

Memorandum

To: Wells, Rocky Reach, and Rock Island HCP Hatchery Committees and Priest Rapids Coordinating Committee Hatchery Subcommittee Document Date: January 10, 2024

From: Tracy Hillman, HCP Hatchery Committees Chairman and PRCC Hatchery Subcommittee Facilitator

cc: Larissa Rohrbach, Anchor QEA, LLC

Re: Minutes of the November 15, 2023, HCP Hatchery Committees and PRCC Hatchery Subcommittee Meetings

The Wells, Rocky Reach (RR), and Rock Island (RI) Hydroelectric Projects Habitat Conservation Plan Hatchery Committees (HCP-HCs) and Priest Rapids Coordinating Committee's Hatchery Subcommittee (PRCC HSC) meetings were held virtually on Wednesday, November 15, 2023, from 10:00 a.m. to 3:15 p.m.

Attendees are listed in Attachment A to these meeting minutes.

Action Item Summary

Long-Term

Joint HCP Hatchery Committees and PRCC Hatchery Subcommittee

- Keely Murdoch and Mike Tonseth will obtain estimates of pre-spawn mortality (PSM) from Andrew Murdoch to update the retrospective analysis for Wenatchee spring Chinook Salmon (Item I-A). *(Note: This item is ongoing; expected completion date to be determined.)*
- Members of the HCP-HCs and PRCC HSC will provide feedback to the Washington Department of Fish and Wildlife (WDFW)-revised version of questions on recalculation for Policy Committees (Item I-A). *(Note: This item is ongoing.)*
- Chelan PUD, Grant PUD, and WDFW will develop recommendations for reducing stress and mortality from disease for individual rearing groups at Eastbank Hatchery. (Item I-A). *(Note: This item is ongoing.)*

Near-Term (to be completed by next meeting)

Joint HCP Hatchery Committees and PRCC Hatchery Subcommittee

- Catherine Willard will research feasibility questions around planning for potential emergency Okanogan Sockeye Salmon broodstock collection, including the following (Item I-A) (*Note: This item is ongoing.*):
 - Flexibility around quarantine requirements for transporting adult fish into Canada
 - Minimum feasible program size under an emergency scenario
- Tracy Hillman will obtain decision item approval from representatives not in attendance, including meeting minutes and Hatchery Management and Evaluation (M&E) Implementation Plans (Items I-A, IV-A, and V-A). (*Note: approvals were obtained via email on November 29, 2023.*)
- Authors of the 10-Year Comprehensive Report will respond to comments by November 29, captured in the version distributed following the meeting by Tracy Hillman on November 17, 2023, and Hillman will distribute to the HCP-HCs and PRCC HSC for final approval the spring Chinook Salmon summary (Item II-A).
- Brett Farman will review the National Oceanic and Atmospheric Administration National Marine Fisheries Service (NOAA Fisheries) biological opinion for the Wenatchee spring Chinook Salmon programs to support interpretation of language around proportionate natural influence (PNI) targets (Item II-A). (*Note: This item is ongoing.*)
- Authors of the 10-Year Comprehensive Report will generate steelhead program M&E outcomes tables (Item II-A). (*Note: This item is ongoing.*)
- Rod O'Connor and Tom Kahler will coordinate with Brett Farman on a proposed shift in collection dates documented in the 2024 Broodstock Collection Protocols for the Methow-Okanogan summer Chinook Salmon program (Item II-B).
- WDFW and PUD representatives will provide revisions to the draft 2024 Broodstock Collection Protocols by January 8, 2024, using the version distributed by Larissa Rohrbach on November 20, 2023 (Item II-B).
- Tom Kahler and Mike Tonseth will coordinate between Methow-Okanogan summer Chinook Salmon programs to move any surplus wild-by-wild progeny with low or moderate bacterial kidney disease (BKD) levels into the Wells Hatchery program (Item III-D). (*Note: an update from John Rohrbach was provided via email on December 11, 2023.*)
- Rod O'Connor will provide an update to the PRCC on how the passive integrated transponder (PIT)-tagging of wild spring Chinook Salmon subyearlings inform the Chiwawa and Nason programs (Item V-A).
- Rod O'Connor will meet with Brett Farman to understand NOAA Fisheries' position on the ecological risks of the proposed change to Priest Rapids Hatchery fall Chinook Salmon release timing (Item V-B).

Decision Summary

- The RI/RR HCP-HC approved Chelan PUD's draft 2024 Hatchery M&E Implementation Plan.
- The PRCC HSC approved Grant PUD's draft 2024 Hatchery M&E Implementation Plan.
- The Wells HCP-HC approved Douglas PUD's draft 2024 Hatchery M&E Implementation Plan.

Agreements

- The RI/RR HCP-HC agreed to continue the evaluation of air-spawning female steelhead at Eastbank Hatchery for an additional year.
- No meeting will be convened in December.

Review Items

- Grant PUD's draft 2023 Priest Rapids Hatchery Annual report was distributed on November 14 for 30-day review, with comments due to Todd Pearsons by December 14, 2023.
- The most recent version of the draft 10-Year Summary Report was distributed on November 17, 2023.
- A revised version of the Douglas PUD's draft 2024 Hatchery Implementation M&E Plan was distributed on November 20 for approval via email no later than December 1.

Finalized Documents

- None.

I. Welcome

A. Agenda, Approval of Past Minutes, Action Item Review

Tracy Hillman welcomed the HCP-HCs and PRCC HSC and reviewed the agenda.

Revised meeting minutes from October 18, 2023, were reviewed and approved by parties that attended that meeting. Kirk Truscott was absent from that meeting and abstained.

Action items from the HCP-HCs and PRCC HSC October 18 meeting were reviewed. *(Note: Italicized text below corresponds to action items from the previous meeting.)*

Long-Term

Joint HCP-HCs and PRCC HSC

- *Keely Murdoch and Mike Tonseth will obtain estimates of PSM from Andrew Murdoch to update the retrospective analysis for Wenatchee spring Chinook Salmon (Item I-A). (Note: This item is ongoing; expected completion date to be determined.)*

Tonseth said this item is close to completion. Conversations with Mike Hughes (WDFW) are ongoing to obtain PSM values from the ongoing relative reproductive success (RRS) study to support Wenatchee spring Chinook Salmon management. Estimating PSM for males has been a challenge. A solution may be to change the management plan approach to manage for female PSM rather than total PSM because better estimates are available for females. K. Murdoch said recreating the original analysis supporting the management plan to use only females might be difficult but could be done. She also noted that re-consultation over permits is in the near future and the split between safety-net and conservation program fish may be reconsidered based on results of the RRS study. Rod O'Connor asked for clarification on whether there is a direct connection between reanalyzing the management plan with new PSM estimates and the presentation of the RRS study results. Tonseth said there may be a linkage, but reworking the analysis for the management plan with updated PSM values is a separate expectation. Todd Pearsons noted this has been an action item for some time and asked how the currently used PSM values were determined. Murdoch said that at the time the analysis was developed, an estimate of PSM was based on best judgement, although the science was starting to emerge. Pearsons noted that there is much more data available now and suggested that there may not be a perfect value for PSM and asked whether the available information can inform how the programs are being managed. Murdoch said she agrees that there may be better data coming from the RRS study, refined by tributary and fish origin, that was not available in the past. Pearsons asked whether an interim presentation could be offered with the understanding that it may not be a final product. Tonseth said the people working with the data are not comfortable releasing results yet and that he would not pressure those analysts to provide a value. They understand that there may be management decisions made based on those analyses; values that become included in a report ultimately may be the basis for "take" in a permit, and there is a need to have confidence in those values.

- *Members of the HCP-HCs and PRCC HSC will provide feedback to the WDFW-revised version of questions on recalculation for Policy Committees (Item I-A). (Note: This item is ongoing.)*

Hillman said this item is ongoing.

- *Chelan PUD, Grant PUD, and WDFW will develop recommendations for reducing stress and mortality from disease for individual rearing groups at Eastbank Hatchery. (Item I-A). (Note: This item is ongoing.)*

Catherine Willard said this item is ongoing.

Near-Term (to be completed by next meeting)

Joint HCP-HCs and PRCC HSC

- *Catherine Willard will research feasibility questions around planning for potential emergency Okanagan Sockeye Salmon broodstock collection, including the following (Item III-B) (Note: This item is ongoing.):*

- *Flexibility around quarantine requirements for transporting adult fish into Canada*
- *Minimum feasible program size under an emergency scenario*

Willard said this item is ongoing.

- *Rod O'Connor will share data on interannual variability in survival from the Priest Rapids Hatchery fall Chinook Salmon release timing evaluation (Item II-A).*

This item will be discussed in today's meeting. This item is complete.

- *Tracy Hillman will make revisions to the 10-Year Comprehensive Report on PNI targets for spring Chinook Salmon, as discussed in today's meeting (Item III-A).*

This item will be discussed in today's meeting. This item is complete.

- *PUD Authors of the 10-Year Comprehensive Report will generate steelhead program M&E outcomes tables (Item III-A).*

This item will be discussed in today's meeting. This item is ongoing.

- *Brett Farman will review the NOAA Fisheries biological opinion for the Wenatchee spring Chinook Salmon programs to support interpretation of language around PNI targets (Item III-A).*

Farman was not present in the meeting. This item is ongoing.

- *Bill Gale will identify a representative from U.S. Fish and Wildlife Service (USFWS) Ecological Services to coordinate on limits to Bull Trout encounters during the spring Chinook Salmon broodstock collection at the Chiwawa Wier in 2024 (Item III-D).*

Gale said Stuart Fetty (USFWS) will be the USFWS representative serving in this role and would like to attend a meeting to meet the group. This item is complete.

- *O'Connor will identify revisions to the 2024 Broodstock Collection Protocols needed for consistency on broodstock collection at Wells Hatchery Volunteer Channel (Item III-D).*

This was distributed on November 8, 2023, and will be discussed in today's meeting. This item is complete.

- Tom Kahler will lead development of contingency language for broodstock collection at the Wells Dam East Ladder (Item III-D).

Kahler said it does not appear that there will be interference with access in 2024. This item is complete.

II. Joint HCP-HCs and PRCC HSC

A. 10-Year Comprehensive Review – Spring Chinook Salmon/Steelhead

Tracy Hillman, in response to last month's discussion about how to report on PNI in the comprehensive review, prepared a spreadsheet to show PNI calculations for previous management approaches and the current approach relative to targets after spawn year 2012 (Attachment B).

Hillman compared two approaches for calculating PNI. The approximate PNI, which is calculated using the equation from the Hatchery Science Review Group (HSRG) guidance,¹ is generally smaller than PNI calculated with Mike Ford's (NOAA Fisheries) equations.² However, the differences were small—on the order of 0.01 to 0.02 using the different equations. These PNI data tables are now set up for reporting more accurately and consistently in annual reports.

Unfortunately, unadjusted Wenatchee steelhead escapement datasets were used in the PUDs' 10-year comprehensive reports. Since those reports were completed, the HCP-HCs approved WDFW's adjustments to the steelhead spawning escapement estimates in the Wenatchee River. Hillman is in the process of reanalyzing the data using the recently approved datasets.

Hillman reviewed and resolved comments from WDFW and USFWS on the spring Chinook Salmon portion of the 10-year review report during the meeting.

In response to a question from Matt Cooper, Hillman noted that 2022 is the last year that White River program fish would have an effect on Wenatchee spring Chinook Salmon population productivity and natural-origin return (NOR) recruits. The last year these fish would influence total abundance was 2018. These years are documented in the report.

B. 2024 Broodstock Collection Protocols Revisions, Schedule

The group identified the following components of the Broodstock Collection Protocols (BCPs) that require revision for 2024:

- Routine program edits will be made by WDFW and PUD authors.

¹ Hatchery Science Review Group, 2009. Appendix A of *Columbia River Hatchery Reform System-Wide Report*. February 2009.

² Ford, M. J., 2002 "Selection in Captivity During Supportive Breeding May Reduce Fitness in the Wild." *Conservation Biology* 16:815–825.

- The Wenatchee steelhead release plan is being prepared by Chelan PUD. It is working on the analysis and will share the results in an upcoming meeting to help inform the new release strategy.
- Yakama Nation (YN) staff will provide their Coho Salmon BCPs appendix.
- Language will be refined around guidance for collection of summer/fall Chinook Salmon at the Wells Hatchery Volunteer Channel.
- Language will be refined to clarify scenarios when the PRCC HSC and HCP-HCs should be informed or involved in decisions about culling for BKD.

Mike Tonseth said there are already guidelines in place in Section 10 permits and biological opinions for Wenatchee and Methow spring Chinook Salmon programs about BKD management and BKD culling. Those guidelines can be used as the template to start the conversations; however, the guidance is more ambiguous for summer Chinook Salmon. In Appendix A, Table 1, the first two columns have culling criteria for all of the summer Chinook Salmon programs. Biological assumptions around a predicted cull rate are based on past observations. Tonseth will make preliminary revisions based on guidelines carried over from the biological opinions.

Rod O'Connor has worked with John Rohrback to revise sections for collection of summer/fall Chinook Salmon to be consistent between both of the Methow-Okanogan integrated programs (Attachment C). For the Carlton program, the Wells Hatchery Volunteer Channel would only be used as a contingency, with flexibility in the collection schedule at the ladders if it is predicted to be challenging to meet targets based on run projections. Kahler said the BCPs state that summer Chinook Salmon collection occurs from July 1 to September 15; however, the program is never collecting fish in September, and they are seeing many summer Chinook Salmon in mid-June. He suggested revising collection dates to June 15 to August 15. Tonseth said he disagrees because that would be inconsistent with the permit and Hatchery Genetic Management Plans, which define the collection period as July 1 to September 15. In years past, when collecting for both Okanogan and Methow programs, broodstock were collected until September 15. In past years, a variance has been requested to start retention of summer Chinook Salmon encountered prior to July 1, concurrent with spring Chinook Salmon collection, at the Wells Dam west ladder and east ladder. A more permanent change in collection dates needs to be justified and documented appropriately as a deviation from the permit. NOAA Fisheries will need to be engaged with approving this in the BCPs. Kahler said he thought of this as an exception already obtained from NOAA Fisheries. Kahler said fish that are of adequate quality for inclusion in broodstock are not arriving as late as September 15; there has been a shift in the run distribution, the program should adjust to it, and we should engage with NOAA Fisheries to obtain permission to do so.

III. Wells HCP-HC

A. 2024 Douglas PUD Wells Hatchery M&E Implementation Plan

John Rohrback introduced the changes to the Douglas PUD's draft 2024 Wells Hatchery M&E Implementation Plan (distributed on November 14, 2023) and said the only updates were to dates and some nomenclature.

Mike Tonseth said there should be language in the plan that captures monitoring of the Methow summer Chinook Salmon program for Douglas PUD under module 15 or 16.

Charles Frady asked about WDFW staff presence at all surplussing activities and whether that is specific to Douglas PUD-owned facilities. Rohrback said, because this implementation plan is specific to Douglas PUD, it is only specific to Douglas PUD-owned facilities.

Tracy Hillman asked when a vote is needed to approve this plan. Tom Kahler said approval of the contract for WDFW is contingent on approval of this plan in the last board meeting of the year, on December 6.

Rohrback will revise the plan by the end of this week (November 17) to include in-hatchery M&E of Methow-Okanagan summer Chinook Salmon. Douglas PUD will seek approval by the Wells HCP-HC via email no later than December 1.

B. Brood Year 2023 Columbia Safety Net Steelhead Thiamine Levels

The results of the first of 3 planned years of the egg thiamine level study were provided in a memorandum distributed on November 8 (Attachment D). Tom Kahler reminded the committees of the methods and described results.

Eggs from six individual females fell below the LD50 threshold of 6.54 nanomoles per gram (nmol/g) thiamine, to be interpreted that 50% of these progeny would not survive. This LD50 comes from Futia and Rinchar (2019), which examined thiamine deficiency in steelhead collected from Lake Ontario; there are no local studies of steelhead populations to confirm whether this is indeed an LD50 for Upper Columbia populations. Researchers are finding differences in responses for different sampling locations. Below the 10 nmol/g level may result in sublethal impacts (e.g., neurological effects); this has been observed in Lake Trout in the Great Lakes.

The proposal submitted last spring was to study thiamine levels over 3 years to establish a baseline for this population. Last year's early sampling (prior to this study) was based on only 10 individuals. There are 2 more years of study, and Douglas PUD will provide a more substantial interpretation at that time as the basis for any management decisions.

Alf Haukenes asked whether the samples represented by dots on the chart are random grab samples of different females. Betsy Bamberger answered that the dots represent samples taken from all 40 females from the Columbia Safety Net (CSN) program. Mike Tonseth asked whether there were any differences in the ages of the fish or differences in thiamine levels between earlier or later spawners. Bamberger said those data are not available; these fish were not labeled at collection or tracked after collection. That could be possible in the future. Megan Finley asked whether there are hatchery-origin returns (HOR) and NOR in this program and whether differences in origin could be tracked in the future. Kahler said these are all HOR. There may be an occasion where some NOR fish are incorporated; however, those all tend to go to the Methow Safety Net program.

Tonseth suggested that a comparison with the natural-origin population could be made with the Winthrop National Fish Hatchery (WNFH) program, which is technically the conservation program. Bill Gale said, at this time, there is no reason to suspect that the NOR and HOR populations would be different. USFWS took a risk-averse approach to supplement the wild brood by injecting females with thiamine, which precludes the ability to take egg samples of that population. Injections are the best approach at this time; progeny become replete with thiamine once they begin to feed. The CSN samples should inform the WNFH program of deficiencies. Kahler said taking egg samples from a safety-net program was a more palatable request than from a small conservation program.

Bamberger said females could be tracked more closely, but this would require more thought about logistics. Currently, after shock and pick, the eggs are spread across trays, and family groups could be tracked out to ponding. So far, we have been looking at survival on a population level. Finley said tracking survival of eggs and early stages by individual female would make the data more relevant to identifying whether the deficiency below the expected LD50 does translate to lower survival. Finley said differences in egg survival between HOR and NOR might be related to thiamine as well. Tonseth asked whether it would be feasible to track eggs from individual females up to the eyed-egg stage for these 2 additional years, understanding that mixing occurs after that stage. Bamberger agreed that would probably be possible with some better labeling and tracking. There is a philosophical question about whether to assess this on a population level or individual level. Rohrback said we would not lose the population-level metrics by tracking individuals.

Bamberger said there were no other exceptional pathology issues or potential clinical manifestations of thiamine deficiency in this cohort.

Gale said when all female steelhead were supplemented at WNFH, they did not take a sample of eggs to test whether the supplementation was effective. There was bacterial cold-water disease and early mortality in some offspring, and it is unknown whether this was related to thiamine levels. The USFWS is still evaluating the plan for this year to determine whether eggs should be collected to verify that they were replete with thiamine. Gale said they are also considering tracking egg survival

through incubation by female for spring Chinook Salmon this year. There was an idea to pond fish into nursery tanks based on thiamine level tiers (the very lowest individuals would be ponded together) to compare and identify any performance differences early in rearing.

C. Methow Conservation Program Steelhead Escape

John Rohrback summarized a memorandum sent on October 31, 2023 (Attachment E), reporting on a loss of 3,650 unaccounted for steelhead in the circular tanks. The suspected cause was water pressure on the sump screen, which appears to have created a gap through which fish were able to escape. This has been corrected by bolting the sump screens in place. Keely Murdoch asked whether there was some foam added around the opening to fill the gap. Rohrback said there is foam around the opening already, but they suspect that some compression of that foam may have happened over time. The screens are now bolted tightly, and they do not anticipate a gap developing in the future.

D. Bacterial Kidney Disease Results for Methow-Okanogan Summer Chinook Salmon Program

Tom Kahler summarized that, in the past, the Wells summer Chinook Salmon program did not require many NOR brood to produce 320,000 yearling smolts and 484,000 subyearlings. The BCPs now direct the program to include NOR brood, based on 2009 HSRG recommendations to include a small component of NOR fish into segregated programs to avoid complete genetic divergence of populations. It is a small component, less than 10% of brood collected. Since recalculation, there is also a 35,000-smolt yearling Methow-Okanogan summer Chinook program for release into the Methow River using NOR brood collected and held at Wells rather than at Eastbank Hatchery with Grant PUD's Carlton program brood. This year, of the total NOR brood for both the Wells summer Chinook and Douglas PUD Methow-Okanogan programs, eggs from 39 females had high or moderate BKD levels. Typically, the moderate and high-level eggs would be culled from the program; however, that would leave no sources to backfill the Methow-Okanogan program. There are only six wild-by-wild crosses with low BKD levels, but nine are needed for the Methow-Okanogan program. Douglas PUD is proposing, for consideration by the Wells HCP-HC, to use three wild-by-hatchery crosses that have low BKD levels to backfill up to the nine pairs necessary.

Mike Tonseth asked whether the hatchery component is Wells stock and whether eggs from different females would be reared separately to the eyed-egg stage. Kahler answered yes to both questions. Tonseth said he is not inclined to approve culling until eggs reach the eyed stage so we can get an accurate count. He added that we need to see what portion of Grant PUD's Carlton program eggs have high BKD. For instance, at Eastbank Hatchery, recent BKD data showed few groups that need culling. Some wild-by-wild progeny could be transferred from the Carlton program to the Methow-Okanogan summer Chinook Salmon program if there are extra eggs.

Brandon Kilmer said the only concern about transferring between programs would be differences in accumulated temperature units, and he recommended making this decision as soon as possible to minimize differences.

Betsy Bamberger and Kahler agreed to wait until the egg inventorying process. All other Wells HCP-HC parties present supported the proposed approach.

Tonseth and John Rohrback will coordinate between programs to identify any overages, and if possible, shift wild-by-wild progeny from the Carlton program to the Wells Hatchery program.

IV. RI/RR HCP-HC

A. DECISION: 2024 Chelan PUD Hatchery M&E Implementation Plan

Chelan PUD's draft 2024 Hatchery M&E Implementation Plan was distributed on October 10, 2023, for a 30-day review. No comments were received, and no other comments or revisions were shared in the meeting. The RI/RR HCP-HC approved Chelan PUD's Hatchery M&E Implementation Plan (approvals from the Confederated Tribes of the Colville Reservation [CTCR] and NOAA Fisheries were obtained following the meeting).

B. Air-Spawning Wenatchee Steelhead

Catherine Willard reminded the committee about the background leading up to this year's activities to live-spawn the 2023 steelhead broodstock at Eastbank Hatchery, in coordination with YN Fisheries, to test whether Eastbank Hatchery females may be incorporated into a future kelt reconditioning program led by YN staff. Willard said Chelan PUD will be asking the RI/RR HCP-HC to evaluate these data and is proposing to continue to evaluate air-spawning again for an additional year.

Katy Shelby summarized the study methods and program (Attachment F). Efficacy of the methods was tested on both HOR and NOR females; however, in the future, a kelt reconditioning program would focus on NOR or conservation program females. Shelby summarized the broodstock and collection timeline. The study defines "air-spawn eggs" as eggs that came out of the female during the spawning, and "residual eggs" are those that were extracted after the female was sacrificed and cut open. Metrics included fecundity, number of air-spawn eggs, number of residual eggs, and green-to eyed-egg survival. Safety-net and conservation program fish were compared. Methods are documented in detail as background information, based on YN protocols. Shelby summarized results describing PSMs, egg take, and egg survival as follows:

- PSM was higher than in previous years.

- Air-spawning achieved 87.5% of the total green egg-take target. To replace the residual eggs not collected, 7.4 additional pairs of safety-net or conservation program fish would have needed to have been collected.
- There was high variation in the number of residual eggs per spawning female.
- Survival was higher in air-spawn eggs than residual eggs; no differences in egg survival were observed between conservation and safety-net fish.
- Twelve females had 0% survival in either their air-spawn or residual eggs, but it was more common for the mortality to occur in the residual eggs.
- Air-spawning produced fewer green eggs overall than lethal collection.

Clint Deason summarized techniques that were changed in response to observations in the field. Those changes are summarized in the memorandum and included changes to sedation methods in response to higher-than-expected PSM.

Cory Kamphaus asked whether some of the residual eggs were left in the cavity because of the positioning of the fish on the spawning deck. Shelby confirmed that staff improved methods with practice.

Shelby said an additional year of evaluation would be beneficial, and she would recommend tracking survival beyond the eyed-egg stage. It may not be possible at Eastbank Hatchery, but it may inform whether there really would be a need to collect additional females if survival is better in some groups than others.

Betsy Bamberger asked about the source for the air pressure levels used for egg evacuation. Matt Abrahamse said the protocols came from the Warm Springs Tribe program. He said that use of lower pressures requires longer sedation times and more manual stripping; however, higher pressure can result in difficulty pushing all eggs and air out while trying to assist the fish with recovery. The pressures used are based on trial and error and what seems most effective.

Mike Tonseth asked whether there is any advantage to using compressed oxygen or whether there are any disadvantages of using compressed atmospheric air. Deason said regulating pressure with compressed air might be more variable than using compressed oxygen. Abrahamse said that is an interesting idea, but it has not been considered. There is a logistical need to use equipment that is easily transported. Tonseth asked whether there is any effect on adult survival of reabsorbing oxygen or atmospheric air or any attempt to adjust the temperature of air used for spawning if the cold air might affect gamete or adult survival. Deason said that had not been considered, but there may be a way to track survival relative to the air temperatures in comparison to the water temperatures.

Cory Kamphaus noted that eggs are exposed to that same atmospheric air when fish are lethally spawned. Tonseth said the Wenatchee steelhead program struggles with relatively low fertilization

rates and has struggled to meet the brood targets for the program, but there is a high variance among brood years. With live-spawning, if that shortfall will have to be made up with NOR fish, small steps to improve survival may help chip away at the need to offset lower egg collection.

Tim Taylor asked whether there was any consideration about how the orientation of the air compressor needle affected egg expression. Abrahamse said needles are oriented to avoid hitting critical organs or going through muscle tissue, which results in death.

Shelby said there appears to be a pattern that air-spawn survival was lower in the first half of the spawning period; there was no pattern in the survival rates over time with the residual eggs. Megan Finley said there is lower egg survival in the beginning of the spawning period in general. Abrahamse said WNFH also had poorer survival below their typical standard in the first couple of weeks. This could be related to physiology. Shelby said these fish are spawned in a hatchery 2.5 to 3 months earlier than spawning in the river. These fish may not be reproductively mature during those first few weeks. Deason said those earlier spawners are HOR fish and would not affect the conservation programs.

K. Murdoch agreed that there seems to have been a learning curve and lessons were learned throughout the season, and that the green-egg take may change with typical year-to-year variability. For those reasons, she supports another year of study.

All members in attendance support another year of evaluation of live-spawning Wenatchee steelhead at Eastbank Hatchery. No members requested a more formal proposal from the hatchery program. Tonseth said a few changes to the methodology should be made because the program is still on a learning curve with the current methods.

V. PRCC HSC

A. DECISION: 2024 Grant PUD Hatchery M&E Implementation Plan

Grant PUD's draft 2024 Hatchery M&E Implementation Plan was distributed on October 11, 2023, for a 30-day review.

Mike Tonseth revised citations to refer to the most recent version of the 5-year M&E Plan, updated in 2019.

Tonseth asked whether Nason spring Chinook Salmon winter emigration rate data from the PIT-tag mark-recapture study are ready for presentation to the PRCC HSC in 2024. Rod O'Connor said he was not familiar with the data. Keely Murdoch and Cory Kamphaus said this is related to remote PIT-tagging to monitor the movement of fish during the overwinter period. Language was added to confirm that Grant PUD will provide an update on this task in 2024.

Katy Shelby said this relates to work being performed by WDFW using electrofishing gear to collect and PIT-tag 3,000 fish in the Chiwawa River and Nason Creek to evaluate overwinter emigration. WDFW is contracted to do the work through the end of 2024. Kamphaus agreed because YN assists with this work and is contracted to do that work through 2025. O'Connor confirmed that Grant PUD is continuing to support that work.

Grant PUD's Hatchery M&E Implementation Plan was approved by all PRCC HSC members present. (CTCR and NOAA Fisheries approved after the meeting).

B. Priest Rapids Hatchery Fall Chinook Salmon Release Timing

Rod O'Connor presented a summary of responses to questions about shifting to an earlier fall Chinook Salmon release timing at Priest Rapids Hatchery (PRH). This was discussed in the October 18, 2023, meeting (Appendix F). Results were shown by year to review interannual variation within each release group. One risk that had been identified is that the median travel time after release would increase and potentially exceed one of the proxies used by NOAA Fisheries in the biological opinion for reducing ecological interactions. O'Connor concluded with Grant PUD's proposal and potential tradeoffs for release dates in 2024 and 2025, noting Grant PUD is aware of the tradeoffs. A decision will be requested in January to prepare content for the BCPs and to prepare the hatchery staff.

Keely Murdoch asked for a reminder why this earlier release time was being tested, because it was not a study to improve smolt-to-adult returns (SARs). O'Connor said one reason was to respond to a future with warmer water temperatures due to climate change, and another reason was a successful shift to earlier releases at Wells Hatchery. Mike Lewis, who was the complex manager at PRH, had spoken with Grant PUD about releasing fish earlier to improve survival. Grant PUD brought it before the committee to determine whether there would be support for testing this to improve program performance.

K. Murdoch said she has concerns about fish taking longer to travel through the reach and potential ecological interactions. It is an interesting decision because there appears to be higher hatchery-origin survival with the earlier release groups. There is an unexpected trend in the data; typically, when hatchery fish have slower travel times it is associated with higher mortality, yet these results show the opposite. She wondered what could be happening to those later release groups and whether an increase in piscine predation or some other factor is driving that lower survival that could be another issue to manage. O'Connor agreed that it is counterintuitive. Juvenile arrival date at Bonneville Dam was the metric used to estimate travel time; however, perhaps estuary arrival timing or early ocean arrival timing played a role. Todd Pearsons asked whether similar patterns were observed at Wells Dam with juvenile in-river survival rates that do not translate over to survival to

adulthood. Pearsons said that ultimately, Grant PUD considered better survival to adulthood the more important metric.

K. Murdoch said that under the section on juvenile releases in the biological opinion, it states that “adaptive management will be used for hatchery releases.” She said that doing this action to maximize SARs could be viewed as a good thing, but the potential for negative interactions with natural-origin fish could also increase. She said she is not sure at this time how to address those concerns.

Pearsons said hatchery staff would need a decision in December, and an email vote may be necessary if groups are uncomfortable approving this change today. K. Murdoch said she and Kamphaus need to have internal discussions with others at YN and other agencies to understand what should be done to minimize impacts to natural-origin fish in the reach.

K. Murdoch said that in last month’s meeting, this request also included a proposal to release fish at an even smaller size, which might also contribute to a slower travel time. Pearsons noted that when rearing fish to achieve 50 fish per pound, fish had to be overfed, and it is theorized that a more natural feed regime might produce fish that smolt better.

Mike Tonseth said WDFW shares concerns about ecological interactions. He said there are questions about where survival advantages are being gained; a comparison in smolt-to-smolt survival to McNary or to Bonneville may help show where that survival advantage may be taking place. O’Connor said that since the change in spill conditions in 2017, it has been difficult to evaluate smolt-to-smolt survival.

O’Connor proposed that a potential resolution for this year is to continue with the release strategy that has been done over the past few years. K. Murdoch said she would commit to talking about this issue with others at YN and supports O’Connor’s proposed approach if an answer is needed soon for hatchery operations. O’Connor said he will discuss this within Grant PUD and with WDFW to confirm that will be a suitable solution going forward. Grant PUD will reach out to Brett Farman to determine what NOAA Fisheries’ perspective may be on this proposal and requested that Tracy Hillman join the call with Farman.

Obtaining approval to continue with the existing release strategy will be brought as a decision item in the next meeting.

C. Draft Priest Rapids Hatchery Annual M&E Report – Upcoming Review

Grant PUD’s PRH 2023 Annual M&E Report was distributed for a 30-day review on November 14, 2023. Todd Pearsons noted the main highlight in this year’s report is an adjustment to the PNI value,

which was 0.426, falling short of the 0.67 target. Comments and edits from the PRCC HSC and Fall Chinook Work Group are due by December 14.

VI. Administration

A. Next Meetings

All Committees members agreed to cancel the regular meeting in December.

The next meetings of the HCP-HCs and PRCC HSC will be held on January 17, February 21, and March 20, 2024. Meetings will be held virtually until March.

VII. Attachments

Attachment A List of Attendees

Attachment B Wenatchee Spring Chinook Salmon Proportionate Natural Influence Calculations

Attachment C Proposed edits to the Broodstock Collection Protocols for Carlton program Methow Summer Chinook Salmon

Attachment D Columbia Safety Net Steelhead Egg Thiamine Results and Interpretation Brood Year 2023

Attachment E Methow Conservation Program Steelhead Escape at Wells Hatchery

Attachment F Evaluation of Eastbank Hatchery Steelhead Live-Spawning Brood Year 2023

**Attachment A
 List of Attendees**

Name	Organization
Larissa Rohrbach ^o	Anchor QEA, LLC
Tracy Hillman ^o	BioAnalysts, Inc.
Catherine Willard ^{*o}	Chelan PUD
Betsy Bamberger ^o	Douglas PUD
Tom Kahler ^{*o}	Douglas PUD
Brandon Kilmer ^o	Douglas PUD
John Rohrbach ^o	Douglas PUD
Rod O'Connor ^{‡o}	Grant PUD
Deanne Pavlik-Kunkel ^o	Grant PUD
Todd Pearsons ^{‡o}	Grant PUD
Tim Taylor ^o	Grant PUD
Megan Finley ^o	Washington Department of Fish and Wildlife
Ben Goodman ^o	Washington Department of Fish and Wildlife
Alf Haukenes ^o	Washington Department of Fish and Wildlife
Katy Shelby ^o	Washington Department of Fish and Wildlife
Mike Tonseth ^{*‡o}	Washington Department of Fish and Wildlife
Matt Cooper ^{*‡o}	U.S. Fish and Wildlife Service
Charles Frady ^o	U.S. Fish and Wildlife Service
Bill Gale ^{*‡o}	U.S. Fish and Wildlife Service
Matt Abrahamse ^o	Yakama Nation
Keely Murdoch ^{*‡o}	Yakama Nation
Cory Kamphaus ^{*‡o}	Yakama Nation

Notes:

* Denotes HCP-HCs member or alternate

‡ Denotes PRCC HSC member or alternate

^o Joined remotely

Spring Chinook Salmon PNI Calculations

Chiwawa River spring Chinook Salmon PNI

NP = no program; NT = no PNI management target

Spawn year	Spawners			Broodstock			Approximate PNI ^a	PNI ^a	Sliding-scale PNI target	5-year PNI mean
	NOS	HOS	pHOS	NOB	HOB	pNOB				
1989	713	0	0.00	28	0	1.00	1.00	1.00	NT	NT
1990	571	0	0.00	18	0	1.00	1.00	1.00	NT	NT
1991	242	0	0.00	27	0	1.00	1.00	1.00	NT	NT
1992	676	0	0.00	78	0	1.00	1.00	1.00	NT	NT
1993	231	2	0.01	94	0	1.00	0.99	0.99	NT	NT
1994	123	61	0.33	8	4	0.67	0.67	0.68	NT	NT
1995	0	33	1.00	NP	NP	NP	NP	NP	NT	NT
1996	41	17	0.29	8	10	0.44	0.60	0.62	NT	NT
1997	60	122	0.67	32	79	0.29	0.30	0.32	NT	NT
1998	59	32	0.35	13	34	0.28	0.44	0.47	NT	NT
1999	87	7	0.07	NP	NP	NP	NP	NP	NT	NT
2000	233	113	0.33	9	21	0.30	0.48	0.50	NT	NT
2001	506	1,219	0.71	113	259	0.30	0.30	0.32	NT	NT
2002	254	453	0.64	20	51	0.28	0.30	0.33	NT	NT
2003	168	102	0.38	41	53	0.44	0.54	0.55	NT	NT
2004	574	277	0.33	83	132	0.39	0.54	0.56	NT	NT
2005	139	460	0.77	91	181	0.33	0.30	0.32	NT	NT
2006	113	414	0.79	91	224	0.29	0.27	0.29	NT	NT
2007	155	1,141	0.88	43	104	0.29	0.25	0.27	NT	NT
2008	190	968	0.84	83	220	0.27	0.24	0.26	NT	NT
2009	297	1,050	0.78	96	111	0.46	0.37	0.39	NT	NT
2010	419	675	0.62	77	98	0.44	0.42	0.43	NT	NT
2011	801	1,231	0.61	80	93	0.46	0.43	0.45	NT	NT
Average^b	289	364	0.45	54	80	0.52	0.54	0.56	NT	NT
Median^b	231	113	0.38	43	53	0.44	0.44	0.47	NT	NT
2012	574	904	0.61	66	45	0.59	0.49	0.50	0.80	
2013	422	956	0.69	68	2	0.97	0.58	0.59	0.80	
2014	523	452	0.46	58	12	0.83	0.64	0.65	0.80	
2015	337	630	0.65	64	0	1.00	0.61	0.61	0.67	
2016	389	156	0.29	57	42	0.58	0.67	0.68	0.80	0.61
2017	160	271	0.63	50	18	0.74	0.54	0.55	Any	0.62
2018	167	454	0.73	30	57	0.34	0.32	0.34	Any	0.57
2019	146	296	0.67	28	33	0.46	0.41	0.42	Any	0.52
2020	147	167	0.53	63	21	0.75	0.59	0.60	Any	0.52
2021	310	330	0.52	73	0	1.00	0.66	0.66	0.67	0.51
2022	424	476	0.53	72	7	0.91	0.63	0.64	0.80	0.53
Average^c	327	463	0.57	57	22	0.74	0.56	0.57	--	0.55
Median^c	337	452	0.61	63	18	0.75	0.59	0.60	--	0.53

^a PNI was calculated using PNI approximate equation 11 (HSRG 2009; their Appendix A) and by iterating Ford's (2002) equations 5 and 6 to equilibrium using a heritability of 0.3 and a selection strength of three standard deviations. C. Busack, NOAA Fisheries, 21 March 2016, provided the model for calculating PNI.

^b Descriptive statistics represent the program before recalculation in 2011.

^c Descriptive statistics represent the current program, which began in 2012. Origin determinations should be considered preliminary pending scale analyses.

Upper Wenatchee Spring Chinook Salmon PNI

NP = no program; NT = no PNI management target

Spawn year	Spawners			Broodstock			Approximate PNI ^a	PNI ^a	Sliding-scale PNI target	5-year PNI mean
	NOS	HOS	pHOS	NOB	HOB	pNOB				
1989	1,461	0	0.00	28	0	1.00	1.00	1.00	NT	NT
1990	1,003	0	0.00	18	0	1.00	1.00	1.00	NT	NT
1991	585	0	0.00	27	0	1.00	1.00	1.00	NT	NT
1992	1,098	0	0.00	78	0	1.00	1.00	1.00	NT	NT
1993	896	296	0.25	94	0	1.00	0.80	0.80	NT	NT
1994	214	66	0.24	8	4	0.67	0.74	0.74	NT	NT
1995	23	36	0.61	0	0		0.39	0.39	NT	NT
1996	135	47	0.26	8	10	0.44	0.63	0.64	NT	NT
1997	205	183	0.47	32	79	0.29	0.38	0.41	NT	NT
1998	149	35	0.19	13	34	0.28	0.60	0.62	NT	NT
1999	121	12	0.09	0	0		0.91	0.91	NT	NT
2000	486	276	0.36	9	21	0.30	0.45	0.48	NT	NT
2001	985	1,971	0.67	118	259	0.31	0.32	0.34	NT	NT
2002	732	881	0.55	38	51	0.43	0.44	0.46	NT	NT
2003	383	212	0.36	48	53	0.48	0.57	0.59	NT	NT
2004	1,012	589	0.37	89	132	0.40	0.52	0.54	NT	NT
2005	302	1,171	0.79	194	254	0.43	0.35	0.37	NT	NT
2006	303	635	0.68	282	359	0.44	0.39	0.41	NT	NT
2007	386	1,621	0.81	297	110	0.73	0.47	0.48	NT	NT
2008	362	1,778	0.83	199	220	0.47	0.36	0.38	NT	NT
2009	585	1,610	0.73	334	111	0.75	0.51	0.52	NT	NT
2010	536	1,223	0.70	167	98	0.63	0.47	0.49	NT	NT
2011	1,217	1,774	0.59	386	93	0.81	0.58	0.59	NT	NT
Average^b	573	627	0.41	107	82	0.61	0.60	0.62	NT	NT
Median^b	486	276	0.37	48	51	0.48	0.52	0.54	NT	NT
2012	932	1,579	0.63	456	45	0.91	0.59	0.60	0.80	
2013	614	1,408	0.70	471	7	0.99	0.59	0.59	0.50	
2014	779	575	0.42	79	12	0.87	0.67	0.68	0.67	
2015	617	775	0.56	124	63	0.66	0.54	0.55	0.50	
2016	618	221	0.26	127	108	0.54	0.68	0.69	0.50	0.62
2017	259	369	0.59	120	82	0.59	0.50	0.51	Any	0.60
2018	225	656	0.74	83	111	0.43	0.37	0.38	Any	0.56
2019	206	679	0.77	75	118	0.39	0.34	0.35	Any	0.50
2020	272	466	0.63	118	82	0.59	0.48	0.50	Any	0.49
2021	503	964	0.66	137	61	0.69	0.51	0.52	0.40	0.45
2022	650	1,252	0.66	137	61	0.69	0.51	0.52	0.67	0.45
Average^c	516	813	0.60	175	68	0.67	0.53	0.54	--	0.53
Median^c	614	679	0.63	124	63	0.66	0.51	0.52	--	0.50

^a PNI was calculated using PNI approximate equation 11 (HSRG 2009; their Appendix A) and by iterating Ford's (2002) equations 5 and 6 to equilibrium using a heritability of 0.3 and a selection strength of three standard deviations. C. Busack, NOAA Fisheries, 21 March 2016, provided the model for calculating PNI.

^b Descriptive statistics represent the program before recalculation in 2011.

^c Descriptive statistics represent the current program, which began in 2012. Origin determinations should be considered preliminary pending scale analyses.

Summer/fall Chinook

Beginning in 2022, the summer/fall Chinook mitigation programs in the Methow River include the Carlton Program for Grant PUD, and the Douglas PUD's program with two components. The Carlton Program utilizes adult broodstock collections at Wells Dam and incubation/rearing at Eastbank Fish Hatchery. The total production target is 164,533 summer/fall Chinook smolts for acclimation and release from the Carlton Acclimation Facility. The 35,437-smolt Douglas PUD program utilizes adult broodstock collected at Wells Hatchery or Dam; spawning, incubation, and early rearing at Wells Hatchery; and direct release to the Methow River concurrent with releases from the Carlton Acclimation Facility. The two components of the Douglas PUD program differ in acclimation strategy, with half remaining full term at Wells Hatchery, and the other half overwinter acclimated at Methow Hatchery. Both components will be truck planted directly to the Methow River concurrently at the same location.

The following broodstock collection protocol for the Methow summer Chinook program was developed based on initial run expectations of summer Chinook to the Columbia River, program objectives, and program assumptions (Appendix A).

For 2023, up to 130 natural-origin summer Chinook will be targeted at Wells Dam west (and east, if necessary) ladder(s). ~~for the Carlton Program, and The Wells Hatchery volunteer channel will be used as a contingency option if broodstock collection targets appear as if they will not be met at the Wells Dam ladders. -at Wells Hatchery volunteer channel or Wells Dam for the Douglas PUD program.~~ The 130 broodstock include 53 females for the Carlton summer Chinook program, and 12 females for the Douglas PUD program (Table 6). Collection will be approximately proportional to return timing between 01 July and 15 September. At the discretion of broodstock-collecting personnel, broodstock may be collected in excess of the weekly proportion in order to reduce the likelihood of a failure to meet the overall broodstock collection goal. Summer Chinook stock assessment will run concurrent with summer Chinook broodstock collection at the west ladder trap. Each day, fish needed for broodstock will be collected prior to those used for stock assessment. Trapping may occur up to 3-days/week, 16 hours/day (48 cumulative hours per week). Age-3 males ("jacks") will not be collected for broodstock unless needed to pair with females.

~~Should use of Wells Dam be needed to meet any shortfalls in Chief Joseph Hatchery broodstock for summer/fall Chinook programs, the CCT will notify the HCP-HC and Wells HCP Coordinating Committee/PRCC-HSC and coordinate with Douglas PUD, Grant PUD, and WDFW to facilitate additional broodstock collection effort. Summer Chinook broodstock collection efforts at Wells Dam, should they be required to meet CJH program objectives, may be conducted concurrent with the Methow (Carlton) summer Chinook collection at Wells Dam.~~

If the probability of achieving the broodstock goal is reduced based on passage at the west ladder or natural-origin escapement levels, broodstock collections may be expanded to the east ladder trap, the Wells Hatchery Volunteer Channel, and/or origin composition will be adjusted to meet the broodstock collection objective. If collection of adults from the east ladder trap is necessary, access will be coordinated with staff at Wells Dam due to multiple projects potentially interfering with access to the east ladder, such as the ongoing rotor rewind project.

In-season data for fish age, size, and estimated fecundity may be used to adjust the number of broodstock collected to meet program production needs. Adjustments made to broodstock collection targets based on pre-spawn mortality exceeding current year assumptions will require review and concurrence on the additional number and composition of the broodstock necessary to backfill shortfalls.

Should use of Wells Dam be needed to meet any shortfalls in Chief Joseph Hatchery broodstock for summer/fall Chinook programs, the CCT will notify the HCP-HC and Wells HCP Coordinating Committee/PRCC-HSC and coordinate with Douglas PUD, Grant PUD, and WDFW to facilitate additional broodstock collection effort. Summer Chinook broodstock collection efforts at Wells Dam, should they be required to meet CJH program objectives, may be conducted concurrent with the Methow (Carlton) summer Chinook collection at Wells Dam.

Table 4. Number of broodstock needed for Grant and Douglas PUD Methow summer Chinook production obligation of 199,970 smolts, collection location, and mating strategy.

Program	Production target	Number of Adults		Total	Collection location	Mating protocol
		Hatchery	Wild			
GPUD	164,533		53F/53M	106	Wells Dam/Wells Hatchery	1:1
DPUD	35,437		12F/12M	24		
Total	199,970		130	130		

Table 4. Summary of broodstock collection, spawner escapement tagging, adult management, run composition sampling, and/or reproductive success activities anticipated to be conducted at Wells Dam in 2023 (and 2024 for steelhead). Blue = steelhead, brown = spring Chinook, pink = summer Chinook, orange = sockeye, and green = Coho.

Activity	Month											
	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sep	Oct	Nov	Dec
East/West Ladders												
Sp Chinook BS collection					1 May	30 Jun						
Sp Chinook run comp					1 May		15 Jul					
Sockeye SA tagging ¹						Late June		Early Aug				
Su. Chin BS collection ²						15 June			15 Sep			
Coho BS collection ³									15 Sep		15 Nov	
Wells Volunteer Trap												
Su SHD BS/pHOS mgt. ⁴		15 Feb				15 June			1 Sep			15 Dec
Su. Chin BS collection ⁵						15 June			15 Sep			
Su. Chin Surplussing							1 Jul			30 Oct		

- CRITFC trapping of sockeye for stock assessment and tagging typically begins the last week of June and extends through the third week of August, following an up to 3d/week 16hr/day (48 cumulative hours) coordinated with WDFW/DPUD spring or summer Chinook broodstock collection and stock assessment trapping, preferring to trap on the East ladder.
- Summer Chinook broodstock collection for the Methow (Carlton and Douglas PUD) programs -will be targeted at Wells Dam west (and east, if necessary) ladder(s). The Wells Hatchery volunteer channel will be used as a contingency option if broodstock collection targets appear as if they will not be met at the Wells Dam ladders will be prioritized at the West ladder trap. However, if broodstock objectives cannot be met at the West ladder then trapping may occur at the East ladder. Trapping at the west and/or East ladders for summer Chinook broodstock will follow an up to 3d/week 16hr/day (48 cumulative hours) trapping schedule and may run concurrent with other broodstock collection, run sampling, or adult management activities.
- Coho trapping may be conducted at both East and/or West ladders. Trapping at Wells Dam ladder traps for Coho broodstock prior to September 27, will follow up to 3d/week 16hr/day (48 cumulative hours) coordinated with WDFW steelhead broodstock collection and stock assessment trapping; from September 27 through October 9, an up to 5d/week 9hr/day trapping schedule and may run concurrent with other broodstock collection, run sampling, or adult management activities, and 7 days per week/16 hours per day beginning October 10. Trapping at the Wells Dam ladder will cease no later than November 15.
- Adult management of the 2023/2024 steelhead return will end in June 2024. However, it is anticipated that adult management will occur for the 2023/2024 return beginning as early as 1 September or earlier if conducted in conjunction with broodstock collection activities at the Wells Hatchery volunteer channel for other species. Emergency collection of 2024 brood steelhead may also occur in the fall if the 2023/2024 return is deemed inadequate to support spring collection of broodstock.
- Summer Chinook broodstock collection for the Wells Hatchery programs released into the Columbia River Mainstem below Wells Dam will be prioritized at the Wells Hatchery volunteer trap. Trapping at the volunteer channel may occur up to 7 days per week, 24 hours per day and may include broodstock collection and/or adult management.



Memorandum

TO: Wells HCP Hatchery Committee
FROM: Betsy Bamberger, Douglas PUD
SUBJECT: Egg Thiamine Results and Interpretation BY23
DATE: November 8, 2023

In the spring of 2023, roughly five grams of green eggs were collected from each of 40 Columbia Safety Net (CSN) summer steelhead at Wells Fish Hatchery (WFH) and shipped to the United States Geological Survey (USGS) Columbia Environmental Research Center for total thiamine analysis. This effort is part of a three-year monitoring study to determine baseline thiamine concentrations in Interior Columbia Basin salmon runs believed to be susceptible to thiamine deficiency complex (TDC) (see previous memorandum distributed on February 22, 2023 to the Wells HCP Hatchery Committee, Attachment 1).

The egg total thiamine results were analyzed and compared to published reference ranges. Research suggests that the concentration of thiamine needed in eggs to avoid TDC-induced mortality of hatched fry is varied, even in studies involving multiple sample locations and years. This inconsistency may be influenced by familial or population-level effects (adaptive genetics or epigenetic variation) or environmental conditions that could impact egg development or maturation (such as maternal diet) (Harder et al., 2018). Interpretations of results should therefore be approached with caution.

Of the 40 egg samples analyzed in 2023, six (15%) fell below the 6.54 nmol/g total thiamine (TTH) LD50* threshold widely held to be the standard (and only) benchmark for TDC in steelhead (Futia et al. 2019) (Figure 1). By contrast, 24 of the 40 females sampled (60%) produced eggs with at least 10 nmol/g of TTH, the amount required to be considered fully thiamine replete in lake trout (Fitzsimons et al. 2009).

There is evidence that suboptimal thiamine levels can impact fry development even if overt mortality is avoided. One study involving lake trout concluded that sublethal effects (such as reduced foraging effectiveness and predator avoidance) can be impactful at an EC50[†] of 7.4-10 nmol/g TTH (Ivan et al. 2018); sixteen of the fish tested (40%) here produced eggs with TTH of less than 10 nmol/g. The average TTH of all 40 females tested was 10.86 nmol/g.

* The concentration of a given substance that would be considered lethal to 50% of the population. In this context, the estimated level of thiamine present in eggs that would result in the expiration of half of hatched-out fry.

† In this context, the concentration of thiamine that can induce nonlethal (but adverse) effects. More technically, the concentration of a given substance that elicits half-maximal effective response.

Further action at the hatchery level will be informed by the scheduled sampling of CSN eggs and evaluation of thiamine and survival data in 2024 and 2025. These results should further support our understanding of the baseline and range of thiamine levels in eggs used in upper Columbia Basin steelhead broodstock including within-year and multi-year variation.

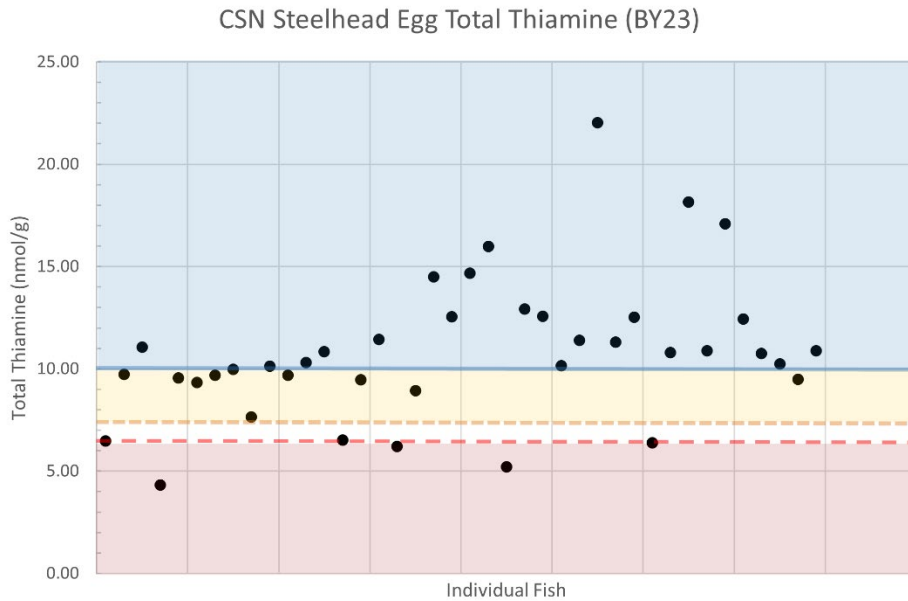


Figure 1. Total thiamine results of BY23 CSN steelhead eggs tested at WFH. Shaded areas correspond to categories defined by published reports in steelhead and lake trout: replete (blue with solid line at lower threshold, n=24), insufficient with possible sublethal effects (yellow with dashed line at lower threshold, n=10), and deficient (red with dashed line at upper threshold, n=6).

References

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Futia M.H., Rinchar J. (2019) Evaluation of Adult and Offspring Thiamine Deficiency in Salmonine Species from Lake Ontario. *J. Gt. Lakes Res.* 45: 811–820.

Harder A.M., Ardren W.R., Evans A.N., Futia M.H., Kraft C.E., Marsden J.E., Richter C.A., Rinchar J., Tillitt D.E., Christie M.R. (2018) Thiamine deficiency in fishes: causes, consequences, and potential solutions. *Reviews in Fish Biology and Fisheries* 28: 865–886

Ivan L.N., Schmitt B.R., Rose K.A., Riley S.C., Rose J.B., Murphy C.A. (2018) Evaluation of the thiamine dose-response relationship for lake trout (*Salvelinus namaycush*) fry using an individual based model. *Journal of Great Lakes Research* 44: 1393–1404.

ATTACHMENT 1

**MEMORANDUM REGARDING EGG THIAMINE TESTING PREPARED BY
DOUGLAS PUD FOR DISTRIBUTION TO THE WELLS HCP HATCHERY
COMMITTEE ON FEBRUARY 22, 2023**



Memorandum

TO: Wells HCP Hatchery Committee
FROM: Betsy Bamberger, Douglas PUD
SUBJECT: Egg Thiamine Testing
DATE: February 21, 2023

Thiamine deficiency complex (TDC) in salmonids is a vitamin B1 deficiency that results in various neurological problems and overt mortality in both adults and their progeny. It is hypothesized that shifts in the relative abundance and consumption of certain oceanic prey species is causing an increase in TDC in the Pacific Northwest. The incidence and severity of TDC in the Interior Columbia Basin is not currently well understood and regional data is limited. Douglas PUD proposes a monitoring effort not to exceed three years to determine baseline thiamine concentrations in the eggs of summer run Steelhead, a species believed to be particularly susceptible to TDC and locally protected under the Endangered Species Act. Initial sampling efforts will target hatchery-origin fish from the Columbia Safety Net (CSN) program at Wells Hatchery. Successful thiamine supplementation, via hen injection or egg immersion bath, is possible in hatchery-reared populations if concentrations are deemed low or deficient.

Douglas PUD has contracted with the United States Geological Survey (USGS) Columbia Environmental Research Center to test for total thiamine levels in eggs. Douglas PUD will collect and ship roughly 5 grams (or roughly 50) of green eggs from each of at least thirty and up to 60 females annually, starting in calendar year 2023 through 2025. Douglas PUD will reassess the number of necessary eggs per female following the results of the 2023 testing, and prior to spawning of the CSN Steelhead in 2024. Sample collection and shipment will be in accordance with USGS protocols.

USGS Columbia Environmental Research Center will assess free thiamine (TH), thiamine monophosphate (TMP), and thiamine pyrophosphate (TPP) as described by Futia et al. (2017) on samples shipped annually for Douglas PUD. For thiamine extraction, approximately one gram of unfertilized egg tissue will be extracted and be run in duplicate. Thiamine levels will be determined using a high-performance liquid chromatograph (HPLC) system (Agilent Technologies 1100 series). Concentrations from the three thiamine vitamers will be summed to determine total thiamine concentrations in nmol/g. This information will be reported to Douglas PUD no later than 6 months from date of email notification of shipment details. Results will be shared with the Wells HCP Hatchery Committee. Further action at the hatchery level will be informed by the interpretation of the results and the observations of egg and fry survival from 2023 through 2025.



DEPARTMENT OF NATURAL RESOURCES

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Memorandum

TO: Wells HCP Hatchery Committee Reps
FROM: John Rohrback, Douglas PUD
SUBJECT: MetCon Steelhead Escape at Wells Hatchery
DATE: October 31, 2023

Observation

Public Utility District No. 1 of Douglas County (Douglas PUD) receives the Methow River Conservation (MetCon) steelhead program from Winthrop National Fish Hatchery as eyed eggs. These fish are reared and tagged at Wells Hatchery until their release into the Methow River. Tagging of these fish began on or about October 17, 2023. At the initiation of tagging, Douglas PUD assumed an inventory of 20,980 MetCon steelhead within the circular tank based on the eyed egg transfer count and subsequently recorded mortality. The MetCon program tagging concluded on October 23, 2023, and provided a firm count of 17,330 steelhead. This number resulted in 3,650 unaccounted summer steelhead and leaves the current inventory for MetCon steelhead below the release target of 20,000 smolts (17,111 NNI + 2,889 inundation¹).

Identified Cause

Following tagging, as the circular tank in which the steelhead had been held was being dewatered, Douglas PUD staff observed a gap at the base of the side screen of the overflow sump (Figure 1). Douglas PUD staff concluded that this gap provided an escape route for the missing MetCon steelhead, which were determined to have been inadvertently released to the mainstem Columbia River through the Wells Hatchery plant drain. Approximately ten steelhead were observed swimming in the sump behind the screen upon drawdown of the circular tank, further reinforcing the conclusion that fish were able to get behind the screen.

¹ Wells HCP Hatchery Committee Statement of Agreement. Douglas PUD Hatchery Compensation, Release Years 2024-2033. Approved July 20, 2022



Figure 1. Gap at the base of the side screen of the overflow pump.

Modification or Fix

Douglas PUD staff have rectified the issue by bolting together aluminum plating on either side of the base of the sump screen (see Figures 2 and 3). Prior to installation of this fix, the sump screen had been affixed to the circular tank only vertically, on both sides. The compressive force of these new plates is anticipated to prevent future development of a gap at the base of the screen and eliminate the possibility of escape through this route. This fix will be implemented on all the circular tanks.



Figure 2. Sump screen fix viewed from the inside of a circular tank.



Figure 3. Sump screen fix viewed from the outside of a circular tank.

EASTBANK LIVE-SPAWNING STUDY

Summary of Results and Responses to Anticipated Questions

This document is intended to be an “at a glance” resource for providing some key information gleaned from WDFW’s data.

BACKGROUND

- Live-spawning took place between 12/27/2022 and 3/7/2023.
- A total of 52 females were spawned.
- Females were air-spawned first and then lethally spawned to collect any retained eggs.

METHODS

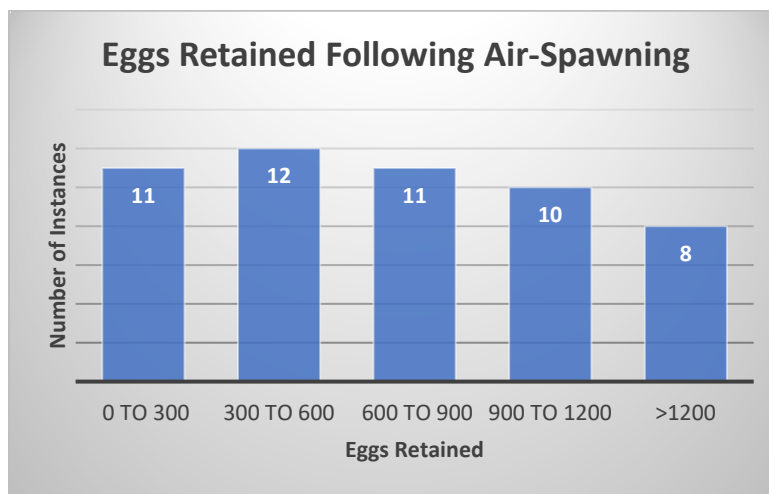
- Air-spawning works best when females are at peak gravidity.
 - Egg take may be improved with we could refine how maturation is assessed
 - Would it be possible to categorize females by ripeness?
 - Even two categories would be informative
 - Example – Definitely ripe and somewhat ripe or fully soft and partially soft
 - When we spawned at Methow Salmon Hatchery, they would often hold fish another week if they weren’t sure it was fully gravid, with good results
- On January 31, 2023, there was a change in where the fish were spawned and how they were held
 - Early on, fish were held by staff standing in pond
 - Made it difficult hold vertical
 - Harder to get the eggs higher up in skein
 - Shift was made to hold fish standing on the deck
- Equipment changes after the first few spawns
 - WDFW initially used a different type of compressor and regulator
 - Switched to using our equipment have several spawns of them having difficulty controlling air pressure
- Air spawning was done by multiple different staffers
 - It’s understandable that staff needs to train with the method as it is new to many
 - However, it may add variability to the data
 - For study purposes, WDFW might consider using same staff for air spawning
 - Alternatively, staff conducting spawning could be included in the data collection
 - Could use ID numbers
 - One way to reduce an unknown variable

GREEN EGGS

- The total number of green eggs and the median eggs per female value summarized for each spawning method below. Median was chosen over mean because lethally spawned egg take data was not normally distributed.

Green Eggs	Air Spawned Egg Take	Lethal Spawned Egg Take	Combined
Total	268,522	39,006	307,528
Median	5,276	660	5975

- There was a wide range in the number of eggs retained following air-spawning.



- The lowest number of retained eggs was **30** and the highest was **1,947**.

EYED EGGS

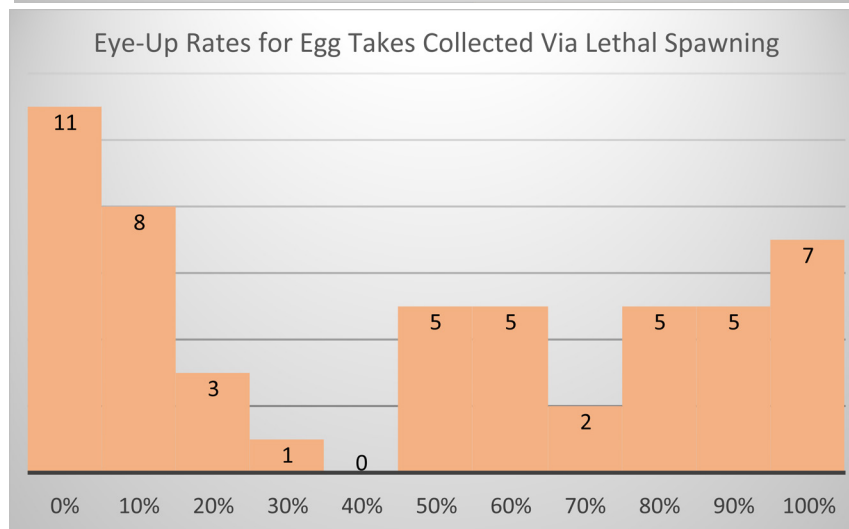
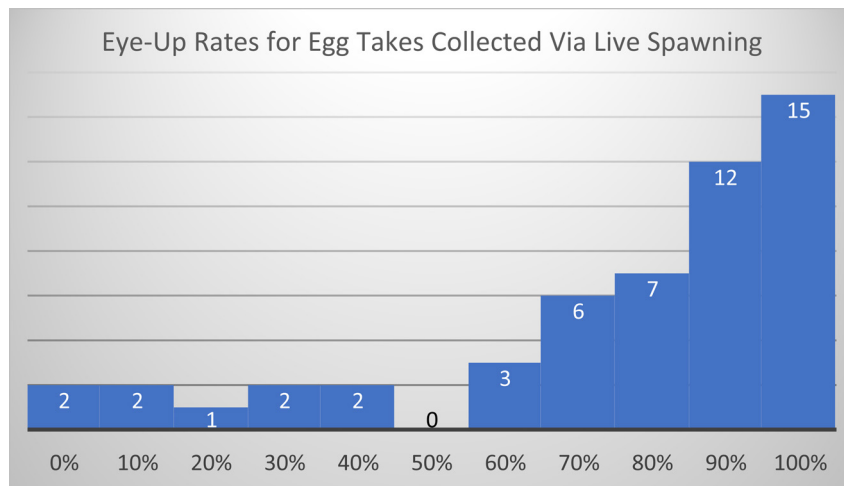
- Eggs collected via air-spawning were incubated separately from those collected via lethal spawning.
- The total and median number of eyed eggs for each spawning method are below. Median was chosen over mean because lethally spawned egg take data was not normally distributed.

Eyed Eggs	Air Spawned Egg Take	Lethal Spawned Egg Take	Combined
Total	188,605	19,969	208,574
Median	3,979	211	4,116

- There were two females whose air spawn take produced zero eyed eggs.
- There were 11 females whose lethal spawn take produced zero eyed eggs.
- Eggs collected via air-spawning had a higher rate of eye up than retained eggs collected via lethal spawning.

- I've often suspected that eggs trapped in the skin or high up in the abdomen, one that are most often missed during air-spawning, were not fully ripe or mature and are more likely to die.

Eyed Up Rate	Air Spawned Egg Take	Lethal Spawned Egg Take	Combined
Total	70.2%	51.2%	67.8%
Mean	70.4%	42.1%	67.8%



- There were 10 females where the eye up rate was greater in the lethally collected eggs
- The difference in eye up rate was $\geq 10\%$ in only five of those females

CONCLUSIONS

- Air-spawning will likely reduce overall egg take
- The fact that air-spawned eggs have a higher green to eyed-egg survival mitigates some of the egg loss
- It may be possible to reduce egg retention with experience and technique refinement
- Future studies may benefit from including additional variables in the data collection and analysis
 - Categorizing female gravidity
 - Changes in technique
 - Changes in equipment
 - Participating personnel

WDFW BY2023 Wenatchee Steelhead Air-Spawning Evaluation

November 14, 2023



**Prepared by WDFW:
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Introduction

Chelan County Public Utility District in coordination with Yakama Nation Fisheries have proposed a kelt reconditioning program for the conservation component of Wenatchee steelhead.

Incorporation of kelt reconditioning for the natural-origin component of the Wenatchee steelhead program would provide an opportunity for natural-origin broodstock to contribute directly to natural production. The most effective method of live spawning females for kelt reconditioning and subsequent release is air-spawning or extracting eggs from live females using compressed air. Air-spawning is a minimally invasive method to express ripe eggs from live females for rearing in the hatchery environment as opposed to the more traditional method of lethal spawning. The air-spawn method has successfully been implemented on other upper Columbia conservation steelhead programs to allow for the broadest genetic contribution of a given natural-origin individual possible and not be relegated solely to hatchery production.

The Wenatchee steelhead conservation program differs from other locally operated conservation programs in a multitude of ways, which may affect the success of utilizing the air-spawn method for kelt reconditioning. Wenatchee steelhead broodstock are collected 2 to 7 months prior to hatchery spawning, increasing the amount of time that adults reside in the hatchery ponds. Other differences potentially affecting the naturally occurring maturation processes of natural spawn timing include altered water temperatures due to Eastbank Hatchery aquifer water source, and modified photo periods due to pond coverage. Wenatchee steelhead are spawned 2 to 3 months earlier in the hatchery than their counterparts in the natural environment (Figure 1). In order to meet hatchery spawn timing needs, Lutening Hormone-Release Hormone (LHRHa) is occasionally used to synchronize maturation for spawn.

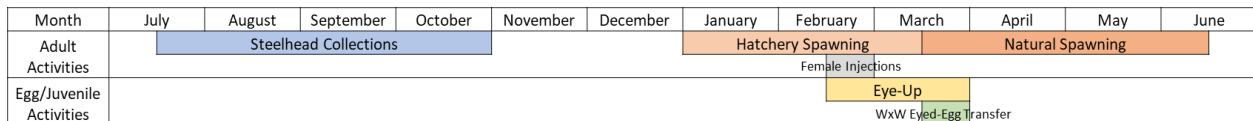


Figure 1. Wenatchee summer steelhead broodstock collection and spawning timeline.

For brood year 2023, WDFW was requested to evaluate the practice of Wenatchee steelhead air-spawning at Eastbank Hatchery. If adopted, only natural-origin females will undergo kelt reconditioning using the air-spawn method. However, this evaluation included both natural and hatchery-origin females to increase the sample size while allowing us to compare the efficacy of this method. Throughout the report, natural-origin and conservation program are used interchangeably, as is hatchery-origin and safety net program.

Our 2023 evaluations include enumeration of total green-eggs and eyed-eggs collected by air-spawning compared to residual eggs (remaining eggs collected after air-spawning). Fecundity per female was compared between eggs collected via air-spawn and total eggs (air-spawn + residual eggs). Additionally, we gathered data on green to eyed-egg survival from air-spawned eggs collected and compared them to survival of residual eggs. We compared these metrics (egg take and egg survival) between both the safety net and conservation programs. We also address the total eggs potentially lost due to incomplete spawning and pre-spawn mortalities. Green-egg take for both the conservation and safety net programs were compared to adjusted targets to better understand the impact of implementing the air-spawn method may have on achieving mitigation production requirements. Finally, we estimated the number of additional broodstock pairs required to produce the same egg take using only the air-spawn method.

Methods

2023 Wenatchee River steelhead broodstock were collected at Dryden and Tumwater Dams from 13 July to 1 November 2022. Natural and hatchery-origin fish are identified by marks and tags. Hatchery-origin steelhead are targeted for collection at Dryden Dam, while natural-origin steelhead are targeted for collection at Tumwater-Dam. Natural and hatchery-origin broodstock were held at Eastbank Hatchery in a single pond until spawning. Spawning operations commenced on 27 December 2022 and fish were inspected weekly for spawning readiness until 22 March 2023 (Figure 1). Females of natural and hatchery-origin steelhead were of similar size (N= 52; Range 1.05 – 3.55 kg).

The LHRHa injections were used to accelerate gonadal maturation of males and females to compress the spawning season to achieve a smolt release target size of 6 fpp (fish per pound) at release. A portion of the males and females were injected with LHRHa into the dorsal sinus. The number of injections and the total dose varied between males and females. Within each sex a maximum dose of 125 ug/kg was utilized. Males were injected throughout the spawning season with individual injections ranging from one to three times. Females were injected in the final weeks of the spawning period and nearly all ovulated following a single injection.

On each spawn date, male and female steelhead were sorted to assess spawning maturity. Hatchery staff crowded all fish in the pond, each fish was then placed into a tank and lightly sedated using Aqui-S (20 mg/L) to determine spawn maturation. If ready, ripe females were placed into a holding net pen. All males were placed into a separate net pen to await spawn. Once all fish were checked, presumptive female spawners were completely sedated in Aqui-S solution and checked a second time by the most experienced hatchery staff to confirm readiness to spawn.

Ripe females were brought to the spawning platform one at a time adjacent to the pond where the air-spawn process was initiated. Air-spawning methods generally followed those developed by the Yakama Nation Fisheries kelt reconditioning program as mentioned in “Upper Columbia River Steelhead Kelt Reconditioning Project” by Abrahamse and Murdoch (2015). Female steelhead were held over a bucket and a 16-gauge, 2.54 cm syringe needle was inserted perpendicularly at the mid-point between the two pelvic fins. Compressed air was released at 5-7 psi to express the eggs for approximately 3 seconds. (See Appendix A for equipment description.) The hatchery specialist holding the fish massaged the abdomen in a downward motion to remove air and any additional eggs from the fish. These steps were repeated until eggs were no longer expressed. Air voided eggs were placed into a zip-lock bag labeled with fish origin, fish identification number, and the label “Air”. The bag was stored in a cooler until egg collection from all females was completed. Following the air spawn process, each female was lethally spawned to remove any residual eggs for enumeration. Females were opened ventrally using a Wyoming knife and any eggs collected in a bucket. These eggs were also placed in zip-lock bags and labeled with fish origin, fish identification number, and the label “Residual”. They were then placed in a cooler until eggs from all females had been completed. Once female egg quality was examined, the appropriate number of mature males were assessed for milt expression. Milt was manually expressed into zip-lock bags with oxygen and stored in coolers until combined with eggs.

Spawning crosses for air-spawn and residual eggs used the same combination of gametes from male(s) and females. Slight differences between the conservation program and the safety net program were implemented. The conservation program employed a reciprocal approach of a 2x2

factorial cross (two males crossed with two different females). The safety net program employed a 1x1 factorial cross (one male crossed with one female). Hormone injected males were all assessed for motility prior to crosses. For fertilization, milt is poured in the zip-lock bag containing the eggs and hand mixed, then allowed to fertilize for approximately 2 minutes. Fertilized eggs were then placed in Heath trays in an iodophor bath while half-way suspended out of the stack for approximately 1 hour. The trays were slid into the stack with water running through the stack to flush the iodophor treatment. A single tray for each female was used for air-spawned eggs. Residual eggs were reared in divided trays to maintain separation. The first group of eggs were incubated on chilled water while later spawn groups were incubated on warmer water.

After roughly 350-400 TU, or when a good visible eye was present, the eggs were shocked. Two days post-shocking, any non-viable eggs were removed from the tray and number of nonviable eggs were recorded. The remaining viable eyed-eggs were run through an optical counter two times to confirm the eyed-egg count. Once confirmed, the eyed-eggs for the Conservation program were transferred to Chelan PUD FH for final incubation and rearing until acclimation. The Safety Net program remains at Eastbank FH until transfer to acclimation.

Data Analysis

Air-spawned and total (air-spawned and residual) green-egg were recorded as the sum of non-viable eggs plus live eyed-eggs remaining. Total eyed-egg count was determined by the optical counting method and was summed across all females for both conservation and safety-net program. Percent of total eggs that were extracted via air-spawn method was calculated. Fecundity was calculated as eggs per female between air-spawned, residual, and total green-eggs. Differences in fecundity between air-spawned and total green-eggs were compared using a Wilcoxon signed rank test. Percent green to eyed-egg survival of air-spawn and residual components were calculated for each female. Differences in percent survival among air-spawned eggs and residual eggs were compared using a Wilcoxon signed rank test. Significant differences among metrics were determined using $p < 0.05$. Number of females needed to replace the egg take lost by utilizing only air-spawn methods was calculated by dividing number of residual eggs by median fecundity per female. Although minimal differences were observed between the conservation and safety-net programs, data was presented for both because different methods are utilized for each program, and M&E evaluates these programs separately.

Results

Broodstock collected, spawned, and pre-spawn mortalities

Table 1. Number of collected, spawned, and pre-spawn mortalities by program. Collection target from 2022 Broodstock Collection Protocol.

Program	# Collected (includes green fish released)	Collection Target	# Spawn Females	# Spawn Males	# Female Pre-spawn Mortalities	# Male Pre-spawn Mortalities
Conservation	60 (29 F/ 31 M)	62 (31 F/ 31 M)	23	25	6	6
Safety Net	54 (30 F/24 M)	60 (30 F/ 30 M)	29	23	1	1
Total	114 (59 F/ 55 M)	122 (61 F/ 61 M)	52	48	7	7

- BY 2023 broodstock collection target not met for conservation program or safety-net program
- 52 females crossed with 48 males across both programs
- 7 female pre-spawn mortalities and 1 female released account for difference between collection number and number spawned
- Due to the low number of adipose present cwt fish available 14 (5 females, 9 males) adipose clipped CWT were taken for broodstock
- Safety net collections took place at Tumwater and Dryden dams due to the low number of hatchery-origin fish

Green-egg Take: Air-spawn, residual, and total eggs

Table 2. Adjusted green-egg take target, green-egg take for air-spawn, residual, and total eggs, percent of air-spawn and residual eggs to total eggs collected for conservation and safety-net program.

Program	Adjusted Egg Take Target ¹	Air-Spawn Egg Take	Residual Egg Take	Total Egg Take	% of Air-Spawn Eggs	% of Residual Eggs
Conservation	128,754	113,187	16,081	129,268	87.6%	12.4%
Safety Net	165,358	155,335	22,925	178,260	87.1%	12.9%
Total	294,112	268,522	39,006	307,528	87.3%	12.7%

¹Adjusted green-egg take target calculated using number of BY2023 females spawned x average fecundity from the 2022 Broodstock Collection Protocol.

What percent of total eggs were extracted via air-spawn?

- Air-spawning achieved 87.3% of total green-egg take combined
- Median air-spawned green-egg take per female for both programs combined was 5,276
- Median air-spawned green-egg take per female for conservation program was 4,762
- To produce the same combined (conservation + safety-net) green-egg take (307,528) using just the air-spawned method we would need to collect and air-spawn an estimated 7.4 ($39,006/5,276=7.4$) additional pairs
- To produce the same green-egg take for the conservation program (129,268) using just the air-spawned method we would need to collect and air-spawn an estimated 3.4 ($16,081/4,762=3.4$) additional pairs
- There was no difference in percent of air-spawn egg take or residual egg take to total egg take between conservation and safety net programs

Was the adjusted green-egg take target achieved?

- Adjusted green-egg take target was met for safety-net air-spawned eggs ($\pm 10\%$ of adjusted egg take target)
 - Air-spawn 94%
 - Total eggs 108%
- Adjusted green-egg target was not met for conservation air-spawned eggs, but was met with total eggs ($\pm 10\%$ of adjusted egg take target)
 - Air-spawn 88%
 - Total eggs 100%

Fecundity: Air-spawn and total eggs per female



Figure 2. Box and whisker plot of green-egg fecundity for air-spawned and total take per female.

- Proportionally most of the eggs are extracted via air-spawn method
- The total spawn egg take was significantly larger than the air-spawn method (Wilcoxon signed rank test: $p < 0.001$)
- Residual eggs ranged from 30 to 1,947 among all females (Figure 3)

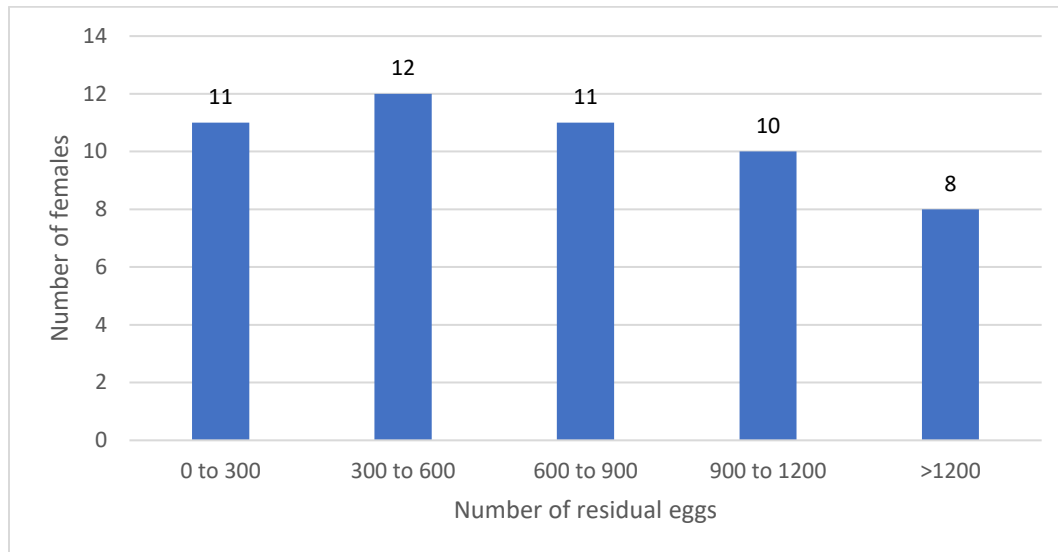


Figure 3. Number of females with residual green eggs.

Eyed-Egg Take: Air-spawn, residual, and total eggs

Table 3. Eyed-egg take target, eyed-egg take for air-spawn, residual, and total (air-spawn + residual) counts for the conservation and safety net programs. Eyed-egg targets are from the 2022 Broodstock Collection Protocol.

Program	Eyed-Egg Target	Air-spawn	Residual	Total
Conservation	138,641	80,025	7,137	87,162
Safety-net	133,309	108,580	12,832	121,412
Total	271,950	188,605	19,969	208,574

- Air-spawning achieved 90.4% of total eyed-egg take
- Median air-spawned eyed-egg take per female for both programs combined was 3,979
- Median air-spawned eyed-egg take per female for conservation program was 3,666
- To produce the same combined (conservation + safety-net) eyed-egg take (208,574) using just the air-spawned methods we would need to collect and air-spawn an estimated 5.0 ($19,969/3,979=5.0$) additional pairs
- To produce the same eyed-egg take for the conservation program (87,162) using just the air-spawned methods we would need to collect and air-spawn an estimated 1.9 ($7,137/3,666=1.9$) additional pairs

Percent Survival: Green-egg to eyed-egg

Table 4. Median percent of green-egg to eyed-egg survival for air-spawn, residual, and total (air-spawn + residual) eggs for the BY 2023 conservation, safety-net and combined (total) programs. Median (min/max) percent for the BY 2011-2021. Survival target is from the 2022 M&E Annual Report.

Program	Survival Target	Air-spawn	Residual	Total	BY2011- 2021
Conservation	92%	82%	43%	75%	85% (67%-91%)
Safety-net	92%	80%	50%	68%	88% (74%-96%)
Total	92%	81%	43%	77%	86% (67%-96%)

- Percent survival was higher in air-spawn eggs vs residual eggs ($p < 0.001$; Wilcoxon signed rank test)
- Variability in percent survival was larger for residual eggs vs air-spawned eggs (Figure 4)
- Survival of air-spawn eggs and residual eggs was similar between the conservation and safety-net programs
- Green- to eyed-egg survival target not achieved for BY2023 air-spawn or residual eggs in either program
- BY2023 median survival was lower than the 11-year median survival
- Conservation program has not met the target in the last 11 years (BY2011- BY2021)
- Safety-net program has not met target 7 out of the last 11 years (BY2011- BY2021)

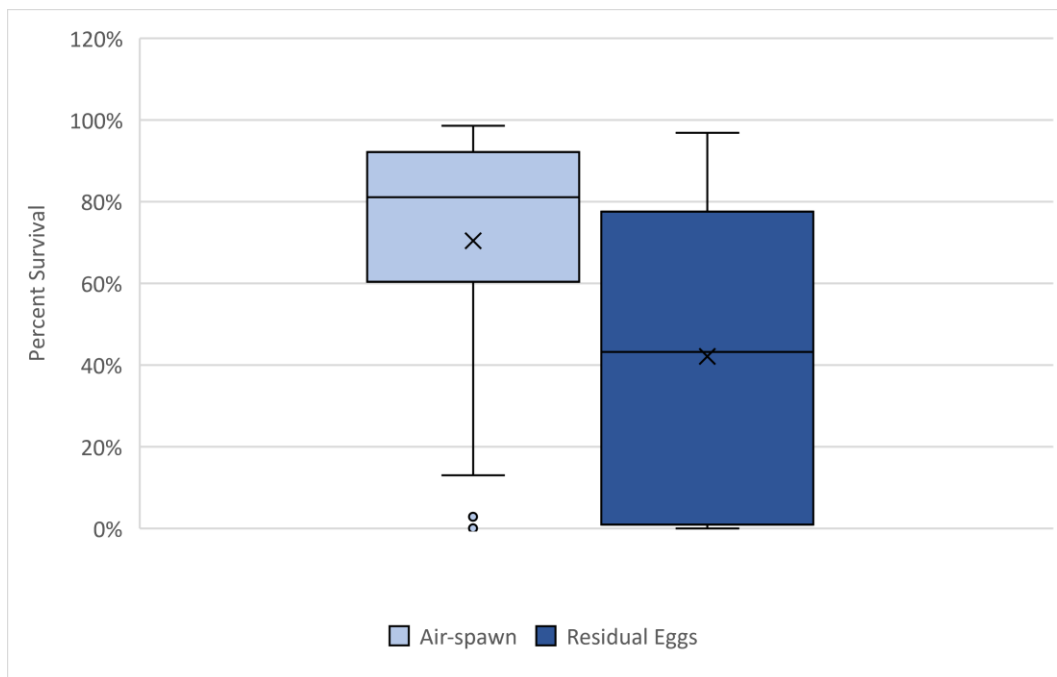


Figure 4. Box and whisker plot of percent green to eyed-egg survival between air-spawn and residual eggs.

Percent survival of eggs by female

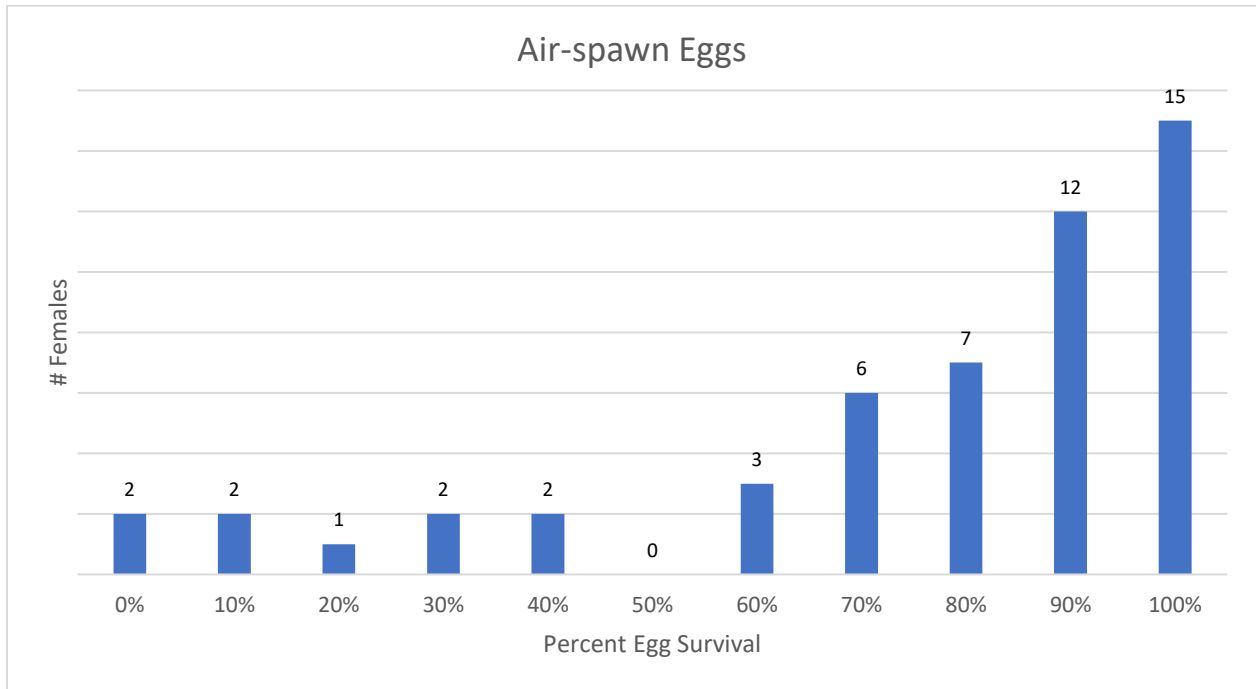


Figure 5a

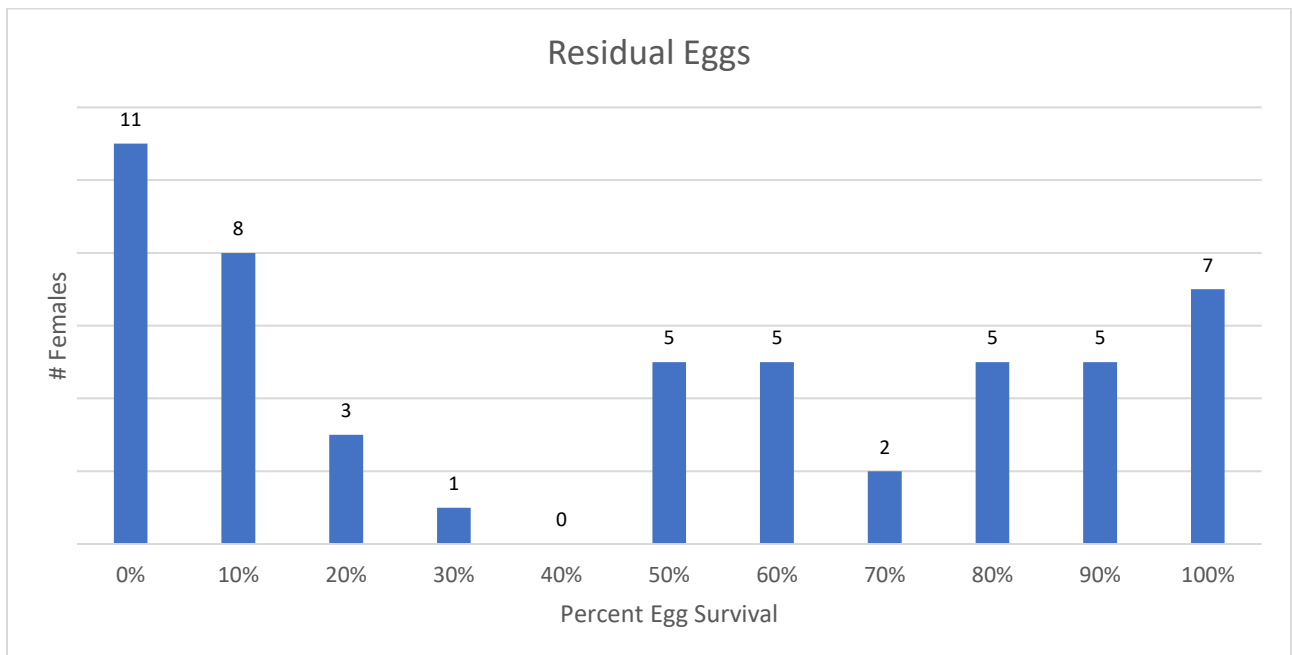


Figure 5b

Figure 5a and 5b. Distribution of percent survival for air-spawn eggs (5a) and residual eggs (5b) by number of females.

- 12 females had 0% survival in either air-spawn or residual eggs
 - One female had 0% survival for both air-spawn and residual eggs which is counted twice in both the air-spawn and residual egg (Figure 5a and 5b)
 - One female had higher percent survival for residual eggs than air-spawn eggs
 - 10 females had higher survival for air-spawn eggs than residual eggs
- Residual egg group had more eggs with 0% survival than air-spawn egg group
 - Residual eggs from 19% (10 of 52) females had 0% survival
 - Air-spawn eggs from 4% (2 of 52) females had 0% survival

Conclusions

Overall, the air-spawning method contributed the majority of eggs to total green-egg take (87.3%) and to total eyed-egg take (90.4%). The air-spawning method produced 39,006 fewer green-eggs overall and a median of 660 (range 30 to 1,947) fewer green-eggs per female than would have been collected via traditional lethal spawn methods (i.e. total egg take). This trend translated to eyed-egg take, where air-spawning produced 19,969 fewer eyed-eggs and a median of 210 (range 0 to 1,743) fewer eyed-eggs per female than total eyed-egg take. The percentage of air-spawned eggs to total eggs increased from green- to eyed-egg because the egg survival was greater with air-spawned eggs (81%) than residual eggs (43%). Both air-spawned and residual eggs had variability in survival, with higher variability in survival of residual eggs. These results indicate variable egg survival among individual females, especially for the residual eggs. Despite extracting fewer eggs via the air spawn method compared to the traditional lethal method, the air spawned eggs did have higher survival than residual eggs.

To produce the same combined green-egg take (307,528) using just the air-spawned method we would need to collect and air-spawn an estimated 7.4 additional pairs. To produce the same green-egg take for the conservation program (129,268) using just the air-spawned method we would need to collect and air-spawn an estimated 3.4 additional pairs. To produce the same combined eyed-egg take (208,574) using just the air-spawned methods you would need to collect and air-spawn an estimated 5.0 additional pairs. To produce the same eyed-egg take for the conservation program (87,162) using just the air-spawned method we would need to collect and air-spawn an estimated 1.9 additional pairs.

The adjusted green-egg take target was met with air-spawned eggs for the safety-net program (94% of target) and total eggs for the conservation program (air-spawned + residual; 100% of target). The adjusted green-egg take target was not met for the conservation program with air-spawned eggs (88% of target). The green- to eyed-egg survival target was not met for either the air-spawned eggs or the residual eggs and was lower than the previous 11-year median. Variables noted which may have influenced total egg take included not reaching broodstock collection target and higher than usual pre-spawn mortality. A total of 7 female pre-spawn mortalities (1 safety net, 6 conservation) limited available green-egg take by an estimated 34,374 eggs. While this affected total egg take numbers, it does not influence the evaluation of the air-spawning method.

Appendix A

<i>Equipment</i>	<i>Details/Specifications</i>	<i>What We Use</i>
Air Compressor	120V AC, 150psi max pressure	Ridgid 6 gallon (Model OF60150HB)
Regulator	¼ in NPT, 14 cfm, 5 psi to 125 psi	Black Diamond (Model BD019-0167RP)
Filter	Pressure gauge, up to 150 psi,	Forney (Model 75545)
Gauge	Pressure gauge, up to 150psi,	Forney (Model 75565)
Air gun	Air gun, thumb lever grip, ¼ in NPT female	Husky, Air Gun Kit (Model 1000-386-948)
Barb Fittings	Misc. ¼ in NPT male and female fittings and barbs to fit air gun outlet	
Hose	Air hose, length from compressor regulator & another from regulator to air gun	
Surgical Tubing	Surgical Tubing, sized to fit snug over barb fitting and syringe base	
Needle and syringe	16-gauge, 1 inch needle; 3 ml syringe. Cut base off syringe to allow tubing to stretched over it.	
Various hose fittings	Misc. quick coupler fittings	
Timer	Stopwatch to record time fish is out of water	

Observations from the field

- Changed compressor and regulator to get more consistent PSI
- Changes to sedation methods
 - Lower concentrations of Aqui-S
 - Refreshed sedation tank more often
 - Minimize number of times each fish is sedated
 - Minimize time that each fish was sedated
 - Some mortalities occurred after the first spawn
- Faster spawning on the deck than in the pond (position of fish)
- Bag in the bucket to minimize egg impact
- Improved handling technique through spawning events
- Motility measured for every male spawned, not just those that were LHRH injected (take into account low milt supply)