

Date: August 21, 2019

Memorandum

To: Wells, Rocky Reach, and Rock Island HCPs Hatchery

Committees and Priest Rapids Coordinating

Committee Hatchery Subcommittee

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

Facilitator

cc: Larissa Rohrbach, Anchor QEA, LLC

Re: Final Minutes of the July 17, 2019 HCP Hatchery Committees and PRCC Hatchery

Subcommittee Meetings

The Wells, Rocky Reach, and Rock Island Hydroelectric Projects Habitat Conservation Plans (HCPs) Hatchery Committees (HCs) and Priest Rapids Coordinating Committee Hatchery Subcommittee (PRCC HSC) meetings were held in Wenatchee, Washington, on Wednesday, July 17, 2019, from 9:00 a.m. to 2:00 p.m. Attendees are listed in Attachment A to these meeting minutes.

Action Item Summary

Joint HCP-HCs and PRCC HSC

- Tracy Hillman will review aspects of the Independent Scientific Advisory Board's Review of Spring Chinook Salmon in the Upper Columbia River under HCP-HCs' purview (Item I-A). (Note: this item is ongoing.)
- Mike Tonseth will coordinate with Andrew Murdoch (Washington Department of Fish and Wildlife [WDFW]) to present pre-spawn mortality modeling results for spring Chinook salmon at an upcoming HCP-HC meeting (Item I-A). (Note: this item is ongoing.)
- Catherine Willard will update the genetics section of the *Monitoring and Evaluation (M&E) Plan* for PUD Hatchery Programs (Update to the 2017 Plan) based on the genetics panel recommendations and will append the recommendations from the panel to the plan (Item I-A). (Note: this item is ongoing.)
- Kirk Truscott will discuss with Colville Confederated Tribes (CCT) biologists whether elemental signature analysis could differentiate natural-origin Okanogan spring Chinook salmon from other natural-origin Chinook salmon during broodstock collection at Wells Dam for Methow Fish Hatchery programs (Item I-A). (Note: this item is ongoing.)
- Brett Farman will discuss with Charlene Hurst and Mike Tonseth the potential use of a multipopulation model for estimating proportionate natural influence (PNI) for the Nason and Chiwawa spring Chinook salmon programs (Item I-A). (Note: this item is ongoing.)

- Mike Tonseth will revise the Relative Reproductive Success (RRS) Study extension agreement memorandum for clarity (Item I-A). (Note: this item is ongoing.)
- Larissa Rohrbach will add sizing of upper Columbia River conservation programs as a periodic agenda item (Item I-A). (Note: this item is ongoing.)
- Tracy Hillman and Larissa Rohrbach will maintain a list of outstanding topics, as follows, for consideration in HCP-HCs and PRCC HSC meetings prior to development of the 2020 Broodstock Collection Protocols (Item I-A). (Note: this item is ongoing.)
 - Use of age-3 males in broodstock
 - Use of alternative mating strategies
 - Establishing ranges around broodstock collection targets
 - Source for Chiwawa spring Chinook salmon broodstock
- Tracy Hillman and Larissa Rohrbach will add review of the Broodstock Collection Protocols to the September meeting agenda to help the HCP-HCs and PRCC HSC identify co-authors and opportunities to discuss major revisions in advance of 2020 deadlines (Item I-A). (Note: this item is ongoing.)
- Mike Tonseth will revise and redistribute the HCP-HCs Annual Broodstock Collection Protocols
 development timeline Statement of Agreement (SOA; Item I-A). (Note: this item is ongoing.)
- Mike Tonseth will ask Michael Humling (U.S. Fish and Wildlife Service [USFWS]) and Charlie Snow (WDFW) to estimate the number of Methow returns that are likely to return to Winthrop National Fish Hatchery to inform a translocation discussion in a future HCP-HCs meeting (Item I-A). (Note: this item is ongoing.)
- Mike Tonseth will revise and redistribute the 2017 Out-planting Surplus Methow Composite Spring Chinook Salmon Adults memorandum (Item I-A). (Note: this item is ongoing.)
- Larissa Rohrbach will add HCP Policy Committee guidance on policy-level issues to the HCP-HC Meeting Protocols (Item I-B). (Note: this language was added to the protocols under the section titled "Disputes and Conflicts." The updated version was saved to the HCP-HC Extranet Site and PRCC HSC SharePoint sites, dated July 17, 2019.)
- Betsy Bamberger and Greg Mackey will distribute a draft 2020 study plan for *The Control of Saprolegnia Sp. Growth on Summer Chinook (Oncorhynchus tshawytscha) Eggs* (Item II-E).
- Greg Mackey will distribute a white paper reviewing broodstock composition and mating strategies for conservation programs, focusing on spring Chinook salmon at the Methow Hatchery (Item II-D). (Note: this item is ongoing.)

Wells HC

Mike Tonseth will prepare a proposal for the Wells HCP-HC on the use of surplus summer
Chinook collected from the Wells Volunteer Trap for the production of subyearling smolts to
support the Southern Resident Killer Whale population (Item III-B).

PRCC Hatchery Subcommittee

- HSC representatives will submit a list of minimum data or information needs for making a
 decision on the White River spring Chinook salmon hatchery program to Tracy Hillman (Item V-B).
 (Note: this item is ongoing.)
- Keely Murdoch and Peter Graf will ask the PRCC whether members of the HSC can participate
 in the PRCC meeting when Jeff Jorgensen (National Marine Fisheries Service [NMFS]) discusses
 the Wenatchee life-cycle model and data needs (Item V-B).
- Tracy Hillman will compile questions from the PRCC HSC for Jeff Jorgensen during the August 21, 2019 meeting (Item V-B).

Decision Summary

- The Wells HCP-HC voted to approve the *Implementation of Comprehensive Monitoring and Evaluation of Wells Hatchery Complex Programs in 2019* in today's meeting (Item I-A).
- The PRCC HSC voted to approve the revised *Grant County PUD Hatchery Monitoring and Evaluation Implementation Plan for Spring and Summer Chinook in the Wenatchee Basin and Summer Chinook in the Methow Basin 2020* in today's meeting (Item V-C).

Agreements

 The Wells HCP-HC agreed to recommend to the Wells HCP-CC that Douglas PUD mark subyearling summer Chinook used for the Douglas PUD 2020 Survival Verification Study with coded wire tags (CWTs) with a unique code, in addition to passive integrated transponder (PIT) tags and adipose clips (Item III-A).

Review Items

There are no items available for review.

Finalized Documents

- The Wells HCP-HC-approved plan for *Implementation of Comprehensive Monitoring and Evaluation of Wells Hatchery Complex Programs in 2019* was finalized in the meeting on July 17, 2019, and distributed via email by Larissa Rohrbach on July 22, 2019 (Item I-A).
- The PRCC HSC-approved Grant County PUD Hatchery Monitoring and Evaluation
 Implementation Plan for Spring and Summer Chinook in the Wenatchee Basin and Summer
 Chinook in the Methow Basin 2020 was finalized in the meeting on July 17, 2019, and
 distributed via email by Larissa Rohrbach on July 22, 2019 (Item V-C).

I. Welcome

A. Review Agenda, Review Last Meeting Action Items, and Approve the May 15, 2019 Meeting Minutes (Tracy Hillman)

Tracy Hillman welcomed the HCP-HCs and PRCC HSC and asked for any additions or changes to the revised agenda. Hillman requested the addition of a Policy Committee Update to the agenda to follow the review of past minutes and action items. Catherine Willard requested a change to the Joint HCP-HCs and PRCC HSC topics to move an update on genetics monitoring to next month and to add a discussion on the film "Artifishal." The HCP-HCs and PRCC HSC approved revisions to the agenda.

The HCP-HCs and PRCC HSC representatives reviewed the revised draft May 15, 2019 meeting minutes. Larissa Rohrbach said there were some minor revisions that the representatives then reviewed. The HCP-HCs and PRCC HSC representatives approved the draft May 15, 2019 meeting minutes as revised.

Action items from the HCP-HCs and PRCC HSC meeting on May 15, 2019, and follow-up discussions were addressed (note: italicized text below corresponds to agenda items from the meetings on May 15, 2019):

Joint HCP-HCs and PRCC HSC

- Tracy Hillman will review aspects of the Independent Scientific Advisory Board's Review of Spring Chinook Salmon in the Upper Columbia River under HCP-HCs' purview (Item I-A).
 Hillman said this item is ongoing.
- Greg Mackey will continue researching broodstock composition and mating strategies for conservation programs, focusing on spring Chinook salmon at the Methow Hatchery (Item I-A). Mackey said he will provide an update in today's meeting. This item is ongoing.
- Mike Tonseth will coordinate with Andrew Murdoch (Washington Department of Fish and Wildlife [WDFW]) to present pre-spawn mortality modeling results for spring Chinook salmon at an upcoming HCP-HC meeting (Item I-A).
 Tonseth said this item is ongoing.
- Catherine Willard will update the genetics section of the Monitoring and Evaluation (M&E) Plan for PUD Hatchery Programs (Update to the 2017 Plan) based on the genetics panel recommendations and will append the recommendations from the panel to the plan (Item I-A). Willard said this item is ongoing.
- Kirk Truscott will discuss with Colville Confederated Tribes (CCT) biologists whether elemental signature analysis could differentiate natural-origin Okanogan spring Chinook salmon from

other natural-origin Chinook salmon during broodstock collection at Wells Dam for Methow Fish Hatchery programs (Item I-A).

Truscott said this item is ongoing.

- Brett Farman will discuss with Charlene Hurst and Mike Tonseth the potential use of a multipopulation model for estimating proportionate natural influence (PNI) for the Nason and Chiwawa spring Chinook salmon programs (Item I-A).
 - Farman said this item is ongoing. Tracy Hillman said that Ford et al.'s¹ (NMFS) iterative approach for estimating PNI was used in the *Monitoring and Evaluation of the Chelan and Grant County PUDs Hatchery Programs Draft 2018 Annual Report* (Chelan and Grant PUD's M&E Report) rather than the simple approach that is identified in the permits. Catherine Willard and Hillman asked if NMFS would concur that use of this approach is suitable. Farman said, yes, he concurs that the approach taken in the Chelan and Grant PUDs M&E report is suitable.
- Mike Tonseth will confirm with Andrew Murdoch (WDFW) that Wenatchee spring Chinook DNA sampling of the 2018 to 2023 returns is still consistent with the original Relative Reproductive Success (RRS) Study extension agreement and provide an update to the extension (Item I-A). The RRS study extension agreement memo was revised by Mike Tonseth and distributed to the HCP-HCs and PRCC HSC via email by Rohrbach on July 16, 2017. Bill Gale noted that the memo requests that the Rock Island HCP-HC approve the updated memo. This will be added as a decision item will be added to the August agenda. This item is ongoing.
- Larissa Rohrbach will add sizing of upper Columbia River conservation programs as a periodic agenda item (Item I-A).
 - Rohrbach said this item is ongoing.
- Tracy Hillman and Larissa Rohrbach will maintain a list of outstanding topics for consideration in HCP-HCs and PRCC HSC meetings prior to development of the 2020 Broodstock Collection Protocols (Protocols) (Item I-A).
 - Use of age-3 males in broodstock
 - Use of alternative, non-random mating strategies
 - Establishing ranges around broodstock collection targets
 - Source for Chiwawa spring Chinook salmon broodstock

Hillman said this item is ongoing. Greg Mackey will discuss the use of alternative mating strategies in today's meeting.

• Tracy Hillman and Larissa Rohrbach will add review of the Broodstock Collection Protocols to the September meeting agenda to help the HCP-HCs and PRCC HSC identify co-authors and opportunities to discuss major revisions in advance of 2020 deadlines (Item II-A).

Hillman said this item is ongoing.

¹ Ford, M. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. Conservation Biology 16:815-825.

- Mike Tonseth will revise and redistribute the HCP-HCs Annual Broodstock Collection Protocols development timeline SOA (Item II-A).
 - Tonseth said this item is ongoing.
- Mike Tonseth will ask Michael Humling (U.S. Fish and Wildlife Service) and Charlie Snow (WDFW) to estimate the number of Methow returns that are likely to return to Winthrop National Fish Hatchery to inform a translocation discussion in a future HCP-HCs meeting (Item II-B). Tonseth said this item is ongoing.
- Mike Tonseth will revise and redistribute the 2017 Out-planting Surplus Methow Composite Spring Chinook Salmon Adults memorandum (Item II-B).
 Tonseth said this item is ongoing.
- Tracy Hillman and Larissa Rohrbach will distribute HCP-HC and PRCC HSC-approved distribution lists and meeting protocols (Item II-C).
 Rohrbach distributed the updated meeting protocols and distribution lists by email on May 20,
- Tracy Hillman will present the HCP-HC and PRCC HSC-approved distribution lists to the HCP-CC for their approval (Item II-C).
 - The HCP-CC approved the updated HCP-HC meeting protocols and distribution lists on May 21, 2019. This item is complete.
- Emi Kondo will confirm the status of the draft Wells Hatchery Methow Steelhead Program permit with Charlene Hurst (Item II-D).
 - Brett Farman said he will provide an update in today's meeting. This item is complete.

Wells HCP Hatchery Committee

2019. This item is complete.

- Greg Mackey will provide a revised version of the Implementation of Comprehensive Monitoring and Evaluation of Wells Hatchery Complex Programs in 2019 (Douglas PUD's 2019 M&E Plan) for HCP-HC approval by email (Item I-A).
 - Mackey said Douglas PUD would like to finalize a version of the plan that excludes edits regarding potential changes to methods for estimating steelhead spawner abundance and distribution [that currently rely on detections of upstream-migrating steelhead that are PIT tagged at the Priest Rapids Dam Off-ladder Adult Fish Trap (OLAFT)]. Mike Tonseth said steelhead sampling at the OLAFT is still taking place this year. Keely Murdoch said the edits were written to alert the committee that changes to the run-size modeling approach should be retained so they can be revisited in Douglas PUD M&E Plan for 2020 (next year's plan). Mackey said the language in the plan should be more specific and said the edits provided were too vague. Edits were made during the meeting to specify the run-size modeling methods for brood year 2020 fish (to be carried out in 2019). Tracy Hillman called for the Wells HCP-HC to vote to approve Douglas PUD's 2019 M&E Plan and all parties approved.

PRCC Hatchery Subcommittee

• HSC representatives will submit a list of minimum data or information needs for making a decision on the White River spring Chinook salmon hatchery program to Tracy Hillman (Item V-B). Hillman said there has been no feedback and this item is ongoing.

B. Policy Committee Update

Tracy Hillman provided a summary of HCP Policy Committees guidance directed toward the HCP Hatchery Committees regarding the process and criteria for elevating topics from the HCs to the Policy Committees. Hillman summarized a situation regarding a dispute in the HCP Tributary Committees where a project was deemed biologically relevant, but a policy-level consideration overrode the selection criteria at the technical level. Hillman said a formal dispute was pulled back because the parties acknowledged they would not come to resolution by following the HCP dispute resolution process. The Yakama Nation (YN) asked to convene the HCP Policy Committees to determine a path forward.

Hillman said the HCP Policy Committees provided guidance to all technical groups, including the HCP Hatchery Committees and HCP Coordinating Committees. Hillman said that HCP Hatchery Committees will base decisions on the technical merits of the issue. Any policy-level concerns impeding decision making will be elevated to the HCP Policy Committees for review and a decision.

Bill Gale asked if these recommendations from the HCP Policy Committees will be documented somewhere. Hillman said they will be recorded in the Policy Committees meeting minutes. Gale asked if this guidance changes the dispute resolution direction given in the HCPs. Hillman said no, this guidance does not pertain to official disputes. Gale said this could result in HCP Hatchery Committees members asking for an issue to bypass the Coordinating Committees and go directly to the Policy Committees. Hillman said if it's disagreement or dispute regarding a technical issue, it should still go to the HCP Coordinating Committees. Kirk Truscott said this guidance was developed by the Policy Committees because if it's a policy issue, meaning if it's a top-down issue with direction from existing policy, it is a waste of time to take the issue through the HCP dispute resolution process. Truscott gave the example when recalculation of the Chiwawa program size was not agreed to on a policy level due to *US v. Oregon*, the issue was resolved at the policy level first in order for changes to go forward on the technical level. Gale asked if the HCP Hatchery Committees are solely technical committees and if policy should not be discussed. Hillman said the guidance from the Policy Committees is to evaluate issues based on technical merits. Gale said he wants to ensure the guidance provided by the HCP Policy Committees is aligned with the HCPs. Hillman said the Policy



Committees do not want to change the HCPs. Tom Kahler said the intent of the HCP Policy Committees decision was not to change the operation of the technical committees, but was intended to provide a means to resolve policy conflict resolution outside of the technical committees.

Gale asked how the HCP Tributary Committees issue was resolved. Hillman said the issue is to be resolved by asking leadership of the member parties in conflict to meet and come to resolution with the biological merits in mind. Hillman said the Policy Committees intend to educate tribal council members on the purpose and objectives of the HCPs. Gale said the solution to any issue that may rise to the HCP Policy Committees could be rooted in both groups (technical and policy) by providing a technical workaround. Keely Murdoch agreed but said that providing a technical workaround, in this case, did not get to the core policy issue that had no nexus to the resource and was going to continue to be a problem if the Policy Committees were not engaged.

Murdoch suggested memorializing this development in a place that is more accessible for new committee members and future uses. She said it should be incorporated into the operating protocols. Larissa Rohrbach will add the HCP Policy Committee guidance to the HCP-HC and PRCC HSC protocols files (*Note: this language was added to the protocols under the section titled "Disputes and Conflicts." The updated version was saved to the HCP-HC Extranet Site and PRCC HSC SharePoint sites, dated July 17, 2019.*)

II. Joint HCP-HCs and PRCC HSC

A. Goat Wall Acclimation Site Performance Update

Tracy Hillman welcomed Rick Alford (YN) who gave the presentation, "Goat Wall Acclimation Site, Methow Valley" (Attachment B). Alford shared a PowerPoint presentation on operations and outcomes of acclimation activities for spring Chinook salmon at the Methow Valley Goat Wall site in the upper Methow River from 2017–2019.

Slides 1–8: Alford presented background information about the Goat Wall Acclimation Site. It is located 25 miles up the Methow River on Cold Creek, a disconnected side channel of the Methow River. Todd Pearsons asked if it is Methow River water; Alford said yes. Water temperatures range from the 30s°F to 40s°F, depths 1 to 1.5 meters, and capacity is about 30,000 fish with a conservative stocking density. The habitat is more complex in the downstream area than upstream area of the side channel. Snow and access can be challenging.

Slides 9–16: Alford described the activities to set up the acclimation site in spring. Snorkel surveys are conducted to identify other fish. Pearsons asked if it is completely enclosed; Alford said yes, the sites have custom nets. Best efforts are made to secure the nets so no fish escape. Fish transport trucks

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bring juveniles. Methow Fish Hatchery staff support the collection of the fish from the hatchery. Snorkel surveys are done to monitor fish and three PIT-tag detection systems are used to monitor for escapees. During rearing, staff visit up to five times a day to feed and haze predators. Growth monitoring and fish health samples are taken. All activities are done by boat. Feed is kept off site and the site is maintained in a clean condition to meet the landowners' needs. The landowners have been very supportive. Pearsons asked if the side channel has been blocked off. Alford said they have to maintain connectivity of the side channel to the Methow River.

Slides 17–18: Alford summarized the outcomes of three years of activities (2017–2019). The beginning of acclimation depends on flow and ability to access the site through snow. Releases are timed to coincide with Methow Fish Hatchery releases to compare survival between these two groups. Pearsons asked if fish are ushered out of the side channel at the time of release. Alford said release is completely volitional. Fish may move upstream or downstream once the nets are dropped. PIT-tag arrays are monitored for 2 to 3 days following release to ensure all fish have moved out. Net security was good with 1.2% fish escaping pre-release.

Slides 19–21: Alford summarized in-river survival and travel time results. Survival is calculated using PIT tags and the Cormack-Jolly-Seber mark-recapture model. Survival of Goat Wall-acclimated fish to Rocky Reach Dam is similar to Methow Fish Hatchery releases. This year, survival to McNary Dam may not be as good as other programs due to low flow conditions [in the Methow River].

Slide 22: Alford showed adult return data for the first returning cohort from brood year 2015 based on PIT tags detected at Wells Dam this spring. He said 2019 is the first year of 4-year-old fish returning from this acclimation site to the Methow Basin. Estimated smolt-to-adult return rates to Wells Dam were presented in comparison to other production groups.

Hillman asked if the acclimation site is spring-fed. Alford said the geology in the area is interesting. The side channel is fed by a type of underground river. When the water level in the Methow River goes down, water level in this site goes down and vice versa.

Bill Gale asked if the YN is monitoring site-fidelity to observe whether the project encourages more upstream distribution of spawners. Alford said yes, that adult distribution monitoring will start this year. Alford said PIT tags will not be monitored upstream of Winthrop. Keely Murdoch said data will be collected from CWTs collected in spawner surveys. Murdoch added there will be a small run this year and data may be limited. Gale said it may be difficult to observe a shift in distribution due to small samples sizes. Gale added the combination of small releases, low return rates, and low observation rates in the carcass sampling may make it difficult to have confidence in a potential shift in distribution.



Pearsons asked if there is a model to estimate predation effects on survival during acclimation. Alford said yes, they use a model based on literature and predators observed on site. Pearsons said the survival rates presented are unusually high for a natural site. Alford said yes, they haven't seen many predators on site.

Catherine Willard asked how long it takes for fish to leave the site volitionally. Alford said spring Chinook leave quickly, in approximately 5 days. Alford added the fish overwhelm the PIT-tag readers and PIT-tag detection efficiency can be low during the release period [due to tag collisions].

Pearsons asked about the duration of the study. Murdoch said it is a 5-year study; however, the HCP-HCs will need to evaluate the preliminary outcomes of the study prior to the complete adult return record to decide on the future of the site. Murdoch said the HCs should discuss whether they should move forward with evaluating site performance with a partial return dataset or wait for a more complete set. Gale said the understanding site fidelity of returning adults is important, but work can proceed without the complete dataset of returns as long as juvenile survival estimates are available to evaluate.

B. 2018 Egg Treatment Study Update

Betsy Bamberger presented the results of the study (Attachment C) and said the 2018 Egg Treatment study plan was originally brought to the HCP-HCs in July 2018 as a proposed pilot study to test various treatment methods for preventing infestation with the water mold *Saprolegnia spp*. with the goal of reducing formalin use [by Douglas PUD programs to preemptively address potential future regulation and health and safety issues].

Bamberger said the treatments tested were formalin, salt, hydrogen peroxide, and no treatment (water). She said no difference was observed between the treatments, indicating that all treatment methods were equally effective. She hypothesized that the pathogen load was too low in the water source [at Methow Fish Hatchery] to observe a difference if one exists. She said follow-up work is needed to determine the best egg treatment protocol at Methow Fish Hatchery.

Bamberger said some differences between the pilot study and real world uses are that summer Chinook salmon were used in the study instead of the target species, spring Chinook salmon, due to limitations on spring Chinook salmon availability and permitting issues. Bamberger said another difference was that incubation trays were used that are different from the incubation isobuckets used for rearing spring Chinook salmon embryos.

Greg Mackey said the experiment was run until embryos reached the eyed-egg stage, per study design. Further, the HCP-HCs later approved observing a subset of the fish to the alevin stage to



examine if treatment effects carried past hatch. However, no additional effects were observed at the alevin stage. Bamberger said salt had an effect on the alevin stage in other published studies.

Bamberger said staff reported that non-treated eggs were stickier than formalin treated eggs.

Mike Tonseth asked if the treated eggs were subjected to the same shocking and handling as eggs in the spring Chinook salmon production program. Bamberger said yes, in fact they were probably handled more because eggs from different females were mixed to reduce familial effects on egg survival.

Bill Gale asked if the intent is to repeat the study in a place where there is a higher *Saprolegnia* load. Bamberger said yes, they intend to repeat the study at Wells Fish Hatchery with summer Chinook salmon this fall (2019). Tonseth said that implementing the study in a hatchery with a low *Saprolegnia* load was an original critique of the study, but due to facility modifications, Wells Hatchery wasn't prepared to house the study in 2018. Mackey said implementation of this first year was an opportunity to develop the methods.

Gale asked if the problem with hydrogen peroxide is that it's difficult to procure. Bamberger said yes, the Department of Homeland Security has to be informed, but it's easier if there is a plan to use it on a short timeline and it is easier to acquire in smaller volumes.

C. 2019 Egg Treatment Study Plan

Betsy Bamberger said the Egg Treatment Study will be repeated in 2019 using summer Chinook salmon with the addition of another treatment group. Greg Mackey said they plan to eliminate the use of hydrogen peroxide because it is difficult to handle.

Mike Tonseth asked if the plan is to use the existing broodstock or to collect additional broodstock for the study. Mackey said if a treatment doesn't work, an egg stack could be lost to *Saprolegnia*, but if the risk is low, existing program production fish could be used. Mackey said formalin is already used on the production fish so for formalin, production fish would be used as per the usual treatment at the hatchery. He added that at this time the study will be kept small to make it easier to collect enough eggs.

Bamberger and Mackey will provide another draft study plan in August for review and approval by the Wells HCP-HC.

Tracy Hillman asked if there will be replication at other sites. Bamberger said no, not at this time. Tonseth said implementation of these types of studies will depend on the relative risk to each program.

D. Alternative Broodstock Composition and Mating Strategies

Greg Mackey summarized how this topic was originally raised. Mackey said the original question was whether to use jacks in the broodstock, focusing on spring Chinook in the Methow basin. He said the policy so far has been to exclude jacks in the broodstock, but that in the recent Broodstock Protocol allowance was made to use wild jacks if wild male broodstock were in short supply. Mackey said in researching this topic he determined that the question is actually about constructing the mating strategies overall, of which jacks are one component.

Mackey said the following pertains mostly to the conservation programs. Mackey said there are two major themes for maintaining a conservation broodstock:

- 1. A "genetically benign" approach that attempts to collect random sample to match the genetic composition of the broodstock with the wild stock. Mating is random to the extent possible. The male to female ratio could be 1:1 or a spawning matrix can be used. There are efforts made to minimize domestication selection. There are guidelines to cull (equalize) families to maximize effective population size. The goal is to have no effect on the natural population with what is done with the hatchery population.
 - Mackey noted two relevant publications. He said Don Campton (USFWS) wrote a paper² about sperm competition, though much of the paper discusses the genetically benign approach in broader terms. Tom Quinn wrote a response paper³ comparing what hatcheries actually do compared to what fish do in the wild.
- 2. Dave Hankin's paper⁴ focuses on counteracting the directional selection for younger age at maturity in hatchery populations, especially if there is a fishery that tends to select the older, larger fish. Hankin modelled an approach using non-random mating. Hankin prescribed an approach where jacks are not used. Instead, for each female, a male that is equal size or greater is used to fertilize eggs, which [practically] guarantees that the male and the female are the same age or the male is older. This practice would drive the population toward older age at maturity, countering the effects of hatchery production and fisheries.

Mackey said the Methow Fish Hatchery uses a spawning matrix for spring Chinook. Mackey said the Busack and Knudsen paper⁵ does a great job describing matrix-based spawning methods. The

² Campton, D. E. 2004. "Sperm Competition in Salmon Hatcheries: The Need to Institutionalize Genetically Benign Spawning Protocols." *Transactions of the American Fisheries Society* 133:1277–1289.

³ Quinn, T. P, 2005. "Comment: Sperm Competition in Salmon Hatcheries: The Need to Institutionalize Genetically Benign Spawning Protocols." *Transactions of the American Fisheries Society* 134:1490–1494.

⁴ Hankin, D. G., J. Fitzgibbons, and Y. Chen, 2009. "Unnatural Random Mating Policies Select for Younger Age at Maturity in Hatchery Chinook Salmon (Oncorhynchus Tshawytscha) Populations." *Canadian Journal of Fisheries and Aquatic Sciences* 66(9):1505–1521.

⁵ Busack, C., and C.M. Knudsen, 2007. "Using factorial mating designs to increase the effective number of breeders in fish hatcheries." *Aquaculture* 273:24–32.



question remains whether jacks should be used. Warm Springs National Fish Hatchery is using an approach where they use jacks in proportion to their natural occurrence in the wild, but decrease their use in the hatchery in proportion to their relative reproductive success as compared to large males in the wild. One way to approach this would be to estimate their contribution [to the offspring] as done in the Berejikian paper⁶ and multiply the average contribution of jacks by their occurrence [in the total population?].

The Committees discussed the current genetic health of the Methow Spring Chinook population. Mackey said there are often spinal deformities in hatcheries. Deformities have been observed over the years at Wells Hatchery for summer Chinook, but the reporting has been spotty. Betsy Bamberger looked into the causes of spinal deformities and there are different types and the causes are not well-understood. Mackey talked to Ron Hardy (University of Idaho; animal nutrition expert), who says it's always a nutrition issue (a phosphorous limitation). Douglas PUD contracted with the WDFW Molecular Genetics Laboratory to genotype a sample of 50 of the deformed fish at Methow Hatchery for the 2018 brood to determine whether it could be a genetic problem. Mackey said Sewel Young et al. (WDFW 2019)⁷ used the results of this analysis to compare the homozygosity of hatchery-origin Twisp River spring Chinook salmon with all of the Snake River basin spring Chinook salmon populations. He found that the Twisp River hatchery-origin fish had lower homozygosity than the entire Snake River population. By this preliminary measure it would appear that the Twisp River component of the Methow Hatchery program has done a good job for maintaining genetic integrity.

Mackey said the Methow River basin hatchery program appears to be doing a good job but there could be other, better ways for doing things. Bill Gale said there may be some programs that follow Hankin's concept and it should be contemplated but programs should avoid being overly selective for a few spawners that meet the criteria. Gale said there is value in maintaining the criteria for random mating. Mackey said Jennifer McLean (University of British Columbia) studied the effectiveness of program random mating and found that unconscious bias results in mating that actually isn't very random.⁸

Mackey said he will provide a white paper to the HCP-HCs and PRCC HSC and a presentation during the August meeting to summarize his conclusions. (Note: Mackey's white paper will be distributed following PUD review.)

⁶ Berejikian, B.A., D.M. Van Doornik, R.C. Endicott, T.L. Hoffnagle, E.P. Tezak, M.E. Moore, and J. Atkins, 2010. "Mating success of alternative male phenotypes and evidence for frequency-dependent selection in Chinook salmon, Oncorhynchus tshawytscha." *Canadian Journal of Fisheries and Aquatic Sciences* 67(12):1933-1941.

⁷ Young, S. F., A. Terepocki, and C. Bowman. 2019. Parentage analysis of deformed Chinook salmon juveniles from brood year 2018, Methow Hatchery. WDFW Molecular Genetics Laboratory. Report to Douglas PUD, 2019.

⁸ McLean, J.E., P. Bentzen, and T.P. Quin, 2005. "Nonrandom, Size- and Timing-Biased Breeding in a Hatchery Population of Steelhead Trout." *Conservation Biology* 19:446–454.

E. Out-planting Spring Chinook Salmon Adults in Chewuch 2019 Status Update

Greg Mackey said this year the Methow spring Chinook salmon run was predicted to be quite small. Mackey said out-planting depends upon whether there are extra fish available and whether adult management should be done this year following the protocols for surplusing fish. Mackey said the Methow Fish Hatchery has collected all their broodstock for this year. Bill Gale said broodstock is available at Winthrop but is not meeting the Methow Hatchery-origin adult target for the Winthrop program. Mackey said fish continue to trickle in at Methow Hatchery. Keely Murdoch asked if USFWS knows how many more fish are needed to meet natural-origin fish targets. Matt Cooper said they have less than half of the target of Methow Hatchery-origin brood pairs but have plenty of WNFH-origin returns to backfill the program.

Rick Alford asked where the out-planting sites on the Chewuch River are located. Mackey said they were based on preliminary surveys to identify sites that were not already occupied by wild fish and allowed access for planting trucks. Tom Kahler said release sites were identified at river kilometer (RKM) 29 and RKM 14 on the Chewuch River.

Tracy Hillman summarized that it is unlikely that the out-planting of spring Chinook salmon will occur this year.

The HCP-HCs will continue to move forward with updating the Methow spring Chinook salmon translocation plan. Murdoch said this was originally intended as an alternative to acclimation. Murdoch said the intent was to compare the results of out-planting with acclimation. Mike Tonseth said if there are not sufficient conservation program fish, it may be worth experimenting with Winthrop National Fish Hatchery HOR program fish to study behavior and success, though it would affect PNI on the spawning ground.

Gale asked how much of the Methow Spring Chinook Salmon Conservation program fish are remote acclimated. Mackey said of the approximately 224,000 juveniles, more than half are acclimated remotely. Gale said, so only half of the program is being acclimated at the Methow Fish Hatchery because they are being remotely acclimated. Murdoch said Twisp acclimation sites are not necessarily remote acclimation.

Gale asked if the Methow program is trying to do too much with a small number of adults returning to Methow Fish Hatchery. Gale said even in an average return year there may not be enough fish for translocation. Gale suggested reviewing smolt-to-adult return rates (SARs) to determine what would actually be needed to implement the translocation plan. Tonseth said the determination may not be on total Methow spring Chinook SARs, but rather a comparison of the number of adults returning to the out-plant sites versus the hatchery.



F. Artifishal

Catherine Willard said that the Cascade Columbia Fisheries Enhancement Group (CCFEG) is showing the movie "Artifishal" at the Snowy Owl Theater in Leavenworth on August 8. CCFEG asked if local entities would be willing to participate on a panel discussion after the movie.

Willard said the movie is quite biased. Bill Gale said USFWS asked for the ability to watch the movie and determine who would be on the panel first. Gale said USFWS has not responded yet but is willing to consider participating. Keely Murdoch said YN would require approval from their media committee and the Tribal Council to participate. Murdoch said YN is discussing it and have told CCFEG that a person will likely participate but has not determined who that will be. Murdoch said they will likely review the movie first and determine their approach to answering questions. Gale said a screening in the Methow community went well (in Winthrop, WA). Tom Kahler said he questioned whether the panel will happen with or without their participation. Gale said he is concerned about the screening of the film without a panel to provide context.

Tracy Hillman asked if the CCT will participate. Kirk Truscott said they would have to ask approval of their media council. Truscott said the CCT response was that it was a bad idea to give any time to the film because it appears to be propaganda. Gale said it is unfair to be asked to be on a panel without being able to view the material for discussion. Todd Pearsons said Grant PUD has no plan whether or not to participate at this time. Brett Farman said NMFS had an internal screening but no formal response that he is aware of. Willard said Chelan PUD prefers that CCFEG do not show the movie because it is biased and does not accurately reflect the hatchery programs in the upper Columbia, but feels they need to participate to provide a balanced view. However, Chelan PUD feels there will not be enough time during the panel discussion to describe how these programs are different from what is being shown in the movie. Gale said they conflate net-pen rearing of Atlantic salmon with hatchery rearing across the entire landscape. Gale asked if anyone knows CCFEG's motivation for bringing this movie to the valley. Chelan PUD read from an email that CCGEG feels this provides an opportunity to have a balanced discussion. Mike Tonseth said screening the film and panel time are not an adequate way to provide a balanced discussion. Greg Mackey said asking to stifle the film is worse that participating in a biased discussion.

Willard asked if the participants should prepare a letter asking for better explanation of the intent of showing the film and an opinion on a joint response for preparing a more balanced discussion. Peter Graf suggested asking for some speaking time to introduce how the HCP programs are different from what is shown in the film. Willard agreed it would be helpful to be able to explain the programs before taking questions. Hillman asked if the HCP-HCs and PRCC HSC would like support to facilitate participation of members. Potential participants will coordinate outside of the committees.

G. Wenatchee Spring Chinook Salmon Broodstock Collection Update

Mike Tonseth gave an update on the number of adults that have been retained for Wenatchee spring Chinook salmon broodstock. Tonseth said there are currently:

- Nason Creek spring Chinook salmon: 52 NOR, 77 HOR (129 total)
- Chiwawa River spring Chinook salmon: 37 NOR, 40 HOR (77 total)

The program is 25 fish below target numbers for Chiwawa NOR. Collection of NOR is continuing at Tumwater Dam and any that assign genetically to the Chiwawa population with 95% surety will be retained. Ten fish collected at Tumwater Dam are awaiting assignments. The numbers arriving at Tumwater Dam are declining rapidly for the season.

Tonseth said this year the number of fish captured at Tumwater Dam that did not assign to Nason nor Chiwawa populations was almost double the number sent back to the river compared to previous years due to non-assignment to either population. Tonseth said there were 14 non-Nason/non-Chiwawa-assigning fish and an unusually high number of those assigned genetically back to Leavenworth National Fish Hatchery. Bill Gale said there could have been a problem with the cutoff for the genetic stock identification criteria. Peter Graf asked for clarification whether fish that do not assign with at least 95% surety to either program would be allocated to the safety net program. Tonseth said no, they would be released back to the river.

Tracy Hillman summarized that both Nason and Chiwawa programs have met the overall brood goals, but the NOR target has not been met for the Chiwawa program.

H. National Marine Fisheries Service Consultation Update

Brett Farman said that the Steelhead and Summer and Fall Chinook Salmon Environmental Assessments Finding of No Significant Impact (FONSI) is still in internal review. Farman said the Winthrop Steelhead permit is waiting for a signature. Farman said the permit for the Methow Steelhead program was sent out by Charlene Hurst at the end of May, comments were received by mid-June, and it is awaiting signature when the FONSI is finalized. Farman said there are no hard dates for completion once permits go into internal review.

III. Wells HC

A. Wells Hatchery Summer Chinook Salmon Tagging for the 2020 Survival Study

Tom Kahler said that every 10 years Douglas PUD implements a survival verification study (survival study). The next study will be conducted in 2020 using Wells Hatchery summer Chinook salmon. In May 2009, the Wells HCP-HC decided not to tag the 2010 survival study fish with CWTs because they would already be PIT tagged for the survival study. The primary difference between then and now

was that in 2010, the entire study fish group was in addition to the hatchery production numbers. In 2020, the study fish (110,000 fish) would be taken from the total yearling summer Chinook hatchery production of 320,000 fish. The study fish would receive PIT tags and would be adipose fin-clipped (ad-clip). The remaining 210,000 production fish would receive CWTs and would be ad-clipped. The HCP Coordinating Committee asked whether there would be any adverse effect on the monitoring and evaluation activities and US v. Oregon agreements due to not tagging 110,000 fish with CWTs. The HCP Coordinating Committee was interested in getting input from the HCP-HC on this issue.

Kahler said CWTs are used by Douglas PUD for determining age structure, harvest, and stray-rates. An assumed mark-retention rate is applied to the numbers. One challenge would be calculating a different mark-retention rate for 2020 because there would be a different mark rate.

Mike Tonseth said ocean harvest cannot be calculated without CWTs. Tonseth said the concern is whether PIT tags would suffice as a surrogate for CWTs. The upper Columbia is the only place where PIT tags are interrogated in the recreational fishery.

Kirk Truscott said the Wells Hatchery stock is the indicator stock for harvest on upper Columbia summer Chinook salmon, including in the Alaskan and Canadian fisheries. In approximately 2005, the HCP-HCs determined that 100% tagging is needed to provide sufficient rigor to estimate ocean harvest.

Bill Gale said fish frequently dip into numerous different tributaries. The problem with PIT tags is that you don't know if they stay. Gale asked why Douglas PUD is using production fish for the survival study and not additional fish as in 2010. Kahler said the Coordinating Committee decided this in February 2019.

Tonseth said the consultation with NMFS is moving forward without the additional fish. He said that in the US v Oregon tables, the survival study fish were removed.

Kahler said that of the 110,000 survival study fish, 50% will be released downstream from Wells Dam from a barge (the release methodology used in the survival studies to ensure similar fish handling and release protocols for all release groups, above and below the dam), 25% will be released at the mouth of the Methow River, and 25% will be released at the mouth of the Okanogan River (approximately 50,000 fish released upstream from Wells Dam).

It was asked if the HCP-HC is subordinate to the HCP-Coordinating Committee. Truscott said in the case of planning the survival studies they are.



Tonseth said that not tagging production fish with CWTs would deviate from *US v. Oregon*. This would require a revision to the *US v. Oregon* tables, then revising them back in the future. Gale said that *US v. Oregon* also stipulates fish be released on station.

Keely Murdoch said there is a need to CWT 100% of the fish to achieve adequate sample sizes to determine ocean harvest rates. Gale said there are two problems. One is the deviation from *US v. Oregon*, and the second is the deviation from the preferred tagging method determined by the HCP-HC.

Kahler said a decision on double-tagging has not been made; it is up to the HCP-HC to provide that determination.

Tonseth said it would be prudent for the survival study fish to have a unique CWT code. Tonseth said in looking ahead to 2030 to track spring Chinook salmon survival, PIT tagging production fish may be necessary, but they should have similar performance as non-PIT tagged fish. A unique CWT number would allow for estimating differential survival of PIT-tagged fish.

Kahler asked whether the Comparative Survival Study found differential survival of PIT-tagged fish (reported in the 2018 annual report). Kahler said the HCP Coordinating Committee did agree that spring Chinook salmon should be the study fish in 2030.

AGREEMENT: The direction from the HCP Hatchery Committee to the HCP Coordinating Committee is to tag all 2020 survival study fish with CWTs that have a unique group code in addition to tagging with PIT tags and ad-clipping.

Kahler will report the agreement back to the HCP Coordinating Committee.

Peter Graf asked if using a unique CWT code for a smaller number of fish still meets the need of an adequate sample size for estimating harvest. Tonseth said harvest requires a smaller number; estimating SARs requires a larger number.

B. Request for Broodstock to Expand Wells Hatchery Subyearling Production

In an email on July 15, 2019, Mike Tonseth outlined a request to the Wells HCP-HC to support the collection and spawning of an additional 350 adult hatchery summer Chinook salmon from the Wells volunteer channel to support an additional production of 500,000 subyearlings in brood year 2019 for the benefit of Southern Resident Killer Whales.

Tonseth said the decision being requested does not pertain to how to allocate surplus fish, but rather asks the Wells HCP-HC to approve that there is capacity at the Wells Fish Hatchery to accommodate rearing of the additional subyearling summer Chinook salmon.

Keely Murdoch said there are three issues with the request:

- 1. This should be a Joint Fisheries Parties (JFP) discussion.
- 2. The YN feels that existing programs such as the Yakima Basin summer Chinook salmon program should be filled prior to meeting new program needs. Earlier this year, the HC declined a request to send surplus summer Chinook salmon to the Yakima Basin program that perhaps should have been discussed not in the HC but in the JFP. The YN feels allocation of additional fish to the Yakima Basin is a better use for meeting the need of providing prey to Southern Resident Killer Whales.
- 3. The management considerations of additional fish returning to the upper Columbia Basin have not been considered, which is also a JFP issue.

Tracy Hillman asked if this is still an HC issue or should it be discussed and resolved by the JFP. Murdoch said there is inconsistency about how allocating surplus is handled in the HC and that is creating a conflict. Greg Mackey said the HC is responsible for approving documents and making decisions related to the PUDs' HCP programs. In the case of the Yakima Reintroduction program for instance, it is not an HCP-HCs or PRCC HSC program and allocation to the program should not be decided within the Committees setting. Tonseth said with regard to the HCs the nexus is that the fish would be reared at Wells Fish Hatchery. The allocation of surplus adult returns should be decided outside the HCP process.

Murdoch said that last week there was a discussion over email about planning for the approved uses of surplus fish from Wells Fish Hatchery, and now there is a sudden higher need outside of the approved surplus fish uses. Tonseth said this is not a higher need but an additional need that is a new legislative mandate. Murdoch said the intent of the mandates should be met, and can be met by allocating fish to the Yakima Basin, which has been permitted for this action. Wells Fish Hatchery has not been permitted for this action.

Bill Gale said the issue is about hatchery production at Wells Fish Hatchery. Gale said a concern is that there were proposals developed for rearing fish at Wells Hatchery without involvement of the HC. The proposals that were developed by Douglas PUD and WDFW should have gone through the HC prior to the time of broodstock collection. Proposals need to be discussed in Committee prior to planning.

Tonseth disagreed. Tonseth said WDFW reached out to multiple facilities to identify places where additional capacity would be available for rearing the fish. Tonseth said WDFW does not need approval to implement the program. Gale said they do if it involves Wells Fish Hatchery. Murdoch said only the element of whether there is capacity requires HC approval. Gale said it's not just about rearing capacity, it's also about whether this proposed program impacts the existing HCP programs.

FINAL

HCP Hatchery Committees Meeting Date: July 17, 2019 Document Date: August 15, 2019 Page 20

Gale said a proposal should be created with more consideration than the existing email request.

Murdoch said there has to be concurrence by the JFP that the new proposed use of the surplus fish is the most beneficial use for the program.

Gale asked about bull trout permit compliance. Tonseth said that would be part of the permitting process. Gale asked if this is going to be proposed as a new program under *US v. Oregon*. Tonseth said no, because there is only funding for two years, so it is not WDFW's intent to include this program under *US v. Oregon*. Hillman asked if Brett Farman had a comment. Farman declined to comment on the issue of use of surplus fish. Murdoch said NMFS has been engaged regarding obtaining permits. Kirk Truscott asked what would happen if permits are not approved and there is a surplus at the juvenile stage. Tonseth said juveniles would be out-planted to non-anadromous waters.

Truscott said allocation of surplus adults to the tribes and collection of broodstock for this new proposal can be done concurrently. Tonseth agreed there appears to be enough surplus for all programs this year.

Catherine Willard asked whether the 500,000 juveniles needed is for one year or two years. Tonseth said the funding is for \$175,000 per year, which roughly equates to rearing 500,000 subyearlings annually.

Truscott said last week over email he asked the HC to concur that there is now a surplus at Wells Fish Hatchery to allow for fairness in allocation to the programs receiving surplus.

Hillman said the issue is that there is no proposal for the HCP-HC to evaluate whether there is an effect on HCP production programs. Murdoch said the issue is that a proposal was developed but wasn't brought forward formally to the Wells HC. Hillman asked how quickly this needs to be resolved. Tonseth said the critical piece is having adults on hand and suggested having a brief discussion after the HCP HC meeting among WDFW, Douglas PUD, CCT, and YN members to establish a temporary path forward to ensure broodstock are not lost during a period in which WDFW develops a more formal proposal.

Murdoch said at Wells Hatchery there hasn't been space in the past and a formal resolution to that problem hasn't been provided. Tom Kahler noted that the hatchery has been under construction for approximately 5 years, and has limited space.

Todd Pearsons asked how this would affect downstream programs. For instance, how this would affect recalculation. Tonseth said smolts produced for the orca program should not be added into the mitigation obligation for operating the hydro-projects. Recalculation looks at SARs and total



adult returns. Returns from the orca program would need to be subtracted from the number used for calculating SARs, otherwise they would count against survival through the projects.

Truscott said he has confidence that Douglas PUD has done a complete evaluation about rearing space; however, he hasn't heard whether this proposal would require removing a certain number of potential adult returns. The expectation is that it would, but this calculation hasn't been formalized.

WDFW, Douglas PUD, YN, and CCT convened during the lunch break to establish a short-term path forward for retaining broodstock.

Tonseth said WDFW will prepare a 1-page proposal for the Wells HC to outline the impacts of the additional subyearling production, which are expected to be negligible. Hillman said the Wells HC will then evaluate the proposal to ensure the proposal does not adversely affect the HCP production programs. Once there is agreement to the proposal, the JFP will determine the allocation of surplus summer Chinook salmon broodstock.

Truscott said CCT will expect advance notification from WDFW if a surplussing day will occur. Tonseth agreed that coordination will occur.

IV. Rock Island/Rocky Reach HC

A. Relative Reproductive Success Study Extension Memo Update

Catherine Willard said that in 2014, WDFW asked the Rocky Island HC to extend the scope of the Relative Reproductive Success (RRS) Study to include brood years 2014 through 2018. Mike Tonseth clarified that DNA sampling of adults would be required through 2023 for NOR fish; the juvenile DNA sampling would be terminated after 2019.

Bill Gale asked if the intent would be to sample 100% of the adults passing over Tumwater Dam. Willard said trapping at Tumwater would be consistent with the approved trapping operation plan at Tumwater Dam. Tonseth said this is mostly done during broodstock collection and adult management. Tonseth said fish are sampled for DNA, are PIT tagged, and then passed above the dam. Tonseth said although the Operating Plan allows for trapping 24 hours a day, trapping is not actually carried out 24 hours a day; there would still be some passage at night. Tonseth said this year's activities would be indicative of what will be done through 2023 for the RRS study.

Todd Pearsons asked how many NOR fish would be sampled. Tonseth said he didn't know. The intent is to sample as many as possible within the operating constraints. Pearsons said the issue is to ensure there is representative sampling.

Gale asked if the activities are contingent on funding. Tonseth said no, it is fully funded through 2023.

Peter Graf asked if juvenile sampling is continuing at the Wenatchee River screw trap. Tonseth said yes, sampling would occur through 2020 to capture the outmigrants.

The current RRS study extension memo requests the HCP-HC to approve the revised memo. Tonseth said there are additional clarifications needed in the memo. He will retract the memo, revise it, and resend it for review and approval during the August meeting.

V. PRCC HSC

A. Approve the May 15, 2019 Meeting Minutes, Committee Updates, and Meeting Summary Review (Todd Pearsons)

The PRCC HSC representatives approved the May 15, 2019 meeting minutes as revised.

B. White River Memorandum Progress Update

Tracy Hillman reminded the PRCC HSC that he asked Denny Rohr for specific direction from the PRCC on the intent of the White River memorandum. Hillman has not yet received direction from the PRCC.

Keely Murdoch said the issue has been discussed in the PRCC but there remains some confusion over the original intent of the PRCC request to the HSC.

Scott Carlon (NMFS representative to the PRCC) invited Jeff Jorgensen (NMFS life-cycle modeler) to the PRCC meeting to discuss data needs for life-cycle modeling; however, Jorgensen is unavailable until September so the issue will be delayed until the September PRCC meeting. Murdoch suggested that members of the HSC attend to view Jorgensen's presentation and ask questions of him.

Bill Gale asked if anyone has heard from Craig Busack regarding his ability to re-engage with the group on this topic. Brett Farman will follow up with Busack.

Todd Pearsons asked if the HSC should develop some questions for Jorgensen for a more productive discussion. For instance, asking what data needs there are, to understand why certain existing data are insufficient.

Murdoch and Graf will make a request to the PRCC that HSC members participate in the September meeting for Jorgensen's presentation and that they are able to identify specific questions for Jorgensen prior to the meeting. Hillman will compile the questions to be sent to Jorgensen.

C. Finalize the Grant PUD 2020 M&E Implementation Plan for Wenatchee and Methow Basins

Todd Pearsons reviewed minor changes made to the draft "Grant County PUD Hatchery Monitoring and Evaluation Implementation Plan for Spring and Summer Chinook in the Wenatchee Basin and Summer Chinook in the Methow Basin 2020."

Pearsons identified one substantive change. Pearsons said forced releases limit their ability to use PIT tags to monitor releases at Carlton Pond and Nason Acclimation Facility. Kirk Truscott asked if there are predation problems that affect calculation of survival of the fish reared at Carlton Pond. Pearsons said no.

Mike Tonseth asked to review a passage on residualism. Pearsons updated a section of estimating residualism by monitoring PIT-tag detections post-release. Tonseth said there should be language consistent with draft permits for pre-release sampling; an edit may not be needed in this document as long as there is an understanding that what was written in the permits will be implemented.

Tracy Hillman asked the PRCC HSC to vote on the Priest Rapids M&E plan; all parties approved.

VI. Administration

A. Next Meetings

The next HCP-HCs and PRCC HSC meetings are August 21, 2019, September 18, 2019, and October 16, 2019, at Grant PUD in Wenatchee, Washington.

Bill Gale asked if there are agenda items for the PRCC HSC meeting in August such as broodstock protocol issues. Mike Tonseth said there was not a proposal for real-time otolith reading to be discussed, and there is now an alternative strategy in use so PRCC HSC broodstock protocols will not be modified.

VII. List of Attachments

Attachment A List of Attendees

Attachment B Presentation: Goat Wall Acclimation Site, Methow Valley

Attachment C Control of Saprolegnia sp. Growth on Summer Chinook (Oncorhynchus Tshawytscha)

Eggs

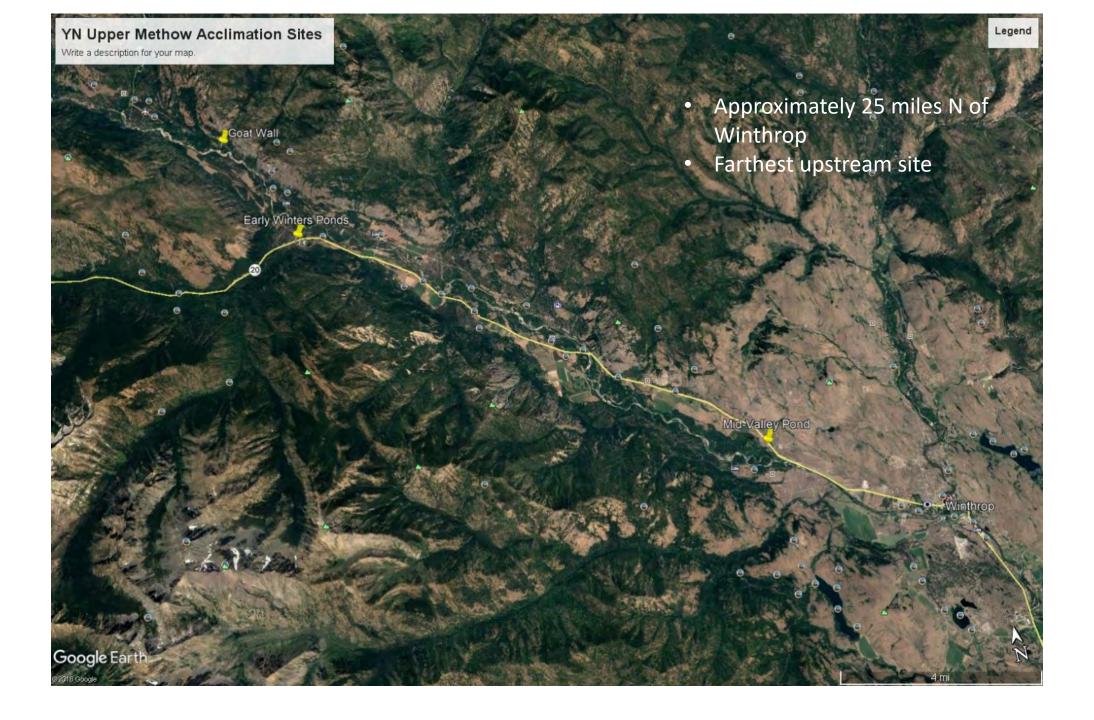
Attachment A List of Attendees

Name	Organization
Tracy Hillman	BioAnalysts, Inc.
Larissa Rohrbach	Anchor QEA, LLC
Catherine Willard*	Chelan PUD
Kirk Truscott*‡	Colville Confederated Tribes
Betsy Bamberger	Douglas PUD
Greg Mackey*	Douglas PUD
Tom Kahler*	Douglas PUD
Peter Graf‡	Grant PUD
Deanne Pavlik-Kunkel	Grant PUD
Todd Pearsons‡	Grant PUD
Brett Farman*‡	National Marine Fisheries Service
Matt Cooper*‡	U.S. Fish and Wildlife Service
Bill Gale*‡	U.S. Fish and Wildlife Service
Mike Tonseth*‡	Washington Department of Fish and Wildlife
Rick Alford	Yakama Nation
Keely Murdoch*‡	Yakama Nation

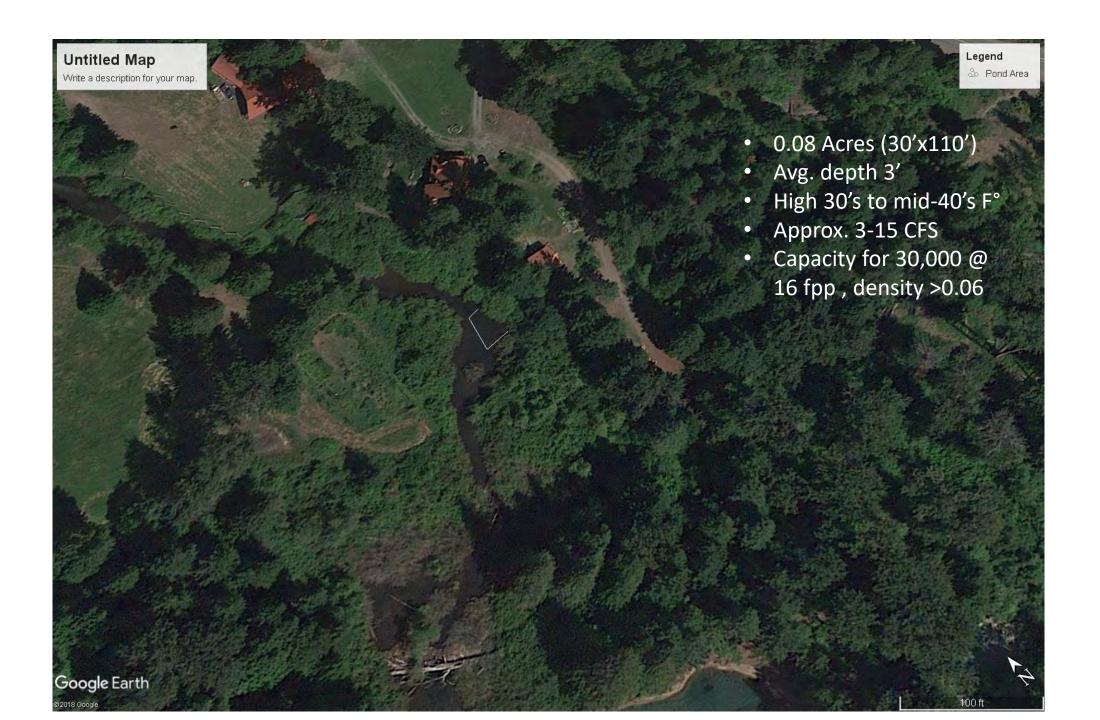
Notes:

- * Denotes HCP-HC member or alternate
- ‡ Denotes PRCC HSC member or alternate
- ^o Joined by phone



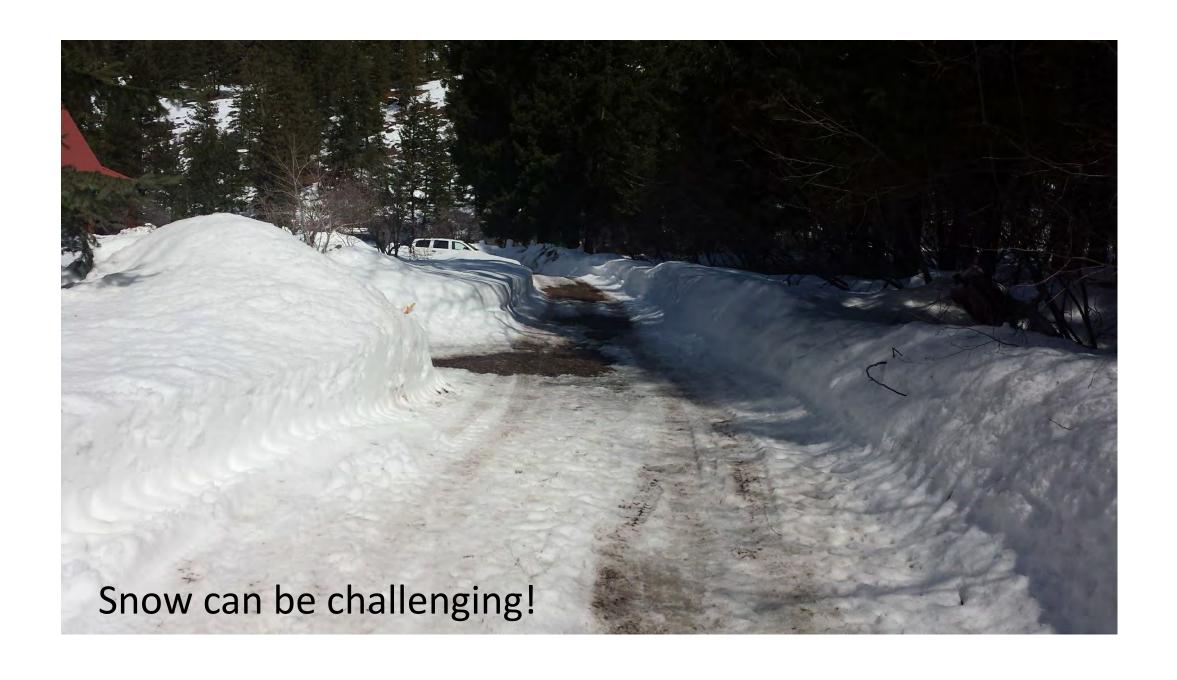






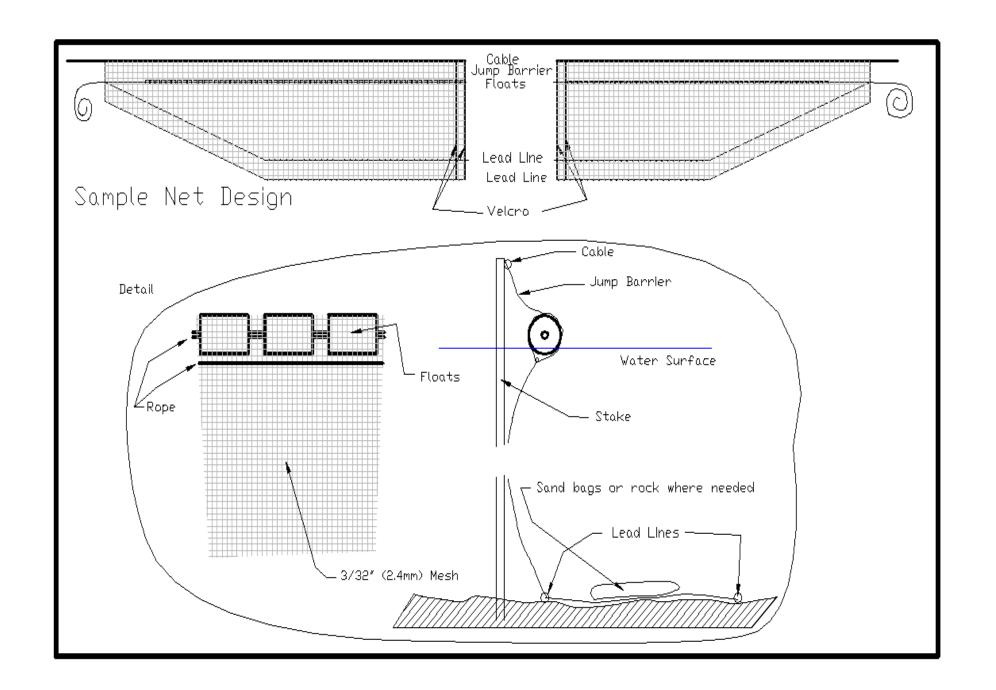
















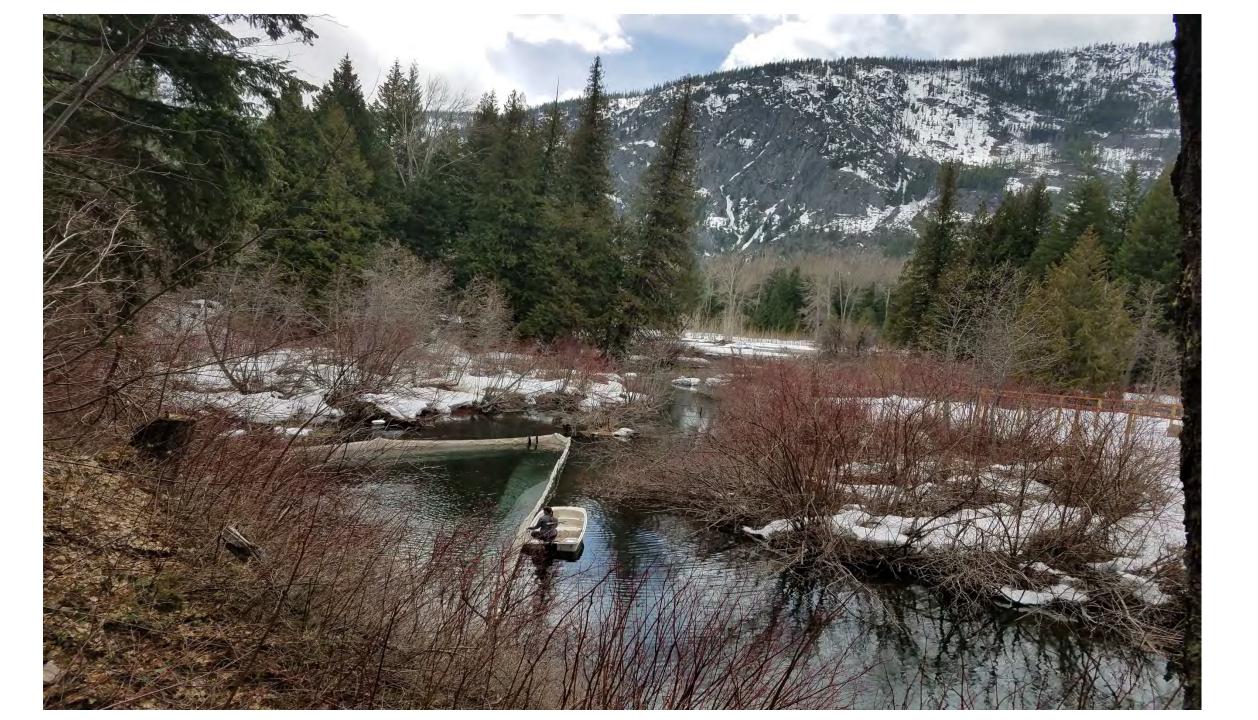


Acclimation activities

- Fed 2 to 3 times per day
- DO and Temp
- Growth samples and health monitoring
- Periodic snorkel surveys
- Predator hazing





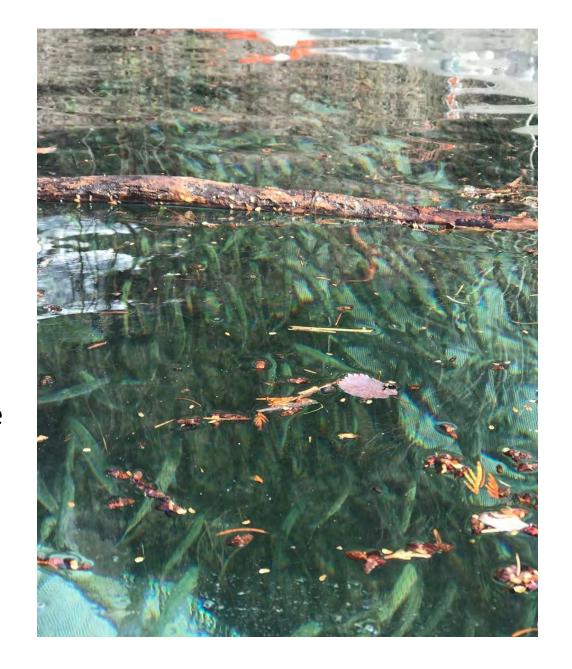


Release Summary 2017 - 2019

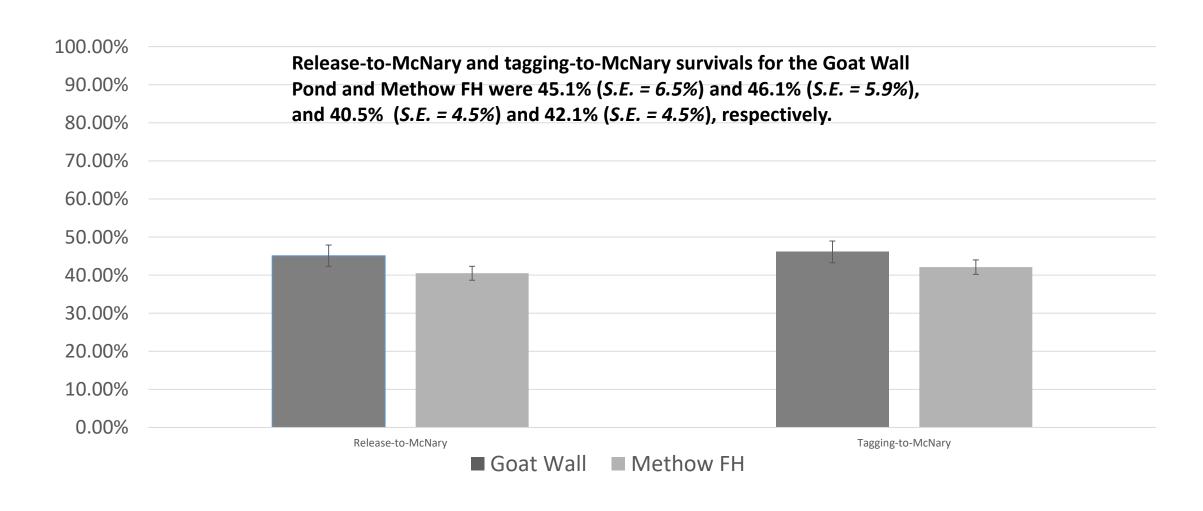
Release Year	# Received	# Released	Transfer Date	Start Release	End Release	PIT tags Released
2017	25,978	25,894	3/30	4/17	4/26	4,934
2018	28,535	27,970	3/15	4/18	4/29	4,425
2019	29,810	29,777	4/2	4/22	4/30	4,971

Results so far?

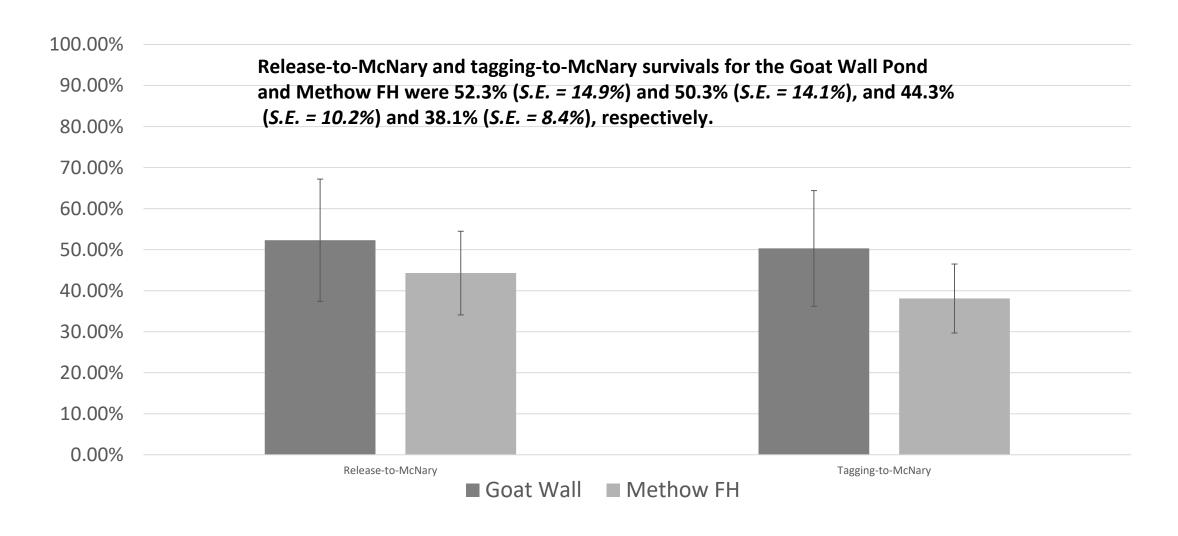
- Meet target size 15-18 fpp
- High in-pond survival
 - 2017 99.7%
 - 2018 98.0%
 - 2019 99.9%
- Minimal pre-release escapement 1.2 %
- Similar outmigration survival to other release groups
- Similar travel time



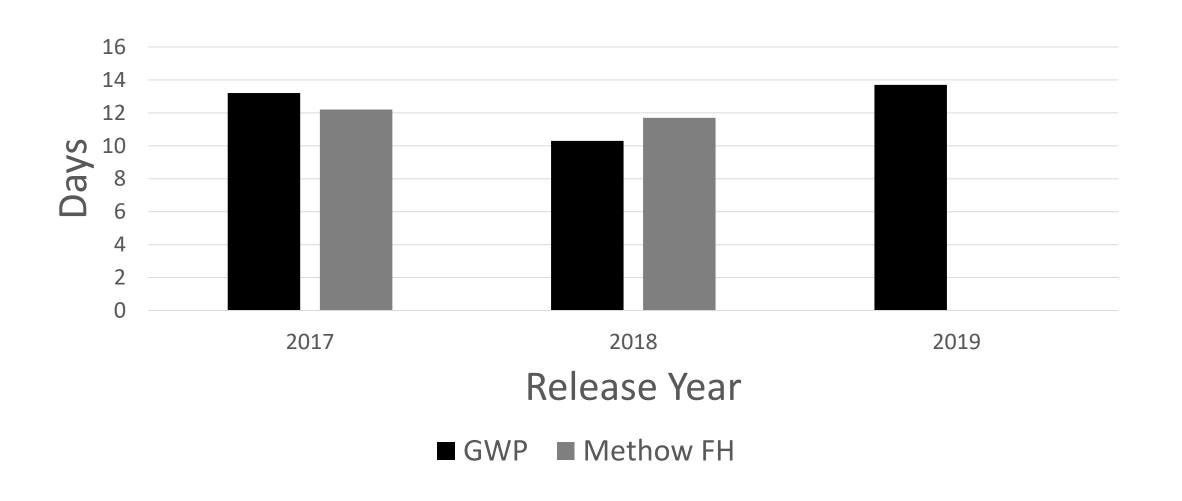
2017 Outmigration Survival



2018 Outmigration Survival

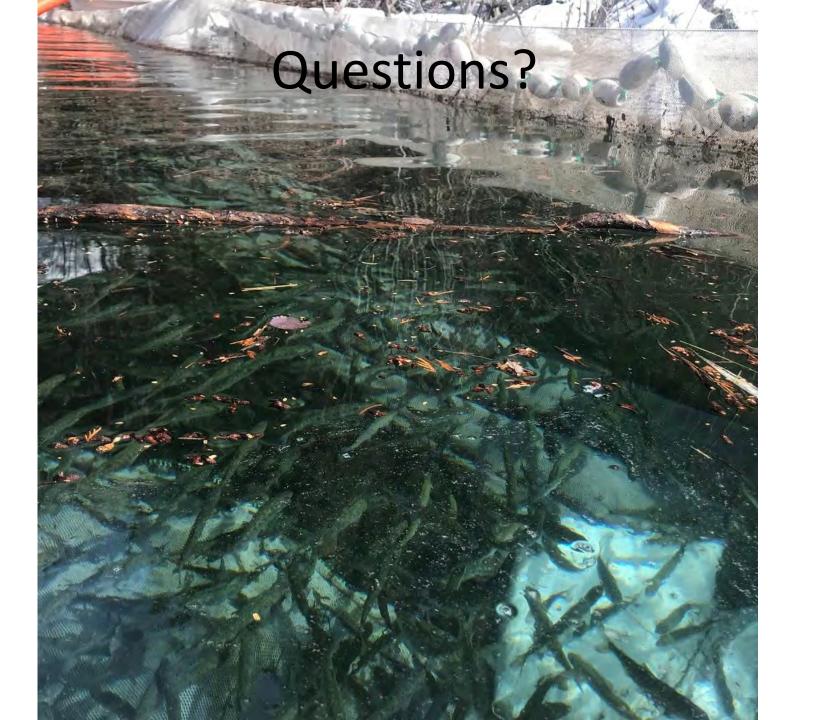


Mean Travel Time to Rocky Reach



Available adult return data for BY2015 based on PITs over Wells Dam

Release Location	# Released	Tag Rate %	Est. over Wells	SAR to Wells(%)
Goat Wall Pond	25,894	19.1	79	.30
Winthrop NFH	424,591	4.7	1364	.32
Methow FH	59,260	8.4	166	.28
Chewuch AF	65,621	7.6	210	.32
Twisp Weir	40,351	12.4	65	.16



CONTROL OF SAPROLEGNIA SP. GROWTH ON SUMMER CHINOOK (ONCORHYNCHUS TSHAWYTSCHA) EGGS

EXPERIMENTAL PROTOCOL - PILOT STUDY

WELLS HYDROELECTRIC PROJECT FERC NO. 2149

May 21, 2019

Prepared by:
Dr. Betsy Bamberger
DCPUD Fish Health and Evaluation Specialist

Public Utility District No. 1 of Douglas County East Wenatchee, Washington 98802

ABSTRACT

Summer Chinook (*Oncorhynchus tshawytscha*) eggs were treated with hydrogen peroxide, sodium chloride (salt), formalin, and ambient water for the prophylactic management of Saprolegniasis (water mold infestation) at Methow Fish Hatchery (MFH) in Winthrop, Washington. This pilot study detected no difference in apparent effect on viability or survivability of eggs and alevins among the treatments described. However, the relatively few number of water mold-infected eggs in the treatment groups suggests *Saprolegnia* sp. was present in the water supply in insufficient amounts to cause substantial infection, pathology, and/or loss. This result questions the historic and future need for preventative measures to be implemented for "fungus" control during egg incubation at MFH.

1.0 INTRODUCTION

Water mold (*Saprolegnia* sp.) is a common pathogen of salmonid eggs in fish hatcheries. Traditionally, hatcheries have used formalin for prophylactic management of *Saprolegnia* sp. infection in incubating eggs. However, formalin has long been associated with worker safety and environmental hazards, and is expected to be met with increasing scrutiny by regulatory agencies in the immediate future. In this study, we investigate the efficacy of purported alternatives to formalin that can be used as safe therapeutic substitutes. These alternatives include hydrogen peroxide (H₂O₂) and salt (NaCl), as well as no treatment (i.e., ambient water only), in controlling water mold infestations during salmonid egg incubation under typical hatchery conditions at the Methow Fish Hatchery (MFH) (located in Winthrop, Washington). The study described here is a pilot study to develop and evaluate the mechanics of delivering treatment and to gain preliminary results to guide future work. We tested the null hypothesis that there will be no difference found among test treatment groups (hydrogen peroxide, sodium chloride, no treatment [water]) and the formalin group, using egg mortality caused by Saprolegniasis as the basis for the analysis.

2.0 METHODS

Twenty-four (24) females and fourteen (14) males of Wells Hatchery-origin (hatchery) summer Chinook were collected at Wells Fish Hatchery (WFH) in mid to-late July, 2018. Once sexually mature, the adult fish were stripped of eggs and milt at WFH on October 16, 2018. All gametes were harvested on the same day to eliminate temporal bias. Once all were harvested, green eggs and ovarian fluid from spawned females were collected directly into a communal, approximately 65 L plastic tub, and gently mixed to control for potential maternal effects across the treatments. Eggs were then divided into twenty-four approximately equal portions (each deposited into a separate, numbered large Ziploc® bag) and placed in an ice-filled cooler lined with burlap. Milt from each male was collected in separate, small Ziploc® bags and stored in the same chilled cooler. Later that same day, all gametes were transported to MFH, approximately a one hour drive away. Upon arrival at MFH, the unfertilized eggs were split into two sets of twelve (each bag of eggs deposited into a separate plastic bucket) to accommodate the hatchery staff's preferred work methods. The eggs were combined with the milt from an individual male, used for primary fertilization, and then milt from a second male used as backup several minutes after initial fertilization by the primary male. Each male served as a primary male for at least one female and a backup male for one or more females, thus providing greater probability of successful fertilization and allowing identification of an individual male with reduced viability or non-viable gametes. All eggs were mixed prior to fertilization so detection of an individual female with reduced viability or non-viable eggs was not possible.

Eggs and milt were stirred together, gently rinsed with water, and then placed in a designated individual Heath vertical incubator tray and within a stack assigned to one of four treatment groups (formalin, salt, hydrogen peroxide, and water [no treatment]).

Eight incubation stacks were used, two for each of the four treatment groups. The topmost tray of each stack was kept empty to allow for ease of chemical introduction; three staggered trays below were reserved for eggs (see treatment-specific information and schematic representation

of experimental set-up in Figure 1, below). The formalin stacks were located in a separate incubation room to avoid potential adverse chemical reactions between formalin and hydrogen peroxide, but otherwise the stacks and trays were identical to the other treatments. Each tray was numbered in advance to identify treatment type and tray position in the stacks. Egg clutches were placed within trays in sequential order until all trays were occupied. For all treatments, the fertilized eggs in the trays within the stacks were water-hardened and disinfected in a 100 ppm buffered iodophor (Ovadine®) solution (static bath) for 60 minutes. Following water hardening and surface disinfection, fresh well water (averaging 8°C, [47°F]) was introduced into the stacks, effectively draining away the used iodophor solution from each tray. Flow was set at 3 gallons per minute (gpm) except in the salt treatment stacks, where it was set at 3.2 gpm to accommodate the added volume of saline solution to be introduced into the system.

Formalin, salt, and hydrogen peroxide were added to the topmost (empty) tray of the incubation stacks and delivered via a metered peristaltic pump (INTLLABTM or MasterFlex easy-load® II). Dosages of hydrogen peroxide, formalin, and salt were calculated to consider flow rate, treatment time, final desired concentration of chemical treatment, and chemical strength. As such, treatments were consistent with FDA-label instructions or previously published data (see Figure 1). Salt was pre-dissolved before administration; salinity was monitored during treatment with an Apera 5052 saltwater salinity tester with the probe placed in the topmost empty tray and recorded at multiple time points during administration (0, 5, 10, 15, and 20 minutes).

Daily 15-minute flow-through treatments with hydrogen peroxide, well water, and salt were initiated on the day following fertilization (October 17, 2018). Treatments continued on alternate days until November 22, 2018, providing 19 days of treatment, and ceased just prior to the initiation of hatching. Formalin treatments were administered on the second day following fertilization (October 18, 2018) to avoid undesirable exposure to other oxidizing compounds used in this study, and continued on alternate days until November 23, 2018, providing 19 days of treatment.

A total of 975 mL of hydrogen peroxide and 1,539 mL of formalin were used for each treatment day (volume accounts for both treated stacks). Salt concentrations varied during treatments before stabilizing but reached above 30 ppt on Oct 17th, Oct. 19th, Oct. 27th, Oct 29th, Nov 6th, and Nov 18th for 5-10 minutes.

On November 24, 2018, the incubator trays in the hydrogen peroxide, well water, and salt stacks were opened and eggs photographed; the eggs were shocked by mechanical agitation within the trays and then dead eggs were removed and counted before trays were returned to the stacks. The same occurred on November 25, 2018 for the formalin group (photographed on November 26, 2018). On November 27, 2018, five live eggs from each treatment group (trays 1-4) were fixed in 10% neutral buffered formalin and sent to the Washington Animal Disease Diagnostic Laboratory (WADDL) for histological analysis.

On December 1, 2018, the trays for all groups were again opened and any dead and *Saprolegnia*-infected eggs were removed by hand and counted. An average individual egg weight was estimated from a 100 egg sample of the remaining live eggs in each tray that were carefully dried (via Wypall shop towels) and weighed. This total dried egg weight from each female was divided

by the average egg weight to estimate the number of live eggs for each tray (note: total weights were reduced by 3% to approximate the weight of residual water). All trays were disinfected with Ovadine® for 10 minutes at 100 ppm before being placed back in the stacks.

Room 3		Roo	Room 3		Room 3			Room 1	
Stack 1	Stack 2	Stack 3	tack 3 Stack 4		Stack 5	Stack 6		Stack 7	Stack 8
Empty	Empty	Empty	Empty		Empty	Empty		Empty	Empty
1	5	2	6		3	7		4	8
Empty	Empty	Empty	Empty		Empty	Empty		Empty	Empty
9	13	10	10 14		11	15	12	16	
Empty	Empty	Empty	Empty Empty		Empty	Empty		Empty	Empty
17	21	18	18 22		19	23		20	24
Empty	Empty	Empty	Empty		Empty	Empty		Empty	Empty
Empty	Empty	Empty	Empty		Empty	Empty		Empty	Empty
No Trea	No Treatment		Hydrogen Peroxide		Sodium Chloride			Formalin	
100% Well Water		35% PER	35% PEROX-AID®		Diamond Crystal® Solar Naturals®			Parasite-S [®]	
		mg/L for 15 (~488 mls Hz continuous f once per day alternate day	Dosing regimen: 1000 mg/L for 15 minutes (~488 mls H202) in a continuous flow system once per day on alternate days until day 39 (consistent with FDA label).		Dosing regimen: 20,000 ppm for 15 minutes (~8 lbs salt per stack) in a continuous flow system once per day on alternate days until day 39 (based on findings in Waterstrat, 1995 and Edgell, P. and D. Lawseth, 1993).			Dosing regimen: 1/600 (1,666 ppm) for 15 minutes (~770 ml per stack) in a continuous flow system once per day on alternate days until day 40 (consistent with FDA label).	
	;		e (or 0.54				51.3 ml/minute (or 0.85 ml/second)		

Figure 1: Schematic representation of incubation room, stack and tray assignment, and dosing regimen per treatment group.

Fifty (50) eggs from each tray were combined into one tray for each treatment group (5^{th} tray from the top in stack 7, and 7^{th} tray from the top in stacks 1, 3, and 5) on December 8, 2018 and incubated until the alevin stage (50 eggs x 6 trays per treatment group = 300 per group x 4 groups = 1200 eggs total). All other remaining eggs were destroyed, per the study design.

Mortality from eyed egg to unfed fry for each treatment was assessed when the unfed fry were determined to be near ponding readiness on January 22, 2019. Staff checked for discernable morphological differences in unfed fry from each group to determine if histological analysis was warranted.

All alevins were destroyed before the first feeding, per the study design.

The criteria used to evaluate the efficacy of each compound was mortality from fertilization through eyed egg (which includes both water mold-infected eggs and dead uninfected eggs throughout the 40 day incubation period and after shocking) and eyed egg to unfed fry survivability. In addition, the extent of water mold infection was qualitatively (via photography) and quantitatively (via the number of eggs that appear infected) estimated and enumerated, respectively.

3.0 STATISTICAL ANALYSIS

One-way ANOVA was used to examine similarities or differences between treatment groups. We verified that the assumptions (equal sample sizes, independence, etc.) were maintained in order to justify the application of the ANOVA. Further, in order to verify that tray position had little or no influence on experimental results, we compared mean egg survival to the eyed egg stage (%) against tray position number within each stack using a one-way ANOVA. Further, to insure there was no interaction between tray position and treatment, we ran a 2-WAY ANOVA using these predictors and percent egg loss as the response variable. P-values were assessed at $\alpha = 0.05$. Had the response variable not satisfied the assumption of homogeneous variance, an alternative analysis such as beta regression would have been used. All statistical analysis was conducted in JMP (8.0.2 SAS Institute Inc.).

4.0 RESULTS

All treatments had high average egg survival in excess of 92.9%, suggesting that there was little difference among the treatments (Figures 2-8 and Table 1). The vast majority of egg mortality occurred between fertilization and the eyed egg stage, accounting for approximately 95% of total egg loss. Conversely, less than 5% of all egg loss occurred between the eyed egg stage and emersion from the egg (first and second picks).x Similarly, the survival of alevins revealed no difference among treatments (Table 2). Notably, the no treatment (water only) group was not statistically different from the other treatment groups during the egg or the alevin stages of the study.

Upon opening the trays to assess mortality and perform the first pick, a scant amount of grey flocculent material (*Saprolegnia* sp. mycelia) was found surrounding white-to-tan (nonviable) individual eggs in trays 1 (two eggs), 9 (two eggs), 17 (four eggs), and 21 (four eggs) of the no treatment group; 10 (three eggs), 18 (three eggs), and 22 (one egg) of the hydrogen peroxide group; and 19 (one egg) and 23 (one egg) of the salt group (see Figure 6). These eggs were readily apparent at first glance and situated on top of the egg pile; the trays were not mixed or otherwise disturbed to ascertain if any affected eggs were hidden. No eggs were observed to be infected in the formalin group.

Total mortality per sample (tray) ranged from 4.22-9.28% regardless of treatment. One-way ANOVA verified that there were no significant differences between treatment groups when we examined average percent loss in each treatment (p = 0.66, df = 3, fratio = 0.55; Figure 2).

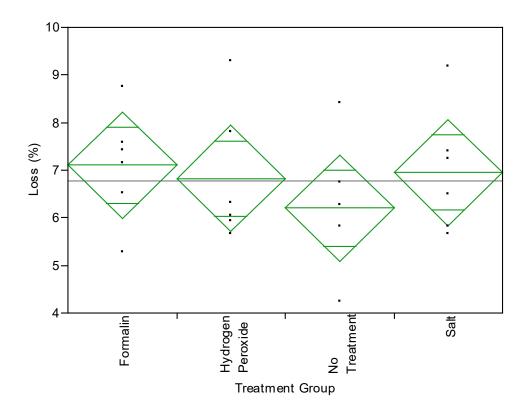


Figure 2. One-way ANOVA comparing total egg loss as a percentage of available eggs (y-axis) for each treatment group (x-axis). Green triangles represent 95% confidence intervals, the middle green line represents the group mean, and the gray horizontal line represents the mean of all replicates regardless of treatment. Results were somewhat variable within each treatment group and, as such, no significant differences were observed. (p = 0.66, df = 3, fratio = 0.55).

To test that our handling procedures, or in this case the order of tray placement, did not influence egg survival, we examined tray location category with average egg loss at those positions (%). This ANOVA yielded no relationship (p = 0.59, df = 2, fratio = 0.55, $\eta^2 = 0.082$; Figure 3), giving us confidence that tray position was unimportant in predicting egg loss and our husbandry of trays was appropriate. Finally, our 2-way ANOVA that used tray position and treatment as predictor variables that may interact with each other to have an effect on percent egg survival yielded no interaction (p = 0.48, df = 6, fratio = 0.96, $\eta^2 = 0.75$).

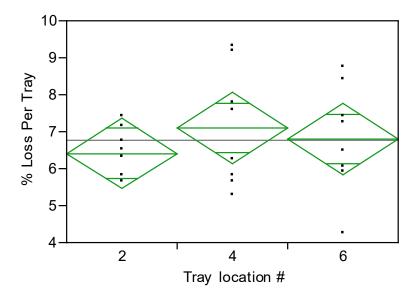


Figure 3. One-way ANOVA comparing average total egg loss as a percentage of available eggs (y-axis) relative to tray location (x-axis). Green triangles represent 95% confidence interval, the middle green line represents the group mean, and the gray horizontal line represents the mean of all replicates regardless of treatment. Results suggest tray location did not influence egg loss (p = 0.59, df = 2, f ratio = 0.55, $\eta^2 = 0.052$).



Figure 4: Pictures of Egg Trays (No Treatment Group); tray numbers in upper right corner.



Figure 5: Pictures of Egg Trays (Hydrogen Peroxide [35% PEROX-AID®]); tray numbers in upper right corner.



Figure 6: Pictures of Egg Trays (Sodium Chloride [Diamond Crystal®]); tray numbers in upper right corner.



Figure 7: Pictures of Egg Trays (Formalin [Paraside-S@]); tray numbers in upper right corner.

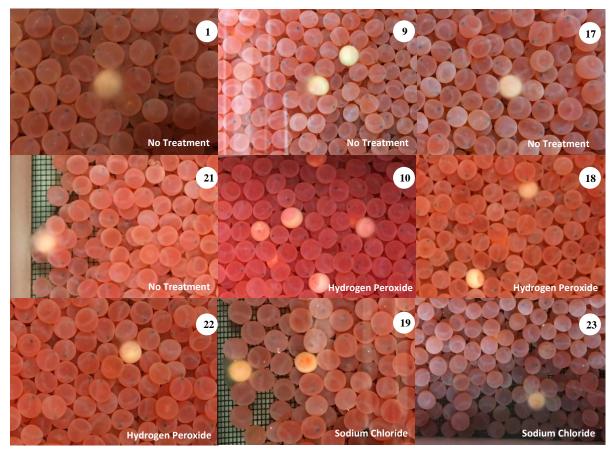


Figure 8: Saprolegnia sp.-infected eggs on the day of the first pick; tray numbers in upper right corner.

Table 1: Egg loss per tray from fertilization to eyed egg (first pick) and during the eyed egg stage (after second pick).

			tuge (uitei	. 50002	ia pien).					
Treatment Group	Female #	Shock Date	1st Pick Date	Egg Loss	2nd Pick Date	Egg Loss	Total Loss	Total Eggs	% Loss Per Tray	Average % Loss
No Treatment	1	24-Nov	25-Nov	251	1-Dec	2	253	3,758	6.73	
No Treatment	9	24-Nov	25-Nov	266	1-Dec	3	269	4,636	5.80	
No Treatment	17	24-Nov	25-Nov	156	1-Dec	3	159	3,770	4.22	6.200781296
No Treatment	5	24-Nov	25-Nov	238	1-Dec	3	241	4,147	5.81	0.200/81290
No Treatment	13	24-Nov	25-Nov	218	1-Dec	9	227	3,633	6.25	
No Treatment	21	24-Nov	25-Nov	324	1-Dec	13	337	4,016	8.39	
Hydrogen Peroxide	2	24-Nov	25-Nov	250	1-Dec	13	263	4,173	6.30	
Hydrogen Peroxide	10	24-Nov	25-Nov	310	1-Dec	8	318	4,086	7.78	
Hydrogen Peroxide	18	24-Nov	25-Nov	234	1-Dec	7	241	3,998	6.03	6.826897426
Hydrogen Peroxide	6	24-Nov	25-Nov	220	1-Dec	7	227	4,018	5.65	
Hydrogen Peroxide	14	24-Nov	25-Nov	410	1-Dec	8	418	4,503	9.28	
Hydrogen Peroxide	22	24-Nov	25-Nov	239	1-Dec	6	245	4,142	5.92	
Salt	3	24-Nov	25-Nov	240	1-Dec	2	242	4,175	5.80	
Salt	11	24-Nov	25-Nov	226	1-Dec	15	241	4,276	5.64	
Salt	19	24-Nov	25-Nov	284	1-Dec	11	295	4,560	6.47	6.948034887
Salt	7	24-Nov	25-Nov	278	1-Dec	6	284	3,841	7.39	0.948034887
Salt	15	24-Nov	25-Nov	308	1-Dec	7	315	3,437	9.17	
Salt	23	24-Nov	25-Nov	264	1-Dec	3	267	3,694	7.23	
Formalin	4	25-Nov	26-Nov	247	1-Dec	4	251	3,863	6.50	
Formalin	12	25-Nov	26-Nov	218	1-Dec	3	221	4,192	5.27	7.100103437
Formalin	20	25-Nov	26-Nov	360	1-Dec	5	365	4,178	8.74	
Formalin	8	25-Nov	26-Nov	314	1-Dec	1	315	4,414	7.14	
Formalin	16	25-Nov	26-Nov	261	1-Dec	2	263	3,478	7.56	
Formalin	24	25-Nov	26-Nov	232	1-Dec	5	237	3,204	7.40	

Table 2: Total egg/alevin loss in combined trays before destruction.

Treatment Group	Pick Date	Egg/Alevin Loss	% Loss per Tray
No Treatment	22-Jan	0	0
Hydrogen Peroxide	22-Jan	3	6
Salt	22-Jan	2	4
Formalin	22-Jan	1	2

5.0 DISCUSSION

There was no significant difference in survival among treatment groups to the eyed egg or the alevin stages. The spatial pattern of loss within trays, as evidenced by the photos taken after shocking, was more or less consistent between trays. Most often, nonviable eggs were evenly distributed throughout the tray, indicating that mortality was ostensibly sporadic and not necessarily associated with pathogen epicenters or water flow irregularities. The two exceptions were trays 10 (hydrogen peroxide—treated group) and 20 (formalin-treated group) with dead eggs clumped in the center of the trays. The phenomenon may have been coincidental. The egg/alevin losses were almost negligible and consistently low among all groups. The relatively few numbers of water mold-infected eggs suggests *Saprolegnia* sp. was present in the water supply in insufficient amounts to cause substantial infection, pathology, and/or loss. This result questions the historic and future need for preventative measures to be implemented for "fungus" control during egg incubation at MFH.

In 2015, the percent survival of unfertilized egg-eyed was 96.1 and 98.8 for the Methow Composite spring Chinook and Twisp spring Chinook, respectively, while in the Wells summer Chinook yearling program (reared at WFH and the source of the eggs used in this study) the percent survival of unfertilized egg-eyed was 90.0. The mean percent survival of brood years 1999 through 2015 was 95.0 and 94.2 for the Methow Composite spring Chinook and Twisp spring Chinook, respectively. The egg survival percentage in this study (on average equal to or in excess of 92.9% for all treatments) is comparable to those historically recorded at Methow, if slightly lower. This disparity may be attributable to the difference in stock (summer versus spring-run Chinook), incubation vessel (trays versus the standard isolation buckets used at the hatchery), or quality of eggs (study eggs were attained near the tail-end of the spawning season at Wells Hatchery and underwent additional handling during transport to Methow Hatchery). Compared to the egg mortality rates at Wells, the eggs in this study had slightly higher survival, but it is difficult and perhaps unproductive to compare losses across facilities with different water sources, set-ups, and other variables.

The chemicals themselves appear to have made little impact on the eggs in terms of survivability. Indeed, the eggs collected and submitted on November 27, 2019 for histological analysis were found to have no differences in cellular structure (see Attachment 1 for the report from the Washington Animal Disease Diagnostic Laboratory). Hatchery staff did notice that the formalintreated eggs felt the "hardest" (re: most rigid) among all groups and that the salt-treated eggs were perceived to be the tackiest. Additionally, when trays were tapped (presumably to better sift

through and inspect the eggs within), the eggs in the hydrogen peroxide, salt, and no treatment groups did not move within the trays as effortlessly as was thought to be normal for formalintreated eggs. The hatchery staff also noted that the eggs in the salt treatment group hatched a day or two early; it is theorized that the saline mixture dissolved in hot water the day before may have warmed the ambient well water in the salt-treated stacks and influenced the rate of development in treated eggs. This seems unlikely but no other explanation to account for this observation is readily apparent.

6.0 CONCLUSION

There was no appreciable difference in egg mortality or hatch-out viability between treatment groups. There was no apparent effect on viability or survivability of eggs and alevins associated with the treatments described. Notably, the use of ambient water (i.e., no treatment) was as effective as treating with any of the three chemicals tested, suggesting that in some situations chemically treating eggs may not be necessary to achieve high survival during egg incubation at MFH. However, it is unclear if the treatments attempted in this pilot would control *Saprolegnia* sp. infestations if tried in a system with infectious levels of pathogen present.

Future work should include conducting a similar study in a facility with a greater likelihood of high levels of *Saprolegnia* sp. A follow-up pilot study is planned for the fall of 2019 at MFH. Untreated spring or summer Chinook eggs (if available) will be incubated in isolation buckets and compared to formalin-treated eggs of the same run reared in similar containers. Based on the findings found here, it is hypothesized that there will be no difference in egg mortality and hatchout survivability between the two groups. These findings could help inform future egg management strategies and reduce chemical use at MFH.

Another follow-up study will be attempted on summer Chinook in the fall of 2019 at WFH, where levels of *Saprolegnia* sp. may be higher. The study protocol will greatly resemble this one with provisions and accommodations appropriate for that facility.

7.0 ACKNOWLEDGEMENTS

Special thanks to the Methow Fish Hatchery staff (Brandon Kilmer, Dave Dinsmore, Matt Moore, and Emily Vinge); Greg Mackey and Andrew Gingerich for their help in completing this pilot study; and review by Tracy Hillman.

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Betsy Bamberger Douglas County PUD 1151 Valley Mall Parkway East Wenatchee, WA 98802 Case#: **2018-14837**Report Date: 11 Dec 2018
Received: 30 Nov 2018

Owner: Methow Fish Hatchery Animal: Experimental Eggs Species: Chinook Salmon

Breed: Sex/Age: ,

Histopathology Report

Embryonated summer chinook salmon eggs from 4 treatment groups are received fixed in formalin. Eggs are bisected and processed into 4 slides for histologic examination as follows:

- Slide 1. Hydrogen peroxide
- Slide 2. No treatment
- Slide 3. Formalin
- Slide 4. Salt

All eggs are histologically similar and unremarkable.

HISTOLOGIC DIAGNOSIS:

1. Histologically normal embryonated eggs (see comments)

COMMENTS: There was no discernable difference between the treatment groups on histologic examination. However, the lack of histologic changes does not necessarily rule out functional or physiological differences or viability between groups. Chemical changes (e.g. on yolk proteins and lipid) associated with different treatments may not result in a corresponding histologically appreciable change.

WORK PENDING: None

Case#: 2018-14837 Report Date: 11 Dec 2018

Histopathology Report

Pathologist: Dr. Allan Pessier

Report authorized by: Dr. Allan Pessier, Senior Pathologist