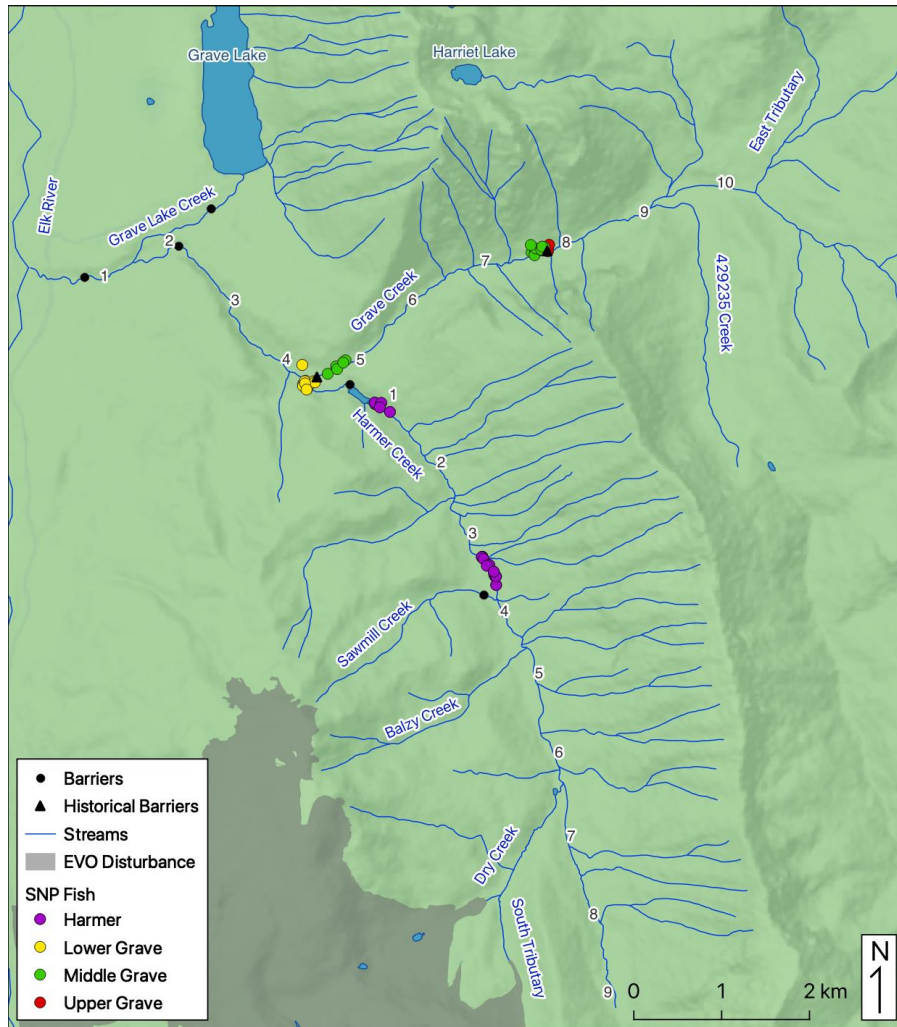


SUBJECT MATTER EXPERT REPORT: SMALL POPULATION SIZE. EVALUATION OF CAUSE – REDUCED RECRUITMENT IN THE HARMER CREEK WESTSLOPE CUTTHROAT TROUT POPULATION

FOR USE IN EVALUATION OF CAUSE

November 23, 2022



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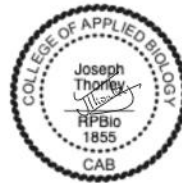
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EXECUTIVE SUMMARY

The Harmer Creek population likely experienced reduced recruitment for the 2017 to 2019 spawn years and recruitment failure for the 2018 spawn year. Small population size can lead to reduced recruitment due to vulnerability to natural disasters, demographic stochasticity, inbreeding depression and/or maladaptation. Demographic stochasticity is variation in population abundance due to chance outcomes of individual survival and/or reproduction. Inbreeding depression, which is explained in more detail in the Genetics Primer in the Background, is a decline in reproduction, growth, or survival due to the expression of recessive deleterious alleles. Maladaptation is the inability to adapt to changing environmental conditions due to a lack of genetic variation. Natural disasters and demographic stochasticity were considered to have made a negligible contribution to the reduced recruitment and recruitment failure as no landslides, fires or floods have been documented in Harmer Creek since the flood in 2013 and the number of adults was estimated to have been in excess of 200 individuals in all three years.

Single-nucleotide polymorphism (SNP) data, which were collected from fish in the Harmer and Grave Creek populations in 2016, were used to compare the genetic diversity of both populations to each other and to other pure (non-admixed), isolated stream-dwelling populations. The SNP data were also used to estimate the genetic differentiation between the two populations as well as their effective population sizes. Key genetic concepts are explained in the Genetics Primer in the Background and described in the Glossary of Terms.

The genetic diversity was lower in the Harmer Creek population than in the Grave Creek population (allelic richness of 1.4 versus 1.6 alleles/loci and an expected heterozygosity of 0.09 versus 0.12). However, these differences are relatively minor and there is little to no genetic differentiation between the populations (F_{ST} of -0.05%). In addition, estimates of F_{IS} (the inbreeding coefficient) showed no evidence for inbreeding. Consequently, any contribution from inbreeding depression or maladaptation to the reduced recruitment or recruitment failure was considered to be at most minor. This conclusion was further supported by comparison with other non-admixed, isolated, lotic Westslope Cutthroat Trout populations almost half of which have lower genetic diversity.

ACKNOWLEDGEMENTS

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GLOSSARY OF TERMS

| Term | Description |
|---------------------------|--|
| 50/500 Rule | A rule which proposes that an effective population size of 50 is required to avoid inbreeding depression in the short-term while an effective population size of 500 is required to maintain evolutionary potential in the long-term. |
| Allelic richness | The average number of alleles per loci for a sample of individuals. |
| Demographic stochasticity | Variation in abundance due to chance outcomes of individual survival and/or reproduction in a finite population. |
| Diploid | Diploid individuals like Westslope Cutthroat Trout have two copies of each chromosome in a cell. |
| Effective population size | Number of individuals in an ideal population with equal sex ratios, random mating, non-overlapping generations, and a Poisson distributed family size that would experience the same amount of genetic drift as the observed population. In salmonid populations the effective population size is often assumed to be one-fifth the adult population size. |
| Expected heterozygosity | The average proportion of genotypes that would be heterozygous under Hardy Weinberg equilibrium in a sample. |
| Extinction vortex | Positive feedback loop between inbreeding depression and maladaptation due to small population size and further reduction in the population size. |
| Fixation index | The proportional decrease in the heterozygosity relative to the Hardy-Weinberg expectation due to population structuring. |
| Genetic adaptation | The translocation of individuals to increase the long-term adaptive potential. |
| Genetic bottleneck | Loss of genetic diversity due to a period of very small effective population size. |
| Genetic differentiation | The extent to which populations differ genetically. See pairwise fixation index for an example. |
| Genetic diversity | The amount of genetic variation among individuals of a population. |
| Genetic drift | Random changes in allele frequencies in a population between generations due to stochastic processes. |
| Genetic load | The decrease in the mean fitness of individuals in a population compared with the theoretical mean fitness if all individuals had the most favored genotype. Caused in part by the frequency of recessive deleterious alleles in a population. |
| Genetic rescue | The translocation of individuals into an isolated population to mitigate the effects of inbreeding depression. |

| | |
|----------------------------|--|
| Hardy-Weinberg Equilibrium | The expected allele frequencies in the absence of mutation, natural selection, non-random mating, genetic drift, and gene flow. |
| Heterozygosity | The average proportion of the loci that are heterozygous. |
| Heterozygous | A diploid individual is heterozygous at a locus if the two alleles are different. |
| Inbreeding coefficient | The extent to which the observed heterozygosity differs from the expected heterozygosity under Hardy-Weinberg equilibrium in a sample. |
| Inbreeding depression | A reduction in fitness (e.g., growth, survival and/or reproduction) of progeny from mating between genetically similar individuals. |
| Linkage disequilibrium | Non-random association of alleles at different loci within a population. |
| Locus | A region or position on a chromosome of a gene or other genetic marker. |
| Maladaptation | The inability to adapt to changing environmental conditions due to a lack of variation. |
| Natural Selection | Differential contribution of genotypes to the next generation due to differences in survival and reproduction. |
| Non-admixed | A population that has experienced no to negligible interbreeding with another species. |
| Observed heterozygosity | The average proportion of the loci that are heterozygous in a sample. |
| Outbreeding depression | A reduction in fitness (e.g., growth, survival and/or reproduction) of progeny from mating between genetically dissimilar individuals. |
| Pairwise fixation index | The proportional decrease in the heterozygosity relative to the Hardy-Weinberg expectation due to division into two subpopulations. |
| Paralogous genes | Genes that are related due to duplication events. |
| Polymorphic loci | Loci with two or more different alleles for the individuals sampled. |
| Recruitment failure | A negligible number of new individuals added to the population. |

MATHEMATICAL SYMBOLS

| Symbol | Term |
|--------------|--|
| A_R | Average allelic richness |
| D | Coefficient of linkage disequilibrium |
| F | Fixation index |
| F_{IS} | Inbreeding coefficient |
| F_{ST} | Pairwise fixation index |
| H_E | Expected heterozygosity |
| ΔH_E | Relative proportional loss of heterozygosity |
| H_O | Observed heterozygosity |
| LD | Linkage disequilibrium |
| N_A | Adult population size |
| N_e | Effective population size |
| P | Percent polymorphic loci |

ACRONYMS

| Acronym | |
|---------|--|
| CI | Confidence Intervals |
| FL | Fork Length |
| RB | Rainbow Trout |
| SNP | Single Nucleotide Polymorphism |
| SSR | Simple Sequence Repeats or Microsatellites |
| 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 ʔqapi 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 watershed¹, 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 areas³ from 2017 to 2021 (Thorley et al. 2022; Chapter 4, Evaluation of Cause), and several patterns related to recruitment⁴ were identified:

- *Reduced Recruitment*⁵ occurred during the 2017, 2018 and 2019 spawn years⁶ in the Harmer Creek population and in the 2018 spawn year in the Grave Creek population.
- The magnitude of Reduced Recruitment in the Harmer Creek population in the 2018 spawn year was significant enough to constitute *Recruitment Failure*⁷.
- Recruitment was *Above Replacement*⁸ for the 2020 spawn year in both the Harmer and Grave Creek populations.

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

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

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

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

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

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

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

How the Evaluation of Cause Was Approached

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

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

The Overarching Question the Team Investigated

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

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

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

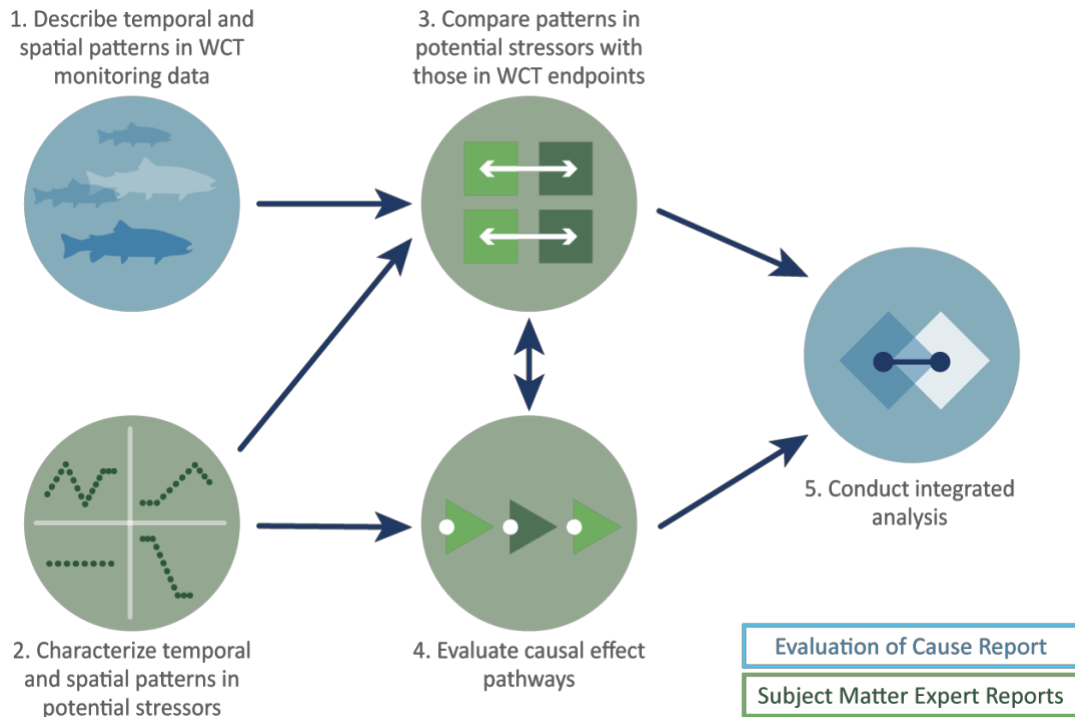
1. Individual Subject Matter Expert reports (such as the one that follows this Note). Potential stressors were evaluated by SMEs and their co-authors using the available data. These evaluations were documented in a series of reports that describe spatial and temporal patterns associated with the potential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

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

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

The Evaluation of Cause report:

- a. Provides readers with context for the SME reports and describes Harmer and Grave Creeks, the Grave Creek watershed, the history of development in the area and the natural history of WCT in these creeks
- b. Presents fish monitoring data, which characterize the Harmer Creek and Grave Creek populations over time
- c. Uses an integrated approach to assess the role of each potential stressor in contributing to Reduced Recruitment in the Harmer Creek population area.



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

Participation, Engagement & Transparency

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

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

Citations for Evaluation of Cause Team Reports

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

HARMER EVALUATION OF CAUSE: SMALL POPULATION SIZE

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

HARMER EVALUATION OF CAUSE: SMALL POPULATION SIZE

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

INTRODUCTION

Teck Coal undertakes aquatic monitoring programs in the Elk Valley, including fish population monitoring. Using data collected from 2017 to 2019 in Harmer and Grave Creeks, Cope & Cope (2020) reported low abundance of juvenile Westslope Cutthroat Trout (WCT; *Oncorhynchus clarkii lewisi*), which indicated apparent recruitment failure in Harmer Creek. Teck Coal initiated an Evaluation of Cause — a process to evaluate and report on what may have contributed to the apparent recruitment failure. Data were analyzed from annual monitoring programs in the Harmer and Grave Creek population areas¹ from 2017 to 2021 (Thorley et al. 2022; Chapter 4, Evaluation of Cause), and several patterns related to recruitment² were identified:

- *Reduced Recruitment*³ occurred during the 2017, 2018 and 2019 spawn years⁴ 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*⁵.
- Recruitment was *Above Replacement*⁶ for the 2020 spawn year in both the Harmer and Grave Creek populations.

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

The Evaluation of Cause Project Team investigated one overarching question: **What potential stressors can explain changes in the Harmer Creek Westslope Cutthroat Trout population over time, specifically with respect to patterns of Reduced Recruitment?** To investigate this question, the Team evaluated trends in WCT population parameters, including size, condition and recruitment, and in the potential stressors⁷ that could impact these parameters. They evaluated the trends in WCT population parameters based on monitoring data collected from 2017 to 2021 (reported in Thorley et al., 2022 and Chapter 4, Harmer Creek Evaluation of Cause Team, 2023). The Grave Creek population area was used as a reference area for this evaluation.

The approach for analyzing potential stressors for the Evaluation of Cause was to, (1) characterize trends in each stressor for the Harmer and Grave Creek populations, (2) compare the trends between the two population areas, (3) identify any changes in Harmer Creek during the period of Reduced Recruitment, including the 2018 Recruitment Failure of the 2018 spawn year where appropriate, and (4) evaluate how each stressor trended relative to the fish population parameters. The Team then identified mechanisms by which the potential stressors could impact WCT and determined if the stressors were present at a sufficient magnitude and duration to have an adverse effect on WCT during the period of Reduced Recruitment. Together, these analyses were used in the Evaluation of Cause report to support conclusions about the relative contribution of each potential stressor to the Reduced Recruitment observed in the Harmer Creek population area.

BACKGROUND

OVERALL BACKGROUND

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

SMALL POPULATION SIZE

Small population size can cause Reduced Recruitment through four possible mechanisms: 1) a restricted spatial distribution that makes the population vulnerable to natural disasters; 2) demographic stochasticity (Lande 1993); 3) inbreeding depression (Wang et al. 2002) and 4) maladaptation. Natural disasters can be excluded as a contributor to the Reduced Recruitment as no landslides, fires or floods have been documented in Harmer Creek since the flood in 2013 (Robinson, M. pers. comm.). Demographic stochasticity is population-level fluctuations in abundance due to a large proportion of the individuals having higher or lower survival and/or reproductive success than expected given the environmental conditions (Lande 1993). Demographic stochasticity is most important at very low population sizes (Lande 1993). As the Harmer Creek adult population was estimated to be 200 fish (95% CI 90-410) in 2019 (Thorley et al. 2022), demographic stochasticity is also considered to have made a negligible contribution to the three successive years of Reduced Recruitment. However, small genetic population size can also lead to inbreeding depression and maladaptation which are the focus of the remainder of this report. Inbreeding depression is a reduction in growth, survival and/or reproduction due to the expression of recessive deleterious alleles (Wang et al. 2002), while maladaptation is the inability to adapt to changing environmental conditions due to a lack of genetic variation. In summary, small population size can lower the genetic diversity resulting in inbreeding depression and maladaptation, both of which could be reducing early life-stage growth and survival. This process can lead to a positive feedback loop sometimes referred to as “the extinction vortex”.

The effects pathway is plotted as a flowchart in Figure 1. Early life-stage growth is important for survival because the WCT in the Harmer Creek population have a relatively short period of time to grow above the threshold size required to survive the winter. In Colorado Cutthroat trout (*Oncorhynchus clarkii pleuriticus*) the threshold is estimated to be 30-35 mm (Coleman and Fausch 2007a).

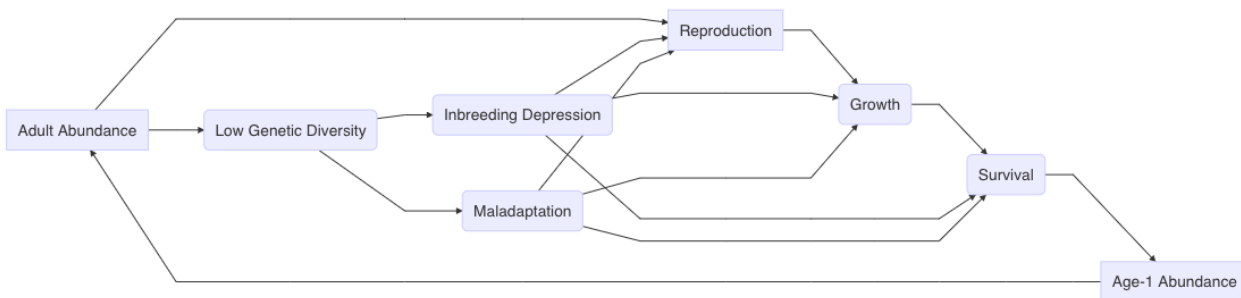


Figure 1. The effects pathway for the linkage between population size and recruitment failure.

GENETICS PRIMER

Like many other species, WCT are diploid (have paired chromosomes), which means that they have two DNA sequences (alleles) for each location on a chromosome (locus). If the alleles at a locus are identical, then the individual is said to be homozygous for that locus. Mutation introduces new alleles into a population while natural selection (differential survival and reproduction) and genetic drift (random changes in the relative proportions of alleles between generations) alter the frequency of the alleles. Recessive alleles only have an effect (influence the phenotype or behaviour) when homozygous, while dominant alleles are expressed when homozygous or heterozygous (just one copy). Natural selection quickly eliminates dominant deleterious alleles, but recessive deleterious alleles persist in heterozygotes. The frequency of recessive deleterious alleles in a population is referred to as its genetic load. Such alleles are a concern because as the genetic diversity of a population decreases, the probability of an individual being homozygous for one or more recessive deleterious alleles increases, resulting in a reduction in growth, survival and/or reproduction (Wang et al. 2002). A lack of genetic variation can also result in a reduction in growth, survival and/or reproduction as a population fails to adapt to changing environmental conditions.

The genetic diversity of a population can be quantified in terms of the percent polymorphic loci (P), average allelic richness (A_R), observed heterozygosity (H_O) and expected heterozygosity (H_E) for a given set of loci. A locus is polymorphic if two or more alleles are present while the allelic richness is the number of alleles present in a sample. The observed heterozygosity is the average proportion of the loci that are heterozygous in the sample of individuals while the expected heterozygosity is the average proportion of the loci that would be heterozygous if the alleles were in Hardy-Weinberg equilibrium (randomly distributed among individuals). P and A_R give equal weight to common and rare alleles but must be scaled by the sample size. H_O and H_E , in contrast, do not need to be scaled by the sample size but are relatively insensitive to the frequency of rare alleles. Rare alleles are important for the long-term adaptive potential of the population (Greenbaum et al. 2014). The set of loci can be DNA fragments that are separated by gel electrophoresis, Simple Sequence Repeats (SSRs), which are also known as microsatellites, or Single Nucleotide Polymorphisms (SNPs).

The expected heterozygosity for a single locus is given by the following equation where p_i is the frequency of the i^{th} of k alleles.

$$H_E = 1 - \sum_{i=1}^k p_i^2 \quad (1)$$

The inbreeding coefficient (F_{IS}) quantifies the extent to which the observed heterozygosity in a population differs from the expected heterozygosity. The inbreeding coefficient can be calculated from the observed and expected heterozygosity using the following equation.

$$F_{IS} = 1 - \frac{H_O}{H_E} \quad (2)$$

Positive inbreeding coefficient values indicate lower observed than expected heterozygosity while negative inbreeding coefficient values indicate the opposite. Pairwise F_{ST} , or pairwise fixation index, measures the proportional decrease in the expected heterozygosity due to division into two subpopulations.

EFFECTIVE POPULATION SIZE

Effective population size is the number of individuals in an ideal population with equal sex ratios, random mating, non-overlapping generations, and a Poisson distributed family size that would experience the same amount of genetic drift as the observed population.

Expected heterozygosity is lost due to genetic drift at a relative rate of

$$\Delta H_E = \frac{1}{2N_e} \quad (3)$$

per generation where N_e is the effective population size. For the purposes of this report we assume that the isolated, lotic WCT populations have a generation time of four years (Fisheries and Oceans Canada 2017, 2019). In an ideal population, $N_e = N_A$, where N_A is the adult population size. However due to non-random mating, iteroparity and high variance in family size in salmonid populations, N_e is typically assumed to be one fifth of N_A (Allendorf et al. 1997; Hastings et al. 2008).

$$N_e = \frac{N_A}{5} \quad (4)$$

LINKAGE DISEQUILIBRIUM

Populations with smaller effective population sizes undergo more genetic drift (stochastic changes in allele frequencies) than larger populations (Waples et al. 2016). Genetic drift not only results in the loss of genetic diversity but also causes associations between alleles at different loci – a phenomenon known as linkage disequilibrium (*LD*) - at a rate inversely proportional to N_e (Waples et al. 2016). As result, in the absence of selection the *LD* between independently segregating loci can be used to estimate N_e .

INBREEDING DEPRESSION

Inbreeding depression is a reduction in growth, survival and/or reproduction associated with the expression of deleterious recessive alleles (Wang et al. 2002). Inbreeding depression can occur if closely related individuals interbreed and/or if randomly mating populations have low levels of genetic diversity. The genetic diversity of an isolated population depends on the number and genetic diversity of the founding individuals, the number of generations the population has been isolated, and whether there have been any subsequent genetic bottlenecks (loss of genetic diversity due to a period of very small population size) as well as the size of the population.

MALADAPTATION

Maladaptation is a reduction in growth, survival and/or reproduction due to an inability to adapt to changing environmental conditions associated with a lack of genetic variation – the raw material of natural (and sexual) selection.

50/500 Rule

The 50/500 rule for managing population genetic diversity states that in general an N_e of 50 is required to avoid inbreeding depression in the short-term (10s of generations), while an N_e of 500 is required to maintain adaptive potential over the long-term (Franklin 1980). It is important to note that the N_e of 500

is not a definite threshold that predicts extinction risk but rather a viable target for maintaining genetically robust populations (Jamieson and Allendorf 2012). Based on equation 4, this rule of thumb is typically translated into an adult salmonid population size of 250 adults to avoid inbreeding depression in the short-term and 2,500 adults to maintain genetic diversity in the long-term (Hilderbrand and Kershner 2000).

RAINBOW TROUT

Hybridization of Rainbow Trout (RB) with WCT, which are sister species, has resulted in the loss of many non-admixed (pure) WCT populations and is an ongoing conservation concern (Yau and Taylor 2013). Due to their close relationship, RB, which are one of the most accessible and widely studied fish species (Thorgaard et al. 2002), provide a reasonable surrogate for WCT when relevant studies on WCT or other subspecies of Cutthroat Trout are unavailable.

INBREEDING EXPERIMENTS

Inbreeding experiments often involve repeated sibling crosses to produce offspring with a high probability of homozygosity (Wang et al. 2002). First generation full sibling crosses have an inbreeding coefficient F of 25%, which indicates that there is a probability of 0.25 that two alleles in the same individual will be identical by descent, while third generation full sibling crosses have an F of 50%. In populations with low genetic diversity an individual may be homozygous without the two alleles being identical by descent.

AUGMENTED GENE FLOW

Genetic rescue is the translocation of individuals into an isolated population to reduce the effects of inbreeding depression (Bell et al. 2019) while genetic adaptation is the translocation of individuals into an isolated population to increase the long-term adaptive potential (Weeks et al. 2011).

METHODS

STUDY AREA

GRAVE HARMER POPULATIONS

The primary study area was the Grave and Harmer population areas in the Grave watershed above the barrier falls at 2.1 km (Figure 2). Historically, the WCT in the Grave watershed constituted connected populations, but in 1972 construction of the Harmer Sedimentation Pond and Dam prevented fish from moving upstream at 0.55 km on Harmer Creek. Prior to construction of the Harmer Sediment Pond and Dam a culvert was placed at 4.6 km on Grave Creek above the confluence with Harmer Creek preventing the upstream passage of fish. This lower culvert was replaced by a passable bridge in November 2017. In 2013 upstream fish movement was limited further by installation of a second culvert at 7.8 km. This second upper culvert was replaced by a passable bridge in October 2018. For the purposes of the current report the Grave Creek fish were assumed to be a single population. This assumption may cause the genetic diversity within the Grave Creek population to be overestimated. It is important to note that in Lamson (2016) and Lodmell et al. (2017) ‘lower Grave’ refers to samples in the 1 km of stream below barrier 1 which are excluded from the current analysis while ‘upper Grave’ refers to samples above the lower

historical barrier which corresponds to middle and upper Grave in the current analysis. For more information on the primary study area see Harmer Creek Evaluation of Cause Team (2023). All river kilometres are based on the BC Freshwater Atlas.

BRITISH COLUMBIAN AND ALBERTAN POPULATIONS

Genetic diversity values and barriers for genetically non-admixed, isolated, lotic WCT populations in British Columbia and Alberta (Figure 3) were extracted from the peer-reviewed and grey literature for comparison with the Grave and Harmer populations. Following Fisheries and Oceans Canada (2019) only populations with a mean genetic admixture proportion $\geq 99\%$ were considered to be pure (non-admixed). Reported barriers were individually checked by plotting them on Google Earth and where necessary correcting them by hand. In cases where the locations of fish barriers were not provided, the BC HabitatWizard, the World Waterfall Database, the elevational profile of the stream and visual identification of the falls from aerial photographs were used to identify the mostly likely barrier location.

Other populations were included solely to provide more data on the relationship between H_E estimated using SSRs versus SNPs (see below). An isolated, lotic population's location was defined to be its most downstream extent. In the case of a connected or lentic population, the most downstream extent was considered to be the mouth of the river or outflow of the lake.

The population locations were individually checked by plotting them on Google Earth and where necessary correcting them by hand. In cases where the locations of fish barriers were not provided, the elevational profile of the stream and visual confirmation of a falls were used to identify the locality. The Google Earth API was used to assign each location an elevation. The total length of upstream WCT-bearing habitat was extracted from reports and where necessary confirmed and/or corrected by manually tracing the stream segments using the Google Earth path tool.

EFFECTIVE POPULATION SIZE

Two estimates of contemporary effective population size (N_e) were calculated for the Harmer and Grave Creek populations. The first was a demographic estimator based on the adult population size in 2019, which used equation 4, while the second was a genetic estimator based on the amount of linkage disequilibrium (LD) in the SNP data. $LD N_e$ was estimated under a monogamy model with confidence intervals generated by jack-knifing over individuals using NeEstimator v. 2 (Do et al. 2014) after excluding singleton alleles (alleles that only occur in a single heterozygote). The adult population size was based on removal estimation of backpack electrofishing data with adults considered to be those with fork lengths greater than or equal to 170 mm (Thorley et al. 2022).

The LD based N_e value provides an average estimate of the effective population size over the last several generations and assumes that the alleles are segregating independently and are unaffected by selection. The adult population size-based value, in contrast, provides an estimate based only on the generations present in the population estimate and assumes that the effective population size is one-fifth the adult population size. Demographic estimators may overestimate the true N_e since they don't include factors such as variance in reproductive success which can reduce N_e compared to the N_C (Luikart et al. 2010).

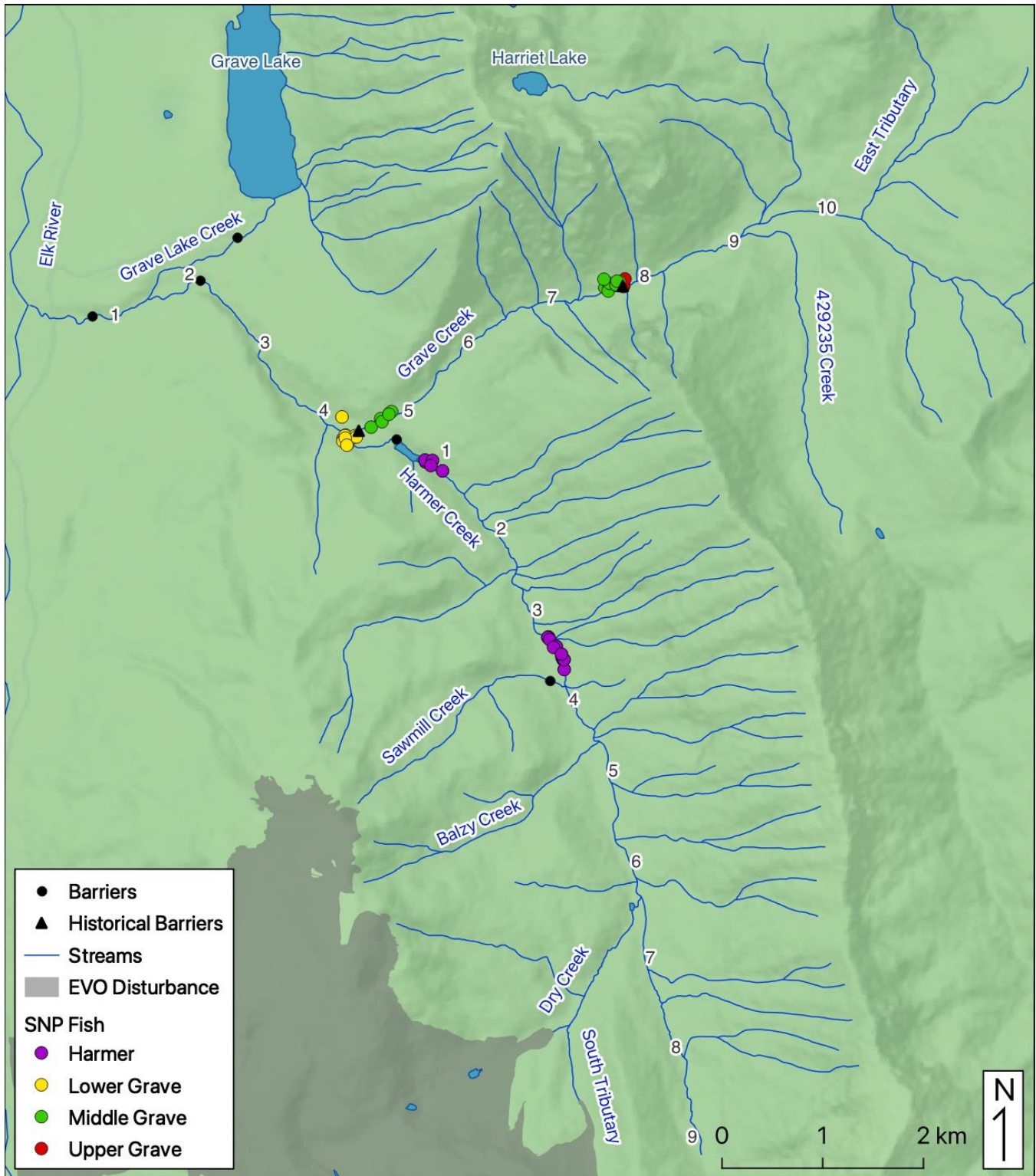


Figure 2. The reported locations of the 15 fish from the Harmer Population and 34 fish from the Grave population (15 from lower, 16 from middle and 3 from upper Grave) that were genetically sampled between July and September 2016 to determine their alleles at 34 Single Nucleotide Polymorphisms (SNPs) loci obtained from Lamson (2016).

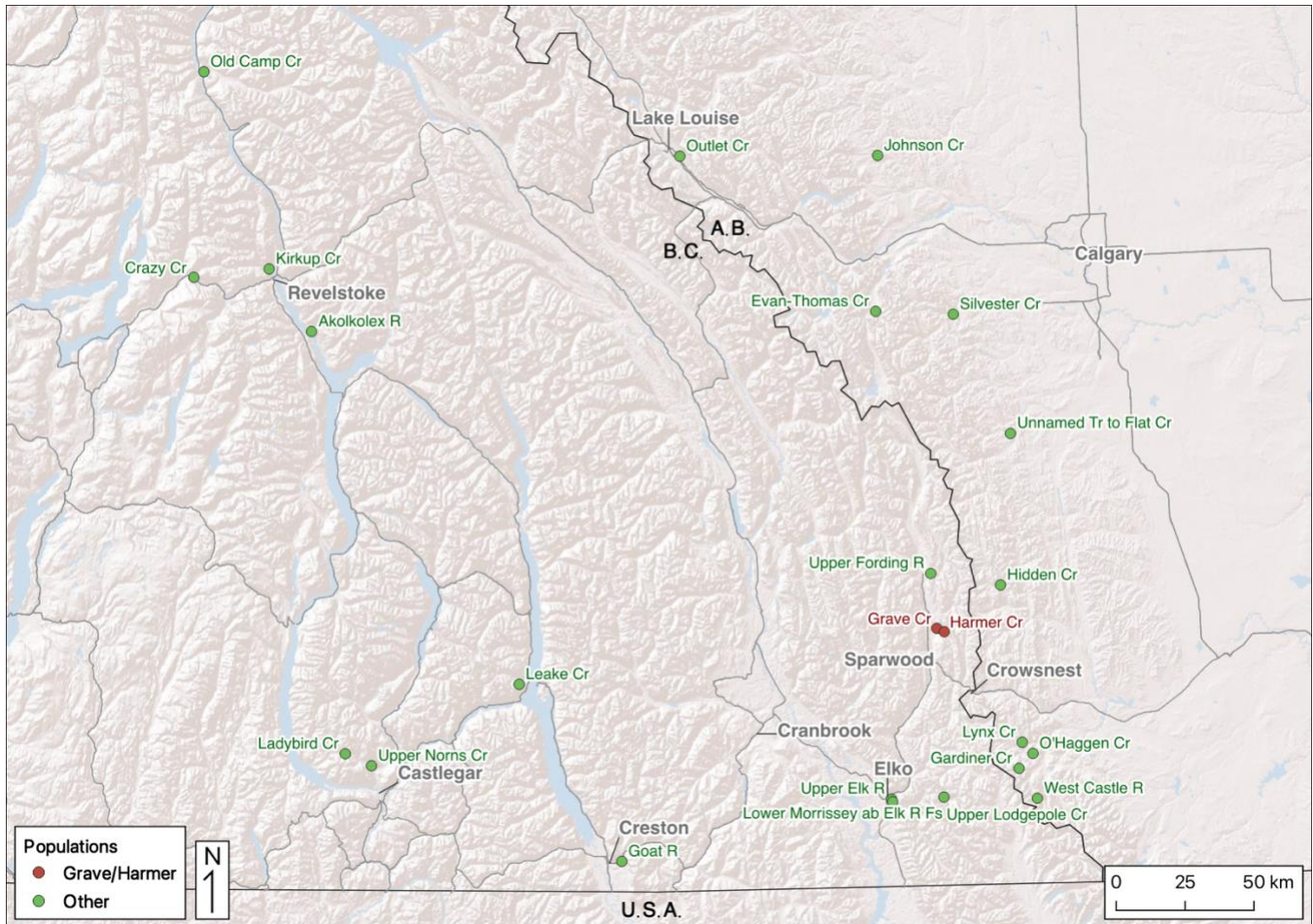


Figure 3. The locations of non-admixed, isolated, stream-dwelling WCT populations in British Columbia and Alberta. The Harmer and Grave Creek populations are colored red.

GENETIC DIVERSITY

SNP DATA

Raw SNP data for 34 loci were obtained from Lamson (2016) for 16 populations, including the Grave and Harmer Creek populations, with a sample size of at least 14 individuals. The SNP data included 15 individuals from Harmer Creek and 34 individuals from Grave Creek of which 15 were from lower Grave, 16 from middle Grave and 3 from upper Grave (Figure 2). The genetic samples for the Grave and Harmer populations were collected between July and September 2016 (Lodmell et al. 2017). P and A_R values, corrected to a sample size of 14 (by resampling without replacement), were calculated from the SNP data using R 4.1.0 (R Core Team 2021). H_O , H_E and F_{IS} values were calculated from the same data using GENEPOP v. 4.0 (Rousset 2008). H_O , H_E and F_{IS} values for the remaining three populations in Lamson (2016) were taken directly from the report. Missing H_O values were estimated using equation 2 and the H_O , H_E and F_{IS} values were averaged (mean) by population.

SSR DATA

H_O , H_E and F_{IS} values were also taken from studies that used SSRs with at least 6 common loci and up to 4 unique loci (Taylor et al. 2003; Taylor 2008; Yau and Taylor 2013). To adjust for the differences between methods, the population H_E values from the SNP study (Lamson 2016) were regressed (through the origin) on the corresponding population H_E values from the SSR studies. The estimated slope was then used to adjust the SSR H_E and H_O values for which SNP-based values were not available.

GENETIC DIFFERENCE

The genetic difference between the Harmer and Grave Creek populations was evaluated from the pairwise F_{ST} using hierfstat v. 0.5-7 (Goudet 2005) following Weir and Cockerham (1984).

POPULATION EFFECTS

INBREEDING EXPERIMENTS

Inbreeding experiments provide insight into the range of possible effects of inbreeding depression. The measured effects of inbreeding on the survival and growth of RB early life-stages were plotted from data in the published literature (Aulstad and Kittelsen 1971; Kincaid 1976, 1983; Gjerde et al. 1983). The survival effects are reported in terms of the log odds ratio (Bland and Altman 2000) while growth is reported in terms of the percent weight difference.

GENETIC AUGMENTATION

The possible magnitude of inbreeding effects on early WCT life-stage growth and survival was also estimated by reviewing the literature on the effects of genetic augmentation on small lotic salmonid populations that had recently been isolated by barriers.

RESULTS

EFFECTIVE POPULATION SIZE

Based on Thorley et al.'s (2022) estimated population size of 200 (90-410, 95% CI) adults in 2019 and equation 4 the Harmer Creek population was calculated to have a demographic N_e of 40 (18 – 82, 95% CI). The Grave population with an adult population size of 310 (130-630 95% CI) was calculated to have a demographic N_e of 62 (26 – 126, 95% CI). Based on equation 3, the H_E of the Harmer population would therefore be expected to have declined by 1.3% (0.6 – 2.8%, 95% CI) between 2016 when the samples were taken and 2020 (based on a generation time of four years) compared to just 0.8% (0.4 – 1.9%, 95% CI) for the Grave population (Table 1).

The linkage disequilibrium based N_e estimate was just 23 (4 – ∞ , 95% CI) for the Harmer population and 43 (14 – 855, 95% CI) for the Grave population. Based on the amount of linkage disequilibrium the expected heterozygosity of the Harmer population would therefore be expected to change at a rate of 2.2% (0 - 25 %, 95% CI) per generation compared to 1.2% (0.1– 3.6, % 95% CI) per generation for the Grave population (Table 1).

Table 1. The estimated number of adults in 2019 (N_A), the population based effective population size ($N_A N_e$), the population based expected percent change in the genetic diversity per generation ($N_A \Delta H_E$), the linkage disequilibrium based effective population size ($LD N_e$) and the linkage disequilibrium based expected percent change in the genetic diversity per generation ($LD \Delta H_E$) by population with 95% CIs in brackets.

| Population | N_A | $N_A N_e$ | $N_A \Delta H_E$ | $LD N_e$ | $LD \Delta H_E$ |
|------------|---------------|---------------|------------------|--------------------|-----------------|
| Grave | 310 (130-630) | 62 (26 – 126) | 0.8 (0.4 – 1.9) | 43 (14 – 855) | 1.2 (0.1 – 3.6) |
| Harmer | 200 (90-410) | 40 (18 – 82) | 1.3 (0.6 – 2.8) | 23 (4 – ∞) | 2.2 (0.0 - 25) |

GENETIC DIVERSITY

Based on the SNP data, 32% of the loci are polymorphic in fish from the Harmer population compared to 47% for the Grave population when corrected for a sample size of 14 (Figure 4). Similarly, the sample size corrected allelic richness was 1.40 for Harmer population fish compared to 1.60 for Grave population fish. As expected, there was a close relationship between the allelic richness and the percent polymorphic loci (Figure 4).

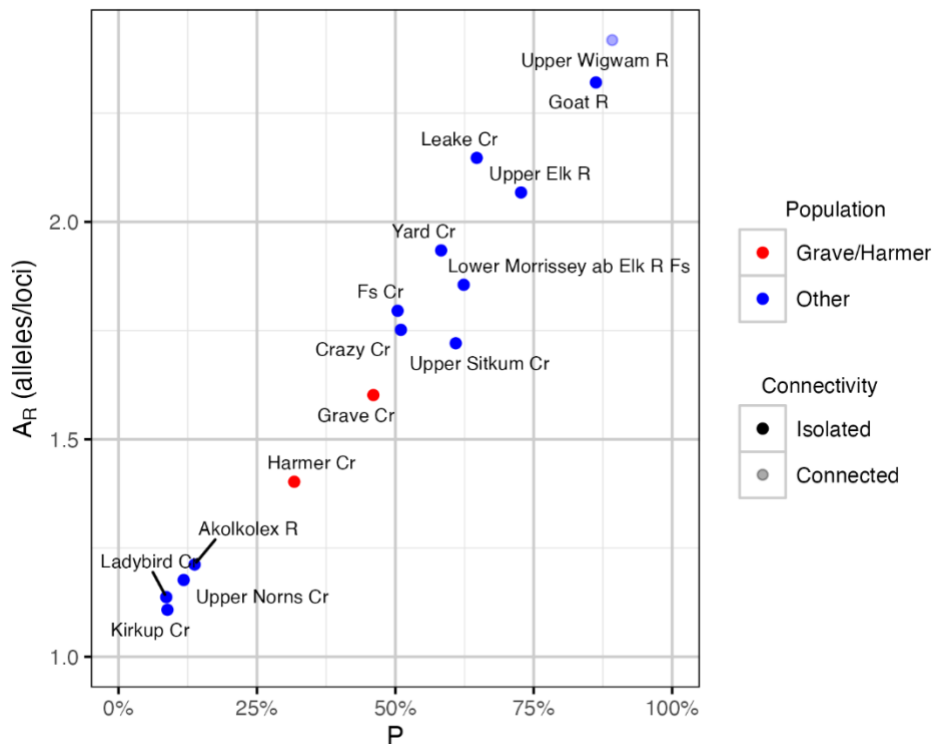


Figure 4. The allelic richness (A_R) by percent polymorphic (P) loci (corrected for a sample size of 14) for the 15 populations from Lamson (2016) for which raw SNP data was provided. All 15 population are from British Columbia. Cr = Creek, Fs = Falls, R = River.

The estimated slope of 0.50 (0.42 – 0.57) for the expected heterozygosity from the SNP data to the expected heterozygosity for the Taylor et al. (2003) SSR data explained 92% of the variation in the SNP-based H_E values (Figure 5).

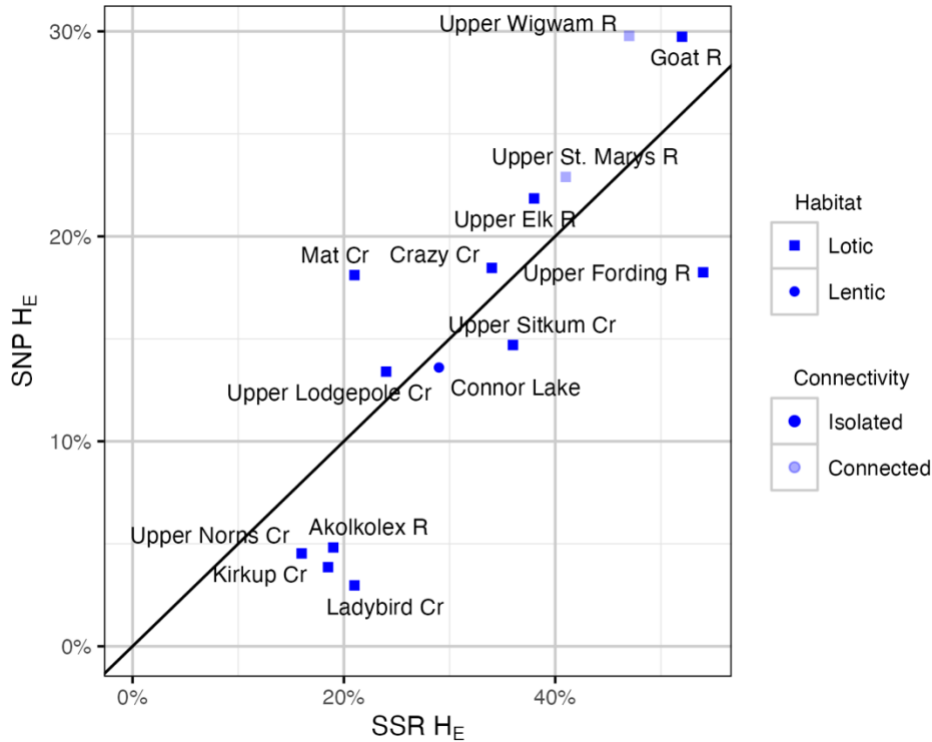


Figure 5. Population expected heterozygosity (H_E) by method where the Single Nucleotide Polymorphism (SNP) values are from Lamson (2016), and the Simple Sequence Repeat (SSR) values are from Taylor et al. (2003). Cr = Creek, Fs = Falls, R = River.

The mean standardized (in terms of SNPs) expected heterozygosity (H_E) for the Harmer (9%) and Grave (12%) populations are plotted by habitat length with values for other non-admixed, isolated, stream-dwelling populations in Alberta and British Columbia (Figure 6). The results indicate that the expected heterozygosities for the Harmer and Grave populations are in the lower 46th to 50th percentiles, respectively.

The mean inbreeding coefficients (F_{IS}) for the Harmer (-0.067) and Grave (-0.093) populations are plotted by the expected heterozygosity (H_E) with values for other non-admixed, isolated, stream-dwelling populations (Figure 7). Both the Harmer and Grave populations have negative inbreeding coefficients which indicates that the observed heterozygosity (H_O) is greater than the expected heterozygosity (H_E). Positive F_{IS} values from inbreeding between closely related individuals was not observed. The negative F_{IS} suggests individuals in the sample collections are less related than expected, possibly from non-random sampling of the population due to small sample size and/or restricted movement.

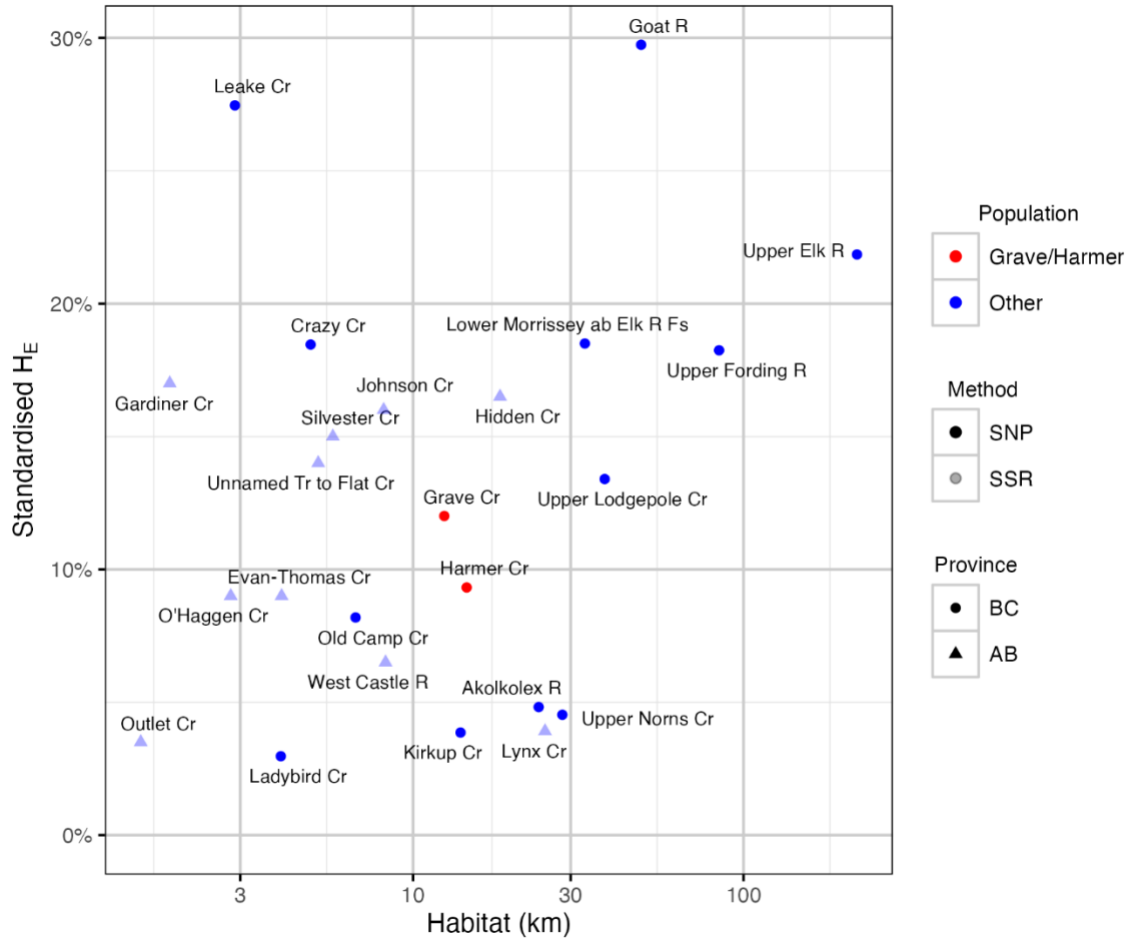


Figure 6. The standardised (in terms of SNPs) expected heterozygosity (H_E) for non-admixed, isolated, stream-dwelling populations by habitat length, population, method, province, and focus. The habitat lengths are on a log scale. Cr = Creek, Fs = Falls, R = River.

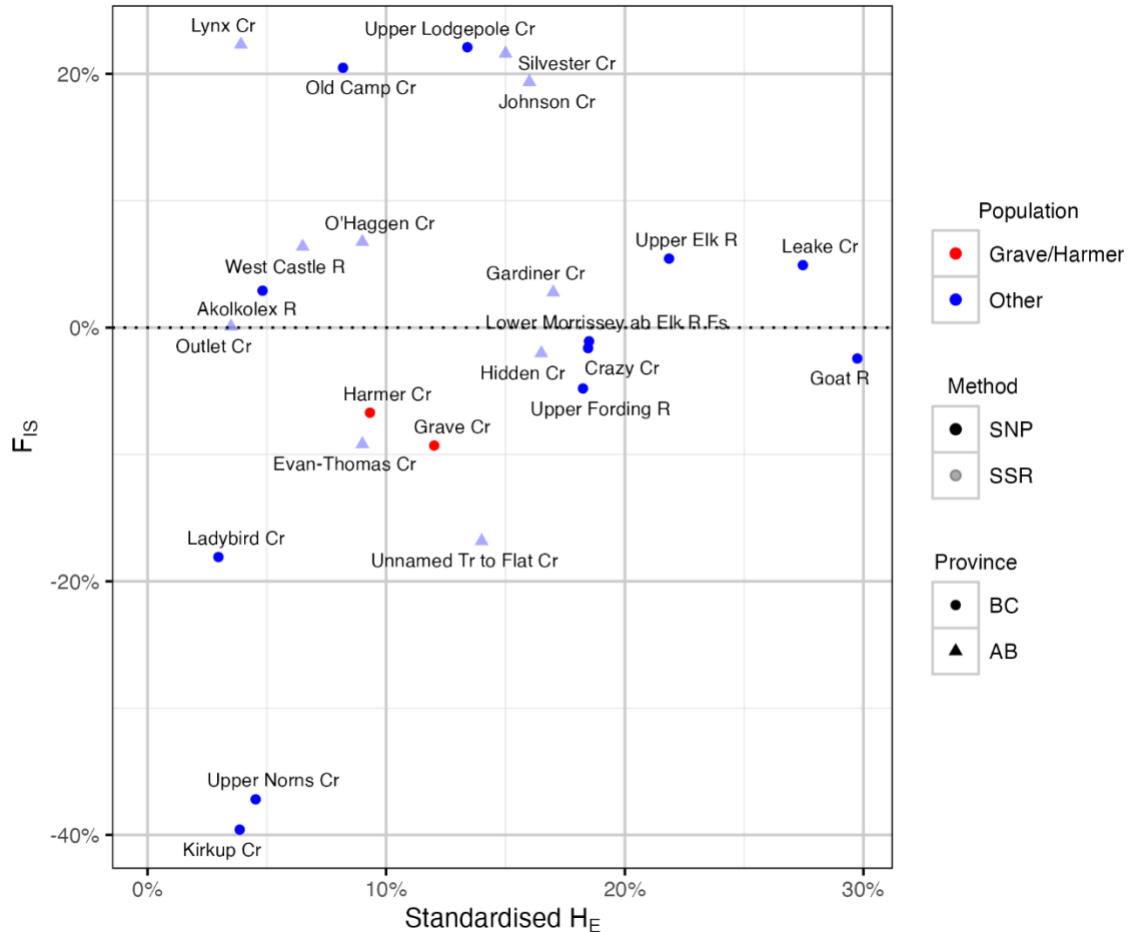


Figure 7. The inbreeding coefficient (F_{IS}) for non-admixed, isolated, stream-dwelling populations by mean standardised (in terms of SNPs) expected heterozygosity (H_E), population, method, province, and focus. Cr = Creek, Fs = Falls, R = River.

GENETIC DIFFERENTIATION

The pairwise F_{ST} value of -0.05% (-0.24% - 0.18% 95% CI) indicates that there is more variance within the populations than between them. This is not an unexpected result given the historical and current patterns of gene flow. Upstream gene flow from the Grave to Harmer population was only blocked 50 years ago, while downstream gene flow from the Harmer to Grave population has likely continued.

POPULATION EFFECTS

INBREEDING EXPERIMENTS

Inbreeding experiments (Aulstad and Kittelsen 1971; Kincaid 1976, 1983; Gjerde et al. 1983) suggest that RB early life-stage survival tends to decrease relatively linearly with inbreeding to a log odds ratio of approximately 1 at an inbreeding coefficient of 50% (Figure 8). In other words, the experiments suggests that the survival odds are approximately three times lower after three generations of full sibling crosses compared to the control.

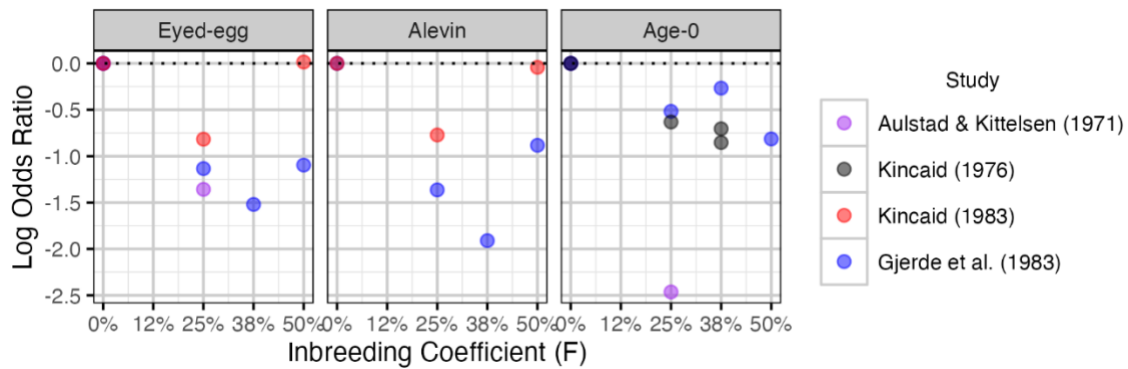


Figure 8. The early life-stage Rainbow Trout survival as the log odds ratio relative to no inbreeding by the inbreeding coefficient.

RB early life-stage growth also tends to change relatively linearly with inbreeding with a reduction of approximately 15% in the weight of age-0 individuals and a reduction of approximately 40% in the weight of age-1 individuals following three generations of full sibling crosses (Figure 9).

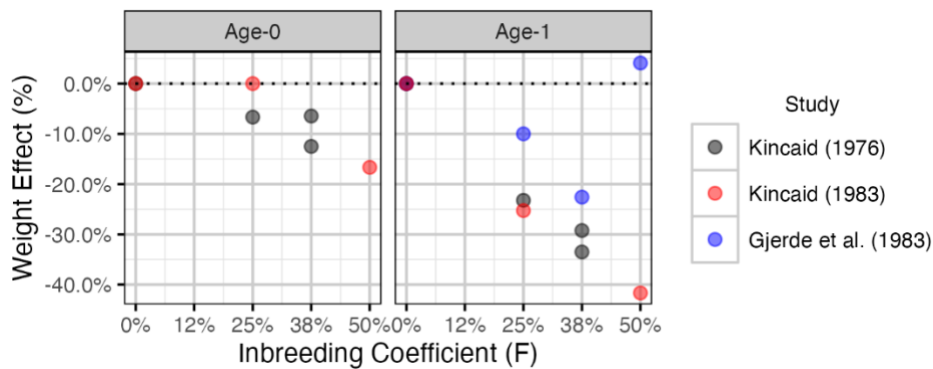


Figure 9. The early life-stage Rainbow Trout growth effect relative to no inbreeding by the inbreeding coefficient.

If Rainbow Trout shape is assumed to remain relatively constant during the first year, then the above weight effects are equivalent to length effects of 5% and 12%, respectively. It is however worth noting that the rate at which the inbreeding crosses are performed can influence the magnitude of the effect. For example Pante et al.'s (2001) results indicate that Rainbow Trout inbred over six generations had higher body weights than those inbred to the same extent over a single generation (Gjerde et al. 1983). Inbreeding in natural populations is likely to proceed even more slowly which allows for a reduction in the genetic load through the gradual purging of deleterious recessive alleles with a resultant reduction in the effects of inbreeding depression (Wang et al. 2002).

GENETIC AUGMENTATION

Genetic augmentation has proven a successful strategy for the recovery of isolated fish populations impacted by low levels of genetic diversity (Yamamoto et al. 2006; Andrews et al. 2016; Robinson et al. 2017; Bell et al. 2019). Yamamoto et al. (2006) translocated 20 anadromous female White-Spotted Charr (*Salvelinus leucomaenis*) above dams in two populations. They only translocated females to ensure mating between the translocated individuals and the resident fish. The A_R and H_E values above the dams increased

by 45% and 95% respectively after the translocation (based on four SSR loci) to below-dam levels. Yamamoto et al. (2006) did not attempt to estimate early life-stage growth or survival before or after the translocation.

Andrews et al. (2016) translocated 35,000 WCT embryos from five sources into vacant habitats in Montana. Although not a form of genetic augmentation (as the habitat was vacant), the results nonetheless demonstrate the benefits of genetic diversity for survival. Andrews et al. (2016) discovered that survival to age-1 was twice as high for the more genetically diverse population. They concluded that

“Continued monitoring over several generations will be necessary to determine the eventual contributions of each source population and the relevance of these initial findings.”

In a test of genetic rescue (Bell et al. 2019), Robinson et al. (2017) translocated five female and five male Brook Trout (*Salvelinus fontinalis*) at the earliest expected onset of spawning upstream into each of four populations that had been isolated by a barrier for an average of 50 years. Thirty-one of the 40 translocated individual reproduced successfully, resulting in an increase in A_R of 45% and an increase in H_E of 25% (based on eight SSR loci). More importantly, hybrids were on average approximately 5% longer relative to resident offspring in the populations. Robinson et al (2017) concluded that

“Until future cohorts are examined, we cannot rule out the potential for outbreeding depression during subsequent generations. However, results to date are consistent with genetic rescue and are highly promising for this underused management option.”

ESTIMATED RESPONSE

Taken together the inbreeding experiments and genetic augmentation studies suggest that increasing genetic diversity would be expected to increase the length of age-0 fish by at most 5%. However, as the genetic diversity in the Harmer Creek is only moderately low, and no inbreeding was detected in the 2016 sample (e.g. negative F_{IS}), a more limited length effect is expected. The inbreeding experiments suggest that the survival odds are approximately three times lower after three generations of full sibling crosses while the genetic augmentation studies suggest that survival to age-1 was approximately twice as high for the more genetically diverse population.

DISCUSSION

As the number of adults did not experience a sharp decline during the period of interest the genetic diversity was not expected to have declined by more than 2.8% in the Harmer population. Furthermore, although the genetic diversity is lower in the Harmer population than the Grave population, the differences (an allelic richness of 1.4 versus 1.6 and an expected heterozygosity of 0.09 versus 0.12), appear insufficient to explain a non-negligible contribution to the Reduced Recruitment in the Harmer population with no apparent reduction in recruitment in the Grave population. This argument is further supported by the F_{ST} of -0.05% which indicates that the frequencies of common alleles are indistinguishable between the two populations. In order for maladaptation to be making even a minor contribution to the Reduced Recruitment (with Above Replacement Recruitment in the Grave population), the fish in the Harmer population would have to be experiencing changing and very different environmental conditions to those in the Grave population. Assessing whether this is the case is beyond the current report but should be evaluated in the integrative report (Harmer Creek Evaluation of Cause Team 2023).

Due to a presumed single tetraploid event of an ancestral salmonid, approximately 50% of the structural gene loci are duplicated in RB and Pacific salmon (Allendorf 1978; Allendorf and Utter 1979; Berthelot et al. 2014). Such a large proportion of duplicated loci may make salmonids less sensitive to deleterious mutations and inbreeding depression than other diploid fish species. An analysis of 12 small, isolated WCT populations from Montana failed to detect a relationship between genetic diversity and population growth rates (Carim et al. 2017). This could be evidence that salmonids genome duplication allows them to tolerate higher levels of inbreeding due to paralogous genes, or alternatively could reflect that purging of deleterious alleles has already occurred in these populations. However, the inbreeding experiments suggest that despite their tetraploid ancestry RB undergoing high levels of rapid inbreeding exhibit reduced fitness.

Comparison with other WCT populations in Alberta and British Columbia suggests that the standardized expected heterozygosity in Harmer is in the lower 45th percentile. Although relatively little is known about recruitment in the 10 populations with lower genetic diversity they are considered to have been self-sustaining at least in the recent past. It is also worth noting that the Harmer and Grave populations both have small negative F_{IS} values indicating no evidence of inbreeding among relatives in the 2016 samples. The small sample size may have affected the result for Harmer Creek, while the Grave Creek sample collection F_{IS} may have been biased low due to the presence of upstream and downstream barriers.

Although we find no evidence suggesting low genetic diversity has contributed directly to the Reduced Recruitment, the census-based effective population size of 40 and the linkage disequilibrium based estimate of 23 are below the threshold of 50 for avoiding inbreeding depression and well below the threshold of 500 for avoiding maladaptation (Franklin 1980). This suggests that inbreeding depression could become a concern if genetic losses continue for multiple generations and rapid major environmental change may result in maladaptation in both populations.

Based on the inbreeding and genetic augmentation experiments, the potential effects of inbreeding depression on the age-0 length were estimated. The resultant responses suggests that if the Harmer and Grave populations were genetically much more diverse then the age-0 fish may have been at most 5% longer and recruitment at best twice current levels in both populations. Furthermore, the fact that the potential effects are expected to be similar for both populations means that when considered in isolation they can explain at most a minor proportion of the Reduced Recruitment and Recruitment Failure patterns. However, age-0 Cutthroat Trout must attain a threshold size to survive the winter (Coleman and Fausch 2007a). Consequently, inbreeding depression occurring in the presence of other growth-related stressors such as water temperature, (Coleman and Fausch 2007b) could tip the balance in a negative direction in particular years. Consistent with this hypothesis, age-0 fish tend to be smaller in Harmer than Grave Creek (Thorley et al. 2022) which has lower summer rearing water temperatures. The relationship between size and survival and interactions with others stressors for the 2017 to 2019 spawn years is evaluated in the integrative report (Harmer Creek Evaluation of Cause Team 2023).

Given the genetic similarities between the Harmer and Grave Creek populations, the comparable genetic diversity to other non-admixed, isolated, stream-dwelling populations, the recent Above Replacement recruitment, and the relatively small potential for adverse impacts, the contributions of inbreeding depression and maladaptation to the Reduced Recruitment in the Harmer Creek population are considered to be at most minor. New genetic samples that include at least some of the current spawners and a larger sample size (e.g., $n > 30$) have been collected in 2021 to confirm that inbreeding has not occurred since 2016, and that the 2016 genetic diversity estimates accurately reflect the population. At the time of writing, these 2021 data were not available for use in the Harmer Creek Evaluation of Cause. Data from sampling in 2021 will be reported and interpreted by Teck Coal through the fish population monitoring program.

REFERENCES

- Allendorf, F.W. 1978. Protein polymorphism and the rate of loss of duplicate gene expression. *Nature* **272**(2): 76–78.
- Allendorf, F.W., Bayles, D., Bottom, D.L., Currens, K.P., Frissell, C.A., Hankin, D., Lichatowich, J.A., Nehlsen, W., Trotter, P.C., and Williams, T.H. 1997. Prioritizing Pacific salmon stocks for conservation. *Conserv. Biol.* **11**(1): 140–152. doi:10.1046/j.1523-1739.1997.95248.x.
- Allendorf, F.W., and Utter, F. 1979. Population Genetics. *In* *Fish Physiology*. Elsevier, Burlington. Available from http://www.123library.org/book_details/?id=38217 [accessed 9 June 2021].
- Andrews, T.C., Shepard, B.B., Litt, A.R., Kruse, C.G., Lee Nelson, M., Clancey, P., Zale, A.V., Taper, M.L., and Kalinowski, S.T. 2016. Performance of Juvenile Cutthroat Trout Translocated as Embryos from Five Populations into a Common Habitat. *North Am. J. Fish. Manag.* **36**(4): 926–941. doi:10.1080/02755947.2016.1165774.
- Aulstad, D., and Kittelsen, A. 1971. Abnormal Body Curvatures of Rainbow Trout (*Salmo gairdneri*) Inbred Fry. *J. Fish. Res. Board Can.* **28**(12): 1918–1920.
- Bell, D.A., Robinson, Z.L., Funk, W.C., Fitzpatrick, S.W., Allendorf, F.W., Tallmon, D.A., and Whiteley, A.R. 2019. The Exciting Potential and Remaining Uncertainties of Genetic Rescue. *Trends Ecol. Evol.* **34**(12): 1070–1079. doi:10.1016/j.tree.2019.06.006.
- Berthelot, C., Brunet, F., Chalopin, D., Juanchich, A., Bernard, M., Noël, B., Bento, P., Da Silva, C., Labadie, K., Alberti, A., Aury, J.-M., Louis, A., Dehais, P., Bardou, P., Montfort, J., Klopp, C., Cabau, C., Gaspin, C., Thorgaard, G.H., Boussaha, M., Quillet, E., Guyomard, R., Galiana, D., Bobe, J., Volff, J.-N., Genêt, C., Wincker, P., Jaillon, O., Crollius, H.R., and Guiguen, Y. 2014. The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. *Nat. Commun.* **5**(1): 3657. doi:10.1038/ncomms4657.
- Bland, J.M., and Altman, D.G. 2000. The odds ratio. *BMJ* **320**(7247): 1468–1468. doi:10.1136/bmj.320.7247.1468.
- Carim, K.J., Vindenes, Y., Eby, L.A., Barfoot, C., and Vøllestad, L.A. 2017. Life history, population viability, and the potential for local adaptation in isolated trout populations. *Glob. Ecol. Conserv.* **10**: 93–102. doi:10.1016/j.gecco.2017.02.001.
- Coleman, M.A., and Fausch, K.D. 2007a. Cold Summer Temperature Regimes Cause a Recruitment Bottleneck in Age-0 Colorado River Cutthroat Trout Reared in Laboratory Streams. *Trans. Am. Fish. Soc.* **136**(3): 639–654. doi:10.1577/T05-288.1.
- Coleman, M.A., and Fausch, K.D. 2007b. Cold Summer Temperature Limits Recruitment of Age-0 Cutthroat Trout in High-Elevation Colorado Streams. *Trans. Am. Fish. Soc.* **136**(5): 1231–1244. doi:10.1577/T05-244.1.
- Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., and Ovenden, J.R. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Mol. Ecol. Resour.* **14**(1): 209–214. doi:10.1111/1755-0998.12157.
- Fisheries and Oceans Canada. 2017. Management plan for the westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), British Columbia population, in Canada. Available from http://publications.gc.ca/collections/collection_2017/mpo-dfo/En3-5-75-2016-eng.pdf [accessed 20 July 2020].
- Fisheries and Oceans Canada. 2019. Recovery strategy and action plan for the westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) Alberta population (also known as Saskatchewan-Nelson River populations) in Canada. Available from http://epe.lac-bac.gc.ca/100/201/301/weekly_acquisitions_list-ef/2020/20-

-
- 01/publications.gc.ca/collections/collection_2020/mpo-dfo/En3-4-182-2019-eng.pdf [accessed 25 March 2021].
- Franklin, I.R. 1980. Evolutionary change in small populations. *In* Conservation Biology: An Evolutionary-Ecological Perspective. Sinauer Associates, Sunderland, Mass. pp. 135–40.
- Gjerde, B., Gunnes, K., and Gjedrem, T. 1983. Effect of inbreeding on survival and growth in rainbow trout. *Aquaculture* **34**(3–4): 327–332. doi:10.1016/0044-8486(83)90212-0.
- Goudet, J. 2005. hierfstat, a package for r to compute and test hierarchical F-statistics. *Mol. Ecol. Notes* **5**(1): 184–186. doi:10.1111/j.1471-8286.2004.00828.x.
- Greenbaum, G., Templeton, A.R., Zarmi, Y., and Bar-David, S. 2014. Allelic Richness following Population Founding Events – A Stochastic Modeling Framework Incorporating Gene Flow and Genetic Drift. *PLoS ONE* **9**(12): e115203. doi:10.1371/journal.pone.0115203.
- Harmer Creek Evaluation of Cause Team. 2023. Evaluation of Cause - Reduced Recruitment in the Harmer Creek Westslope Cutthroat Trout Population. Teck Coal Limited, Sparwood, BC.
- Hastings, K., Frissell, C.A., and Allendorf, F.W. 2008. Naturally Isolated Coastal Cutthroat Trout Populations Provide Empirical Support for the 50/500 Rule. *In* Status, Management, Biology, and Conservation. Oregon Chapter of the American Fisheries Society, Portland, OR. p. 2.
- Hilderbrand, R.H., and Kershner, J.L. 2000. Conserving Inland Cutthroat Trout in Small Streams: How Much Stream is Enough? *North Am. J. Fish. Manag.* **20**(2): 513–520. doi:10.1577/1548-8675(2000)020<0513:CICTIS>2.3.CO;2.
- Jamieson, I.G., and Allendorf, F.W. 2012. How does the 50/500 rule apply to MVPs? *Trends Ecol. Evol.* **27**(10): 578–584. doi:10.1016/j.tree.2012.07.001.
- Kincaid, H.L. 1976. Inbreeding in Rainbow Trout (*Salmo gairdneri*). *J. Fish. Res. Board Can.* **33**(11): 2420–2426. doi:10.1139/f76-288.
- Kincaid, H.L. 1983. Inbreeding in fish populations used for aquaculture. *Aquaculture* **33**(1–4): 215–227. doi:10.1016/0044-8486(83)90402-7.
- Lamson, H. 2016. Evaluation of Current Westslope Cutthroat Trout Hybridization Levels in the Upper Kootenay Drainage. Fish and Wildlife Compensation Program.
- Lande, R. 1993. Risks of Population Extinction from Demographic and Environmental Stochasticity and Random Catastrophes. *Am. Nat.* **142**(6): 911–927. Available from <http://www.jstor.org/stable/2462690>.
- Lodmell, A., Luikart, G., and Amish, S. 2017. 2016 Report on Admixture and Genetic Variation in Grave and Harmer Creek, BC Westslope Cutthroat Trout sample collections. unknown institution.
- Luikart, G., Ryman, N., Tallmon, D.A., Schwartz, M.K., and Allendorf, F.W. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conserv. Genet.* **11**(2): 355–373. doi:10.1007/s10592-010-0050-7.
- Pante, Ma.J.R., Gjerde, B., and McMillan, I. 2001. Effect of inbreeding on body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **192**(2–4): 201–211. doi:10.1016/S0044-8486(00)00467-1.
- R Core Team. 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available from <https://www.R-project.org/>.
- Robinson, Z.L., Coombs, J.A., Hudy, M., Nislow, K.H., Letcher, B.H., and Whiteley, A.R. 2017. Experimental test of genetic rescue in isolated populations of brook trout. *Mol. Ecol.* **26**(17): 4418–4433. doi:10.1111/mec.14225.
-

-
- Rousset, F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol. Ecol. Resour.* **8**(1): 103–106. doi:10.1111/j.1471-8286.2007.01931.x.
- Taylor, E. 2008. DNA Species Identification Report. A UBC Zoology Report, Selkirk Power Company Ltd., Nelson, BC.
- Taylor, E.B., Stamford, M.D., and Baxter, J.S. 2003. Population subdivision in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) at the northern periphery of its range: evolutionary inferences and conservation implications. *Mol. Ecol.* **12**(10): 2609–2622. doi:10.1046/j.1365-294X.2003.01937.x.
- Thorgaard, G.H., Bailey, G.S., Williams, D., Buhler, D.R., Kaattari, S.L., Ristow, S.S., Hansen, J.D., Winton, J.R., Bartholomew, J.L., Nagler, J.J., Walsh, P.J., Vijayan, M.M., Devlin, R.H., Hardy, R.W., Overturf, K.E., Young, W.P., Robison, B.D., Rexroad, C., and Palti, Y. 2002. Status and opportunities for genomics research with rainbow trout. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **133**(4): 609–646. doi:10.1016/S1096-4959(02)00167-7.
- Thorley, J.L., Kortello, A.K., Brooks, J., and Robinson, M. 2022. Grave and Harmer Creek Westslope Cutthroat Trout Population Monitoring 2021. A Poisson Consulting, Grylloblatta Consulting and Lotic Environmental Report, Teck Coal Ltd, Sparwood, BC.
- Wang, S., Hard, J.J., and Utter, F. 2002. Salmonid inbreeding: a review. *Rev. Fish Biol. Fish.* **11**: 301–319.
- Waples, R.K., Larson, W.A., and Waples, R.S. 2016. Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity* **117**(4): 233–240. doi:10.1038/hdy.2016.60.
- Weeks, A.R., Sgro, C.M., Young, A.G., Frankham, R., Mitchell, N.J., Miller, K.A., Byrne, M., Coates, D.J., Eldridge, M.D.B., Sunnucks, P., Breed, M.F., James, E.A., and Hoffmann, A.A. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective: Translocations in changing environments. *Evol. Appl.* **4**(6): 709–725. doi:10.1111/j.1752-4571.2011.00192.x.
- Weir, B.S., and Cockerham, C.C. 1984. Estimating F-Statistics for the Analysis of Population Structure. *Evolution* **38**(6): 1358–1370. doi:10.1111/j.1558-5646.1984.tb05657.x.
- Yamamoto, S., Maekawa, K., Tamate, T., Koizumi, I., Hasegawa, K., and Kubota, H. 2006. Genetic evaluation of translocation in artificially isolated populations of white-spotted charr (*Salvelinus leucomaenis*). *Fish. Res.* **78**(2–3): 352–358. doi:10.1016/j.fishres.2005.11.011.
- Yau, M.M., and Taylor, E.B. 2013. Environmental and anthropogenic correlates of hybridization between westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*O. mykiss*). *Conserv. Genet.* **14**(4): 885–900. doi:10.1007/s10592-013-0485-8.
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