Subject Matter Expert Report: Infectious Disease. Evaluation of Cause - Decline in Upper Fording River Westslope Cutthroat Trout Population

July 27, 2021

Prepared for:

Teck Coal Limited

Suite 1000 – 205 9th Street SE

Calgary, AB, T2G 0R3

Prepared by:

TKB Ecosystem Health Services Ltd

Published by TKB Ecosystem Health Services Ltd., PO Box 216, Dundurn, SK, S0K 1K0

Citation:

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.

USE AND LIMITATIONS OF THIS REPORT

This report has been prepared by TKB Ecosystem Health Services Ltd for the use of Teck Coal Ltd. (the “Client”). In providing this report and performing the services in preparation of this report TKB Ecosystem Health Services Ltd accepts no responsibility in respect of the site described in this report or for any business decisions relating to the site.

This report and the assessments and recommendations contained in it are intended for the sole and exclusive use of the Client. Any use of, reliance on, or decision made by a third party based on this report, or the services performed by TKB Ecosystem Health Services Ltd in preparation of this report is expressly prohibited, without prior written authorization from TKB Ecosystem Health Services Ltd. Without such prior written authorization, TKB Ecosystem Health Services Ltd accepts no liability or responsibility for any loss, damage, or liability of any kind that may be suffered or incurred by any third party as a result of that third party’s use of, reliance on, or any decision made based on this report or the services performed by TKB Ecosystem Health Services Ltd in preparation of this report.

The findings contained in this report are based, in part, upon information provided by others. In preparing this report, TKB Ecosystem Health Services Ltd has assumed that the data or other information provided by others is factual and accurate. If any of the information is inaccurate, site conditions change, new information is discovered, and/or unexpected conditions are encountered in future work, then modifications by TKB Ecosystem Health Services Ltd to the findings, conclusions and recommendations of this report may be necessary.

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.

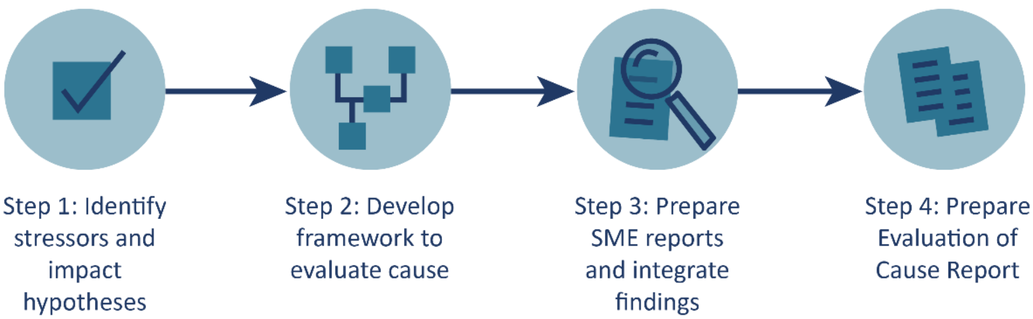
The report that follows this Reader's Note is one of those Subject Matter Expert Reports.

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.



1 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:

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).

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.

Table of Contents

EXECUTIVE SUMMARY

An ~ 90% reduction in predominately adult Westslope Cutthroat Trout (WCT) was reported from the upper Fording River (UFR) over the time period from September 2017 to September 2019 (Cope 2020). As no die-off events were identified during the population decline and only a few carcasses were found during this time period, none of which underwent complete necropsies, there is no information on diseases or chronic health issues within the WCT population on the UFR, if present. Disease in the broad sense is any impairment of the normal physiology of an individual that adversely affects its response to biological and environmental factors. The specific hypothesis that was addressed in this report was that infectious disease was the sole or contributing cause of the WCT population decline in the upper Fording River. Although carcasses can be lost due to scavenging and decomposition and may be difficult to detect during certain times of the year, even in a river that is visited relatively frequently, the fact that accumulations of carcasses were not reported during the decline window suggested that specific infectious diseases were unlikely to have occurred during this time period. The decline was also characterized by affecting predominately adult aged fish which ruled out other diseases. Finally, based on typical clinical signs, seasonality and age classes affected, plus expected lesions, infectious disease is considered not a likely sole cause of the population decline although infectious agents cannot be ruled out as the direct cause of mortality in circumstances where fish are immunosuppressed due to other stressors.

Introduction

Background

Overall Background

This document is one of a series of Subject Matter Expert (SME) reports that support the overall Evaluation of Cause into the upper Fording River Westslope Cutthroat Trout (WCT) population decline (Evaluation of Cause Team, 2021). For general information, see the preceding Reader's Note.

Report-specific Background

Stressor investigated: infectious diseases

Disease in the broad sense is any impairment of the normal physiology of an individual that adversely affects its response to biological and environmental factors. Disease varies in severity from its most severe form resulting in mortality, to mild or subclinical disease. Mild or subclinical disease may become significant if the host experiences additional stressors, the outcome of which may result in exacerbation of the original disease, enhancement of the effects of the new stressor, or both. Disease is multifactorial, which in its simplest form can be described as the interplay of factors inherent to the pathogen, host and environment. These interacting factors can be visually represented as a web of causation (Figure 1). This figure depicts some of the potential stressors acting on the WCT population on the UFR and how they may interact to cause disease or mortality. Many of these stressors are discussed in detail by other SMEs. How these factors may have contributed to the population decline will be explored in more detail in the Evaluation of Cause report (Evaluation of Cause Team, 2021).

Although there are various ways of describing disease progression, terms used commonly in epidemiology are direct and indirect causes. The direct cause is the most recent factor that has contributed to a change in the health of an individual and the indirect cause or causes are the contributing or historical factors that have led to this new state. The outcome from exposure to a direct cause is dependent on the underlying indirect causes. The potential for disease to explain the WCT population decline will be described in this context but the term "impact hypothesis" will be used for consistency with other SME reports to refer to both the direct and indirect effects of a particular disease or etiology.

This report addresses the impact hypothesis that infectious disease either caused or contributed to the Upper Fording River Westslope cutthroat trout population decline.

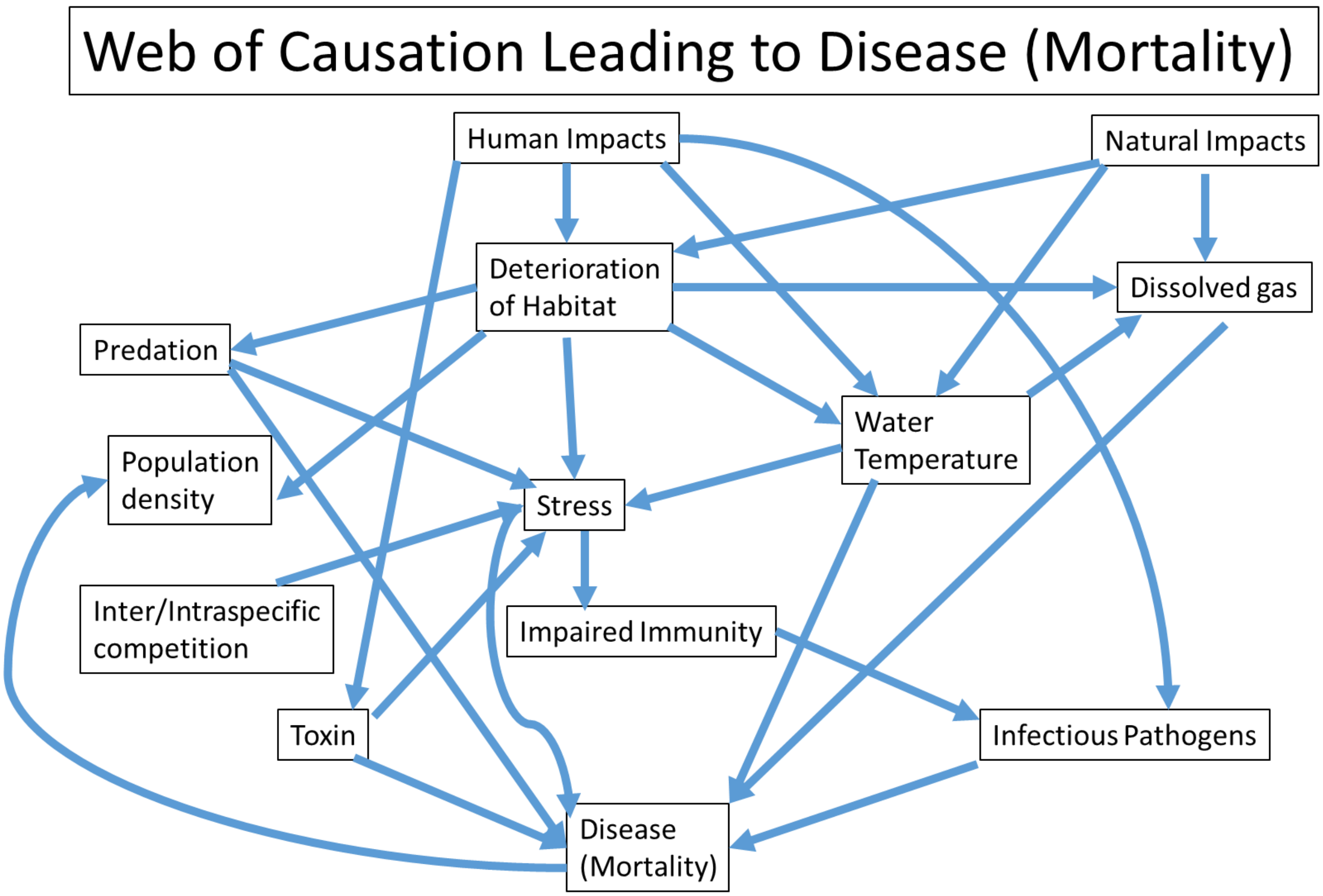


Figure 1. Conceptual web of causation leading to disease for the WCT population in the UFR.

Qualifications of SME

Trent Bollinger has a BSc (Hon) in Biology from the University of Saskatchewan, a Doctor of Veterinary Medicine, Western College of Veterinary of Veterinary, U of S, and a Doctor of Veterinary Sciences, specializing in wildlife pathology and epidemiology, Ontario Veterinary College, University of Guelph, Ontario. He has been a wildlife diagnostic pathologist and researcher for 28 years, working on fish and wildlife diseases. He has published extensively on wildlife health issues, including publications on Columnaris disease and *Myxobolus neurophilus* infections in fish. He is a Professor in the Department of Veterinary Pathology, WCVM, Saskatoon, where he teaches graduate and undergraduate courses related to wildlife and fish health. He also works as an independent consultant with TKB Ecosystem Health Services Ltd.

Objectives:

The objective of this report is to evaluate the evidence for various infectious diseases to explain the decline in the UFR WCT population.

Approach:

The assessment of infectious disease as a potential cause of the population decline is based on a review of the literature of pathogens of trout that have been reported to cause die-offs and population declines in wild fish. As well five fish that died of entrapment during the spring of 2020 were necropsied to look for underlying disease (see appendix). Potential causes of disease were divided into broad categories such as bacterial, viral and parasitic diseases, and then within these categories specific etiologies were discussed based on those perceived as having the highest potential for being the sole cause, or a contributing cause, of the population decline. This was based on personal experience, electronic searches of Web of Science and Google Scholar, as well as investigating appropriate references in bibliographies. The pathology, clinical signs and epidemiology of the diseases are reviewed and then are contrasted with what is known of the UFR WCT population and the population decline.

The only data available on causes of mortality on the UFR was from Cope and is based on monitoring radio-tagged fish from 2012 to 2015 (Cope et al. 2016). In this study predation accounted for 44% of the mortality, followed by post-spawning mortality 36%, and winter ice 16%. Post-spawning mortality could also include losses due to predation or be the result of fish weakened by the stress of spawning. The precise cause of mortality under the category of winter ice could not be determined in this study so should be considered undefined winter mortality. Infectious disease was not reported as a cause of mortality in this study but unfortunately necropsies were not performed to determine cause of death or presence of underlying health issues. In some cases of mortality, no carcass was observed and in others the carcasses were likely unsuitable for necropsy due to autolysis.

Prospective surveys for various fish pathogens could be undertaken on the UFR to determine their presence, but even if detected their relevance to the decline would be highly speculative. Environmental DNA, DNA shed from an organism and present in environmental substrates, has been used to detect invasive species and levels of biodiversity in aquatic environments (Harper et al. 2019) and could potentially be used for pathogen detection, but the technique has limitations and would require extensive validation.

Impact Hypothesis 1: Infectious Disease

The hypothesis investigated was that infectious disease was the cause of UFR WCT decline – either as part of over-arching hypothesis 1 (sole cause) or 2 (contributory).

The role of infectious disease in the population dynamics of wild fish is poorly understood. Research on infectious diseases of fish has been stimulated by the emergence of diseases in the fish farming industry, which has been expanding over the last several decades. The potential for spill-over and spill-back has resulted in additional studies of infectious diseases in wild fish stocks. New pathogens are being discovered (Mordecai et al. 2019) and the role of previously known pathogens in disease outbreaks and population declines are being clarified, both in freshwater and marine environments. Many of the bacterial and fungal diseases of fish in natural environments are opportunistic pathogens, which means they are present in the environment, or are present at low levels in some individuals within the population, but only cause significant disease when fish become stressed and immunocompromised (Figure 1). High stocking densities, poor water quality, temperature stress are examples of factors that can precipitate disease outbreaks. If many fish are similarly stressed, disease causing organisms can increase in numbers on carrier fish resulting in spread to other individuals through the water and substrate and by direct contact. This can result in large die-offs over variable periods of time. Introduction of a novel pathogen into a naïve population can also cause die-offs and population declines (Johnson and Paull 2011).

Viral diseases:

Trout are susceptible to a variety of viral agents. Some viruses produce no obvious effects or mild disease, others cause localized skin lesions, and others can cause systemic disease with clinical signs that include one or more of: pallor, edema, darkening, multifocal hemorrhage, erratic swimming, and lethargy. Many of these viral diseases, such as infectious pancreatic necrosis virus which has a wide host range, cause acute and severe disease in young fish, but only mild or subclinical disease in older age classes (McAllister 1993). Infectious hematopoietic necrosis virus (IHNV) is another example. This virus is endemic among populations of wild salmonids along the west coast of North America. A wide range of species are considered susceptible to varying degrees, including rainbow and cutthroat trout (OIE 2019). Severe disease and mortality occur most commonly in fish up to 2 months age. Adults typically become infected without showing clinical signs but mortalities do occasionally occur (Dixon et al. 2016). Disease is more likely to develop when fish are stressed, and viruses are more readily transmitted in situations of high stocking density. These diseases are typically more significant in hatcheries or fish farm facilities.

Viral diseases were viewed as being a highly unlikely cause of the UFR population decline and therefore none were reviewed in detail. This was based on the absence of reports of viruses being a cause of wild trout population declines elsewhere in western North America, the lack of sick fish being detected in the UFR, and the propensity for viral diseases to affect younger age classes most severely, which was not consistent with the reported decline of primarily adult WCT in the UFR from 2017-19.

Bacterial diseases:

Bacteria are ubiquitous in freshwater environments and many species are resident on skin, gills and mucosal surfaces. Some of these bacteria are opportunistic pathogens, which means they proliferate and cause disease when fish are stressed due to declining water quality, temperatures outside thermal optimal ranges, rapid temperature changes, hormonal changes, etc. Disease can vary from mild clinical disease to acute die-offs involving large numbers of fish (Scott and Bollinger 2014). Bacterial diseases are important in aquaculture, but several can cause die-offs in wild fish as well. Bacterial and fungal pathogens also have optimal temperatures for growth and proliferation. These can be at temperatures that are suboptimal for fish to mount an effective immune response. Disease causing agents may be associated with warm water or cold-water conditions depending on thermal optimums for the host and preferred growth conditions of the bacterium. Sudden changes in water temperature can also favor pathogens as fish immune responses are slower to respond to these changes. Two of the more common bacteria that cause fish die-offs in wild fish are *Flexibacter columnaris* the cause of columnaris disease and *Aeromonas salmonicida*, the cause of furunculosis (Herman 1990).

Columnaris outbreaks tend to occur under warm water conditions when temperatures rise above 15° C. *Flexibacter columnaris* attacks skin and gills, starting as white foci of tissue necrosis surrounded by a red zone of hyperemia. The infection extends under the skin causing expanding areas of ulceration. Similar necrotizing lesions occur on the gills. The bacteria then invade the bloodstream resulting in septicemia. The septicemia and osmotic stresses caused by skin and tissue damage are the cause of death (Wakabayashi 1993).

Furunculosis gets its name from the “boil-like” lesions caused by necrosis and swelling of body musculature that sometimes occur in infected fish. *Aeromonas salmonicida* is an obligate pathogen of fish and has limited survival in the environment. The bacterium invades susceptible hosts through skin, gills or mucosal surfaces and a septicemia develops. Fish can die acutely with few necropsy findings or the disease can become more chronic with development of hemorrhage at the base of fins, lethargy, darkening of the skin and swellings. Outbreaks are typically stress-related and mortality rates can be high, especially in farmed fish. All ages of fish are affected but younger fish appear to be less susceptible. Latent infections in carrier fish appear to be responsible for spread of the disease (Munro and Hastings 1993).

Bacterial diseases are very unlikely the sole cause of the WCT trout population decline as these infections typically occur in warm summer months when fish are stressed due to spawning or there is decline in water quality. The trout in the UFR are observed relatively frequently and no skin lesions or edema in sick or dead fish, which would suggest a bacterial infection, have been reported. However, because lesions may be difficult to detect especially during certain times of the year, bacterial diseases may have contributed to mortality associated with post-spawning, winter mortality and predation reported by Cope (Cope et al. 2016).

Oomycete diseases (Saprolegniosis):

Oomycetes, or water molds, are a frequent cause of mortalities in individual wild fish, or groups of fish, and in some circumstances can contribute to population declines (Neitzel, Elston, and Abernethy 2004). They have a world-wide distribution and are endemic in freshwater habitats. They normally feed saprophytically on dead organic matter and are typically opportunistic pathogens, infecting sites of previous skin or gill injury, or infecting immunosuppressed fish. Outbreaks often occur after a drop in temperature or during the winter when fish are thermally stressed. Many species of oomycetes grow optimally at cooler temperatures and water molds are often the direct cause of winter kills in fish. Other reports describe die-offs associated with low river flows, warmer temperatures and poor water quality (referenced report could not be verified) (van West 2006).

Most fish and animal pathogenic oomycetes are within the order *Saprolegniales* which contains three main genera *Saprolegnia*, *Achlya* and *Aphanomyces*, all of which can infect fish or shellfish. *Saprolegnia* and *Aphanomyces* species are responsible for infections of fish in aquaculture and aquariums. *Saprolegnia diclina* and *S. parasitica* are the two most common species isolated, hence the name saprolegniosis for this condition. *Saprolegnia parasitica* is the more pathogenic of the two and can be a primary pathogen (van West 2006).

The life cycle of oomycetes requires water and consists of various stages, including hyphae, zoospores and encysted forms. Motile primary zoospores are produced from the ends of hyphae and once released they swim a short distance before encysting. The encysted spore then produces a secondary zoospore which is more persistent and motile. If the secondary zoospore lands on suitable substrate it germinates to produce hyphae which grow together to form mats called mycelia. The secondary zoospores are the stage that infects fish. Water molds are ubiquitous saprophytes in freshwater and substrates. Most infections are probably acquired from sporulating fungi on decaying organic matter (Noga 1996).

Saprolegnia can infect eggs and all ages of fish. Mycelia can cover and invade egg masses causing their death. Mycelia also develop on the skin of fish, invading the epidermis and extending into underlying connective tissue. Rarely do they extend into muscle and blood vessels. Growth often begins on the head or at the base of fins and can spread from there to involve varying amounts of the body surface. The mycelia appear as white to tan, fuzzy, cotton-like, whorled mats and can be discolored due to algae, sediments and other detritus in the water column. Destruction of large areas of epidermis causes the loss of electrolytes and protein, and imbibition of water in freshwater systems. The resulting osmotic imbalances can result in death. If the lesions are not severe fish can recover from the disease (Roberts 2012).

There have been no reports of Saprolegnia infections in the UFR, although infected fish may have been missed. If fish develop this disease under the ice or in other secluded settings it could go undetected. Due to the ubiquitous nature of water mold it is likely to have been the direct cause of death of some WCT in the UFR but would not be a major case of the WCT population decline. Since Saprolegnia is not typically a primary pathogen, if present, it would have been the result of other more significant indirect disease processes or stressors.

Parasitic diseases:

Trout are hosts to several different species of helminth, metazoan and protozoan parasites, acting as either definitive, intermediate or paratenic hosts. The majority of these parasites do not cause dramatic population declines but can act as an additional stressor during periods of starvation or reduced metabolism. Two myxosporean parasites have been reported as causing population declines in trout and will be evaluated here as a cause of the UFR population decline.

Whirling disease:

Whirling disease, caused by the microscopic metazoan parasite *Myxobolus cerebralis*, is thought to have evolved in native European trout. It was first identified as a significant pathogen when it caused severe morbidity and mortality in rainbow trout imported into Germany for fish farming (Bartholomew and Reno 2002). It spread to several European countries within cultured fish facilities. In North America *M. cerebralis* was first reported in a fish hatchery in 1958 and subsequently spread to other hatchery facilities. Beginning in the 1990s it spread to wild populations causing severe declines in wild trout in watersheds of Montana and other western states. Canada was considered free of whirling disease until it was detected in brook trout with abnormal swimming behaviour in Johnson Lake, within Banff National Park, in August of 2016. The disease has since been confirmed in the Bow, Oldman and Red Deer River watersheds (Figure 2). Given the proximity of documented cases of whirling disease in Canada, the resistance of infectious stages to environmental conditions which facilitates its spread, and its history of producing severe population reductions in trout in other watersheds in western North America, whirling disease was considered a potential cause of WCT populations decline in the UFR and therefore reviewed.

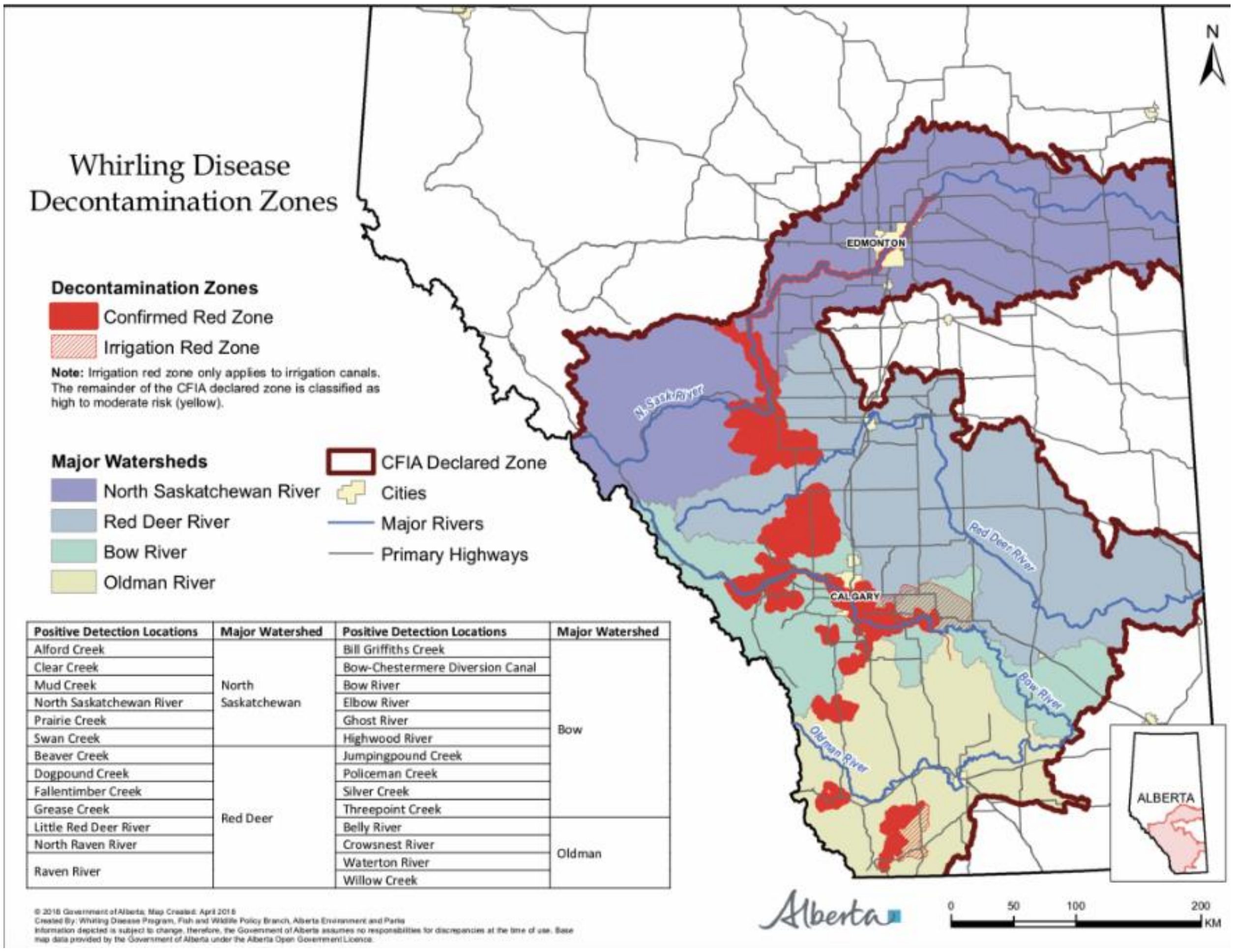
Myxobolus cerebralis has an obligate two-host life cycle involving the oligochaete worm *Tubifex tubifex* and salmonid fish (Gilbert and Granath 2003). The definitive host is *T. tubifex*. Myxospores, after being released from fish, are ingested by the worm which then penetrate the intestinal epithelium using a polar filament. Once in the epithelium asexual reproduction occurs, followed by sexual reproduction involving two gametes which fuse to form a zygote which subsequently divides and develops to produce a single triactinomyxon (TAM). The TAM is released into the water column via the worm's feces. The TAM, when it contacts a fish, will attach, and penetrate the skin using their polar filaments, before moving into nerves and the central nervous system and meninges. From there the parasite migrates into adjacent cartilage where it matures into a large plasmodium, which feeds on the cartilage. After approximately 80 days in the fish the parasite undergoes sporogeny producing myxospores, a resistant stage which persist in the fish. Myxospores are released from the fish when it is predated, or when it dies and decays. Myxospores can survive passage through the digestive tract of avian and terrestrial predators and can survive adverse conditions in the environment, such as periods of drying and freezing.

Destruction of cartilage by feeding stages of *M. cerebralis* causes skeletal deformities such as deviated and twisted vertebral columns and misshapen skulls. Inflammation associated with infection can cause compression of adjacent nerves, which can affect melanocyte function resulting in blackened tails. Damage and inflammation of the auditory and vestibular system results in fish being unable to remain oriented in the water column and erratic, spiral swimming behaviour; hence the name "whirling disease".

Although whirling disease has not yet been detected in British Columbia the parasite is present in adjacent Alberta and Montana. The approximate straight-line distance from locations where whirling disease has been confirmed to the Upper Fording River is less than 100 km. Appropriate intermediate hosts are likely present throughout the drainage, but this has not been confirmed.

As a potential cause for population decline of WCT in the UFR, I would assess it as very unlikely. Fish are monitored regularly by visual counts and capture and there have been no reports fish with blackened tails, deformed vertebral columns or fish with abnormal swimming behaviour to suggest whirling disease is present. A total of 5 WCT from the UFR have been necropsied along with light microscopic evaluation of nervous and skeletal system with no evidence of myxosporean infection (Appendix). Alternative surveillance and testing options, such as skull digests of all fish mortalities followed by polymerase chain reaction testing for *M. cerebralis* should be considered for future whirling disease monitoring of this population.

The Upper Fording River may be predisposed to introduction of this disease into the drainage due to localized alterations in macrophyte and periphtyon abundance experienced during stable low flow periods (Larratt and Self, 2021), changes in relative concentrations of nitrogen and other water parameters, and as a result of altered habitat causing increased fish densities during various life stages (Bartholomew et al. 2005).



Positive Detection Locations	Major Watershed	Positive Detection Locations	Major Watershed
Alford Creek	North Saskatchewan	Bill Griffiths Creek	Bow
Clear Creek		Bow-Chestermere Diversion Canal	
Mud Creek		Bow River	
North Saskatchewan River		Elbow River	
Prairie Creek		Ghost River	
Swan Creek		Highwood River	
Beaver Creek	Red Deer	Jumpingpound Creek	
Dogpound Creek		Policeman Creek	
Fallentimber Creek		Silver Creek	
Grease Creek		Threepoint Creek	
Little Red Deer River		Belly River	Oldman
North Raven River		Crowsnest River	
Raven River		Waterton River	
		Willow Creek	

Figure 2. Distribution of whirling disease in Canada, April 2018 (https://open.alberta.ca/publications/where-is-whirling-disease-in-alberta).

Proliferative Kidney Disease

Proliferative kidney disease (PKD) is widely distributed in Europe and North America and is an important disease of wild and farmed salmonids. Its detection in new locations, changing transmission dynamics and its impact on populations indicate it is an emerging disease. Warmer temperatures promote disease development, increases the abundance of its intermediate host and enhances transmission. Eutrophication and environmental degradation have also been shown to promote disease and these combined factors likely explain its emergence.

PKD is caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. Like other myxozoan parasites it has an invertebrate definitive host, which in the case of PKD, are species of freshwater bryozoans. Bryozoans are microscopic organisms that form variably sized and shaped, large, clonal colonies with a gelatinous or chitinous exoskeleton. They are benthic filter feeders and are found in a wide range of aquatic environments. Despite their common occurrence they are often overlooked in limnologic surveys due to problems with taxonomy and difficulties in species identification (Ricciardi and Reiswig 1994). Genera most commonly associated with PKD outbreaks are *Fredericella* and *Plumatella* (Okamura and Wood 2002). There is virtually nothing published on the distribution of bryozoans in western Canada, in particular the distribution of suitable hosts for *T. bryosalmonae*.

Spores of *T. bryosalmonae* are passed in the urine of infected fish. These spores initially establish a covert infection in bryozoans consisting of single cell stage associated with the body wall. Transformation to overt infections involves the development of sac-like structures containing several thousand spores which are then released into the water column. Bryozoans remain infected indefinitely shifting back and forth between covert and overt infections depending on season and other environmental factors (Okamura et al. 2011). Spores released from bryozoans use eversible filaments within polar capsules to attach and penetrate gills and skin of fish. Amoeboid cells released from the spores enter the blood stream where they replicate before establishing infections in the interstitium of the kidneys and other tissues. Proliferation of this extrasporogenic stage, and the associated inflammation, causes swelling and damage to the kidney which, if severe, can kill the fish. Fish can also clear this stage of infection and repair the damage. Extrasporogenic stages can migrate into the lumen of kidney tubules where they differentiate into pseudoplasmodia which remain attached to the epithelium. Each pseudoplasmodium produces a single spore, with two polar capsules, which is passed with urine (Morris and Adams 2008).

Fish if severely infected with *T. bryosalmonae* die of renal failure. Clinical signs include edema and, lethargy. Less severely infected fish, due to their weakened state, are prone to secondary bacterial and fungal infections and unless necropsies are performed, which includes histopathology, the underlying disease may go undetected. Peak prevalence of disease occurs in the summer as transmission, rates of development of various life stages, and immune response in fish are temperature dependant. Studies done on rainbow trout showed clinical disease developed at temperatures between 12-18° C, but did not develop at 9° C (Clifton-Hadley, Richards, and Bucke 1986). Mortality rates were higher at higher temperatures. Juvenile fish are most likely to develop clinical disease, but all ages can be affected. Subclinical infections can persist in older age classes.

Proliferative kidney disease is thought to have played a major role in the decline of wild brown trout populations in Switzerland (Rubin et al. 2019) and is found throughout North America in a variety of fish species, including WCT (Macconnell and Peterson 1992). Losses in wild fish can be severe. For example, a media report from 2016 described the parasite as devastating the whitefish population and spreading to trout in the Yellowstone River in Montana (Yong 2016).

Given the short-time period of population decline in the UFR involving primarily adult fish, and in the absence of any detectable sick or dead fish, it is very unlikely PKD was responsible for the UFR WCT population decline.

Bibliography

Bartholomew, J.L., and P.W. Reno. 2002. 'The History and Dissemination of Whirling Disease', *American Fisheries Society Symposium*, 26: 1-22.

Bartholomew, JerriL, BillieL Kerans, RonaldP Hedrick, StuartC Macdiarmid, and JamesR Winton. 2005. 'A Risk Assessment Based Approach for the Management of Whirling Disease', *Reviews in Fisheries Science*, 13: 205-30.

Clifton-Hadley, R. S., R. H. Richards, and D. Bucke. 1986. 'Proliferative kidney disease (PKD) in rainbow trout Salmo gairdneri: Further observations on the effects of water temperature', *Aquaculture*, 55: 165-71.

Cope, S. 2020. "Upper Fording River Westslope Cutthroat Trout Population Monitoring Project 2019." In *Report Prepared for Teck Coal Limited*, 48. Sparwood, BC: Westslope Fisheries Ltd.

Cope, S., C.J. Schwarz, A. Prince, and J. Bisset. 2016. "Upper Fording River Westslope Cutthroat Trout Population Assessment and Telemetry Project: Final Report." In *Report Prepared for Teck Coal Limited*, 266. Sparwood, B.C.

Dixon, Peter, Richard Paley, Raul Alegria-Moran, and Birgit Oidtmann. 2016. 'Epidemiological characteristics of infectious hematopoietic necrosis virus (IHNV): a review', *Veterinary Research*, 47: 63.

Gilbert, Michael A., and Willard O. Granath. 2003. 'WHIRLING DISEASE OF SALMONID FISH: LIFE CYCLE, BIOLOGY, AND DISEASE', *Journal of Parasitology*, 89: 658-67, 10.

Harper, Lynsey R., Andrew S. Buxton, Helen C. Rees, Kat Bruce, Rein Brys, David Halfmaerten, Daniel S. Read, Hayley V. Watson, Carl D. Sayer, Eleanor P. Jones, Victoria Priestley, Elvira Mächler, Cesc Múrria, Sandra Garcés-Pastor, Cecilia Medupin, Katherine Burgess, Gillian Benson, Neil Boonham, Richard A. Griffiths, Lori Lawson Handley, and Bernd Hänfling. 2019. 'Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds', *Hydrobiologia*, 826: 25-41.

Herman, R.L. 1990. 'The Role of Infectious Agents in Fish Kills.' in F.P. Meyer and L.A. Barclay (eds.), *Field Manual for the Investigation of Fish Kills* (U.S. Government Printing Office: Washington, DC).

Johnson, Pieter T. J., and Sara H. Paull. 2011. 'The ecology and emergence of diseases in fresh waters', *Freshwater Biology*, 56: 638-57.

Larratt, H. and J. Self. 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.

Macconnell, Elizabeth, and James E. Peterson. 1992. 'Proliferative Kidney Disease in Feral Cutthroat Trout from a Remote Montana Reservoir: A First Case', *Journal of Aquatic Animal Health*, 4: 182-87.

McAllister, Philip E. 1993. 'Salmonid Fish Viruses.' in M.K. Stoskopf (ed.), *Fish Medicine* (W.B. Saunders Company: Philadelphia, Pennsylvania).

Mordecai, Gideon J., Kristina M. Miller, Emiliano Di Cicco, Angela D. Schulze, Karia H. Kaukinen, Tobi J. Ming, Shaorong Li, Amy Tabata, Amy Teffer, David A. Patterson, Hugh W. Ferguson, and Curtis A. Suttle. 2019. 'Endangered wild salmon infected by newly discovered viruses', *eLife*, 8.

Morris, D. J, and A. Adams. 2008. 'Sporogony of Tetracapsuloides bryosalmonae in the brown trout Salmo trutta and the role of the tertiary cell during the vertebrate phase of myxozoan life cycles', *Parasitology*, 135: 1075-92.

Munro, A.L.S., and T.S. Hastings. 1993. 'Furunculosis.' in V. Inglis, R.J. Roberts and N.R. Bromage (eds.), *Bacterial Diseases of Fish* (Halstead Press: New York).

Neitzel, D.A., R.A. Elston, and C.S. Abernethy. 2004. "Prevention of Prespawning Mortality: Cause of Salmon Headburns and Cranial Lesions." In, 99. Portland, Oregon: U.S. Army Corps of Engineers.

Noga, E.J. 1996. 'Problem 33: Typical water mold infection (Saprolegniosis, Oomycte infection).' in, *Fish Disease: Diagnosis and Treatment* (Mosby: St. Louis, Missouri).

OIE. 2019. 'Infection with infectious haematopoietic necrosis virus.' in, *Manual of Diagnostic Tests for Aquatic Animals*.

Okamura, B, and T S Wood. 2002. 'Bryozoans as hosts for Tetracapsula bryosalmonae, the PKX organism', *Journal of Fish Diseases*, 25: 469-75.

Okamura, Beth, Hanna Hartikainen, Heike Schmidt-Posthaus, and Thomas Wahli. 2011. 'Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease', *Freshwater Biology*, 56: 735-53.

Ricciardi, Anthony, and Henry M. Reiswig. 1994. 'Taxonomy, distribution, and ecology of the freshwater bryozoans (Ectoprocta) of eastern Canada', *Canadian Journal of Zoology*, 72: 339-59.

Roberts, R.J. 2012. 'The Mycology of Teleosts.' in R.J. Roberts (ed.), *Fish Pathology* (Wiley-Blackwell: West Sussex, UK).

Rubin, AuréLie, Pauline De Coulon, Christyn Bailey, Helmut Segner, Thomas Wahli, and Jean-Franà§Ois Rubin. 2019. 'Keeping an Eye on Wild Brown Trout (Salmo trutta) Populations: Correlation Between Temperature, Environmental Parameters, and Proliferative Kidney Disease', *Frontiers in Veterinary Science*: NA.

Scott, Steven J., and Trent K. Bollinger. 2014. 'Flavobacterium columnare: an important contributing factor to fish die-offs in southern lakes of Saskatchewan, Canada', *Journal of Veterinary Diagnostic Investigation*, 26: 832-36.

Team, Evaluation_of_Cause_. 2020. "Evaluation of Cause — Decline in Upper Fording River Westslope Cutthroat Trout Population. Report prepared for Teck Coal Limited by Evaluation of Cause Team. XmonthX 2020." In.

van West, Pieter. 2006. 'Saprolegnia parasitica, an oomycete pathogen with a fishy appetite: new challenges for an old problem', *Mycologist*, 20: 99-104.

Wakabayashi, H. 1993. 'Columnaris Disease.' in V. Inglis, R.J. Roberts and N.R. Bromage (eds.), *Bacterial Diseases of Fish* (Halstead Press: New York).

Yong, Ed. 2016. "A Tiny Jellyfish Relative Just Shut Down Yellowstone River." In *The Atlantic*.

APPENDIX

History:

Five Westslope Cutthroat trout, that had become trapped in a fish occlusion fence on Smith Pond on April 16, 2020, were placed in 10% neutral buffered formalin shortly after being found . They They were kept in formalin for several days until preserved at which time the excess formalin was poured off and fish were placed in plastic bags containing formalin saturated paper towel. Samples were shipped to TKB in Saskatoon where they were necropsied. Sections of formalin fixed fish were placed in tissue cassettes and submitted to the Prairie Diagnostic Services (Saskatoon, SK) for routine histological preparation. Four micrometer thick sections of tissue were mounted on glass slides and stained with haematoxylin and eosin. The slides were examined by an experienced fish and wildlife pathologist.

Necropsy Findings: The fish were all approximately 10 cm in length. No abnormalities were observed on gross examination of the fish.

Histopathology Findings:

No significant histological lesions were detected. A summary of the tissues examined with the light microscope and results are provided below.

Table 1. List of tissue examined with the light microscope for each of the submitted fish. Cells containing the word "notes" have more detailed descriptions below.

Teck Sample ID	Gill	Cartilage	Brain	Eyes	Kidney	spinal cord	Liver	skeletal muscle	skin	thyroid	heart
FR_SP1-WCT-1	NAF	NAF	NAF	NAF	NAF	NAF	UN	NAF	UN	UN	UN
FR_SP1-WCT-2	NAF	NAF	NAF	NAF	NAF	NAF	UN	NAF	UN	UN	NAF
FR_SP1-WCT-3	Notes	NAF	NAF	NAF	NAF	NAF	Notes	NAF	NAF	NAF	NAF
FR_SP1-WCT-4	UN	NAF	NAF	NAF	NAF	NAF	UN	NAF	NAF	UN	UN
FR_SP1-WCT-5	NAF	NAF	NAF	NAF	NAF	NAF	UN	Notes	NAF	NAF	NAF

NAF = no abnormal findings.

UN = unknown, tissue not present in the sections examined.

Notes = additional information provided below.

Histopathology notes:

Teck Sample id: FR_SP1-WCT-3_2020-04-16 (PDS20-16278B)

Liver: There is periacinar degeneration and necrosis of hepatocytes (likely related to hypoxia; Figure 1).

Gills: There is mild multifocal to locally extensive epithelial hyperplasia predominately on distal aspects of filaments (normal).

Teck Sample id: FR_SP1-WCT-5_2020-04-16 (PDS20-16278E)

Skeletal muscle: Bilaterally ventral paravertebral muscles adjacent to the kidney are pale amorphous and fragmented (peracute degeneration and necrosis).

Interpretation: No significant diseases were detected in these fish.

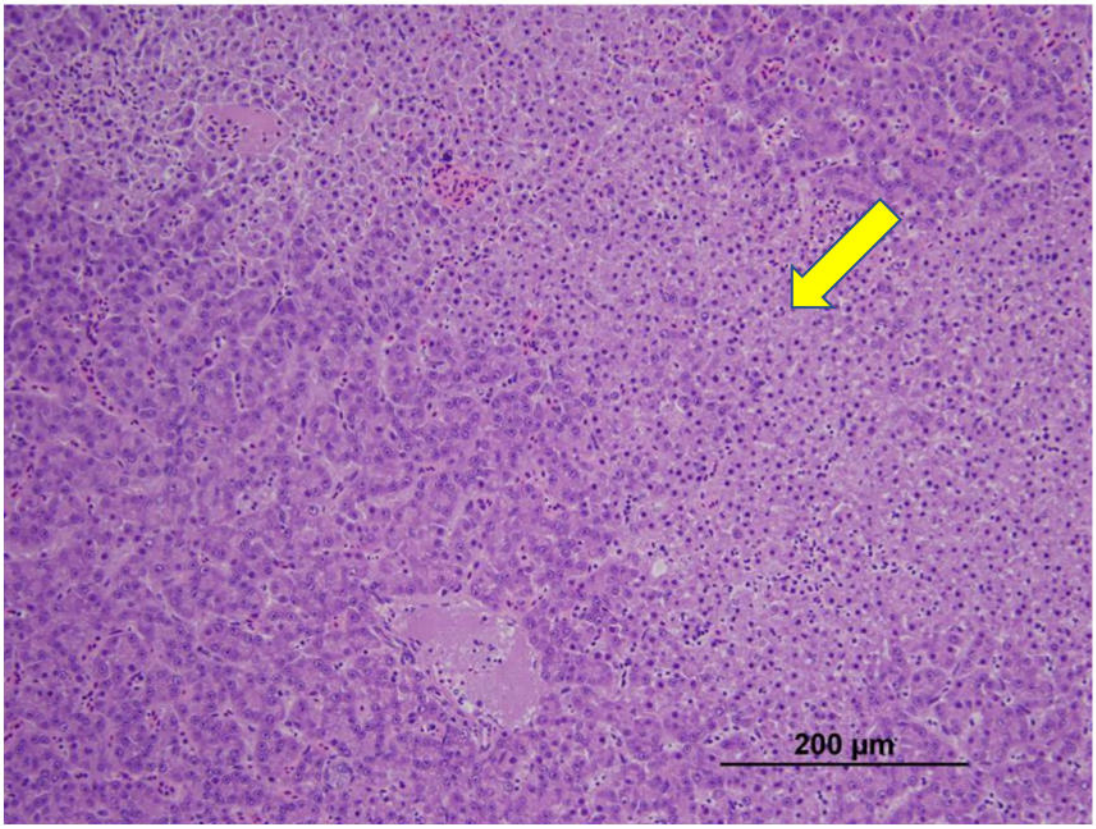


Figure 1 Liver from fish FR_SP1-WCT-3_2020-04-16 showing areas of periacinar hepatic necrosis, likely related to hypoxia. Arrow shows zone of hepatocellular degeneration and necrosis.

Figure 2. Normal gills from one of the examined fish. The lifting of the epithelium off the basement membrane of gill lamellae is due to delayed fixation.