Subject Matter Expert Report: DISSOLVED OXYGEN

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

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December 1, 2022

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Published by Ecofish Research Ltd., 600 Comox Rd., Courtenay, B.C., V9N 3P6

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Citation:

Abell, J., X. Yu, J. Braga, and T. Hatfield. 2022. Subject Matter Expert Report: Dissolved Oxygen. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population. Consultant's report prepared for Teck Coal Limited by Ecofish Research Ltd., December 1, 2022.

Certification: *Certified - stamped version on file.*

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EXECUTIVE SUMMARY

Abundance of age-1 Westslope Cutthroat Trout (WCT) was lower in 2018, 2019, and 2020 (spawning cohorts 2017, 2018, and 2019) in the Harmer Creek WCT population in comparison to previous years and the adjacent Grave Creek population. Teck Coal Ltd. (Teck Coal) initiated an "Evaluation of Cause" to assess potential stressors responsible for the reduced recruitment in the Harmer Creek population. This report evaluates whether adverse dissolved oxygen (DO) conditions may have caused or contributed to the Reduced Recruitment.

In fishes, maintenance of appropriate blood oxygen levels is essential to sustain life, and low DO concentrations can cause chronic and acute effects. Like other salmonids, WCT are adapted to cold well-oxygenated water and they are sensitive to reductions in DO concentrations, such as due to increased oxygen demand from organic material or infiltration of fine sediments into spawning gravels, which can reduce DO availability to buried eggs and alevins. Changes in water temperature may also affect DO concentrations because oxygen solubility declines with increasing water temperature.

Additional analysis was undertaken of the DO dataset presented by Warner *et al.* (2022). The dataset comprises data for nine reaches in the two population areas, although data were most extensive for Reach 2 of Dry Creek (DC-R2; Harmer Creek population area) and Reach 1 of Harmer Creek (HRM-R1), downstream of the Harmer Creek Sedimentation Pond (Grave Creek population area). Recent data were much more limited for other reaches of Harmer Creek and the mainstem of Grave Creek, which constrained the scope of the analysis. The assessment focused on data collected since 2014, which spans periods of variable recruitment success in Harmer Creek.

The assessment broadly focused on three lines of evidence:

1. Comparison of DO measurements to applicable provincial guidelines and supporting literature regarding the physiological effects of low DO concentrations on salmonids;
2. Spatial variability in DO concentrations between the two population areas; and
3. Temporal variability in DO concentrations between periods of variable recruitment success in the Harmer Creek population area.

Key results and conclusions from the assessment are as follows:

- Available data indicate that adverse DO conditions are not expected to have caused acute or chronic adverse effects to free swimming life stages of WCT (fry, parr, and adults) in the Harmer Creek population area.
- Available data indicate that DO concentrations at the outlet of Dry Creek Sedimentation Pond were sufficiently low at times to cause chronic effects to buried eggs and alevins, although DO concentrations were likely not sufficiently low to be the sole cause of the recruitment failures. Measurements collected in Dry Creek reflect conditions in DC-R2 and, to an extent, DC-R1 immediately downstream. Dry Creek is generally unsuitable for spawning due to impairment

of spawning habitat by calcite concretion, with spawning activity in the Harmer Creek population area largely confined to the Harmer Creek mainstem.

The limited data that are available for Reach 5 of Harmer Creek downstream of Dry Creek (data generally only available for 2019 and 2020) indicate that DO concentrations were higher than those in Dry Creek, although comparison with provincial guidelines indicates that DO concentrations were sufficiently low to also potentially cause chronic effects (e.g., reduced growth) to buried eggs and alevins during at least the 2019 and 2020 incubation periods in the Harmer Creek mainstem downstream of Dry Creek. Reduced Recruitment occurred for the 2019 spawn year but not the 2020 spawn year.

- The lowest DO concentrations were measured in Dry Creek in the Harmer Creek population area. Statistical analysis of spatial variability between sites in the two population areas provided only a weak line of evidence for this assessment due to low statistical power and lack of suitable data for multiple sites (suitable data for the Harmer Creek population area were only available for Dry Creek, whereas suitable data for the Grave Creek population area were only available for Reach 1 of Harmer Creek, not the Grave Creek mainstem). Nonetheless, the analysis undertaken of spatial differences did not support a conclusion that low DO concentrations in the Harmer Creek population area were the sole cause of the Reduced Recruitment.
- Relatively low DO concentrations occurred more frequently in Dry Creek during the incubation periods that were associated with the periods of reduced or failed recruitment, than during a "Before" period when recruitment was higher. These low concentrations included the lowest DO concentration (8.54 mg/L) measured at any site in the period analyzed (data available post 2014); this value was slightly (5%) less that the provincial guideline instantaneous minimum value (9 mg/L) for the protection of buried life stages (eggs/alevins), which is intended to avoid "slight" impairment of fishery resources, assuming a constant exposure level. The observation of relatively low minimum DO concentrations in Dry Creek during the incubation periods in years that included 2018 and 2019 suggests a possible link between DO and the Reduced Recruitment, assuming that low DO concentrations extended to Harmer Creek mainstem, where spawning in the Harmer Creek population area generally occurs. However, the timing of the Reduced Recruitment period and the general occurrence of low DO concentrations were not clearly synchronous.
- DO saturation in Dry Creek approximated 100% throughout the period applicable to the recruitment failure, indicating that the measurements of low DO concentration were due to relatively warm water temperatures during summer, rather than an increase in biochemical oxygen demand, e.g., associated with a change to substrate quality or water pollution.

Overall, the results of this assessment suggest that low DO concentrations were not the sole cause of the Reduced Recruitment period for WCT in the Harmer Creek population area, but low DO concentrations during the incubation period may have interacted additively or synergistically with other stressors to reduce juvenile WCT recruitment in the Harmer Creek population area. Confidence in the conclusions is influenced by several uncertainties; a key uncertainty is the lack of information about the difference between DO concentrations in interstitial waters (most relevant to buried life stages) and concentrations in the surface waters previously sampled.

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
BC	British Columbia
BC WQG-AL	British Columbia Water Quality Guidelines for the Protection of Aquatic Life
DO	Dissolved Oxygen
EoC	Evaluation of Cause
SME	Subject Matter Expert
WCT	Westslope Cutthroat Trout

READER'S NOTE

Background

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

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

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

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

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

The Evaluation of Cause Process

The Process Was Initiated

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

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

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

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

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

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

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

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

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

How the Evaluation of Cause Was Approached

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

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

The Overarching Question the Team Investigated

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

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

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

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

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

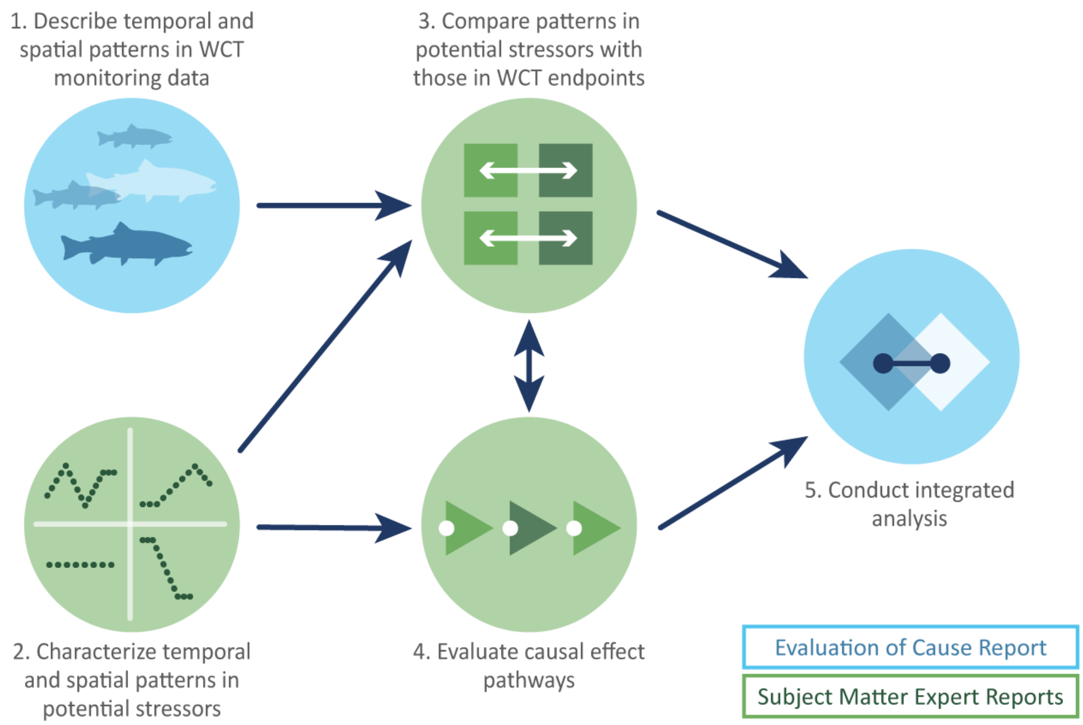
2. The Evaluation of Cause report. The SME reports provided the foundation for the Evaluation of Cause report, which was prepared by a subset of the Team and included input from SMEs.

   The Evaluation of Cause report:

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

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

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



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

Participation, Engagement & Transparency

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

- Ktunaxa Nation Council
- BC Ministry of Forests,
- BC Ministry of Land, Water and Resource Stewardship

- BC Ministry Environment & Climate Change Strategy
- Ministry of Energy, Mines and Low Carbon Innovation
- Environmental Assessment Office

Citations for Evaluation of Cause Team Reports

Focus	Citation
Harmer Creek Evaluation of Cause report	Harmer Creek Evaluation of Cause Team. (2023). *Evaluation of Cause - Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited.
Calcite	Hocking, M. A., Cloutier, R. N., Braga, J., & Hatfield, T. (2022). *Subject Matter Expert Report: Calcite. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Dissolved oxygen	Abell, J., Yu, X., Braga, J., & Hatfield, T. (2022). *Subject Matter Expert Report: Dissolved Oxygen. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Energetic Status	Thorley, J.L. & Branton, M.A. (2023) *Subject Matter Expert Report: Energetic Status at the Onset of Winter Based on Fork Length and Wet Weight. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population*. Report prepared for Teck Coal Limited. Prepared by Poisson Consulting Ltd and Branton Environmental Consulting.

Focus	Citation
Food availability	Wiebe, A., Orr, P., & Ings, J. (2022). *Subject Matter Expert Report: Food Availability. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Minnow Environmental Inc.
Groundwater	Canham, E., & Humphries, S. (2022). *Evaluation of Groundwater as a Potential Stressor to Westslope Cutthroat Trout in the Harmer and Grave Creek Watersheds.* Memo prepared for Teck Coal Limited. Prepared by SNC-Lavalin Inc.
Habitat availability (instream flow)	Wright, N., Little, P., & Hatfield, T. (2022). *Subject Matter Expert Report: Streamflow and Inferred Habitat Availability. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Sediment quality	Wiebe, A., Orr, P., & Ings, J. (2022). Subject Matter Expert Report: Sediment Quality. *Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population*. Report prepared for Teck Coal Limited. Prepared by Minnow Environmental Inc.
Selenium	de Bruyn, A., Bollinger, T., & Luoma, S. (2022). *Subject Matter Expert Report: Selenium. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by ADEPT Environmental Sciences Ltd, TKB Ecosystem Health Services, and SNL PhD, LLC.
Small population size	Thorley, J. L., Hussein, N., Amish, S. J. (2022). *Subject Matter Expert Report: Small Population Size. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Poisson Consulting and Conservation Genomics Consulting, LLC.

Focus	Citation
Telemetry analysis	Akaoka, K., & Hatfield, T. (2022). *Harmer and Grave Creeks Telemetry Movement Analysis.* Memo prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Total suspended solids	Durston, D., & Hatfield, T. (2022). *Subject Matter Expert Report: Total Suspended Solids. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.
Water quality	Warner, K., & Lancaster, S. (2022). *Subject Matter Expert Report: Surface Water Quality. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by WSP-Golder.
Water temperature and ice	Hocking, M., Whelan, C. & Hatfield, T. (2022). *Subject Matter Expert Report: Water Temperature and Ice. Evaluation of Cause – Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population.* Report prepared for Teck Coal Limited. Prepared by Ecofish Research Ltd.

1. INTRODUCTION

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

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

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

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

[10] Recruitment refers to the addition of new individuals to a population through reproduction. For the EoC, recruitment is defined as the estimated number of age-1 fish in the fall (i.e., late-September/early October) following the first full overwintering period.

[11] For the purposes of the Evaluation of Cause, Reduced Recruitment is defined as a probability of >50% that annual recruitment was < 100% of that required for population replacement (see Chapter 4, Evaluation of Cause, Harmer Creek Evaluation of Cause Team 2022).

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

[13] For the purposes of the Evaluation of Cause, Recruitment Failure is defined as a probability of >50% that annual recruitment is <10% of that required for population replacement (see Chapter 4 Evaluation of Cause, Harmer Creek Evaluation of Cause Team 2022).

[14] For the purposes of the Evaluation of Cause, recruitment Above Replacement is defined as a probability of >50% that annual recruitment is >100% of that required for population replacement (see Chapter 4 Evaluation of Cause, Harmer Creek Evaluation of Cause Team 2022).

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

This report provides an assessment of whether adverse (i.e., low) dissolved oxygen (DO) concentrations caused or contributed to Reduced Recruitment in the Harmer Creek population area, including the Recruitment Failure of the 2018 spawn year. This assessment comprised two key tasks: 1) a focused review of applicable literature, including the technical basis for relevant DO water quality guidelines, and; 2) analysis of spatial and temporal variability in DO concentrations undertaken in the context of the outcomes of the literature review, and spatiotemporal variability in WCT recruitment success. This report will be considered alongside assessments of other stressors to support conclusions about the relative contribution of each potential stressor to the Reduced Recruitment observed in the Harmer Creek population area. This document is therefore one of a series of Subject Matter Expert reports that supports the integrated Harmer Creek Westslope Cutthroat Trout Evaluation of Cause (Harmer Creek Evaluation of Cause Team 2022). For more information, see the preceding Reader's Note.

2. METHODS

2.1. Literature Review

A focused review was undertaken of the technical basis for relevant DO water quality guidelines, as applicable to WCT. The review focused on the provincial water quality guidelines for the protection of aquatic life (BC WQG-AL; Table 1) for DO, although DO guidelines from other jurisdictions were also considered. The review also considered additional literature relevant to the tolerance of WCT to low DO that was identified using Google Scholar or by searching Ecofish files.

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

Table 1. Dissolved oxygen guidelines for the protection of aquatic life in British Columbia (BC MOE 1997).

	Life Stages Other Than Buried Embryo/Alevin	Buried Embryo/Alevin[1]	Buried Embryo/Alevin[1]
Dissolved Oxygen Concentration	Water column mg/L O_2	Water column mg/L O_2	Interstitial Water mg/L O_2
Instantaneous minimum[2]	5	9	6
30-day mean[3]	8	11	8

[1] For the buried embryo / alevin life stages these are in-stream concentrations from spawning to the point of yolk sac absorption or 30 days post-hatch for fish; the water column concentrations recommended to achieve interstitial dissolved oxygen values when the latter are unavailable. Interstitial oxygen measurements would supersede water column measurements in comparing to criteria.

[2] The instantaneous minimum level is to be maintained at all times.

[3] The mean is based on at least five approximately evenly spaced samples. If a diurnal cycle exists in the water body, measurements should be taken when oxygen levels are lowest (usually early morning).

2.2. Spatial and Temporal Variability

2.2.1. Spatial Variability

Spatial variability in DO data collected at sampling locations in Grave Creek and Harmer Creek population areas (Table 2; Map 1) and reported by Warner *et al.* (2022) was evaluated. The following were considered in this assessment:

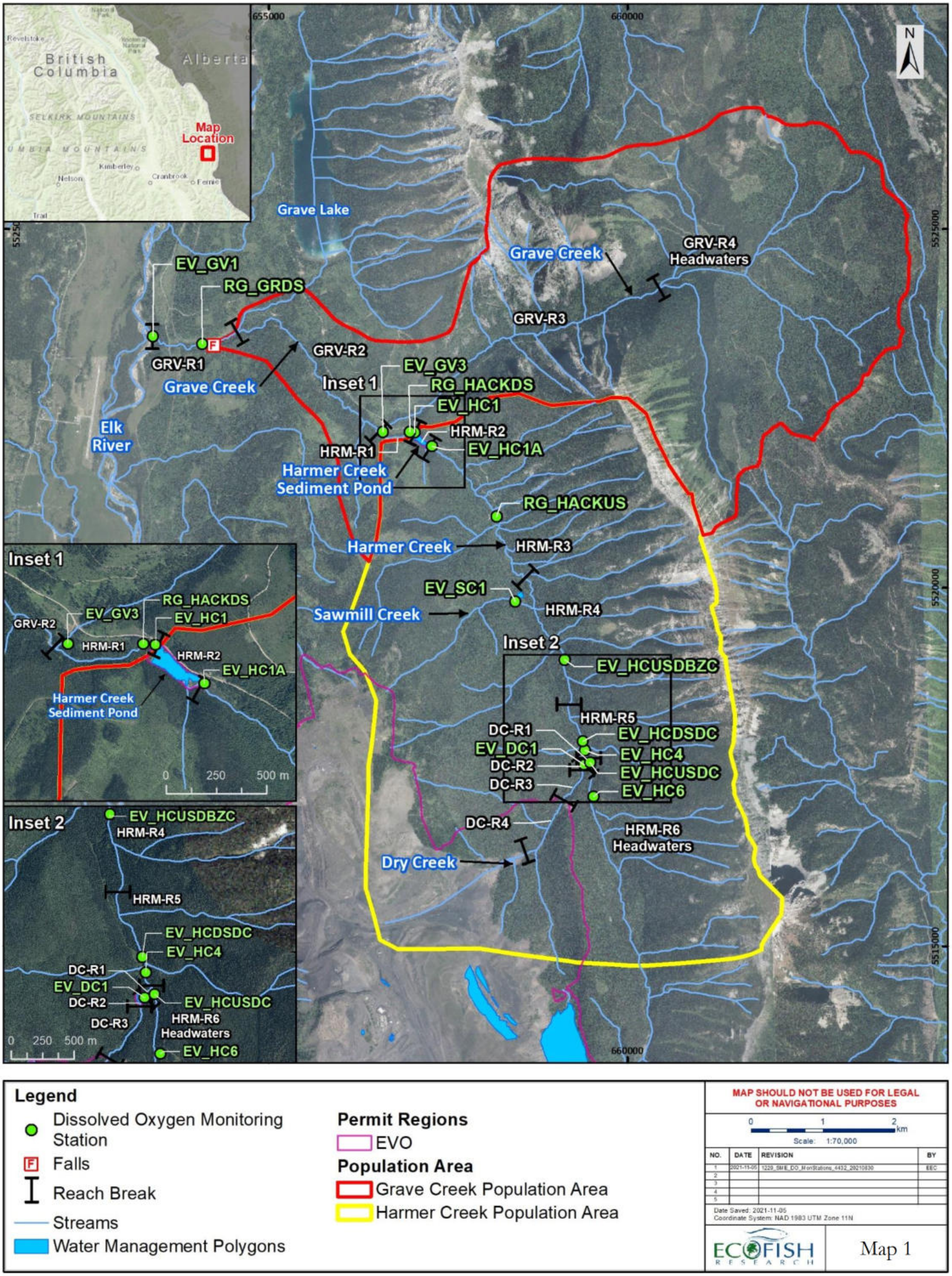
- Spatial variability in DO concentrations between Grave and Harmer population areas, evaluated in the context of BC WQG-AL; and
- Spatial variability in DO concentrations within Harmer Creek population area, evaluated in the context of information about the spatial distribution of fish.

Furthermore, a statistical test was used to compare DO concentrations between the Grave and Harmer population areas, as described in the sub-section below.

Table 2. Reaches and stations with available dissolved oxygen data.

Area	Creek	Reach	Station	Reference or Mine-Influenced
Harmer Creek Population Area	Dry Creek	DC-R2	EV_DC1	Mine Influenced
	Harmer Creek (upstream of Harmer Sediment Pond spillway)	HRM-R6	EV_HC6 EV_HCUSDC	Reference
		HRM-R5	EV_HC4	Mine Influenced
		HRM-R4	EV_HCUSDBZC	Mine Influenced
		HRM-R3	RG_HACKUS EV_HC1A	Mine Influenced
	Sawmill Creek	SM-R1	EV_SC1	Mine Influenced
Grave Creek Population Area	Harmer Creek (downstream of Harmer Sediment Pond spillway)	HRM-R1	EV_HC1 RG_HACKDS	Mine Influenced
	Grave Creek	GRV-R3	EV_GV3	Reference
		GRV-R1	RG_GRDS EV_GV1	Mine Influenced

Grave Harmer EoC - Dissolved Oxygen Monitoring Locations



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2.2.2. Temporal Variability

Temporal variability in DO concentrations was evaluated in the context of the assumed life history activity periods defined by the EoC Subject Matter Expert team (Table 4), as well as periods of variable recruitment success. This analysis also considered spatial variability of DO in the Grave Creek Harmer Creek population areas. The aims of this analysis were to 1) evaluate which life-history periods (if any) may have been affected by low DO concentrations; 2) to assess whether DO concentrations were lower during relevant periods for recruitment of WCT in Harmer Creek; and 3) to assess whether there were differences in DO concentrations between the Harmer Creek and Grave Creek population areas that might contribute to the spatial variability in recruitment.

To support these aims, a statistical test (see below) was used to compare DO concentrations measured at individual sampling locations between two different periods (Table 3):

1. A "Before Period" prior to the period of Reduced Recruitment of WCT in the Harmer Creek population area. This Before Period started on the assumed start date of the spawning period (June 8; Table 4) in either 2013 (HRM-R1 in the Grave Creek population area) or 2014 (DC-R1 in the Harmer Creek population area). The initial year in the Before Period was selected as the first year when data were collected at sufficiently high frequency (typically at least monthly) to complete the analysis (the availability of data differed between sites). The Before Period ended on June 7, 2017, immediately prior to the assumed start of the spawning period in the first year of Reduced Recruitment in the Harmer Creek population area.
2. A Period of Concern, which corresponds to the period of Reduced Recruitment (Section 1) when there was concern regarding low recruitment. The Period of Concern started on June 8, 2017, which is the assumed start of the spawning period in the first year of the three consecutive years (2017, 2018, and 2019) when there was concern regarding low recruitment in the Harmer Creek population area in association with the progeny of the cohort of fish that spawned that year (Section 1). The Period of Concern ended on 7 June, 2020, prior to the start of spawning in 2020 when recruitment was above replacement in both population areas (see Reader's Note). Thus, the Period of Concern was configured to consider conditions experienced by age-1 fish that were associated with the 2017, 2018, and 2019 spawning cohorts. The Period of Concern therefore encompassed the 2018 spawning period and subsequent sampling of age-1 fish from the 2018 spawning cohort that are associated with particularly low recruitment success (i.e., recruitment failure; see Reader's Note)[16].

[16] As described in the Reader's Note, we recognize there was also Reduced Recruitment in the Grave Creek population area in the 2018 spawn year, although the magnitude of Reduced Recruitment was greatest that year in the Harmer Creek population area. The statistical analysis described here was not designed to consider variability in recruitment success in the Grave Creek population area as the focus of this assessment is the Reduced Recruitment in the Harmer Creek population area. However, evaluation of data described in Section 3.2.2.2 nonetheless provides insight into the suitability of DO conditions in the Grave Creek population area during 2018.

DO concentrations were analyzed independently for two WCT life stages: 1) during the incubation stage (i.e., from June 8 to October 31), which also largely overlaps with the summer rearing period (Table 4); and 2) during the remainder of the year that encompasses the overwintering period and the initial weeks of the summer rearing period (i.e., from November 1st to June 7th; Table 4). The incubation stage was analyzed independently because this was the only period for which DO observations were below minima prescribed by the BC WQG-AL and therefore it was desirable to focus analysis on this potentially sensitive period. Furthermore, as is typically observed in temperate streams, there was a strong seasonal trend in the DO data, reflecting seasonal differences in temperature and the dependence of oxygen solubility on temperature (Figure 1). Thus, it was desirable to analyze data collected during the warmest period of the year separately.

Temporal variability in DO concentrations was analyzed separately for individual sampling locations in two reaches: 1) HRM-R1 within Harmer Creek but within the Grave Creek population area, downstream of the barrier below the Harmer Creek Sedimentation Pond; and 2) and DC-R2 within Dry Creek, within the Harmer Creek population area (Table 2, Map 1). There were insufficient data to complete the analysis for other sampling locations.

We compared DO concentrations between the two periods using a general additive mixed model (GAMM) with the identity function with a normal distribution, parameterized as follows:

$$DO\ concentration \sim Reach + Period + Reach \times Period + s(Sample\ date) + s(Sample\ date, \text{Reach}) + s(Sample\ date, Period) + (1 \mid Year)$$

The term "Reach" was a categorical variable that refers to the two reaches (HRM-R1 and DC-R2) and estimated the average effect attributed to differences between the reaches on DO concentrations. The term "Period" was a categorical variable referring to the periods of variable recruitment ("Before" and Period of Concern) and estimated the average effect of each period on DO concentrations. The interaction term "Reach × Period" was included to assess if DO concentrations varied between reaches to different degrees between periods of variable recruitment.

To enhance model accuracy, another component tested was DO seasonality. Seasonality was considered with a non-linear component of the model composed of three smoother terms: a global smoother term "Sample date" that estimated the overall DO seasonality across both sites and periods, and two factor-smoother terms that estimated and tested for deviations from the overall smoother for each "Reach" or during each "Period". The addition of the smoother term "Sample date" also had the advantage of resolving issues related to temporal autocorrelation, which was detected during data exploration, and may affect statistical analyses by inflating type I error. The random effect term "$1 \mid Year$" was a categorical variable referring to the year of sampling and accounted for annual stochasticity when modelling DO[17].

[17] We recognize that inclusion of this term might reduce our ability to detect differences among the two periods. To evaluate this possible effect, the analysis was run separately without including the random term; this modification did not change the results in a way that would affect the conclusions.

To test for differences between reaches and period, an analysis of variance (ANOVA) was performed using the GAMM model. Three null hypotheses were tested:

- **$H_0$1:** The DO concentration did not vary between stream reaches (i.e., the Reach term is not statistically significant);
- **$H_0$2:** The DO concentration did not vary by period (i.e., the Period term is not statistically significant); and
- **$H_0$3:** The DO concentration did not vary by period and stream reach (i.e., the Reach × Period interaction is not statistically significant).

Thus, the three null hypotheses listed above were evaluated to provide lines of evidence to assess whether low DO concentrations may have contributed to the Reduced Recruitment in the Harmer Creek population area. If low DO concentrations caused the Reduced Recruitment , then we hypothesize there would have been clear differences in space and time in DO concentrations, with the lowest concentrations observed in the Harmer Creek population area during the Reduced Recruitment. It is acknowledged that use of HRM-R1 to represent the Grave Creek population area is confounded by conditions in the Harmer Creek population area, i.e., HRM-R1 is at the downstream end of Harmer Creek (Map 1) and is therefore not an independent representation of conditions in the Grave Creek population area, and HRM-R1 does not provide an indication of DO concentrations in reaches 3 and 4 (reference reaches) of Grave Creek, upstream of the Harmer Creek confluence. However, constraints with the availability of data (described in Section 3.2.1) meant it was not possible to complete the statistical analysis using data collected at a mainstem site in Grave Creek. Accordingly, it was necessary to consider this potentially confounding influence when evaluating the results and including data for HRM-R1 in the analysis was deemed preferable to omitting the site from the analysis[18].

To examine statistical power, we conducted a separate *post hoc* test for each non-statistically significant result to quantify the effect size that could theoretically be detected using the test. The tests involved repeating the analysis with synthesized datasets in which values were reduced by increments of 0.05 mg/L, thereby reducing the average DO concentration for a specific treatment, while maintaining the variance and seasonality in the data. The values that were adjusted varied depending on the null hypothesis being considered: for $H_0$1, values for DC-R2 were reduced; for $H_0$2, values from both sites

[18] If a clear change in DO concentrations were observed at HRM-R1, then further evaluation would be required to understand whether such a change solely reflects conditions experienced by fish in the Grave Creek population area, or whether the change reflects variability in DO concentrations in the Harmer Creek population area upstream.

For further context, Dry Creek contributes a minority (~15–25%) of the discharge observed at HRM-R1, based on discharge estimates reported in Table 3.19 of Cope and Cope (2020). Thus, including HRM-R1 in the analysis could at least provide a check of whether a clear change in DO concentrations hypothetically observed at Dry Creek was also observed elsewhere, thereby potentially helping to understand the cause of such a change, if it were observed.

corresponding to the Period of Concern were reduced; and for H_03, values from DC-R2 corresponding to the Period of Concern were reduced. For each test, progressively greater reductions in DO concentrations were applied until the test returned a *p-value* < 0.05, thus indicating the magnitude of change required to yield a statistically significant result. All modelling analyses were completed using the mgcv packages in the R Statistical Language Program (R Core Team 2021). Prior to modelling, two outlier values were removed from the analysis due to anomalously high DO concentrations that were presumed to be erroneous (16.85 mg/L, recorded at DC-R2 on March 3, 2015 and 91.70 mg/L, recorded at HRM-R1 on October 17, 2016).

Table 3. Periods analyzed using analysis of variance.

Period	Site	Incubation		Other Life Stages	
		Start	End	Start	End
Before	HRM-R1	08-Jun-13	31-Oct-16	01-Nov-13	07-Jun-17
	DC-R1	08-Jun-14	31-Oct-16	01-Nov-14	07-Jun-17
Period of Concern	HRM-R1 and DC-R1	08-Jun-17	31-Oct-16	01-Nov-17	07-Jun-20

Figure 1. Relationship between dissolved oxygen concentration and water temperature in freshwater, assuming 100% saturation. The relationship is based on equation 19.32 presented by Chapra (2014), sourced from APHA (1992).

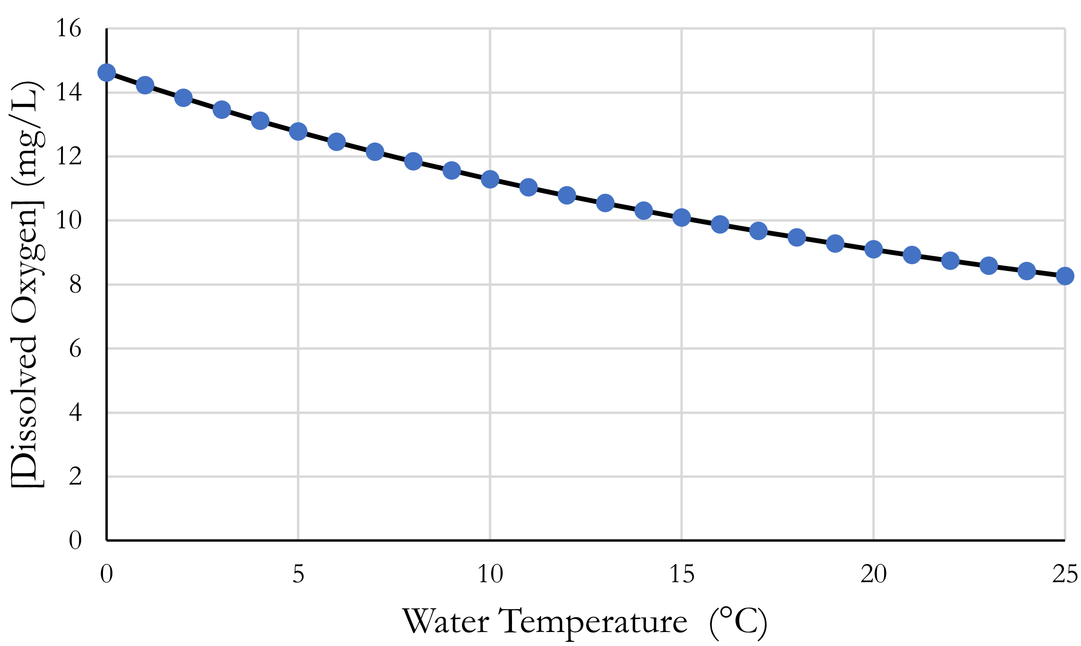


Table 4. WCT life history periodicity table for the Grave Creek Watershed. Within the incubation periodicity, two scenarios are provided based on observations of redd construction and WCT spawning habits: incubation for eggs spawned early in the spawning period (dark grey) and incubation for eggs spawned late in the period (light grey).

Life History Activity	Jan				Feb				Mar				Apr				May				Jun				Jul				Aug				Sep				Oct				Nov				Dec			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Spawning																																																
Incubation (egg and alevin)																																																
Summer Rearing (>5°C)																																																
Over-wintering																																																

3. RESULTS

3.1. Review

3.1.1. Overview

In fishes, maintenance of appropriate blood oxygen levels is essential to sustain life, and low DO concentrations have been shown to adversely affect growth rate, food conversion efficiency, and feeding, in addition to enhancing the lethal effects of toxicants that may be present (reviewed by Davis 1975). Like other salmonids, WCT inhabit cold well-oxygenated water and they are sensitive to reductions in DO concentrations (COSEWIC 2006), e.g., due to increased oxygen demand from organic material or infiltration of fine sediments into spawning gravels, which can reduce DO availability to buried eggs and alevins (Greig *et al*. 2007).

Section 3.1.2 describes the applicable DO guidelines and summarizes key supporting information that was used for guideline development. Section 3.1.3 summarizes the outcome of a review of literature specific to the DO tolerance of Cutthroat Trout.

3.1.2. Water Quality Guidelines and Supporting Literature

Water quality guidelines are scientifically derived quantitative thresholds or narrative statements that are considered protective of values such as aquatic life (BC MOECC 2019a). Water quality guidelines do not have direct legal standing and exceedance of guidelines does not mean that adverse effects will occur, but rather that further investigation may be warranted (BC MOECC 2019b).

For DO, water quality guidelines for the protection of aquatic life have been established at a federal level by the Canadian Council of Ministers of the Environment (CCME) (1999). At a provincial level, the BC government (a member of the CCME) has also developed DO water quality criteria for the protection of aquatic life (Table 1; BC MOE 1997). These provincial guidelines (BC MOE 1997) were developed to specifically protect life in BC waters and are therefore considered the most applicable DO guidelines in relation to Harmer and Grave creeks[19]. No site-specific water quality objectives have been identified for the two streams, based on reviewing objectives listed by BC MOECC (2021).

Provincial DO water quality criteria are prescribed in relation to instantaneous minimum concentrations as well as 30-day mean values (Table 1). Guidelines based on instantaneous minima are most applicable for assessing the potential for acute (short term and severe) effects, whereas the 30-day mean values apply to assessing the potential for chronic effects (long term effects such as reduced growth or reproduction success). Individual measurements of DO from Harmer and Grave creeks are considered directly comparable with guideline instantaneous minimum water column

[19] For context, the federal guidelines for "cold water" ecosystems (applicable to salmonid-bearing streams such as Harmer and Grave creeks) prescribe a minimum acceptable DO concentration of 9.5 mg/L for buried life stages (embryos and alevins) and 6.5 mg/L for other life stages.

values[20]. Our view is that the 30-day mean values prescribed by provincial guidelines (BC MOE 1997) provide context for this assessment, but the available data are not directly comparable with the mean guideline values because the sampling frequency (typically monthly or less) is insufficient to appropriately calculate 30-day mean values (see footnote 3 in Table 1 for recommended calculation method).

Guidelines applicable to buried embryos and alevins, as well as guidelines applicable to other life stages (e.g., WCT fry), are both relevant to this assessment, reflecting that the Reduced Recruitment of WCT could be caused by impacts to buried life stages (embryos or alevins) and/or impacts to free-swimming juvenile life stages (Table 5). When considering potential effects to buried WCT life stages, conditions in interstitial water are more relevant than conditions in surface water; however, for this assessment, the DO dataset relates to measurements collected in stream surface water and therefore it is not possible to directly compare interstitial measurements with the guidelines for interstitial waters (Table 1). The BC surface water DO guidelines to protect buried embryos/alevins are based on the guidelines for interstitial water, with the assumption that interstitial DO concentration is 3 mg/L lower than the DO concentration of the overlying water due to reduced permeability and oxygen consumption in the hyporheic region (BC MOE 1997). The differential of 3 mg/L is based on the value used in the US EPA (1986) DO guidelines, which identify a differential of 3 mg/L based on reviewing two studies that considered streams in Pennsylvania and Oregon. It is uncertain whether it is accurate to expect a 3 mg/L difference in DO concentrations between surface and interstitial waters in Grave and Harmer creeks. However, in the Fording River watershed, Wright *et al.* (2018) showed that the difference between DO concentration measured at a depth of 50 cm in the substrate was typically (but not consistently) less than 3 mg/L lower than DO concentration measured in surface water in Greenhills, LCO Dry, and Henretta creeks, which are affected by calcite accretion to varying degrees. Thus, if it is assumed that stream water quality and substrate in those three creeks are broadly similar to those in Harmer and Grave creeks, the results of Wright *et al.* (2018) suggest that the 3 mg/L differential that is used in the BC guidelines may be an overestimate for the study streams, meaning that use of the surface water guidelines to assess the potential for effects to buried embryos/alevins is precautionary. This assumption is uncertain as information about substrate porosity and interstitial water quality in the Grave Creek watershed was unavailable to inform this assessment.

[20] A qualifier is that the direct comparison of spot measurements with guideline values assumes that diurnal fluctuations of DO in the study streams are negligible. Due to the expected low primary productivity of the study streams, it is likely that diurnal variability of DO is generally absent or negligible and therefore this assumption is reasonable; however, validation of the assumption would require multiple measurements collected over a 24-hour period, which are unavailable. BC MOE (1997) stresses the importance of designing a sampling strategy that accounts for diurnal fluctuations in DO concentrations in productive waters, where DO concentrations typically reach daily minimum values in the morning and daily maximum values late in the day.

The provincial DO criteria (Table 1) are largely based on the US EPA (1986) information on fishery production impairment levels, modified to be sufficiently conservative for protecting cold-water fisheries in BC (BC MOE 1997). For embryo/alevin life stages, the instantaneous minimum guideline in interstitial waters of 6 mg/L (9 mg/L in the water column) was selected to be slightly more conservative than the 1-day minimum value of 5 mg/L prescribed by US EPA (1986) for cold-water fisheries. BC MOE (1997) states that prolonged exposure to the instantaneous minimum guideline of 9 mg/L in the water column would result in minor loss of production, corresponding to the US EPA's "slight" impairment level that is defined as "representing a high level of protection of important fishery resources, risking only slight impairment of production in most cases", assuming a constant exposure level. The 30-day mean guideline in interstitial waters of 8 mg/L (11 mg/L in the water column) for the protection of embryos/alevins was selected to be consistent with the US EPA (1986) impairment level of "none" (Table 5). For juvenile and adult life stages, the instantaneous minimum guideline of 5 mg/L was selected to correspond to the US EPA's "moderate" impairment level (deemed acceptable as a short-term non-lethal stress level), whereas the 30-day mean guideline of 8 mg/L was selected to be consistent with the US EPA (1986) impairment level of "none".

The provincial DO criteria (Table 1), in addition to the US EPA (1986) guidelines, are based on multiple studies in the laboratory and under field conditions that considered fish species that included salmonids and other cold-water fishes such as sculpins. Species studied included multiple species of the *Oncorhynchus* genus, such as Rainbow Trout (*O. mykiss*), Chinook Salmon (*O. tshawytscha*), and Sockeye Salmon (*O. nerka*); however, the technical appendix that supports the provincial DO criteria does not reference studies of Cutthroat Trout (BC MOE 1997), reflecting a paucity of studies relating to the DO requirements of the species (see Section 3.1.3). Nonetheless, it is reasonable to expect that studies of the DO tolerance of other *Oncorhynchus* species have broad relevance to WCT, given that *Oncorhynchus* species occupy habitats of similar water quality (i.e., cool and well-oxygenated), and the distribution of Cutthroat Trout overlaps with the distributions of other *Oncorhynchus* species. Regarding acute effects, the literature reviewed by BC MOE (1997) shows that lethal DO concentrations are ~1.5–3.0 mg/L for free swimming juvenile salmonids, as well as buried embryos/alevins (for which the lethal range applies to interstitial waters, implying a lethal range of ~4.5–6.0 mg/L in overlying surface water, assuming a 3 mg/L difference between interstitial and water column DO). Regarding chronic effects, BC MOE (1997) considers literature pertaining to a range of effects, including impairment to swimming performance, metabolism, and feeding. Figure 2 and Figure 3 below are reproduced from BC MOE (1997) and provide useful context to assess the potential effect of reduced DO concentrations on WCT, with the qualifier that the information is based on studies conducted on other fish species that are assumed to have similar tolerance to DO reductions, as discussed above. Key points from these two figures that are pertinent to this assessment are:

- Relative to growth at an ambient DO concentration of 9 mg/L, a decline in DO concentration to 8.5 mg/L (approximately the minimum value in the dataset; Section 3.2.1) is expected to cause no reduction or a minor reduction (~0–2%) to the growth of free swimming life stages of cold-water fishes (Figure 2).

- Based on the slopes of the linearly interpolated lines in Figure 3, a decline in DO concentration from ~9 mg/L to ~8 mg/L is expected to generally have a similarly minor effect on the development of salmonid embryos, based on measurements of growth at hatch (Figure 3). The results of one study suggest a slightly greater effect (a growth reduction of ~5%; see red line for "Steelhead-b" study in Figure 3), although this inference is based on extrapolation between data points across a wide range of DO concentrations and is therefore considered uncertain.

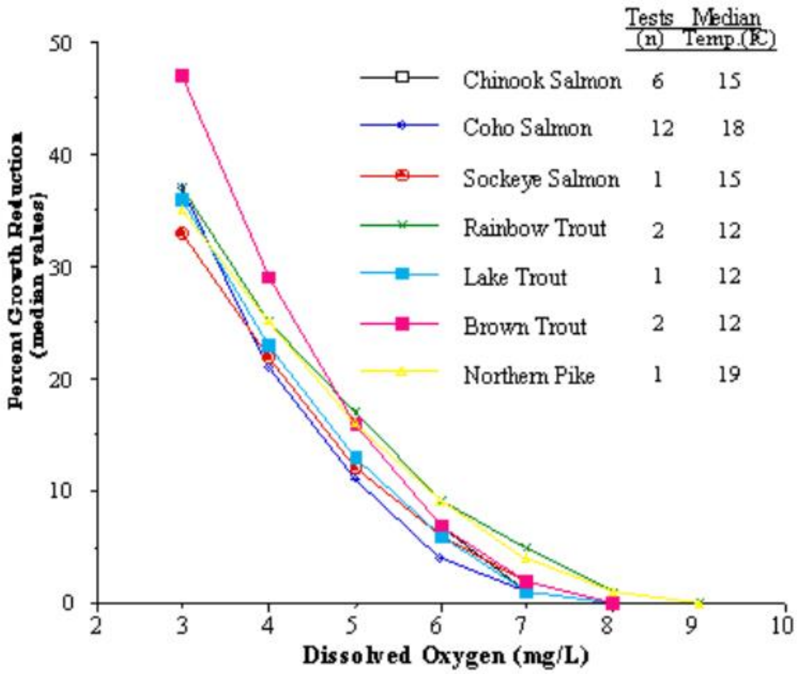
- Based on Figure 3, the growth of embryos could be reduced by ~10–35% due to a decline in interstitial DO concentrations from an approximate optimum value of 11 mg/L to a concentration of ~5.5 mg/L (i.e., 3 mg/L less than the approximate minimum value of ~8.5 mg/L measured in surface water at DC-R2).

Table 5. US EPA (1986) production impairment levels for dissolved oxygen. The format of the table is based on Table 10 in BC MOE (1997).

Impairment	Salmonid Early Life Stages (mg O_2/L)[1]	Salmonid Other Life Stages (mg O_2/L)
None	11 (8)	8
Slight	9 (6)	6
Moderate	8 (5)	5
Severe	7 (4)	4
Limit to avoid acute mortality	6 (3)	3

[1] Numbers in parentheses represent interstitial oxygen concentrations (a 3 mg/L differential relative to overlying water is assumed).

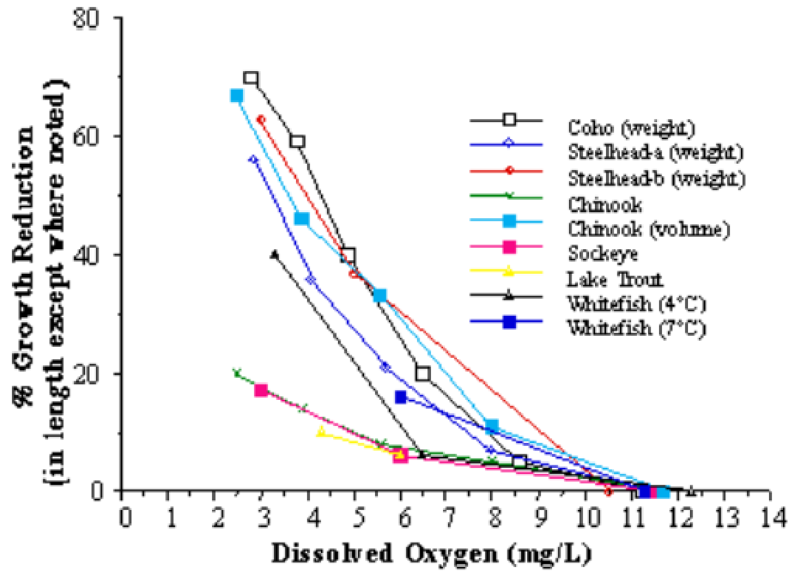
Figure 2. Reduction (%) in growth[21] of salmonid/salmonid-like fishes at dissolved oxygen concentrations, relative to growth at 8–9 mg/L. Figure reproduced from BC MOE (1997), which provides details of the supporting studies.



[21] The figure is based on data presented in Table 1 of US EPA (1986), although values for Brown Trout and Lake Trout seem to have been swapped. Values for Chinook Salmon are obscured on the figure by the values presented for Lake Trout. Growth rates were calculated based on changes in fish weight, e.g., as described in Brett and Blackburn (1981), which is one of the source studies.

As reported in US EPA (1986), the dataset used to prepare Figure 2 was prepared by JRB Associates (1984), who analyzed data from >30 experimental tests to prepare a standardized dataset that was adjusted to account for variability among the tests in factors such as water temperature and food type. Accordingly, the data shown are directly comparable values of growth reduction (based on fish weight), relative to growth at an ambient dissolved concentration (8 mg/L or 9 mg/L) that yields no impairment to growth.

Figure 3. Effects of reduced dissolved oxygen concentration on the growth of salmonid embryos at hatch. Figure reproduced from BC MOE (1997), which provides details of the supporting studies.



3.1.3. Additional Literature

Given that the BC MOE (1997) guidelines were published >20 years ago, a focused literature review was undertaken with the aim to identify studies specific to the DO tolerance of Cutthroat Trout. Only a single, partly relevant study was identified (described below).

Wagner *et al.* (2001) studied hypoxia tolerance in six stocks of Cutthroat Trout that comprised free-swimming juveniles from the following sub-species: Bonneville (*O. c. utah*), Yellowstone (*O. c. bouvbieri*), and fine spotted Snake River (*O. c.* subsp.). Fish acclimated to 13°C were subject to tests in tanks that involved either reducing the DO concentration over 24 hours, or subjecting fish to constant DO concentrations for 24-hours in the range 1.85–3.34 mg/L. Mortality began when DO concentration declined below 2.6 mg/L and there was no difference among the stocks in tolerance to hypoxia. The threshold for mortality was therefore lower than the instantaneous minimum guideline value for free-swimming life stages (5 mg/L) prescribed by BC WQG-AL (Table 1). The study did not specifically consider the WCT sub-species, nor did it evaluate chronic effects or effects to embryos/alevins.

3.2. Variability in Dissolved Oxygen Concentrations

3.2.1. Data Summary

Available DO data for Grave Creek and Harmer Creek watersheds are presented in Figure 4. Data are the same as those presented by Warner *et al.* (2022) and are provided here for context, with plots re-drawn to better show temporal variability, as well as the instantaneous (acute) guidelines, which are directly comparable with the spot measurements.

For the Harmer Creek population area, data are most extensive for DC-R2 (Map 1), where DO has been measured at monthly frequency since 2014, with less frequent measurements available for the period 2009–2013. All data analyzed for Dry Creek were collected at EV_DC1, which is at the outlet of Dry Creek Sedimentation Pond and reflect conditions in DC-R2 and, to an extent, DC-R1 immediately downstream; DO measurements collected in Dry Creek do not necessarily reflect conditions upstream of the sedimentation pond.

For the Grave Creek population area, data are most extensive for reach HRM-R1 (which is downstream of the Harmer Creek Sedimentation Pond; Map 1), where DO has generally been measured at monthly or greater frequency since at least 2009. The availability of data for other sampling locations is limited and insufficient to compare DO concentrations between periods before and after the Reduced Recruitment period (Figure 4).

Figure 4. **Dissolved oxygen concentrations measured in Grave Creek and Harmer Creek watersheds compared to BC water quality guidelines; see Map 1 for site locations.**

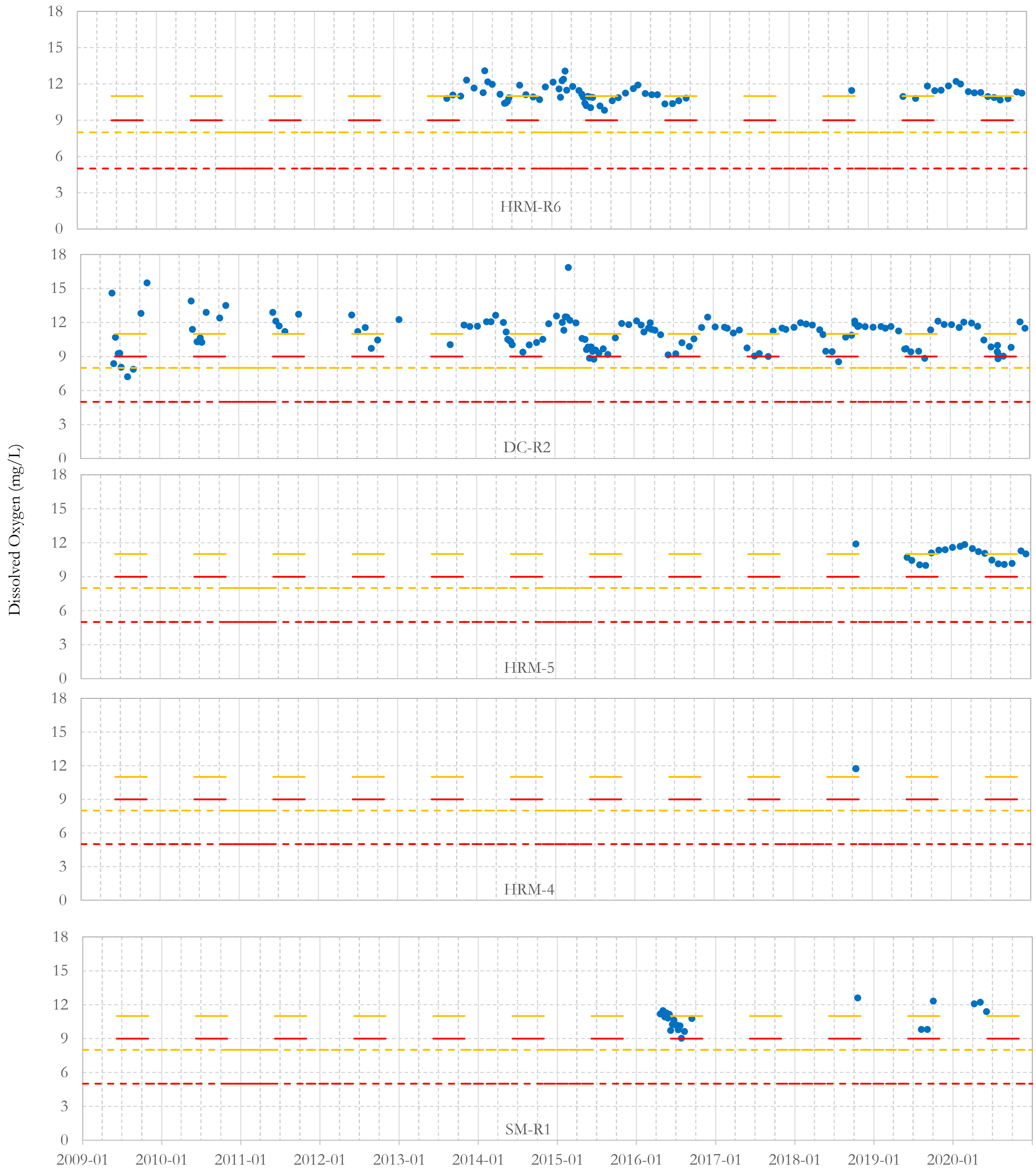
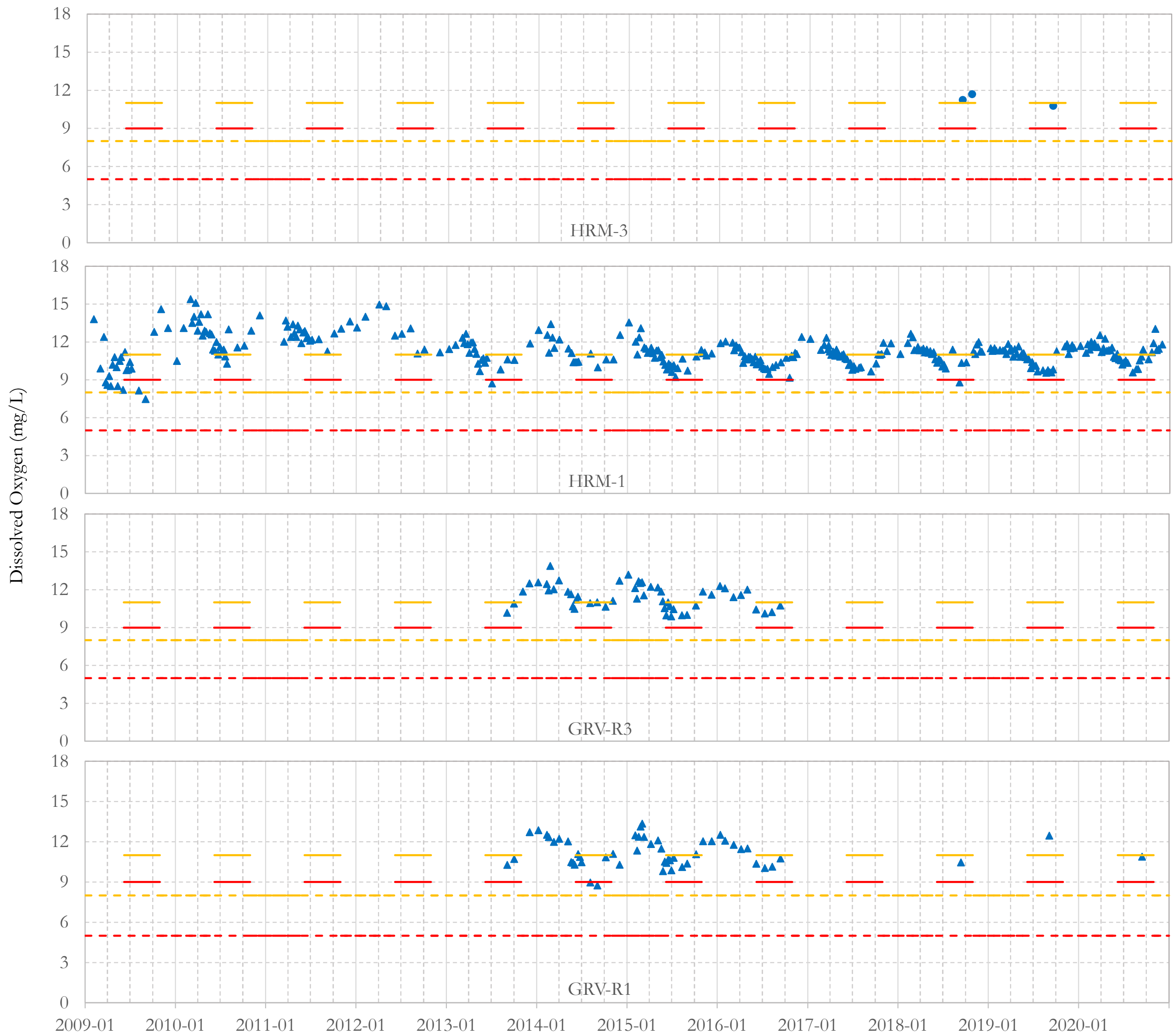


Figure 4. Continued (2 of 2).



● DO measurements in the Harmer Creek population area

▲ DO measurements in the Grave Creek population area

—— Instantaneous DO criteria for buried embryo/alevin life stages

—— Chronic DO criteria for buried embryo/alevin life stages

- - - Instantaneous DO criteria for all life stages other than buried embryo/alevin

- - - Chronic DO criteria for all life stages other than buried embryo/alevin

Chronic guidelines are shown for context only and are not directly comparable with spot measurements.

3.2.2. Spatial Variability

3.2.2.1. Spatial Variability Within Harmer Creek Population Area

DO concentrations varied among sampling locations within the Harmer Creek WCT population area (HRM-R6, DC-R2, HRM-R5, HARM4, SAWM1, HARM3), although there were differences in the temporal extents of the datasets, which limited the extent to which data could be directly compared among sites for matching periods (Figure 4). Paired DO concentrations for Harmer Creek sampling locations are available for 2019 and 2020; DO statistics are presented in Table 6 based on available data for the incubation period, i.e., the most sensitive period of the year that also coincides with the period when annual minimum values occur. During the incubation period, DO concentrations in Dry Creek were lower than in Reach 6 (headwaters) of Harmer Creek during periods with paired data (Figure 4). In Reach 5 of Harmer Creek downstream of the confluence with Dry Creek, DO concentrations during the incubation periods in 2019 and 2020 were intermediate between those in Reach 6 of Harmer Creek (higher values) and those in Dry Creek (lower values) (Figure 4; Table 6).

DO saturation was calculated for each spot measurement collected in Dry Creek using the equations for determining the equilibrium oxygen concentration at non-standard temperatures and pressures (Benson and Krause 1984). DO saturation (%) is a measure of the DO concentration of a water sample, relative to the theoretical DO concentration based on water temperature (see Figure 1). Thus, DO saturation provides useful information about the balance between oxygen consuming and oxygen generating processes, whereby sub-saturated conditions (i.e., DO saturation <100%) indicate net oxygen consumption due to biochemical oxygen demand. DO saturation is less relevant than DO concentration to fish physiology as transfer of oxygen across fish gills is controlled by diffusion down a concentration gradient (BC MOE 1997).

For the period with regular monthly data (2014 to 2020), DO saturation values in Dry Creek remained generally constant around 100%, even when DO concentrations were lower in the incubation period during the summer (Figure 5). Furthermore, the periods with lower DO concentration coincided with the period with higher water temperature (Figure 6). These DO saturation and water temperature characteristics indicate that lower DO concentrations during the incubation period in Dry Creek were caused by higher background water temperatures in this period, e.g., rather than DO consumption due to processes such as decomposition of organic material caused by factors such as pollution or decay of dense communities of periphyton. Data for Dry Creek were collected at the outlet of the Dry Creek Sedimentation Pond and therefore water temperatures downstream of the pond are expected to be influenced by warming in the pond in the summer. Prior to 2014, DO was frequently supersaturated (100-140%); the reason for this is uncertain, although it could be due to sampling late in the day during periods with high algal productivity, or due to measurement error. Uncertainty associated with this potential data quality issue supports our approach of focusing on the period after 2014.

In Sawmill Creek and mainstem Harmer Creek, DO concentrations during the incubation period were not less than (i.e., were compliant with) the instantaneous guideline minimum value of 9 mg/L that applies to embryos/alevins, indicating that acute effects on early life stages would not be expected in these areas. In Dry Creek, five DO measurements were below the guideline instantaneous minimum (9.0 mg/L) for buried eggs and alevins (8.85 mg/L on 9 June 2015, 8.77 mg/L on 29 June 2015, 8.54 mg/L on 2 August 2018, 8.84 mg/L on 4 September 2019, and 8.80 mg/L on 7 August 2020) during the period of interest. However, these low DO concentrations were only slightly (2–5%) less than the guideline instantaneous minimum. All DO concentrations measured in the Harmer Creek population area (including Dry Creek) were above the guideline instantaneous minimum (5.0 mg/L) that applies to life stages other than buried eggs and alevins.

Consideration of potential chronic effects to fish caused by low DO concentrations is critical for evaluating whether DO is a stressor that may have caused lower WCT recruitment. The extent of DO measurements is insufficient for a full evaluation of chronic effects, which requires calculation of 30-day mean values. However, consistent with Warner *et al.* (2022), we evaluated monthly DO spot measurements in the context of the chronic effect guidelines to provide an indication of the potential for low DO concentrations to have caused chronic effects to WCT. Most DO concentrations measured during the incubation period were lower than the long-term minimum guideline water column value of 11 mg/L for buried eggs and alevins in Dry Creek, and available DO measurements in reach 5 of Harmer Creek (HRM-R5) showed the same trend (Figure 4). Several DO measurements collected at HRM-R6 (headwaters) were also below the guideline value of 11 mg/L during the incubation period, although available data indicate that DO measurements during the incubation period were higher at HRM-R6 than at either HRM-R5 or Dry Creek (Figure 4).

Figure 5. Dissolved oxygen concentrations and saturation in Dry Creek (DC-R2).

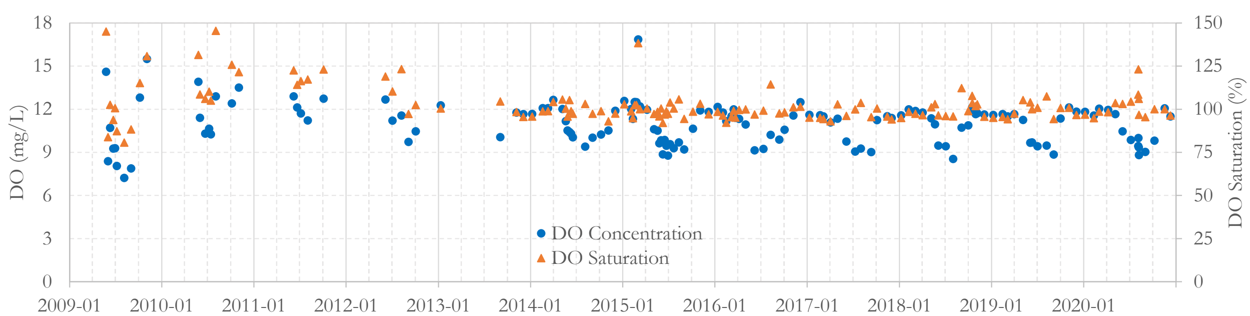


Figure 6. Dissolved oxygen concentrations and water temperature in Dry Creek (DC-R2).

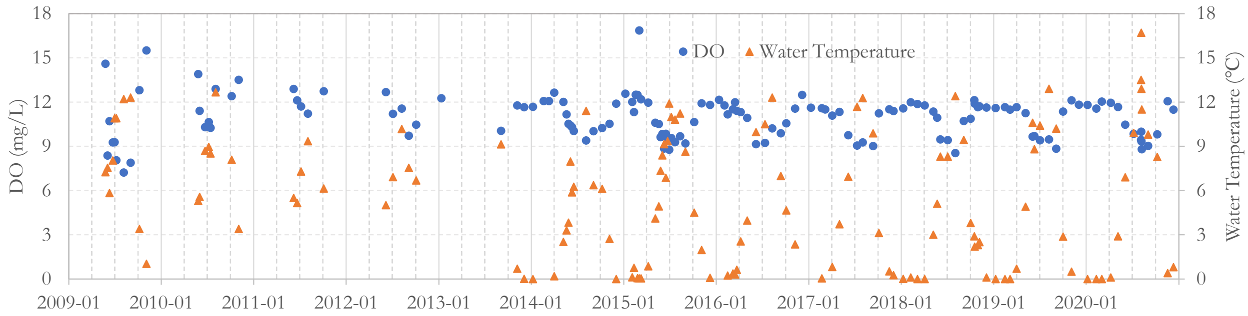


Table 6. Summary statistics for dissolved oxygen concentrations during incubation periods in 2019 and 2020, which are the years when paired measurements collected at the Harmer Creek sites were available.

Year	Statistic (mg/L)	Reach			
		Reference	Mine Influenced		
		HRM-R6	DC-R1	HRM-R5	HRM-R1
2019	Mean	11.19	9.75	10.47	10.02
	Minimum	10.80	8.84	10.00	9.56
	Maximum	11.82	11.35	11.11	11.26
	Standard Deviation	0.55	0.95	0.46	0.50
2020	Mean	10.82	9.46	10.22	10.56
	Minimum	10.67	8.80	10.09	9.59
	Maximum	10.95	9.99	10.48	11.90
	Standard Deviation	0.12	0.45	0.18	0.60

3.2.2.2. Spatial Variability Between the Grave and Harmer Population Areas

Very few DO concentrations were measured in Grave Creek mainstem sampling locations after September 2016 (Figure 4) so it is necessary to consider data for reach 1 of Harmer Creek (HRM-R1) to characterize DO conditions in the Grave Creek population area during the Reduced Recruitment period, although we acknowledge that HRM-R1 does not fully reflect conditions in the mainstem of Grave Creek, and that conditions at HRM-R1 are affected by conditions in the Harmer Creek population area upstream (Section 2.2.2). Based on available data for sampling locations in the Harmer population area (HRM-R5, HRM-R6, and DC-R2) for 2019 and 2020, average and minimum DO concentrations were highest at HRM-R6 (reference) and lowest at DC-R2 (mine-affected) (Table 6, Figure 4).

One DO measurement at HRM-R1 during the incubation period in the period of interest was below the instantaneous minimum guideline for buried eggs and alevin (8.79 mg/L on 4 September 2018). No measurements collected at HRM-R5 or HRM-R6 were below this guideline, whereas five measurements were below the 9 mg/L guideline at DC-R2 (see Section 3.2.2.1). DO concentrations at all sampling locations were above the instantaneous minimum guideline (5 mg/L) that applies to all stages other than buried eggs/alevin. For Dry Creek and HRM-R1, DO concentrations during the incubation period were lower than the long-term minimum guideline value (11 mg/L) for buried eggs and alevin during most years, including every year since 2014 (Figure 4).

Differences between DO concentrations measured at DC-R2 (Harmer Creek population area) and HRM-R1 (Grave Creek population area) are further examined in Section 3.2.3 based on ANOVA results.

3.2.2.3. Correspondence with Fish Distribution

It is necessary to consider spatial variability in DO measurements in the context of information about fish distribution in the watershed. Based on the summary by Cope and Cope (2020) of observations during 2018 and 2019, WCT have access to all reaches for which DO data were available (Table 2). However, WCT spawning does not widely occur in Dry Creek due to habitat impairment by calcite: surveys in 2018 and 2019 detected a single redd in Dry Creek, located in roadbed gravels unaffected by calcite formation where the stream channel had avulsed and flowed along the road (Cope and Cope 2020)[22]. Redds were distributed throughout the Harmer Creek mainstem, except for in Reach 6 upstream of the confluence with Dry Creek (Map 1), where water temperatures are coolest. Thus, DO concentrations measured at DC-R2 in Dry Creek (i.e., the primary site in the Harmer Creek population area that is considered in this assessment), are used to represent DO conditions for WCT present in Dry Creek, and DO conditions for WCT present downstream in the Harmer Creek mainstem (Reaches 2 to 5) where the majority of spawning occurs. A qualifier is that DO concentrations in the mainstem are expected to be higher (i.e., more optimal) than those in Dry Creek due to mixing with water from Reach 6 where DO concentrations are generally higher (Figure 4).

In the Grave Creek population area, observations during 2018 and 2019 show that redds were distributed throughout the mainstem up to Reach 3 (Map 1), as well as in HRM-R1. Thus, DO concentrations measured at HRM-R1 (i.e., the primary site in the Grave Creek population area that is considered in this assessment) reflect DO conditions in HRM-R1, and provide an indication of DO conditions in Reaches 1 and 2 of Grave Creek, for which available (albeit limited) historical paired measurements suggest that DO concentrations are similar to those at HRM-R1 (Figure 4). Historical paired measurements for Reach 3 of Grave Creek suggest that DO concentrations in Reach 3 of Grave Creek may be slightly higher than those at HRM-R1 (Figure 4). Thus, DO concentrations

[22] Furthermore, calcite index values for Dry Creek reported by Hocking *et al.* (2022) support the view that Dry Creek is unsuitable for spawning, based on a draft spawning suitability curve developed by Hocking *et al.* (2020).

measured at HRM-R1 are expected to provide a reasonable proxy for DO concentrations in Reaches 1 and 2 of Grave Creek, but they may be slightly lower than DO concentrations in Reach 3 of Grave Creek, i.e., our use of data for HRM-R1 to represent DO conditions in the Grave Creek population area is likely to be precautionary.

3.2.3. Temporal Variability

3.2.3.1. Incubation

As described in Section 2.2.2, temporal variability was analyzed by comparing data collected at HRM-R1 (Grave Creek population area) and Dry Creek (Harmer Creek population area) between two periods during June 2013 through June 2020. In Dry Creek during the incubation period, the magnitude and pattern of seasonal variability in the modelled average DO concentrations was highly similar between the Before Period and the Period of Concern (left panel on Figure 7). Likewise, at HRM-R1, DO concentrations were also similar between the periods of variable recruitment (right panel on Figure 7) when the modelled averages are considered. Accordingly, differences in seasonality were not statistically significant between periods ($\alpha = 0.05$; Table 7). However, seasonal differences were statistically significant between sites, whereby DO concentrations in Dry Creek tended to increase faster through September and October than in HRM-R1. The significance of this interaction supports our approach of modelling seasonality separately for each reach-period combination, rather than assuming that the seasonal pattern of DO concentrations is consistent at both sites and during both periods.

Based on the ANOVA results (Table 7), differences in the overall DO average concentrations between the sites were not statistically significant ($\alpha = 0.05$), nor were differences between the periods at either site. Thus, the three null hypotheses listed in Section 2.2.2 are retained, based on the ANOVA using data collected during the incubation periods. A potential temporal signal in the data based on considering annual minimum values is discussed in Section 4 – note that the overall minimum concentration was measured in Dry Creek in 2018 (2018 data are depicted separately in Figure 7).

The *post hoc* assessment of statistical power showed that, to detect differences between stream reaches (reject H_01), it was necessary to reduce concentrations at DC-R2 by an average of 0.35 mg/L to yield a statistically significant result. To detect statistically significant differences among periods (reject H_02), it was necessary to reduce average concentrations for the Period of Concern by approximately 0.75 mg/L. Similarly, to detect statistically significant differences between period and stream reach (reject H_03), it was necessary to reduce the average DO concentrations for the Period of Concern DC-R2 treatment by 0.50 mg/L.

To understand statistical power, it is relevant to evaluate the results of the *post hoc* assessment in the context of the magnitude of change that is considered biologically significant. Such a change is challenging to define; however, we suggest that the differences required to reject H_02 and H_03 (i.e., reductions in average DO concentration of 0.50–0.75 mg/L) exceed the magnitude of change that is biologically significant, e.g., based on the rationale that a reduction of this magnitude at DC-R2 would result in numerous additional periods when DO concentrations are below the BC MOE (1997) instantaneous minimum guideline of 9 mg/L that is applicable to the incubation period. Based on this rationale, we suggest that the difference required to reject H_01 (i.e., a reduction in average DO concentration of 0.35 mg/L) is approximately equal to the threshold for biological significance, whereby biological significance is considered to be a measurable adverse effect on growth/development of buried embryos and alevins (i.e., a lower threshold than recruitment failure). Based on the above, we qualitatively consider the ANOVA analysis to have moderate power to test H_01, but low power to test H_02 and H_03 for the incubation period.

Figure 7. Dissolved oxygen concentrations during the incubation period in Dry Creek (DC-R2; Harmer Creek population area) and Harmer Creek (HRM-R1; Grave Creek population area) during a Before period (green line) and a Period of Concern (red line). The coloured shaded areas depict 95% confidence intervals. The overlap between the 95% confidence intervals of each period indicates that differences are not statistically significant. Values for the 2018 spawn year (anomalous year) are shown separately.

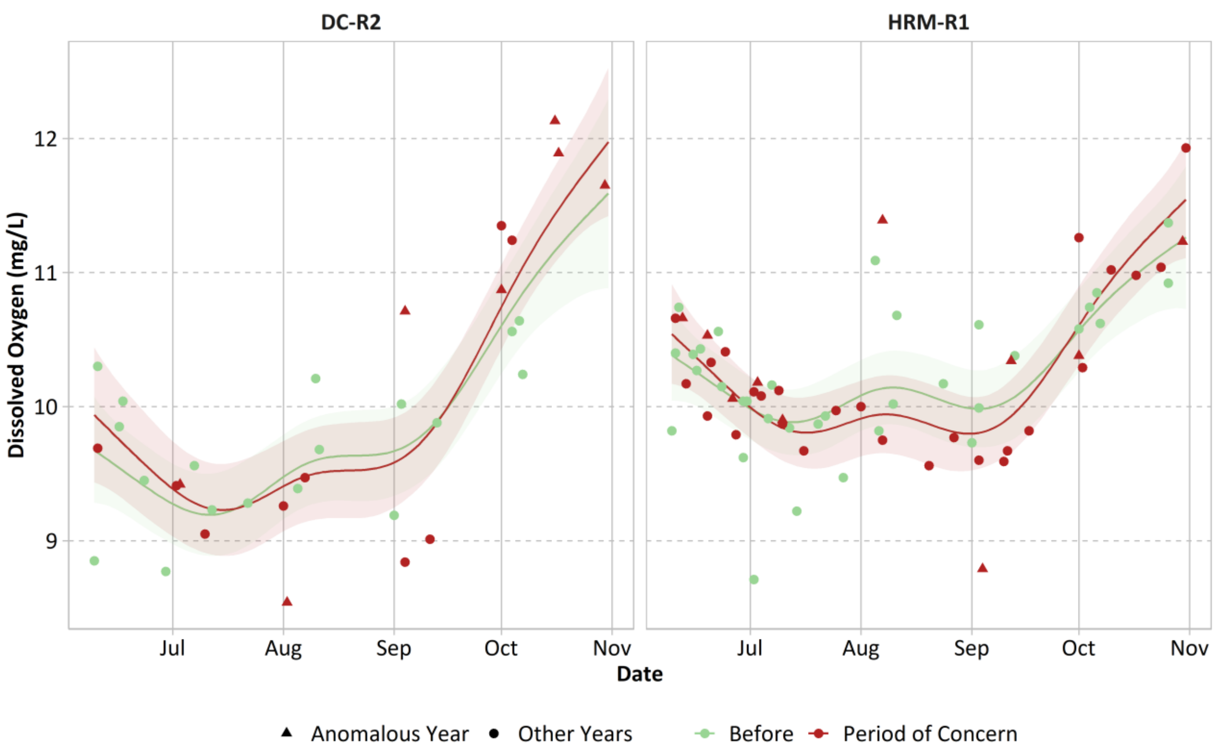


Table 7. ANOVA results for the non-linear component and the parametric components of the analysis of dissolved oxygen concentrations measured during incubation periods.

Term	Degree of Freedom	*F-score*	*p-value*
s(Sample date)	5.99	4.61	< 0.001
s(Sample date, Period)	17	0.23	0.09
s(Sample date, Reach)	17	0.84	< 0.001
Reach	1	1.57	0.21
Period	1	0.18	0.67
Period x Reach	1	0.28	0.60

3.2.3.2. Other Life Stages

When dates outside of the incubation period are considered, seasonal patterns in the modelled average DO concentrations varied between the two periods at both sites (Table 8; Figure 8). This difference is particularly noticeable between December and early February, when modelled average DO concentrations were higher during the Before Period than the Period of Concern, although DO concentrations at these times exceeded (i.e., were compliant with) water quality guidelines (Section 3.1.2).

As for the incubation period (Section 3.2.3.1), our analysis suggested that seasonal patterns in DO concentrations vary between the two reaches (Table 8), whereby DO concentrations in Dry-Creek tended to decrease faster in early summer than in HRM-R1. However, based on the ANOVA results (Table 8), differences in overall average DO concentrations between the sampling locations were not statistically significant ($\alpha = 0.05$), nor were the differences among the periods at either sampling location. The three null hypotheses (Section 2.2.2) were thus not rejected based on the ANOVA results.

Considering all dates outside of the incubation period, the *post hoc* assessment of statistical power showed that, to detect differences between stream reaches (reject $H_0$1), it was necessary to reduce concentrations at DC-R2 by an average of 1.55 mg/L to yield a statistically significant result. Similarly, to detect statistically significant differences among periods (reject $H_0$2), it was necessary to reduce average concentrations for the Period of Concern period by approximately 0.30 mg/L. To detect statistically significant differences between period and stream reach (reject $H_0$3), it was necessary to reduce the average DO concentrations for the Period of Concern DC-R2 treatment by 0.35 mg/L. The magnitude of the difference required to reject $H_0$1 (1.55 mg/L) is biologically significant and therefore we consider the ANOVA analysis to have low statistical power to test $H_0$1in relation to free-swimming life stages. We suggest that the magnitude of the differences required to reject the other two null hypotheses (i.e., reductions in average DO concentration of 0.30–0.35 mg/L) are generally less than the magnitude of change that is considered biologically significant, e.g., based on the rationale that a reduction in DO concentrations of this magnitude would not result in additional periods when DO concentrations are below either the BC MOE (1997) instantaneous minimum guideline of 5 mg/L, or the chronic guideline of 8 mg/L that is applicable free-swimming life stages. Accordingly, we consider the ANOVA analysis to have moderate to high power to test $H_0 2$ and $H_0 3$ in relation to free-swimming life stages.

Figure 8. Dissolved oxygen concentrations measured outside of the WCT incubation period in Dry Creek (DC-R2; Harmer Creek population area) and Harmer Creek (HRM-R1; Grave Creek population area) during a Before period (green line) and a Period of Concern (red line). The coloured shaded areas depict the 95% confidence intervals. Values applicable to the 2018 spawn year ("anomalous year") are shown separately.

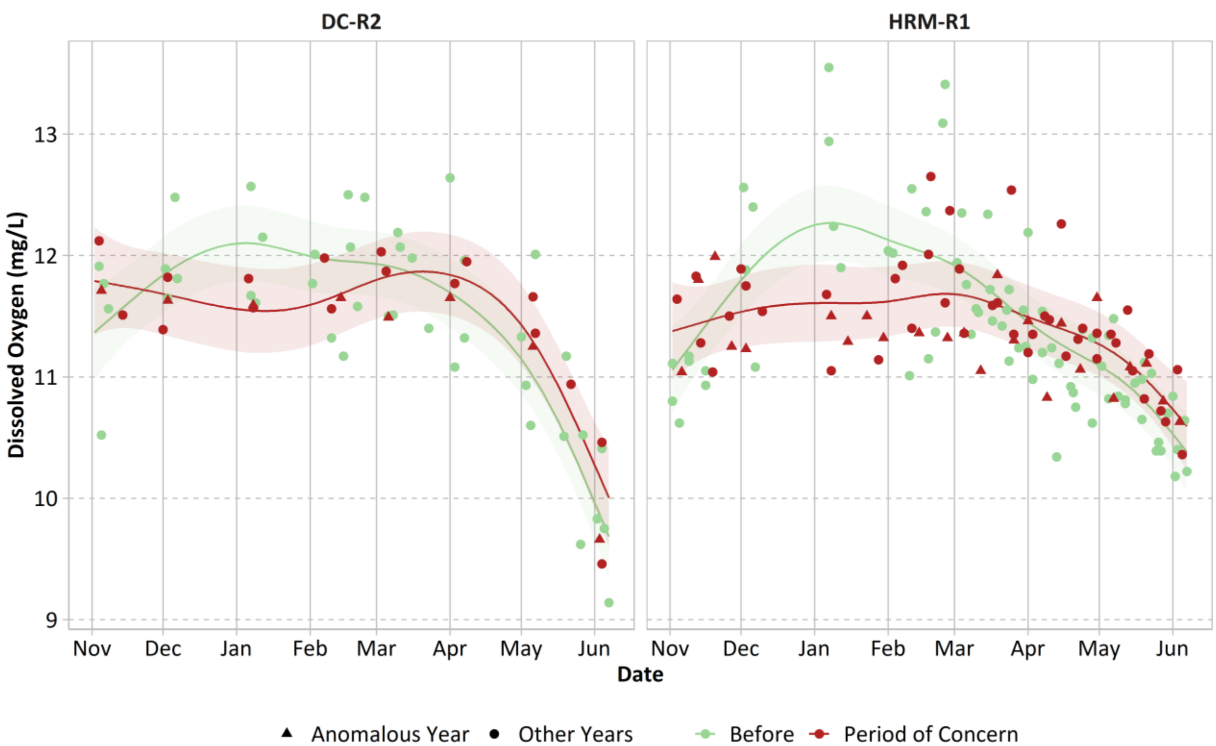


Table 8. ANOVA results for the non-linear component and the parametric components of the analysis of dissolved oxygen concentrations measured outside of incubation periods.

Term	Degree of Freedom	*F-score*	*p-value*
s(Sample date)	2.98	0.66	0.60
s(Sample date, Period)	17	1.86	< 0.001
s(Sample date, Reach)	17	1.48	< 0.001
Reach	1	0.13	0.72
Period	1	2.33	0.13
Period x Reach	1	0.74	0.39

4. DISCUSSION

Outcomes of the review and analysis described above support the following lines of evidence relevant to understanding whether low DO concentrations may have contributed to the Reduced Recruitment of WCT in the Harmer Creek population area:

- Available data (Figure 4) for the Harmer Creek population area were predominantly collected in Dry Creek (mine-influenced), as well as the Harmer 5 (mine-influenced) and Harmer 6 reaches (reference). These data indicate that adverse DO conditions are not expected to have caused acute or chronic adverse effects to free swimming life stages of WCT (fry, parr, and adults) in the Harmer Creek population area. This conclusion is based on comparing measurements with BC WQG-AL (Table 1) and considering other literature and supporting studies (e.g., Figure 2), which indicate that chronic adverse effects to free swimming life stages of salmonids are not expected to occur at DO concentrations in the range > 8 mg/L, which bounds the minimum DO concentrations measured in either population area.

- Available data (Figure 4) show that water column DO concentrations in Dry Creek (DC-R2) during the WCT incubation period were occasionally below the instantaneous guideline minimum (9 mg/L; Table 1) applicable to the protection of buried life stages (embryos/alevins). This observation applies to incubation periods relevant to the Reduced Recruitment period as DO concentrations <9 mg/L were measured in Dry Creek during August 2018 and September 2019 when minimum DO concentrations of 8.54 mg/L and 8.84 mg/L were measured respectively, i.e., slightly (2–5%) below the guideline. The lowest of these two values was the lowest DO concentration measured at any site in the period analyzed (post 2014) and reflected conditions during incubation of the cohort of fish that was age-1 during sampling in September 2019[23]. Data for Dry Creek were collected at the outlet of the Dry Creek Sedimentation Pond and reflect conditions in DC-R2 and, to an extent, DC-R1 immediately downstream; DO measurements collected in Dry Creek do not necessarily reflect conditions upstream of the sedimentation pond.

The occurrence of low DO concentrations in Dry Creek during the incubation period was driven by the occurrence of relatively warm water temperatures during summer; water temperature measured during DO sampling in August 2018 was 12.4°C, which is slightly above the optimum temperature range for Cutthroat Trout incubation (9–12°C; Oliver and Fidler 2001) and, at the time, was the highest water temperature measured in Dry Creek since 2010 (Figure 6; higher temperatures have been subsequently measured). Summer water temperatures in DC-R2 are likely influenced by warming in the Dry Creek Sedimentation Pond.

Furthermore, the available data strongly indicate that DO concentrations were occasionally below the long-term (chronic) guideline minimum (11 mg/L) that is applicable to the protection of buried life stages during the WCT incubation period at all or most sampling locations throughout the two population areas (Figure 4). This observation reflects that spot measurements at most sampling locations were frequently <11 mg/L during the incubation period, although insufficient data are available to make direct comparisons with the long-term guideline, which is based on a 30-day mean value (Table 1).

Thus, available data indicate that DO conditions in the Harmer Creek population area were sub-optimal for WCT incubation. The effect of the low observed DO concentrations on the development of eggs and alevins is uncertain, although it is likely that DO concentrations were temporarily sufficiently low to cause chronic effects (e.g., impair development), but likely not sufficiently low to be the sole cause of the Reduced Recruitment. Uncertainty partly relates to the lack of information about how DO concentrations in interstitial water (not measured but most relevant to eggs/alevins) relate to measurements in surface water. Based on Figure 3, an estimated worst-case prediction for Dry Creek is that low DO concentrations could have caused a reduction in the growth of embryos of up to ~10–35% (based on length), relative to growth at a DO concentration of 11 mg/L, although a key qualifier is that spawning does not widely occur in Dry Creek due to calcite concretion (Section 3.2.2.3). This estimate is predicated on the assumption that the DO concentration in interstitial water was ~3 mg/L lower (BC MOE 1997) than the minimum surface water DO concentration measured in August 2018 (8.54 mg/L), although the estimate is considered worst-case as such low interstitial DO concentrations (i.e., ~5.5 mg/L) potentially only occurred during part of the incubation period. Furthermore, DO concentrations in the mainstem of Harmer Creek where

[23] DO concentrations (e.g., 9.01 mg/L and 9.05 mg/L) approximating the guideline (9.0 mg/L) were also measured at Dry Creek during the WCT incubation period in 2017, which corresponds to the incubation period for the cohort of fish that was age-1 in 2018.

spawning generally occurs (Cope and Cope 2020) are expected to have been higher than those in Dry Creek, e.g., based on comparing measurements for DC-R2 and HRM-R5 in Figure 4. Nonetheless, given the short summer rearing period between fry emergence and overwintering (Table 4), it might be expected that a small reduction in embryo growth could have a disproportionately large adverse effect on overwinter survival of age-0 fry. Therefore, while it seems unlikely that the low measured DO concentrations could have solely caused the Reduced Recruitment, it is possible that low DO concentrations during incubation in Harmer Creek mainstem might have interacted with other stressors to have caused a larger cumulative adverse effect on recruitment. As a hypothetical example, it is plausible that low interstitial DO concentrations acted additively or synergistically with a stressor such as unusually cool water temperatures during part of the incubation period (not evaluated here) to cause chronic effects to eggs and alevins in Harmer Creek mainstem downstream of Dry Creek, leading to poor overwinter survival of age-0 fry.

- If low DO concentrations were the sole cause of the Reduced Recruitment Period, then we hypothesize there would have been a clear difference in DO concentrations between the Harmer Creek population area (Reduced Recruitment observed in multiple years, with recruitment failure observed in the 2018 spawn year) and the Grave Creek population area (no recruitment failure observed and Reduced Recruitment only observed in one year). ANOVA showed no statistically significant difference in DO concentrations between the Grave Creek (in HRM-R1) and Harmer Creek (DC-R2) population areas during the periods of reduced or failed recruitment in Harmer Creek; however, the statistical power of this test was low for analysis of the incubation periods (Section 3.2.3), and therefore the ANOVA provides a weak line of evidence in this case. Inspection of the data shows that the lowest DO concentrations during the incubation period were measured in Dry Creek (see above) and DO concentrations were generally lower at DC-R2 (Harmer Creek population area) than in HRM-R1 (Grave Creek population area) during the incubation period (Figure 7), although not necessarily during other times of the year (Figure 8). Intuitively, the differences between DO concentrations measured in the two population areas do not seem sufficiently large to suggest that low DO concentrations were the sole cause of the Reduced Recruitment in the Harmer Creek population area, e.g., in both population areas, a single DO concentration measurement was recorded below the 9 mg/L guideline during the Reduced Recruitment period, with both measurements of similar magnitude (Figure 7). However, limitations of this analysis are that it is based on comparing only a single site in each population area (HRM-R1 vs. DC-R2), and the sampling locations are not independent as HRM-R1 is downstream of DC-R2, and therefore the analysis does not consider conditions in the Grave Creek mainstem. Both sampling locations are mine-affected and available historical data indicate that the analysis is conservative in the sense that DO concentrations at each site are expected to be similar or lower than at other sampling locations in the population areas[24].

Overall, statistical analysis of spatial variability is a weak line of evidence due to the data constraints described above; however, the analysis undertaken of spatial differences does not support a conclusion that low DO concentrations in the Harmer Creek population area were the sole cause of the Reduced Recruitment.

- Relatively low DO concentrations occurred more frequently in Dry Creek during the incubation periods associated with the periods of reduced or failed recruitment, than during the "before" period (see values < 9.5 mg/L in Figure 7 and discussion above). When considering potential effects of low DO concentrations (particularly acute effects), it is at least as important to consider minimum values as to consider average values, consistent with the basis of the acute DO guidelines prescribed by the BC WQG-AL (Table 1). Thus, the observation of relatively low minimum DO concentrations in Dry Creek during the incubation periods in 2018 and 2019 suggests a possible link between the occurrence of low DO concentrations during the WCT incubation period and the reduced or failed recruitment, assuming that low DO concentrations extended to Harmer Creek mainstem where spawning in the Harmer Creek population generally occurs. There was no statistically significant difference in DO concentrations in Dry Creek among the two periods of variable recruitment success, although this is a weak line of evidence as the statistical power of the test was low (quantified in Section 3.2.3). However, visual inspection of data in Figure 7 does not show clear differences among the two periods. If low DO concentrations were the sole cause of the Reduced Recruitment, then we hypothesize there would have been a clearer difference in DO concentrations between the periods of variable recruitment in the Harmer Creek population area. Thus, the absence of a clear temporal change in DO concentrations does not support a view that low DO concentrations were the sole cause of the Reduced Recruitment, although the occurrence of low minimum DO concentrations in Dry Creek in summer 2018 and 2019 indicates that a partial contribution cannot be discounted.

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[24] We understand there have been incidental observations of high algal biomass ("blooms") in Harmer Creek Sedimentation Pond, which is upstream of HRM-R1. Specifically, a bloom was incidentally observed by Teck during fieldwork in July 2021 (Mike Moore to EoC Team, pers. comm. 2021), although it is possible that blooms occurred at other times. Algal blooms can increase the range of diel fluctuations in DO concentrations, as well as cause declines in DO following algal senescence (e.g., Kalff 2002). Algal blooms in the pond could not affect DO concentrations in the Harmer Creek population area because the pond is at the downstream end of Harmer Creek (Map 1). It is possible that algal blooms could have influenced DO concentrations at HRM-R1 during the incubation period. If so, the effect would likely have been to depress measured DO concentrations during the incubation period because measurements have typically been collected during recent years in the morning, when respiration overnight can reduce DO concentrations. Thus, if algal blooms in the pond influenced DO concentrations, then this would further support our contention that the analytical focus on HRM-R1 is precautionary.

- Analysis of DO saturation and water temperature indicates that low DO concentrations measured in Dry Creek during the incubation period solely reflected warmer temperatures during the summer, e.g., rather than changes to the substrate or organic pollution caused by anthropogenic activities. This observation does not necessarily mitigate the potential for low DO concentrations to adversely affect incubation success; however, it suggests there has not been a marked change in biochemical oxygen demand in the watershed.

Based on the lines of evidence presented above, we conclude that low DO concentrations were not a sole cause of the WCT Reduced Recruitment period in the Harmer Creek population area, although it is possible that low DO concentrations during the incubation period interacted additively or synergistically with other stressors to reduce juvenile WCT recruitment.

The following key uncertainties limit confidence in the conclusions of this assessment:

- DO concentration data are available for surface water but not interstitial water, which is most relevant to incubation conditions. BC WQG-AL assume that the DO concentration in interstitial water is 3 mg/L lower than in surface water; however, the applicability of this assumption to Harmer and Grave creeks is uncertain. Furthermore, it is plausible that changes to substrate quality (e.g., calcite formation or fine sediment accumulation) have occurred in the Harmer Creek population area that have caused a decline in DO concentration in interstitial water via reduced exchange between the water column and the shallow hyporheic zone.

- There is a paucity of information about the specific tolerance of WCT to low DO and therefore it is necessary to make inferences about effects to WCT based on information from studies of other cold-water species (Section 3.1.2).

- The availability of data for the Grave Creek population area was limited (Figure 4) and it was necessary to analyze data collected in the HRM-R1 reach (Map 1) to characterize conditions in the Grave Creek population area. Accordingly, DO concentrations in the mainstem of Grave Creek during the Reduced Recruitment period are uncertain, although this lack of data does not constrain analysis of DO concentrations in the Harmer Creek population area.

- There is good availability of DO data for Dry Creek during the period of interest. The availability of data for other reaches in the Harmer Creek population area was more limited, although monthly data were available for multiple mainstem reaches in Harmer Creek during 2020 and part of 2019 (Figure 4).

5. CONCLUSION

Low DO concentrations are not expected to have been the sole cause of the Reduced Recruitment of WCT in the Harmer Creek population area. This conclusion is based on considering the magnitude of measured DO concentrations in the context of applicable guidelines and supporting studies, in addition to analysis of spatiotemporal variability in measured DO concentrations considered in the context of the location and timing of the Reduced Recruitment period. However, it is possible that low DO concentrations during the incubation period (primarily associated with relatively warm water temperatures during the summer) interacted additively or synergistically with other stressors to reduce juvenile WCT recruitment, thereby contributing to the Reduced Recruitment. Uncertainties were identified (Section 4) that limit confidence in this conclusion.

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