STATEMENT OF WORK

Quantifying the relatedness of Similkameen River Rainbow Trout to Anadromous Steelhead in the Upper Columbia River DPS

Background: Steelhead (*Oncorhynchus mykiss*) in the Upper Columbia Distinct Population Segment (DPS) may soon have the opportunity to access the Similkameen River, the largest tributary of the Okanogan River, providing over 1500 km of habitat that has been unavailable for over a century. Such a restoration effort would be unprecedented in the Upper Columbia steelhead DPS, which has been ESA listed as threatened since 1997, with Okanogan River adult abundance hovering between 300 and 800 fish for the last decade. However, a cascade near the mouth of the Similkameen River may have historically blocked some species at some flows from accessing the Similkameen River, so the amount of anadromous behavior in the watershed historically is unclear. Steelhead recolonization has been aided in other systems by re-emergence of anadromy (oceangoing behavior) that has remained in resident rainbow trout trapped behind impoundments. Therefore, establishing the degree of relatedness between rainbow trout above the cascade and steelhead populations in the Upper Columbia could have profound implications for steelhead reintroduction in the Similkameen River watershed. We propose to compare a panel of genetic markers from fin clips collected from rainbow trout in several locations in the Similkameen River watershed, with known ancestral resident inland Redband Rainbow Trout and steelhead from other Upper Columbia populations to determine the degree of relatedness (F_{st}). If F_{st} values between sample collections and other Upper Columbia steelhead are high (e.g., >0.02), there is fairly strong evidence that anadromous steelhead were not prevalent in the watershed historically. However, if F_{st} values are low (≤ 0.01), it would indicate that there were likely anadromous steelhead present historically and their lineage remains in the upper watershed, which may aid recolonization. The provenance of Similkameen river trout would help determine the prevalence of historical anadromy in the system, and the potential for local rainbow trout to boost the prevalence of anadromous phenotypes following reintroduction.

Statement of Work: NWFSC, along with The Confederated Tribes of the Colville Reservation, have collected and compiled ~560 fin clips from juvenile steelhead in a dozen tributaries of the Similkameen River. The samples will be removed from the filter paper archive, DNA extracted, and evaluated for 384 single nucleotide polymorphism (SNPs) loci that are mostly neutral markers and have been highly vetted to be useful for determining population-level differences in the Columbia River basin. The resulting SNP data will be compared to an extensive baseline that includes individuals from nearly all other Columbia River steelhead populations and known resident ancestral redband rainbow trout. Measures of genetic differentiation (e.g., F_{st}) between our Similkameen collections and the pre-existing baseline will be performed to determine the likely lineage of Similkameen River collections.

Tasks: Key tasks include the following:

- Processing fin clip samples to extract DNA and evaluate with the 384 SNP panel.
- Analyze the resulting data to determine genetic relatedness within the Similkameen River locations, and between the Similkameen River collections and others in the baseline *Oncorhynchus mykiss* dataset from previous collections to test hypotheses of former connectivity of populations up- and downstream of Enloe Dam.

Deliverables: The contractor will provide a final report with a summary of lab and analysis methods and results, including analysis of relatedness.

Timeline: Period of Performance: 1 month after the contract is signed through 9/1/2027.

560 Samples delivered to WDFW MGL with run list	30 days after signing
Laboratory work completed by	03/01/2027
Completed Final Report	09/01/2027

[redacted]

Billing: One bill will be submitted in the federal government's IPP system by 9/15/2027.