Subject Matter Expert Report: Pathophysiology of Stressors on Fish. Evaluation of Cause - Decline in Upper Fording River Westslope Cutthroat Trout Population

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READER'S NOTE

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

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

Background

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

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

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

Evaluation of Cause

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

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

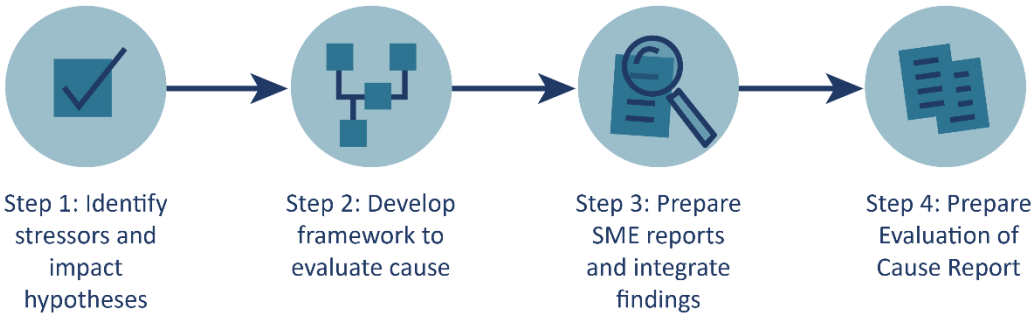
Monitoring conducted for Teck Coal in the fall of 2019 found that the abundance of Westslope Cutthroat Trout adults and sub-adults in the upper Fording River had declined significantly since previous sampling

in fall 2017. In addition, there was evidence that juvenile fish density had decreased. Teck Coal initiated an *Evaluation of Cause* process. The overall results of this process are reported separately (Evaluation of Cause Team, 2021) and are supported by a series of Subject Matter Expert reports such as this one. The full list of SME reports follows at the end of this Reader's Note.

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

How the Evaluation of Cause was approached

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



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

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

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

[1] Implies September 2017 to September 2019.

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

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

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

The Evaluation of Cause process produced two types of deliverables:

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

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

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

Participation, Engagement & Transparency

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

Ktunaxa Nation Council

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

BC Ministry Environment & Climate Change Strategy

Ministry of Energy, Mines and Low Carbon Innovation

Environmental Assessment Office

Citation for the Evaluation of Cause Report

When citing the Evaluation of Cause Report use:

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

Citations for Subject Matter Expert Reports

Focus	Citation for Subject Matter Expert Reports
Climate, temperature, and streamflow	Wright, N., Greenacre, D., & Hatfield, T. (2021). *Subject Matter Expert Report: Climate, Water Temperature, Streamflow and Water Use Trends. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Ice	Hatfield, T., & Whelan, C. (2021). *Subject Matter Expert Report: Ice. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Ltd. Report Prepared by Ecofish Research Ltd.
Habitat availability (instream flow)	Healey, K., Little, P., & Hatfield, T. (2021). *Subject Matter Expert Report: Habitat availability. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited by Ecofish Research Ltd.
Stranding – ramping	Faulkner, S., Carter, J., Sparling, M., Hatfield, T., & Nicholl, S. (2021). *Subject Matter Expert Report: Ramping and stranding. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited by Ecofish Research Ltd.
Stranding – channel dewatering	Hatfield, T., Ammerlaan, J., Regehr, H., Carter, J., & Faulkner, S. (2021). *Subject Matter Expert Report: Channel dewatering. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited by Ecofish Research Ltd.

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

Focus	Citation for Subject Matter Expert Reports
Water quality (all parameters except water temperature and TSS [Ecofish])	Costa, EJ., & de Bruyn, A. (2021). *Subject Matter Expert Report: Water quality. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Golder Associates Ltd. Healey, K., & Hatfield, T. (2021). *Calculator to assess Potential for cryoconcentration in upper Fording River.* In Costa, EJ., & de Bruyn, A. (2021). *Subject Matter Expert Report: Water quality. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Golder Associates Ltd.
Industrial chemicals, spills and unauthorized releases	Van Geest, J., Hart, V., Costa, EJ., & de Bruyn, A. (2021). *Subject Matter Expert Report: Industrial chemicals, spills and unauthorized releases. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Golder Associates Ltd. Branton, M., & Power, B. (2021). *Stressor Evaluation – Sewage.* In Van Geest et al. (2021). *Industrial chemicals, spills and unauthorized releases. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Golder Associates Ltd.
Wildlife predators	Dean, D. (2021). *Subject Matter Expert Report: Wildlife predation. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by VAST Resource Solutions Inc.
Poaching	Dean, D. (2021). *Subject Matter Expert Report: Poaching. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by VAST Resource Solutions Inc.
Food availability	Orr, P., & Ings, J. (2021). *Subject Matter Expert Report: Food availability. Evaluation of Cause – Decline in upper Fording River Westslope Cutthroat Trout population.* Report prepared for Teck Coal Limited. Prepared by Minnow Environmental Inc.

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

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Introduction

The work that follows is different from the other Subject Matter Expert (SME) reports. It is meant to augment the information found in those reports, specifically expanded on the pathophysiology of the various abiotic and biotic stressors present on the upper Fording River (UFR) during the decline window and how they may have interacted to affect the health of Westslope Cutthroat Trout (WCT). The literature on these topics is extensive and therefore the treatment of each topic is limited and intentionally uneven, to target key questions or discussion points. The coverage is based on what was is considered relevant and is commensurate with my understanding of other SME reports. As no dead fish were available to be necropsied during the decline window, the discussion is based on a review of the literature and speculation on what stressors may have contributed to the decline.

Stress

Fish are routinely exposed to multiple environmental stressors which trigger a complex cascade of biochemical, physiological and behavioral responses to maintain homeostasis and enhance survival (Figure 1). The stress response is part of the normal physiology of fish, but if excessive and persistent can be detrimental. The response is typically biphasic. The initial rapid phase is in response to severe acute stressors such as the threat of capture or predation or marked reduction in blood oxygen. Direct stimulation of chromaffin tissue in the kidney by the sympathetic nervous system, modulated by hormones such as serotonin and adrenocorticotrophic hormone, results in the release of preformed catecholamines, adrenaline and noradrenaline. The effects of these catecholamines includes: increased cardiac output, increased oxygen affinity by hemoglobin, and increased plasma glucose levels from metabolism of glycogen stores in the liver. Low or moderate levels of stress do not elicit a catecholamine response (Pankhurst 2011).

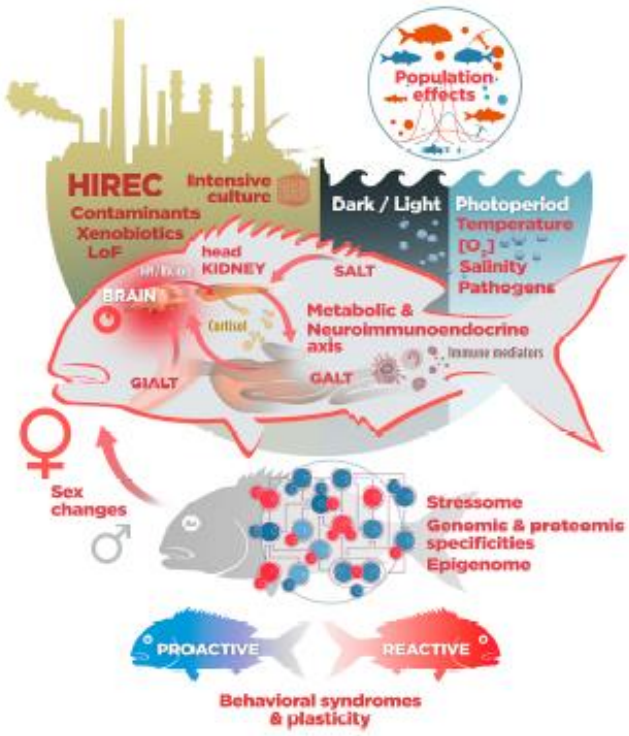
More persistent or chronic stressors activate the second phase of the stress response mediated by the hypothalamic-pituitary-interrenal axis which results in release of cortisol into circulation. A stressor, or stressors, is detected by the sensory system which activates neurons in the hypothalamus whose axons project to the pituitary gland causing release of corticotropin releasing factor (CRF). Corticotropin releasing factor stimulates production of various hormones, such as adrenocorticotrophic hormone (ACTH), β-endorphins and α-melanocyte stimulating hormone, depending on the stimulus. Among the effects of CRF is suppressed appetite, reduced feeding and other behavioral changes (Pankhurst 2011). Adrenocorticotrophic hormone released into circulation stimulates inter-renal cells, lying adjacent to major blood vessels of the kidney, to release cortisol. Increased levels of plasma cortisol have a range of effects which include: gluconeogenesis resulting in increased glucose in circulation, anaerobic metabolism of muscles with production of lactate, increased protein turnover and amino acid metabolism, increased ammonia production, and increased lipolysis. Cortisol also affects osmoregulation by stimulating Na^+/K^+ATPase activity of gill membranes (Mommsen, Vijayan, and Moon 1999).

Although there is a latency period in this second phase compared to the catecholamine response, cortisol levels can still be increased within minutes in species such as trout, depending on the stimulus. In other species, and in other circumstances, cortisol levels in circulation make take hours to increase. The cortisol response does not appear to be influenced by temperature. Once the stimulus is removed cortisol level return to normal within hours to days.

The generalized stress response influences a wide range of physiological processes and interacts with the direct biochemical and physiological effects of other stressors such as temperature, oxygen, and contaminants (Figure 1). These interactions are complex and often non-linear. Predicting the outcome of these cumulative stressors under field conditions is challenging and is an area of active research. Although the physiological mechanisms of the stress response are relatively well understood under laboratory conditions, demonstrating its effect on fish and fish populations under field conditions has been more difficult. For example, Binder et al (Binder et al. 2015) artificially increased circulating levels of cortisol in fish under field condition during winter to determine if stimulation of the hypothalamic-pituitary-interrenal axis from chronic stress affects survival and found no effects.

Secondary and tertiary effects of chronic stress, such as changes in heat shock proteins, histopathology and impaired immune response, have been proposed as markers of severe stress with potential impacts on survival. As well, the concept of energy limited tolerance to stress has been proposed as a mechanistic framework to integrate changes in molecular, physiological and behavioral processes to understand implications for fitness of the whole organism. The concept focuses on the bio-energetic consequences of trade-offs between energy allocated to basal metabolism, stress response, detoxification, reproduction, growth etc. (Sokolova 2013).

Figure 1. A schematic illustration of some of the stresses on fish and their physiological and behavioral implications from Balasch and Tort (Balasch and Tort 2019).



Stressors can be natural and anthropogenic. They operate against the backdrop of local evolutionary adaptation to the environment, prior stress in parents and during development, along with recent behavioral responses to changing conditions (Figure 1). The remainder of this report will focus on

aspects of the pathophysiology of specific stressors as they relate to the WCT population decline on the UFR. The discussion however needs to be considered in the context of these broader physiological stress responses and the evolution and adaptation of populations to local conditions.

"Natural" Environmental Factors

Under natural conditions the environment in which fish live can change dramatically. Seasonal environmental changes are the most predictable but can vary in severity from year to year. Local fish populations have evolved and adapted to these variable environmental conditions (Drinan et al. 2012; Carim et al. 2017), but rare extremes can stress or even kill large numbers of fish. When environmental factors change, fish move to find more suitable conditions. They move in response to factors such as flow rates, temperature, oxygen levels, pH, particulates, food availability, predator avoidance, etc. A diversity of connected habitat types is required for fish to be able to move to find optimal conditions.

Environmental changes can occur on much shorter time scales as well. For example, a heavy cold rain, or hailstorm, can rapidly change surface water temperatures resulting in disruption of existing thermal stratification and a sudden upwelling, or turnover, of deep anoxic waters. This sudden turnover events can kill large numbers of fish (Herman and Meyer 1990). Rapid, large die-offs of fish can overwhelm scavengers in the area resulting in accumulations of carcasses, which is often detected by people if present in an area. Lower rates of mortality over a longer time scale can be more difficult to detect as scavengers remove carcasses before they can accumulate.

Winter Mortality

It is commonly believed that winter conditions strongly influence fish survival (Alexiades, Peacock, and Al-Chokhachy 2012) and population size; however, this topic has been poorly studied due to the difficulty of collecting data during this time period (Huusko et al. 2007). Conditions under which winter mortality occurs are even less well known but can be broadly separated into cold, starvation, predation and disease (Hurst 2007b). Since winter conditions can be highly variable from year-to-year, the relative effect of these factors on winter survival also varies. Due to altered environmental conditions fish losses can be extreme during some winters (Templeman 1965). Starvation may be a factor in some situations, but in others, losses are likely the result of physiological stress or altered habitat availability resulting in increased predation and disease (Annear et al. 2002). Hoffsten (Hoffsten 2003) reported a 77% reduction in trout density, as well as marked reductions in macroinvertebrate abundance and species richness, in nine medium-sized streams in central Sweden after an extremely cold winter with low snow fall.

Cold Stress

The direct effect of low temperatures is responsible for fish mortalities in several settings. Fish species have differing lower lethal temperatures. In warm water or eurythermic (adapted to wide temperature ranges) fish lethal temperatures are generally above freezing, and if temperatures go below these thresholds fish are unable to maintain homeostasis and can die. Temperate, cold or stenothermic fish, such as trout, have lower temperature limits approximating zero Celsius (Beitinger, Bennett, and McCauley 2000), but they cannot survive temperatures below which plasma freezes, which is from -0.5 to -1.0 ^{o}C (Hurst 2007a). As fish approach their lower lethal temperatures they are unable to maintain

osmotic balance and concentrations of ions and electrolytes begin to equilibrate with those in adjacent water (Donaldson et al. 2008).

Osmoregulation is the result of passive down-gradient diffusion of electrolytes across epithelial membranes and active ion-pumping which is dependant on Na^+ and K^+ ATPase activity (Hochachka 1988). Cold adapted fish minimize osmotic stresses of declining temperatures by reducing membrane permeability and increasing Na-K ATPase activity (Schwarzbaum, Wieser, and Niederstätter 1991). Cold adaptation involves incorporating relatively higher proportions of unsaturated fatty acids into the membrane which increases the disorder of the lipid bilayer and counteracts the ordering effect of cold (Cossins and Macdonald 1989). This is referred to as homeoviscous adaptation and is only one aspect of the adaptation of membranes to decreased temperatures (Hazel 1995). Polyunsaturated phospholipids are at higher risk of lipid peroxidation from reactive oxygen species (ROS) generated during oxidative stress (Cosgrove, Church, and Pryor 1987). Since selenium (see below) and other stressors can induce oxidative stress there is the potential for membranes under cold conditions to be more prone to lipid peroxidation and damage. Other antioxidant mechanisms may mitigate this risk as evidenced by a lack of increased lipoperoxidation in eurythermic fish under experimental conditions (Grim, Miles, and Crockett 2010). Research on other fish species is needed.

Fish are thought to cope with cold temperature in two general ways. Cool and warm water species tend to tolerate cold by depressing metabolism and reducing physical activity in order to minimize metabolic requirements (Speers-Roesch, Norin, and Driedzic 2018) whereas cold-water fish tend to be more active during winter (Shuter et al. 2012). For most species of fish individuals accumulate energy stores during spring, summer and fall to be used during winter when food is limited (Fernandes and McMeans 2019). As larger fish have greater capacity for energy storage, cold associated mortality is reported to be greater in juvenile age classes.

Energy depletion is not the only explanation for mortalities during winter. For example, “cold shock” appeared to be responsible for winter mortality of juvenile fish and high rates of impingement on cooling water intake screens at the Quad Cities Nuclear Station on the Mississippi River in the central US (Bodensteiner and Lewis 1992). This winter die-off affected many different species, but freshwater drum (*Aplodinotus grunniens*) were studied in detail because of their abundance and wide geographic range (includes Manitoba up to Hudson Bay). Fish were observed floating incapacitated on the surface of the water in main channel areas of the river where temperatures were 0 ^oC. Temperatures were the same in side-channels but in pockets of backwaters temperatures were above 1 ^oC and these areas were heavily used by drum and other fish species. It was postulated that variation in water flow and depletion of oxygen in some of the backwater thermal refuges caused fish to move into the colder main and side channels causing them to become incapacitated. Malnutrition was ruled out and the clinical signs of incapacitation was demonstrated under controlled conditions in the lab when drum from the river were exposed to water temperature of 0 ^oC after a period of acclimation and gradual temperature reduction (Bodensteiner and Lewis 1992).

Freezing or ice-crystal propagation within fish has been reported in temperatures above the freezing point of plasma when ice crystals are present in the water column. Since skin is a good barrier to ice crystal propagation this presumably occurs across gill membranes (Valerio, Kao, and Fletcher 1992). Cold adapted marine fish can survive temperatures down to -1.7 ^oC due to the evolution of antifreeze substances in these species. Ice crystals generated at the periphery of the fish can be transported via the

blood to other tissues such as spleen (Praebel et al. 2009). Whether this occurs during conditions of frazil ice occurrence in super-cooled water in lotic environments (see below) is unknown. Ice crystal formation in tissues is detrimental to fish.

Frazil Ice

Frazil ice is a common occurrence in lotic environments during the winter and is reported to cause adverse effects on fish. There is limited evidence that frazil ice directly kills fish, but this has been poorly studied. Mortality has been reported in radio-tagged juvenile rainbow trout within 3 days of a frazil-ice episode, but only a few carcasses were found, and the cause of death in these fish could not be determined (Simpkins, Hubert, and Wesche 2000). Brown et al (Brown, Stanislawski, and Mackay 1993) cites a 1938 publication from Germany that describes frazil ice forming in the upper layer of a pond during a windy, cold, clear night causes a die-off of fish due to suffocation. It was speculated that suspended ice crystals impeded respiration by physically obstructing the oral cavity and/or gills. Brown has also speculated that suspended ice crystals could abrade the gill epithelium causing hemorrhage and erosions (Brown, Stanislawski, and Mackay 1993). An analogy was made to the damage to gill epithelial produced by barbed spines on species of diatoms. The referenced paper describe the diatoms causing excess mucus production and hemorrhage, which overtime led to hypertrophy and hyperplasia of gill epithelium (Yang and Albright 1992). Although the spines from this particular diatom have barbs that anchor it to the gill epithelium and are therefore likely to produce different and more severe lesions than ice crystals, the pathogenesis might be similar; however, gill lesions associated with ice has never been reported. In fact, some Antarctic fish are known to spend considerable time in water under sea ice, which contains suspended ice crystals, with no reported adverse effects (Praebel et al. 2009). If abrasions did occur to gill epithelium it could potentially affect gas exchange and make fish more prone to hypoxia. It could also affect acid-base balance, osmoregulation and excretion of ammonia (Speare and Ferguson 2006).

To my knowledge there is only one published report of experimental exposure of fish to frazil ice (Brown et al. 1999). In this experiment they exposed juvenile and adult rainbow trout to super-cooled temperatures, frazil ice, and anchor ice in a refrigerated flume. They found that plasma chloride, sodium and potassium levels were significantly reduced in juvenile rainbow trout after 6.5 hours under these conditions. These changes were attributed to a generalized stress response (Wendelaar Bonga 1997). Plasma glucose was increased (not statistically significant) but lactate had not changed. Changes in blood parameters of adult fish were not significantly different, but their swimming activity was reduced. No mortalities were observed. The duration of exposure to frazil ice in this experiment was limited to 6.5 hours and therefore the effects of more prolonged or repeated exposures is unknown.

The most significant effect of frazil ice in rivers and streams is displacement of fish due to its accumulation in the water column or on the substrate (anchor ice), and the formation of unstable ice dams that form and collapse causing dramatic shifts in water levels. Frazil ice can cause fish to congregate at high densities in suboptimal habitat. Conditions during the winter of 2018-19 had the potential to produce excessive amounts of frazil ice and it may have affected the movement of fish in the UFR. This is discussed in other SME reports (Wright, Greenacre, and Hatfield 2021; Whelan and Hatfield 2021).

Hypoxia

A common cause of fish kills is oxygen depletion or hypoxia. This condition occurs when the oxygen demand of all aquatic organisms in an area depletes oxygen dissolved in the water to a level that causes mortality in fish. Species have varying tolerances to oxygen depletion. Warm water adapted species tend to be more tolerant of low oxygen levels and are often able to survive in water with 2-3 mg/L dissolve O_2, whereas cold water adapted species need at least 4-5 mg/L (Tucker 1993).

Although commonly stated that large fish of a particular species die first whereas smaller fish may survive (Herman and Meyer 1990; Noga 1996b) this is not always the case and in fact may not generally hold true. Earlier studies investigating the effect of body size on tolerance to low dissolved oxygen were plagued by poor experimental design making interpretation and comparisons of results difficult (Nilsson and Östlund-Nilsson 2008). Many of the studies focus on embryos, larval stages and juveniles which is likely not applicable to adult fish. A recent review of the literature draws a couple of conclusions based on physiological mechanisms (Nilsson and Östlund-Nilsson 2008) . The first is that body size has little or no influence on a fish's ability to take up oxygen during hypoxic conditions because the respiratory surface area of gills matches the metabolic rate over a wide size range. Secondly, during severe hypoxia and anoxia fish generally must rely on anaerobic metabolism to produce adenosine triphosphate (ATP), the energy source for cellular metabolism. Anaerobic metabolism is inefficient and rapidly depletes glycogen stores. Small fish have less stored glycogen than larger fish and therefore will deplete stores faster. As well, lethal levels of acidosis, a by-product of anaerobic glycolysis, will be reached sooner in small fish compared to large (Nilsson and Östlund-Nilsson 2008). In a study on the tolerance of various stocks of cutthroat trout to hypoxia and other water quality parameters, larger fish were not more susceptible to hypoxic conditions than small fish (Wagner, Arndt, and Brough 2001). Under natural conditions fish will often move to avoid water with low oxygen tension and larger fish are more likely to seek out water with higher oxygen concentrations (Burleson, Wilhelm, and Smatresk 2001).

There is an inverse relationship between the amount of oxygen that can be dissolved in water and its temperature, so hypoxia in fish is more common in the summer. The main inputs of oxygen into water comes from the water surface, which can be enhanced by turbulence, and via photosynthesis by aquatic plants and algae. Fish kills often occur in calm lakes just before sunrise when the combined respiration from all aquatic organisms has depleted the oxygen created by photosynthesis during the day. Warm eutrophic lakes with high rates of decomposition can rapidly deplete oxygen levels at night. If dissolved oxygen levels are measured during the day they are often high and the hypoxic conditions that occurred at night may be missed (Noga 1996b).

Ice and snow cover prevent oxygen exchange at the water surface and can diminish or block sunlight, preventing photosynthesis. Cold temperatures reduce the metabolic rate of poikilothermic animals which slows oxygen demand, but if animal densities are high, and there is decomposition of macrophytes, periphyton, algae, and other aquatic organisms, oxygen levels can decline to levels where fish start to die (Barica and Mathias 1979). Although less well documented in lotic environments, these conditions can occur in pools and oxbows where water flow is reduced or absent, and water volume is reduced due to ice. Decomposition of organic matter, plus the respiration of fish and other aquatic organisms if in high enough densities, can deplete oxygen levels to the point where fish die.

Although speculative, given that winter is typically the most stressful season for fish in temperate zones, the frequency at which winter die-offs have been reported as a cause of sudden and dramatic

population declines, and the difficulty of finding carcasses during winter, a winter kill event is a plausible explanation for the UFR WCT population decline.

Anthropogenic Causes

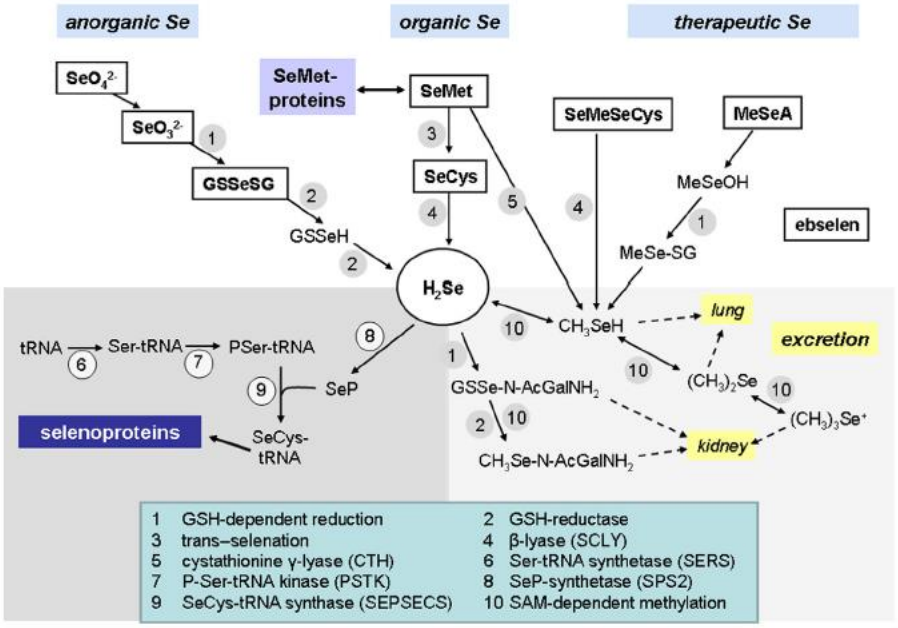
As part of the discussion on pathophysiology, I have evaluated water quality parameters and anthropogenic factors identified by other SMEs as being potentially contributory to the population's decline.

Selenium

The literature on selenium toxicity is extensive and will not be reviewed in detail here. More information can be found in reviews by Janz et al (Janz et al. 2010) and others (Hamilton 2004). As well some of the results and interpretations are controversial (Hamilton 2003). Effects vary depending on species of fish, the concentrations and types of selenium and the parameters measured. Much remains to be learned regarding the pathophysiology of selenium toxicity in fish. This section will focus on information relevant to the WCT UFR population decline and discuss the lesions expected if selenium was a likely contributing factor in the decline. As well, the pathophysiology of selenium toxicity will be discussed briefly to evaluate whether selenium, in conjunction with other stressors, may have contributed to mortality of fish.

Selenium is an essential micronutrient which is ingested by vertebrates as inorganic selenate and selenite, or as selenomethionine (Se-Met). Other seleno-compounds are much less commons sources of selenium. Selenomethionine has similar chemical properties to methionine and may replace methionine in proteins of plankton, vegetation, and other organisms. Selenomethionine is the main dietary source of selenium in aquatic species. Within fish Se-Met may be trans-selenated to produce selenocysteine, converted to selenide, which is then incorporated into selenoproteins. Selenoproteins are biologically active proteins such as glutathione peroxidase, which is involved in antioxidant defense, and deiodinases involved in thyroid function (Hesketh 2008). Excess selenium is detoxified and excreted via methylation, or is bound to sugars (Hoefig et al. 2011) (Figure 2). The liver is the primary site of selenium metabolism, but selenium is present in all tissues to varying degrees.

Figure 2. A schematic representation of the metabolism of various forms of selenium in mammals from Hoefig (Hoefig et al. 2011).



The range between toxic levels of selenium and normal requirements for optimal health is relatively narrow. At least three mechanisms have been proposed to explain the toxicity and observed lesions. 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 in fish results in increased production of reactive oxygen species (ROS) which overwhelms antioxidant capacities of the cell resulting in oxidative injury to tissues. In addition, in some studies, alterations in the metabolism of glycogen and triglycerides has been implicated as the cause of toxicity 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 reactive oxygen species is thought to play an important role in selenium toxicity. Reactive oxygen species 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 reactive oxygen species 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 reactive oxygen species (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 glutathione (GSH) to the oxidized form of glutathione (GSSG) is an important pathway for removal of reactive oxygen species. A reduced ratio of GSH to GSSG is indicative of oxidative stress. Excess selenium and the production of reactive oxygen species can deplete antioxidant defenses leading to oxidative stress and tissue damage (Birnie-Gauvin et al. 2017).

Researchers have reported altered energy stores in fish exposed to elevated selenium (Table 1). Genomic, proteomic and metabolomic analysis indicated alteration in metabolic pathways, but responses often varied among species and tissues. In a metabolomic study of Atlantic salmon (*Salmo salar*) exposed to diets containing 15 mg selenite/kg WW or 15 mg Se-Met/kg WW there were marked changes in metabolites affecting pathways of lipid catabolism, endocannabinoid synthesis and antioxidant levels. Changes were greatest in selenite exposed fish (Berntssen et al. 2017). Muscle Se concentrations at the end of this study were approximately 12 mg/kg dry weight. Although these studies are still too preliminary to identify tissue selenium thresholds where altered energy metabolism might affect survival, levels of greater than approximately 15-20 mg/kg DW in muscle or whole body appear relevant. In these experiments there was no affect of elevated dietary selenium on fish survival, but this mechanism could have implications for fish under natural conditions where energy is being mobilized during periods of low food availability or in conjunction with other stressors.

In the UFR, muscle selenium concentrations in 2018 ranged from 5.2 to 15 mg/kg dry weight and those in 2019 ranged from 9 – 30 mg/kg DW (Table 1;(Costa and de Bruyn 2021)). At muscle selenium concentrations greater than 15 mg/kg DW, alterations in energy metabolism will likely be occurring. The impact that these tissue concentrations would have on survival of WCT under prevailing conditions in the UFR is unknown. The potential role of selenium and other water quality constituents is discussed further in the Evaluation of Cause report (EoC Team, 2021).

Table 1. Summary of literature on effects on energy stores related to selenium exposure to 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 dry matter) 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
Water: 5 µg/l selenate& selenite Diet: Se-Met: 5 µg/g: 180 days: 20 °C & 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
Natural UFR (2018)	WCT					Muscle: 5.2 – 15 mg/kg DW	Costa and de Bruyn, 2021 Table A7

Natural UFR (2019)	WCT					Muscle: 9-30 mg/kg DW: 8/28 >20.8 mg/kg DW; 15/28 > 16.9 mg/kg DW	Costa and de Bruyn, 2021 Table A7

DW – dry weight
Juv. – juvenile
Mon. - months

Mechanistically there is evidence that high levels of selenium can alter metabolic pathways of glucose and triglycerides. Experiments in rodents demonstrated that high selenium levels activated selenoproteins and depleted chromium leading to increased lipolysis of adipose tissue and accumulation of triglycerides in hepatocytes of the liver. By various pathways this resulted in the relative proportion of reactive oxygen species being altered causing insulin resistance (Wang et al. 2014). Epidemiological studies have also revealed exposure to high levels of selenium in humans is associated with higher rates of type 2 diabetes (Stranges et al. 2010). A recent study in fish found that individuals exposed to high levels of selenium were unable to clear intraperitoneally injected glucose, compared to control fish, and the fish had increased levels of methylglyoxal (Pettem et al. 2018), a toxic metabolite of glucose present in diabetics (Kalapos 2013). As well, cataracts were observed in the high selenium treatment groups (Pettem et al. 2018) which is a lesion associated with hyperglycemia in other species.

Exposure to high doses of selenomethione under experimental conditions have resulted in changes in behavior and swim response (Table 2). The effect depended on species exposed and the dose. Implications of these findings to fish under field conditions remains to be determined.

Table 2. Summary of laboratory experiments examining the effect of selenium on behavior and neuromuscular systems.

Pathophysiological effect	**Species**	**Exposure**	**Tissue concentration**	**Reference**
Highest dose group: Impaired socially-learned predator avoidance, induction of oxidative stress, alterations in serotonergic system	Zebrafish	Diet: control, 3.6, 12.8, 34.1 µg/g Se-Met DW for 90 days	Whole body Se: 1.0, 2.2 and 4.27 µg/g WW, respectively. Highest dose group ~17 µg/g DW	Attaran et al, 2020
No significant effect on visual response to threat or burst-swimming behavior	Fathead minnow (*Pimephales promelas*)	Diet: Se-Met 0.09 (control), 2.9, 6.8 µg Se/g WW; 35 or 70 days.	Whole body Se: 35 days: ~ 0.05, 0.5, 1.1 µg/g WW. 70 days: ~0.05, 0.75, 1.0 µg/g WW. Highest dose groups ~ 4 – 5 µg/g DW	Anderson et al, 2019
Higher dosages (>15-60) disruption of dopamine and serotonin metabolism in brain, damage of neuromuscular system in skeletal muscle (histology)	Juvenile steelhead trout	Diet: Se-Met: 1.09 (control), 8.79, 15.37, 30.79, 61.58 mg Se/kg DW	Not determined	Lee et al, 2020
Mortality %: 15 (1.3 dose), 20 (3.4), 27 (9.8), 35 sig. dif. (27.5) Impaired swim performance: reduced critical swimming speed in fish diets > 3 µg Se/g and increased oxygen consumption.	Zebrafish adult	Diet: Se-Met 1.3 (control), 3.4, 9.8, 27.5 µg Se/g DW for 90 days	Whole fish: 1.6, 4.0, 7.6, 11.2 µg Se/g DW	Thomas et al, 2013

DW – dry weight
WW – wet weight
Sig. dif. – significantly different

In aquatic organisms, eggs and larvae are the life stages most sensitive to selenium. Although adult mortality can occur at high exposure concentrations, effects on early life stages, and the presence of deformities, is a more sensitive indicator of selenium levels impacting fish populations. Accumulation of selenium in tissues of adult fish results in maternal transfer to the egg, primarily the yolk. The metabolism of yolk containing high levels of selenium during development can cause embryo mortality, edema and deformities (Rudolph, Andreller, and Kennedy 2008; Holm et al. 2005). Deformities in larvae consist of stunting and deviation in the notochord (vertebral column) as well as craniofacial abnormalities including absent or deformed jaws. Differences among species in sensitivity to selenium have been reported and some researchers have reported cold water fish appear to be less sensitive to selenium than warm water fish. These conclusions are controversial (Hamilton 2003). The reproductive effects of dietary selenium also vary among species which may be related to the levels of selenium transferred to the eggs (Holm et al. 2005).

The teratogenic effects of selenium in fish are well substantiated; however skeletal deformities are not specific to selenium exposure. Rates of deformities in salmonids spawned in the laboratory range from <1-5% (Werner et al. 2006) and are slightly higher in wild salmonids (Holm et al. 2005). The reported causes of skeletal deformities in fish include: metacercarial stages of trematodes (Villeneuve et al. 2005), myxosporean parasites (Gilbert and Granath 2003), genetics (Afonso et al. 2000), elevated temperatures (Georgakopoulou et al. 2007) and other contaminants (Lindesjöö et al. 1994), to name a few. The increased prevalence of skeletal deformities in fish populations can have a variety of causes.

The occurrence of deformities and other external lesions in WCT from the UFR, and in WCT and other species of fish from other watersheds in the area, have been documented for several years and have been part of the Regional Aquatic Effects Monitoring Program since at least 2015 (Minnow 2018a, 2020a, 2020b, 2018b). The range of lesions recorded include: abnormal jaw, growth, scars, gashes, nasal dents, shortened operculums (unilateral and less frequently bilateral), curvature of the spine, and messed up jaws. Many of these lesions can be attributed to trauma, either recent or in the past, while others may be developmental defects. More recent reports have separated the external lesions into four categories: deformities, erosions, lesions and tumors (DELT), to allow some distinction between potential causes. Within the category of deformities, operculums shortened to varying degrees are most commonly reported; most often these occur unilaterally, but occasional they are bilateral. For example, in 2017 of the 110 WCT examined from Greenhills Creek 12% had deformities and all but one were described as having a shortened operculum (Minnow 2018b).

Gill cover or operculum defects can have different causes. Operculum defects are relatively common in farmed fish, but less so in wild fish (Beraldo et al. 2003). For example, the prevalence of gross abnormalities in hatchery-bred milkfish (*Chanos chanos* Forsskal) juveniles varied from 3-26% with branchiostegal membrane, branchiostegal ray and operculum defects reported as being most common, and of almost equal proportions, often occurring together (Hilomen-Garcia 1997). Operculum defects reduced survival, depending on their severity. Similarly, in hatchery reared European sea bass (*Dicentrarchus labrax*) operculum defects were the largest category of defect reported (Barahona-Fernandes 1982; Ortiz-Delgado et al. 2014). High levels of selenium in eggs can cause opercular defects but most appear to occur in conjunction with other craniofacial deformities (Holm et al. 2003). Fish that survive will carry these abnormalities into adulthood. Attributing a cause to opercular defects detected in the wild can be challenging. Elevated tissue selenium concentrations may be useful in identifying a cause, but they do not necessarily reflect concentrations present during larval development. Better

descriptions of gill and operculum lesions from fish with well characterized selenium exposure, including histology and examination of bony plates, may be useful in defining specific lesion characteristics associated with selenium toxicity. Although these are unlikely to be unique to selenium exposure, lesions with a similar pathogenesis should have common features (Beraldo et al. 2003; Ortiz-Delgado et al. 2014).

The pathogenesis of the operculum lesion in a WCT captured in Coal Creek near Fernie, BC, that has appeared in photographs in two recent publications (Lemly 2014, 2019), is unclear. No tissue selenium concentrations are provided, and in the photos there is virtually complete loss of one gill cover with a sharp linear margin. No other craniofacial abnormalities are visible. Attributing a cause of operculum lesions in individual fish, as shown in photos in Lemley (2014, 2015), is highly speculative.

Other gross and histological lesions have been attributed to selenium, primarily in older fish; however, in many cases evidence for causation is lacking or controversial. Sorensen et al. (Sorensen et al. 1984) describes various lesions in wild, green sunfish (*Lepomis cyanellus*) from Belews Lake, North Carolina, a lake with elevated selenium and arsenic levels due to fly ash contaminated sluice water from a coal generated power plant being discharged into the lake. The following lesions were reported: swollen and vacuolated gill epithelium, reduced hematocrits, increased numbers of Kupffer cells in the liver and vacuolation of hepatocytes around central veins, lymphocyte infiltration of the hepatopancreas, focal intra-capillary proliferative glomerulonephritis, pericarditis, and necrotic and rupture ovarian follicles. Sorensen et al. refers back to literature from the 1930s on selenium exposures in rodents and other mammals for comparable lesions (Smith, Stohlman, and Lillie 1937). These reports have several deficiencies including lack of detail on source of experimental animals, low numbers of individuals examined, lack of controls, and absence of a dose response.

Histopathological evaluation of fish exposed to high levels of selenium under experimental conditions have reported a variety of lesions. Rainbow trout (*Salmo gairdneri* Richardson) fed high levels of selenite were reported to develop nephrocalcinosis (Hicks, Hilton, and Ferguson 1984). Sacramento splittail (*Pogonichthys macrolepidotus*) fed varying levels of selenium in their diet, ranging from 12.6 to 57.6 µg Se/g dry weight for 5 and 9 months, showed liver pathology consisting of macrophage aggregates, glycogen depletion, single cell necrosis, fatty vacuolar degeneration and eosinophilic protein droplets (Teh et al. 2004). Splittails from this previous experiment allowed to undergo a period of depuration showed hypertrophy and necrosis of tubular epithelial cells, renal tubular casts but no significant liver lesions except for basophilic foci in two fish (Deng, Hung, and Teh 2007). Juvenile steelhead trout (*Oncorhynchus mykiss*) fed elevated levels of selenomethionine for 2 and 4 weeks showed increased lymphocyte infiltration, boundary loss and homogeneity of hepatocyte cytoplasm, and single cell necrosis (Lee et al. 2020). Histopathology can be a good biomarker for toxicological effects of selenium under natural conditions but additional studies are still needed to better characterize lesions and understand their pathogenesis.

Telangiectasis of gill lamellae has been reported in bluegill (*Lepomis macrochirus*) fish experimentally exposed to selenium (Lemly 1993). Lemly (Lemly 2002) subsequently includes a description and photograph of this lesion in a review of selenium toxicity in fish and reports this lesion was described in green sunfish from Belews Lake including a photograph referencing Sorensen et al. (Sorensen et al. 1984). There is no reference to this lesion in the Sorensen et al paper and the photograph is identical to the photograph in Lemly's original bluegill experimental trial paper. To my knowledge gill lamellar

telangiectasis has not been described in any other field or experimental trials examining selenium toxicity.

Telangiectasis of gill lamellae can be caused by rough handling during euthanasia (Ferguson 1989). In the experiment reported by Lemly, fish were “anesthetized, and killed by severing the spine”(Lemly 1993). As well, the lesion description and photographs do not correspond with what is known of the pathogenesis and progression of this lesion. Telangiectic lesions in gill lamellae often thrombose and later undergo fibrosis and resorption over time (Ferguson 1989). Given that this was a chronic selenium exposure experiment under laboratory conditions, lesions of varying age in the gills examined, including subacute to chronic lesions with thrombosis and fibrosis, respectively, would be expected. Further research is needed to verify this finding before an association between selenium exposure and gill telangiectasis can be made.

Based on water quality data collected during the decline window (Costa and de Bruyn 2021) elevated selenium levels by themselves are unlikely to be cause of the population decline. They could potentially be an indirect cause of mortality in conjunction with other stressors.

Winter Stress Syndrome

The concept of “winter stress syndrome” was proposed by Lemly (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^{o}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. Reportedly these stressors, in combination, create a metabolic deficit resulting in severe lipid depletion and mortality (Table 3). These findings have not yet been replicated under experimental or field conditions.

McIntyre et al (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 ^{o}C followed by a weekly decline of 2 ^{o}C/week until reaching 4 ^{o}C in exposure system one (ES1) and two (ES2) and until reaching 9 ^{o}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 ^{o}C and the other two were lowered to 4 ^{o}C over the remaining weeks. In ES1 and ES2 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 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 ug/g DW in the McIntyre study. However, in the other experimental exposures, as whole-body Se concentrations reached 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 ~ day 45 at temperatures of 14 ^{o}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 highest treatment group (ES1), there were 11 fish found dead 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 20-9 °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. The toxicity of selenium to bluegill sunfish was 1.9 times less in the McIntyre study.

The high mortality rates in bluegill sunfish at whole body selenium concentrations of 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 appears to be significant differences among fish species in response to selenium and/or temperature.

Table 3. 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

Mortality was different between the Lemly and McIntyre et al. 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. study, mortalities, which only occurred in the high treatment groups, began before temperatures reached 4 °C and 9 °C. In ES2, meant to replicate the Lemly 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 already ~ 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. (DeForest, Brix, and Adams 1999), without cold stress. They reviewed existing literature and estimated an EC50 for larval mortality of 19 mg/kg DW dietary selenium and estimated 100% mortality at 40 mg/kg DW. This study also estimated the EC10 for larval mortality at 10 mg selenomethionine/kg DW in the diet. In the McIntyre et al. 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 and 9 °C in ES1 and ES3, respectively. For comparison, the Lemly "winter stress" study and the replicate by McIntyre et al. exposed fish to 5 mg/kg DW dietary selenomethionine.

Attempts to demonstrate winter stress syndrome under field conditions have been unsuccessful. Driedger et al. (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 dry wt. Bennett and Janz (Bennett and Janz 2007) similarly showed 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 contaminants in the water; however, exposure levels were well 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.

Under controlled experimental conditions, excess selenium has been reported to alter energy metabolism in fish. Adult zebrafish fed Se-Met at concentrations from 3.7, 9.6 and 26.6 ug/g dry weight for ~ 100 days had elevated whole body triglycerides and glycogen concentrations compared to fish fed a control diet of 1.3 ug/g dry weight. Critical swimming speeds were reduced in treatment groups and fish in the high dose group also had increased whole body cortisol (Thomas and Janz 2011). Rainbow trout fed high but environmentally relevant levels of Se-Met for prolonged periods had elevated concentrations of glycogen and triglycerides in their livers compared to controls (Pettem et al. 2018). An observed down-regulation of liver citrate synthase mRNA was hypothesized to be the cause. Citrate synthase is a key component of the citric acid cycle of cellular respiration, and if reduced, hepatocytes

could accumulate glycogen and lipids. Trout in the high dose group were also unable to properly metabolize intraperitoneally injected glucose and had excess levels of the toxic glucose metabolite, methylglyoxal. Since mobilization of triglyceride stores are required for energy during the winter, these selenium induced alterations may have implications for over winter survival of fish.

In an in vitro study examining the effect of selenomethionine on trout intestinal epithelial cells at different temperatures researchers found cells at 4^0C remained viable when exposed to high doses (500 and 1000 µM) whereas cell viability was significantly reduced at 14, 18 and 26^0 C. Energy metabolism was markedly reduced in the intestinal epithelial cells at 4 ^{o}C, which may explain the difference in response to Se-Met (Kim et al. 2018).

Although the term "winter stress syndrome" appears frequently in the aquatic contaminants' literature, especially in relation to selenium exposure, there is currently limited direct evidence supporting this hypothesis. More research needs to be done to determine if or when this occurs. For the purposes of the Evaluation of Cause, the approach taken (EoC Team, 2021) was to evaluate stressors that could contribute to the population decline in WCT from first principles, rather than focusing on any particular combination as a syndrome, such as that described by Lemly. As a result, the Evaluation of Cause looked at the same stressors as Lemly evaluated, in addition to several others.

Cyanobacteria

Another SME report (Larratt and Self 2021) evaluates the potential for cyanobacteria in the UFR to be contributory to the WCT population decline. This review is intended to augment that work.

Although cyanobacteria can be toxic to mammals documentation of direct toxicity of fish is less well characterized (Tucker 1993). Large fish kills are occasionally associated with cyanobacterial blooms but many of these incidents have been attributed to oxygen depletion or increased ammonia concentrations (Zurawell et al. 2005), indirect effects of the bloom. Phillips et al (PHILLIPS et al. 1985) reported *Microcystis aeruginosa*, a common fresh water cyanobacterium, was non-toxic with whole body immersion but caused mortality when exposure occurred via intraperitoneal inoculation. They concluded that these findings, along with the lack of reports of *M. aeruginosa* causing mortality in wild fish despite its abundance, indicated this organism is not generally toxic under normal conditions. The difficulty in determining direct and indirect effects of cyanobacterial blooms is well described in a recent paper by Landsberg et al (Landsberg et al. 2020). They describe the investigation of a multispecies fish kill that lasted for 6 weeks along a 50 km stretch of the Lower St. Johns River in Florida during 2010, associated with a cyanobacterial bloom caused by *Aphanizomenon flos-aquae*. The decomposing bloom triggered a complex series of events with several possible scenarios for a cause of the fish mortality. The pathological changes in the fish were not consistent with acute cyanotoxin exposure but were attributable to chronic lethal hemolysis likely caused by other organisms, including hemolytic bacteria, which were more abundant due to altered environmental conditions.

Nevertheless, there are reports that implicate cyanobacterial hepatotoxins in fish die-offs. A large cyanobacterial bloom caused by *Anabaena flos-aquae* in a shallow eutrophic freshwater lake in Scotland was associated with dead and dying brown trout over a period of 2 days. In this case histopathological lesions were consistent with cyanotoxins. The lesions included liver degeneration and necrosis and necrosis of gill lamellar epithelium. Microcystins, the most common and best studied cyanobacterial hepatoxins was identified and its toxicity demonstrated via mouse inoculation (Rodger et al. 1994). In

general acute toxicities due to microcystins in fish are quite rare and even under experimental conditions of oral and immersion exposure fish are relatively resistant to adverse effects (Zurawell et al. 2005). Confirming that cyanobacteria are a direct or indirect cause of fish mortality requires and in-depth investigation including histopathology, toxin identification and testing as well as ruling out other causes of mortality.

Cyanobacterial blooms occur in freshwater and estuarine ecosystems and are most often associated with eutrophic conditions. Stability of the water column both vertically and horizontally allow for the buildup and persistence of blooms and therefore large blooms are most often associated with lakes and estuaries during periods of calm conditions (Paerl 1988). In these systems blooms typically occur in the summer or early fall when temperatures and nutrient levels are optimal for growth.

In the UFR 20 different cyanobacteria capable of producing cyanotoxins have been identified, of which *Phormidium autumnale* (synonym *Microcoleus autumnalis*) is considered of most concern (Larratt and Self 2021). There is a reported recent rise in the proliferation of toxin producing *Phormidium* in rivers worldwide and it has been suggested factors such as changes to riparian zones, increased nitrate and fine sediment, and alterations in water flow are among those contributing to this increase (McAllister, Wood, and Hawes 2016). Anatoxins appear to be the most common cyanotoxin produced by *Phormidium autumnale* (Wood et al. 2020). Anatoxins bind to and activate neuromuscular nicotinic receptors of the neuromuscular junction and because they are not degraded by cholinesterase they continually stimulate myofibers causing twitching, fatigue and paralysis. Anatoxins produced by *Phormidium* have been reported as the cause of mortality in dogs which had ingested periphyton mats (Wood et al. 2007) but there don't appear to be reports of toxicity in other mammalian species under field conditions. Exposure to anatoxins have been reported to give fish a musty taste (Wood et al. 2017) but to my knowledge there are no reports of anatoxins being associated with mortalities.

No die-offs were detected during the population decline time period in the UFR. This combined with the relative resistance of fish to cyanotoxins, suggest that cyanobacteria are a very unlikely cause of the WCT population decline.

Ammonia, Nitrite and Nitrate

Ammonia toxicity is a common problem in cultured fish when stocking densities are high and there is a build up of waste and decaying organic material. In established culture systems and under natural conditions ammonia is converted to nitrite (NO_2^-) by bacteria, primarily *Nitrosomonas* sp. which is then subsequently converted to nitrate (NO_3^-) by bacteria, primarily *Nitrobacter* sp. In ponds, ammonia levels increase during the day, and toxicity is most likely to occur around sunset when pH and temperature are highest. Ammonia levels can also increase in natural ponds in the fall and winter potentially due to decreased algal and bacterial metabolism of ammonia.

Anthropogenic sources of ammonia to surface and groundwater include sewage effluent, agricultural run-off and residues from blasting at mine sites.

Ammonia is present in two forms, unionized ammonia (NH_3) and ionized ammonium (NH_4^+). The un-ionized form is most toxic to fish as it readily moves across gill membranes. Cold increases the toxicity of unionized ammonia to fish. In rainbow trout the 96-h LC50 for un-ionized ammonia nitrogen was 0.47 mg/L at 3-5 ^oC compared to 0.76 mg/L at 13-15 ^oC (Reinbold and Pescitelli 1982). The relative concentrations of un-ionized ammonia to ammonium ion is dependent on temperature and pH, with

each unit rise in pH increasing the former by 10-fold and each 10 ^{o}C rise increasing it 2 -fold (Erickson 1985). This does not adequately explain toxicity under various conditions and a joint toxicity model is proposed (Erickson 1985).

Acute ammonium toxicity in fish results in hyperventilation, hyperexcitability and coma. The mechanism by which these clinical signs occur is poorly understood. Fish excrete ammonia across gill membranes down a concentration gradient. When concentrations are high in water excretion rates are reduced.

Although some reports have associated chronic ammonia exposure with gill hypertrophy and hyperplasia chronic experimental exposures have failed to demonstrate histopathological changes in the gill (DAOUST and FERGUSON 1984).

The combined effect of ammonia and other water quality parameters have been investigated. Zhao et al (Zhao et al. 2020) reported in large mouth bass under experimental conditions a combination of hypoxia (1.2 mg/L O_2) and ammonia (6.0 mg/L: 0.13 mg/L NH_3) induced an antioxidant response and oxidative stress, decreased liver glycogen, and induced genes related to apoptosis and inflammation. Each treatment group (ammonia and hypoxia alone, and both simultaneously) had histological changes in gills, including hyperplasia of lamellar epithelium and chloride cells, epithelial necrosis as well as lamellar fusion. The combined group had the most severe gill lesions. In another study the combination of unionized ammonia (0.05 mg/L) and total suspended solids (25 mg/L) in rainbow trout had no adverse affects.

Nitrite

Ammonia in the presence of oxygen is converted to nitrite by species of *Nitrosomonas* bacteria. Nitrite is further metabolized to nitrate by species of *Nitrobacter* bacteria. The relative proportions of ammonia, nitrite and nitrate is determined by numbers and metabolic rates of these bacteria. Nitrite poisoning can occur when water temperature are reduced due to differences in temperature optima of *Nitrosomonas* and *Nitrobacter* (Noga 1996a).

Nitrite changes hemoglobin to methemoglobin which is unable to carry oxygen. Blood with a high concentration of methemoglobin is brown in color. If in high enough concentrations nitrite can be acutely toxic. These concentrations are affected by the dissolved oxygen levels of the water, exacerbating the hypoxia. Chloride, bicarbonate and calcium can reduce the toxicity of nitrite (Lewis and Morris 1986).

Nitrate

Mortality of larvae of some salmonid species occurs at nitrate concentrations of approximately 2.3 – 7.6 mg/L (Kincheloe, Wedemeyer, and Koch 1979). The long term effects of nitrates at low concentrations is poorly understood but there is some evidence that nitrate can affect steroidogenesis and disrupt endocrine function (Guillette and Edwards 2005). Although nitrate levels were increased in some areas, at sometimes, during the decline window, these levels were not considered to be significant and are discussed in more detail by Costa and de Bruyn (2021).

Sulphate

Sulphate concentrations in the Upper Fording River are increased relative to baseline levels due to mine related activities, but the majority (over 80%) of measured concentrations during the decline window were below the long-term BC WQG (Costa and de Bruyn 2021). Sulphate concentrations that were

above guidelines were spatially restricted and therefore were determined to impact only a small proportion of WCT in the UFR. Although not considered directly responsible for the decline, the literature on the pathophysiology of sulphate was briefly reviewed to assess whether it had the potential to be a contributory stressor to this population.

The physiological and toxic effects of sulphate cannot be considered in isolation as sulphate creates its effect through ionic imbalances. Total dissolved solids (TDS) are an integrative measure of the concentrations of common ions (sodium, potassium, calcium, magnesium, chloride, sulfate and bicarbonate) in fresh water, and is generally an adequate predictor of toxicity. Sulphate is considered to be the least toxic of these ions in freshwater systems (Mount et al. 1997).

The physiology of osmoregulation and ionoregulation in freshwater animals, as it relates to toxicopathology, has been recently reviewed (Griffith 2017). The gills of freshwater fish appear to be impermeant to sulphate (de Renzis and Maetz 1973) due to the absence of transporters and intercellular tight junctions that prevent its passage. Loss of epithelium due to cell death or alterations of epithelial tight junctions due to sublethal injury, making them more porous, can result in passive influx of sulphate down a concentration gradient (Hobe 1987). Although not extensively studied, sulphate transporters have been identified in renal and intestinal epithelium of some freshwater fish which may play a role in osmoregulation (Oikari and Rankin 1985; Nakada et al. 2005).

Based on the current literature sulphate would only be a contributory stressor if there was injury to epithelial surfaces, such as those found on the gills, allowing it to diffuse into the fish, potentially causing osmotic stress or ionic imbalances. Injury to the epithelium would also affect rates of diffusion of water and regulation of other ions.

Anthropogenic Noise

Anthropogenic noise in aquatic environments, from activities such as seismic blasts, detonation of explosives, boat traffic, wind turbines and pile-driving, can have detrimental effects on fish and other aquatic organisms. Some underwater high intensity sources of sound, such as pile driving, can be detected several thousand kilometers from its source. Effects of sound on fish can vary from avoidance behaviour; which could affect feeding, breeding and reproduction, to injury affecting auditory tissues resulting in hearing loss, to acute mortality associated with barotrauma type injuries (Popper and Hawkins 2019).

Onshore construction work (blasting, pile driving, etc.) can also produce sound in adjacent aquatic environments. The most significant effect is from shock waves travelling through the substrate to water where it continues to move as a pressure wave through the water column. Sound waves in air can also transmit into water but is less significant.

Pile driving and blasting are particularly intense forms of impulsive aquatic noise that have the potential to injure or kill fish. One mechanism of reported injury is the result of pressure waves causing rapid movement of the walls of the swim bladder, causing hemorrhage of the swim bladder and adjacent tissues. Fish without swim bladders do not sustain this type of injury. Fish with a closed (physoclistous) swim bladder have more frequent and severe injuries, including mortality, and those with an open (physostomous) swim bladder have similar but less severe injuries, particularly at higher sound levels. Physostomous swim bladders, those with a pneumatic duct connecting the swim bladder to the gut, are found in salmonids, sturgeons and other similar fish. Injuries reported under experimental conditions

consist of deflated swim bladders and hemorrhage of swim bladder, kidney and intestine. External injuries are not typically observed (Dahl et al. 2020).

Noise was raised as potential stressor on the UFR during the 2017-19 time period because blasting can occur relatively close to the river, specifically at Eagle Mountain (2.6 – 4 km from the UFR), Lake Mountain (0.4 -1.1 km) and Swift Mountain (0.9 - 1.6 km). Blasting occurs at all operations in the Elk Valley at a rate roughly proportional to the operation's production and since Fording River Operations is the largest Teck Coal operation in the Elk Valley it requires the most blasting. Blasting occurs during the day (7:00 am to 7:00 pm). Between 2013 -2016, there were approximately 190-220 blasts per year (or a monthly average of ~15.8 – 18.3 blasts). In 2017, 2018, and 2019, there were 262, 259, and 270 blasts respectively (or a monthly average of ~21.6 – 22.5 blasts). Specifically, within the Decline Window (September 2017-September 2019 inclusive), there was a total of 552 blasts at FRO (an average of 22 blasts monthly). All new blasting activity started prior to the start of the Decline Window, where the most recent changes were: 1) blasting activity in Lake Mountain, which began in September of 2016 after a hiatus since ~1992, and 2) blasting activity in Swift which began in February of 2017 after a hiatus since ~1990 (Sword, G and Fitzgerald, R. personal communications 2020).

Each blast involves the detonation of 600 kgs of ammonium nitrate in 15 m deep blastholes and on average there are 300 blastholes per blast (Sword, G and Fitzgerald, R. personal communications 2020). Canadian guidelines for setback of blasting activity from fish habitat uses a overpressure threshold of 100 kPa at the location where fish may be present in order to prevent damage to swim bladders. The setback distance is calculated by multiplying the square root of the charge weight by a "K" factor, which for rock is 5.03 (Wright and Hopky 1998). Using 600 kg charges for the calculation, the minimum distance to fish habitat for the UFR is 123.3 m. Although there are several blast holes along a line that could add to the pressure field at any one point, the blast distances to the UFR are well within guidelines.

Noise levels and ground vibrations are not currently monitored on the UFR in units that can be interpreted for effects on fish. Thus, it is not possible to directly measure the effect of this activity on WCT; however, because WCT are physostomes (defined above), the potential for direct mortality is low. Blasting noise could have resulted in movement of fish away from affected areas during the blasting period. As well, anthropogenic noise can induce stress in fish causing elevated cortisol and elevation of other stress associated parameters as well as impaired reproduction (Sierra-Flores et al. 2015; Celi et al. 2016). Initially these stress responses can be adaptive, but if chronic or severe they may alter physiological processes and be detrimental to the fish (Popper and Hawkins 2019).

Although there is still a lot to learn about the effects of anthropogenic noise in aquatic environments, particularly as it pertains to fish, there is no evidence pile driving or explosive detonations onshore would have been directly responsible for fish mortality, and although it may have affected fish behaviour and movement, it is unlikely this could have contributed to the population decline.

Cumulative Effects

Since no single stressor was identified as the sole cause of population decline of WCT on the UFR it is possible that multiple stressors may have combined to reduce survival of fish. Since the specific combinations of stressors present on the UFR have not been evaluated under experimental or field

conditions the potential for this to occur is hypothetical but is based on the underlying mechanisms by which these stressors affect the physiology of the host.

There are two main pathophysiological mechanisms by which the various stressors could have combined to contribute to the population decline. The first is increasing oxidative stress and the second is related to fish energetics.

Firstly, increased oxidative stress can cause damage to membrane lipids, both plasma membranes and intracellular membranes, leading to loss of intracellular ionic homeostasis. Stressors that can set up an oxidizing environment include elevated levels of selenium, hypoxia and ammonia (Misra, Hamilton, and Niyogi 2012) (Zhao et al. 2020).

The ratio of reduced to oxidized glutathione is a measure of oxidative stress. This ratio has been demonstrated to decline in a dose-dependent manner in cultured trout hepatocytes exposed to increasing levels of Se-Met (Misra, Hamilton, and Niyogi 2012). Similarly, hypoxia and ammonia both induced oxidative stress in largemouth bass and when fish were exposed to both the deleterious effects were greater than the individual stressors alone. Cold can increase the oxidative stress of fish with the potential for cell damage (Lu et al. 2019). Cold also results in lipid remodelling characterized by increased levels of unsaturated fatty acids in cell membranes. Unsaturated fatty acids are more susceptible to oxidative damage which could potentially increase the risk of lipid peroxidation from increased levels of reactive oxygen species (Grim, Miles, and Crockett 2010).

Secondly, winter is a stressful time when temperatures are low, food resources are limited and there are varied demands on energy stores. Although metabolism and physical activity are generally reduced during this time, adverse conditions caused by ice, low oxygen levels and other factors may cause fish to move which would increase energy demands. Research shows that high selenium exposure can affect triglyceride and glycogen metabolism under laboratory conditions. If this translates to significant effects under field conditions this, in conjunction with natural stressors of cold and varying water parameters, could impact survival of fish.

The winter of 2019 on the UFR was long and cold. Two of three temperature data-loggers on the UFR recorded over 100 days where water temperatures were <1 ^{o}C, which was longer than those reported in previous years. This extended period of cold would have been an additional stress affecting physiological processes such as metabolism and oxidative stress.

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