	148	27.2
	EV_DCSP_DS1	28-Aug-21	<0.010	0.066	0.066	1.29	131	12.9
	EV_DC1	28-Aug-21	<0.010	0.022	0.022	0.313	41.3	8.6
	EV_DC1	02-Sep-21	<0.010	0.070	0.070	1.29	164	13.7
	EV_DC1	01-Oct-21	<0.010	0.046	0.046	0.358	149	10.4
	EV_DC1	01-Oct-21	<0.010	0.036	0.036	1.05	145	10.4
	EV_DC1	14-Oct-21	<0.010	0.026	0.026	0.842	154	9.2
	EV_DC1	09-Nov-21	<0.010	<0.010	<0.010	0.530	105	6.5
	EV_DC1	09-Dec-21	<0.010	0.013	0.013	0.493	106	8.1
HRM-R5	RG_HARM5	22-Sep-20	0.027	0.022	0.049	0.346	44.7	11.5
	RG_HARM5	22-Sep-20	0.027	0.022	0.049	0.336	43.6	11.5
	EV_DCSP_DS2	28-Aug-21	<0.010	<0.010	<0.010	0.313	41.3	6.9
HRM-R4	EV_DCSP_DS3	28-Aug-21	<0.010	0.014	0.014	0.340	40.2	8.1
HRM-R3	RG_HACKUS	15-Sep-20	0.013	0.014	0.027	0.221	29.1	9.8
	EV_DCSP_DS4	28-Aug-21	<0.010	0.014	0.014	0.294	32.8	8.2
	EV_HASP_US	28-Aug-21	<0.010	0.023	0.023	0.306	30.8	8.9
HRM-R2	EV_HC1	21-Feb-17	<0.010	0.020	0.020	0.147	32.4	9
	EV_HC1	06-Jun-17	<0.010	<0.010	<0.010	0.094	18.2	7.8
	EV_HC1	11-Sep-17	0.037	0.033	0.070	0.300	29.2	13.4
	EV_HC1	01-Dec-17	<0.010	0.020	0.020	0.206	41.8	9.0
	EV_HC1	07-Feb-18	<0.010	<0.010	<0.010	0.046	10.7	8.1
	EV_HC1	05-Jun-18	<0.010	<0.010	<0.010	0.126	17.5	7.9
	EV_HC1	04-Sep-18	0.021	0.046	0.067	0.300	25.3	13.3
	EV_HC1	03-Dec-18	<0.010	<0.010	<0.010	0.128	26.5	7.6
	EV_HC1	14-Feb-19	<0.010	<0.010	<0.010	0.100	28.5	7.4
	EV_HC1	04-Jun-19	<0.010	0.012	0.012	0.104	10.9	9.2
	EV_HC1	03-Sep-19	0.029	0.033	0.062	0.344	29.7	12.8
	EV_HC1	03-Dec-19	0.013	0.013	0.026	0.220	40.4	9.5

Reach	Location	Sample Date	[DMSeO]	[MeSe(IV)]	Total OrganoSe	[Se(IV)]	[Se(VI)]	Modelled BI [Se]
	EV_HC1	11-Feb-20	<0.010	<0.010	<0.010	0.084	18.1	7.8
	EV_HC1	12-May-20	<0.010	0.015	0.015	0.171	29.9	8.8
	EV_HC1	01-Sep-20	0.034	0.030	0.064	0.322	30.9	12.9
	EV_HC1	01-Dec-20	<0.010	0.013	0.013	0.187	45.2	8.3
RM-R1	RG_HACKDS	16-Sep-20	0.014	0.029	0.043	0.291	27.6	11.3
	EV_HASP_DS1	28-Aug-21	<0.010	0.028	0.028	0.343	29.5	9.3
GRV-R1	RG_GRDS	13-Sep-20	<0.010	0.014	0.014	0.243	21.7	9.1
	EV_HASP_DS2	29-Aug-21	<0.010	0.019	0.019	0.343	25.7	8.4
	EV_HASP_DS3	29-Aug-21	<0.010	0.017	0.017	0.269	20.7	8.3
	EV_HASP_DS4	01-Sep-21	<0.010	0.021	0.021	0.310	26.4	9.0

Note: Species concentrations are in µg/L; modelled benthic invertebrate selenium concentrations (BI [Se]) are in mg/kg dw. Following Golder (2021), shading indicates total organoselenium (as the sum of [DMSeO] and [MeSe(IV)]) <0.025 µg/L (no shading, unlikely to affect bioaccumulation), 0.025 to 0.05 µg/L (blue, may affect bioaccumulation but unlikely to increase risk), 0.05 to 0.1 µg/L (yellow, likely to affect bioaccumulation and may increase risk), and >0.1 µg/L (orange, likely to affect bioaccumulation and likely to increase risk). Modelled BI [Se] were calculated using the de Bruyn and Luoma (2021) bioaccumulation model.

The data summarized in Table 5 illustrate the changes in aqueous selenium speciation that can occur in sedimentation ponds. Concurrent data indicate a 2.4-fold (September 2020) to 3.5-fold or greater (August 2021) increase in total organoselenium between monitoring locations upstream (EV_DC3, EV_DCSP_UP) and downstream (EV_DCOUT, EV_DCSP_DS1) of Dry Creek Sedimentation Pond. Similarly, concurrent data indicate a 1.6-fold (September 2020) to 1.2-fold (August 2021) increase in organoselenium between monitoring locations upstream (RG_HACKUS, EV_HASP_US) and downstream (RG_HACKDS, EV_HASP_DS1) of Harmer Creek Sedimentation Pond. Concentrations of DMSeO are generally close to the detection limit (0.010 µg/L) and more variable than MeSe(IV), making it more difficult to describe spatial patterns in individual species. However, the changes in total organoselenium are consistent with patterns described in Golder (2021) that are currently understood to reflect organoselenium generation via algal and/or microbial activity occurring in sedimentation ponds.

Beyond the immediate vicinity of sedimentation ponds, concentrations of organoselenium decline as a function of dilution, degradation, and uptake by periphyton (Golder 2021). The analysis of longitudinal patterns of speciation illustrated in Golder (2021; Figure 5 therein) indicates that DMSeO and MeSe(IV) are rapidly lost from the aqueous phase, with half lives on the order of one to a few hours. The data in Table 5 do not support a quantitative calculation of loss rate, but it is apparent that concentrations of organoselenium species decline more rapidly than total selenium in Harmer Creek downstream of Dry Creek and in Grave Creek downstream of Harmer Creek, indicating that there are loss processes beyond the effect of dilution. Work is currently underway under Teck Coal's Selenium Speciation Monitoring Program to quantitatively describe these longitudinal patterns by sampling additional locations in Dry Creek (EV_DCSP_DS1, DS2, DS3, and DS4), Harmer Creek (EV_HASP_DS1), and Grave Creek (EV_HASP_DS2, DS3, and DS4).

The implications of changes to aqueous selenium speciation for bioaccumulation are described in de Bruyn and Luoma (2021) and Golder (2021). Golder (2021; Figure 9 therein) assessed the effect of increasing concentrations of organoselenium as follows:

- In lotic monitoring areas in the Elk Valley with no detectable organoselenium, selenium bioaccumulation is strongly inhibited by sulphate and composite benthic invertebrate selenium concentrations rarely exceed tissue-based effects benchmarks[16] even in the most mine-affected tributaries.

- <0.025 µg/L organoselenium (shown with no highlighting in Table 5) does not have a discernible effect on bioaccumulation.
- 0.025 to 0.05 µg/L organoselenium (highlighted blue in Table 5) is sometimes associated with a discernible increment in bioaccumulation but rarely has been associated with tissue selenium concentrations exceeding benchmarks.
- 0.05 to 0.1 µg/L organoselenium (highlighted yellow in Table 5) is often associated with a discernible increment in bioaccumulation and sometimes has been associated with tissue selenium concentrations exceeding benchmarks.
- >0.1 µg/L organoselenium (highlighted orange in Table 5) is consistently associated with a discernible increment in bioaccumulation and often has been associated with tissue selenium concentrations exceeding benchmarks.

The pattern of organoselenium concentrations shown in Table 5 is reflected in the pattern of modelled (Table 5) and measured benthic invertebrate tissue selenium concentrations (discussed further in Section 3.4). The highest concentrations in benthic invertebrate tissue occur immediately downstream of Dry Creek Sedimentation Pond, where organoselenium concentrations were greater than 0.1 µg/L (orange in Table 5), and in Harmer Creek Sedimentation Pond, where seasonal peak organoselenium concentrations were between 0.05 and 0.1 µg/L (yellow in Table 5). Reaches HRM-R3 and HRM-R5 of Harmer Creek had organoselenium concentrations between 0.025 and 0.05 µg/L (blue in Table 5), which would be expected to cause a discernible increment in benthic invertebrate tissue selenium concentrations relative to areas with no organoselenium but would not necessarily cause exceedance of a tissue-based effects benchmark.[17]

In addition to the spatial patterns discussed above, Table 5 indicates large seasonal and interannual variability in organoselenium concentrations immediately downstream of Dry Creek Sedimentation Pond (DC-R1). Consistent with the seasonal patterns described by Golder (2021), peak organoselenium concentrations occur in late summer of each year, coincident with peak productivity and/or post-growing season senescence. Golder (2021) also showed that organoselenium concentrations can vary from year to year for reasons that are not yet fully understood. Maximum measured organoselenium concentrations in DC-R1 were 0.173 µg/L in 2018 (the year of recruitment failure), 0.104 µg/L in 2020, and 0.234 µg/L in 2021. Although these data indicate that peak organoselenium concentrations were higher in 2021 compared to 2018, this comparison is based on only a single value in 2018. It cannot be ruled out that higher organoselenium concentrations occurred in 2018 that were not captured in the July 2018 sampling. This uncertainty is somewhat reduced by the analysis provided in Attachment A, which concluded that organoselenium concentrations in Dry Creek Sedimentation Pond in 2021 coincided with a distinctly warmer and earlier growing season than other years in the period of record, but that

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[16] Tissue-based effects benchmarks for selenium are described in Annex E of the Elk Valley Water Quality Plan (Teck Coal 2014). In brief, these benchmarks are tissue selenium concentrations that indicate a potential for chronic, sublethal effects on the most sensitive species and life stages of aquatic life in the Elk Valley.

[17] In addition to the monitoring data shown in Table 5, it is possible to estimate the speciation that would have occurred in HRM-R5 in July 2018 from measured values in DC-R1. Applying an approximate dilution ratio of 0.3 between these two reaches (calculated from concurrent measurements of selenate and sulphate as conservative tracers) gives an organoselenium concentration of 0.052 µg/L and a modelled benthic invertebrate selenium concentration of 12 mg/kg dw. A similar estimation for HRM-R3 (applying an approximate dilution ratio of 0.25 calculated in the same way) gives an organoselenium concentration of 0.043 ug/L and a modelled benthic invertebrate selenium concentration of 11 mg/kg dw, which is close to the concentration of 10 mg/kg dw measured in September 2018.

2018 was not distinct from other years in the period of record in terms of temperature, flow, or nutrient availability.

3.4 Benthic Invertebrate and Sediment Selenium Data

Tissue selenium monitoring in the Harmer Creek population area has focused on sampling of benthic invertebrates at routine monitoring locations upstream (RG_HACKUS), downstream (RG_HACKDS, EV_HC1), and within (RG_HA7) the Harmer Creek Sedimentation Pond. Benthic invertebrate selenium data are also available for one or more years at monitoring locations in Dry Creek, Harmer Creek upstream and downstream of Dry Creek, and Grave Creek upstream and downstream of Harmer Creek. All available benthic invertebrate selenium data are plotted in Figure 13 of Warner and Lancaster (2022). Data from the Harmer Creek population area, which are the focus of this assessment, are plotted in the middle row of Figure 4 and are provided in Attachment B.

Spatial patterns of benthic invertebrate selenium concentrations on Figure 4 align with the interpretation of aqueous selenium concentrations and speciation discussed in the previous subsections. Benthic invertebrate selenium concentrations are highest in Dry Creek Sedimentation Pond and immediately downstream (DC-R2 and -R1), reflecting the highest aqueous total selenium (up to 200 µg/L) and organoselenium (>0.1 µg/L) concentrations in the population area, as well as abundant annelids (aquatic worms) inhabiting the depositional sediment in these reaches (Nupqu and Hemmera 2020). Measured concentrations (Figure 4) were several-fold higher than modelled concentrations (Table 5), which may reflect the presence of annelids in tissue selenium samples. As discussed in Section 2.1, annelids can exhibit higher bioaccumulation of selenium than other benthic invertebrate taxa and the presence of one or more annelids in a tissue sample can have a large influence on composite selenium concentrations (Golder 2021). Benthic invertebrate selenium concentrations in these reaches are also more variable than elsewhere in the population area, ranging from ~35 to 100 mg/kg dw. This underprediction and variability would be consistent with variable proportions of annelids in composite tissue samples, or may reflect spatial heterogeneity in organoselenium concentrations. Recent sediment data evaluated in Wiebe and Orr (2022) similarly reflect conditions of high selenium bioavailability[18] immediately downstream of Dry Creek Sedimentation Pond (DC-R1), with a concentration of ~80 mg/kg dw in sediment reported in 2020.

Benthic invertebrate selenium concentrations in Harmer Creek are 5- to 10-fold lower than concentrations measured in lower Dry Creek. Long-term monitoring in HRM-R3 (RG_HACKUS) reported benthic invertebrate selenium concentrations between 7 and 10 mg/kg dw from 2012 to 2019. Sampling in HRM-R5 in 2020 also reported concentrations near 10 mg/kg dw (Nupqu and Hemmera 2020). Although few data are available, these concentrations are consistent with what would be predicted by the de Bruyn and Luoma (2021) bioaccumulation model (Table 5) and with the patterns described in Golder (2021), given the concentrations of organoselenium present (0.025 to 0.05 µg/L).[17] Benthic invertebrate selenium concentrations in this range do not indicate an influence of annelids on tissue data from Harmer Creek. A condition of lower bioavailability in HRM-R5 compared to Dry Creek is also reflected in sediment data, with a concentration of <2 mg/kg dw reported in 2020 (Wiebe and Orr 2022).

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[18] Concentrations of selenium in sediment are strongly influenced by deposition of aqueous selenium by sediment-associated microbes and attached algae. Thus, observation of high sediment selenium concentrations implies high bioavailability of aqueous selenium.

Benthic invertebrate selenium data are only available in HRM-R5 for 2020 and 2021 and in HRM-R4 in 2021, which results in uncertainty about whether selenium exposure conditions in the period of interest may have contributed to reduced recruitment and/or recruitment failure in 2018. This uncertainty is somewhat reduced by the observation that benthic invertebrate selenium concentrations measured in 2021 were similar in HRM-R5 (median 10 mg/kg dw), HRM-R4 (median 12 mg/kg dw), and HRM-R3 (median 13 mg/kg dw). The similarity of concentrations across reaches in Harmer Creek is consistent with the similar aqueous selenium concentrations (typically 40-60 µg/L; Figure 4) and speciation (≤0.05 µg/L; Table 5) in these reaches. It may be reasonable to assume that benthic invertebrate selenium concentrations were also similar across reaches of Harmer Creek during the period of interest, and therefore that monitoring data from HRM-R3 would provide an estimate of conditions in HRM-R5 and -R4. Concentrations in HRM-R3 were roughly stable between 2012 and 2019 (7 to 10 µg/L; Figure 4), suggesting that concentrations in HRM-R5 and -R4 were also likely stable over this period. Given the limited data and the complexity of processes that cause organoselenium generation in a pond environment, it cannot be ruled out there could have been greater exposures in HRM-R5 and HRM-R4 than is indicated by data from HRM-R3. However, none of the (limited) available data support this conjecture. In the absence of any indication for a mechanism causing higher dietary selenium concentrations in HRM-R5 compared to HRM-R3, it seems reasonable to assume that data from HRM-R3 approximate exposure conditions in HRM-R5. Uncertainty stemming from the lack of data from HRM-R4 is somewhat reduced for the same reasons. In the absence of any indication that bioaccumulation conditions in HRM-R4 are distinct from the rest of Harmer Creek, it seems reasonable to assume that benthic invertebrate selenium concentrations in this reach are intermediate between HRM-R5 upstream and HRM-R3 downstream.

Harmer Creek Sedimentation Pond has lower concentrations of aqueous total selenium (≤50 µg/L) and organoselenium (0.05 to 0.1 µg/L) than Dry Creek Sedimentation Pond, and consequently has benthic invertebrate selenium concentrations ranging from ~15 to 35 mg/kg dw (as compared to ~35 to 70 mg/kg dw in Dry Creek Sedimentation Pond). As discussed above for Dry Creek Sedimentation Pond, the magnitude and variability of these tissue concentrations clearly reflect speciation conditions and may also in part reflect the presence of annelids in the depositional sediments of the pond. Speciation conditions in Harmer Creek Sedimentation Pond are also reflected in sediment data, with concentrations ranging from ~20 to 30 mg/kg dw in 2019 (Wiebe and Orr 2022).

The benthic invertebrate selenium data on Figure 4 are plotted in comparison to benchmarks derived in the EVWQP (Teck Coal 2014) to indicate potential effects to juvenile growth of sensitive fish species. As discussed in Section 2.2, these benchmarks reflect the best available scientific information on how selenium can affect growth of juvenile fish, but there is residual uncertainty in the interpretation of these values for juvenile WCT. Notably, the level 1 and 2 benchmarks are the 10% effects concentration (IC_{10}, 11 mg/kg dw) and lowest observed effects concentration (LOEC, 18 mg/kg dw) from a chronic feeding study of chinook salmon fry (Hamilton et al. 1990, IC_{10} calculated by DeForest et al. 1999). The level 1 benchmark is equal to a reported no observed effect concentration (unbounded NOEC) for survival and growth from a 2.5-year feeding study with juvenile Yellowstone cutthroat trout (Hardy et al. 2010), indicating that cutthroat trout may be less sensitive to selenium than chinook salmon. However, both studies (and all other published studies of juvenile growth effects of selenium) initiated feeding trials on fry several weeks after swim-up. It is unknown whether younger fry may differ in sensitivity from the ages used in these studies.

In relation to these benchmarks, the monitoring data plotted on Figure 4 and the inferred benthic invertebrate selenium concentrations discussed above indicate potential high-magnitude effects (>50%) on growth if fry feed in Dry Creek Sedimentation Pond, the reach of Dry Creek downstream of the pond, or Harmer Creek Sedimentation Pond. Dietary selenium concentrations greater than the level 3 benchmark may also approach or exceed a threshold for lethality in sensitive fish species (Teck Coal 2014). These conditions would likely have occurred before, during, and following the year of reduced recruitment. The potential for fry mortality is discussed further in Section 4.3.

In contrast to the pond-influenced reaches, the limited data on measured and inferred benthic invertebrate selenium concentrations in Harmer Creek indicate lower risk of growth effects throughout the period of record. Benthic invertebrate selenium concentrations measured between 2012 and 2019 upstream of Harmer Creek Sedimentation Pond (HRM-R3) were less than the estimated threshold for effects on juvenile growth of sensitive fish species and less than the Hardy et al. (2010) NOEC for Yellowstone cutthroat trout, indicating that effects on juvenile growth may be unlikely. Benthic invertebrate selenium concentrations measured in 2020 and 2021 in Harmer Creek downstream of Dry Creek (HRM-R5, -R4, and -R3) were on average similar to or slightly higher than the Hardy et al. (2010) NOEC for Yellowstone cutthroat trout, indicating that it is possible that low-level growth effects could have occurred in this species in more recent years. As noted above, it also cannot be ruled out that fry may be more sensitive immediately after swim-up compared to a few weeks later when the growth effects studies were conducted. There is also uncertainty about whether residual maternal selenium could contribute to growth effects. The potential for fry growth effects at dietary concentrations close to the level 1 benchmark is discussed further in Section 4.4.

3.5 Fish Tissue Selenium Data

Monitoring of selenium concentrations in fish tissue is not routinely conducted in the Harmer Creek population area. As a result, few data exist to directly characterize the exposure of WCT in this population to bioaccumulated selenium. Available data are plotted on the top row of Figure 4 and are provided in Attachment B.

As discussed in Section 2.1, fish bioaccumulate selenium via their diet, which in this assessment is represented by benthic invertebrate selenium concentrations. Westslope Cutthroat Trout exhibit selenium concentrations in muscle tissue that are similar to or slightly higher than their averaged dietary exposure, and egg/ovary concentrations that are on average 1.6× higher than muscle or 1.6× to 2× higher than diet (Teck Coal 2014). To the extent that fish move around to feed, their bioaccumulated egg/ovary concentrations would reflect a spatial averaging of dietary concentrations over a period of several months prior to spawning.

The few available data plotted on Figure 4 generally conform to the spatial patterns of benthic invertebrate selenium concentrations discussed above. Of two fish captured in Dry Creek Sedimentation Pond in 2013, one had a muscle selenium concentration of 8 mg/kg dw, reflecting feeding on dietary concentrations lower than any reported in that reach and more likely reflecting feeding in some other low-selenium area such as the adjacent reach of Harmer Creek or Dry Creek upstream of the Dry Creek Sedimentation Pond. The other fish captured in 2013 reflected feeding on the low end of the range of benthic invertebrate selenium concentrations in lower Dry Creek and/or the sedimentation pond (34 mg/kg dw), or potentially an average of conditions in these reaches and nearby reaches with lower dietary selenium concentrations. The 4-fold difference in concentration between these two fish reflects the large variability in exposure conditions in this area.

A single fish captured in Harmer Creek Sedimentation Pond in 2006 reflected a dietary selenium concentration consistent with that measured in the pond. No other fish data are available from Harmer Creek Sedimentation Pond because despite intensive sampling over more than a decade, no other fish have been collected from the pond. Fish sampling was undertaken in the Harmer Creek Sedimentation Pond in July 2002 (82 trap hours of minnow trapping and 8.6 hours of gill netting; Minnow 2003), May 2006 (minnow traps and fyke nets, effort not specified; Minnow et al. 2007), August 2008 (500 seconds of electrofishing; Interior Reforestation 2008), May and July 2012 (31 rod-hours of angling; Minnow 2014), and June 2013 (1,400 trap hours of hoop netting; Lotic 2015). No fish were captured or observed in 2002, 2008, 2012, or 2013.

Fish muscle selenium concentrations have only been collected in reach HRM-R3 of Harmer Creek. All fish sampled in this reach in 2012 (*n*=5), 2018 (*n*=8), and 2021 (*n*=8) reflect a dietary selenium concentration consistent with benthic invertebrates collected in Harmer Creek (HRM-R3, -R4, and -R5) in the same year, further supporting the interpretation that there is little to no movement of these fish to feed in Harmer Creek Sedimentation Pond. Similar concentrations in fish muscle were observed in 2012 and 2018, consistent with the stable concentrations observed in benthic invertebrate sampling between 2012 and 2019. Higher concentrations were observed in both benthic invertebrates and fish muscle in 2021 compared to previous years (Figure 4).

No fish data are available from HRM-R5 or -R4, but because the measured and inferred dietary selenium concentrations in these reaches (as discussed in Section 3.4) are similar to those in HRM-R3, it would be reasonable to expect that fish tissue concentrations in these reaches were also similar to HRM-R3. It is unknown whether some feeding may occur in the lower reaches of Dry Creek, although abundances of benthic invertebrates in Reach 1 of Dry Creek are lower than in Harmer Creek (Wiebe et al. 2022).

The data on Figure 4 are plotted in relation to benchmarks for 10% (level 1), 20% (level 2), and 50% (level 3) effects of selenium on reproduction of WCT. The benchmarks shown on Figure 2 are muscle equivalents for egg selenium effects concentrations derived from combined data from two studies of reproductive selenium effects in WCT (Nautilus and Interior Reforestation 2011). Interpretation of these benchmarks has low uncertainty because of the species-specific and site-specific testing used to derive them.

In relation to these benchmarks, the monitoring data from 2012 and 2018 plotted on Figure 4 indicate that reproductive effects would be expected on fish feeding in the lower reaches of Dry Creek and in Harmer Creek Sedimentation Pond, but would not be expected on fish feeding in Harmer Creek. This interpretation can be tested by considering the spatial pattern of benthic invertebrate selenium concentrations from 2012 to 2019 shown on Figure 4, in that these dietary concentrations are expected to result in similar or slightly higher concentrations in muscle tissue of fish feeding in these reaches, and thus can be approximately compared to the same muscle equivalent reproductive effects benchmarks. Such a comparison would result in the same interpretation: selenium exposures in these years inferred from benthic invertebrate data are sufficient to cause reproductive effects in fish feeding in the lower reaches of Dry Creek and in Harmer Creek Sedimentation Pond (noting that this does not seem to be an operable exposure pathway), but effects are not indicated based on the limited data in Harmer Creek. In contrast, both benthic invertebrate and WCT selenium concentrations from HRM-R3 in 2021 indicate a potential for reproductive effects on fish with the highest exposures.

Estimated effects on WCT reproduction are discussed further in Section 4.1.

4.0 EVALUATION OF IMPACT HYPOTHESES

The objective of this section is to interpret relevant site-specific information to test the validity of each of the impact hypotheses identified in Section 2.3. The intended outcome is a conclusion, for each impact hypothesis and overall, of the strength of evidence that selenium contributed to reduced recruitment.

4.1 Embryo-larval Toxicity from Maternally Derived Selenium

Impact Hypothesis

The impact hypothesis is that adult female WCT accumulated sufficient dietary selenium to result in egg selenium concentrations exceeding a threshold for embryo-larval toxicity. Resulting embryo-larval deformity and/or mortality was severe enough to cause or contribute to reduced recruitment.

Criteria for Evaluation

The impact hypothesis would be supported by evidence that egg selenium concentrations could have exceeded a threshold for embryo-larval toxicity. The strength of evidence for contribution would be proportional to the magnitude of potential effects and the fraction of WCT in the Harmer Creek population area exposed to such concentrations. The strength of evidence would be increased if there was an indication that the greatest potential for effects occurred in late 2017 or early 2018, which would support a linkage to recruitment failure in the 2018 spawning cohort.

Nautilus and Interior Reforestation (2011) calculated a 10% effects concentration (EC_{10}) for embryo-larval mortality in WCT of 25 mg/kg dw in egg/ovary using the combined results of their study and a previous study by Rudolph et al. (2008). This egg/ovary threshold concentration would be associated with approximately 16 mg/kg dw in muscle and 14 mg/kg dw in benthic invertebrate prey (Teck Coal 2014). US EPA (2016) reanalyzed data from this study and calculated a slightly higher EC_{10} of 27.7 mg/kg dw from the Nautilus and Interior Reforestation (2011) results. The 50% effect level (EC_{50}) for embryo-larval toxicity in WCT is 33 mg/kg dw (Nautilus and Interior Reforestation 2011), associated with approximately 21 mg/kg dw in muscle and 18 mg/kg dw in benthic invertebrate prey (Teck Coal 2014). Tissue selenium concentrations in this range (or greater) would support the impact hypothesis.

The aqueous selenium concentrations associated with these tissue-based effects concentrations depend on selenium speciation (de Bruyn and Luoma 2021; Golder 2020, 2021). In lotic areas with no detectable organoselenium, selenium bioaccumulation is strongly inhibited by sulphate and mean composite benthic invertebrate selenium concentrations do not exceed 14 mg/kg dw even in the most mine-affected tributaries (Golder 2020, 2021). However, areas with 0.05 to 0.1 µg/L organoselenium sometimes exceed 14 mg/kg dw and areas with >0.1 µg/L organoselenium often exceed 18 mg/kg dw (see Figure 9 in Golder 2021). To the extent that such areas contribute to selenium exposure in WCT (i.e., proportional to their use for feeding by adults), the presence of organoselenium concentrations in this range would support the impact hypothesis.

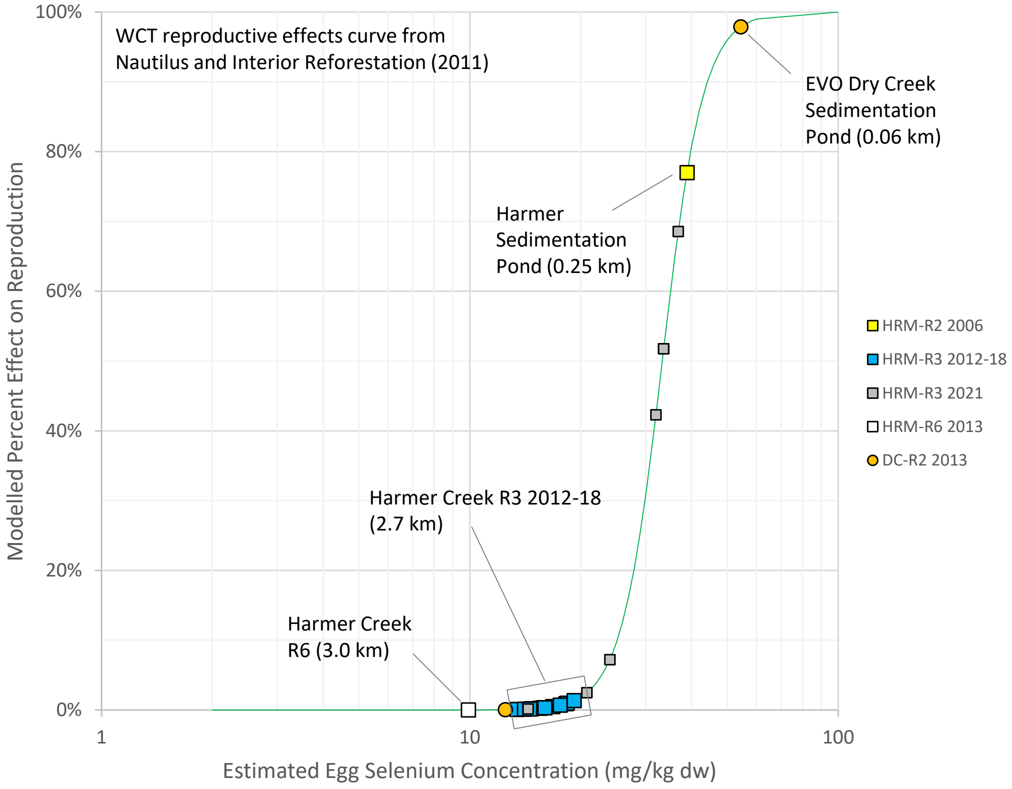
Available Evidence

Potential embryo-larval effects were evaluated by comparing measured selenium concentrations in WCT and benthic invertebrates (as plotted on Figure 4) to muscle-equivalent embryo-larval effects concentrations for WCT, as described above.

Selenium concentrations in WCT and benthic invertebrates were higher than the EC_{10} for embryo-larval toxicity in Dry Creek Sedimentation Pond (DC-R2), the reach immediately downstream (DC-R1), and Harmer Creek Sedimentation Pond (HRM-R2) in all years with data. Selenium concentrations in WCT and benthic invertebrates were lower than the EC_{10} for embryo-larval toxicity in Harmer Creek between 2012 and 2020 (HRM-R3 and -R5) but near (HRM-R5 and -R4) or higher than (HRM-R3) the EC_{10} for embryo-larval toxicity in 2021.

The magnitude of modelled reproductive effects is shown on Figure 5 as a function of egg selenium concentrations estimated from measured muscle tissue selenium concentrations in WCT collected from each reach. Data from HRM-R3 are plotted separately for sampling in 2012 to 2018 (blue symbols) and 2021 (grey symbols) to illustrate the apparent recent change in tissue selenium concentrations in this area.

Figure 5: Modelled effect on embryo-larval survival by reach



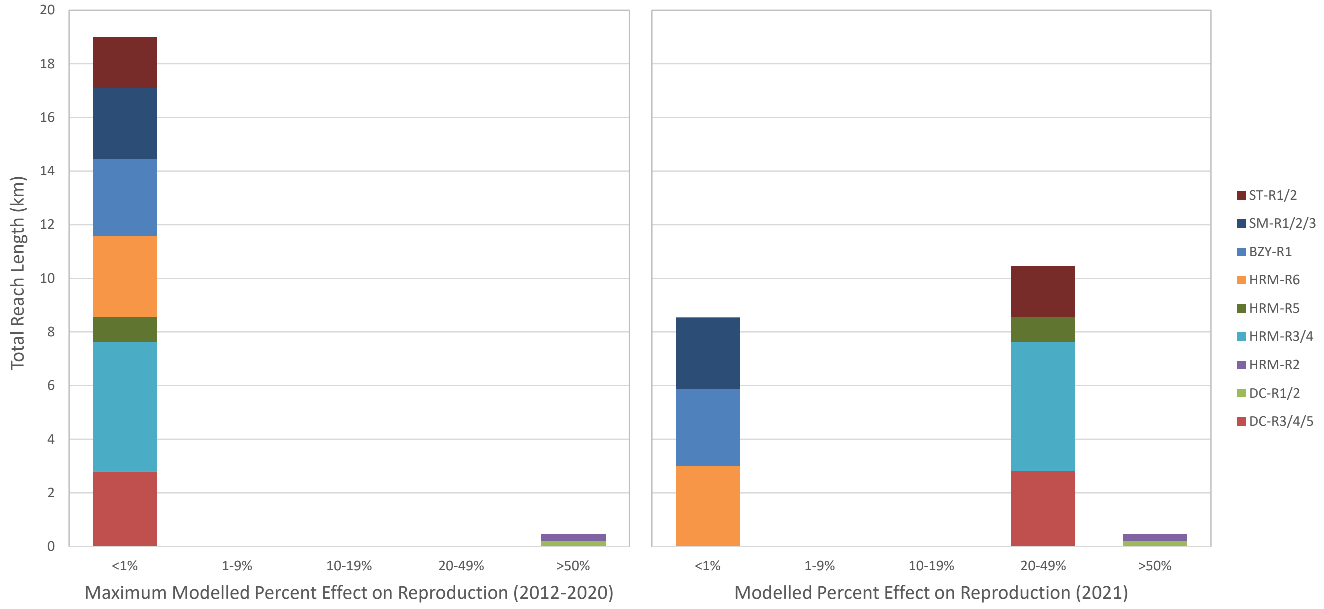
The proportion of the Harmer Creek WCT population exposed to selenium speciation immediately downstream of sedimentation ponds is expected to be small given the small proportion of overall habitat that these areas represent (0.21 km in the lower portion of Dry Creek and 0.25 km in Harmer Creek Sedimentation Pond, out of a total of 19.4 km in the Harmer Creek population area). Also, as summarized in Section 3.5, there is abundant evidence that WCT are not usually found in Harmer Creek Sedimentation Pond during spring, summer, or fall, although one fish was collected there in 2006. There is evidence that WCT occur in Dry Creek, although prey abundances there are lower than in other areas of the Harmer Creek population area (Wiebe et al. 2022). However, any fish that did feed in these reaches could be exposed to selenium concentrations sufficient to cause greater than 50% embryo-larval mortality.

Selenium concentrations in WCT (HRM-R3) and benthic invertebrates (HRM-R5 and -R3, DC-R4) were less than thresholds for embryo-larval toxicity between 2012 and 2020 in all sampled reaches of Harmer Creek (Figure 4) and reaches of Dry Creek upstream of the sedimentation pond, and are expected to be less than thresholds in Sawmill Creek and Balzy Creek given low or no mine influence on selenium concentrations in these tributaries. Harmer Creek and Dry Creek upstream of the Dry Creek Sedimentation Pond constitute the majority of aquatic habitat use by fish in the Harmer Creek population area and are expected to characterize selenium exposure of the majority of the Harmer Creek WCT population in these years. As noted above, sampling in 2021 reported higher selenium concentrations in Harmer Creek than previously observed in both WCT (HRM-R3) and benthic invertebrates (HRM-R3, -R4, -R5), along with higher organoselenium concentrations than previously observed near the outlet of Dry Creek Sedimentation Pond (DC-R1). Higher concentrations than previous years were also observed in 2021 in benthic invertebrates in Dry Creek upstream of the sedimentation pond (16 mg/kg dw as a median of 11 samples collected between August and October 2021, compared to a single value of 8.4 mg/kg dw measured in September 2020).

There was no indication in the available data that selenium exposures were higher in 2018 compared to other years. However, data collected in 2018 included only one paired sampling of benthic invertebrates and aqueous speciation in DC-R1, one sample of benthic invertebrates in HRM-R3, and eight WCT collected in HRM-R3. Observations in 2021 (summarized above) indicate that this population area can exhibit high variability in organoselenium concentrations (Table 5) and resulting concentrations in biota (Figure 4). It cannot be ruled out that conditions may have occurred in 2018 that are not fully reflected in the available speciation, benthic invertebrate tissue, and WCT muscle selenium data from that year. As discussed in Section 3.3, this uncertainty is somewhat reduced by the analysis provided in Attachment A, which concluded that 2018 was not distinct from other years in the period of record in terms of conditions expected to affect selenium bioavailability.

The spatial distribution of modelled embryo-larval effects is illustrated on Figure 6 as the total amount of aquatic habitat (expressed as the sum of reach lengths) associated with categories of estimated effects calculated using the highest modelled effects from data collected between 2006 and 2020 (left panel) and in 2021 (right panel). In reaches with tissue selenium data, estimated effects were modelled from egg selenium concentrations calculated from WCT muscle (where available) or benthic invertebrate selenium data, whichever was higher. In reaches without tissue selenium data, estimated effects were assumed to be the same as reaches with similar selenium exposure conditions: HRM-R4 was assumed to be intermediate between HRM-R5 and HRM-R3 (supported by speciation data shown in Table 5); DC-R4 and -R5 and ST-R1 and -R2 were assumed to be similar to DC-R3; and reaches without mine influence (SM-R1, -R2, and -R3; BZ-R1) were assumed to be similar to HRM-R6.

Figure 6: Spatial distribution of maximum modelled effects on embryo-larval survival in 2012-2020 (left panel) and 2021 (right panel)



Data collected between 2012 and 2020 indicate <1% modelled effects in 98% of stream length in the Harmer Creek population area, including all of Harmer Creek upstream of Harmer Creek Sedimentation Pond. In contrast, data collected in 2021 indicate on average 28% modelled effects on embryo-larval survival in HRM-R3 (based on eight WCT muscle samples)[19] and 28% modelled effects on embryo-larval survival in DC-R4 (based on 11 benthic invertebrate samples). The spatial distribution plotted on Figure 6 assumes that these modelled effects extend to HRM-R4 and -5 and all reaches of Dry Creek upstream of the Dry Creek Sedimentation Pond (DC-R3 and -R5; ST-R1 and -R2).

As noted above, the available selenium data from the period of interest indicate conditions as depicted in the left panel of Figure 6, which would not support selenium as a significant contributor to reduced recruitment. However, because of the relative paucity of data from the period of interest, and in light of the high variability evident in data from 2021, it cannot be ruled out that conditions may have occurred during the period of interest as depicted in the right panel of Figure 6. The spatial distribution of modelled effects shown on the right panel of Figure 6 would support selenium as a significant contributor to reduced recruitment.

Conclusion

This impact hypothesis is not supported by available evidence from the period of interest as the sole cause or a meaningful contributor to reduced recruitment in the period of interest nor to recruitment failure in 2018. Tissue selenium concentrations in most areas of the Harmer Creek population area indicated <1% embryo-larval

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[19] WCT muscle selenium concentrations measured in HRM-R3 in 2021 would translate into estimated egg selenium concentrations ranging from 12 to 37 mg/kg dw. Half of the eight WCT have estimated egg selenium concentrations less than the reproductive EC_{10}, indicating low potential for embryo-larval mortality. The other four WCT would have modelled embryo-larval mortality between 40 and 70%. The average modelled embryo-larval mortality across these eight WCT was 28%.

effects. On the basis of these data, this impact hypothesis is not supported as a contributor to reduced recruitment.

This impact hypothesis would be supported if conditions occurred during the period of interest that were similar to data collected in 2021.

Confidence and Uncertainty

The major sources of uncertainty in testing this impact hypothesis are: 1) the small dataset of measured selenium concentrations in Harmer Creek in the period of interest; and 2) the unknown proportion of fish that may feed in the lower reaches of Dry Creek.

The first uncertainty is reduced by the understanding of selenium bioaccumulation outlined in Section 2.2 and the interpretation of monitoring data outlined in Section 3.0. Specifically, the overview of available information presented on Figure 4 supports a cohesive characterization of exposure conditions across the Harmer Creek population area, including in reaches and years with few or no monitoring data. Multiple lines of evidence point to high exposures in DC-R1, DC-R2, and HRM-R2. In HRM-R5, -R4, and -R3, the interpretation relies on the predictable spatial pattern of aqueous selenium concentrations (Section 3.2), an understanding of spatial patterns and effects of selenium species (Section 3.3), similar concentrations in benthic invertebrates over time (Section 3.4), and the observation that the WCT collected in HRM-R3 in 2012 and 2018 reflected dietary concentrations (Section 3.5). Notably, WCT collected in HRM-R3 in 2018 (the year of recruitment failure) did not indicate a risk of embryo-larval mortality. Considered in combination, these lines of evidence lend weight to the conclusion that selenium exposures in Harmer Creek were not high enough to contribute to reduced recruitment in HRM-R5 to -R3 via embryo-larval mortality. However, there is uncertainty in the extent to which the available data characterize conditions in 2018, especially in light of the variability apparent in 2021 data. It cannot be ruled out that conditions in 2018 may not be fully reflected in the available data. If selenium exposures in 2018 were higher than reflected in the available data, the potential for selenium to have contributed to the recruitment failure would be proportionately higher.

The second uncertainty is unlikely to affect the overall conclusion outlined above. The reaches with high exposure constitute approximately 2% of total aquatic habitat in the Harmer Creek population area. Therefore, this impact hypothesis is not supported as a meaningful contributor to reduced recruitment.

4.2 Alevin/Fry Mortality from Direct Toxicity of Aqueous Selenium

Impact Hypothesis

The impact hypothesis is that alevin and/or fry were exposed to aqueous concentrations of reactive selenium species exceeding a threshold for direct toxicity. Resulting alevin and/or fry mortality was severe enough to cause or contribute to reduced recruitment and/or recruitment failure in 2018.

Criteria for Evaluation

The impact hypothesis would be supported by evidence that aqueous concentrations of reactive selenium species exceeded an effects threshold. The strength of evidence for contribution would be proportional to the magnitude of potential effects and the fraction of spawning and/or rearing areas in the Harmer Creek population area that were exposed to such conditions. The strength of evidence would be increased if there was an indication that the greatest potential for effects occurred in 2018, which would support a linkage to recruitment failure in the 2018 spawning cohort.

Direct toxicity of waterborne selenium exposure has been studied in rainbow trout (*Oncorhynchus mykiss*). Hodson et al. (1980) evaluated effects on plasma osmolarity as an integrative measure of osmotic stress and found no effects in post-hatch rainbow trout at up to 53 µg/L selenite for 44 weeks. Hunn et al. (1987) exposed rainbow trout fry to waterborne selenite for 90 days and reported effects on growth at ≥100 µg/L, effects on survival at ≥47 µg/L, and changes in bone calcium at ≥12 µg/L selenite. Miller et al. (2007) reported that exposure to up to 100 µg/L selenite for 30 days activated the physiological stress response in fish but did not impair cortisol secretion. Selenite concentrations greater than these effects concentrations would support the impact hypothesis.

Available Evidence

The highest selenite concentrations measured in the Harmer Creek population area (Table 5) are near 2 µg/L, which is five-fold lower than the lowest sublethal effects concentration summarized above. The concentrations in Table 5 were measured at a time (September) and location (downstream of Dry Creek Sedimentation Pond) that would be expected to result in relatively high production of selenite. Selenite concentrations in Harmer Creek (HRM-R5, -R4, and -R3) were near 0.3 µg/L in 2020 and 2021. There is no known mechanism by which materially higher selenite concentrations could occur elsewhere in the Harmer Creek population area.

Conclusion

This impact hypothesis is not supported by the available evidence as the sole cause or a contributor to reduced recruitment nor to recruitment failure in 2018.

Confidence and Uncertainty

There have been few studies of direct waterborne toxicity of selenite to fish and none were conducted with WCT. This uncertainty is reduced by the analysis presented in Appendix B of Costa and de Bruyn (2020), which indicated that WCT and rainbow trout, which are sister species in the genus *Oncorhynchus*, have similar sensitivity to six constituents for which acute toxicity data were identified for both species. WCT and rainbow trout also have similar sensitivity to reproductive effects of selenium, and were combined by US EPA (2016) to calculate a genus-mean chronic value for derivation of a selenium criterion value.

There remains uncertainty in the extent to which the available data characterize conditions in 2018, especially in light of the large interannual variability apparent in 2021 data. However, even selenite concentrations measured in 2021 (the highest concentrations in the available dataset) do not approach the effects concentrations summarized above, indicating that fry mortality would not be expected.

4.3 Fry Mortality from Metabolic Stress of Dietary Selenium

Impact Hypothesis

The impact hypothesis is that fry were exposed to dietary selenium concentrations, potentially in combination with residual maternal selenium, exceeding a threshold for lethality. Resulting fry mortality was severe enough to cause or contribute to reduced recruitment and/or recruitment failure in 2018.

Criteria for Evaluation

The impact hypothesis would be supported by evidence that dietary and/or residual maternal concentrations of selenium exceeded a lethal effects threshold. The strength of evidence for contribution would be proportional to the magnitude of potential effects and the fraction of spawning and/or rearing areas in the Harmer Creek population affected. The strength of evidence would be increased if there was an indication that the greatest potential for effects occurred in 2018, which would support a linkage to recruitment failure in the 2018 spawning cohort.

Dietary thresholds for juvenile mortality in sensitive fish species such as Sacramento splittail (Teh et al. 2004) and zebrafish (Thomas and Janz 2011) have been reported to be near 26 mg/kg dw. Hamilton et al. (1990) reported no effects on survival of chinook salmon fry fed a dietary selenium concentration of 18.2 mg/kg dw but DeForest et al. (1999) subsequently reanalyzed the data from that study and estimated a dietary LC_{10} of 19 mg/kg dw, LC_{20} of 27 mg/kg dw, and LC_{50} of 41 mg/kg dw. In contrast, feeding studies with rainbow trout fry reported no mortality at dietary selenium concentrations up to 32 mg/kg dw (Knight et al. 2016) or 48 mg/kg dw (Pettem et al. 2018). Benthic invertebrate selenium concentrations greater than the estimated LC_{10} for chinook salmon (19 mg/kg dw) would provide uncertain support for the impact hypothesis. Benthic invertebrate selenium concentrations greater than 26 mg/kg dw would provide stronger support.

Available Evidence

Benthic invertebrate selenium concentrations (Figure 4) were higher than 26 mg/kg dw in Dry Creek Sedimentation Pond (DC-R2), the reach immediately downstream (DC-R1) and Harmer Creek Sedimentation Pond (HRM-R2). Benthic invertebrate selenium concentrations in DC-R2 and -R1 were also higher than the unbounded no-effect concentrations for rainbow trout discussed above. The proportion of the Harmer Creek WCT population exposed to these conditions is expected to be small given the small proportion of overall habitat that these areas represent (0.21 km in Dry Creek and 0.25 km in Harmer Creek Sedimentation Pond, out of a total of 19.4 km in the Harmer Creek population area). As summarized in Section 3.5, WCT are not usually found in Harmer Creek Sedimentation Pond during spring, summer, or fall. Although one adult fish was collected there in 2006, Harmer Creek Sedimentation Pond is not suitable habitat for spawning or juvenile rearing. There is evidence that WCT occur in Dry Creek, although prey abundances there are lower than elsewhere in the Harmer Creek population area (Wiebe et al. 2022). However, any fry that did feed in these reaches could be exposed to dietary selenium concentrations that may be sufficient to cause mortality in sensitive species, potentially including WCT.

Benthic invertebrate selenium concentrations were less than 26 mg/kg dw in all sampled years in Harmer Creek (Figure 4) and reaches of Dry Creek upstream of the sedimentation pond, and are expected to be lower in Sawmill Creek and Balzy Creek. These reaches constitute the majority of aquatic habitat in the Harmer Creek population area and are expected to characterize selenium exposure of the majority of the Harmer Creek WCT population.

With respect to the possible contribution of residual maternal selenium, the estimated threshold for fry mortality in sensitive fish species discussed above (26 mg/kg dw) is similar to the egg/ovary EC_{10} for embryo-larval effects in WCT (25 to 28 mg/kg dw). Therefore, the analysis provided in Section 4.1 of embryo-larval effects in relation to egg/ovary selenium concentrations is also relevant to this impact hypothesis and the overall conclusion applies here also. Tissue selenium concentrations in most areas of the Harmer Creek population area indicated <1% embryo-larval effects, and would therefore indicate no risk of fry mortality. As discussed in Section 2.1, fry will biodilute residual maternal selenium very quickly after swim-up; therefore, this evaluation is expected to be conservative.

Conclusion

This impact hypothesis is not supported by the available evidence as the sole cause or a major contributor to reduced recruitment nor to recruitment failure in 2018. It cannot be ruled out that some WCT fry may be exposed to dietary selenium concentrations in the lower reaches of Dry Creek that could cause fry mortality in sensitive fish species. However, dietary selenium concentrations in most areas of the Harmer Creek population area did not indicate a potential for fry mortality. Therefore, this impact hypothesis is not supported as a meaningful contributor to reduced recruitment.

Confidence and Uncertainty

The major sources of uncertainty in testing this impact hypothesis are: 1) the paucity of measured benthic invertebrate selenium concentrations in Harmer Creek during the years of reduced recruitment; 2) the uncertain sensitivity of WCT fry to dietary selenium; and 3) the unknown proportion of fish that may feed in the lower reaches of Dry Creek where dietary selenium concentrations are highest.

The first uncertainty is somewhat reduced by the understanding of selenium bioaccumulation outlined in Section 2.2 and the interpretation of monitoring data outlined in Section 3.0. Specifically, the overview of available information presented on Figure 4 supports a cohesive characterization of exposure conditions across the Harmer Creek population area, including in reaches and years with few or no monitoring data. Multiple lines of evidence point to high exposures in DC-R1, DC-R2, and HRM-R2. In HRM-R5, -R4, and -R3, the interpretation relies on the predictable spatial pattern of aqueous selenium concentrations (Section 3.2), an understanding of spatial patterns and effects of selenium species (Section 3.3), and the apparently stable concentrations in benthic invertebrates over time (Section 3.4). Considered in combination, these lines of evidence lend weight to the conclusion that selenium exposures in Harmer Creek are lower than thresholds for fry mortality in sensitive species. There remains uncertainty in the extent to which the available data characterize conditions in 2018, especially in light of the large interannual variability apparent in 2021 data. However, even benthic invertebrate selenium concentrations collected in 2021 (the highest concentrations in the available dataset) do not exceed 26 mg/kg dw, indicating that fry mortality would not be expected.

The second uncertainty is highlighted by the apparent difference in sensitivity to juvenile mortality effects between chinook salmon and rainbow trout, which both belong to the genus *Oncorhynchus*. It is uncertain which species is a better surrogate for WCT.

The third uncertainty is unlikely to affect the overall conclusion outlined above. The reaches with high exposure constitute approximately 2% of total aquatic habitat in the Harmer Creek population area and available information on these reaches suggests disproportionately low use by WCT relative to this percentage. Therefore, this impact hypothesis is not supported as a meaningful contributor to reduced recruitment.

4.4 Impaired Fry Growth from Metabolic Stress of Dietary Selenium

Impact Hypothesis

The impact hypothesis is that fry were exposed to dietary selenium concentrations, potentially in combination with residual maternal selenium, exceeding a threshold for growth effects, leading to impaired growth and contributing to a failure of fry to reach minimum size to survive winter. Resulting overwinter mortality of fry was severe enough to cause or contribute to reduced recruitment and/or recruitment failure in 2018.

The context for this impact hypothesis is described further in the Hocking et al. (2022) evaluation of temperature response curves in relation to size-dependent overwinter survival of age 0 WCT. In brief, the analysis of Hocking et al. (2022) indicates that the Harmer Creek population area is characterized by cold temperatures and a short growing season, resulting in age 0 fish that enter their first winter at the low end of body sizes that are required for overwinter survival. In the context of a fish population that is living in such a marginal temperature regime, low-level effects on growth may have a greater potential to contribute to reduced recruitment than would otherwise be the case.

Criteria for Evaluation

The impact hypothesis would be supported by evidence that dietary and/or residual maternal concentrations of selenium exceeded a growth effects threshold. The strength of evidence for contribution would be proportional to the magnitude of potential growth effects and the fraction of spawning and/or rearing areas in the Harmer Creek population affected. The strength of evidence would be increased if there was an indication that the greatest potential for effects occurred in 2018, which would support a linkage to recruitment failure in the 2018 spawning cohort.

Table 6 summarizes the technical basis for the growth effects benchmarks shown as dashed lines on the middle row of Figure 4. Studies published after these benchmarks were derived are discussed in Section 2.2. Notably, Knight et al. (2016) reported a statistically significant ~21% effect on weight of rainbow trout fry at a dietary concentration of 19.5 mg/kg dw, which is near the concentration of 18 mg/kg dw that resulted in statistically significant ~22% effects on weight of chinook salmon fry in the Hamilton et al. (1990) study. Rainbow trout (*Oncorhynchus mykiss*) and chinook salmon (*Oncorhynchus tshawytscha*) are closely related to WCT (*Oncorhynchus clarki lewisi*), indicating that the level 2 benchmark may be a reliable effects concentration for WCT. Estimated 10% effects concentrations for these species are more uncertain because they are less than minimum detectable effects concentrations (Hamilton et al. 1990; Knight et al. 2016). The level 1 benchmark of 11 mg/kg dw was shown by Hardy et al. (2010) to have no effects on growth of Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*). The analysis that follows relies on the growth effects model from Hamilton et al. (1990).

Table 6: Selenium benchmarks for juvenile fish (from Annex E of Teck Coal 2014)

Dietary Selenium Benchmark (mg/kg dw)	Rationale	Studies Supporting Rationale
7	• Lowest reported effect concentration (secondary study)	• Lee et al. (2010; Study JF-17) observed a 23% reduction in specific growth rate of a marine flounder species at 7.4 mg/kg dw (LOEC). Given the disparity between this value and all reviewed studies with freshwater species, it is uncertain whether this value is relevant to fish species inhabiting the Elk Valley.
11	• Level 1 benchmark based on 10% effects on larval chinook salmon growth • No-effect concentration for juvenile Yellowstone cutthroat trout survival and growth	• Hamilton et al. (1990; Study JF-10) generated growth data for swim-up chinook salmon larvae, which DeForest et al. (1999) used to derive an IC_{10} of 11 mg/kg dw. • Hardy et al. (2010; Study JF-15) found no significant effects (NOEC) on survival and growth in juvenile cutthroat trout at 11.2 mg/kg dw.
18	• Level 2 benchmark based on 22% effects on larval chinook salmon growth • Lower than LC_{10} for larval chinook salmon and LOECs for bluegill survival • Lower than no-effects concentrations for juvenile beluga sturgeon survival and growth and larval razorback sucker survival and growth	• Hamilton et al. (1990; Study JF-10) found significant effects on growth in swim-up chinook salmon larvae at 18.2 mg/kg dw, with 22% reduction in weight; DeForest et al. (1999) used the weight data to calculate an IC_{20} of 17 mg/kg dw. • DeForest et al. (1999) used data from Hamilton et al. (1990; Study JF-10) to calculate an LC_{10} of 19 mg/kg dw. • Arshad et al. (2011; Study JF-1) found no significant effects (NOEC) on survival or growth of juvenile beluga sturgeon at 20.3 mg/kg dw. • Beyers and Sodergren (2002; Study JF-4) found no significant effects (NOEC) on survival or growth of larval razorback suckers at 21.8 mg/kg dw. • McIntyre et al. (2008; Studies JF-22 and JF-24) derived a NOEC and LOEC for survival in two experiments with juvenile bluegills. The magnitude of effects were 50% and 56% at the LOECs and <3% at the NOECs; MATC calculated using these data were 19.1 and 19.6 mg/kg dw.
26	• Level 3 benchmark based on 44% effects on juvenile Sacramento splittail growth • Equal to LC_{10} for juvenile Sacramento splittail • Lower than NOECs and LOECs for juvenile bluegill survival and growth • Lower than LC_{20} for larval chinook salmon	• Teh et al. (2004; Study JF-26) found significant effects on growth of Sacramento splittail at 26 mg/kg dw (weight was 44% lower compared with the control). • Teh et al. (2004; Study JF-26) observed 10% mortality of Sacramento splittail at 26 mg/kg dw. • Cleveland et al. (1993; Study JF-6) found no effects on survival (<20% difference from control) or growth (8% reduction in condition factor) at 18.1 and 36 mg/kg dw. • DeForest et al. (1999) used Hamilton et al. (1990; Study JF-10) data with larval chinook salmon to calculate LC_{20} of 27 mg/kg dw.

Abbreviations: LOEC = lowest observed effect concentration; NOEC = no observed effect concentration.

Units: mg/kg dw = milligrams per kilogram dry weight.

The effect of residual maternal selenium on fry growth was evaluated in the Nautilus and Interior Reforestation (2011) study by rearing WCT for 28 days after swim-up. Nautilus and Interior Reforestation (2011) found no effect of egg selenium concentrations from 3 to 20 mg/kg dw on length, weight, or growth rate of fry. Fry from the Connor Lake reference area (and the eggs from which they hatched) were initially larger than those from the Fording River with similar egg selenium concentrations. However, fry from the Fording River and Clode Sedimentation Pond had higher growth rates than those from Connor Lake.

Available Evidence

Benthic invertebrate selenium concentrations were higher than the level 3 benchmark for juvenile growth effects in Dry Creek Sedimentation Pond (DC-R2) and the reach immediately downstream (DC-R1) in all sampled years, and were higher than the level 1 benchmark in Harmer Creek Sedimentation Pond (HRM-R2) in all sampled years (middle row of Figure 4). Benthic invertebrate selenium concentrations were less than or equal to the level 1 benchmark in Harmer Creek between 2012 and 2020 (HRM-R3 and -R5) but were equal to or greater than the level 1 benchmark in 2021 (HRM-R3, -R4, and -R5).

The magnitude of modelled growth effects[20] is shown on Figure 7 as a function of benthic invertebrate selenium concentrations in each reach. Modelled effects indicated a potential 15 to 60% reduction in growth within Harmer Creek Sedimentation Pond and >60% reductions in growth in Dry Creek Sedimentation Pond and the reach of Dry Creek downstream. The proportion of Harmer Creek WCT fry exposed to these conditions is expected to be small given the small proportion of overall habitat that these areas represent (0.21 km in Dry Creek and 0.25 km in Harmer Creek Sedimentation Pond, out of a total of 19.4 km in the Harmer Creek population area) and the low suitability of these reaches for fry rearing.[21] As summarized in Section 3.5, WCT are not usually found in Harmer Creek Sedimentation Pond during spring, summer, or fall. Although one adult fish was collected there in 2006, Harmer Creek Sedimentation Pond is not suitable habitat for spawning or juvenile rearing. There is evidence that WCT occur in Dry Creek, although prey abundances there are lower than elsewhere in the Harmer Creek population area (Wiebe et al. 2022). Notwithstanding these factors, any fry that did feed in these reaches could be exposed to dietary selenium concentrations sufficient to cause severe growth effects.

Benthic invertebrate selenium concentrations in HRM-R3, HRM-R5, and DC-R4 between 2012 and 2020 indicated potential 5 to 10% reductions in fry growth (Figure 7). The chinook salmon growth effects model has elevated uncertainty in estimating potential effects <20% (the lowest statistically significant effect on growth, associated with a dietary selenium concentration near 18 mg/kg dw). However, these modelled effects indicate that dietary selenium exposures could have resulted in low-level effects on growth of WCT fry in the indicated reaches. These reaches are estimated to represent the majority of fry rearing habitat in the Harmer Creek population area (Harmer Creek EoC Team 2022). As discussed in Section 4.1, benthic invertebrate selenium concentrations were higher in 2021 than previous years in Harmer Creek (HRM-R3, -R4, and R5) and in Dry Creek above the sedimentation pond (DC-R4); resulting modelled growth effects in 2021 ranged from 5 to 40% in these areas. The average modelled growth effect was 14% in Harmer Creek (HRM-R3, -R4, and R5) and 20% in Dry Creek above the sedimentation pond (DC-R4).

Benthic invertebrate selenium concentrations indicated low potential for juvenile growth effects in Harmer Creek upstream of Dry Creek (HRM-R6) and are expected to be less than thresholds in Sawmill Creek and Balzy Creek given low or no mine influence on selenium concentrations in these tributaries.

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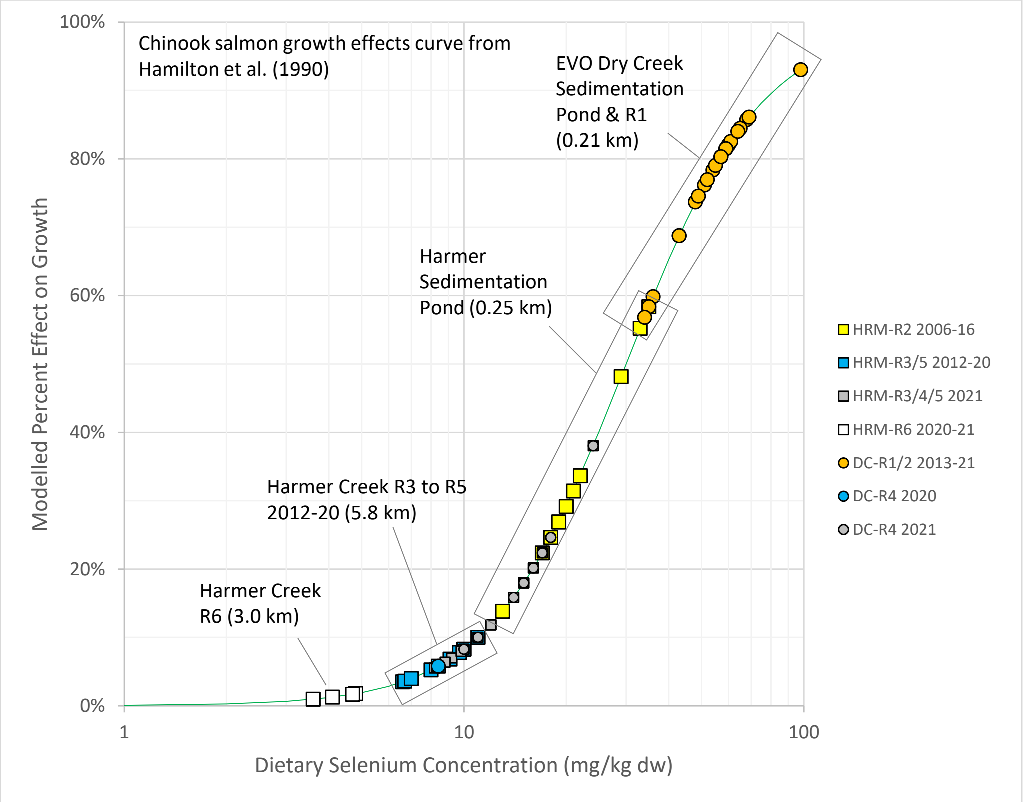
[20] Modelled growth effects were calculated using the fitted growth effects model for chinook salmon (Hamilton et al. 1990). See Section 3.4 for further discussion of this model.

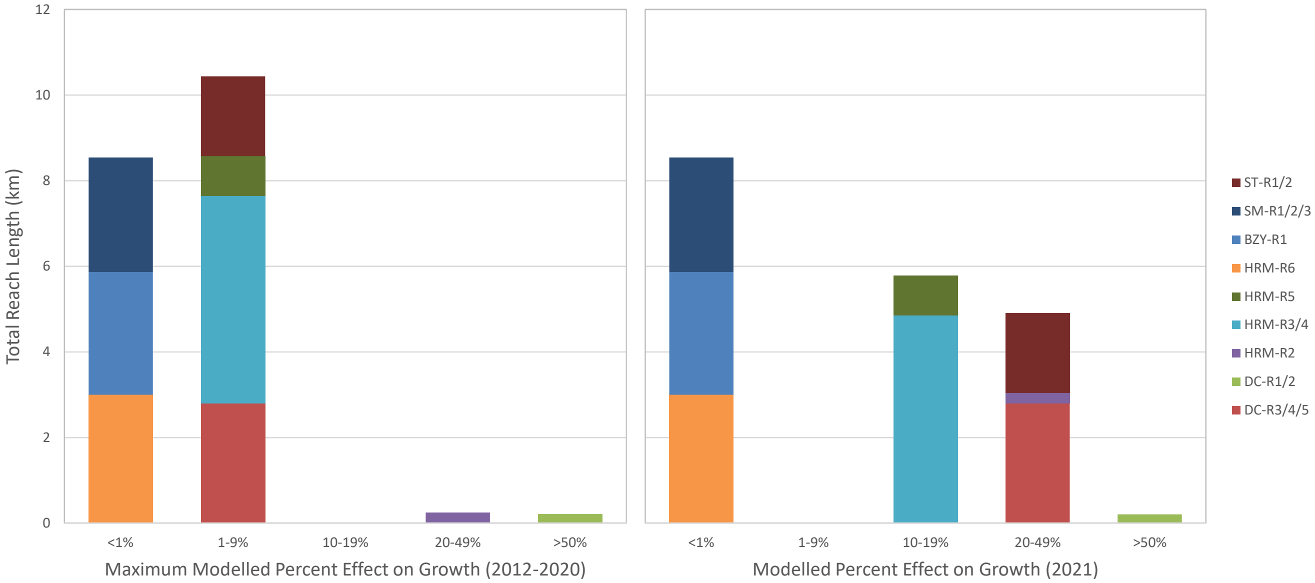
[21] See Chapter 4 of the Evaluation of Cause Report for further discussion of reaches used for fry rearing.

Figure 7: Modelled effect on fry growth by reach



The spatial distribution of modelled growth effects is illustrated on Figure 8 as the total amount of aquatic habitat (expressed as the sum of reach lengths) associated with categories of estimated effects calculated using the highest modelled effects from data collected between 2006 and 2020 (left panel) and 2021 (right panel). Estimated effects were modelled from benthic invertebrate selenium data (where available) or assumed to be the same as reaches with similar selenium exposure conditions: HRM-R4 was assumed to be intermediate between HRM-R5 and HRM-R3; DC-R4 and -R5 and ST-R1 and -R2 were assumed to be similar to DC-R3; and reaches without mine influence (SM-R1, -R2, and -R3; BZ-R1) were assumed to be similar to HRM-R6.

Figure 8: Spatial distribution of modelled effects on fry growth in 2012-2020 (left panel) and 2021 (right panel)



Data collected between 2012 and 2020 indicate 5 to 10% modelled growth effects in Harmer Creek upstream of Harmer Creek Sedimentation Pond and Dry Creek upstream of Dry Creek Sedimentation Pond. In contrast, data collected in 2021 indicate on average 14% modelled effects on fry growth in Harmer Creek (11% in HRM-R5, 12% in HRM-R4, 16% in HRM-R3) and 20% modelled effects on fry growth in Dry Creek upstream of the sedimentation pond (DC-R4). The spatial distribution plotted on Figure 6 assumes that modelled effects in DC-R4 extend to all reaches of Dry Creek upstream of the Dry Creek Sedimentation Pond (DC-R3 and -R5; ST-R1 and -R2).

As noted above, the available selenium data from the period of interest indicate conditions as depicted in the left panel of Figure 8, which would support selenium as a potential contributor to reduced recruitment in the context of other factors affecting fry growth. However, because of the relative paucity of data from the period of interest, and in light of the high variability evident in data from 2021, it cannot be ruled out that conditions may have occurred during the period of interest as depicted in the right panel of Figure 8. The spatial distribution of modelled effects shown on the right panel of Figure 8 would support selenium as a more significant potential contributor to reduced recruitment.

Conclusion

The impact hypothesis is supported by data collected in the period of interest as a potential contributor to reduced recruitment that should be evaluated further in the context of multiple stressors that could affect fry growth. Modelled growth effects from dietary selenium were 5 to 10% in the period of interest in the majority of reaches that are estimated to be used for fry rearing.[22] Effects of this magnitude would not ordinarily be considered to have the potential for population-level effects, but these findings indicate that dietary selenium exposure could have contributed to a cumulative effect on fry growth. As discussed by Hocking et al. (2022),

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[22] See Chapter 4 of the Evaluation of Cause Report for further discussion of the analysis of reaches used for fry rearing.

such an effect could, in combination with other factors affecting fry growth in the Harmer Creek population, have increased the potential for overwinter fry mortality.

This impact hypothesis is more strongly supported as a potential contributor to reduced recruitment by data collected in 2021. Modelled growth effects from dietary selenium were 14 to 20%, which would increase the potential that selenium, in combination with other factors affecting fry growth in the Harmer Creek population, may have increased the potential for overwinter fry mortality.

Confidence and Uncertainty

The major sources of uncertainty in testing this impact hypothesis are: 1) the paucity of data to characterize benthic invertebrate selenium concentrations in Harmer Creek during the period of interest; 2) the uncertain sensitivity of WCT fry to dietary selenium at concentrations less than the level 1 benchmark; and 3) the limited basis for evaluating the potential for residual maternal selenium to contribute to growth effects.

The first uncertainty is somewhat reduced by the understanding of selenium bioaccumulation outlined in Section 2.2 and the interpretation of monitoring data outlined in Section 3.0. Specifically, the overview of available information presented on Figure 4 supports a cohesive characterization of exposure conditions across the Harmer Creek population area, including in reaches and years with few or no monitoring data. Multiple lines of evidence point to high exposures in DC-R1, DC-R2, and HRM-R2. In HRM-R5, -R4, and -R3, the interpretation relies on the predictable spatial pattern of aqueous selenium concentrations (Section 3.2), an understanding of spatial patterns and effects of selenium species (Section 3.3), and the apparently stable concentrations in benthic invertebrates over time (Section 3.4). Considered in combination, these lines of evidence support the interpretation that dietary selenium exposures in Harmer Creek in the period of interest ranged from 6 to 11 mg/kg dw, with the resulting modelled potential effects on growth depicted on Figure 7.

The interpretation above relies heavily on estimation of exposure conditions in reaches and years with no monitoring data. There is uncertainty in the extent to which the available data characterize conditions in 2018, especially in light of the variability apparent in 2021 data. It cannot be ruled out that conditions in 2018 may not be fully reflected in the available data. If selenium exposures in 2018 were higher than reflected in the available data, the potential for selenium to have contributed to the recruitment failure would be proportionately higher. As discussed in Section 3.3, this uncertainty is somewhat reduced by the analysis provided in Attachment A, which concluded that 2018 was not distinct from other years in the period of record in terms of conditions expected to affect selenium bioavailability.

The second uncertainty is reduced by the summary of growth effects data provided in Table 6 and associated text, which suggests that chinook salmon may be a reasonable surrogate for the sensitivity of other *Oncorhynchus* species, including WCT, at concentrations near the level 2 benchmark. However, there remains uncertainty about whether dietary selenium concentrations less than the level 1 benchmark can cause low-level growth effects in these species. The analysis herein assumed that such exposures could cause low-level effects.

The third uncertainty is relatively high and cannot be reduced at the current state of toxicological understanding for selenium. The rearing study conducted by Nautilus and Interior Reforestation (2011) indicates that residual maternal selenium alone does not affect fry growth up to at least 20 mg/kg dw in eggs. Estimated egg selenium concentrations in Harmer Creek (13 to 19 mg/kg dw in HRM-R3; Section 4.1) are within this studied range. However, the potential contribution of residual maternal selenium to growth effects from dietary selenium exposure cannot be calculated with confidence because it is unknown how efficiently or how quickly fry depurate or biodilute selenium obtained from the yolk. It is similarly unknown how long it takes for fry to reach an internal steady state concentration relative to their diet once they swim up and begin feeding. If maternal selenium does persist in fry tissues for an extended period of time (e.g., 30 days) and if fry growth is as sensitive to bioaccumulated selenium as it is to the dietary selenium exposures studied by authors in Table 6, it cannot be ruled out that residual maternal selenium exposure could exacerbate dietary growth effects.

5.0 SUMMARY

5.1 Conclusions of Assessment

The available evidence supports a potential effect of dietary selenium on fry growth (5 to 10% in the period of interest; 14 to 20% in 2021) as a mechanism by which selenium could have contributed to reduced recruitment in the Harmer Creek WCT population. A <10% effect on a chronic, sublethal endpoint such as growth would not usually be interpreted to indicate a potential for population-level changes (US EPA 1999, 2013; Suter et al. 1995; Mebane 2010). However, because there are indications that the Harmer Creek WCT population may be unusually sensitive to the size of pre-winter fry (Hocking et al. 2022), and in light of 2021 data indicating that growth effects could potentially have been greater, this impact hypothesis warrants further evaluation in the context of other factors that can affect fry growth. Modelled effects on growth presented herein are evaluated in combination with other factors in Harmer Creek EoC Report (Harmer Creek EoC Team 2022).

The available evidence also supports a possible contribution of embryo-larval mortality from maternally-derived selenium if conditions similar to 2021 occurred in the period of interest. This impact hypothesis is not directly supported by available data from the period of interest (<1% modelled embryo-larval effects), but it cannot be ruled out in light of 2021 data.

The other impact hypotheses considered herein are less likely to have had a material effect on recruitment in the Harmer Creek WCT population for reasons discussed in Section 4. In brief, evidence for exposure conditions that could cause fry mortality is restricted to the reaches within and immediately downstream of the Dry Creek Sedimentation Pond, where multiple lines of evidence suggest there is limited exposure of fish to such conditions. It cannot be ruled out that some fish may have been exposed to these conditions, but it is implausible that enough of the Harmer Creek population could be exposed to materially contribute to a population-wide effect on recruitment.

5.2 General Summary of Confidence and Uncertainty in Assessment

Key elements of confidence and uncertainty in evaluating selenium exposure are discussed in Sections 2.1 (general principles) and 3.0 (site-specific exposure data). In general, the assessment rests on a large dataset of exposure information, but these data are unevenly distributed in space and time. In some reaches, the available data provide a reliable and robust characterization of exposure, whereas in other reaches the data are more indicative and conditions must be inferred from adjacent reaches, other years, or other types of data. The assessment attempted to overcome these limitations by considering all available data in an integrated way, with consideration of how this inference would be consistent with the available science on selenium fate and bioaccumulation. However, there are important reaches, notably in Harmer Creek downstream of Dry Creek, where the evaluation of exposure relied heavily on estimation. In addition, data collected in 2021 indicated higher selenium concentrations the previous years and exhibited higher variability than previous years. The 2021 data show that selenium speciation (Table 5) and tissue selenium concentrations (Figure 4) in the Harmer Creek population area can be highly variable, which increases uncertainty that the available data fully characterize selenium exposures during the period of interest. As discussed in Section 3.3, this uncertainty is somewhat reduced by the analysis provided in Attachment A, which concluded that 2018 was not distinct from other years in the period of record in terms of conditions expected to affect selenium bioavailability.

Key elements of confidence and uncertainty in the assessment of selenium toxicity are discussed in Sections 2.2 (general principles) and 4.0 (site-specific effects modelling). Notably, the assessment of potential embryo-larval effects was supported by species-specific toxicity data from three separate studies conducted in the Elk Valley (Table 1), the data from which have been independently analyzed by US EPA (2021). In contrast, the assessment of potential effects of dietary selenium on survival and growth of fry relied mainly on studies of related species in the genus *Oncorhynchus* (Table 2). Extrapolating toxicity data between species is common practice in risk assessment and management, for example in setting water quality guidelines. However, this approach necessarily assumes that species of interest (in this case, WCT) are not markedly more sensitive than the species used in toxicity studies. This assumption is supported in the present analysis by the results of Hardy et al. (2010) that indicate WCT are not more sensitive than the surrogate *Oncorhynchus* species used to model growth effects.

A third element of the selenium assessment was the consideration of potential exposure of fish to different reaches of the Harmer Creek population area with different levels of potential effects. In particular, the conclusions about impact hypotheses are sensitive to assumptions about the extent to which adults (for embryo-larval effects) or fry (for growth and mortality effects) might feed in lower Dry Creek. The assumptions of fish use made herein were informed by analyses in other EoC reports of available telemetry data, redd counts, consideration of habitat quality and prey abundance, and the size of these reaches relative to the remainder of the population area, all of which lend weight to the interpretation.[23] Ultimately, uncertainty in this element is unlikely to have a material effect on the overall conclusions of the assessment because the reaches in question are not large enough to support feeding of a large fraction of the Harmer Creek population.

5.3 Information Gaps and Residual Uncertainty

The most important data gap in the exposure assessment is the incomplete characterization of selenium bioaccumulation and resulting dietary selenium exposure in HRM-R5 and HRM-R4 in 2018. These reaches receive water flowing out of Dry Creek Sedimentation Pond and therefore are exposed to concentrations of organoselenium that can cause increases in bioaccumulation. Sampling in September 2020 and August 2021 indicated that this effect was relatively small, consistent with the expected dilution of Dry Creek flows after entering Harmer Creek and the understanding that organoselenium species are labile. September is when organoselenium concentrations tend to exhibit a seasonal peak (Golder 2021), suggesting that conditions in other months would likely result in lower bioaccumulation. However, organoselenium export from Dry Creek varies both within and among years (Table 5). It is unclear how well the available data from HRM-R5 characterize the range of conditions across that reach. It cannot be ruled out that peak organoselenium concentrations in 2018 were higher than the available measured value.

The uncertainty described above will be reduced by ongoing studies undertaken by Teck Coal under the Selenium Speciation Monitoring Program. One component of that program involves sampling selenium speciation and benthic invertebrate tissue selenium concentrations along a longitudinal gradient extending from upstream of Dry Creek Sedimentation Pond to the mouth of Dry Creek and into Harmer Creek. The objective of this study is to characterize the spatial extent over which organoselenium from the pond persists and affects bioaccumulation. Stations upstream and downstream of the pond are also being sampled monthly over at least one year (and biweekly during the growing season) to characterize the seasonal pattern of organoselenium concentrations. It is anticipated that the data from these studies will help understand how well the data

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[23] See Chapter 4 of the Evaluation of Cause Report for further detail on these analyses.

considered in this assessment characterized the range of exposures present in lower Dry Creek and Harmer Creek across months, between years (in comparison to 2020 and 2021), and across each reach.

The most important data gap in the effects assessment is the incomplete understanding of how dietary selenium concentrations near the level 1 benchmark, potentially in combination with residual maternal selenium, might translate into low-level growth effects. It may not be possible to reduce this uncertainty because of limitations inherent in such dietary toxicity studies. In particular, endpoints like growth tend to be highly variable among individual test organisms, and as a result it can be very difficult to detect and model low-level responses with confidence (e.g., Hamilton et al. 1990; Knight et al. 2016; Pettem et al. 2018). It is recommended that Teck Coal evaluate potential ways to study such low-level effects and, if a feasible approach can be identified, undertake appropriate studies to reduce this uncertainty.

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6.0 REFERENCES

Bailey FC, Knight AW, Ogle RS, Klaine SJ. 1995. Effect of sulfate level on selenium uptake by *Ruppia maritima*. Chemosphere 30: 579-591.

Bennett PM, Janz DM. 2007. 'Bioenergetics and growth of young-of the-year northern pike (*Esox lucius*) and burbot (*Lota lota*) exposed to metal mining effluent', Ecotoxicology and Environmental Safety, 68: 1-12.

Berntssen MHG, Sundal TK, Olsvik PA, Amlund H, Rasinger JD, Sele V, Hamre K, Hillestad M, Buttle L, Ørnsrud R. 2017. Sensitivity and toxic mode of action of dietary organic and inorganic selenium in Atlantic salmon (*Salmo salar*). Aquatic Toxicology 192: 116-26.

Besser JM, Canfield TJ, La Point TW. 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. Environ. Toxicol. Chem. 12: 57-72.

Birnie-Gauvin K, Costantini D, Cooke SJ, Willmore WG. 2017. A comparative and evolutionary approach to oxidative stress in fish: A review. Fish and Fisheries 18: 928-42.

Cain DJ, Luoma SN, Carter JL, Fend SV. 1992. Aquatic insects as bioindicators of trace element contamination in cobble-bottom rivers and streams. Canadian Journal of Fisheries and Aquatic Sciences 49: 2141-2154.

Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP (editors). 2010. Ecological Assessment of Selenium in the Aquatic Environment. CRC Press, Boca Raton, Florida.

Conley JM, Funk DH, Buchwalter DB. 2009. Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. Environ. Sci. Technol. 43: 7952-7957.

Conley JM, Funk DH, Cariello NJ, Buchwalter DB. 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly *Centroptilum triangulifer*. Ecotoxicol. 20: 1840-1851.

Conley JM, Funk DH, Hesterberg DH, Hsu L-C, Kan J, Liu Y-T, Buchwalter DB. 2013. Bioconcentration and biotransformation of selenite versus selenate exposed periphyton and subsequent toxicity to the mayfly *Centroptilum triangulifer*. Environ. Sci. Technol. 47:7965-7973.

Cope S, Cope A. 2020. Harmer and Grave Creek Westslope Cutthroat Trout Habitat and Population Assessment: Final Report. Report Prepared for Teck Coal Limited, Sparwood, BC. Report Prepared by Westslope Fisheries Ltd., Cranbrook, BC. (121 p. + 2 app.).

Costa E-J, de Bruyn A. 2020. Subject Matter Expert Report: Water Quality. Evaluation of Cause – Decline in Upper Fording River Westslope Cutthroat Trout Population. Report prepared for Teck Coal Limited by Golder Associates Ltd.

Covington SM, Naddy RB, Prouty AL, Werner SA, Lewis MD. 2018. Effects of in situ selenium exposure and maternal transfer on survival and deformities of brown trout (*Salmo trutta*) fry. Environ Toxicol Chem 37: 1396-408.

Cutter GA, Cutter LS. 2004. Selenium biogeochemistry in the San Francisco Bay estuary: changes in water column behavior. Estuarine, Coastal Shelf Sci. 61: 463–476.

deBruyn A, Hodaly A, Chapman P, DeForest D, Canton S, Baker S, Janz D, Muscatello J. 2008. Selenium Tissue Thresholds: Tissue selection criteria, threshold development endpoints, and potential to predict population or community effects in the field. North America Metals Council - Selenium Working Group.

DeForest DK, Adams WJ. 2011. Selenium accumulation and toxicity in freshwater fishes. in W. Nelson Bey and James P. Meador (eds.), Environmental Contaminants in Biota (CRC Press: Boca Raton).

DeForest DK, Brix KV, Adams WJ. 1999. Critical Review of Proposed Residue-Based Selenium Toxicity Thresholds for Freshwater Fish. Human and Ecological Risk Assessment: An International Journal 5: 1187-228.

Driedger K, Weber LP, Rickwood CJ, Dubé MG, Janz DM. 2009. Overwinter alterations in energy stores and growth in juvenile fishes inhabiting areas receiving metal mining and municipal wastewater effluents. Environmental Toxicology and Chemistry 28: 296-304.

Dubois M, Hare L. 2009. Selenium assimilation and loss by an insect predator and its relationship to Se subcellular partitioning in two prey types. Environmental Pollution 157: 772-777.

Golder 2021. Elk Valley Selenium Speciation Program (State of the Science Report). Report prepared for Teck Coal Limited.

Hamilton SJ, Buhl KJ, Faerber NL, Wiedmeyer RH, Bullard FA. 1990. Toxicity of organic selenium in the diet to chinook salmon. Environmental Toxicology and Chemistry 9: 347-358.

Hardy RW, Oram LL, Möller G. 2010. Effects of dietary selenomethionine on cutthroat trout (*Oncorhynchus clarki bouvieri*) growth and reproductive performance over a life cycle. Archives of Environmental Contamination and Toxicology 58: 237-245.

Harmer Creek Evaluation of Cause Team. 2023. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population. Report prepared for Teck Coal Limited.

Hocking M, Whelan C, Hatfield T. 2022. Subject Matter Expert Report: Water Temperature and Ice. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population. Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.

Hodson PV, Spry DJ, Blunt BR. 1980. Effects on rainbow trout (*Salmo gairdneri*) of a chronic exposure to waterborne selenium. Canadian Journal of Fisheries and Aquatic Science 37:233-240.

Hunn JB, Hamilton SJ, Buckler DR. 1987. Toxicity of sodium selenite to rainbow trout fry. Water Research 21(2):233-238.

Janz DM, DeForest DK, Brooks ML, Chapman PM, Gilron G, Hoff D, Hopkins WA, McIntyre DO, Mebane CA, Palace VP, Skorupa JP, Wayland M. 2010. Selenium toxicity to aquatic organisms. in P.M. Chapman, W.J. Adams, M.L. Brooks, C.G. Delos, S.N. Luoma, W.A. Maher, H.M. Ohlendorf, T.S. Presser and D.P. Shaw (eds.), Ecological Assessment of Selenium in the Aquatic Environment (Society of Environmental Toxicology and Chemistry (SETAC): Pensacola, Florida).

Kennedy C J, McDonald LE, Loveridge R, Strosher MM. 2000. The effect of bioaccumulated selenium on mortalities and deformities in the eggs, larvae, and fry of a wild population of Cutthroat Trout (*Oncorhynchus clarki lewisi*). Archives of Environmental Contamination and Toxicology 39: 46-52.

Knight R, Marlatt VL, Baker JA, Lo BP, deBruyn AMH, Elphick JR, Martyniuk CJ. 2016. Dietary selenium disrupts hepatic triglyceride stores and transcriptional networks associated with growth and Notch signaling in juvenile rainbow trout. Aquatic Toxicology 180: 103-14.

Kupsco A, SchlenkD. 2016. Molecular mechanisms of selenium-Induced spinal deformities in fish. Aquatic Toxicology 179: 143-50.

Lee, B-G, Lee J-S, Luoma SN. 2006. Comparison of selenium bioaccumulation in the clams *Corbicula fluminea* and *Potamocorbula amurensis*: a bioenergetic modeling approach. Environmental Toxicology and Chemistry 25: 1933-1940.

Lemly AD. 1996. Assessing the toxic threat of selenium to fish and aquatic birds. Environmental Monitoring and Assessment 43: 19-35.

Lemly, AD. 1999. Selenium transport and bioaccumulation in aquatic ecosystems: a proposal for water quality criteria based on hydrologic units. Ecotoxicology and Environmental Safety 42: 150-156.

Lemly AD. 1985. Toxicology of selenium in a freshwater reservoir: implications for environmental hazard evaluation and safety. Ecotoxicol. Environ. Saf. 10: 314–338.

Lemly DA. 1993. Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicology 27: 133-58.

Lorax (Lorax Environmental Services Ltd.) 2019. Dry Creek and Harmer Creek Local Flow and Water Quality Investigation. 04 June 2019.

Lotic (Lotic Environmental Ltd.) 2015. Elkview Operations Baldy Ridge Extension Project: Fish and Fish Habitat Baseline Report. Prepared for Teck Coal Ltd., Sparwood, BC.

Luoma SN, Johns C, Fisher NS, Steinberg NA, Oremland RS, Reinfelder J. 1992. Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. Environmental Science and Technology 26: 485-491.

Luoma SN, Rainbow PS. 2008. Metal contamination in Aquatic Environments: Science and Lateral Management. Cambridge University Press, Cambridge. 571pp.

Maier KJ, Knight AW. 1993. Comparative acute toxicity and bioconcentration of selenium by the midge *Chironomus decorus* exposed to selenate, selenite, and seleno-DL-methionine. Archives of Environmental Contamination and Toxicology 25: 365–370

McIntyre DO, Pacheco MA, Garton MW, Wallschlager D, Delos CG. 2008. Effect of Selenium on Juvenile Bluegill Sunfish at Reduced Temperature. Washingtion, DC: Health and Ecological Criteria Division, Office of Water, U.S. Environmental Protection Agency.

Mebane, C.A. 2010. Cadmium risks to freshwater life: Derivation and validation of low-effect criteria values using laboratory and field studies (version 1.2): U.S. Geological Survey Scientific Investigations Report 2006 5245, 130 p.

McPhee DL, Janz DM. 2014. Dietary selenomethionine exposure alters swimming performance, metabolic capacity and energy homeostasis in juvenile fathead minnow. Aquatic Toxicology 155: 91-100

McPhee DL. 2014. 2014 Sublethal effects of dietary selenium exposure on juvenile fishes. MSc Thesis, Toxicology Graduate Program, University of Saskatchewan.

Miller LL, Wang F, Palace VP, Hontela A. 2007. Effects of acute and subchronic exposures to waterborne selenite on the physiological stress response and oxidative stress indicators in juvenile rainbow trout. Aquatic Toxicology 83: 263-271.

Minnow (Minnow Environmental Inc.) 2018. Elk Valley Watershed Regional Aquatic Effects Monitoring Program (RAEMP), 2015-2016. Prepared for Teck Coal Limited.

Mo, A, Wang X, Yuan Y, Liu C, Wang J. 2020. Effects of waterborne exposure to environmentally relevant concentrations of selenite on reproductive function of female zebrafish: A life cycle assessment. Environmental Pollution 270: 116-237.

Nautilus (Nautilus Environmental) and Interior Reforestation. 2011. Evaluation of the Effects of Selenium on Early Life Stage Development of Westslope Cutthroat Trout from the Elk Valley, BC. Final Revised Report. Prepared for Teck Coal Ltd. November 2011.

Nautilus. 2015. Fording River Westslope Cutthroat Trout Gamete Study: Final Report. Prepared for Teck Coal Limited. March 2015.

Oremland RS, Steinberg NA, Maest AS, Miller LC, Hollibaugh JT. 1990. Measurement of in situ rates of selenate removalby dissimilatory bacterial reduction in sediments. Environ. Sci.Technol. 24: 1157–1164.

Oremland RS, Hollibaugh JT, Maest AS, Presser TS, Miller LG, Culbertson CW. 1989. Selenate reduction to elemental selenium by anaerobic bacteria in sediments and culture: biogeochemical significance of a novel, sulfate-independent respiration. Applied and Environmental Microbiology 55: 2333-2343.

Palace VP, Halden NM, Young P, Evans RE, Sterling GI. 2007. Determining residence patterns of rainbow trout using laser ablation inductively coupled plasma spectrometry analysis of selenium in otoliths. Environ. Sci. Technol. 41: 3679-3683

Palace VP, Spallholz JE, Holm J, Wautier K, Evans RE, Baron CL. 2004. Metabolism of selenomethionine by rainbow trout (*Oncorhynchus mykiss*) embryos can generate oxidative stress. Ecotoxicology and Environmental Safety 58: 17-21.

Pettem CM, Weber LP, Janz DM. 2017. Cardiac and metabolic effects of dietary selenomethionine exposure in adult zebrafish. Toxicological Sciences 159: 449-60.

Pettem C, Briens J, Janz D, Weber L. 2018. Cardiometabolic response of juvenile rainbow trout exposed to dietary selenomethionine. Aquatic Toxicology 198.

Presser TS Naftz DL. 2020. Understanding and Documenting the Scientific Basis of Selenium Ecological Protection in Support of Site-Specific Guidelines Development for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada, USGS Open File Report 2020-1098. 41pp.

Presser TS, Luoma SN. 2010. A methodology for ecosystem-scale modeling of selenium. Integr. Environ. Assess. Manag. 6: 685–710.

Reading BJ, Andersen LK, Ryu Y-W, Mushirobira Y, Todo T, Hiramatsu N. 2018. Oogenesis and egg quality in finfish: yolk formation and other factors influencing female fertility. Fishes 3: 45.

Rudolph B-L, Andreller I, Kennedy CJ. 2008. Reproductive success, early life stage development, and survival of westslope cutthroat trout (*Oncorhynchus clarki lewisi*) exposed to elevated selenium in an area of active coal mining. Environmental Science & Technology 42: 3109-14.

Schlekat CE, Purkerson DG, Luoma SN. 2004. Modeling selenium bioaccumulation through an arthropod food webs in San Francisco Bay. Environmental Toxicology and Chemistry 23: 3003-3010.

Stewart R, Grosell M, Buchwalter D, Fisher N, Luoma S, Mathews T, Orr P, Wang W-X. 2010. Selenium Toxicity to Aquatic Organisms. Chapter 6 (Pages 47-93) in P.M. Chapman, W.J. Adams, M.L. Brooks, C.G. Delos, S.N. Luoma, W.A. Maher, H.M. Ohlendorf, T.S. Presser, and D.P. Shaw (editors). Ecological Assessment of Selenium in the Aquatic Environment. CRC Press, Boca Raton, Florida.

Stewart AR, Luoma SN, Schlekat CE, Dobin MA, Hieb KA. 2004. Food web pathway determines how selenium affects aquatic ecosystems. Environmental Science and Technology 38: 4519-4526.

Suter GW II, Cornaby BW, Hadden CT, Hull RN, Stack M, Zafran FA. 1995. An approach for balancing health and ecological risks at hazardous waste sites. Risk Analysis 15: 221-231.

Thomas JK, Janz DM. 2011. Dietary selenomethionine exposure in adult zebrafish alters swimming performance, energetics and the physiological stress response. Aquatic Toxicology 102: 79-86.

Thomas JK, Wiseman S, Geisy JP, Janz, DM. 2013. Effects of chronic dietary selenomethionine exposure on repeat swimming performance, aerobic metabolism and methionine catabolism in adult zebrafish (*Danio rerio*). Aquatic toxicology 130-131: 112-122.

Thorley JL, Kortello AK, Robinson M. 2021. Grave and Harmer Creek Westslope Cutthroat Trout Population Monitoring, 2020. A Poisson Consulting report prepared by Poisson Consulting, Grylloblatta and Lotic Environmental for Teck Coal Ltd., Sparwood, BC.

Thorley JL, Kortello AK, Brooks J, Robinson M. 2022 Grave and Harmer Creek Westslope Cutthroat Trout Population Monitoring 2021. A Poisson Consulting, Grylloblatta and Lotic Environmental report prepared for Teck Coal Ltd., Sparwood, BC.

Tokunaga TK, Pickering IJ Brown GE. 1996. Selenium transformations in ponded sediments. Soil Science Society of America Journal 60: 12-24.

Tokunaga TK, Sutton SR, Bajt S, Nuessle P, Shea-McCarthy G. 1998. Selenium diffusion and reduction at the water-sediment boundary: micro-XANES spectroscopy of reactive transport. Environmental Science and Technology 32: 1092-1098.

Unrine, JM, Jackson BP, Hopkins WA, Romanek C. 2006. Isolation and partial characterization of proteins involved in maternal transfer of selenium in the western fence lizard (*Sceloporus occidentalis*). Environmental Toxicology and Chemistry 25: 1864-67.

United States Environmental Protection Agency (US EPA). 1999. 1999 Update of Ambient Water Quality Criteria for Ammonia. EPA-822-R-99-014. National Technical Information Service, Springfield, VA.

US EPA. 2013. Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater – 2013. Office of Water, Office of Science and Technology, Washington, DC. April 2013. EPA-822-R-13-001.

Van Derveer WD, Canton SP. 1997. Selenium sediment toxicity thresholds and derivation of water quality criteria for freshwater biota of western streams. Environmental Toxicology and Chemistry 16: 1260-1268.

Van Geest J, de Bruyn AMH, Elphick JE, Lo BP. 2016. A Combined laboratory and field analysis of the sulphate dependence of selenium bioaccumulation in algae. Annual Meeting of the Society of Environmental Toxicology and Chemistry, Orlando, FL.

Velinsky DJ, Cutter GA. 1991. Geochemistry of selenium in a coastal salt marsh. Geochimica et Cosmochimica Acta 55: 179-191.

Vidal D, Bay SM, Schlenk D. 2005. Effects of dietary selenomethionine on larval rainbow trout (*Oncorhynchus mykiss*). Archives of Environmental Contamination and Toxicology 49: 71-75.

Wang H, Chen H, Chernick M, Li D, Ying G-G, Yang J, Zheng N, Xie L, Hinton DE, Dong W. 2020. Selenomethionine exposure affects chondrogenic differentiation and bone formation in Japanese medaka (*Oryzias latipes*). Journal of Hazardous Materials 387: 121720.

Wang W-X, Fisher NS, Luoma SN. 1996. Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. Marine Ecology Progress Series 140: 91-113.

Warner K, Lancaster S. 2022. Subject Matter Expert Report: Water Quality. Evaluation of Cause – Recruitment Failure in the Harmer Creek Westslope Cutthroat Trout Population. Report prepared for Teck Coal Limited. Prepared by Golder Associates Ltd.

Wiebe A. Orr P. 2022. Subject Matter Expert Report: Sediment Quality. Evaluation of Cause – Recruitment Failure in the Harmer Creek Westslope Cutthroat Trout Population. Report prepared for Teck Coal Limited. Prepared by Minnow Environmental Inc.

Wiebe A, Orr P, Ings J. 2022. Subject Matter Expert Report: Food Availability. Evaluation of Cause – Recruitment Failure in the Harmer Creek Westslope Cutthroat Trout Population. Report prepared for Teck Coal Limited. Prepared by Minnow Environmental Inc.

Wiseman S, Thomas JK, McPhee L, Hursky O, Raine JC, Pietrock M, Giesy JP, Hecker M, Janz DM. 2011. Attenuation of the cortisol response to stress in female rainbow trout chronically exposed to dietary selenomethionine. Aquatic Toxicology 105: 643-51.

Wrench JJ. 1978. Selenium metabolism in the marine phyoplankters *Tetraselmis tetrathele* and *Dunaliella minuta*. Mar. Biol. 49: 231–236.

Xu Y, Wang W-X. 2002. Exposure and potential food chain transfer factor of Cd, Se and Zn in marine fish *Lutjanus argentimaculatus*. Marine Ecology Progress Series 238: 173-186.

Zhang Y-Q, Moore JN. 1996. Selenium fractionation and speciation in a wetland sediment. Environmental Science and Technology 30: 2613-2619.

Zhang Y-Q, Moore, JN. 1997. Environmental conditions controlling selenium volatilization from a wetland system. Environmental Science and Technology 31: 511-517.

Attachment A

Evaluation of Historical Conditions in Dry Creek Sedimentation Pond

Attachment A. Evaluation of Historical Conditions in Dry Creek Sedimentation Pond

The following analysis provides an interannual comparison of monitoring data from Dry Creek Sedimentation Pond to attempt to discern whether conditions may have occurred in the period of reduced recruitment (2017 – 2019) that could have resulted in organoselenium generation resembling that observed in 2021.

The processes by which selenate and selenite are converted to organoselenium have been linked to biological productivity both in natural lentic areas (Stewart et al. 2010) and sedimentation ponds (Golder 2021). The inferred mechanism is biological reduction of inorganic selenium to organoselenides, followed by degradation and oxidation to form methylated selenium metabolites (Cooke and Bruland 1987; Eswayah et al. 2016; LeBlanc and Wallschläger 2016; Ponton et al. 2020). Stewart et al. (2010) noted that lentic systems are generally characterized by long hydraulic retention times, low oxygen content, and high carbon content, which would be expected to promote the progressive reduction of selenium to more bioavailable organic forms. In an intensive geochemical study of two natural lentic areas in the Elk Valley, Martin et al. (2018) found that aqueous organoselenium occurred at higher concentrations under hypoxic and low redox conditions, primarily near the surface of highly organic sediment with aquatic vegetation. The authors concluded that these low redox conditions facilitated both dissimilatory and assimilatory microbial reduction of selenium. These observations suggest a key role of algal productivity and microbial activity in organoselenium generation.

The specific characteristics of sedimentation ponds that promote organoselenium generation appear to include extensive vegetation cover and organic sediment (Golder 2020; ADEPT 2022), high nutrient availability (Lorax 2020), low oxidation-reduction potential (Lorax 2020; ADEPT 2022), and high growing season water temperature (ADEPT 2022). ADEPT (2022) noted that warmer water could directly promote speciation changes by increasing algal productivity and/or bacterial metabolism, or alternatively (or in addition) could act via an indirect mechanism such as promotion of aquatic vegetation or depletion of dissolved oxygen from enhanced bacterial metabolism. Both possibilities were supported by an observed correlation of water temperature with chlorophyll-*a* and phycocyanins in sedimentation ponds (indicating greater algal productivity) and with abundance of emergent vegetation (indicating structural and biological factors that could further enhance algal productivity and/or bacterial metabolism).

The understanding of processes controlling organoselenium generation outlined above provides a way to evaluate whether selenium exposures during the period of reduced recruitment were reasonably reflected in the speciation and tissue selenium data collected in 2018, or whether conditions may have occurred that would result in exposures similar to those measured in 2021 (the only year with evidence of enhanced organoselenium generation). The approach to this evaluation involved comparing monitored conditions in Dry Creek Sedimentation Pond across years to identify what conditions occurred in 2021 that could have resulted in enhanced organoselenium generation, and whether such conditions also occurred during the period of reduced recruitment. Of the potential drivers of organoselenium generation discussed above, three can be readily evaluated with long-term monitoring data collected by Teck Coal: hydraulic residence time (as reflected in pond discharge), water temperature, and nutrient availability (as reflected in phosphorus concentrations). Year-on-year comparisons of seasonal patterns are shown in Figures A (pond discharge), B (temperature), and C (total phosphorus).

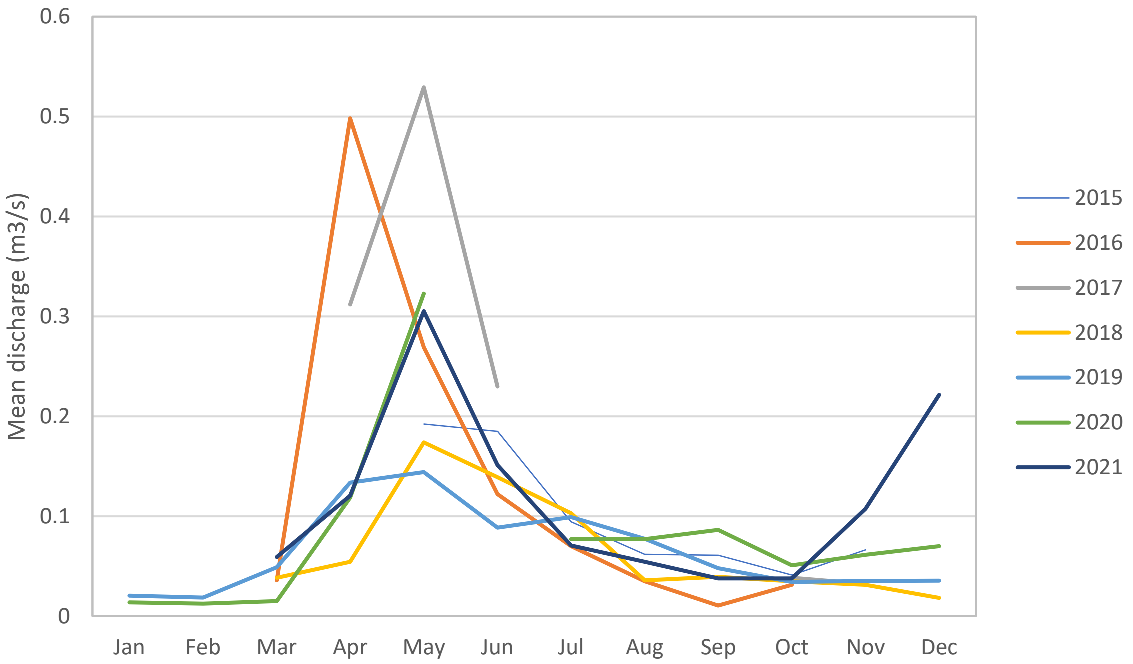

Figure A. Seasonal patterns of mean monthly discharge from Dry Creek Sedimentation Pond, 2015-2021

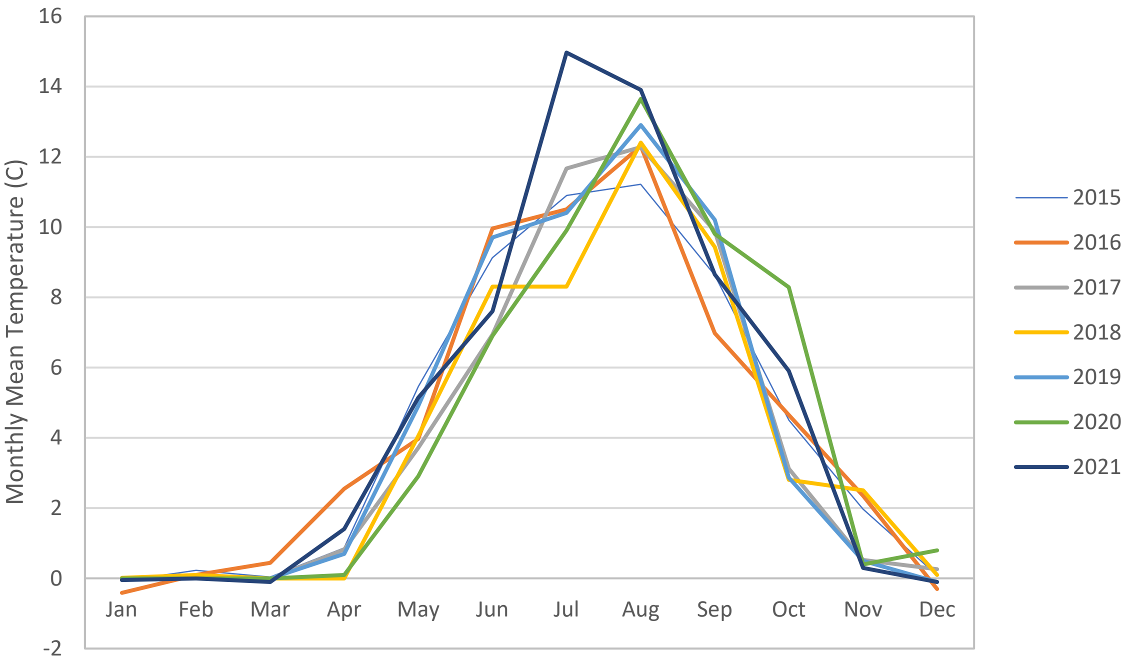

Figure B. Seasonal patterns of mean monthly temperature in Dry Creek Sedimentation Pond, 2015-2021

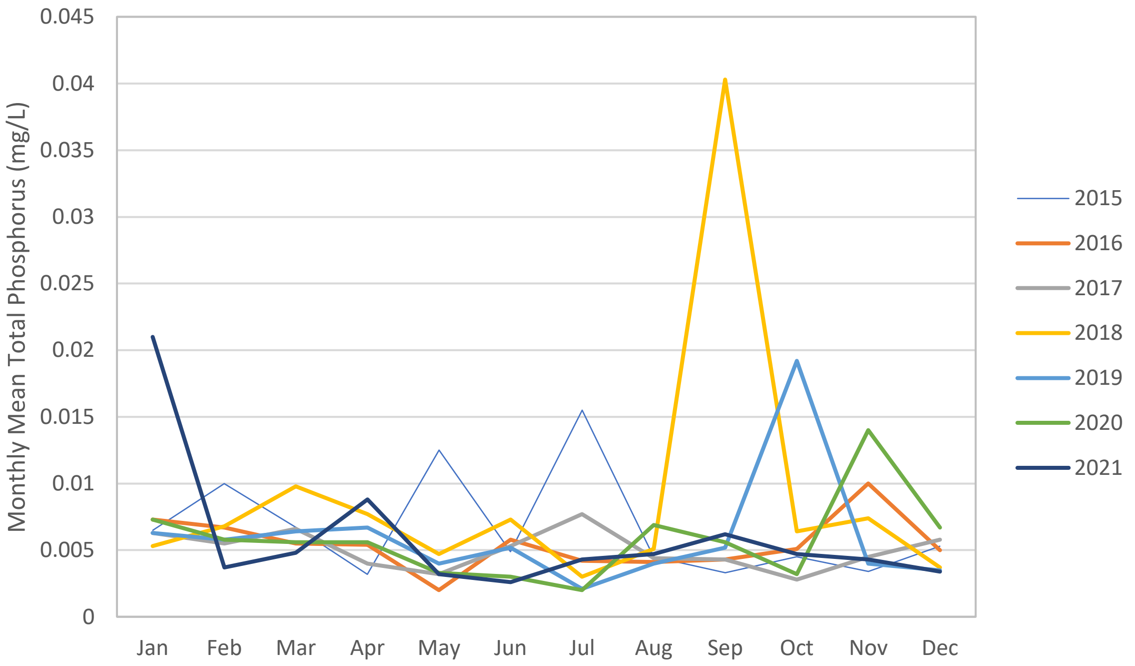

Figure C. Seasonal patterns of mean monthly total phosphorus concentration in Dry Creek Sedimentation Pond, 2015-2021

Figure A indicates that 2021 had higher monthly mean pond discharge in November and December relative to other years, but that discharge during the growing season was similar to previous years and nearly identical to the years of reduced recruitment. This observation suggests that relatively long hydraulic residence time (as reflected in low pond discharge) was not responsible for the elevated organoselenium generation observed in 2021. The years of reduced recruitment also had pond discharge during the active growing season consistent with other years, suggesting that atypical flow conditions did not occur during the period of reduced recruitment that may have contributed to enhanced generation of organoselenium.

Figure B indicates that 2021 had markedly higher monthly mean temperatures in July and August relative to other years, and the seasonal peak temperature occurred a month earlier than in all other years shown. The monthly mean water temperature in July 2021 was about 5 degrees warmer than other years plotted. This observation indicates that higher peak temperatures in the growing season, and possibly earlier timing of those peak temperatures, could have been responsible for the elevated organoselenium generation observed in 2021. The years of reduced recruitment did not exhibit higher-than-typical growing season temperatures, and in fact July 2018 was about 2 degrees cooler than most other years plotted. These observations suggest that atypical temperature conditions did not occur during the period of reduced recruitment that may have contributed to enhanced generation of organoselenium.

Figure C indicates that 2021 had a relatively high total phosphorus concentration in January, but that all other months were near 0.005 mg/L and consistent with other years. This observation suggests that high nutrient availability was not likely responsible for the elevated organoselenium generation observed during the growing season in 2021. Most months in the years of reduced recruitment were also typical of the long-term average near 0.005 mg/L, with the notable exception of September 2018. The reported total phosphorus concentration in September 2018 was the highest value in the plotted years, markedly higher than all other months in 2018. A peak in total phosphorus such as this could indicate high nutrient availability directly (if the phosphorus was present in a highly bioavailable form) or indirectly (if the phosphorus was present as algal biomass, for example following a bloom). The potential for this observation to indicate high nutrient availability was investigated further by evaluating whether other indicators of nutrient availability, such as orthophosphate (reflecting the most bioavailable form of phosphorus) and total organic carbon (reflecting algal biomass leaving the pond) also showed peaks in September 2018.

The evaluation of other water quality parameters in 2018 did not find a correspondence of total phosphorus with other indicators of nutrient availability. Orthophosphate concentrations were consistently low in all months in 2018 (0.001 to 0.005 µg/L), including September (0.0025 µg/L). Nitrate concentrations in 2018 ranged from 3 to 4 mg/L as N and were average in September (3.5 mg/L as N). The parameters that showed peaks in September were total suspended solids (28 mg/L in September compared to 1 to 3 mg/L in other months) and total concentrations of aluminum (35× higher than August), cadmium (5× higher than August), iron (15× higher than August), and manganese (8× higher than August). Similarly large peaks were not observed for dissolved metals (no increase), nor for total organic carbon (1.6× higher than August), indicating that the peaks in metals concentrations in September 2018 were related to high particulate concentrations, and that these particulates were predominantly inorganic in nature. Taken together, these observations suggest that the relatively high total phosphorus concentration in September 2018 likely reflected suspended inorganic material, and did not reflect a large increase in bioavailable phosphorus. This interpretation suggests that atypical nutrient availability did not occur during the period of reduced recruitment that may have contributed to enhanced generation of organoselenium.

The analysis outlined above does not indicate that the years of reduced recruitment had conditions similar to those that resulted in elevated organoselenium generation in 2021. The years of reduced recruitment had conditions of flow, temperature, and nutrient availability consistent with other years that did not show reduced recruitment.

References

ADEPT. 2022. DRAFT Elk Valley Selenium Speciation Monitoring Program 2021 Annual Report. Prepared for Teck Coal Limited. April 2022.

Cooke TD, Bruland KW. 1987. Aquatic chemistry of selenium: evidence of biomethylation. Environ. Sci. Technol. 21: 1214–1219.

Eswayah AS, Smith TJ, Gardiner PHE. 2016. Microbial transformations of selenium species of relevance to bioremediation. Appl. Environ. Microbiol. 82:4848–4859.

Golder. 2020. Updates to the lotic and lentic statistical bioaccumulation models for selenium in the Elk Valley. Prepared for Teck Coal Limited. November 2020.

Golder. 2021. Elk Valley Selenium Speciation Program: State of the Science Report. Prepared for Teck Coal Limited. March 2021.

LeBlanc KL, Wallschläger D. 2016. Production and release of selenomethionine and related organic selenium. species by microorganisms in natural and industrial waters. Environ. Sci. Tec hnol. 50: 6164−6171.

Lorax. 2020. Line Creek Operations Assessment of Selenium Speciation and Bioavailability in Dry Creek. Prepared for Teck Coal Limited. February 2020.

Martin AJ, Fraser C, Simpson S, Belzile N, Chen YW, London J, Wallschläger D. 2018. Hydrological and biogeochemical controls governing the speciation and accumulation of selenium in a wetland influenced by mine drainage. Environ. Toxicol. Chem. 37: 1824–1838.

Ponton DE, Graves SD, Fortin C, Janz D, Amyot M, Schiavon M. 2020. Selenium interactions with algae: chemical processes at biological uptake sites, bioaccumulation, and intracellular metabolism. Plants 9: 528.

Stewart, R., M. Grosell, D. Buchwalter, N. Fisher, S. Luoma, T. Mathews, P. Orr, and W.-X. Wang. 2010. Bioaccumulation and trophic transfer of selenium. In: Ecological assessment of selenium in the aquatic environment. Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP (Eds). SETAC Workshop on ecological assessment of selenium in the aquatic environment. CRC Press. Boca Raton, FL, New York, NY, London. 339 pp.

Attachment B: Aqueous and Tissue Selenium Monitoring Data from the Harmer Creek Population Area

Aqueous Total [Se] (ug/L)			
Reach	Site	Date	Concentration
HRM-R6	EV_HC6	2013-08-06	0.56
HRM-R6	EV_HC6	2013-09-03	0.66
HRM-R6	EV_HC6	2013-10-01	0.66
HRM-R6	EV_HC6	2013-11-06	0.73
HRM-R6	EV_HC6	2013-12-03	0.66
HRM-R6	EV_HC6	2014-01-07	0.73
HRM-R6	EV_HC6	2014-02-18	0.75
HRM-R6	EV_HC6	2014-02-25	0.82
HRM-R6	EV_HC6	2014-03-11	0.85
HRM-R6	EV_HC6	2014-04-01	0.7
HRM-R6	EV_HC6	2014-05-07	0.43
HRM-R6	EV_HC6	2014-05-27	0.3
HRM-R6	EV_HC6	2014-06-03	0.27
HRM-R6	EV_HC6	2014-06-10	0.32
HRM-R6	EV_HC6	2014-06-17	0.38
HRM-R6	EV_HC6	2014-06-24	0.39
HRM-R6	EV_HC6	2014-07-01	0.44
HRM-R6	EV_HC6	2014-08-05	0.55
HRM-R6	EV_HC6	2014-09-03	0.62
HRM-R6	EV_HC6	2014-10-07	0.66
HRM-R6	EV_HC6	2014-11-05	0.71
HRM-R6	EV_HC6	2014-12-02	0.74
HRM-R6	EV_HC6	2015-01-07	0.7
HRM-R6	EV_HC6	2015-02-03	0.7
HRM-R6	EV_HC6	2015-02-10	0.69
HRM-R6	EV_HC6	2015-02-17	0.75
HRM-R6	EV_HC6	2015-02-24	0.74
HRM-R6	EV_HC6	2015-03-10	0.69
HRM-R6	EV_HC6	2015-04-07	0.553
HRM-R6	EV_HC6	2015-05-06	0.311
HRM-R6	EV_HC6	2015-05-19	0.422
HRM-R6	EV_HC6	2015-05-26	0.318
HRM-R6	EV_HC6	2015-06-02	0.303
HRM-R6	EV_HC6	2015-06-09	0.376
HRM-R6	EV_HC6	2015-06-16	0.491
HRM-R6	EV_HC6	2015-06-23	0.501
HRM-R6	EV_HC6	2015-06-29	0.545
HRM-R6	EV_HC6	2015-07-08	0.561
HRM-R6	EV_HC6	2015-08-11	0.634
HRM-R6	EV_HC6	2015-09-01	0.707
HRM-R6	EV_HC6	2015-10-07	0.703
HRM-R6	EV_HC6	2015-11-04	0.698

HRM-R6	EV_HC6	2015-12-07	0.707
HRM-R6	EV_HC6	2016-01-12	0.726
HRM-R6	EV_HC6	2016-02-02	0.832
HRM-R6	EV_HC6	2016-03-07	0.673
HRM-R6	EV_HC6	2016-04-06	0.454
HRM-R6	EV_HC6	2016-05-02	0.448
HRM-R6	EV_HC6	2016-06-06	0.431
HRM-R6	EV_HC6	2016-07-11	0.655
HRM-R6	EV_HC6	2016-08-09	0.689
HRM-R6	EV_HC6	2016-09-12	0.724
HRM-R6	EV_HCUSDC	2018-10-16	0.658
HRM-R6	EV_HCUSDC	2019-06-10	0.606
HRM-R6	EV_HCUSDC	2019-07-02	0.534
HRM-R6	EV_HCUSDC	2019-08-07	0.676
HRM-R6	EV_HCUSDC	2019-09-04	0.608
HRM-R6	EV_HCUSDC	2019-10-01	0.726
HRM-R6	EV_HCUSDC	2019-10-09	0.747
HRM-R6	EV_HCUSDC	2019-11-04	0.694
HRM-R6	EV_HCUSDC	2019-12-03	0.758
HRM-R6	EV_HCUSDC	2020-01-06	0.826
HRM-R6	EV_HCUSDC	2020-02-10	0.703
HRM-R6	EV_HCUSDC	2020-03-02	0.834
HRM-R6	EV_HCUSDC	2020-04-07	0.809
HRM-R6	EV_HCUSDC	2020-05-05	0.414
HRM-R6	EV_HCUSDC	2020-06-03	0.392
HRM-R6	EV_HCUSDC	2020-07-06	0.551
HRM-R6	EV_HCUSDC	2020-08-04	0.648
HRM-R6	EV_HCUSDC	2020-08-04	0.689
HRM-R6	EV_HCUSDC	2020-09-01	0.689
HRM-R6	EV_HCUSDC	2020-10-07	0.613
HRM-R6	EV_HCUSDC	2020-11-17	0.733
HRM-R6	EV_HCUSDC	2020-12-10	0.757
HRM-R5	EV_HCDSDC	2018-10-16	53.2
HRM-R5	EV_HC4	2018-10-17	44.7
HRM-R5	EV_HC4	2019-06-10	31.4
HRM-R5	EV_HC4	2019-07-02	35.5
HRM-R5	EV_HC4	2019-08-07	40.7
HRM-R5	EV_HC4	2019-09-04	44.7
HRM-R5	EV_HC4	2019-10-01	48.3
HRM-R5	EV_HC4	2019-10-09	49.1
HRM-R5	EV_HC4	2019-11-04	53.4
HRM-R5	EV_HC4	2019-12-03	57.2
HRM-R5	EV_HC4	2020-01-06	53
HRM-R5	EV_HC4	2020-02-10	50.7
HRM-R5	EV_HC4	2020-03-02	50.8
HRM-R5	EV_HC4	2020-04-07	52.3
HRM-R5	EV_HC4	2020-05-05	45.2

HRM-R5	EV_HC4	2020-06-03	20.8
HRM-R5	EV_HC4	2020-07-06	32
HRM-R5	EV_HC4	2020-08-04	42.9
HRM-R5	EV_HC4	2020-08-04	49.2
HRM-R5	EV_HC4	2020-09-01	49.2
HRM-R5	RG_HARM5	2020-09-21	48.9
HRM-R5	RG_HARM5	2020-09-21	48.9
HRM-R5	RG_HARM5.1	2020-09-21	49
HRM-R5	EV_HC4	2020-10-07	44.7
HRM-R5	EV_HC4	2020-11-17	58.1
HRM-R5	EV_HC4	2020-12-10	54.3
HRM-R4	EV_HCUSDBZC	2018-10-17	51
HRM-R3	EV_HC1A	2005-08-02	25
HRM-R3	EV_HC1A	2007-07-03	23.4
HRM-R3	RG_HACKUS	2018-09-09	41.7
HRM-R3	EV_HC1A	2018-10-17	38.6
HRM-R3	RG_HACKUS	2019-09-10	40.1
HRM-R3	EV_HC1A	2019-10-08	35.8
HRM-R3	RG_FLA_HM2	2020-05-13	41.1
HRM-R3	RG_FLA_HM3	2020-05-13	43.5
HRM-R3	RG_HACKUS	2020-09-15	31.7
HRM-R3	RG_HACKUS	2020-09-15	38.5
HRM-R3	RG_FLA_HM2	2020-10-07	36.3
HRM-R3	RG_FLA_HM3	2020-10-07	37.2
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HRM-R2	EV_HC1	1998-12-02	29
HRM-R2	EV_HC1	1999-09-13	24
HRM-R2	EV_HC1	2000-01-03	29
HRM-R2	EV_HC1	2000-05-08	32
HRM-R2	EV_HC1	2000-07-03	23
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HRM-R2	EV_HC1	2001-06-05	21
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HRM-R2	EV_HC1	2002-08-06	27
HRM-R2	EV_HC1	2002-09-03	30.2
HRM-R2	EV_HC1	2002-10-02	32.4
HRM-R2	EV_HC1	2002-12-03	27.4

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HRM-R2	EV_HC1	2004-07-06	16.9
HRM-R2	EV_HC1	2004-08-03	24
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HRM-R2	EV_HC1	2006-01-03	40.1
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HRM-R2	EV_HC1	2006-04-04	23.6
HRM-R2	EV_HC1	2006-05-02	13.6
HRM-R2	EV_HC1	2006-06-06	22.6
HRM-R2	EV_HC1	2006-07-04	25.8
HRM-R2	EV_HC1	2006-08-01	26.6
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HRM-R2	EV_HC1	2006-10-03	33
HRM-R2	EV_HC1	2006-11-07	27.9
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HRM-R2	EV_HC1	2007-08-07	24.4
HRM-R2	EV_HC1	2007-09-05	29.8
HRM-R2	EV_HC1	2007-10-03	32.3
HRM-R2	EV_HC1	2007-11-06	18.8
HRM-R2	EV_HC1	2007-12-04	29.5

HRM-R2	EV_HC1	2008-01-03	27.7
HRM-R2	EV_HC1	2008-02-25	25.5
HRM-R2	EV_HC1	2008-03-18	13.7
HRM-R2	EV_HC1	2008-03-25	13.7
HRM-R2	EV_HC1	2008-04-01	29.3
HRM-R2	EV_HC1	2008-05-06	16.8
HRM-R2	EV_HC1	2008-06-03	19.5
HRM-R2	EV_HC1	2008-07-02	26.9
HRM-R2	EV_HC1	2008-08-05	31.2
HRM-R2	EV_HC1	2008-08-15	25.6
HRM-R2	EV_HC1	2008-08-15	30.6
HRM-R2	EV_HC1	2008-09-02	30.6
HRM-R2	EV_HC1	2008-10-07	27.4
HRM-R2	EV_HC1	2008-12-03	32.3
HRM-R2	EV_HC1	2009-02-04	31.3
HRM-R2	EV_HC1	2009-03-03	32.5
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HRM-R2	EV_HC1	2009-03-17	32.3
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HRM-R2	EV_HC1	2009-04-21	21.8
HRM-R2	EV_HC1	2009-04-29	27
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HRM-R2	EV_HC1	2009-06-10	20.8
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HRM-R2	EV_HC1	2009-06-23	23.3
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HRM-R2	EV_HC1	2009-08-05	4.71
HRM-R2	EV_HC1	2009-09-01	22.3
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HRM-R2	EV_HC1	2009-12-01	30.3
HRM-R2	EV_HC1	2010-01-06	24.3
HRM-R2	EV_HC1	2010-02-02	22.6
HRM-R2	EV_HC1	2010-03-02	23.4
HRM-R2	EV_HC1	2010-03-09	20.6
HRM-R2	EV_HC1	2010-03-16	20.3
HRM-R2	EV_HC1	2010-03-23	22.9
HRM-R2	EV_HC1	2010-03-31	22.6
HRM-R2	EV_HC1	2010-04-06	22.4

HRM-R2	EV_HC1	2010-04-13	21.5
HRM-R2	EV_HC1	2010-04-20	15.1
HRM-R2	EV_HC1	2010-04-28	15.7
HRM-R2	EV_HC1	2010-05-04	19.2
HRM-R2	EV_HC1	2010-05-11	23
HRM-R2	EV_HC1	2010-05-19	8.15
HRM-R2	EV_HC1	2010-05-26	14.5
HRM-R2	EV_HC1	2010-06-01	17.7
HRM-R2	EV_HC1	2010-06-08	12.4
HRM-R2	EV_HC1	2010-06-15	11.1
HRM-R2	EV_HC1	2010-06-22	11.4
HRM-R2	EV_HC1	2010-06-29	13.4
HRM-R2	EV_HC1	2010-07-07	17.9
HRM-R2	EV_HC1	2010-07-14	19.7
HRM-R2	EV_HC1	2010-07-20	21.3
HRM-R2	EV_HC1	2010-07-27	22.3
HRM-R2	EV_HC1	2010-08-03	24.5
HRM-R2	EV_HC1	2010-09-07	27.9
HRM-R2	EV_HC1	2010-10-05	27.1
HRM-R2	EV_HC1	2010-11-02	29.1
HRM-R2	EV_HC1	2010-12-07	30.5
HRM-R2	EV_HC1	2011-03-15	27.9
HRM-R2	EV_HC1	2011-03-22	28.6
HRM-R2	EV_HC1	2011-03-29	25.4
HRM-R2	EV_HC1	2011-04-12	26.2
HRM-R2	EV_HC1	2011-04-20	30.3
HRM-R2	EV_HC1	2011-04-26	28.3
HRM-R2	EV_HC1	2011-05-03	26.4
HRM-R2	EV_HC1	2011-05-10	19.6
HRM-R2	EV_HC1	2011-05-17	15.7
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HRM-R2	EV_HC1	2011-06-28	26.8
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HRM-R2	EV_HC1	2011-10-04	32.2
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HRM-R2	EV_HC1	2012-07-03	20.4
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HRM-R2	EV_HC1	2012-09-04	33.3
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HRM-R2	EV_HC1	2012-11-06	33.2
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HRM-R2	EV_HC1	2013-03-05	35.6
HRM-R2	EV_HC1	2013-03-12	35.3
HRM-R2	EV_HC1	2013-03-19	37.1
HRM-R2	EV_HC1	2013-03-26	33.1
HRM-R2	EV_HC1	2013-04-02	25.2
HRM-R2	EV_HC1	2013-04-09	25
HRM-R2	EV_HC1	2013-04-16	33.5
HRM-R2	EV_HC1	2013-04-23	38.8
HRM-R2	EV_HC1	2013-04-30	28.1
HRM-R2	EV_HC1	2013-05-07	14.7
HRM-R2	EV_HC1	2013-05-14	15.9
HRM-R2	EV_HC1	2013-05-21	27.2
HRM-R2	EV_HC1	2013-05-28	22.5
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HRM-R2	EV_HC1	2013-08-06	32.9
HRM-R2	EV_HC1	2013-09-03	37.6
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HRM-R2	EV_HC1	2020-06-09	19.9
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HRM-R2	EV_HC1	2020-08-18	33
HRM-R2	EV_HC1	2020-08-25	32.3
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HRM-R2	EV_HC1	2020-11-09	39.4
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DC-R2	EV_DC1	2006-07-04	143
DC-R2	EV_DC1	2006-09-04	141
DC-R2	EV_DC1	2006-10-03	122
DC-R2	EV_DC1	2007-06-05	96
DC-R2	EV_DC1	2007-09-05	138
DC-R2	EV_DC1	2009-05-25	101
DC-R2	EV_DC1	2009-06-02	107
DC-R2	EV_DC1	2009-06-10	102
DC-R2	EV_DC1	2009-06-23	99.8
DC-R2	EV_DC1	2009-06-30	108
DC-R2	EV_DC1	2009-07-07	125
DC-R2	EV_DC1	2009-08-05	6.1
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DC-R2	EV_DC1	2009-10-06	105
DC-R2	EV_DC1	2009-11-03	104
DC-R2	EV_DC1	2010-05-26	94.2
DC-R2	EV_DC1	2010-06-01	77.8
DC-R2	EV_DC1	2010-06-22	79.9
DC-R2	EV_DC1	2010-07-07	103

DC-R2	EV_DC1	2010-07-14	106
DC-R2	EV_DC1	2010-08-03	112
DC-R2	EV_DC1	2010-10-05	126
DC-R2	EV_DC1	2010-11-02	121
DC-R2	EV_DC1	2011-06-07	104
DC-R2	EV_DC1	2011-06-21	135
DC-R2	EV_DC1	2011-07-06	153
DC-R2	EV_DC1	2011-08-02	154
DC-R2	EV_DC1	2011-10-04	163
DC-R2	EV_DC1	2012-06-05	119
DC-R2	EV_DC1	2012-07-03	130
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DC-R2	EV_DC1	2012-09-04	171
DC-R2	EV_DC1	2012-10-03	186
DC-R2	EV_DC1	2013-01-10	158
DC-R2	EV_DC1	2013-03-26	44.3
DC-R2	EV_DC1	2013-05-01	88.1
DC-R2	EV_DC1	2013-07-24	138
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DC-R2	EV_DC1	2013-09-03	180
DC-R2	EV_DC1	2013-10-02	147
DC-R2	EV_DC1	2013-11-06	174
DC-R2	EV_DC1	2013-12-03	164
DC-R2	EV_DC1	2014-01-07	173
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DC-R2	EV_DC1	2014-04-01	148
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DC-R2	EV_DC1	2014-05-20	107
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DC-R2	EV_DC1	2014-06-04	139
DC-R2	EV_DC1	2014-06-10	147
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DC-R2	EV_DC1	2014-06-24	127
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DC-R2	EV_DC1	2014-08-05	157
DC-R2	EV_DC1	2014-09-03	145
DC-R2	EV_DC1	2014-10-07	165
DC-R2	EV_DC1	2014-11-05	145
DC-R2	EV_DC1	2014-12-02	141
DC-R2	EV_DC1	2015-01-07	155
DC-R2	EV_DC1	2015-02-03	150
DC-R2	EV_DC1	2015-02-10	108
DC-R2	EV_DC1	2015-02-17	104
DC-R2	EV_DC1	2015-02-24	111
DC-R2	EV_DC1	2015-03-03	129
DC-R2	EV_DC1	2015-03-10	135

DC-R2	EV_DC1	2015-04-07	114
DC-R2	EV_DC1	2015-05-05	130
DC-R2	EV_DC1	2015-05-19	134
DC-R2	EV_DC1	2015-05-26	119
DC-R2	EV_DC1	2015-06-02	109
DC-R2	EV_DC1	2015-06-09	101
DC-R2	EV_DC1	2015-06-16	122
DC-R2	EV_DC1	2015-06-23	128
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DC-R2	EV_DC1	2015-08-11	158
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DC-R2	EV_DC1	2015-10-06	156
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DC-R2	EV_DC1	2016-08-09	144
DC-R2	EV_DC1	2016-09-12	144
DC-R2	EV_DC1	2016-10-03	157
DC-R2	EV_DC1	2016-11-07	128
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DC-R2	EV_DC1	2017-05-01	104
DC-R2	EV_DC1	2017-06-05	147
DC-R2	EV_DC1	2017-07-10	162
DC-R2	EV_DC1	2017-08-01	183
DC-R2	EV_DC1	2017-09-11	196
DC-R2	EV_DC1	2017-10-04	197
DC-R2	EV_DC1	2017-11-14	162
DC-R2	EV_DC1	2017-12-01	160
DC-R2	EV_DC1	2018-01-08	194
DC-R2	EV_DC1	2018-02-07	162
DC-R2	EV_DC1	2018-03-05	179
DC-R2	EV_DC1	2018-04-03	170
DC-R2	EV_DC1	2018-05-07	65.3
DC-R2	EV_DC1	2018-06-04	154

DC-R2	EV_DC1	2018-07-03	164
DC-R2	EV_DC1	2018-08-02	172
DC-R2	EV_DC1	2018-09-04	175
DC-R2	EV_DC1	2018-10-01	178
DC-R2	EV_DC1	2018-10-16	174
DC-R2	EV_DC1	2018-10-17	187
DC-R2	EV_DC1	2018-10-30	185
DC-R2	EV_DC1	2018-11-05	183
DC-R2	EV_DC1	2018-12-03	164
DC-R2	EV_DC1	2019-01-08	170
DC-R2	EV_DC1	2019-02-14	179
DC-R2	EV_DC1	2019-03-06	190
DC-R2	EV_DC1	2019-04-01	133
DC-R2	EV_DC1	2019-05-06	132
DC-R2	EV_DC1	2019-06-03	133
DC-R2	EV_DC1	2019-06-10	137
DC-R2	EV_DC1	2019-07-02	133
DC-R2	EV_DC1	2019-08-07	133
DC-R2	EV_DC1	2019-09-04	141
DC-R2	EV_DC1	2019-10-01	160
DC-R2	EV_DC1	2019-10-09	148
DC-R2	EV_DC2	2019-10-09	145
DC-R2	EV_DC3	2019-10-09	154
DC-R2	EV_DC1	2019-11-04	170
DC-R2	EV_DC1	2019-12-03	173
DC-R2	EV_DC1	2020-01-06	159
DC-R2	EV_DC1	2020-02-10	151
DC-R2	EV_DC1	2020-03-02	148
DC-R2	EV_DC1	2020-04-07	154
DC-R2	EV_DC1	2020-05-05	114
DC-R2	EV_DC1	2020-06-03	127
DC-R2	EV_DC1	2020-07-06	129
DC-R2	EV_DC1	2020-08-04	187
DC-R2	EV_DC1	2020-08-05	162
DC-R2	EV_DC1	2020-08-06	149
DC-R2	EV_DC1	2020-08-07	137
DC-R2	EV_DC1	2020-09-01	187
DC-R2	EV_DC3	2020-09-22	172
DC-R2	EV_DCOUT	2020-09-22	181
DC-R2	EV_DC1	2020-10-07	157
DC-R2	EV_DC1	2020-11-17	166
DC-R2	EV_DC1	2020-12-10	176

Attachment B: Aqueous and Tissue Selenium Monitoring Data from the Harmer Creek Population Area

Benthic Invertebrate [Se] (mg/kg dw)			
Reach	Site	Date	Concentration
HRM-R6	EV_HC6	2013-11-06	4.8
HRM-R6	EV_HC6	2021-09-10	4.7
HRM-R6	EV_HC6	2021-09-10	4.1
HRM-R6	EV_HC6	2021-09-10	3.6
HRM-R5	RG_HARM5	2020-11-06	11
HRM-R5	RG_HARM5	2020-11-06	11
HRM-R5	RG_HARM5	2020-11-06	10
HRM-R5	RG_HARM5	2020-11-06	10
HRM-R5	RG_HARM5	2021-09-16	14
HRM-R5	RG_HARM5	2021-09-16	9.2
HRM-R5	RG_HARM5	2021-09-16	16
HRM-R5	EV_DC1_DS2	2021-08-28	8.8
HRM-R5	EV_DC1_DS2	2021-08-28	10
HRM-R5	EV_DC1_DS2	2021-08-28	8.2
HRM-R4	RG_HARM4	2021-09-14	8.3
HRM-R4	RG_HARM4	2021-09-14	8.4
HRM-R4	RG_HARM4	2021-09-14	11
HRM-R4	EV_DC1_DS3	2021-08-28	14
HRM-R4	EV_DC1_DS3	2021-08-28	14
HRM-R4	EV_DC1_DS3	2021-08-28	16
HRM-R3	RG_HACKUS	2012-11-06	8.4
HRM-R3	RG_HACKUS	2015-11-06	6.6
HRM-R3	RG_HACKUS	2016-11-06	8
HRM-R3	RG_HACKUS	2016-11-06	6.7
HRM-R3	RG_HACKUS	2016-11-06	9.1
HRM-R3	RG_HACKUS	2016-11-06	7
HRM-R3	RG_HACKUS	2016-11-06	10
HRM-R3	RG_HACKUS	2018-11-06	10
HRM-R3	RG_HACKUS	2019-11-06	9.7
HRM-R3	EV_DC1_DS4	2021-08-28	10
HRM-R3	EV_DC1_DS4	2021-08-28	12
HRM-R3	EV_DC1_DS4	2021-08-28	24
HRM-R3	RG_HACKUS	2021-09-11	10
HRM-R3	RG_HACKUS	2021-09-11	17
HRM-R3	RG_HACKUS	2021-09-11	15
HRM-R3	RG_HACKUS	2021-08-28	15
HRM-R3	RG_HACKUS	2021-08-28	14
HRM-R3	RG_HACKUS	2021-08-28	15
HRM-R3	RG_HACKUS	2021-10-06	9.8
HRM-R3	RG_HACKUS	2021-10-06	12
HRM-R3	RG_HACKUS	2021-10-06	12
HRM-R2	RG_HA7	2006-08-06	22

HRM-R2	RG_HA7	2009-08-06	29
HRM-R2	RG_HA7	2012-11-06	18
HRM-R2	RG_HA7	2012-11-06	21
HRM-R2	RG_HA7	2013-08-06	19
HRM-R2	RG_HA7	2013-11-06	17
HRM-R2	RG_HA7	2013-11-06	13
HRM-R2	RG_HA7	2015-08-06	19
HRM-R2	RG_HA7	2016-11-06	35
HRM-R2	RG_HA7	2016-11-06	20
HRM-R2	RG_HA7	2016-11-06	33
DC-R2	EV_DCP	2013-11-06	36
DC-R2	EV_DCP	2013-11-06	60
DC-R2	EV_DCP	2013-11-06	51
DC-R2	EV_DCP	2013-11-06	43
DC-R2	EV_DCP	2013-11-06	43
DC-R2	EV_DCP	2013-11-06	68
DC-R2	EV_DC1	2013-11-06	48
DC-R2	EV_DC1	2018-07-05	35
DC-R1	EV_DCOUT	2020-11-06	65
DC-R1	EV_DCOUT	2020-11-06	54
DC-R1	EV_DCOUT	2020-11-06	98
DC-R1	EV_DC1_DS	2021-08-28	49
DC-R1	EV_DC1_DS	2021-08-28	57
DC-R1	EV_DC1_DS	2021-08-28	60
DC-R1	EV_DC1_DS	2021-09-16	55
DC-R1	EV_DC1_DS	2021-09-16	61
DC-R1	EV_DC1_DS	2021-09-16	59
DC-R1	EV_DC1_DS	2021-10-05	59
DC-R1	EV_DC1_DS	2021-10-05	69
DC-R1	EV_DC1_DS	2021-10-05	64
DC-R1	EV_DCOUT	2021-09-09	57
DC-R1	EV_DCOUT	2021-09-09	52
DC-R1	EV_DCOUT	2021-09-09	34

Attachment B: Aqueous and Tissue Selenium Monitoring Data from the Harmer Creek Population Area

WCT Muscle [Se] (mg/kg dw)			
Reach	Site	Date	Concentration
HRM-R6	EV_HC6	2013-08-16	6.2
HRM-R3	RG_HACKUS	2012-05-29	8.4
HRM-R3	RG_HACKUS	2012-05-29	9.9
HRM-R3	RG_HACKUS	2012-05-29	10.5
HRM-R3	RG_HACKUS	2012-05-29	11.4
HRM-R3	RG_HACKUS	2012-05-29	11.5
HRM-R3	RG_HACKUS	2018-09-10	8.8
HRM-R3	RG_HACKUS	2018-09-10	9.2
HRM-R3	RG_HACKUS	2018-09-10	9.7
HRM-R3	RG_HACKUS	2018-09-10	10
HRM-R3	RG_HACKUS	2018-09-10	10
HRM-R3	RG_HACKUS	2018-09-10	11
HRM-R3	RG_HACKUS	2018-09-10	12
HRM-R3	RG_HACKUS	2018-09-12	9.1
HRM-R3	RG_HACKUS	2021-09-01	21
HRM-R3	RG_HACKUS	2021-09-01	9
HRM-R3	RG_HACKUS	2021-09-01	13
HRM-R3	RG_HACKUS	2021-09-01	15
HRM-R3	RG_HACKUS	2021-09-01	21
HRM-R3	RG_HACKUS	2021-09-01	7.8
HRM-R3	RG_HACKUS	2021-09-01	20
HRM-R3	RG_HACKUS	2021-09-01	23
HRM-R2	RG_HA7	2006-05-05	24.3
DC-R2	EV_DC1	2013-06-18	7.8
DC-R2	EV_DC1	2013-08-19	34