2024 Survey Outline for Klamath River Fish Health Monitoring Project

In 2024, the California-Nevada Fish Health Center (Center) plans to obtain samples and provide Quantitative Polymerase Chain Reaction (qPCR) testing of out-migrating juvenile Chinook salmon in the Klamath River. Three rotary screw traps will be sampled in 2024 to estimate *C. shasta* POI during outmigration.

1) Sampling at Rotary Screw Trap locations
    a) Three locations
        i. I-5 RST
        ii. Kinsman RST
        iii. Weitchpec RST

    b) Fish Collection
        i. Target is 30 fish per week at all sampling sites K5-K0 with the understanding that this sampling season bears multiple uncertainties and 30 fish per week at each location may not be achievable.
        ii. The Center is asking that fish be collected Mon-Wed of each week.

2) Hatchery Fish
    a) Schedule
        i. The Fall Creek facility will be conducting releases of unmarked hatchery fish starting in February and presumably continuing through April. Until marked CWT fish are observed and collected in the mainstem Klamath River, all fish collected will be identified as “unknown” origin.
        ii. After marked hatchery fish are released, the focus will shift to collecting coded wire tagged (CWT) fish.

    b) Lower River CWT collection
        i. The Center will communicate with cooperators in the lower river by early June 2024. It will then be determined if sample collection in the lower river is feasible based on hatchery operations (how many fish are released, mark rate, etc.).

3) No Real-time Monitoring – Shasta River to Scott River reach, K4
    a) The Center does not plan to conduct real time qPCR monitoring efforts in 2024.

4) Reporting
    a) Results will be reported by memorandum.
        i. If the minimum number of fish cannot be collected, results will not be reported that week.

b) Results will be sent to the Arcata Fish and Wildlife Office, Bureau of Reclamation, FASTA coordinator, and KFHAT coordinator.

c) Data reported will include:

i. Collection date
ii. Number of fish collected
iii. Number of fish positive for *C. shasta*
iv. *Ceratonova shasta* POI; describes the proportion (percentage) of fish infected on that collection date.
v. DNA copy number range; describes the parasite load within the fish tissue.
vi. Percent of fish with DNA copy number over 3 logs.

5) qPCR assay

a) All *C. shasta* qPCR samples will be assayed using the Quant Studio 6 real-time PCR system.

b) A standard curve will be run in parallel with unknown samples (i.e. standards are included on every assay plate).

c) All samples will be visually inspected using the qPCR software's amplification curve and component view analysis tools.

d) Criteria for positive test results:

i. ≥ 5 copies of *C. shasta* DNA
ii. Samples also produce a minimum change in fluorescent signal (ΔRn) of 100,000 indicating significant amplification above background levels of the instrument.

Collection Schedule: Number of fish collected by week date and river reach.

River Reach (Reach Code)		K5 - Iron Gate Dam to Shasta I-5 RST AFWO	K4 - Shasta to Scott Kinsman RST AFWO/Karuk	K3 - Scott to Salmon Seining Karuk	K2 - Salmon to TR Weitchpec RST Yurok/AFWO	K1 - TR to Blue Creek Seining Yurok	K0 – Blue Creek to Estuary Seining Yurok
1	Mar 18	30	30		30		
2	Mar 25	30	30		30		
3	Apr 1	30	30	30	30		
4	Apr 8	30	30	30	30		
5	Apr 15	30	30	30	30		
6	Apr 22	30	30	30	30		
7	April 29	30	30	30	30		
8	May 6	30	30	30	30		
9	May 13	30	30	30	30		
10	May 20	30	30	30	30		
11	May 27	CWT-30	CWT-30	CWT-30	CWT-30		
12	June 3	CWT-30	CWT-30	CWT-30	CWT-30	CWT-30	
13	June 10			CWT-30	CWT-30	CWT-30	CWT-30
14	June 17			CWT-30	CWT-30	CWT-30	CWT-30
15	June 24			CWT-30	CWT-30	CWT-30	CWT-30
16	July 1			CWT-30	CWT-30	CWT-30	CWT-30
17	July 8			CWT-30	CWT-30	CWT-30	CWT-30
18	July 15				CWT-30	CWT-30	CWT-30
19	July 22				CWT-30	CWT-30	CWT-30
20	July 29					CWT-30	CWT-30
21	Aug 5						CWT-30
22	Aug 12						CWT-30
23	Aug 19						CWT-30