

Genotype Composition of Chinook Salmon Emergent Fry in the Skookumchuck and Newaukum River – 2024 Season



Mara Zimmerman¹, Marisa Litz², Todd Seamons², Rick Coshow³, Phil Peterson⁴, Jarrod Yates⁴, Larry Lestelle⁵, John Ferguson⁶, Nat Kale⁷, Gary Morishima⁸, Tara Livingood-Schott⁹

¹Coast Salmon Partnership, ²Washington Department of Fish and Wildlife, ³Quinalt Indian Nation, ⁴West Fork Environmental, ⁵Biostream Environmental, ⁶Anchor QEA, ⁷Washington Department of Ecology, ⁸Technical Advisor for Natural Resources and the Environment to the President of Quinalt Indian Nation, ⁹Confederated Tribes of the Chehalis Reservation

February 2025

Acknowledgements

We thank West Fork Environmental staff Phil Peterson, Carson Swart, and Jarrod Yates for long hours of field work and the Washington Department of Fish and Wildlife Molecular Genetics Lab staff for lab processing (Cherril Bowman) and contract management (Todd Kassler). The Quinault Indian Nation initiated the Fry Monitoring Study in 2020, laying the groundwork for this study. The 2024 study was possible thanks to the cooperation of private landowners who provided access through their properties to the Skookumchuck and Newaukum trap sites. This study was funded by the Office of the Chehalis Basin at the Department of Ecology.

Recommended citation: Zimmerman et al. 2025. Genotype Composition of Chinook Salmon Fry in the Skookumchuck and Newaukum River – 2024 Season. Report to the Aquatic Species Restoration Plan Steering Committee.

Cover photo: Inclined plane traps operated on the Skookumchuck River, 2024 with insert of Chinook salmon parr (upper) and fry (lower). Photo credit: Mara Zimmerman.

Table of Contents

ACKNOWLEDGEMENTS	II
TABLE OF CONTENTS	III
TABLE OF FIGURES	IV
TABLE OF TABLES	V
TABLE OF APPENDICES	VI
EXECUTIVE SUMMARY	1
INTRODUCTION	2
STUDY GOALS AND OBJECTIVES.....	4
METHODS	4
STUDY SITE	4
TRAP OPERATION.....	5
FISH COLLECTION	7
TRAP EFFICIENCY TRIALS	7
GENOTYPE ASSIGNMENTS.....	8
ANALYSIS.....	8
RESULTS	12
FALL STREAM FLOWS DURING SPAWNING MIGRATION.....	12
SPRING STREAM TEMPERATURES DURING FRY OUTMIGRATION	13
SAMPLED PROPORTIONS OF FRY GENOTYPES	13
FRY GENOTYPE PROPORTIONS (MARK-RECAPTURE).....	15
FRY GENOTYPE PROPORTIONS (CPUE METHOD).....	24
FRY GENOTYPE PROPORTIONS (STREAM METHOD)	26
COMPARISON OF METHODS TO ESTIMATE GENOTYPE PROPORTIONS.....	27
INBREEDING COEFFICIENT	27
DISCUSSION	28
STREAM FLOW TARGETS AND ADULT MIGRATION	28
COMPARISONS AMONG FRY ANALYSIS METHODS	29
COMPARISON AMONG RIVERS AND YEARS	30
DIFFICULTY EVALUATING FLOW HYPOTHESIS.....	32
WHAT HAS BEEN LEARNED	33
NEXT STEPS.....	33
REFERENCES	34
APPENDICES	37

Table of Figures

Figure 1. Skookumchuck Dam at river mile 22.1 of the Skookumchuck River became operational in 1971, creating a four-mile long reservoir that submerged spawning and rearing habitat previously used by spring Chinook salmon. Image: Google Earth image 6/18/2021.	3
Figure 2. Location of fry traps, sonar, and stream flow gages in the Skookumchuck and Newaukum rivers within the Cascade Mountains Ecological Region of the Chehalis River basin.	5
Figure 3. West Fork Environmental staff check the incline plane fry traps on the Newaukum River, 2024. River flows from left to right in this photo. Chinook fry enter the trap on the upstream side (left) and are retained in the live box until processed and released downstream (right).	7
Figure 4. Stream flows in the Skookumchuck River (A) and Newaukum River (B) between June 15th and October 15 th . Graphs show mean daily flow for three time periods: current study (2023), post dam (1971 – 2021), and no dam (1929 – 1968). Current and post-dam flows were measured at the USGS Bucoda Gage #12026400 and the USGS Newaukum River Gage #12025000. No-dam flows for the Skookumchuck were derived from a statistically-based model with input data from the USGS gage located above the dam location because data from the Bucoda gage were not available prior to 1968 (Massmann and Massmann 2023b).	12
Figure 5. Stream temperatures in the Skookumchuck (A) and Newaukum (B) rivers during the period of fry trap operation, January 4 to April 12, 2024 (Newaukum) and January 3 to May 5, 2024 (Skookumchuck). Graphs show daily temperature mean (blue) and range (gray) at both trap sites.	13
Figure 6. Number of Chinook fry (top panel) and trap efficiency (bottom panel) by week for fish produced upstream of the Skookumchuck fry traps, 2024. The total abundance estimate is 839,232 fry with a CV of 9.5%. Error bars and shading around point estimates represent 95% confidence intervals. .	17
Figure 7. Weekly abundance and genetic run-type assignments of Chinook fry migrating past the Skookumchuck River fry traps, 2024.	18
Figure 8. Number of Chinook fry (top panel) and trap efficiency (bottom panel) by week for fish produced upstream of the Newaukum fry traps, 2024. The total abundance estimate is 54,745 with a CV of 34.0%. Error bars and shading around point estimates represent 95% confidence intervals.	21
Figure 9. Weekly abundance and genetic run-type assignments of Chinook fry migrating past the Newaukum traps, 2024.	22
Figure 10. Stream flows in the Skookumchuck River (A) and Newaukum River (B) between June 15th and October 15 th . Graphs show mean daily flow in 2022 (black) and 2023 (blue). Target flow in the Skookumchuck River was 35 to 40 cfs (horizontal gray bar). Flows were measured at the USGS Bucoda Gage #12026400 and Newaukum River Gage #12025000.	29
Figure 11. Proportion of spring (A) and heterozygote (B) genotypes and inbreeding coefficient (C) of Chinook salmon fry migrants in the Skookumchuck River (blue) and Newaukum River (orange), 2021 - 2024. Higher inbreeding coefficient value (C) corresponds to more segregation of genotypes (i.e., greater deficiency of heterozygotes than would be expected if random mating occurred).	30

Table of Tables

Table 1. Trap outings for fry traps in the mainstem Skookumchuck (SKO) and Newaukum (MSN) rivers during scheduled weekdays.	6
Table 2. Genotype assignments for Chinook salmon fry based on run timing SNP loci results, Skookumchuck and Newaukum rivers, 2024.	8
Table 3. Sampled proportions and number of tissue samples (n) of homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes from the Skookumchuck River fry traps, 2024.	14
Table 4. Sampled proportions and number of tissue samples (n) of homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes from the Newaukum River fry traps, 2024.	15
Table 5. Mark-recapture data for Chinook fry in the Skookumchuck River organized by week. Dataset includes total marks released (Total Mark), total marks recaptured (Total Recap), total maiden captures (Total Captures), and the proportion of time fished during the period (Prop Fished). Note that weeks were added to the beginning and end of the dataset to estimate the tails of the run.	16
Table 6. Estimated weekly and total proportions of Chinook fry genotypes passing the Skookumchuck River traps, 2024. Table includes weekly number (n) and proportions (prop) of homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes. Total genotype proportions are the weekly proportions weighted by weekly abundance. Total abundance differs from the sum of weekly totals because each estimate is based on median values of the model outcome and therefore not additive.	19
Table 7. Mark-recapture data for Chinook fry in the Newaukum River organized by week. Dataset includes total marks released (Total Mark), total marks recaptured (Total Recap), total maiden captures (Total Captures), and the proportion of time fished during the period (Prop Fished). Note that one week was added to the end of the dataset to estimate the tail of the run.	20
Table 8. Estimated weekly and total proportions of Chinook fry genotypes passing the Newaukum River traps, 2024. Table includes weekly number (n) and proportions (prop) of homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes. Total genotype proportions are the weekly proportions weighted by weekly abundance. Total abundance differs from the sum of weekly totals because each estimate is based on median values of the model outcome and therefore not additive.	23
Table 9. Weekly catch, effort (hours fished), catch-per-unit-effort (CPUE), and scaled CPUE of Chinook fry passing the Skookumchuck and Newaukum river fry traps, 2024. Scaled CPUE values sum to a total value of 1 across all weeks.	24
Table 10. Estimated weekly and total proportions of each Chinook genotype passing the Skookumchuck and Newaukum river fry traps based on ‘catch-per-unit-effort’ method, 2024.	25
Table 11. Estimated weekly and total proportions of each Chinook genotype passing the Skookumchuck and Newaukum river fry traps based on ‘stream’ method, 2024.	26
Table 12. Estimated genotype proportions of Chinook fry based on three analysis methods for the Skookumchuck and Newaukum rivers, 2024.	27
Table 13. Observed and expected genotype frequencies of Chinook salmon emergent fry in the Skookumchuck and Newaukum rivers, 2024. Expected genotype frequencies were calculated under Hardy-Weinberg Equilibrium. Inbreeding coefficient (F_{IS}) values greater than 1 correspond to spawning	

segregation among genotypes (i.e., greater deficiency of heterozygotes than would be expected if random mating occurred). 27

Table 14. Chinook salmon abundance by spawner run type and fry genotype upstream of fry trap locations in the Skookumchuck and Newaukum rivers. Data are abundance (proportion) correspond to spawning-outmigration years, 2022-23 and 2023-24. Spawner abundance is upstream of the trap. 31

Table of Appendices

Appendix A. Fry Trapping Data Sheet 38

Appendix B. Weekly median and 95% credible intervals for homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes in the Skookumchuck River traps based on weekly modeled mark-recapture abundance and genetic proportions. 40

Appendix C. Weekly median and 95% credible intervals for homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes in the Newaukum River traps based on weekly modeled mark-recapture abundance and genetic proportions. 41

Appendix D. Number of caught and sampled fry, wetted stream width (m), and catch-per-unit-effort of sampled Chinook fry genotypes (sampleCPUE) passing the Skookumchuck and Newaukum river fry traps, 2024. Trap width was 6m (2 traps*3ft/trap). Sample CPUE is the number of samples of each genotype divided by the hours fished. Adjusted CPUE (adjCPUE) is the sampleCPUE expanded by sampling rate (total:sampled fry) and a proxy for trap efficiency (stream:trap width). 42

Appendix E. Estimated genotype proportions of Chinook salmon fry in the Skookumchuck and Newaukum rivers, 2020-2023..... 44

Executive Summary

The number of spring Chinook salmon in the Chehalis River basin has declined over the past 20 years, and a petition for listing under the Endangered Species Act was filed in 2023. Among the potential factors influencing this decline is increased hybridization between spring and fall Chinook coupled with selection against the spring and/or heterozygote Chinook genotypes (e.g., loss of spawning habitat, temperature). A three-year study of Chehalis River Chinook emergent fry, conducted in 2020-2022 by the Quinault Indian Nation, found that the homozygous spring Chinook genotype predominantly occurs in the Newaukum and Skookumchuck sub basins. The proportion of homozygous spring genotype was lower, and the proportion of heterozygous genotype higher, in the Skookumchuck than the Newaukum river.

We hypothesize that the Skookumchuck Dam operation has increased rates of hybridization between spring and fall Chinook due to increased spatial and temporal overlap of spring and fall Chinook spawning since dam operations went into effect in 1971. We initiated this study to determine if reducing late summer and early fall stream flows in the Skookumchuck River to pre-dam levels would decrease the rate of hybridization between spring and fall Chinook and increase the relative frequency of homozygous spring Chinook. The results provided in this report are the second year of a multi-year study. The composition of Chinook fry genotypes in the Newaukum River, a free-flowing river south of the Skookumchuck River, is also monitored as a reference stream.

In this report, we estimate genotype proportions for the 2024 Chinook emergent fry in the Skookumchuck and Newaukum rivers. Genotypes were determined for Single Nucleotide Polymorphism markers which are highly correlated with Chinook run timing (i.e., homozygous spring, homozygous fall, heterozygote). The 2024 emergent fry were produced from 2023 spawners. In late summer of 2023, Skookumchuck River flows were intentionally reduced to 35 – 40 cfs as measured at the USGS Bucoda stream gage (#12026400). Sonar imagery from August to October 2023 showed movements of salmon (presumptive Chinook) into the Skookumchuck River occurred during rain events and were not spread out over the baseflow period.

Inclined-plane traps were operated from January 3 to May 3, 2024 in the Skookumchuck River and January 4 to April 12, 2024 in the Newaukum River. The traps were fished continuously Sunday evening to Friday morning except when conditions were unsuitable for trapping; scheduled trap outages occurred each weekend. Tissue samples were collected for genetic analysis from up to 50 Chinook fry per week at each site. Trap efficiency trials consisted of releasing dye-marked Chinook fry upstream of the trap and then enumerating the number of recaptures and maiden captures following the release event.

Chinook fry migrants were an order of magnitude more abundant at the Skookumchuck River trap ($\hat{n} = 839,232$, 95% credible intervals = 705,366 to 1,018,590) than the Newaukum River trap ($\hat{n} = 54,745$, 95% credible intervals = 31,422 to 106,464). Spring Chinook fry in the Skookumchuck River (47,806, 5.9% CV) were nearly as abundant as the total Chinook fry abundance in the Newaukum River. The Newaukum estimate was based on few recaptures with a high coefficient of variation (33.4%) and therefore greater uncertainty existed in the estimate. Chinook outmigration timing was earliest for homozygous spring fry, intermediate for the heterozygotes, and latest for fall homozygous fry. Proportions of homozygous spring, homozygous fall, and heterozygotes were 5.9%, 69.8%, and 24.3% respectively in the Skookumchuck River and 15.2%, 51.2%, and 33.6% in the Newaukum River.

Similar to 2023, the 2024 analysis included a comparison of three different analytical methods for estimating genotype proportions of emergent fry – mark-recapture, catch-per-unit-effort (CPUE), and the stream method. The three analytic methods yielded similar overall results in 2024 supporting the viability of analyzing trends over time by combining these data with previous years (2020-2022) for which the only available analytical methods were the catch-per-unit effort and stream method.

Introduction

The two types of Chinook salmon managed in the Chehalis River basin are classified as spring-run or fall-run depending on timing of river entry and spawning. Recent research indicates that these differences are strongly associated with a specific gene region (Prince et al. 2017), and a 2019 study in the Chehalis River established a high correlation between genotypes at specific single nucleotide polymorphisms (SNP) markers and the time of year (spring, fall) that Chehalis Chinook salmon entered the Chehalis River basin (Thompson et al. 2019a).

The number of spawning Chinook salmon classified as spring-run in the Chehalis River basin has declined over the past 20 years and a petition for listing under the Endangered Species Act was filed in 2023 (Center for Biological Diversity and Pacific Rivers 2023). In December 2023, the National Marine Fisheries Service announced that Endangered Species Act protections may be warranted and subsequently initiated a status review for Washington coast spring-run and fall-run Chinook. The decline in numbers of returning spring Chinook may be influenced by multiple factors including ocean productivity and/or mortality, spawner escapement methodologies, stream flows and temperature, and hybridization of spring and fall Chinook. Although there is no direct genetic evidence that hybridization rates have changed over time, spawning dates of spring Chinook salmon are later in recent years than those observed in the 1980s (Zimmerman 2017), a finding consistent with the hypothesis that there has been increased prevalence of the hybrid genotype over this time period. In 2020, the Quinault Indian Nation initiated a three-year study of the genotypes of Chinook emergent fry across the upper Chehalis River basin. Their study found that spring Chinook emergent fry predominantly occurred in the Newaukum and Skookumchuck sub basins and that homozygous spring Chinook occurred less frequently than heterozygotes and homozygous fall fry in all areas (Gilbertson 2023; Gilbertson et al. 2021). The study also identified that the timing of fry emergence and migration differed among genotypes – homozygous spring fry emerged first, followed by heterozygotes and then homozygous fall fry.

Hybridization, or interbreeding, of spring and fall Chinook is a natural occurrence. However, in the Chehalis River basin, hybridization may be a major threat to the long-term viability of the spring Chinook salmon if current habitat conditions (e.g., loss of spawning habitat, flow, temperature) result in poorer survival for the spring and heterozygote genotypes than the fall genotype. Access to exclusive habitat (i.e., habitat that is difficult for fall Chinook to access) is hypothesized to be the major evolutionary advantage of spring-run timing that offsets its numerous disadvantages such as smaller body size and lower fecundity (Quinn et al. 2016). Increases in hybridization over time may occur if environmental factors that historically separated spring and fall Chinook salmon are no longer effective (Thompson et al. 2019b). Because of this and other factors, when hybridization rates are high, spring Chinook salmon will disappear as more and more of them spawn with the heterozygote and fall genotypes (Ford et al. 2020). As long as the spring alleles are not lost from the population, spring Chinook can presumably re-emerge because approximately a quarter of the offspring of a spawning pair comprised of heterozygotes would be expected to be the homozygous spring genotype, i.e., spring Chinook.

We hypothesize that managed flows associated with the Skookumchuck Dam operation have increased the rate of hybridization since the dam became operational in 1971 (**Figure 1**). Base flows in the Skookumchuck River typically occur from late August to mid-October, but baseflow rates are two to three times higher under dam operations than the natural hydrograph which occurred prior to dam construction (i.e., pre-dam base flows). Pre-dam base flows in the lower Skookumchuck River were consistently lower than a median daily flow of 40 cfs (Massmann and Massmann 2023a). Massmann and Massmann (2023b) also estimated that, under the current managed flow regime, July and August flows associated with dam operations have been 40 to 45 cfs higher than the pre-dam base flows, and that the September and early October flows

associated with dam operations are approximately 80 cfs higher than the pre-dam base flows. Higher base flows were mandated by the 1998 dam operation agreement between the Washington Department of Fish and Wildlife and the dam owners (update to the 1974 agreement) “to provide spawning flow for Chinook salmon”. Although this boost in stream flows was intended to benefit salmon, the higher than historical flows may have also inadvertently enabled fall Chinook to enter the Skookumchuck River earlier than they would have under historical (and lower) flow conditions. Earlier entry of fall Chinook into the Skookumchuck River, combined with downstream displacement of spawning spring Chinook by dam construction without provision for upstream passage, compressed spring and fall Chinook salmon spawning in space and time thereby increasing hybridization of spring and fall Chinook.

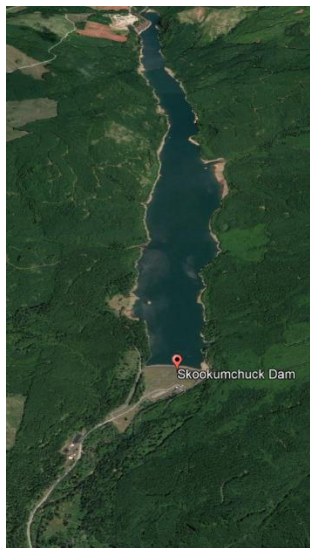


Figure 1. Skookumchuck Dam at river mile 22.1 of the Skookumchuck River became operational in 1971, creating a four-mile long reservoir that submerged spawning and rearing habitat previously used by spring Chinook salmon. Image: Google Earth image 6/18/2021.

This study was initiated to evaluate whether reducing base flows in the Skookumchuck River to pre-dam levels could reduce the rate of hybridization between spring and fall Chinook and increase the relative production of homozygous spring Chinook salmon. Arrangements were made with the Skookumchuck Dam owner, TransAlta, to manipulate flows at the intake to the Trans Alta Stream-Electric Power Plant located near river mile 7.2 so that maximum discharge measured at USGS Bucoda Gage #12026400 would be 40 cfs with a target minimum discharge of either the minimum prescribed under the current operational guidelines or 35 cfs as measured at USGS Gage 12026400, whichever was lower. The intent was to maintain discharge at USGS Gage #12026400 as close to 35 cfs as possible for the two-month period beginning August 15th. Base flows were reduced in this manner in the fall of 2022 – 2024. During the first year of the study, problems with the Bucoda flow gage results in flows that were higher than the target levels.

In this report, we estimate the genotype proportions of Chinook emergent fry (homozygous spring, homozygous fall, heterozygotes) produced from the fall 2023 spawners in the Skookumchuck and Newaukum rivers. As described above, our intent was to reduce river flows to 35 cfs from mid-August to mid-October in the Skookumchuck River to discourage fall Chinook salmon from entering the river during the time that spring Chinook salmon were spawning. The Newaukum River, a free-flowing tributary of the Chehalis River, is located south of the Skookumchuck River and was selected as a reference stream for this study.

This report provides a single year of results and is intended to contribute to a future analysis of the time-series data that makes use of previous and future fry trapping data in the Skookumchuck and Newaukum

rivers. For the three years prior to flow reduction (2020 – 2022), genetic data were collected by the Quinault Indian Nation and serve as the ‘before flow reduction’ data set (before). Estimates of genotype proportions from these initial years are catch-per-unit effort (CPUE) and stream method. However, there were concerns that the expansion methods of the CPUE and stream method may or may not represent weekly abundances of the outmigration which are needed to weight the samples genotypes. Therefore, the 2023 and 2024 studies also included mark-recapture methods to generate weekly estimates of outmigrant fry abundance. Mark-recapture data provide statistically unbiased estimates of abundance (with known precision) as long as the data meet the assumptions of the abundance estimator (Volkhardt et al. 2007). The 2023 and 2024 study years therefore provided an opportunity to compare multiple methods for estimating genotype frequencies and this will help justify future analyses of the multi-year data set.

Study Goals and Objectives

This study evaluated the relative frequencies of spring, fall, and heterozygote Chinook emergent fry in both the Skookumchuck and Newaukum rivers. The objectives were to:

1. Operate each trap for five days per week during the period of fry emergence.
2. Collect up to 50 genetic samples from Chinook fry on a weekly basis.
3. Collect data on a weekly basis needed for CPUE, stream method, and mark-recapture methods, including trap efficiency trials.
4. Test assumptions of the mark-recapture estimator.
5. Compare the genotype proportions estimated by the three methods for weighting genotype frequencies.

Methods

Study Site

The study area is located in the Chehalis River basin, the largest river basin entirely within the borders in Washington State (**Figure 2**). Inclined-plane traps were sited in the Skookumchuck and Newaukum sub-basins of the Cascade Mountains Ecological Region of the Chehalis River basin as defined by the Chehalis Basin Aquatic Species Restoration Plan. The Skookumchuck and Newaukum rivers arise in the Bald Hills, a low-elevation spur of the Cascade Mountains. The Skookumchuck River originates at approximately 3,000 feet in elevation above sea level near Huckleberry Mountain. The South Fork Newaukum River originates at Newaukum Lake also about 3,000 feet in elevation, and the North Fork Newaukum River originates near Windy Knob at about 2,600 feet in elevation.

Stream flows in the Skookumchuck and Newaukum sub-basins are strongly influenced by precipitation. Stream flows in the Skookumchuck are further influenced by operation of the Skookumchuck Dam. Hydrography is mostly rain-dominated with peak flows occurring from late fall to early spring and summer low flows extending into September or October. Annual precipitation varies across the Chehalis River basin and ranges from an annual average of 43 inches along the low-lying valley areas near Centralia and Chehalis to more than 120 inches in the Willapa Hills upstream of the South Fork Chehalis.

The Skookumchuck fry trap was located on private property at river mile 6.2 near the USGS gage 12026400 (Lat: 46.772176; Long: -122.924747). The Newaukum fry trap was located at Stan Hedwall Park at river mile 1.5 approximately six miles downstream from the Interstate-5 crossing (Lat: 46.638594; Long: -122.96714). Sites were selected because of their close proximity to previous trapping locations, reasonable access, and suitable characteristics to maximize trap efficiency throughout the season.

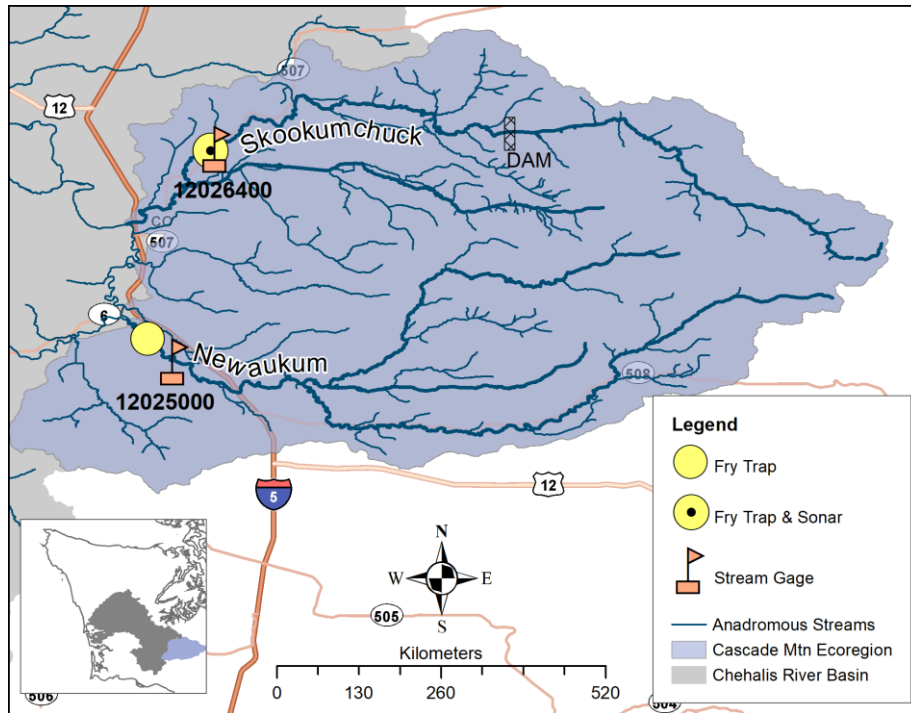


Figure 2. Location of fry traps, sonar, and stream flow gages in the Skookumchuck and Newaukum rivers within the Cascade Mountains Ecological Region of the Chehalis River basin.

Trap Operation

Inclined-plane traps set between aluminum pontoons were operated from January 3 to May 3, 2024 (**Figure 3**). Each trap had a 3-foot wide opening and, once deployed, two traps were fished side-by-side in each river. In some years, panels or wings were used to divert flow and migrating juvenile salmon into the trap; however, there were no panels or wings used in 2024. In the Skookumchuck, two traps were deployed on January 3 and operated through May 3, 2024. In the Newaukum, a single fry trap was deployed on January 4 and a second trap deployed on January 16, 2024, and both traps operated through April 12, 2024. Data from both traps at each site were summed prior to analysis.

The traps were fished continuously from Sunday afternoon to Friday morning with scheduled outages over the weekend. The traps were cleared of fish and debris a minimum of once per day and more frequently when debris or fish densities pose a threat to fish health. There was a short outage (<1 hour) on February 6, 2024 in the Newaukum due to trap repairs. Other unscheduled trap outages occurred in both the Skookumchuck and Newaukum rivers throughout the season due to high water (**Table 1**). Of note, this trap operation protocol differed from the 2020–2022 trapping seasons when weekly trap operation ceased after tissue samples were collected from 50 Chinook fry.

Table 1. Trap outages for fry traps in the mainstem Skookumchuck (SKO) and Newaukum (MSN) rivers during scheduled weekdays.

Site ID	Trap	Stop Time	Start Time	Duration (h)	Comments
MSN	A	1/8/2024 09:16	1/11/2024 10:14	72.97	high water
SKO	A	1/9/2024 07:56	1/11/2024 08:32	48.60	high water
SKO	B	1/9/2024 08:44	1/11/2024 08:37	48.88	high water
MSN	A	1/18/2024 11:29	NA	NA	high water, not reset ¹
MSN	B	1/18/2024 11:36	NA	NA	high water, not reset ¹
SKO	A	1/18/2024 08:24	NA	NA	high water, not reset ¹
SKO	B	1/18/2024 09:28	NA	NA	high water, not reset ¹
SKO	A	NA	1/22/2024 13:46	NA	high water, not set until Mon ²
SKO	B	NA	1/22/2024 13:52	NA	high water, not set until Mon ²
SKO	A	1/23/2024 07:48	1/24/2024 09:15	25.45	high water
SKO	B	1/23/2024 08:45	1/24/2024 09:24	24.65	high water
MSN	A	NA	1/24/2024 10:30	NA	high water, not set until Wed ²
MSN	B	NA	1/24/2024 10:38	NA	high water, not set until Wed ²
MSN	A	2/6/2024 11:20	2/6/2024 12:10	0.83	trap repair
MSN	B	2/6/2024 11:24	2/6/2024 12:10	0.77	trap repair
SKO	A	2/28/2024 08:29	NA	NA	high water, not reset
SKO	B	2/28/2024 09:01	NA	NA	high water, not reset
MSN	A	2/28/2024 07:22	NA	NA	high water, not reset
MSN	B	2/28/2024 07:27	NA	NA	high water, not reset

¹Trap was not reset before planned weekend outage (Friday – Sunday afternoon).

²Trap was out during planned weekend outage but not reset on Sunday afternoon.

During each daily trap event, the following data were collected: Site ID, date, time, crew initials, instantaneous temperature, water clarity, wetted channel width, and any other observations that may have affected trapping efficiency (**Appendix A**). The width of the wetted stream channel was obtained with a range finder device appropriate for the scale of the sample sites.

Stream temperature data were collected at each trap location with a HOBO Pendant® MX-2201 Water Temperature Data Logger, following the Washington Department of Ecology Standard Operating Procedures for continuous temperature monitoring of freshwater rivers and streams (Nelson and Dugger 2022). Protocols included a calibration check, placement of the logger to minimize solar radiation or dewatering, and a post-deployment accuracy check.



Figure 3. West Fork Environmental staff check the incline plane fry traps on the Newaukum River, 2024. River flows from left to right in this photo. Chinook fry enter the trap on the upstream side (left) and are retained in the live box until processed and released downstream (right).

Fish Collection

At each trap check, all fish captured by the traps were identified to species and tallied (**Appendix A**). Photo records were taken as further documentation, and any fish mortalities in the trap were recorded on the data sheet. Chinook were mildly anesthetized with MS-222 in order to measure fork length, evaluate mark status, and collect a tissue sample for genetic analysis. Mark status and tissue samples were recorded for Chinook fry (≤ 45 mm fork length) only. Mark status was recorded as unmarked (no dye, maiden capture) or marked (dyed, recapture from trap efficiency trial). Tissue samples were obtained from up to the first 50 fry sampled each week. A small piece of the upper lobe of the caudal fin was excised for the tissue sample. Fin tissue was immediately placed on a gridded blotter sheet with an identifying number; each sheet with tissue samples had unique identifying numbers for each day of collection. Blotter sheets were completely dried, stored in zip-loc bags with desiccant beads, and held at the West Fork Environmental office under a chain of custody procedure until they were delivered for processing to the Washington Department of Fish and Wildlife genetics lab.

With the exception of Chinook fry to be used for trap efficiency trials (see below), all fish were released to the river at the point of capture.

Trap Efficiency Trials

Trap efficiency trials consisted of releasing dye-marked Chinook fry upstream of the trap and then enumerating the number of recaptures and maiden captures following the release event. The field procedure consisted of marking a known number of Chinook fry for release. Fry were released approximately 0.5 km upstream of the trap above multiple riffle-pool sequences to allow for mixing between marked and unmarked fish prior to recapture in the fry trap.

Chinook fry were marked for trap efficiency trials with Bismark Brown dye. A subset of the dye-marked fish were also marked with an upper caudal clip removed as a genetic sample (i.e., up to 50 fry per week were

used for both genetic sample and trap efficiency trials). The dye solution was 23.2 mg Bismark Brown dye per liter of water. Fry were soaked in 14 L of dye solution in a 5-gallon bucket aerated with bubblers for 60 minutes. Fish were allowed to fully recover for at least half an hour in fresh water before release. Up to 200 Chinook fry per day were marked with Bismark Brown dye and released Monday to Wednesday. Recaptures of marked fish attributed to each weekly release occurred Tuesday to Friday of the same week.

Genotype Assignments

Genomic DNA was isolated from fish tissue with Machery-Nagle silica-based column extraction kits following the manufacturers protocol for animal tissues. Chinook salmon-specific SNPs were genotyped using a cost-effective method based on a custom amplicon sequencing called Genotyping in Thousands (GTseq) (Campbell et al. 2015). For each sample, pools were sequenced, de-multiplexed, and genotyped by generating a ratio of allele counts. The process was divided into four steps: extraction, library preparation, sequencing, and genotyping. The GTseq SNP panel used to infer adult run timing phenotype had 298 autosomal SNP loci, one sex ID SNP locus, and 33 run timing SNP loci. Run timing SNP loci were the same two used in previous genetic analysis of Chehalis Chinook salmon (Thompson et al. 2019a) but also included 31 additional markers identified as highly correlated with run timing by Koch and Narum (2020) and (Thompson et al. 2020).

Genotypes were assigned to seven categories consistent with the two run timing markers developed and validated in the Chehalis River basin (Thompson et al. 2019a). For consistency among study years, genotypes were assigned to three categories: homozygous spring, homozygous fall, and heterozygotes (**Table 2**).

Table 2. Genotype assignments for Chinook salmon fry based on run timing SNP loci results, Skookumchuck and Newaukum rivers, 2024.

Genotype (Thompson et al. 2019a)	Genotype (for analysis)	Description
Homozygous Fall	Homozygous Fall	Fall genotype at both loci
Likely fall	Homozygous Fall	Fall genotype at one locus, no data other locus
Heterozygote	Heterozygote	Heterozygote at both loci
Likely heterozygote	Heterozygote	Heterozygote at one locus, no data other locus
Partial heterozygote	Did not use	One locus heterozygous, other locus spring or fall
Homozygous Spring	Homozygous Spring	Spring genotype at both loci
No data	Did not use	No genotype available

Analysis

Three methods were used to estimate the genotype proportions among outmigrating Chinook salmon fry – mark-recapture, catch-per-unit-effort (CPUE), and the stream method. Estimates were calculated separately for the Skookumchuck and Newaukum rivers. For all three methods, data from the two fry traps operated in parallel on each river were combined, and data were stratified by management week (Sunday – Saturday).

Mark-Recapture

The mark-recapture method calculated a weekly fry abundance, apportioned the weekly fry abundance by the sampled genotype proportions, and summed the weekly genotype-apportioned abundances to a seasonal total abundance for each genotype. Seasonal proportion of a given genotype was the genotype abundance divided by the total abundance.

To estimate abundance of Chinook fry, we used a Bayesian Time-Stratified Population Analysis System (BTSPAS, Bonner and Schwarz 2011; Bonner and Schwarz 2014). This method uses Bayesian P-splines and hierarchical modeling of trap efficiencies to determine abundance with known precision through time. For this analysis, data were stratified by week. The BTSPAS modeling approach allows for estimation during missed trapping days and for periods with minimal efficiency data. Data for input in the analysis included total weekly catch of unmarked fry (i.e., maiden captures), total weekly count of marked fry, total weekly count of recaptured marked fry, and proportion of the week sampled in hours. Prior to analysis, weekly total catch was expanded to adjust for missed catch during trap outages.

BTSPAS model convergence was assessed by visually inspecting the trace plots and using the potential scale reduction statistic, or Rhat. The Rhat statistic measures the ratio of the average variance draws within each chain to the variance of the pooled draws across chains; if all chains are at equilibrium, these will be the same and Rhat will be 1. If the chains have not converged to a common distribution, the Rhat statistic will be > 1 . Models were considered to have converged if Markov Chain Monte Carlo chains were fully mixed based on visual inspection, the smallest number of effective draws was greater than 1,000 and Rhat was less than 1.1 for all parameters (Gelman et al. 2004). As a rule of thumb for mark-recapture studies, a coefficient of variance (CV) of 20% is a reasonable target (Pollock et al. 1990). However, if models converged and CV was $>20\%$, this indicated higher variability and less precision than a model with a CV that was $<20\%$. The BTSPAS analysis was executed in R version 4.2.0 (R Core Team 2022) using the BTSPAS package (Bonner and Schwarz 2014).

The six basic assumptions that need to be met for unbiased estimates in mark-recapture studies include: 1) the population is closed, 2) marks are not lost, 3) marking does not affect behavior, 4) initial capture probabilities are homogenous, 5) the second sample is a random representative sample (i.e., marked and unmarked fish are completely mixed), and 6) mark status is reported correctly (Volkhardt et al. 2007). The assumption that the population was closed (Assumption 1) was likely met as trapping occurred throughout the Chinook fry outmigration period. To support Assumption 1, trap retention trials were conducted throughout the season to ensure fry were not lost from the live box. The assumption that marks were not lost (Assumption 2) was met by using dye marking concentration and duration shown to be effective in other trapping studies and by using a secondary mark (i.e., upper caudal clip) with no possibility of regrowth during the trapping period. The assumption that the second sample was a random representative sample (Assumption 4) was met as the release site for recaptured fish was located ~ 0.5 km upstream (multiple pool-riffle sequences) to allow for mixing of marked and unmarked fish. A month into the trapping season (February 9, and February 23, 2024), mark detection trials were conducted to ensure that marking did not affect behavior or survival after dye application (Assumption 3), and that marks were not missed and were reported correctly (Assumption 6).

Mark detection trial results were 100% for field staff, who both correctly identified an unknown number of marked (upper caudal clip and/or Bismarck Brown dye) and unmarked Chinook fry that were held overnight. In both trials, marked (115 out of 115, 100%) and unmarked (115 out of 115, 100%) Chinook fry had high survival. Trap retention trials also yielded high retention results, indicating that marks were not lost. Trap retention was lowest for Skookumchuck Trap A (average 87% retention over three overnight trials from January 18 to March 15, 2024) and equally high for Skookumchuck Trap B and Mainstem Newaukum Trap B (average 98% retention over seven overnight trials from January 18 to March 15, 2024). Mainstem Newaukum Trap A also had high retention (average 97% over three trials from January 18 to March 15, 2024).

Catch-Per-Unit-Effort

The Catch-Per-Unit-Effort (CPUE) method standardized trap catch by the unit of trapping effort (hours) and then expanded weekly genotype proportions by weekly CPUE calculations. Weekly CPUE values were re-scaled so the seasonal total of each genotype summed to a value of 1, providing a proportional representation of overall fry CPUE each week. Re-scaled CPUE values were apportioned by the sampled genotype proportions in the corresponding week, and the weekly genotype-apportioned CPUEs were summed to a seasonal total proportion for each genotype.

Total genetic samples for week i = fall i + heterozygous i + spring i

Weekly proportion of genetic sample:

- Spring proportion i = spring sample i / total genetic sample i
- Fall proportion i = fall sample i / total genetic sample i
- Heterozygous proportion i = heterozygous sample i / total genetic sample i

Weekly proportion of fry catch:

- CPUE i = fry catch i / hours fished i
- Total CPUE = $\sum_{i=n}^{i=1} CPUE_i$
- Fry catch proportion week i = CPUE week i / Total CPUE

Weekly genotype proportions:

- Spring proportion fry catch i = spring proportion i * fry catch proportion week i
- Fall proportion fry catch i = fall proportion i * fry catch proportion week i
- Heterozygous proportion fry catch i = heterozygous proportion i * fry catch proportion week i

Overall genotype proportions:

- Spring: $\sum_{i=n}^{i=1}$ spring proportion fry catch
- Fall: $\sum_{i=n}^{i=1}$ fall proportion total fry catch
- Heterozygous: $\sum_{i=n}^{i=1}$ heterozygous proportion total fry catch

Based on the CPUE method, genotype proportions in weeks with higher CPUE values are weighted more heavily than weeks with low CPUE values. Results from this method rely on the assumption that CPUE values and overall abundance are correlated which will only be true if the overall catchability (of efficiency) of the trap is the same among weeks. Direct measure of trap efficiencies are needed to test this assumption, and the method may produce inaccurate results if there are wide variations in trap efficiency among weeks.

Stream Method

The stream method accounts for weekly variation in effort and catchability of the trap by standardizing weekly trap catch numbers by both effort (hours) and stream-to-trap width (catchability). Adjusted weekly CPUE for each genotype were then re-scaled so the weekly genotype proportions summed to a value of 1 and the genotype-apportioned CPUEs were summed to a seasonal total proportion for each genotype.

Weekly CPUE of sampled fry was calculated by genotype g and week i and summed for all genotypes on week i . The CPUE calculation standardized the number of sampled fish by different levels of trapping effort among weeks (e.g., trap outage). CPUE of sampled fry:

- $\text{sampleCPUE}_{g,i} = \text{Number sampled genotype}_{g,i} / \text{hours fished}_i$
- $\text{sampleCPUE}_i = \text{Number sampled fry}_i / \text{hours fished}_i$

The adjusted calculation of CPUE was calculated by genotype g and week i and summed for all genotypes on week i . The adjusted CPUE accounted for different sampling rates among weeks (expansion by total-to-sampled ratio) and a proxy for trap efficiency (expansion by stream-to-trap width). Adjusted CPUE:

- $\text{adjCPUE}_{g,i} = \text{sampleCPUE}_{g,i} * \frac{\text{TotalFry}_i}{\text{SampledFry}_i} * \frac{\text{StreamWidth}_i}{\text{Trap Width}_i}$
- $\text{adjCPUE}_i = \sum_{g=3}^{g=1} \text{adjCPUE}_{g,i}$

Re-scaled values of the adjusted CPUEs were necessary so that the genotype proportions g on a given week i summed to a value of 1:

- $\text{EG}_{g,i} = \text{adjCPUE}_{g,i} / \text{adjCPUE}_i$

The overall, seasonal proportions of each genotype g were the re-scaled values of the adjusted CPUE summed across n weeks:

- $\text{EG}_g = \sum_{i=1}^i \text{CPUE}_{g,i}$

Results from this method rely on the assumption that increases or decreases in stream wetted widths are correlated with trap efficiency, although direct measures of trap efficiency are needed to test this assumption. In general, decreases in wetted width should funnel proportionally more water into the trap and increase trap efficiency. However, this assumption should be tested with direct measures of trap efficiency as the correlation between stream width and trap efficiency depends on multiple site-specific factors such as trap position, fry distribution in the stream channel, and geomorphic shape of the stream channel.

An additional method, inbreeding coefficient, was explored to further evaluate effects of flow changes on spawning success of Chinook salmon genotypes.

Inbreeding Coefficient F_{IS}

The inbreeding coefficient (F_{IS}) is a measure of the amount of inbreeding in a subpopulation relative to an individual (Wright 1951). For our use, F_{IS} measures deviations between observed and expected heterozygosity within a population. Expected heterozygosity is based on the conditions of an ideal population, i.e., a population exhibiting Hardy-Weinberg Equilibrium (HWE). Genetic variation (i.e., allele or genotype frequencies) will remain constant (i.e., at HWE) in a population if that population is large, has no genetic mutations, no selection, no geneflow (immigration), and random mating. F_{IS} values can range from -1 to 1 where $F_{IS} = 0$ indicates the observed and expected genotype frequencies are identical, negative values indicate an excess of heterozygotes, and positive values indicate a deficiency of heterozygotes. In this study, if all conditions other than random

mating are met, any changes in F_{IS} among years would indicate changes in mating patterns among the spring, fall, and heterozygote Chinook salmon. If changing the flows reduces interbreeding of spring and fall Chinook, we should see an increasing deficiency in heterozygotes, i.e., an increasingly positive F_{IS} .

Expected genotype frequencies (exp) under Hardy-Weinberg Equilibrium were calculated for spring homozygous (SS), fall homozygous (FF), and heterozygous (SF) genotypes as shown in the equations below. Observed genotype frequencies (obs) in these calculations were the estimated genotype proportions of emergent fry (mark-recapture method).

- $SS_{exp} = (SS_{obs} + 0.5 * SF_{obs})^2$
- $FF_{exp} = (FF_{obs} + 0.5 * SF_{obs})^2$
- $SF_{exp} = 2 * (SS_{obs} + 0.5 * SF_{obs}) * (FF_{obs} + 0.5 * SF_{obs})$

Inbreeding coefficient (FIS) was calculated from the observed and expected heterozygote frequencies as shown below:

- $FIS = 1 - \frac{SF_{obs}}{SF_{exp}}$

Results

Fall Stream Flows During Spawning Migration

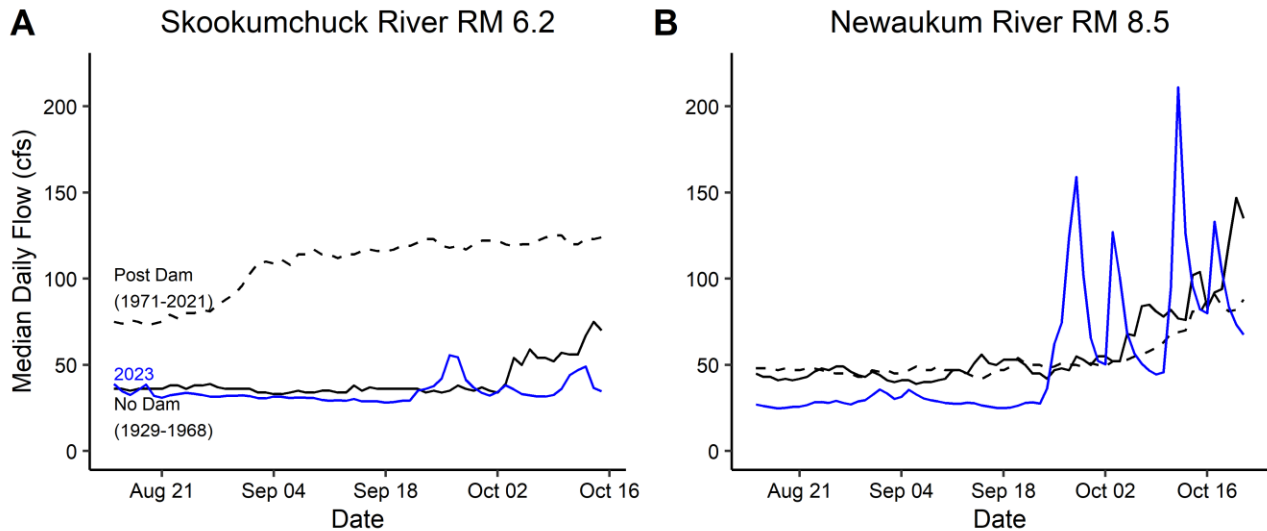


Figure 4. Stream flows in the Skookumchuck River (A) and Newaukum River (B) between June 15th and October 15th. Graphs show mean daily flow for three time periods: current study (2023), post dam (1971 – 2021), and no dam (1929 – 1968). Current and post-dam flows were measured at the USGS Bucoda Gage #12026400 and the USGS Newaukum River Gage #12025000. No-dam flows for the Skookumchuck were derived from a statistically-based gage model with input data from the USGS gage located above the dam location because data from the Bucoda gage were not available prior to 1968 (Massmann and Massmann 2023b).

In the lower Skookumchuck River, daily mean flows between August 15 and October 15, 2023 ranged between 28 and 55 cfs at the USGS Bucoda Gage #12026400 (**Figure 4**). During this time frame, the flows exceeded the target maximum flow of 40 cfs for 11% (7 days) of the time. However, flows were slightly

lower than the median of daily mean flows estimated under no dam scenario by Massmann and Massmann (2023b) (**Figure 4**) except for increased flows on September 26-27 that corresponded to a precipitation events. August to October flows were just ~30% of the median of daily mean flows while the dam has been operational (1971-2021).

In the Newaukum River, daily mean flows between August 15 and October 15, 2023 ranged between 25 and 211 cfs at USGS Newaukum River Gage #12025000 (**Figure 4**). For the purpose of comparison, we organized the flow data in the Newaukum River by the same historical time periods as the Skookumchuck River (1929-1968: no dam, 1971-2021: dam operations, 2023 flow). The Newaukum River flows were similar between the 1929-68 and 1971-2021 time periods. The Newaukum River flows in 2023 were lower than these historical flows until September 26th, after which time stream flows increased in response to precipitation events (**Figure 4**).

Spring Stream Temperatures During Fry Outmigration

Stream temperatures increased over the period of trap operation at both locations (**Figure 5**). Daily mean temperatures in January ranged between 1°C and 9°C in both rivers. A temperature drop on January 15-16 occurred in both rivers and corresponded to a drop in air temperature on these days. A steady increase in temperature began in March and continued to the end of trap operation in April (Newaukum) and May (Skookumchuck).

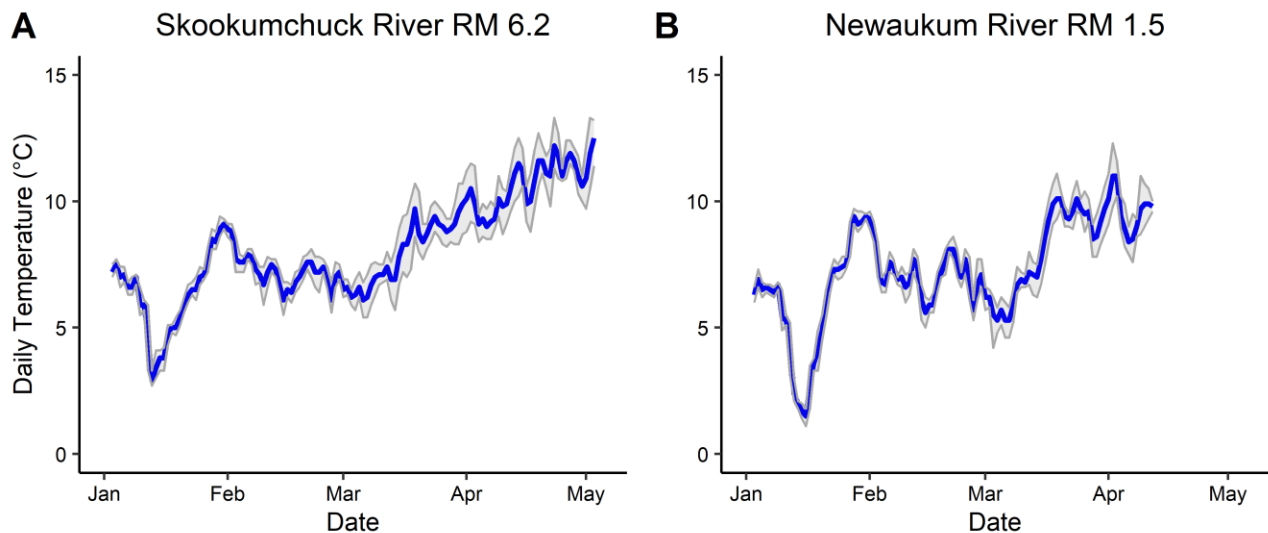


Figure 5. Stream temperatures in the Skookumchuck (A) and Newaukum (B) rivers during the period of fry trap operation, January 4 to April 12, 2024 (Newaukum) and January 3 to May 5, 2024 (Skookumchuck). Graphs show daily temperature mean (blue) and range (gray) at both trap sites.

Sampled Proportions of Fry Genotypes

A total of 1,304 genetic samples were sent to the Washington Department of Fish and Wildlife Molecular Genetics Lab for processing (Skookumchuck = 867 and Newaukum = 437). Of these, 300 samples were removed from the analysis, either because of no data ($n = 294$) or a “partial” run-type assignment ($n = 6$). “No data” meant that useful genetic data were not successfully obtained from the sample. All “likely” run-type assignments ($n = 68$) were included in the final assessment. The final sample size for run-type assignment was 1,004 (Skookumchuck = 684 and Newaukum = 320).

Skookumchuck

Genetic samples were collected between January 4 and May 3, 2024 in the Skookumchuck. A total of 40 samples were homozygous spring from January 4 to February 26, 2024, 508 samples were homozygous fall from January 4 to May 1, 2024, and 136 were heterozygotes from January 4 to April 8, 2024 (**Table 3**). Spring Chinook were sampled in the highest proportions (25.5% and 22.2%) during the second and third weeks of the study (January 7 to 20, 2024). Fall Chinook were sampled each week, but the weekly proportion increased from 45.0% at the start of the study (January 4, 2024) to 100% by the end of the study (May 3, 2024), indicating run-type differences in spring and fall fry outmigration timing. Like spring Chinook, the highest proportion of heterozygous run-types (51.0% and 59.3%) occurred during the second and third weeks of the study.

Table 3. Sampled proportions and number of tissue samples (n) of homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes from the Skookumchuck River fry traps, 2024.

Start	End	Week	Spring	Fall	Heterozygote	n
12/25/2023	12/31/2023	0	NA	NA	NA	NA
1/1/2024	1/6/2024	1	0.150	0.450	0.400	20
1/7/2024	1/13/2024	2	0.255	0.235	0.510	51
1/14/2024	1/20/2024	3	0.222	0.185	0.593	27
1/21/2024	1/27/2024	4	0.116	0.488	0.395	43
1/28/2024	2/3/2024	5	0.158	0.447	0.395	38
2/4/2024	2/10/2024	6	0.060	0.520	0.420	50
2/11/2024	2/17/2024	7	0.071	0.714	0.214	28
2/18/2024	2/24/2024	8	0.028	0.611	0.361	36
2/25/2024	3/2/2024	9	0.033	0.867	0.100	30
3/3/2024	3/9/2024	10	0.000	0.894	0.106	47
3/10/2024	3/16/2024	11	0.000	0.955	0.045	44
3/17/2024	3/23/2024	12	0.000	0.959	0.041	49
3/24/2024	3/30/2024	13	0.000	1.000	0.000	45
3/31/2024	4/6/2024	14	0.000	0.977	0.023	44
4/7/2024	4/13/2024	15	0.000	0.979	0.021	47
4/14/2024	4/20/2024	16	0.000	1.000	0.000	34
4/21/2024	4/27/2024	17	0.000	1.000	0.000	46
4/28/2024	5/4/2024	18	0.000	1.000	0.000	5
5/5/2024	5/11/2024	19	NA	NA	NA	NA

Newaukum

Genetic samples with run timing assignments were collected between January 17 and April 10, 2024 in the Newaukum. A total of 47 Chinook fry samples were homozygous spring from January 8 to

March 12, 2024, 145 samples were homozygous fall from January 17 to April 10, 2024, and 128 were heterozygotes from January 17 to April 1, 2024 (**Table 4**). Spring Chinook were sampled in the highest proportions (6.4% to 100%) during weeks 3 through 7 of the study (January 14 to February 17, 2024) but were not detected after March 12, 2024. This was nearly 3 weeks later than in the Skookumchuck. The proportion of fall Chinook increased from 0% at the start of the study (January 4, 2024) to 100% by the conclusion of the study (April 10, 2024), indicating run-type difference in fry outmigration timing. The highest proportion of heterozygous run-types (66.0%) occurred in week 5 of the study (late January/early February) but were absent by the last week of the study (April 10, 2024).

Table 4. Sampled proportions and number of tissue samples (n) of homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes from the Newaukum River fry traps, 2024.

Start	End	Week	Spring	Fall	Heterozygote	n
1/1/2024	1/6/2024	1	0.000	0.000	0.000	0
1/7/2024	1/13/2024	2	0.000	0.000	0.000	0
1/14/2024	1/20/2024	3	0.250	0.500	0.250	4
1/21/2024	1/27/2024	4	1.000	0.000	0.000	2
1/28/2024	2/3/2024	5	0.064	0.277	0.660	47
2/4/2024	2/10/2024	6	0.205	0.250	0.545	44
2/11/2024	2/17/2024	7	0.316	0.263	0.421	38
2/18/2024	2/24/2024	8	0.111	0.489	0.400	45
2/25/2024	3/2/2024	9	0.172	0.431	0.397	58
3/3/2024	3/9/2024	10	0.097	0.677	0.226	31
3/10/2024	3/16/2024	11	0.056	0.750	0.194	36
3/17/2024	3/23/2024	12	0.000	1.000	0.000	10
3/24/2024	3/30/2024	13	0.000	1.000	0.000	2
3/31/2024	4/6/2024	14	0.000	0.500	0.500	2
4/7/2024	4/13/2024	15	0.000	1.000	0.000	1
4/14/2024	4/20/2024	16	NA	NA	NA	NA

Fry Genotype Proportions (Mark-Recapture)

Median estimated abundance by week and genotype proportions by week were used to determine the contribution of homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes to overall fry abundance outmigrating from the Skookumchuck and Newaukum rivers upstream of the traps in 2024.

Skookumchuck

A total of 20,136 Chinook fry were captured between January 4 and May 3, 2024 (**Table 5**). Of those, 4,606 were marked and released upstream and 194 recaptured. For BTSPAS estimates, a

diagonal model was used as all recaptures were assumed to occur within the same week as release. Model arguments were as follows: number of chains = 4, iterations = 50,000, burn-in = 25,000, simulations = 12,500, and thin rate of 2. Modeled weekly efficiencies ranged from 1.8% to 19.3%. Abundance of Chinook fry outmigrants was estimated to be 839,232 (95% confidence intervals = 705,366 to 1,018,590) with a coefficient of variance (CV) of 9.5% (**Figure 6**). The *Rhat* value was 1.001 and effective sample size 20,000, indicating good model fit.

Table 5. Mark-recapture data for Chinook fry in the Skookumchuck River organized by week. Dataset includes total marks released (Total Mark), total marks recaptured (Total Recap), total maiden captures (Total Captures), and the proportion of time fished during the period (Prop Fished). Note that weeks were added to the beginning and end of the dataset to estimate the tails of the run.

Start	End	Week	Total Mark	Total Recap	Total Capture	Prop Fished
12/25/2023	12/31/2023	0	NA	NA	0	0.001
1/1/2024	1/6/2024	1	0	0	255	0.26
1/7/2024	1/13/2024	2	100 ^a	9	489	0.40
1/14/2024	1/20/2024	3	295 ^b	26	1,019	0.55
1/21/2024	1/27/2024	4	0	0	524	0.46
1/28/2024	2/3/2024	5	300	6	1,623	0.69
2/4/2024	2/10/2024	6	300	15	1,887	0.70
2/11/2024	2/17/2024	7	400	8	1,817	0.71
2/18/2024	2/24/2024	8	600	37	5,784	0.70
2/25/2024	3/2/2024	9	200	12	2,613	0.40
3/3/2024	3/9/2024	10	534	10	784	0.67
3/10/2024	3/16/2024	11	600	10	1,283	0.71
3/17/2024	3/23/2024	12	496	26	916	0.71
3/24/2024	3/30/2024	13	173	5 ^c	237	0.71
3/31/2024	4/6/2024	14	385	13	532	0.68
4/7/2024	4/13/2024	15	129	9	176	0.68
4/14/2024	4/20/2024	16	42	2	119	0.70
4/21/2024	4/27/2024	17	47	6	67	0.69
4/28/2024	5/4/2024	18	5	0	11	0.69
5/5/2024	5/11/2024	19	NA	NA	0	0.001

^aTraps were raised the following day of release for 100 marked fish; 9 were recaptured.

^bTraps were raised the following day of release for 99 of these marked fish.

^c1 of these individuals was captured the following week.

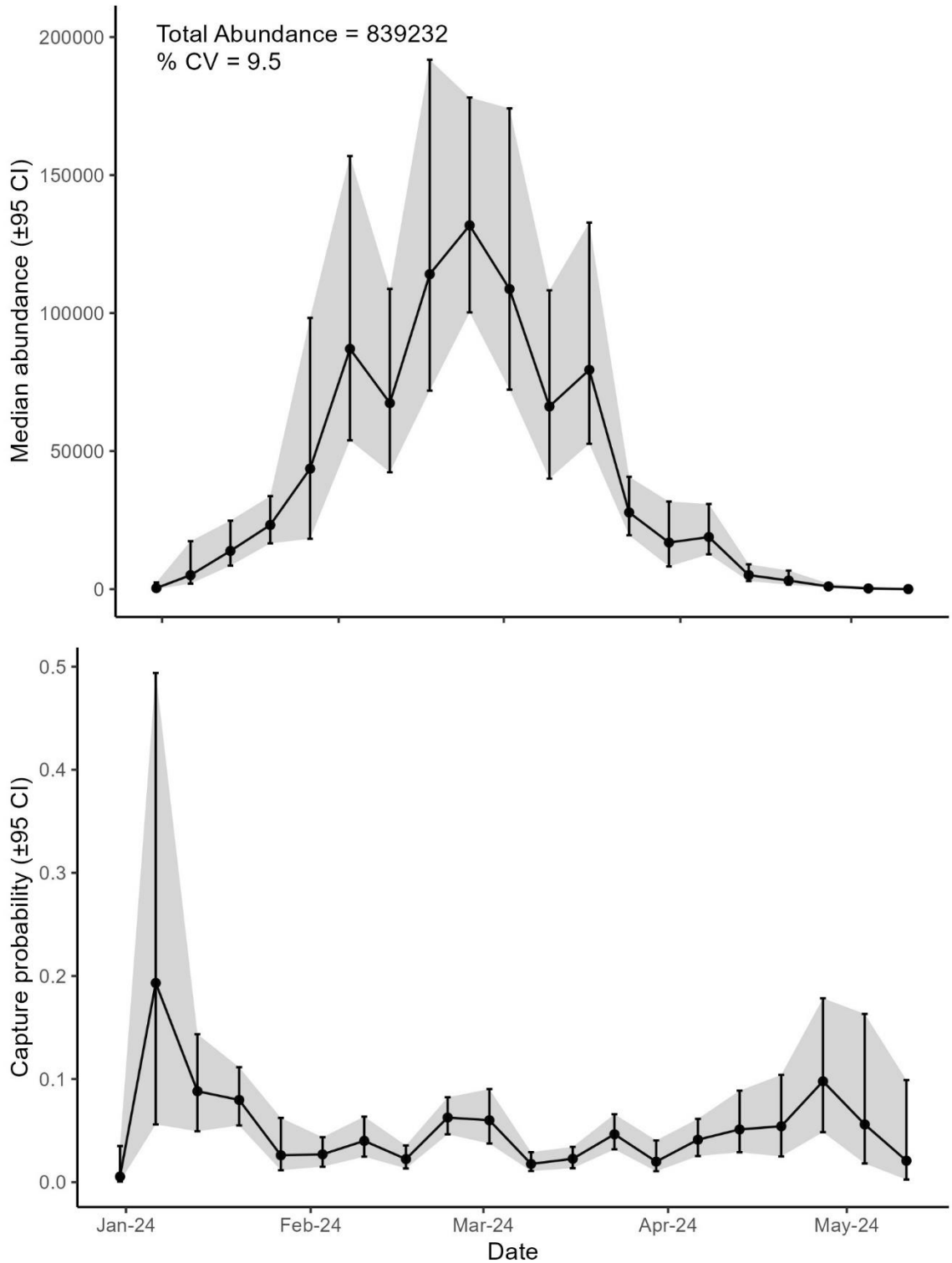


Figure 6. Number of Chinook fry (top panel) and trap efficiency (bottom panel) by week for fish produced upstream of the Skookumchuck fry traps, 2024. The total abundance estimate is 839,232 fry with a CV of 9.5%. Error bars and shading around point estimates represent 95% confidence intervals.

When genotypes were expanded by weekly modeled abundance, spring Chinook accounted for 5.9% (n = 47,806) of the fry outmigration upstream of the fry traps in the Skookumchuck River in 2024 (Table 6 and Figure 7). If proportions were not expanded by weekly modeled abundance, the estimated proportion of spring Chinook would be similar at 5.8% based solely on genetic samples. Fall Chinook accounted for the highest proportion of fry outmigrants at 69.8% (n = 567,894), and heterozygous run-types accounted for 24.3% (n = 198,162) of the run.

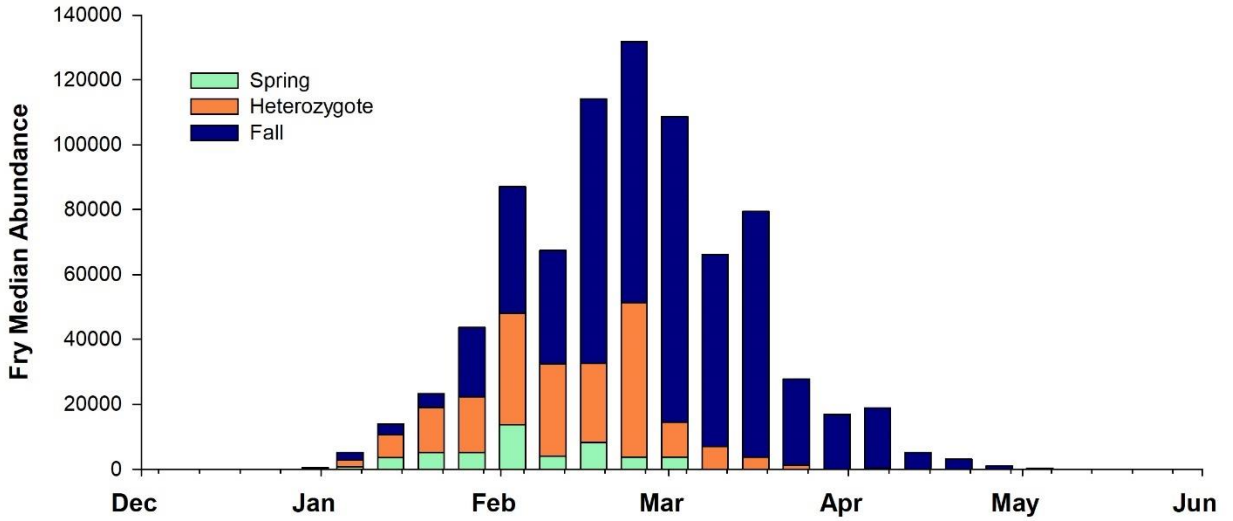


Figure 7. Weekly abundance and genetic run-type assignments of Chinook fry migrating past the Skookumchuck River fry traps, 2024.

Table 6. Estimated weekly and total proportions of Chinook fry genotypes passing the Skookumchuck River traps, 2024. Table includes weekly number (n) and proportions (prop) of homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes. Total genotype proportions are the weekly proportions weighted by weekly abundance. Total abundance differs from the sum of weekly totals because each estimate is based on median values of the model outcome and therefore not additive.

Start	End	Week	Total	Spring		Fall		Heterozygote	
			n	n	prop	n	prop	N	prop
12/25/2023	12/31/2023	0	381	57	0.150	171	0.450	152	0.400
1/1/2024	1/6/2024	1	5,064	760	0.150	2,279	0.450	2,026	0.400
1/7/2024	1/13/2024	2	13,841	3,528	0.255	3,527	0.235	7,056	0.510
1/14/2024	1/20/2024	3	23,246	5,166	0.222	4,305	0.185	13,775	0.593
1/21/2024	1/27/2024	4	43,638	5,074	0.116	21,312	0.488	17,252	0.395
1/28/2024	2/3/2024	5	87,037	13,743	0.158	38,938	0.447	34,357	0.395
2/4/2024	2/10/2024	6	67,409	4,045	0.060	35,053	0.520	28,312	0.420
2/11/2024	2/17/2024	7	114,093	8,149	0.071	81,495	0.710	24,448	0.214
2/18/2024	2/24/2024	8	131,782	3,661	0.028	80,534	0.611	47,588	0.361
2/25/2024	3/2/2024	9	108,720	3,624	0.333	94,224	0.867	10,872	0.100
3/3/2024	3/9/2024	10	66,185	0	0.000	59,144	0.894	7,041	0.106
3/10/2024	3/16/2024	11	79,432	0	0.000	75,822	0.955	3,611	0.046
3/17/2024	3/23/2024	12	27,787	0	0.000	26,653	0.959	1,134	0.041
3/24/2024	3/30/2024	13	16,879	0	0.000	16,879	1.000	0	0.000
3/31/2024	4/6/2024	14	18,883	0	0.000	18,454	0.977	429	0.023
4/7/2024	4/13/2024	15	5,093	0	0.000	4,985	0.979	108	0.021
4/14/2024	4/20/2024	16	3,112	0	0.000	3,112	1.000	0	0.000
4/21/2024	4/27/2024	17	987	0	0.000	987	1.000	0	0.000
4/28/2024	5/4/2024	18	264	0	0.000	264	1.000	0	0.000
5/5/2024	5/11/2024	19	28	0	0.150	28	0.450	0	0.400
		Total	839,232	47,806	0.059	567,894	0.698	189,162	0.243
		%			5.9%		69.8%		24.3%

Newaukum

A total of 791 Chinook fry were captured between January 4 and April 12, 2024 (**Table 7**). Of those, 433 were marked and released upstream and 11 recaptured. For BTSPAS estimates, a diagonal model was used as all recaptures were assumed to occur within the same week as release. Model arguments were as follows: number of chains = 4, iterations = 200,000, burn-in = 100,000, simulations = 50,000, and thin rate of 2. Modeled weekly efficiencies ranged from 2.3% to 3.1%. Abundance of Chinook fry outmigrants was estimated to be 54,745 (95% confidence intervals = 31,422 to 106,464) with a coefficient of variance (CV) of 34.0% (**Figure 8**). The *Rhat* value was 1.002 and effective sample size 4,300, indicating good model fit.

Table 7. Mark-recapture data for Chinook fry in the Newaukum River organized by week. Dataset includes total marks released (Total Mark), total marks recaptured (Total Recap), total maiden captures (Total Captures), and the proportion of time fished during the period (Prop Fished). Note that one week was added to the end of the dataset to estimate the tail of the run.

Start	End	Week	Total Mark	Total Recap	Total Capture	Prop Fished
1/1/2024	1/6/2024	1	0	0	0	0.13
1/7/2024	1/13/2024	2	0	0	1	0.13
1/14/2024	1/20/2024	3	0	0	5	0.41
1/21/2024	1/27/2024	4	0	0	3	0.30
1/28/2024	2/3/2024	5	17	0	57	0.69
2/4/2024	2/10/2024	6	46	1	71	0.70
2/11/2024	2/17/2024	7	131	4	155	0.72
2/18/2024	2/24/2024	8	13	0	96	0.68
2/25/2024	3/2/2024	9	36	1	82	0.39
3/3/2024	3/9/2024	10	51	2	85	0.69
3/10/2024	3/16/2024	11	78	2	146	0.69
3/17/2024	3/23/2024	12	52	1	71	0.69
3/24/2024	3/30/2024	13	1	0	10	0.71
3/31/2024	4/6/2024	14	8	0	8	0.68
4/7/2024	4/13/2024	15	0	0	1	0.68
4/14/2024	4/20/2024	16	1	NA	0	0.001

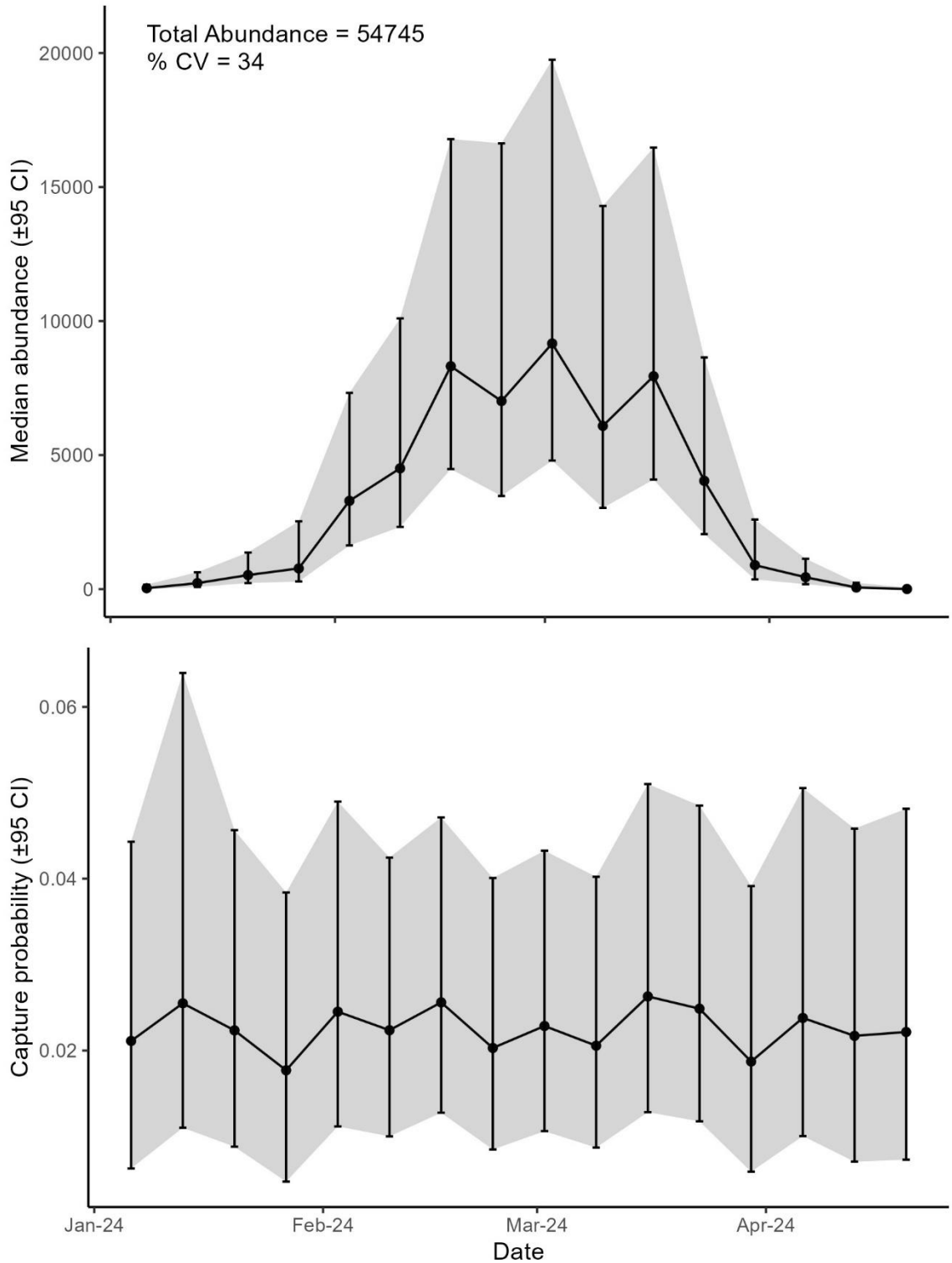


Figure 8. Number of Chinook fry (top panel) and trap efficiency (bottom panel) by week for fish produced upstream of the Newaukum fry traps, 2024. The total abundance estimate is 54,745 with a CV of 34.0%. Error bars and shading around point estimates represent 95% confidence intervals.

When genotypes were expanded by weekly modeled abundance, spring Chinook accounted for 15.2% ($n = 8,118$) of the fry outmigration upstream of the fry traps in the Newaukum River in 2024 (Table 8 and Figure 9). If proportions were not expanded by weekly modeled abundance, the estimated proportion of spring Chinook would be slightly lower at 14.7% based solely on genetic samples. Fall Chinook accounted for the highest proportion of fry outmigrants at 51.2% ($n = 27,307$), and heterozygous run-types accounted for 33.6% ($n = 17,911$) of the run.

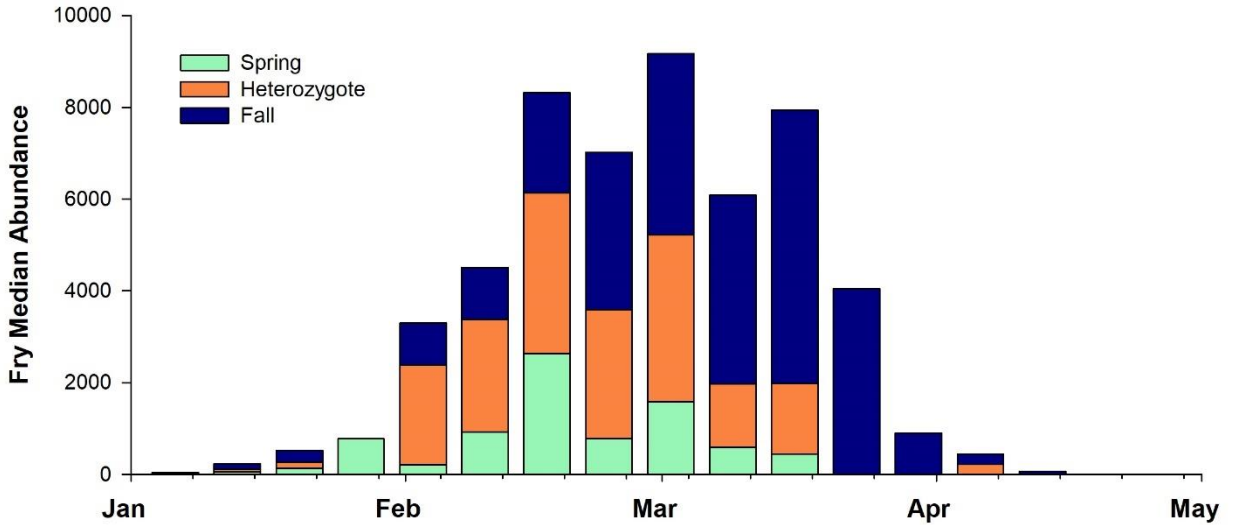


Figure 9. Weekly abundance and genetic run-type assignments of Chinook fry migrating past the Newaukum traps, 2024.

Table 8. Estimated weekly and total proportions of Chinook fry genotypes passing the Newaukum River traps, 2024. Table includes weekly number (n) and proportions (prop) of homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes. Total genotype proportions are the weekly proportions weighted by weekly abundance. Total abundance differs from the sum of weekly totals because each estimate is based on median values of the model outcome and therefore not additive.

Start	End	Week	Total	Spring		Fall		Heterozygote	
			n	n	prop	n	prop	n	prop
1/1/2024	1/6/2024	1	35	9	0.250	18	0.500	9	0.250
1/7/2024	1/13/2024	2	224	56	0.250	112	0.500	56	0.250
1/14/2024	1/20/2024	3	525	131	0.250	263	0.500	131	0.250
1/21/2024	1/27/2024	4	773	773	1.000	0	0.000	0	0.000
1/28/2024	2/3/2024	5	3,294	210	0.064	911	0.277	2,173	0.660
2/4/2024	2/10/2024	6	4,507	922	0.205	1,127	0.250	2,458	0.546
2/11/2024	2/17/2024	7	8,316	2,626	0.316	2,188	0.263	3,501	0.421
2/18/2024	2/24/2024	8	7,016	780	0.111	3,430	0.489	2,806	0.400
2/25/2024	3/2/2024	9	9,165	1,580	0.172	3,950	0.431	3,634	0.397
3/3/2024	3/9/2024	10	6,089	589	0.097	4,125	0.677	1,375	0.226
3/10/2024	3/16/2024	11	7,943	441	0.056	5,957	0.750	1,544	0.194
3/17/2024	3/23/2024	12	4,041	0	0.000	4,041	1.000	0	0.000
3/24/2024	3/30/2024	13	896	0	0.000	896	1.000	0	0.000
3/31/2024	4/6/2024	14	444	0	0.000	222	0.500	222	0.500
4/7/2024	4/13/2024	15	61	0	0.000	61	1.000	0	0.000
4/14/2024	4/20/2024	16	6	0	0.000	6	1.000	0	0.000
Total			54,745	8,118	0.152	27,307	0.512	17,911	0.336
%					15.2%		51.2%		33.6%

Fry Genotype Proportions (CPUE Method)

In the Skookumchuck River, weekly catch per unit effort ranged from 0.05 to 24.67 fry per hour (**Table 9**). Peak CPUE values occurred in the two weeks between February 18 and March 2, 2024.

In the Newaukum River, weekly catch per unit effort ranged from 0 to 0.64 fry per hour. There did not appear to be a defined peak in CPUE values, and the maximum value of 0.64 fry/hour was calculated for week 7 (February 11 to 17, 2024).

Table 9. Weekly catch, effort (hours fished), catch-per-unit-effort (CPUE), and scaled CPUE of Chinook fry passing the Skookumchuck and Newaukum river fry traps, 2024. Scaled CPUE values sum to a total value of 1 across all weeks.

Start	End	Week	Skookumchuck				Newaukum			
			Catch	Hours Fished*	CPUE	CPUE (scale)	Catch	Hours Fished*	CPUE	CPUE (scale)
12/25/2023	12/31/2023	0	---	---	---	---	---	---	---	---
1/1/2024	1/6/2024	1	255	87.2	2.92	0.029	0	21.2	0.00	0.0000
1/7/2024	1/13/2024	2	489	134.1	3.65	0.036	1	42.5	0.02	0.0063
1/14/2024	1/20/2024	3	1,019	183.7	5.55	0.056	5	138.7	0.04	0.0097
1/21/2024	1/27/2024	4	524	155.0	3.38	0.034	3	100.8	0.03	0.0080
1/28/2024	2/3/2024	5	1,623	230.2	7.05	0.071	57	232.8	0.24	0.0660
2/4/2024	2/10/2024	6	1,887	235.4	8.02	0.080	71	236.2	0.30	0.0810
2/11/2024	2/17/2024	7	1,817	237.6	7.65	0.077	155	241.2	0.64	0.1731
2/18/2024	2/24/2024	8	5,784	234.5	24.67	0.247	96	228.4	0.42	0.1132
2/25/2024	3/2/2024	9	2,613	134.3	19.46	0.195	82	130.2	0.63	0.1697
3/3/2024	3/9/2024	10	784	225.4	3.48	0.035	85	230.3	0.37	0.0994
3/10/2024	3/16/2024	11	1,283	238.0	5.39	0.054	146	232.7	0.63	0.1690
3/17/2024	3/23/2024	12	916	238.2	3.85	0.039	71	231.9	0.31	0.0825
3/24/2024	3/30/2024	13	237	239.3	0.99	0.010	10	238.0	0.04	0.0113
3/31/2024	4/6/2024	14	532	229.8	2.32	0.023	8	228.9	0.03	0.0094
4/7/2024	4/13/2024	15	176	229.6	0.77	0.008	1	229.4	0.00	0.0012
4/14/2024	4/20/2024	16	119	235.6	0.51	0.005	---	---	---	---
4/21/2024	4/27/2024	17	67	233.4	0.29	0.003	---	---	---	---
4/28/2024	5/4/2024	18	11	231.7	0.05	0.001	---	---	---	---
5/5/2024	5/11/2024	19	---	---	---	---	---	---	---	---

*Maximum possible hours fished per week is 336 hours (168 hours/week * 2 traps)

In the Skookumchuck River, when genotypes were expanded by the CPUE method, spring Chinook accounted for 6.6% of the fry outmigration upstream of the fry traps (**Table 10**). Fall Chinook accounted for the highest proportion of fry outmigrants at 66.3%, and heterozygotes accounted for 27.2% of the fry outmigrants.

In the Newaukum River, when genotypes were expanded by the CPUE method, spring Chinook accounted for 14.9% of the fry outmigration upstream of the fry traps (**Table 10**). Fall Chinook accounted for the highest proportion of fry outmigrants at 50.8%, and heterozygotes accounted for 33.8% of the fry outmigrants.

Table 10. Estimated weekly and total proportions of each Chinook genotype passing the Skookumchuck and Newaukum river fry traps based on ‘catch-per-unit-effort’ method, 2024.

Start	End	Week	Skookumchuck			Newaukum		
			Spring	Fall	Heterozygote	Spring	Fall	Heterozygote
12/25/2023	12/31/2023	0	---	---	---	---	---	---
1/1/2024	1/6/2024	1	0.0044	0.0132	0.0117	0.0000	0.0000	0.0000
1/7/2024	1/13/2024	2	0.0105	0.0073	0.0186	0.0000	0.0000	0.0000
1/14/2024	1/20/2024	3	0.0123	0.0103	0.0329	0.0024	0.0049	0.0024
1/21/2024	1/27/2024	4	0.0039	0.0165	0.0134	0.0080	0.0000	0.0000
1/28/2024	2/3/2024	5	0.0111	0.0316	0.0278	0.0042	0.0182	0.0435
2/4/2024	2/10/2024	6	0.0048	0.0417	0.0337	0.0166	0.0202	0.0442
2/11/2024	2/17/2024	7	0.0055	0.0546	0.0164	0.0547	0.0456	0.0729
2/18/2024	2/24/2024	8	0.0069	0.1508	0.0891	0.0126	0.0554	0.0453
2/25/2024	3/2/2024	9	0.0065	0.1687	0.0195	0.0312	0.0693	0.0693
3/3/2024	3/9/2024	10	0.0000	0.0311	0.0037	0.0096	0.0674	0.0225
3/10/2024	3/16/2024	11	0.0000	0.0515	0.0025	0.0094	0.1268	0.0329
3/17/2024	3/23/2024	12	0.0000	0.0369	0.0016	0.0000	0.0825	0.0000
3/24/2024	3/30/2024	13	0.0000	0.0099	0.0000	0.0000	0.0113	0.0000
3/31/2024	4/6/2024	14	0.0000	0.0226	0.0005	0.0000	0.0047	0.0047
4/7/2024	4/13/2024	15	0.0000	0.0075	0.0002	0.0000	0.0012	0.0000
4/14/2024	4/20/2024	16	0.0000	0.0051	0.0000	---	---	---
4/21/2024	4/27/2024	17	0.0000	0.0029	0.0000	---	---	---
4/28/2024	5/4/2024	18	0.0000	0.0005	0.0000	---	---	---
5/5/2024	5/11/2024	19	---	---	---	---	---	---
Total Proportion			0.0659	0.6626	0.2715	0.1487	0.5074	0.3376
%			6.6%	66.3%	27.2%	14.9%	50.8%	33.8%

Fry Genotype Proportions (Stream Method)

See **Appendix D** for breakdown of numbers used to calculate weekly genotype proportions based on the stream method.

In the Skookumchuck River, when genotypes were expanded by the ‘stream method’, spring Chinook accounted for 6.6% of the fry outmigration upstream of the fry traps (**Table 11**). Fall Chinook accounted for the highest proportion of fry outmigrants at 66.2%, and heterozygotes accounted for 27.2% of the fry outmigrants.

In the Newaukum River, when genotypes were expanded by the ‘stream method’, spring Chinook accounted for 15.0% of the fry outmigration upstream of the fry traps (**Table 11**). Fall Chinook accounted for the highest proportion of fry outmigrants at 50.9%, and heterozygotes accounted for 43.1% of the fry outmigrants.

Table 11. Estimated weekly and total proportions of each Chinook genotype passing the Skookumchuck and Newaukum river fry traps based on ‘stream’ method, 2024.

Start	End	Week	Skookumchuck			Newaukum			
			Spring	Fall	Heterozygote	Spring	Fall	Heterozygote	
12/25/2023	12/31/2023	0	---	---	---	---	---	---	
1/1/2024	1/6/2024	1	0.150	0.450	0.400	0.000	0.000	0.000	
1/7/2024	1/13/2024	2	0.289	0.200	0.511	0.000	0.000	0.000	
1/14/2024	1/20/2024	3	0.222	0.185	0.593	0.250	0.500	0.250	
1/21/2024	1/27/2024	4	0.116	0.488	0.395	0.000	0.000	0.000	
1/28/2024	2/3/2024	5	0.158	0.447	0.395	0.064	0.277	0.660	
2/4/2024	2/10/2024	6	0.060	0.520	0.420	0.205	0.250	0.545	
2/11/2024	2/17/2024	7	0.071	0.714	0.214	0.316	0.263	0.421	
2/18/2024	2/24/2024	8	0.028	0.611	0.361	0.111	0.489	0.400	
2/25/2024	3/2/2024	9	0.033	0.867	0.100	0.184	0.408	0.408	
3/3/2024	3/9/2024	10	0.000	0.894	0.106	0.097	0.677	0.226	
3/10/2024	3/16/2024	11	0.000	0.955	0.046	0.056	0.750	0.194	
3/17/2024	3/23/2024	12	0.000	0.959	0.041	0.000	1.000	0.000	
3/24/2024	3/30/2024	13	0.000	1.000	0.000	0.000	1.000	0.000	
3/31/2024	4/6/2024	14	0.000	0.977	0.023	0.000	0.500	0.500	
4/7/2024	4/13/2024	15	0.000	0.979	0.021	0.000	1.000	0.000	
4/14/2024	4/20/2024	16	0.000	1.000	0.000	---	---	---	
4/21/2024	4/27/2024	17	0.000	1.000	0.000	---	---	---	
4/28/2024	5/4/2024	18	0.000	1.000	0.000	---	---	---	
5/5/2024	5/11/2024	19	---	---	---	---	---	---	
Total Proportion			0.066	0.6624	0.272	0.150	0.509	0.341	
			%	6.6%	66.2%	27.2%	15.0%	50.9%	34.1%

Comparison of Methods to Estimate Genotype Proportions

Estimates of fry genotype proportions were quite similar among the three analysis methods (Table 12). In the Skookumchuck River, estimates ranged from 5.9 – 6.6% for spring Chinook, 66.3 – 69.8% for fall Chinook, and 24.3 – 27.2% for heterozygous Chinook. In the Newaukum River, genotype proportions ranged from 14.9 – 15.2% for spring Chinook, 50.8 – 51.2% for fall Chinook, and 33.6 – 34.1% for heterozygous Chinook.

Table 12. Estimated genotype proportions of Chinook fry based on three analysis methods for the Skookumchuck and Newaukum rivers, 2024.

Location	Method	Spring	Fall	Heterozygote
Skookumchuck	Mark-Recapture	0.059	0.698	0.243
	CPUE	0.066	0.663	0.272
	Stream Method	0.066	0.662	0.272
Newaukum	Mark-Recapture	0.152	0.512	0.336
	CPUE	0.149	0.508	0.338
	Stream Method	0.150	0.509	0.341

Inbreeding Coefficient

F_{IS} estimates for both rivers were positive (**Table 13**), indicating a heterozygote deficiency. Conditions of HWE were likely met for all but random mating; the populations were large enough genetic drift would be unlikely to have a significant impact on allele frequencies, mutation rates are very small for SNP loci, we already assume the population is closed, and we are assuming that there is no selection on run-timing alleles once spawners reach the spawning grounds. If true, the heterozygote deficiency would indicate that some, but not complete, spawning segregation exists between spring and fall Chinook. The F_{IS} value for the Newaukum was higher than the Skookumchuck, which may indicate more spawning segregation among Chinook salmon spawners in the Newaukum River than the Skookumchuck River. However, these results should be viewed cautiously because point estimates for F_{IS} currently lack a measure of uncertainty (i.e., confidence intervals), and we are not yet certain whether all conditions of HWE, other than random mating, were met.

Table 13. Observed and expected genotype frequencies of Chinook salmon emergent fry in the Skookumchuck and Newaukum rivers, 2024. Expected genotype frequencies were calculated under Hardy-Weinberg Equilibrium. Inbreeding coefficient (F_{IS}) values greater than 1 correspond to spawning segregation among genotypes (i.e., greater deficiency of heterozygotes than would be expected if random mating occurred).

Location	Genotype Frequency (Observed)			Genotype Frequency (Expected)			FIS
	Spring	Fall	Heterozygote	Spring	Fall	Heterozygote	
Skookumchuck	0.059	0.698	0.243	0.033	0.672	0.296	0.179
Newaukum	0.152	0.512	0.336	0.102	0.462	0.435	0.228

Discussion

In 2024, the homozygous spring genotype was the least frequent genotype among emergent Chinook salmon fry in the Skookumchuck and Newaukum rivers, the homozygous fall genotype was the highest frequency, and heterozygotes were intermediate in frequency in both rivers. The proportion of homozygous spring fry in the Skookumchuck River (5.9%) was approximately one third the proportion of homozygous spring fry in the Newaukum River (15.2%). The lower proportion of homozygous spring fry in the Skookumchuck River than the Newaukum River is consistent with, although does not prove, the hypothesis that altered flow and habitat loss may limit production of the homozygous spring genotype in the Skookumchuck River. Results from the 2024 trapping season were consistent with fry monitoring in both rivers between 2020 and 2023 (Gilbertson 2023; Zimmerman et al. 2023).

Genotype proportions estimated for emergent fry offspring can be contrasted with run-type proportions estimated for spawners the previous fall. Spawner surveys of Chinook salmon spawners occur between September and November, and abundances of the spring and fall run-types are derived from visual observation of redd counts, redd timing, and fish condition (Ashcraft et al. 2017). On the Skookumchuck River, spawner survey results from fall 2023 reported that spring Chinook were 40.7% of all Chinook spawners upstream of the fry trap (M. Scharpf, Washington Dept Fish & Wildlife, personal communication). This proportion is substantially greater than the 5.9% emergent fry that were spring homozygotes and the 30.2% emergent fry that were either spring homozygotes or heterozygotes. In the Newaukum River, spawner survey results from fall 2023 reported that spring Chinook were 54.5% of all Chinook spawners upstream of the fry trap (L. Ronne, Washington Dept Fish & Wildlife, personal communication). This proportion is greater than the 15.2% emergent fry that were spring homozygote but close in value to the 48.8% of emergent fry that were either spring homozygotes or heterozygote. The difference in proportions between emergent fry genotypes and adult spawner run types may be due to methodology, differential survival during egg incubation, or some combination of both. For example, visual surveys categorized Chinook salmon spawners as “spring run” or “fall run” but cannot identify genotypes, especially heterozygotes. Even if the genotypes were known at the spawning life stage, the genotype composition of spawners and emergent fry may differ if egg-to-fry survival differs between early (spring) versus late (fall) spawners. Chinook salmon spawn over a 3 to 4 month period, during which time scouring flow events can impact egg-to-fry survival (Devries 1997; Montgomery et al. 1996). If scouring flow events occur early in the spawning season (e.g., early November), egg-to-fry survival of the early (spring) spawners may be lower than the late (fall) spawners, resulting in a lower proportion of the spring genotype in emergent fry offspring than the adult spawner parents. However, if scouring flow events occur after most spawning has been completed (e.g., late December), egg-to-fry survival of early and late spawners may be more similar, resulting in similar genotype proportions between emergent fry offspring and adult spawner parents.

Stream Flow Targets and Adult Migration

In 2023, fall flows in the lower Skookumchuck River met the target discharge of 35 – 40 cfs for the majority of time between August 15 and October 15. Meeting this target was due to in-season coordination with Transalta, who manage flow at the Stream-Electric Power Plant located at river mile 7.2, and US Geological Survey, who maintain the river flow gage at river mile 6.2.

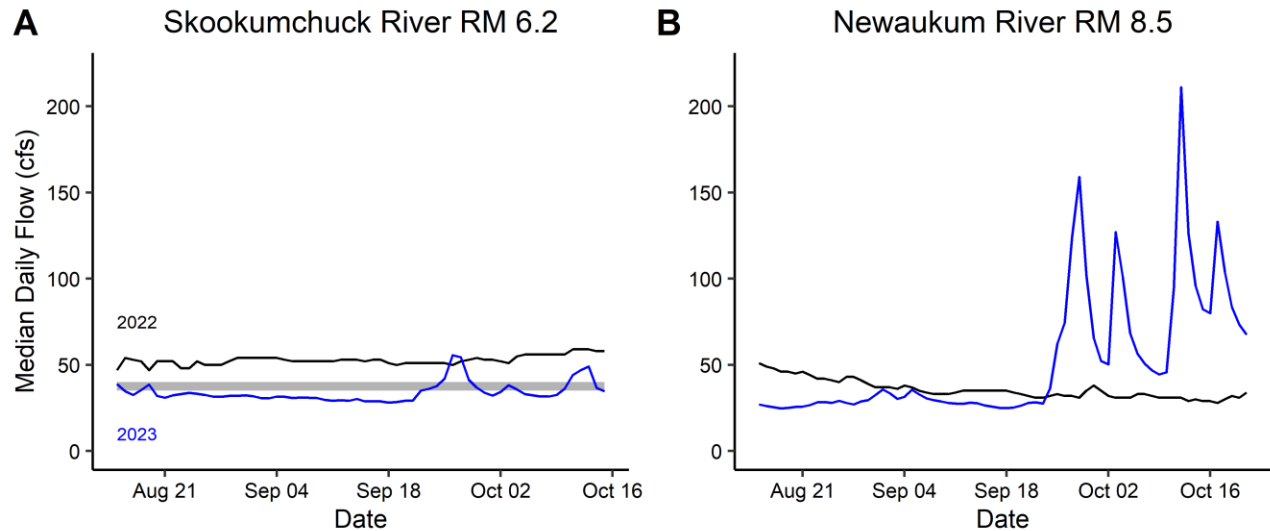


Figure 10. Stream flows in the Skookumchuck River (A) and Newaukum River (B) between June 15th and October 15th. Graphs show mean daily flow in 2022 (black) and 2023 (blue). Target flow in the Skookumchuck River was 35 to 40 cfs (horizontal gray bar). Flows were measured at the USGS Bucoda Gage #12026400 and Newaukum River Gage #12025000.

According to sonar monitoring between August 15 and October 15, 2023, salmon (presumptive Chinook) migration into the Skookumchuck River was concentrated around rain events and there was minimal upstream migration on other days (West Fork Environmental 2023). Migration pulses during rain events are expected behavior in free-flowing rivers, and therefore the migrating adult salmon displayed the typical adult migration behavior during this period.

Comparisons among Fry Analysis Methods

Similar to 2023, we found consistent results among the mark-recapture, stream method, and CPUE analyses (**Table 12**). Although mark-recapture provides a more robust statistical result, the stream method and CPUE method are the only data sets available for the 2020-2022 outmigration years. As a result, the ability to compile time series data to evaluate change over time depends on our confidence in the proportions estimated with the stream method and CPUE method. We recommend the continuation of all three methods in future years.

For the two years when mark-recapture data of Chinook emergent fry were obtained (2023 and 2024), the quality of result differed between the Skookumchuck and Newaukum rivers. Mark-recapture estimates in the Skookumchuck were robust with a well-defined outmigration curve and high precision of the estimate (<10% coefficient of variation). In comparison, mark-recapture estimates in the Newaukum were weak, based on low catch numbers, few recaptures, and large confidence intervals without the defined outmigration curve. Of note, the lack of a defined migration curve is typical of fry migrations in low abundance populations, as observed in other trapping efforts in western Washington (Zimmerman, personal observation). Production of Chinook emergent fry above the Newaukum trap were an order of magnitude less than emergent fry production upstream of the Skookumchuck trap in both 2024 (this report) and 2023 (Zimmerman et al. 2023); as a result, these low abundances may continue to frustrate efforts to solidify a precise estimate.

In an effort to improve catch numbers and abundance estimates, the Newaukum trap was moved to RM 1.5 in 2024, seven miles downstream from the previous years' location at RM 8.5 (2020-2023). This change

encompassed more of the Chinook spawning distribution upstream of the trap in fall 2023 (corresponding to the 2024 trapping season) than fall 2022 (corresponding to the 2023 fry trapping season). In fall 2023, 98.6% of Chinook spawning occurred upstream of the Newaukum trap at RM 1.5. This was a substantial increase from fall 2022 when 55.0% of Chinook spawning occurred upstream of the Newaukum trap at RM 8.5. Interestingly, in 2024, fry catch numbers remained low, the coefficient of variation high, and the change in trap location did not make the desired improvement to the quality of the estimate.

Comparison Among Rivers and Years

Chinook genotypes have been monitored in the Skookumchuck and Newaukum rivers for five years (2020 to 2024). Fry emergence timing can be approximated between the tails of the outmigration curve available for the two years where fry abundances were calculated (Zimmerman et al. 2023; Figure 6 and 8 this report). In those years, the majority of the fry emergence occurred between January 1 and May 15, although emergence appeared to begin later and end earlier in the Newaukum River where overall fry abundance is also lower. In order to compare fry genotype proportions at the population scale, trap operation must span the majority (ideally the entirety) of the fry migration period. This is especially important because the genotypes have different emergence timing and represent different parts of the outmigration curve (Zimmerman et al. 2023; Figure 7 and 9 this report). Trap operation timing in four of the five years (2021-2024) span this emergence timing but the 2020 trap operation commenced in mid-February and likely missed the initial portion of the Chinook emergence (**Appendix E**). For this reason, the 2020 data are not suitable for population-scale comparison of Chinook fry genotypes.

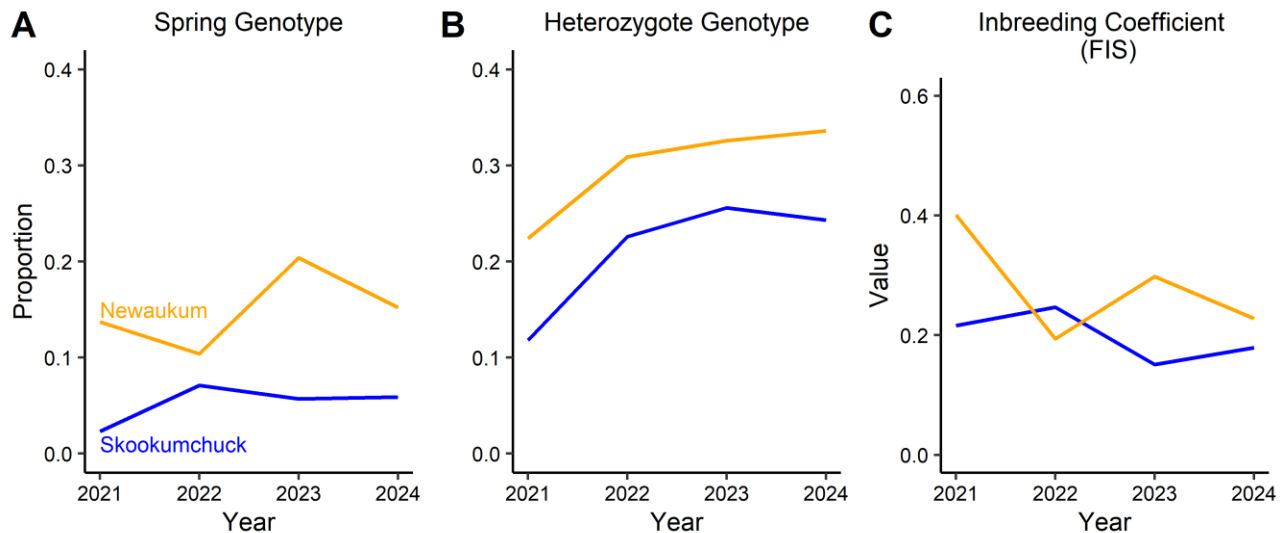


Figure 11. Proportion of spring (A) and heterozygote (B) genotypes and inbreeding coefficient (C) of Chinook salmon fry migrants in the Skookumchuck River (blue) and Newaukum River (orange), 2021 - 2024. Higher inbreeding coefficient value (C) corresponds to more segregation of genotypes (i.e., greater deficiency of heterozygotes than would be expected if random mating occurred).

Between 2021 and 2024, spring genotype proportions among Chinook emergent fry in the Skookumchuck River were consistently lower than the Newaukum River (**Figure 11**). Heterozygote genotype proportions were also consistently lower in the Skookumchuck than the Newaukum river, although this result may be due to the proportionally fewer spring Chinook available for hybridization in the Skookumchuck than the Newaukum river. F_{IS} was greater in the Newaukum than Skookumchuck in three of the four years, suggesting that the spring and fall components of the Chinook returns were more segregated in the

Newaukum River than the Skookumchuck River. However, these results should be viewed cautiously because point estimates for F_{IS} currently lack a measure of uncertainty (i.e., confidence intervals) and we are not yet certain all conditions of HWE, other than random mating, were met.

Chinook fry migrants were an order of magnitude more abundant in the Skookumchuck River than the Newaukum River in both years that fry abundance has been estimated (Zimmerman et al. 2023). The large difference in fry abundance between the rivers cannot be entirely explained by uncertainty in the Newaukum River estimate as there was no overlap in the 95% credible intervals of each abundance estimate. There are multiple factors that could cause such differences between the two rivers including spawner abundance, scouring effects of incubation flows, disease, predation, and available rearing habitat. While spawner abundances may be partially contributing to the differences in resulting fry abundances, spawners alone cannot explain the entirety of the difference between the two rivers. In 2022 and 2023, spawner abundance was 2-4 times higher in the Skookumchuck River than the Newaukum River (**Table 14**) whereas the difference in fry abundance was more than 10-fold.

When contrasting Chinook production in these two rivers, there are limited conclusions to be drawn from abundance of the fry stage alone. Fry migrants are the first of two outmigration pulses of subyearling Chinook salmon in western Washington. Yearling outmigrants are less commonly observed in western Washington and are not typical for the Chehalis River (Campbell et al. 2023; Campbell et al. 2017; West et al. 2021). However, the second outmigration pulse, consisting of subyearling Chinook salmon parr, can be substantial. For example, on the Newaukum River, the 2023 later-timed parr migrants monitored at the Washington Department of Fish & Wildlife rotary screw trap (RM 5.8; Olson et al. 2024) were two times more abundant ($n \sim 64,000$) than the early-timed fry migrants in the same year (Zimmerman et al. 2023). Monitoring studies in other rivers have demonstrated that the relative numbers of early-timed fry migrants and later-timed parr migrants can vary substantially from year to year, and interannual variability in abundance is typically greater for fry than parr (Anderson and Topping 2018; Lamperth et al. 2014; Zimmerman et al. 2015). Interannual variability in abundance is typically greater for fry than parr, and parr migrant abundance is hypothesized to be associated with available rearing habitat (Anderson and Topping 2018; Zimmerman et al. 2015). Therefore, we recommend monitoring of total juvenile Chinook production (i.e., fry and parr) for any future study designed to further investigate differences in fry abundance among rivers.

Table 14. Chinook salmon abundance by spawner run type and fry genotype upstream of fry trap locations in the Skookumchuck and Newaukum rivers. Data are abundance (proportion) correspond to spawning-outmigration years, 2022-23 and 2023-24. Spawner abundance is upstream of the trap.

	Spawners				Fry				Heterozygote
	Year	Total	Spring	Fall	Year	Total	Spring	Fall	
Skookumchuck	2022	1,112	435 (0.39)	677 (0.61)	2023	286,669	16,118 (0.06)	194,020 (0.69)	72,337 (0.25)
	2023	2,705	1,100 (0.41)	1,605 (0.59)	2024	839,232	47,806 (0.06)	567,894 (0.70)	189,162 (0.24)
Newaukum	2022	371	213 (0.57)	158 (0.43)	2023	33,785	6,748 (0.20)	15,528 (0.47)	10,786 (0.33)
	2023	688	375 (0.55)	313 (0.45)	2024	54,745	8,118 (0.15)	27,307 (0.51)	17,911 (0.34)

Difficulty Evaluating Flow Hypothesis

This study was designed to test the hypothesis that experimental reduction of spawning flows in the Skookumchuck River would decrease hybridization among Chinook salmon genotypes, increasing the proportion of the spring genotype and reducing the proportion of heterozygote genotype in the population. Over the five years of study to date, the proportions of the spring genotype among Chinook salmon emergent fry in the Skookumchuck River were consistently lower than the Newaukum River and did not noticeably change 2023 and 2024 after spawning flows were experimentally reduced (**Figure 11**). Although there has been no noticeable change in genotype proportions in response to flow manipulation, there are several reasons to interpret these findings with caution. For ease of communication, this section refers to outmigration years “before” (outmigration 2020-2022) and “after” (outmigration 2023-2024, 2025 is pending) experimental flow reduction.

The most notable issue is the short time series available to evaluate a response. While we acknowledge that any large response should still be visually apparent, the short length of the time series hinders our ability to conduct statistical data analyses and to detect more subtle responses in genotype proportions. Of the three years “before” flow manipulation, the 2020 data not recommended for time series analysis because this year likely missed the initial portion of the outmigration. This leaves just two years of “before” data for comparison. Of the three years “after” flow manipulation (outmigration 2023-2024, 2025 is pending), the flows corresponding to the 2023 outmigration did not achieve the target flow level. Manipulating flows in the Skookumchuck River between August 15 and October 15 has required careful attention to both flow reduction and flow monitoring during this period. In the first year of flow reduction (fall 2022, outmigration 2023), we did not achieve the target flow level due to the incorrect calibration of the stream flow gage (i.e., flow monitoring). As a result, one of the three years “after” flow manipulation is questionable.

A second issue is the ability to isolate the effect of reduced spawning flows on mate selection among Chinook salmon genotypes. Emergent fry was selected as the focal life stage for this study because this life stage most directly represents overall spawning success. Genotype proportions at the fry life stage are the cumulative result of multiple factors, one of which is changes in mate selection (i.e., hybridization) in response to reduced stream flows. However, genotype proportions at the fry life stage may also be influenced by other factors such as the relative abundance of each parental genotype and differential egg-to-fry survival between early and late spawners. As a result, subtle responses in hybridization to reduced spawning flows may be dampened by large interannual variability in either fall-run Chinook salmon abundance or timing of scouring flow events.

Our initial study design accommodated for interannual variability in these other factors through a “Before-After Control-Impact” (BACI) analysis. This design included a reference stream, the Newaukum River, to serve as a comparison to the treatment stream, the Skookumchuck River. The Newaukum River exists in close geographic proximity to the Skookumchuck River with similar interannual variability in environmental conditions, such as spawner escapement and winter precipitation. Using the BACI design, we hypothesized that interannual variability in genotype proportions would be initially correlated between rivers during the “before” phase of study and would diverge from one another after the experimental flow manipulation in the Skookumchuck River. However, use of the BACI design relies on genotype proportions being correlated during the “before” phase, something which cannot be demonstrated with just two years of “before” data in the time series. Use of the BACI design also depends on reliable estimates for the time series, which is problematic given the higher uncertainty in fry abundance estimates for the Newaukum River. Given the data set now apparent after five years of study, the BACI analysis is unlikely to provide an appropriate statistical tool to isolate the effect of the experimental flow reduction on Chinook salmon hybridization.

What Has Been Learned

The flow study has contributed to our understanding of Chinook production in the Chehalis Basin in the following ways:

- There is no indication that flow manipulation has increased the proportion of the spring genotype or decreased the proportion of heterozygote genotype among Chinook emergent fry.
- Sonar data confirm an association between early fall flow events and the migration of adult Chinook into the Skookumchuck River.
- The migration timing of Chinook fry differs among spring, heterozygote, and fall genotypes in both the Skookumchuck and Newaukum rivers.
- The abundance and genotype proportions of Chinook fry migrants differ between the Skookumchuck and Newaukum rivers.
- Improvements have been made to increase performance of inclined-plane traps.

Next Steps

The 2024 fry trap data presented in this report are part of a multi-year study to understand whether operational changes of the Skookumchuck Dam can reduce hybridization of Chinook salmon genotypes. In order for the multi-year study to successfully achieve this goal, we recommend the following:

- Maintain accurate annual monitoring of flows at the USGS Bucoda Gage in order to achieve flow targets each year.
- Continue all three methods of analysis (mark-recapture, CPUE, stream method) in order to justify analysis of time-series data.
- Re-calculate 2020-2022 data to provide genotype proportions by trap location in order to compare weekly proportions among years.
- Investigate the feasibility of operating the Skookumchuck River sonar earlier in the spring and of collecting genetic samples of adults passing the sonar.
- Finalize analytical methods to draw inferences regarding the effect of flow manipulation on genotype production.

This study has raised additional questions about Chinook salmon in the Chehalis River watershed which may warrant further and additional study. For example, why is the proportion of spring genotype so low in the Skookumchuck River? Why is fry abundance and fry-per-spawner in the Newaukum River so much lower than the Skookumchuck River? While beyond the scope of the current study, further exploration of these topics may provide additional, and critical, insight into the factors affecting the status and trends of Chinook salmon in this watershed.

References

- Anderson, J.H., and Topping, P.C. 2018. Juvenile life history diversity and freshwater productivity of Chinook Salmon in the Green River, Washington. *North America Journal of Fisheries Management* **38**: 180-193. doi:<https://doi.org/10.1002/nafm.10013>.
- Ashcraft, S., Holt, C., Scharpf, M., Zimmerman, M., and VanBuskirk, N. 2017. Spawner Abundance and Distribution of Salmon and Steelhead in the Upper Chehalis River, 2013-2017, FPT 17-12. Washington Department of Fish and Wildlife, Olympia, Washington, <https://wdfw.wa.gov/publications/01970/>.
- Bonner, S.J., and Schwarz, C.J. 2011. Smoothing population size estimates for time-stratified mark-recapture experiments using Bayesian P-splines. *Biometrics* **67**: 1498-1507. doi:<https://doi.org/10.1111/j.1541-0420.2011.01599.x>.
- Bonner, S.J., and Schwarz, C.J. 2014. BTSPAS: Bayesian Time Stratified Petersen Analysis System. R package version 2014.0901.
- Campbell, L., Claiborne, A., Anderson, A., Anderson, J., Smith, W., Conway-Cranos, T., and Winkowski, J. 2023. Successful juvenile life history strategies in returning adult Chinook salmon from Western Washington, National Estuary Program Habitat Strategic Initiative Lead, NTA 2018-0809. Washington Department of Fish and Wildlife, <https://coastsalmonpartnership.egnyte.com/dl/5cgkdE6R2G>.
- Campbell, L.A., Claiborne, A.M., Ashcraft, S., Zimmerman, M.S., and Holt, C. 2017. Final Report: Investigating Juvenile Life History and Maternal Run Timing of Chehalis River Spring and Fall Chinook Salmon Using Otolith Chemistry, FPT 17-15. Washington Department of Fish and Wildlife, Olympia, Washington, <https://wdfw.wa.gov/publications/01985/>.
- Campbell, N.R., Harmon, S.A., and Narum, S.R. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources* **15**(4): 855-867. doi:<https://doi.org/10.1111/1755-0998.12357>.
- Center for Biological Diversity and Pacific Rivers 2023. Petition to List the Washington Coast ESU of Spring-Run Chinook Salmon (*Oncorhynchus tshawytscha*) under the Endangered Species Act. <https://www.biologicaldiversity.org/species/fish/pdfs/WA-Spring-Chinook-Petition-05-23-23.pdf>.
- Devries, P. 1997. Riverine salmonid egg burial depths: Review of published data and implications for scour studies. *Canadian Journal of Fisheries and Aquatic Sciences* **54**(8): 1685-1698. doi:<https://doi.org/10.1139/f97-090>.
- Ford, M.D., Nichols, K.M., Waples, R.S., Anderson, E.C., Kardos, M., Koch, I., McKinney, G., Miller, M.R., Myers, J., Naish, K., Narum, S.R., O'Malley, K.G., Pearse, D., Seamons, T.R., Spidle, A., Swanson, P., Thompson, T.Q., Warheit, K.I., and Willis, S. 2020. Reviewing and Synthesizing the State of the Science Regarding Associations between Adult Run Timing and Specific Genotypes in Chinook Salmon and Steelhead: Report of a workshop held in Seattle, Washington, 27–28 February 2020 [Technical Report]. doi:<https://doi.org/10.25923/mv3n-zb79>.
- Gelman, A., Carlin, J., Stern, A., and Rubin, D.B. 2004. *Bayesian Data Analysis*, 2nd Edition. Chapman and Hall/CRC Press, Boca Raton, FL.
- Gilbertson, L. 2023. Memorandum Chehalis Chinook Fry Trapping Project Update - October 20, 2023. Quinault Indian Nation Department of Fisheries, Tahola, Washington.
- Gilbertson, L., Jurasin, T., Coshow, R., and Miller, M.A. 2021. Run-Type Composition of Juvenile Chinook Salmon in the Upper Chehalis River Basin in 2020, Technical Report Series 2021-1. Quinault Indian Nation Department of Fisheries, Tahola, Washington.

- Koch, I.J., and Narum, S.R. 2020. Validation and association of candidate markers for adult migration timing and fitness in Chinook Salmon. *Evolutionary Applications* **13**(9): 2316-2332. doi:<https://doi.org/10.1111/eva.13026>.
- Lamperth, J., Zimmerman, M.S., Claiborne, A.M., Campbell, L.A., and Hildebrandt, A. 2014. Evaluation of Coweeman River Salmonids in 2012 and 2013: Juvenile Production and Other Activities, FPA 14-03. Washington Department of Fish and Wildlife, Olympia, Washington, <https://wdfw.wa.gov/publications/01604>.
- Massmann, A., and Massmann, J. 2023a. Estimating pre-1967 Skookumchuck Streamflow near Bucoda: Technical Memorandum Prepared for the Quinault Indian Nation. Keta Waters, Seattle, Washington.
- Massmann, J., and Massmann, A. 2023b. TransAlta Water Right Acquisition: Phase 1 Feasibility Study Task 3. Prepared for the Quinault Indian Nation. Keta Waters, Seattle, Washington.
- Montgomery, D.R., Buffington, J.M., Peterson, N.P., Schuett-Hames, D., and Quinn, T.P. 1996. Stream-bed scour, egg burial depths, and the influence of salmonid spawning on bed surface mobility and embryo survival. *Canadian Journal of Fisheries and Aquatic Sciences* **53**(5): 1061-1070. doi:<https://doi.org/10.1139/f96-028>.
- Nelson, S., and Dugger, D. 2022. Standard Operating Procedure EAP080, Version 2.2: Continuous Temperature Monitoring of Freshwater Rivers and Streams. Publication 22-03-216. Washington State Department of Ecology, Olympia, WA.
- Olson, D., Litz, M., and Seamons, T. 2024. Newaukum River Smolt Production, 2023, FPA 24-15. Washington Department of Fish and Wildlife, Olympia, Washington, <https://wdfw.wa.gov/publications/02557>.
- Pollock, J.H., Nichols, J.D., Brownie, C., and Hines, J.E. 1990. Statistical inference for capture-recapture experiments. *Wildlife Monographs* **107**: 1-97. doi:<https://www.jstor.org/stable/3830560>.
- Prince, D.J., O'Rourke, S.M., Thompson, T.Q., Ali, O.A., Lyman, H.S., Saglam, I.K., Hotaling, T.J., Spidle, A.P., and Miller, M.R. 2017. The evolutionary basis of premature migration in Pacific salmon highlights the utility of genomics for informing conservation. *Science Advances* **3**(8): e1603198. doi:<https://doi.org/10.1126/sciadv.1603198>.
- Quinn, T.P., McGinnity, P., Reed, T.E., and Bradford, M. 2016. The paradox of “premature migration” by adult anadromous salmonid fishes: patterns and hypotheses. *Canadian Journal of Fisheries and Aquatic Sciences* **73**(7): 1015-1030. doi:<https://doi.org/10.1139/cjfas-2015-0345>.
- Thompson, N.F., Anderson, E.C., Clemento, A.J., Campbell, M.A., Pearse, D.E., Harsey, J.W., Kinziger, A.P., and Garza, J.C. 2020. A complex phenotype in salmon controlled by a simple change in migratory timing. *Science* **370**(6516): 609-613. doi:<https://doi.org/10.1126/science.aba9059>.
- Thompson, T.Q., O'Rourke, S.M., Brown, S.K., Seamons, T.R., Zimmerman, M.S., and Miller, M.R. 2019a. Run-type Genetic Markers and Genomic Data Provide Insight for Monitoring Spring-Run Chinook Salmon in the Chehalis Basin, Washington. Final Report to the Washington Department of Fish and Wildlife, <https://coastsalmonpartnership.egnyte.com/dl/bFQzkDCNHo>.
- Thompson, T.Q., Bellinger, M.R., O'Rourke, S.M., Prince, D.J., Stevenson, A.E., Rodrigues, A.T., Sloat, M.R., Speller, C.F., Yang, D.Y., Butler, V.L., Banks, M.A., and Miller, M.R. 2019b. Anthropogenic habitat alteration leads to rapid loss of adaptive variation and restoration potential in wild salmon populations. *Proceedings of the National Academy of Sciences* **116**(1): 177-186. doi:<https://doi.org/10.1073/pnas.1811559115>.
- Volkhardt, G.C., Johnson, S.L., Miller, B.A., Nickelson, T.E., and Seiler, D.E. 2007. Rotary screw traps and inclined plane screen traps. In *Salmonid field protocols handbook: techniques for assessing status and trends in salmon and trout populations*. Edited by D.H. Johnson and B.M. Shrier and J.S. O'Neal

- and J.A. Knutzen and X. Augerot and T.A. O-Neil and T.N. Pearsons. American Fisheries Society, Bethesda, Maryland. pp. 235-266.
- West, D., Winkowski, J., Seamons, T.R., and Litz, M. 2021. Chehalis River Smolt Production 2020, FPA 21-06. Washington Department of Fish and Wildlife, Olympia, Washington, <https://wdfw.wa.gov/publications/02274>.
- West Fork Environmental. 2023. Assessment of Adult Chinook Salmon Migration Patterns into the Skookumchuck River, 2023. West Fork Environmental, Tumwater, Washington.
- Wright, S. 1951. The genetical structure of populations. *Annals of Eugenics* **15**: 323-354. doi:<https://doi.org/10.1111/j.1469-1809.1949.tb02451.x>.
- Zimmerman, M.S. 2017. Ongoing Studies That Address Status of Spring Chinook Salmon in the Chehalis River. Memo to the Chehalis Aquatic Species Restoration Plan Steering Committee. Washington Department of Fish and Wildlife, Olympia, Washington.
- Zimmerman, M.S., Kinsel, C., Beamer, E., Connor, E.J., and Pflug, D.E. 2015. Abundance, survival, and life history strategies on juvenile migrant Chinook Salmon in the Skagit River, Washington. *Transactions of the American Fisheries Society* **144**: 627-641. doi:<https://doi.org/10.1080/00028487.2015.1017658>.
- Zimmerman, M.S., Litz, M., Seamons, T., Coshow, R., Peterson, P., Yates, J., Lestelle, L., Ferguson, J., Kale, N., Morishima, G., and Livingood-Schott, T. 2023. Run-Type Composition of Chinook Salmon Fry in the Skookumchuck and Newaukum River - 2023 Season., Report to the Chehalis River Basin Aquatic Species Restoration Plan Steering Committee, <https://coastsalmonpartnership.egnyte.com/dl/yn0wLxJ4x3>.

Appendices

Appendix B. Weekly median and 95% credible intervals for homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes in the Skookumchuck River traps based on weekly modeled mark-recapture abundance and genetic proportions.

Start	End	Week	Homozygous Spring		Homozygous Fall		Heterozygotes	
			Median	95% CI	Median	95% CI	Median	95% CI
12/25/2023	12/31/2023	0	57	6-364	171	18-1,091	152	16-970
1/1/2024	1/6/2024	1	760	298-2,605	2,279	893-7,814	2,026	794-6,946
1/7/2024	1/13/2024	2	3,528	2,165-6,326	3,257	1,998-4,329	7,056	4,329-12,652
1/14/2024	1/20/2024	3	5,166	3,684-7,492	4,305	3,070-6,243	13,775	9,825-19,978
1/21/2024	1/27/2024	4	5,074	2,123-11,426	21,312	8,915-47,991	17,252	7,217-38,850
1/28/2024	2/3/2024	5	13,743	8,516-24,771	38,938	24,128-70,185	34,357	21,289-61,928
2/4/2024	2/10/2024	6	4,045	2,542-6,525	35,053	22,029-56,551	28,312	17,792-45,676
2/11/2024	2/17/2024	7	8,149	5,135-13,697	81,495	51,349-136,970	24,448	15,405-41,091
2/18/2024	2/24/2024	8	3,661	2,785-4,948	80,534	61,261-108,845	47,588	36,200-64,318
2/25/2024	3/2/2024	9	3,624	2,410-5,805	94,224	62,658-150,919	10,872	7,230-17,414
3/3/2024	3/9/2024	10	0	0	59,144	35,793-96,738	7,041	4,261-11,516
3/10/2024	3/16/2024	11	0	0	75,822	50,266-126,728	3,611	2,394-6,035
3/17/2024	3/23/2024	12	0	0	26,653	18,714-39,035	1,134	796-1,661
3/24/2024	3/30/2024	13	0	0	16,879	8,243-31,715	0	0
3/31/2024	4/6/2024	14	0	0	18,454	12,345-30,153	429	287-701
4/7/2024	4/13/2024	15	0	0	4,985	2,846-8,810	108	62-192
4/14/2024	4/20/2024	16	0	0	3,112	1,622-6,741	0	0
4/21/2024	4/27/2024	17	0	0	987	534-2,009	0	0
4/28/2024	5/4/2024	18	0	0	264	91-809	0	0
5/5/2024	5/11/2024	19	0	0	28	3-186	0	0

Appendix C. Weekly median and 95% credible intervals for homozygous spring, homozygous fall, and heterozygote Chinook salmon genotypes in the Newaukum River traps based on weekly modeled mark-recapture abundance and genetic proportions.

Start	End	Week	Homozygous Spring		Homozygous Fall		Heterozygotes	
			Median	95% CI	Median	95% CI	Median	95% CI
1/1/2024	1/6/2024	1	9	1-43	18	3-86	9	1-43
1/7/2024	1/13/2024	2	56	20-158	112	40-315	56	20-158
1/14/2024	1/20/2024	3	131	57-342	263	113-684	131	57-342
1/21/2024	1/27/2024	4	773	285-2,529	0	0	0	0
1/28/2024	2/3/2024	5	210	104-467	911	451-2,025	2,173	1,075-4,828
2/4/2024	2/10/2024	6	922	475-2,066	1,127	580-2,525	2,458	1,266-5,509
2/11/2024	2/17/2024	7	2,626	1,414-5,302	2,188	1,179-4,418	3,501	1,886-7,069
2/18/2024	2/24/2024	8	780	386-1,848	3,430	1,700-8,131	2,806	1,391-6,652
2/25/2024	3/2/2024	9	1,580	827-3,405	3,950	2,068-8,512	3,634	1,903-7,831
3/3/2024	3/9/2024	10	589	294-1,383	4,125	2,055-9,683	1,375	685-3,228
3/10/2024	3/16/2024	11	441	227-915	5,957	3,065-12,355	1,544	795-3,203
3/17/2024	3/23/2024	12	0	0	4,041	2,055-8,644	0	0
3/24/2024	3/30/2024	13	0	0	896	363-2,595	0	0
3/31/2024	4/6/2024	14	0	0	222	93-567	222	93-567
4/7/2024	4/13/2024	15	0	0	61	13-241	0	0
4/14/2024	4/20/2024	16	0	0	6	0	0	0

Appendix D. Number of caught and sampled fry, wetted stream width (m), and catch-per-unit-effort of sampled Chinook fry genotypes (sampleCPUE) passing the Skookumchuck and Newaukum river fry traps, 2024. Trap width was 6m (2 traps*3ftm/trap). Sample CPUE is the number of samples of each genotype divided by the hours fished. Adjusted CPUE (adjCPUE) is the sampleCPUE expanded by sampling rate (total:sampled fry) and a proxy for trap efficiency (stream:trap width).

Skookumchuck River

Start	End	Week	Fry (total)	Fry (sample)	Stream Width	sampleCPUE			adjCPUE		
						Spring	Fall	Heterozygote	Spring	Fall	Heterozygote
12/25/2023	12/31/2023	0	---	---	---	---	---	---	---	---	---
1/1/2024	1/6/2024	1	255	20	68.0	0.0344	0.1032	0.0917	4.97	14.91	13.26
1/7/2024	1/13/2024	2	489	45	69.0	0.0969	0.0671	0.1715	12.11	8.39	21.43
1/14/2024	1/20/2024	3	1,019	27	68.1	0.0327	0.0272	0.0871	13.99	11.66	37.31
1/21/2024	1/27/2024	4	524	43	74.3	0.0323	0.1355	0.1097	4.87	20.45	16.55
1/28/2024	2/3/2024	5	1,623	38	70.5	0.0261	0.0738	0.0652	13.08	37.06	32.70
2/4/2024	2/10/2024	6	1,887	50	69.2	0.0127	0.1105	0.0892	5.55	48.08	38.83
2/11/2024	2/17/2024	7	1,817	28	69.4	0.0084	0.0842	0.0253	6.32	63.18	18.95
2/18/2024	2/24/2024	8	5,784	36	68.2	0.0043	0.0938	0.0554	7.79	171.33	101.24
2/25/2024	3/2/2024	9	2,613	30	68.8	0.0074	0.1936	0.0223	7.44	193.35	22.31
3/3/2024	3/9/2024	10	784	47	71.8	0.0000	0.1863	0.0222	0.00	37.20	4.43
3/10/2024	3/16/2024	11	1,283	44	70.4	0.0000	0.1765	0.0084	0.00	60.38	2.88
3/17/2024	3/23/2024	12	916	49	67.6	0.0000	0.1973	0.0084	0.00	41.56	1.77
3/24/2024	3/30/2024	13	237	45	69.0	0.0000	0.1880	0.0000	0.00	11.39	0.00
3/31/2024	4/6/2024	14	532	44	67.0	0.0000	0.1871	0.0044	0.00	25.26	0.59
4/7/2024	4/13/2024	15	176	47	68.0	0.0000	0.2003	0.0044	0.00	8.50	0.18
4/14/2024	4/20/2024	16	119	34	68.0	0.0000	0.1443	0.0000	0.00	5.72	0.00
4/21/2024	4/27/2024	17	67	46	68.1	0.0000	0.1971	0.0000	0.00	3.26	0.00
4/28/2024	5/4/2024	18	11	5	69.9	0.0000	0.0216	0.0000	0.00	0.55	0.00
5/5/2024	5/11/2024	19	---	---	---	---	---	---	---	---	---

Continued on next page

Newaukum

Start	End	Week	sampleCPUE						adjCPUE		
			Fry (total)	Fry (sample)	Stream Width	Spring	Fall	Heterozygote	Spring	Fall	Heterozygote
12/25/2023	12/31/2023	0	---	---	---	---	---	---	---	---	---
1/1/2024	1/6/2024	1	0	0	72.0	0.0000	0.0000	0.0000	0.00	0.00	0.00
1/7/2024	1/13/2024	2	1	0	76.5	0.0000	0.0000	0.0000	0.00	0.00	0.00
1/14/2024	1/20/2024	3	5	4	74.5	0.0072	0.0144	0.0072	0.11	0.22	0.11
1/21/2024	1/27/2024	4	3	2	78.0	0.0198	0.0000	0.0000	0.39	0.00	0.00
1/28/2024	2/3/2024	5	57	47	76.7	0.0129	0.0558	0.1332	0.20	0.87	2.06
2/4/2024	2/10/2024	6	71	44	73.8	0.0381	0.0466	0.1016	0.76	0.92	2.02
2/11/2024	2/17/2024	7	155	38	74.3	0.0498	0.0415	0.0663	2.51	2.09	3.35
2/18/2024	2/24/2024	8	96	45	74.2	0.0219	0.0963	0.0788	0.58	2.54	2.08
2/25/2024	3/2/2024	9	82	49	74.5	0.0691	0.1536	0.1536	1.44	3.19	3.19
3/3/2024	3/9/2024	10	85	31	76.5	0.0130	0.0912	0.0304	0.46	3.19	1.06
3/10/2024	3/16/2024	11	146	36	74.3	0.0086	0.1160	0.0301	0.43	5.83	1.51
3/17/2024	3/23/2024	12	71	10	72.0	0.0000	0.0431	0.0000	0.00	3.67	0.00
3/24/2024	3/30/2024	13	10	2	74.0	0.0000	0.0084	0.0000	0.00	0.52	0.00
3/31/2024	4/6/2024	14	8	2	73.0	0.0000	0.0044	0.0044	0.00	0.21	0.21
4/7/2024	4/13/2024	15	1	1	72.0	0.0000	0.0044	0.0000	0.00	0.05	0.00
4/14/2024	4/20/2024	16	---	---	---	---	---	---	---	---	---
4/21/2024	4/27/2024	17	---	---	---	---	---	---	---	---	---
4/28/2024	5/4/2024	18	---	---	---	---	---	---	---	---	---
5/5/2024	5/11/2024	19	---	---	---	---	---	---	---	---	---

Appendix E. Estimated genotype proportions of Chinook salmon fry in the Skookumchuck and Newaukum rivers, 2020-2023.

Results from 2020-2022 are as reported in Gilbertson (2023) and results from 2023 are mark-recapture analysis included in this report. Location of the Skookumchuck trap was river mile 6.2 in all years. Location of the Newaukum trap was river mile 4.4 (2020) and 8.5 (2021-2023). Comparisons among years should be interpreted with caution as the dates of trapping operation and trap configurations varied among years, and the analysis methods differed between 2020-2022 and 2023.

	Skookumchuck	Newaukum
	2020	
Begin	2/11/2020	02/20/2020
End	5/13/2020	05/06/2020
Total hours fished	421	823
Homozygous spring	0.021	0.119
Homozygous fall	0.799	0.388
Heterozygote	0.180	0.493
	2021	
Begin	01/18/2021	01/08/2021
End	05/20/2021	05/12/2021
Total hours fished	711	1,373
Homozygous spring	0.023	0.137
Homozygous fall	0.859	0.639
Heterozygote	0.118	0.224
	2022	
Begin	01/05/2022	01/04/2022
End	05/05/2022	05/05/2022
Total hours fished	755	1,842
Homozygous spring	0.071	0.104
Homozygous fall	0.703	0.587
Heterozygote	0.226	0.309
	2023	
Begin	01/10/2023	01/10/2023
End	05/12/2023	05/12/2023
Total hours fished	3,725	3,520
Homozygous spring	0.057	0.204
Homozygous fall	0.687	0.470
Heterozygote	0.256	0.326