FINAL

1201 3rd Avenue, Suite 2600
Seattle, Washington 98101
206.287.9130

ANCHOR QEA

Memorandum

To: Wells, Rocky Reach, and Rock Island HCP Coordinating Committees and Priest Rapids Coordinating Committee

Date: June 2, 2026

From: Larissa Rohrbach, PRCC Facilitator
Tracy Hillman, HCP Coordinating Committees Chair

cc: Kristi Geris, HCP Coordinating Committees and PRCC Support

Re: Final Minutes of the April 28, 2026, HCP Coordinating Committees and Priest Rapids Coordinating Committee Subyearling Joint Session 16

The Wells, Rocky Reach, and Rock Island Hydroelectric Projects Habitat Conservation Plan Coordinating Committees (HCP-CCs) and Priest Rapids Coordinating Committee (PRCC) met in person at the Confluence Technology Center in Wenatchee, Washington, on Tuesday, April 28, 2026, from 11:15 a.m. to 12:15 p.m., for a joint session to discuss next steps for subyearling Chinook Salmon survival studies. Attendees are listed in Attachment A to these meeting minutes.

Action Item Summary

1. Anchor QEA and BioAnalysts will coordinate with HCP-CCs and PRCC representatives to formulate questions for Ryan Harnish (Pacific Northwest National Laboratory) and Rebecca Buchanan (Buchanan Statistical Services) after evaluating subyearling tagging effects (Item I-C).
2. Douglas PUD will distribute a revised *Study Design for Subyearling Chinook Salmon Tag Retention Study* by May 13,[1] comments will be due May 20, and Douglas PUD will request approval during the Subyearling Joint Session or HCP-CCs meeting on May 26, 2026[2] (Item II-A). *(Note: The revised study plan was distributed on May 14, and a second revised plan was distributed on May 26.)*
3. The next scheduled Subyearling Joint Session (Session 17) is to be determined[3] (Item II-C).

Review Items

1. Douglas PUD's draft *Study Design for Subyearling Chinook Salmon Tag Retention Study* was distributed to the HCP-CCs and PRCC by Kristi Geris on February 10. The Yakama Nation (YN) provided comments on March 19. A revised study plan[4] and YN Temperature Acclimation and

[1] Douglas PUD will strive to distribute the revised study plan by May 11, 2026, as requested by the Yakama Nation (YN), to provide additional time for review and comment before the YN is away on leave.
[2] The YN will strive to provide a vote via email before going on leave on May 17, 2026.
[3] Tentatively, a subyearling joint session may not be needed next month, and approval of the Douglas PUD study plan would take place during the normal HCP-CCs session. These details will be confirmed closer to the meeting date.
[4] During the meeting, Douglas PUD noted that the revised study plan distributed before today's joint session will be further updated to include Rebecca Buchanan's most recent analysis and additional logistical details.

Thermal Tolerance Literature Review were distributed before the joint session on April 28. The revised study plan for approval was distributed on May 14, 2026 (Item II-A).

Decision Summary

1. There were no decisions approved during today's meeting.

I. Welcome

A. Review Agenda

Larissa Rohrbach (PRCC Facilitator) and Tracy Hillman (HCP-CCs Chair) welcomed the PRCC and HCP-CCs to the sixteenth joint session of the HCP-CCs and PRCC to discuss subyearling survival studies.

B. Joint Session Minutes Approval

The PRCC and HCP-CCs reviewed the draft Subyearling Joint Session 15 (March 24) meeting minutes. Kristi Geris said the draft minutes for review were distributed on April 14. Edits were received from Douglas PUD. Revised minutes for approval were distributed on April 21. There is one outstanding comment to discuss under *Item II-B. Douglas PUD Update* regarding FryID tags. Andrew Gingerich reviewed the comment and accepted the suggested addition of a clarifying footnote. The PRCC members and HCP-CCs representatives present approved the Joint Session 15 minutes, as revised. Kirk Truscott abstained because a Confederated Tribes of the Colville Reservation representative was not present during Joint Session 15.

C. Joint Session Action Items Review

Action items from Joint Session 15 on March 24 and follow-up discussions were as follows *(Note: Italicized text corresponds to agenda items from the meeting on March 24)*:

1. *Anchor QEA and BioAnalysts will coordinate with HCP-CCs and PRCC representatives to formulate questions for Ryan Harnish (Pacific Northwest National Laboratory) and Rebecca Buchanan (Buchanan Statistical Services) after evaluating subyearling tagging effects (Item I-C).*
   This action item will be carried forward.
2. *Douglas PUD will provide a revised study plan for approval, and Wells HCP-CC representatives will be prepared to vote during next month's Joint Session 16 on April 28, 2026 (Item II-A).*
   This will be discussed during today's meeting.

II. Subyearling Joint Session 16

An updated working draft Subyearling Workshop Matrix, which tracks the progression of these joint sessions since the 2024 Subyearling Chinook Salmon Workshop, was distributed to the HCP-CCs and PRCC by Kristi Geris on April 21, 2026.

A. Douglas PUD Update

Tracy Hillman recalled that Douglas PUD's draft *Study Design for Subyearling Chinook Salmon Tag Retention Study* was distributed to the HCP-CCs and PRCC by Kristi Geris on February 10. The YN provided comments on March 19. Last month, the Wells HCP-CC briefly discussed the YN's comments. John Rohrback said a revised study plan was distributed by Geris today that addresses some of the YN's comments, but Douglas PUD plans to provide another revised draft that includes Rebecca Buchanan's most recent analysis and additional logistical details, which Buchanan will present today.

Buchanan shared on Microsoft Teams the presentation, *Tag Retention Study – Sample Size Update* (Attachment B), which was distributed to the PRCC and HCP-CCs by Geris on April 29. Buchanan reviewed each slide and further discussions were as follows.

Compare: Original and Revised Plan (Slide 3)

Hillman asked whether this is still a split-plot design. Buchanan said yes, same design just changes in fish size and sample size.

K. Murdoch recalled one issue she raised with the discrete size classes was with hypothesis testing. The design previously looked at tag retention and mortality in 65-millimeter (mm) fish versus 120-mm fish, so basically binary hypothesis testing. There is higher tag expulsion in smaller fish compared to larger fish, and there is higher tag expulsion at higher temperatures compared to lower temperatures. The way the size classes were broken out worked, but there was an opportunity to use size as a continuous variable to do model-fitting to better understand the relationship between fish size and temperature. Buchanan said a curve will be fitted to fork lengths (FLs) to estimate the regression coefficient. Thus, size will be treated as a continuous variable. Having a larger sample size allows for more variability in FL, so the model will have a greater ability to estimate the regression coefficient.

Fish Sizes Used: Simulated Distribution (Slide 4)

Bill Gale asked why 95 mm for the middle group and not 90 mm? J. Rohrback said target FLs were selected based on available fyke net distribution data and a hatchery growth schedule, but this can be changed to 90 mm if U.S. Fish and Wildlife Service (USFWS) has a preference. Gale said he has no preference, but there is overlap in the tails of the 95-mm and 120-mm groups and no overlap between the 60-mm and 95-mm groups. Is this because the separation is too great to overlap? Andrew Gingerich said the middle group could move down, but then there will be a gap on the other side. Tom Kahler said hatchery staff asked what was needed, and the response was what can be done. The fish need to be fed and will grow over the course of the study according to the recommended feed rate, and he is unsure about what may happen if feed is reduced to manipulate size. Generally, the goal was to match fyke net data.

Study Retention to Day 30: Main Effects Model (Slide 6)

Buchanan modeled probability of study retention to day 30 by temperature and model type to find out whether there is a combination of size and temperature (or interaction effect) the study should not use because it will result in tag loss.

Interaction Effect: Effect Size (Slide 8)

Buchanan modeled various interaction effects and had to decide which to use for the power analysis, so she chose these two (50% reduction and 95% reduction in study retention).

J. Rohrback said these curves are the best guess of what a response might be. This is what was gained by increasing sample size.

Power to Detect Interaction Effect (Slide 10)

Buchanan was looking for 80% power to detect a temperature and FL interaction effect. Option 2 (Logistic Model) had much higher power. For Option 1 (Proportional Hazards Model), it did not matter how many fish were used, the power was abysmal.

Large Interaction Effect: 95% Reduction in Study Retention for Smallest Fish at 20˚C (Slide 11)

Buchanan said a sample size of 50 fish provides good power for FL and temperature, and some good power for interaction effects. Increasing the sample size by a feasible amount does not increase power significantly.

J. Rohrback said he thinks K. Murdoch hoped to be able to apply these results to a survival study in the field and be able to determine the likelihood of losing a tag for each fish tagged. K. Murdoch said her concern was there was no description about how the data would be used to inform a study design. There are different ways the data could be used to identify tagging thresholds. For example, what is the smallest fish threshold, or the highest temperature threshold, or could the data be used to develop a model to adjust study results for a range of sizes and temperatures? J. Rohrback believes Buchanan's power analysis found that this study will not have the statistical power to develop this sort of model. However, increasing the sample size allows the study to estimate the likelihood of tag retention through the 30-day period by looking at temperature and size at the time of tagging (the main effects, see Slide 8), and these data can be used to inform discussions when designing a study. Buchanan said this is correct.

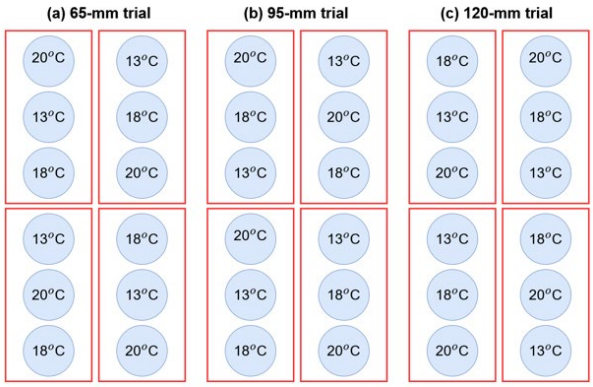
Intermediate Interaction Effect: 50% Reduction in Study Retention for Smallest Fish at 20˚C (Slide 12)

Buchanan said this slide confirms the study should still target the main effects.

Recommendations (Slide 13)

Gingerich noted that an increase in sample size means moving to multiple taggers. This introduces another covariate. The plan will likely involve tagging all fish of a given size and temperature in one day with three taggers at each session. J. Rohrback clarified that this means, within each size class tagging each temperature group in one day. For each target size class, tagging and study treatment will occur over three days – one day at each of the target temperatures. Gingerich said the study will randomly assign taggers to minimize tagger bias, and Buchanan will test for tagger effects. If there is no effect, all data can be considered together in the analysis.

Hillman said the whole plot factor is FL and the subplot is temperature. He asked what the block[5] is in this study? Buchanan explained there are 12 tanks and three temperatures, so four replicates of temperature within each size class, or a spatial block within each size class. Hillman asked, why not analyze the data using a 3x3 ANOVA?[6] What does the blocking gain in this study? Buchanan said the blocking is primarily to get replicates. There is concern that there might be some differences in different parts of the facility (e.g., light, water quality, air quality, or sound). Hillman shared on Microsoft Teams Figure 1 from the draft study plan:



Hillman said if, for example, the first row of red rectangles is a specific tagger or specific lighting, and the second row is a different tagger or different lighting (two blocks), one cannot look at interactions between the blocks (location/tagger) and subfactor (temperature), but one can evaluate interactions between the whole plot (FL) and subplots (temperature), which would address Murdoch's comment. In a typical split-plot design, Buchanan agreed, but this design is not quite consistent with a typical split-plot design because the top level is FL. This limits the type of interactions one can look at. Hillman said if blocking does not provide much contrast, it could be dropped and the data could be analyzed with a 3x3 ANOVA, which still provides the important interaction term. There are different options for analyzing the data from this type of design. Buchanan agreed there is flexibility in this.

[5] In a split-plot design, the "block" is the replication unit (e.g., a field, batch, or location) containing whole plots, the whole-plot factor is applied to the larger experimental units or plots (fork length), and the subplot factor is applied to smaller divisions within the whole-plot (temperature).
[6] Analysis of Variance

Other Comments – Acclimation

K. Murdoch said the YN is still concerned that the acclimation schedule is too rapid. She asked whether Douglas PUD is still proposing 13˚C to 20˚C in 5 days with a 2-day holding period.

J. Rohrback said a YN Temperature Acclimation and Thermal Tolerance Literature Review (Attachment C) was distributed to the PRCC and HCP-CCs by Geris before today's joint session on April 28. Douglas PUD can do what the YN proposed in this literature review, which is ramping up 1˚C per day with a 7-day holding period at the temperature at which fish will be tagged. This adds a little time to the study, but Douglas PUD can make this happen.

K. Murdoch said a particularly applicable study in this literature review is Stewart et al. (2023), which looked at the thermal maxima in Brook Trout (*Salvelinus fontinalis*) and measured how acclimation improves thermal tolerance. This study found there was a point where improvement in thermal tolerance levels out. In Brook Trout, this takes longer than 30 days. Another study (Anttila et al. 2014) evaluated juvenile Atlantic Salmon (*Salmo salar*) of a similar size range and measured thermal tolerance via cardiac output. This study found there is a point where the heart cannot keep up with the oxygen demands resulting from increased temperatures. The study looked at fish acclimated to 12˚C and 20˚C. Fish acclimated to 20˚C for 3 months had an increased thermal tolerance of 3˚C to 6˚C. This study did not measure how long it took to get to this point in the wild. In the Wells Pool, the 10-year average and during last year, temperatures increased from 13˚C to 20˚C in 70 days. This compared to 1 to 2 weeks in a laboratory is not the same thing. The YN is concerned that if fish are not fully acclimated, then this does not truly reflect tag effects at these temperatures. The results will be a mix of tag and thermal acclimation stress that might not be the same in wild fish. In the cardiac example, acclimating a fish for 1 week that has been reared at 13˚C is still physiologically 13˚C fish. Based on this literature review, these PUD studies are a compromise between what is ideal and laboratory logistics. She acknowledged, however, that acclimating study fish for 3 months does not seem feasible.

K. Murdoch said another idea she thought of after conducting this literature review is to have fish on river water and collect study fish every 2 weeks, so fish are fully acclimated, and size and temperature ranges would be the same, with the same photoperiod. This would better mimic a survival study but would likely be more of an inconvenience with a lot of logistics to work out. Kahler agreed this would be ideal, but there would also be the challenge of getting wild fish to eat. Lance Keller also noted biosecurity issues. K. Murdoch said this would provide contrast to the laboratory studies. Gingerich recalled discussing this early on and the HCP-CCs and PRCC landed on laboratory studies because these allow for more control.

Hillman asked in the Brook Trout and Atlantic Salmon studies whether the researchers also measured the production of heat shock proteins. K. Murdoch said the Brook Trout study was testing rate of acclimation, and she does not believe the evaluation included heat shock proteins. She does have

another publication about how heat shock proteins are protective. She added that she has copies of all of the articles cited in the literature review if anyone wants a copy. Hillman said he was just wondering whether the studies acclimated fish slow enough to minimize the production of heat shock proteins. Bill Gale thought there is more than just temperature that affects production of heat shock proteins.

Other Comments – Various

J. Rohrback noted that lights have been installed in Wells Hatchery to match the photoperiod, per K. Murdoch's comments.

K. Murdoch asked whether Douglas PUD has addressed her question about the threshold for a catastrophic disease outbreak. J. Rohrback said yes, if one fish dies per day for 5 consecutive days this would trigger a catastrophic disease outbreak. K. Murdoch said this was based on a 10-fish-per-unit sample size, so will this be updated for the increased sample size? J. Rohrback said yes, the definition of catastrophic disease outbreak is likely to change to accommodate the larger study sample, but because the rule of thumb for a disease outbreak within a hatchery is 0.05% mortality per day, the changes might not be great.

L. Rohrbach recalled one major comment that was similar to revisions requested for the Chelan PUD/Grant PUD study plan was rewriting the objective statements to align with the YN's comments requesting a more direct link showing how study objectives will fill in-river survival study information gaps. J. Rohrback said yes, Douglas PUD is working on this.

Next Steps

Douglas PUD will distribute a revised *Study Design for Subyearling Chinook Salmon Tag Retention Study* by May 13, comments will be due May 20, and Douglas PUD will request approval during the Subyearling Joint Session or HCP-CCs meeting on May 26. *(Note: The revised study plan was distributed on May 14.)*

Douglas PUD will strive to distribute the revised study plan by May 11, as requested by the YN, to provide additional time for review and comment before the YN is away on leave.

The YN will strive to provide a vote via email before going on leave on May 17, 2026.

B. Grant PUD and Chelan PUD Update

No update was shared.

C. Next Meetings

The next scheduled Subyearling Joint Session (Session 17) is to be determined.[7]

List of Attachments

Attachment A List of Attendees

Attachment B *Tag Retention Study – Sample Size Update*

Attachment C YN Temperature Acclimation and Thermal Tolerance Literature Review

[7] Tentatively, a subyearling joint session may not be needed next month, and approval of the Douglas PUD study plan would take place during the normal HCP-CCs session. These details will be confirmed closer to the meeting date.

Attachment A
List of Attendees

Name	Organization
Larissa Rohrbach	Anchor QEA
Kristi Geris	Anchor QEA
Tracy Hillman	BioAnalysts
Rebecca Buchanan○	Buchanan Statistical Services
Lance Keller*	Chelan PUD
Catherine Willard*	Chelan PUD
Bill Towey*	Chelan PUD
Pete McHugh†○	Columbia River Inter-Tribal Fish Commission for the Confederated Tribes of the Umatilla Indian Reservation
Kirk Truscott*†	Confederated Tribes of the Colville Reservation
Tom Kahler*	Douglas PUD
Andrew Gingerich*	Douglas PUD
John Rohrback	Douglas PUD
Rod O'Connor†○	Grant PUD
Tim Taylor†○	Grant PUD
Scott Carlon*†○	National Marine Fisheries Service
Bill Gale*†	U.S. Fish and Wildlife Service
Chad Jackson*†	Washington Department of Fish and Wildlife
Keely Murdoch*†	Yakama Nation

Notes:

* Denotes HCP Coordinating Committees representative or alternate.

† Denotes PRCC representative or alternate.

○ Joined by phone.

Tag Retention Study

Sample Size Update

April 28, 2026

Topics

- Compare: original and revised plan
- Fish sizes used
- Interaction effect
  - Effect size
  - Power curves
- Recommendations

Compare: original and revised plan

Original plan

- 3 target fork lengths
  - 65 mm, 95 mm, 120 mm
- 3 temperatures:
  - 13˚C, 18˚C, 20˚C
- 3 tag types:
  - PIT, sham, control
- 10 fish per group:
  - Target size class X temperature X tag type X replicate
  - 120 fish per temperature X tag type
  - 1,080 fish total

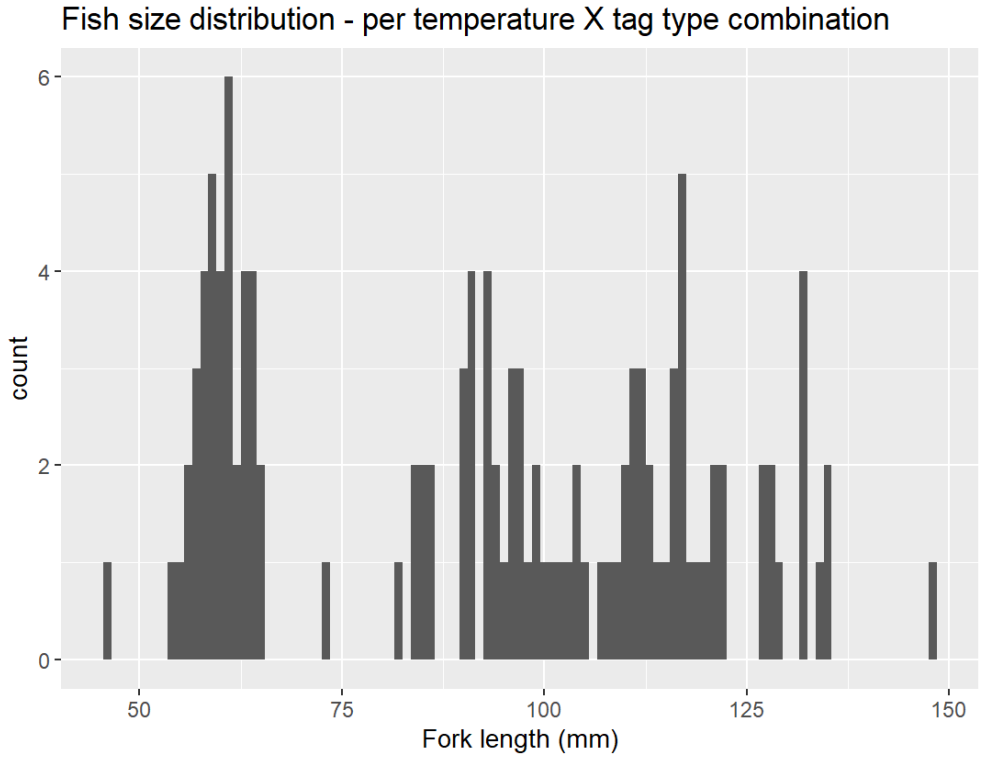
Revised plan

- 3 target fork lengths
  - 60 mm, 95 mm, 120 mm
- 3 temperatures:
  - 13˚C, 18˚C, 20˚C
- 3 tag types:
  - PIT, sham, control
- 50 fish per group:
  - Target size class X temperature X tag type X replicate
  - 600 fish per temperature X tag type
  - 5,400 fish total

Fish sizes used: simulated distribution

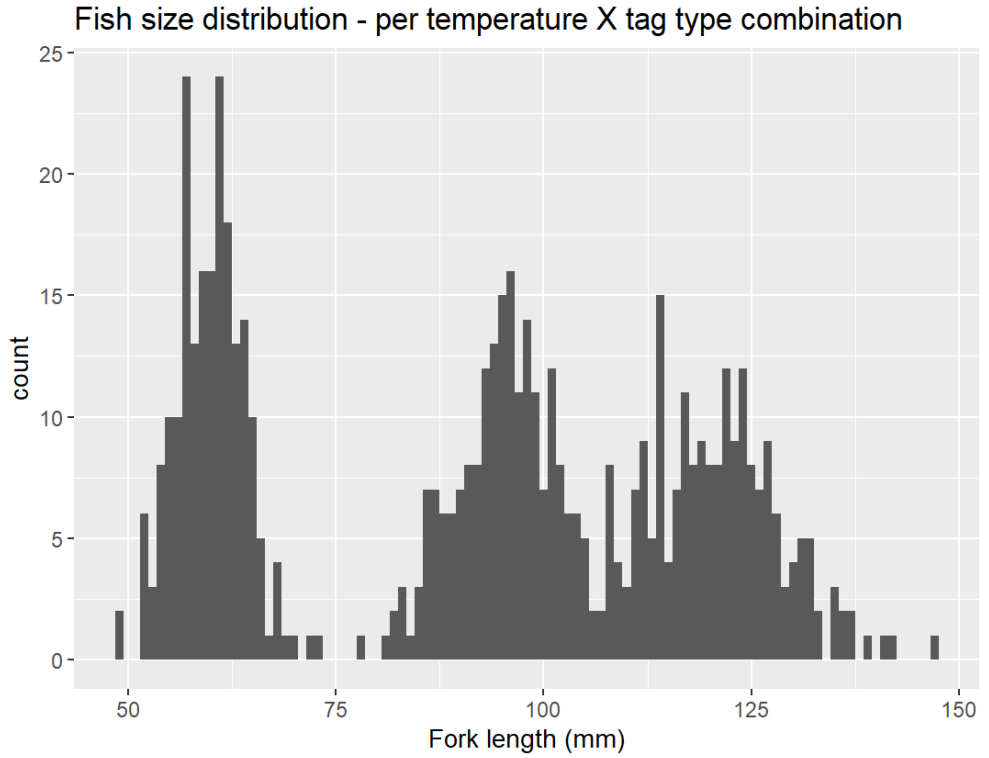
10 fish per group:

- **Target size class X temp X tag type X replicate**
- **120 per temperature X tag type combo**
- **1,080 total**



50 fish per group:

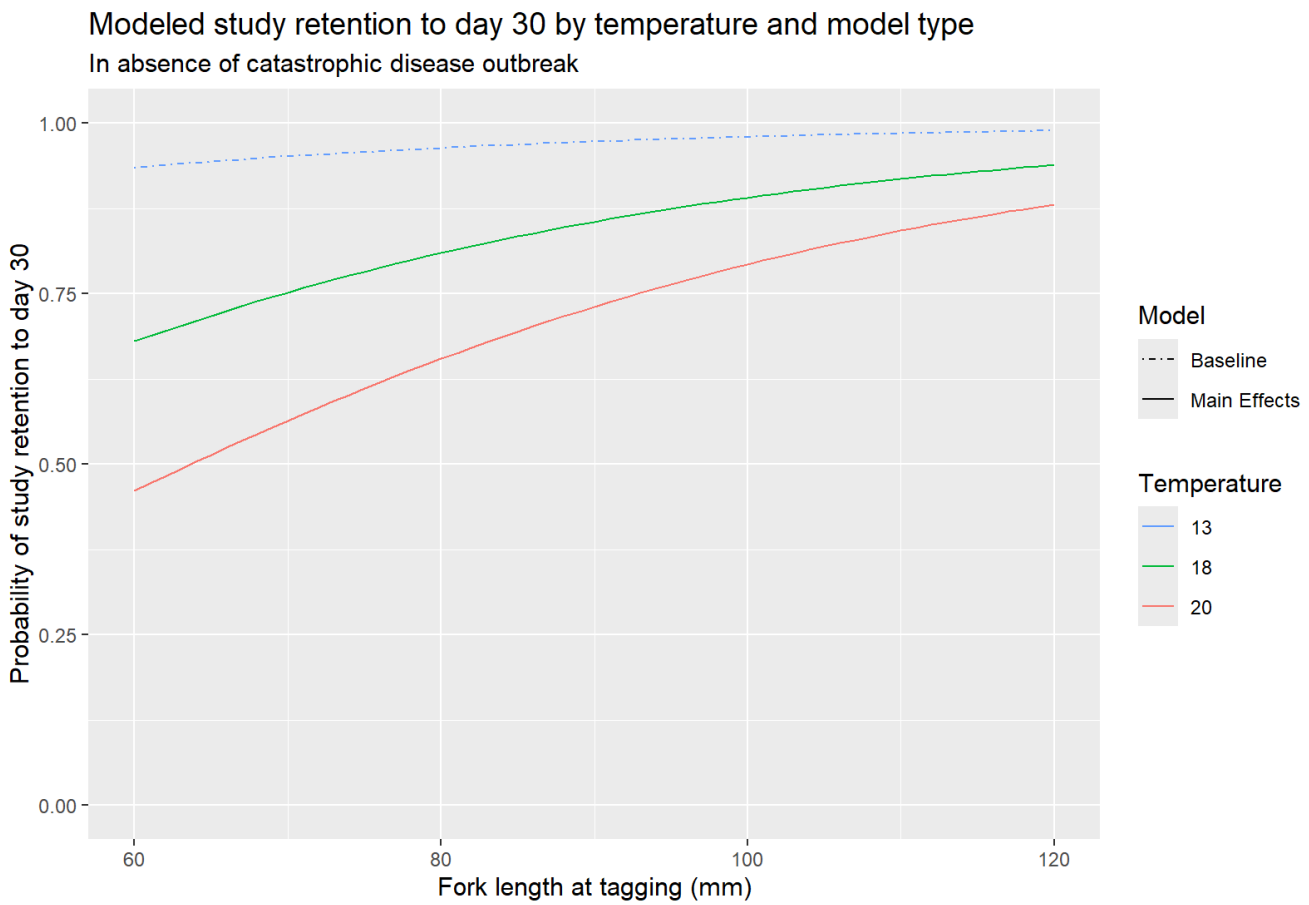
- **Target size class X temp X tag type X replicate**
- **600 per temperature X tag type combo**
- **5,400 total**



Interaction effect

Between fish size and water temperature

Study retention to day 30: main effects model



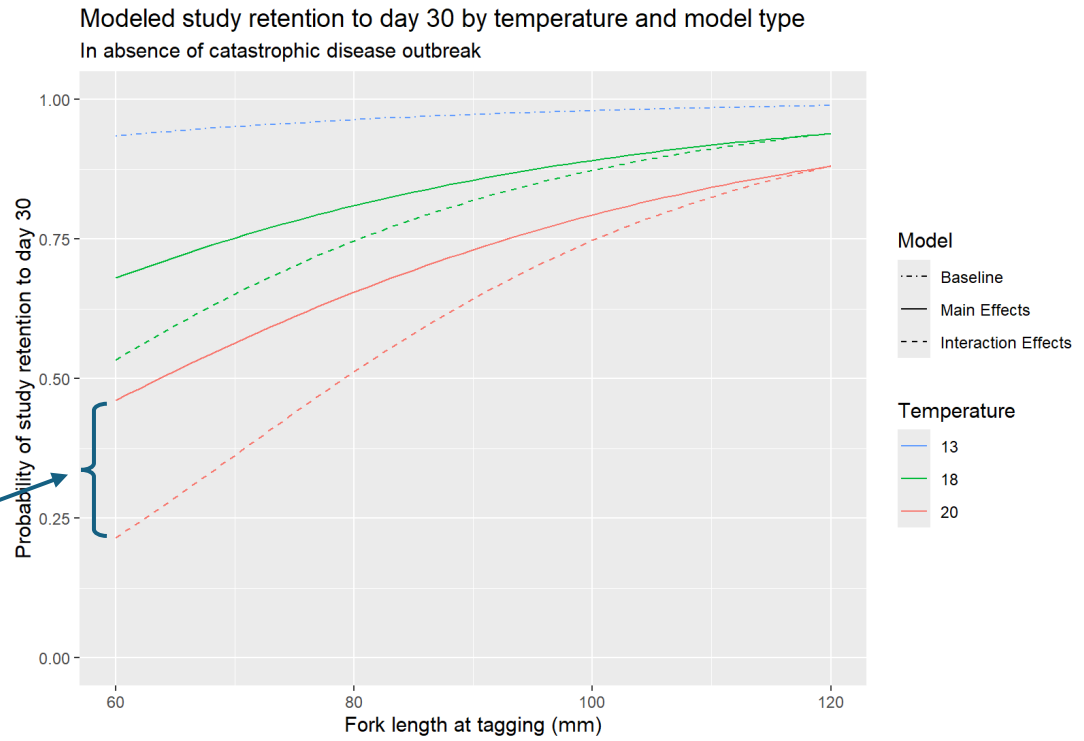
Interaction Effect

Definition

- Relative change in study retention for SMALLEST size class (60mm) at WARMEST temperature (20˚C) compared to modeled result from Main Effects model

Day-30 study retention:

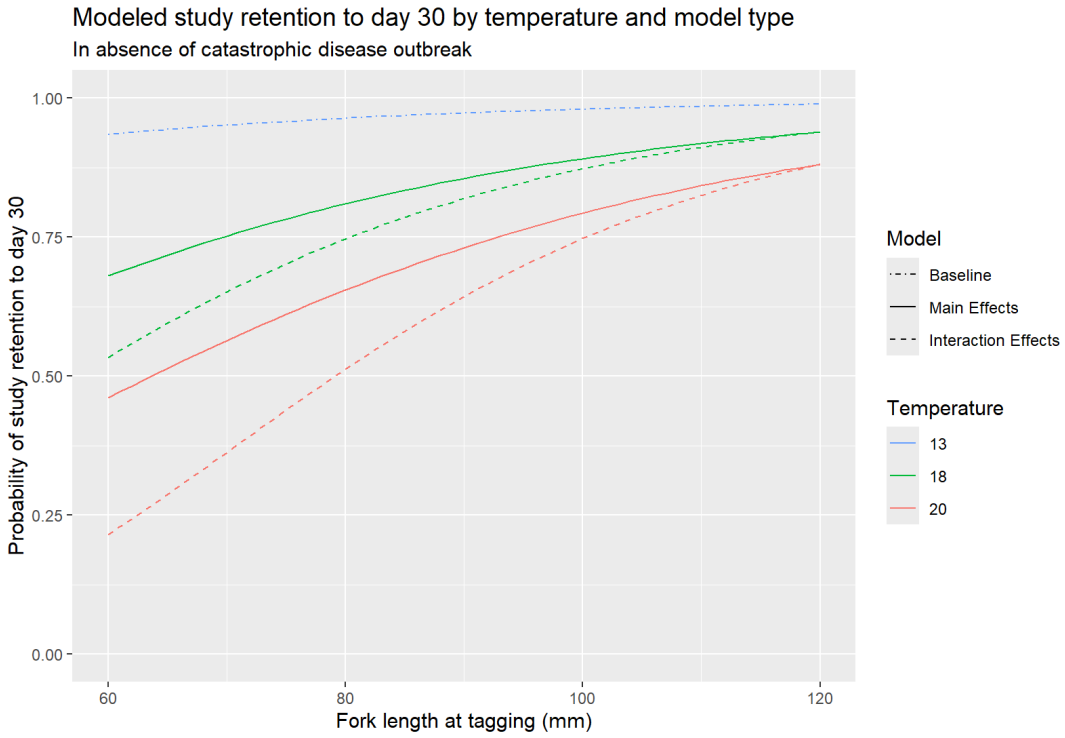
50% reduction



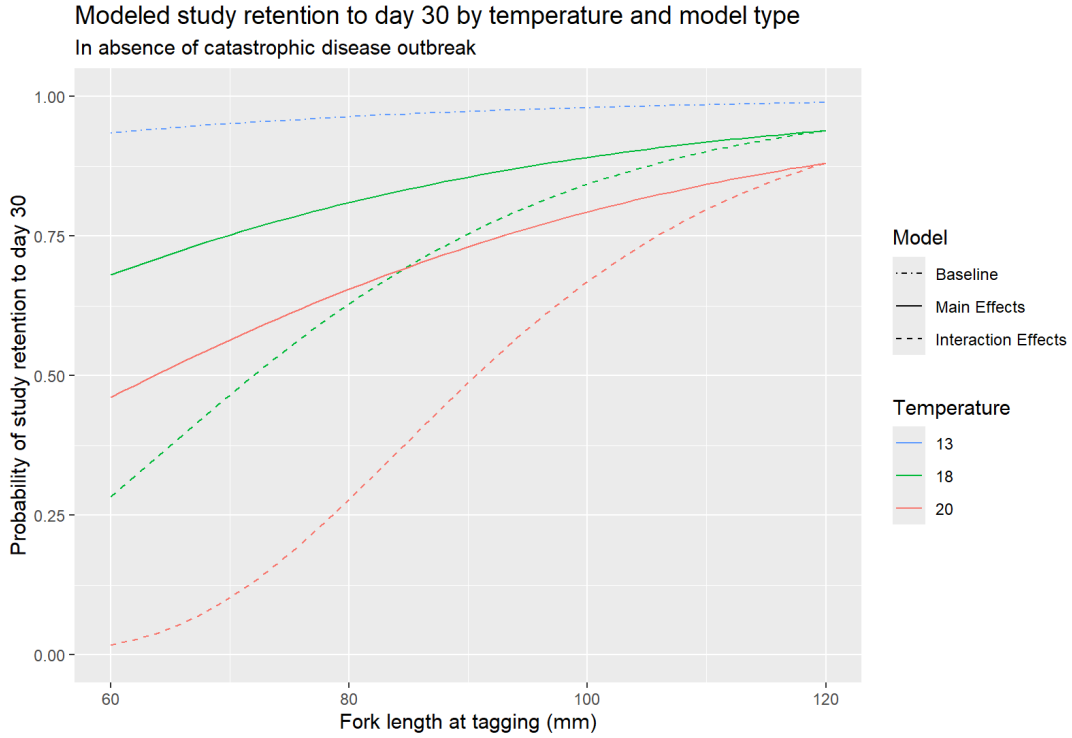
50% difference

Interaction Effect: Effect size

50% reduction in study retention by day 30 for 60mm fish at 20˚C



95% reduction in study retention by day 30 for 60mm fish at 20˚C

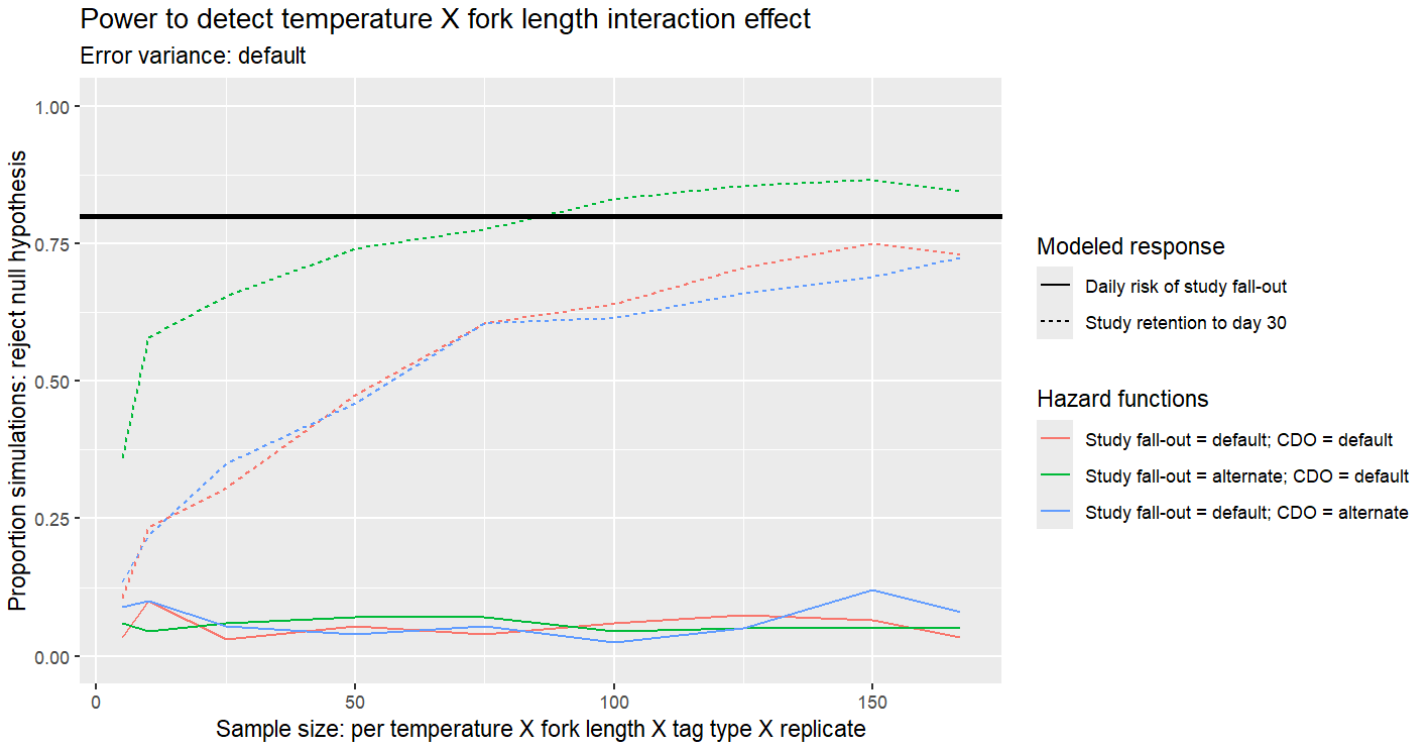


Power Curves

Power to detect interaction effect

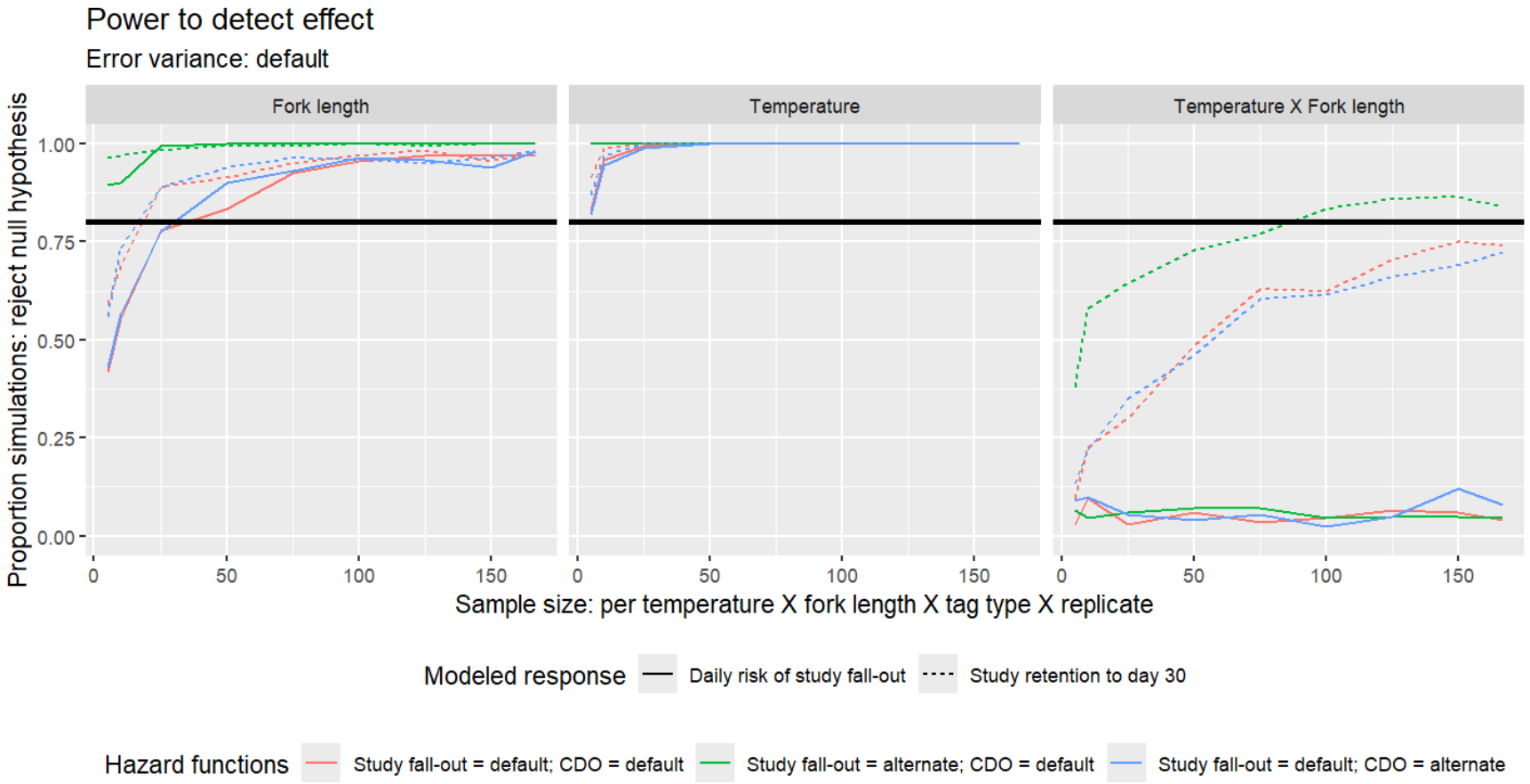
Model options

- Option 1: Proportional hazards model
  - Estimate hazard rate
  - Necessary for applying corrections through time in a tagging study
- Option 2: Logistic model at day 30
  - Estimate probability of study retention to one point in time (day 30)
  - Cannot make corrections in tagging study

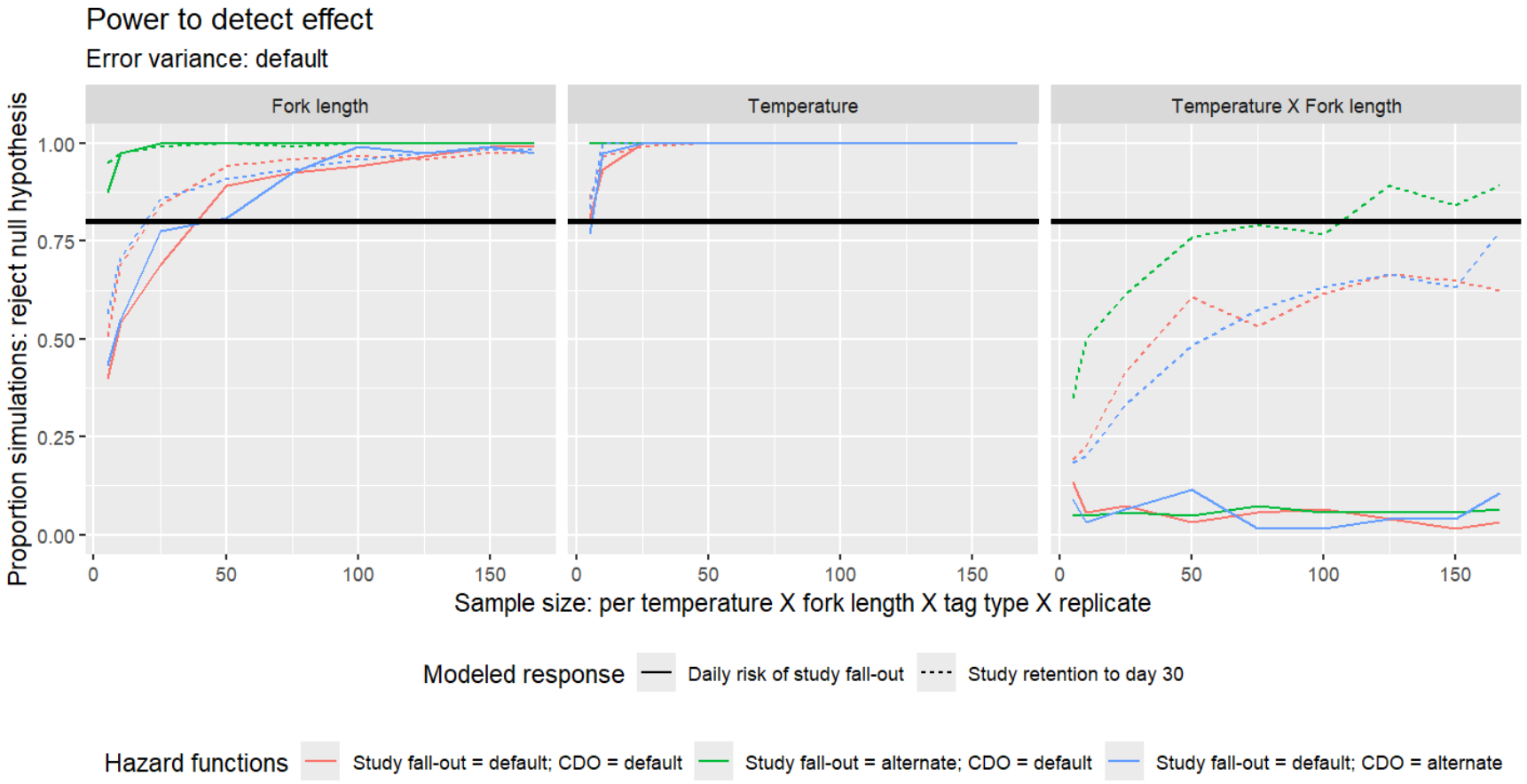


Large Interaction Effect: 95% reduction in study retention for smallest fish at 20˚C

Large Interaction Effect: 95% reduction in study retention for smallest fish at 20˚C



Intermediate Interaction Effect: 50% reduction in study retention for smallest fish at 20˚C



Recommendations

- Target main effect of fish size and water temperature
- Increase per-group sample size to 50
- Total fish per tank = 150
- Total fish per temperature X tag type = 600 (summed over replicates)
- Total fish per replicate within size class = 450
- Total fish per size class = 1,800
- Total fish in study = 5,400

Temperature Acclimation and Thermal Tolerance Literature Review

With Context for the Douglas County PUD tag retention study

1. **Purpose is to understand:**
    a. What does the scientific literature tell us about the role of thermal acclimation on fish condition, temperature tolerance, and wound healing?
    b. What acclimation rates and holding periods are considered adequate in well-designed thermal studies, and how does the proposed DPUD protocol compare? (based on conversation with J Rohrback (1.5 degrees per day over 5 days).

2. **Physiological Basis of Thermal Acclimation**

Thermal acclimation is not instantaneous. The scientific literature describes acclimation as a process with three temporally distinct phases. First, there is a *response induction time* ($t_{induction}$) before any measurable physiological change occurs. Second, there is a progressive adjustment phase during which tolerance improves. Third, there is a *time to full acclimation* (t_{steady}) after which the physiological system reaches a new stable state. A fish that has not completed this process is, by definition, in active physiological transition and is not representative of a fish that has stabilized at the target temperature (De Bonville et al. 2025).

De Bonville et al. (2025) quantified acclimation dynamics across multiple fish species following a +5°C temperature increase. They found that the time to full acclimation (t_{steady}) varied considerably among species but ranged from 4 days (stickleback, flounder, zebrafish) to more than 10 days (goldsinny wrasse), which did not fully acclimate even by the end of the 10-day study period. Critically, the authors emphasized that both the response induction time and time to full acclimation must be explicitly considered in experimental designs, because fish tested before reaching t_{steady} are not physiologically equivalent to fish that have fully acclimated. It is important to note that none of the species studied by De Bonville et al. (2025) were salmonids. The four species studied, wrasse, stickleback, flounder, and zebrafish, represent a range of thermal ecologies, and the authors note that some, particularly stickleback and zebrafish, are adapted to thermally variable shallow water environments with correspondingly rapid acclimation responses.

Stewart et al. (2023) examined acclimation dynamics specifically in brook trout (*Salvelinus fontinalis*), a stenothermic coldwater salmonid with a fundamental thermal niche of 13–17°C. Their results showed that acclimation effects on CT_{max} were detectable from day 1 and continued to progress throughout the entire 30-day study period, with full acclimation not achieved by day 30. This finding suggests that for coldwater salmonids, the 4-10 day range identified by De Bonville et al. (2025) for non-salmonid species likely represents a substantial underestimate of the time required to reach physiological steady state at a new temperature.

The cellular mechanisms underlying this acclimation are well established. Among the most important are changes in membrane lipid composition, enzyme isoform expression, and induction of heat shock proteins. Heat Shock Protein 70 (HSP70) stabilizes damaged proteins during thermal stress, serves as a direct molecular indicator of cellular thermal load. Waterbury et al. (2024) demonstrated in Arctic fishes (broad whitefish and saffron cod) that fish acclimated to 15°C had significantly higher HSP70 protein concentrations in liver, brain, and muscle compared to fish acclimated to 5°C. Acclimated fish also had

measurably higher critical thermal maxima (CT_{max}: 27.3°C and 25.9°C, respectively) compared to cold-acclimated individuals (23.7°C and 23.2°C). These results indicate that the physiological changes underlying improved thermal tolerance is real, measurable at the molecular level, and requires adequate time to complete.

3. Acclimation and Cardiac Function: A Critical Link to Thermal Tolerance

Cardiac function is widely recognized as a central determinant of thermal tolerance in fish. The heart must deliver oxygen to tissues at a rate sufficient to sustain aerobic metabolism, and at high temperatures, the cardiac system can become the limiting factor in the animal's overall performance. Acclimation temperature has a profound effect on how well the heart performs at elevated temperatures.

Anttila et al. (2014) demonstrated this in juvenile Atlantic salmon *Salmo salar* of similar size (77-86mm) as DPUDs draft subyearling Chinook study. Juvenile Atlantic salmon acclimated to 12°C began experiencing cardiac arrhythmia (collapse of normal heart rhythm) at 21–23°C, with a maximum heart rate of approximately 150 beats per minute (bpm). Fish given time to acclimate to 20°C (Anttila et al used 3 months) raised the thermal threshold to 27.5°C and increased their maximum heart rate to approximately 200 bpm, a difference of 5-6°C in cardiac thermal tolerance attributable entirely to acclimation history. This result was consistent across both northern and southern populations, suggesting the cardiac plasticity reflects a fundamental acclimation response rather than local adaptation.

This has direct implications for DCPUD's tag retention study: a subyearling Chinook that has been reared at 13°C and placed in 18°C or 20°C water for only a few days before surgery retains the cardiac physiology of a 13°C fish. Its heart is functioning near or at its performance ceiling at the experimental temperature. The metabolic cost of surgery, anesthesia recovery, and wound healing is superimposed on a cardiac system already operating under thermal stress. Any mortality observed in such fish cannot be cleanly attributed to the tag: it reflects an unresolved combination of surgical burden and inadequate cardiac preparation for the test temperature.

Hvas et al. (2017) reinforced this principle, also in Atlantic salmon, using a rigorous acclimation protocol of 4 weeks at each target temperature before any physiological measurements were taken. Even with this extended preparation, fish held at 23°C experienced 20% mortality during the acclimation period and showed reduced appetite and lower condition factor compared to fish at cooler temperatures. These effects occurred during acclimation, before any experimental manipulation, demonstrating that the physiological challenge of warm temperatures is substantial even under a prolonged acclimation protocol. The fact that 4 weeks of preparation was required before measurements could begin, and that even then thermal stress was measurable, illustrates how inadequate a 2-3 day holding period would be for fish transitioning from 13°C to 18° or 20°C.

4. Acclimation and wound healing: Direct relevance to Tag Retention Studies

Wound healing in fish is mediated by the immune system, which is itself profoundly temperature-dependent. Both the rate and effectiveness of the inflammatory response, cellular repair, and tissue remodeling vary with temperature. In tag retention studies, incision closure and internal wound healing are the primary mechanisms by which tags are retained or expelled: a fish with impaired wound healing is more likely to lose its tag and is more susceptible to post-surgical mortality.

Deters et al. (2010), the study most frequently cited in the Douglas PUD study design to justify temperature treatment levels and acclimation approach, documented these temperature-wound healing relationships directly in subyearling Chinook Salmon implanted with acoustic transmitters and held at 12°C versus 17°C. Fish held at 17°C showed significantly higher wound inflammation, ulceration, lower tag retention, and lower suture retention at 7 and 14 days post-surgery compared to fish at 12°C. The authors cited Finn and Nielsen (1971), who demonstrated that inflammatory responses are slower at 5°C than at 15°C in rainbow trout, and Bunnell and Isely (1999), who found that tag loss rates in rainbow trout were lower at 10°C than at 20°C.

It is critical to note what Deters et al. (2010) did not do: they did not evaluate the adequacy of their acclimation protocol. Their study was designed to test suture type performance. The temperatures they used and the rate at which they transferred fish to those temperatures were study conditions, not findings or recommendations. The paper makes no claim that their acclimation approach was sufficient for full physiological acclimation, and it should not be cited as justification for acclimation adequacy in the Douglas PUD study. This distinction matters because, as Desforges et al. (2023) note in their comprehensive review of thermal tolerance methodology, acclimation periods in laboratory fish studies are "not always disclosed" and fish are "not always acclimated long enough"; a recognized source of variation that makes it difficult to distinguish true temperature effects on physiological performance from artifacts of inadequate acclimation. The finding that wound healing was significantly worse at 17°C than at 12°C in Deters et al. is informative precisely because it demonstrates that temperature effects on post-surgical outcomes are real and substantial even within a relatively narrow thermal range . But whether that effect reflects true performance at 17°C or a combination of temperature and acclimation stress cannot be determined from their study design.

5. Acclimation Rates and Holding Periods: What the Literature Supports

The thermal biology literature provides guidance on what constitutes an adequate acclimation protocol for laboratory thermal tolerance and tag effects studies. Two components are consistently identified as necessary: (1) a ramp rate slow enough to allow physiological compensation to keep pace with temperature change, and (2) a holding period at the target temperature sufficient for the fish to reach a new physiological steady state before experimental manipulation begins.

5.1 Ramp Rate

Selong et al. (2001) developed the Acclimated Chronic Exposure (ACE) method, the most ecologically defensible laboratory approach to thermal tolerance assessment in salmonids. A central design feature of the ACE method is a ramp rate of 1°C per day, which the authors explicitly identified as an environmentally realistic rate that allows fish to fully acclimate to changing conditions and mimics the rate at which fish experience seasonal warming in nature. Selong et al. contrasted this approach with traditional CTM (Critical Thermal Maximum) methods, which change temperature at rates of 1°C per hour or faster, and with the Incipient Lethal Temperature (ILT) method, which transfers fish abruptly from acclimation to test temperatures. The authors found that these faster methods preclude the normal acclimation that occurs in nature and produce results unrepresentative of true thermal tolerance under field conditions. Importantly, Selong et al. documented delayed mortality in their longer-duration ACE trials, with some fish dying up to 31 days after initial exposure to elevated test temperatures. This finding is directly relevant to a 30- or 45-day tag retention study: mortality associated with inadequate acclimation may not appear immediately after surgery but could manifest throughout the observation window, confounding the attribution of mortality to tag effects.

Johnstone and Rahel (2003), in a study of thermal tolerance in Bonneville cutthroat trout (*Oncorhynchus clarkii utah*), acclimated fish to 18°C for 2 to 3 weeks before conducting any thermal tolerance trials. When advancing fish to test temperatures, they used a within-day rate of 1-2°C per hour from the already-established acclimation baseline, but only after the multi-week holding period. The authors specifically noted that this procedure avoids the handling stress and temperature shock that occur when fish are netted and transferred abruptly from acclimation to exposure tanks. The underlying principle, that fish must be genuinely settled at an intermediate temperature before any further thermal challenge, applies directly to multi-step ramp protocols.

Desforges et al. (2023), in a comprehensive review of CT_{max} methodology across fish species, synthesized evidence from dozens of studies and concluded that fish should be fully acclimated prior to conducting CT_{max} trials, and that acclimation should be confirmed using reliable measurable indices. They noted that "recording measurable changes in energy expenditure from one stable state to the next (i.e., from one temperature to another) might require experimental trials to last several months, rather than a few weeks, depending on the magnitude and rate of environmental change" (Beitinger and Lutterschmidt 2011, as cited in Desforges et al. 2023). Their review identified incomplete acclimation as one of the primary sources of variation and uninterpretable results in the fish thermal biology literature, noting that "fish are not always acclimated long enough" and that "details of acclimation are not always disclosed" in published studies.

5.2 Holding Period

The ramp rate and the holding period are complementary requirements, not substitutes for one another. A fish that has arrived at the target temperature via a slow ramp is not the same as a fish that has been held at that temperature long enough to complete physiological acclimation. De Bonville et al. (2025) emphasize that fish tested before reaching their time to full acclimation (t_{steady}) are not physiologically equivalent to fish that have fully acclimated, and that understanding this temporal component of acclimation is critical for interpreting experimental results. Taken together, the literature reviewed here suggests that fish surgically manipulated before reaching physiological steady state will be in active thermal transition at the time of surgery; an experimental condition with no ecological analog that produces results which cannot be interpreted as reflecting the performance of a wild fish at that temperature.

Hvas et al. (2017) used 4 weeks at each target temperature as their standard pre-measurement holding period, and still observed thermal stress effects (mortality, appetite suppression, reduced condition factor) in fish held at 23°C. The 4-week standard they employed reflects scientific judgment about what is required to ensure fish are genuinely acclimated rather than merely surviving at the test temperature.

6. Wild Fish Thermal Context: Wells Forebay Temperature Data

To assess the ecological realism of any laboratory acclimation protocol, we can compare it against the rate at which subyearling Chinook Salmon actually experience temperature change in the Wells Pool. Daily average forebay temperatures at Wells Dam for 2025 and the 10-year average from 2016–2025 were obtained from the Columbia River DART database (Figure 1). Based on the 10-year average, Wells forebay temperatures cross 13°C around June 10 and approach 19-20°C in mid- to late August. This is a transition of approximately 7°C occurring over roughly 70 days. The average rate of warming over this period is approximately 0.09°C per day. By comparison, the Douglas PUD protocol achieves the same 7°C increase in 5 days, at rate of 1.5°C per day, approximately 16 times faster than the rate fish experience in the Wells Pool under natural conditions.

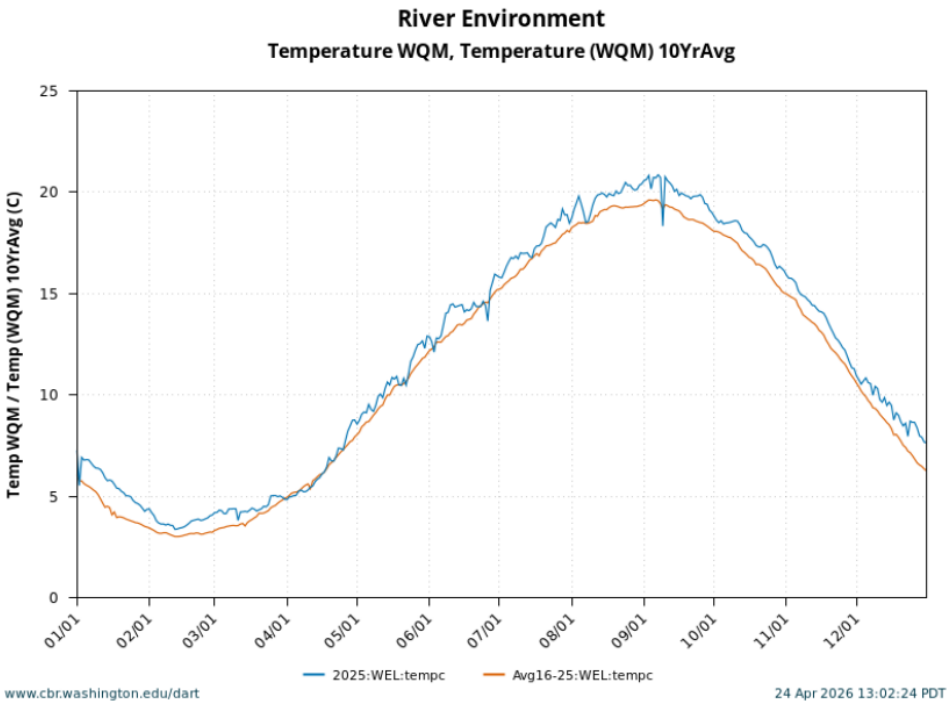


Figure 1. Daily average water temperatures (°C) at Wells Dam forebay for 2025 (blue) and the 10-year average from 2016-2025 (orange). Data source: Columbia River DART (www.cbr.washington.edu/dart).

It is important to note that the DART data represent daily average temperatures. The actual thermal environment experienced by fish in the Wells forebay is considerably more dynamic: temperatures warm during daylight hours and cool overnight, producing diel fluctuations of several degrees around the daily mean. Fish in the forebay are therefore not experiencing a monotonically increasing temperature, rather they are experiencing a thermal cycle in which each day is slightly warmer on average than the last, but with a nightly cooling period that is part of their normal thermal environment.

This difference is important. Morash et al. (2018) demonstrated directly in wild juvenile Atlantic salmon that physiological responses to a stable average temperature are fundamentally different from those observed in fish experiencing a natural diel thermal cycle with the same daily mean. Fish acclimated to a fluctuating cycle of 16-21°C had measurably different routine and maximum metabolic rates and reduced aerobic scope compared to fish held at a stable 18.5°C, the average of that same cycle. The authors conclude that laboratory estimates of performance variables conducted on animals acclimated to a thermally stable environment may not accurately reflect their performance in the wild, where temperature varies. They also found that short-term warming fluctuations during natural diel cycles induce HSP70 expression in Atlantic salmon, indicating that fish in dynamic thermal environments engage cellular stress response machinery as part of their normal thermal management, a physiological state that constant-temperature laboratory conditions do not replicate.

The implication for the Douglas PUD study is twofold. First, fish reared at constant 13°C well water and ramped to a constant 18°C or 20°C are not in a physiological state comparable to wild fish that have arrived at those temperatures through months of natural diel cycling. Second, even if the laboratory daily mean temperature matches the field condition, fish held at a constant temperature are physiologically distinct from fish experiencing the natural thermal variation around that mean. A

laboratory tag effects study conducted at constant elevated temperatures therefore produces results that reflect the performance of fish under an artificial thermal regime, not the performance of wild fish migrating through the Wells Pool under field conditions.

The purpose of the Douglas PUD tag effects study is to generate data that can be used to set tagging thresholds and inform survival study design for subyearling Chinook Salmon under field conditions in the Wells Pool. That purpose depends entirely on the fish being tested in a physiological state comparable to what fish would actually experience at those temperatures in the wild. The Douglas PUD draft protocol of a 5 day ramp from 13°C to 20°C at a constant temperature, proceeding at roughly 16 times the natural rate of warming and without the diel variation that characterizes the actual forebay environment, does not produce that physiological state. As a result, the tag retention and mortality data produced by the study will not reflect what would happen to a wild fish tagged at those temperatures. They reflect what happens to a hatchery fish in active thermal transition, surgically manipulated before its physiology has stabilized under an artificial thermal regime. That is a fundamentally different condition, and results generated under it cannot be used to set defensible tagging thresholds for fish in the field. At best, the data will overstate tag-associated mortality at warmer temperatures because inadequate acclimation adds a thermal stress component that would not be present in properly acclimated fish. At worst, the data will be uninterpretable entirely because the contribution of acclimation stress to observed outcomes cannot be separated from the contribution of the tag itself.

7. Evaluation of the Proposed DPUD Acclimation Protocol

Based on conversations with J. Rohrback (DPUD), the proposed temperature acclimation protocol now proceeds as follows, beginning from a 13°C rearing baseline:

- Monday: 13.0°C → 14.5°C (+1.5°C)
- Tuesday: 14.5°C → 16.0°C (+1.5°C)
- Wednesday: 16.0°C → 17.5°C (+1.5°C)
- Thursday: 17.5°C → 18.0°C (+0.5°C) [18°C treatment group stops here] / 17.5°C → 19.0°C (+1.5°C) [20°C treatment group continues]
- Friday: 19.0°C → 20.0°C (+1.0°C) [20°C treatment group arrives at target temperature]

All treatment groups are then tagged the following Monday.

7.1 Ramp Rate

The proposed ramp rate of 1.5°C per day is 50% faster than the 1°C per day standard established by Selong et al. (2001) as the maximum environmentally realistic rate for salmonid thermal acclimation studies. The total temperature increase of 7°C (13°C to 20°C) is achieved over 5 business days, whereas the same change occurs over approximately 8 to 10 weeks in the natural environment.

For context, the Chelan PUD/Grant PUD Tag Effects Study (Spheros Environmental 2026), which was recently approved by the HCP-CC covers a similar temperature range for summer Chinook Salmon in the same system, proposes a ramp rate of ≤1.0°C per day over 10–14 days, roughly twice the acclimation time. While the Yakama Nation views even that protocol as a practical compromise rather than a fully ideal acclimation design, it represents a more conservative approach than what Douglas PUD has proposed.

7.2 Absence of a Meaningful Holding Period

The proposed protocol includes a short holding period at target temperature before tagging, spanning the weekend between the end of the ramp and Monday tagging. The 18°C treatment group arrives at its target temperature on Thursday and holds through the weekend, reaching Monday tagging with 3 days at target temperature. The 20°C treatment group arrives at its target temperature on Friday and holds through the weekend, reaching Monday tagging with 2 days at target temperature. While this holding period represents an improvement over tagging fish immediately upon arrival at target temperature, the scientific question is whether 2-3 days is sufficient for fish to reach a new physiological steady state before surgery. The literature is unambiguous on this point: it is not.

The adequacy of these holding periods must be evaluated against what the literature tells us about how long it actually takes a fish to reach physiological stability at a new temperature. De Bonville et al. (2025) quantified acclimation dynamics across multiple fish species following a +5°C temperature increase and found that time to full acclimation (t_{steady}) ranged from 4 days in the fastest-acclimating species to more than 10 days, with some species failing to reach a stable new physiological state even by the end of the 10-day study period. These results establish 4 days as a lower bound for full acclimation under a relatively modest temperature increase. Neither the 18°C group nor the 20°C group meets even this minimum threshold. The 18°C group falls one day short at 3 days, and the 20°C group at 2 days falls well below it.

However, this comparison must be interpreted with an important caveat: none of the species studied by De Bonville et al. (2025) were salmonids. All four ,wrasse, stickleback, flounder, and zebrafish, inhabit thermally variable environments and would be expected to acclimate more rapidly than a cold-adapted salmonid. The 4-10 day range from De Bonville et al. therefore likely represents an underestimate of the time required for subyearling Chinook to reach a physiological steady state at a new temperature.

Stewart et al. (2023) studied acclimation dynamics in brook trout (*Salvelinus fontinalis*), which they describe as a stenothermic, coldwater fish with a fundamental thermal niche of 13-17°C, comparable to subyearling Chinook Salmon reared at 13°C well water. Their experiment exposed fish to temperature increases of +3°C and +6°C above ambient (~11°C) and measured CT_{max} at 1, 4, 8, 16, and 30 days of acclimation. In the +6°C treatment group, fish had achieved only 0.66°C of improvement in thermal tolerance after 1 day and 0.74°C after 4 days, compared to a total improvement of 1.87°C still accumulating at day 30, with no plateau reached by the end of the study. The rate of increase, while slowing, remained measurable throughout: 0.016°C per day between days 16 and 30. The authors conclude that "thermal tolerance can continue to change for at least 30 days under sustained acclimation to elevated temperatures" and recommend that future thermal tolerance studies consider this when intending to have organisms fully acclimated. Against this recommendation, the 2-3 days of holding time provided by the Douglas PUD protocol captures only a small fraction of the acclimation benefit that would continue to accrue over weeks, and it does so at temperatures substantially warmer than those used in Stewart et al., where the physiological challenge is greater and acclimation capacity may be more constrained.

Fish surgically manipulated without adequate acclimation and holding time will be in active thermal transition at the time of surgery. Their cardiac function, immune response, and wound healing capacity will reflect their acclimation history, 13°C rearing followed by a rapid 5-day ramp. Importantly, any mortality or tag loss observed under these conditions cannot be attributed cleanly to the tag itself, and results produced under this protocol cannot be used to set defensible tagging thresholds for subyearling Chinook Salmon under field conditions in the Wells Pool.

7.3 Differential Holding Time Between Treatment Groups

Because all treatment groups are tagged on the same day but arrive at their respective target temperatures on different days, the 18°C and 20°C groups differ not only in temperature but also in how long they have been at that temperature before surgery. The 18°C group arrives at target temperature on Thursday (with three days holding before tagging) via a final ramp step of only 0.5°C. The 20°C group arrives at target temperature on Friday (with two days holding before tagging) following the two larger single-day ramp steps (1.5°C on Thursday, 1.0°C on Friday).

This means the 20°C treatment group is disadvantaged relative to the 18°C group in three ways, all of which run in the same direction as the temperature effect the study is designed to measure:

- **Higher target temperature**: the intended experimental variable.
- **Larger and more stressful final ramp steps**: the 20°C group experiences the two larger single-day increments at the end of the ramp, at the highest temperatures, when physiological challenge is greatest.
- **Less time at target temperature before surgery**: two days vs three days for the 18°C group.

Only the first of these differences is intentional. The other two are artifacts of the study design. Because all three differences run in the same direction, any observed difference in tag retention or mortality between the 18°C and 20°C groups cannot be cleanly attributed to temperature alone. This is a confounded comparison that undermines the core purpose of the study.

8. Consequences for Study Interpretation

The Yakama Nation's concern regarding the acclimation rate and holding period is with the interpretability of the results. If fish are inadequately acclimated at the time of tagging, the mortality and tag loss observed in the warmer treatment groups will not represent true tag burden effects on fish at those temperatures. They will represent an unresolved mixture of tag effects and thermal acclimation stress that cannot be separated after the fact.

If inadequate acclimation produces elevated mortality in the warmer groups, the study will *overstate* the risk of tagging at those temperatures, potentially leading to unnecessarily conservative tagging thresholds that restrict the utility of survival studies.

The intent of this study should be to provide the empirical basis for tagging thresholds in compliance survival studies for subyearling summer Chinook Salmon at Wells Project. Generating data from a facility-constrained design with a compromised acclimation protocol and using those data to set tagging thresholds would produce a false empirical basis for decisions affecting survival study design. Generating no new data would be preferable to generating data that cannot be correctly interpreted or provides misleading information.

9. Recommendations

The literature reviewed here makes clear that truly adequate thermal acclimation for a coldwater salmonid may require weeks to months. We recognize this is a timeline that is not practically achievable in most laboratory tag effects study contexts. Our recommendations below represent a pragmatic approach that reflects the best available science while acknowledging the practical constraints of laboratory fish studies.

We note that the Chelan/Grant subyearling tag effects study proposed a ramp rate of ≤1°C per day over 10-14 days with a 24-48 hour holding period. The Yakama Nation approved that study prior to conducting this literature review. This review has enhanced our understanding of acclimation dynamics in salmondis, particularly the findings of Stewart et al. (2023), which indicate that brook trout continue to acclimate measurably for at least 30 days following a temperature increase.

In light of this literature review, we also acknowledge that the Chelan/Grant protocol represents a compromise between scientific ideals and logistical feasibility, and that results from that study should similarly be interpreted with awareness that fish may not be fully acclimated at the time of tagging. That said, the Chelan/Grant protocol is more conservative than what Douglas PUD has proposed in both ramp rate and total acclimation duration.

With that context in mind, the Yakama Nation recommends the following minimum acclimation protocol for the Douglas PUD subyearling Chinook Salmon tag effects study:

- **Ramp rate of ≤1°C per day** from the 13°C rearing baseline to each target temperature, consistent with Selong et al. (2001) and the Chelan/Grant study design.
- **A holding period of 7 days** minimum at the target temperature for all treatment groups before any fish are tagged.
- **Staggered ramp start dates** so that all treatment groups arrive at their respective target temperatures on the same calendar day relative to tagging, ensuring equal acclimation time and equivalent final ramp steps across groups.

If these conditions cannot be met within the constraints of the current facility and schedule, the Yakama Nation recommends that Douglas PUD either seek an alternative facility, delay the study, or formally coordinate with the Chelan/Grant effort so that the resulting data can serve the management needs of all three PUDs under a single, well-designed protocol.

References

Anttila, K., C. S. Couturier, Ø. Øverli, A. Johnsen, G. Marthinsen, G. E. Nilsson, and A. P. Farrell. 2014. Atlantic salmon show capability for cardiac acclimation to warm temperatures. *Nature Communications* 5:4252.

De Bonville, J., A. H. Andreassen, Z.-L. Cowan, L. Silva-Garay, R. H. J. Leeuwis, E. R. Åsheim, B. Speers-Roesch, G. D. Raby, S. A. Binning, and F. Jutfelt. 2025. Dynamics of thermal tolerance plasticity across fish species and life stages. *Journal of Thermal Biology* 127:104024.

Desforges, J. E., K. Birnie-Gauvin, F. Jutfelt, K. M. Gilmour, E. J. Eliason, T. L. Dressler, D. J. McKenzie, A. E. Bates, M. J. Lawrence, N. Fangue, and others. 2023. The ecological relevance of critical thermal maxima methodology for fishes. *Journal of Fish Biology* 102:1000–1016.

Deters, K. A., R. S. Brown, K. M. Carter, J. W. Boyd, M. B. Eppard, and A. G. Seaburg. 2010. Performance assessment of suture type, water temperature, and surgeon skill in juvenile Chinook Salmon surgically implanted with acoustic transmitters. *Transactions of the American Fisheries Society* 139(3):888–899.

Hvas, M., O. Folkedal, A. Imsland, and F. Oppedal. 2017. The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar*. *Journal of Experimental Biology* 220:2757–2764.

Johnstone, H. C., and F. J. Rahel. 2003. Assessing temperature tolerance of Bonneville cutthroat trout based on constant and cycling thermal regimes. *Transactions of the American Fisheries Society* 132:92–99.

Morash, A.J., Neufeld, C., MacCormack, T.J. and Currie, S. 2018. The importance of incorporating natural thermal variation when evaluating physiological performance in wild species. Journal of Experimental Biology 221: jeb164673. doi:10.1242/jeb.164673

Sauter, S. T., J. McMillan, and J. Dunham. 2001. Salmonid behavior and water temperature. Issue Paper 1. Prepared as part of EPA Region 10 Temperature Water Quality Criteria Guidance Development Project. EPA-910-D-01-001. U.S. Environmental Protection Agency, Seattle, Washington.

Selong, J. H., T. E. McMahon, A. V. Zale, and F. T. Barrows. 2001. Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerances in fishes. *Transactions of the American Fisheries Society* 130(6):1026–1037.

Spheros Environmental. 2026. Study Proposal: Tag Effects for Subyearling Summer Chinook Salmon. Study Proposal 25-014. Prepared for Public Utility District No. 2 of Grant County and Public Utility District No. 1 of Chelan County. March 13, 2026.

Stewart, E.M.C., Frasca, V.R., Wilson, C.C., Raby, G.D. 2023. Short-term acclimation dynamics in a coldwater fish. Journal of Thermal Biology 112: 103482.

Waterbury, C. R., G. A. Brill, S. R. Dalton, G. L. Montz, D. A. Larsen, and A. H. Beaudreau. 2024. Effects of temperature acclimation on the upper thermal tolerance of two Arctic fishes. *Conservation Physiology* 12:coae001.