U.S. Fish & Wildlife Service

California-Nevada Fish Health Center Investigational Report:

Ceratonova shasta Prevalence of Infection in Klamath River Juvenile Chinook Salmon, March – August 2024

Ron Stone*, Scott Freund, and J. Scott Foott

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*direct correspondence

US Fish and Wildlife Service
California-Nevada Fish Health Center
24411 Coleman Fish Hatchery Rd
Anderson, CA 96007
(530) 365-4271

Summary

The removal of multiple dams on the Klamath River made 2024 a unique year; a total of 1197 juvenile Klamath River Chinook Salmon (*Oncorhynchus tshawytscha*) collected between March and August 2024 were assayed for *Ceratonova shasta* by quantitative polymerase chain reaction (QPCR). Initial detection occurred on April 23, with the highest proportion of "disease- rated" salmon (37%) collected in the Shasta R. to Scott R. reach. Fall Creek hatchery salmon (coded wire tag fish) had a 73% prevalence of infection, with disease detection occurring within a week of their May releases. Gill tissue testing showed no prognostic advantage over intestinal tissue, as the initial detection date was the same for both tissues. Dam removal operations altered river flow, temperature, and turbidity compared to previous years. Higher river temperatures in March and April likely influenced the earlier occurrence of actinospores and resulting fish infections. This season, the historical prevalence of infection (64%) was similar to what was reported for 2023 (62%).

The correct citation for this report is:

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Notice

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Introduction

The year 2024 was unique for the *Ceratonova shasta* (**CS**) monitoring project. The Klamath dam removal process affected river flow, turbidity, and temperature profiles as well as requiring the movement of Iron Gate hatchery rearing operations to the new Fall Creek hatchery. Monitoring objectives in 2024 were: 1) determine CS prevalence of infection (POI) and infection severity (parasite DNA quantity within fish tissue) in Klamath River juvenile Chinook Salmon during the spring emigration period, 2) compare gill tissue to intestine CS-POI in a subset of fish, and 3) compare CS-POI to previous years (2009-2023).

Methods

Salmon collection - There were 22 weeks of collection throughout the river beginning the week of March 18 and continuing through August 12 (Appendix C). Refer to this table for information relating **week of collection** to actual dates. The Lower Klamath River basin was divided into six reaches defined by major tributaries, with study cooperators collecting fish by either rotary screw trap or beach seine (Table 1, Appendix A map). A pre-liberation sample of 58 smolts was obtained from Fall Creek hatchery on April 4, 2024. Juvenile salmon were collected and categorized into three groups: 1) Natural origin – an unmarked fish collected prior to initial hatchery release (May 15, 2024), 2) Unknown origin - an unmarked fish collected after hatchery release (May 15, 2024), or 3) Fall Creek Hatchery origin salmon – adipose fin clipped salmon with coded wire tag (**CWT**) for this hatchery (codes 062236, 062239, 062240, 062243, 060681). Natural and unknown origin fish were combined in prevalence analysis to increase weekly sample size and due to similar detection conditions. Previous sentinel studies have shown that *C. shasta* trophozoites can be detected in the intestine by QPCR after 4 – 10 days post-exposure (True et al., 2012). Detection in a naïve hatchery fish should be possible after 1 week post-release (~ collection week 10).

On February 26, 2024, 830,000 unmarked fry were released from Fall Creek hatchery into the Klamath River above Iron Gate dam. CDFW reported that the vast majority of these fry died after passage through the dam's discharge tunnel, presumptively due to gas supersaturation (Neumann, 2024). We did not use this release date for the unknown classification. Fall Creek had two other releases (May 15 and 22) with separate tag codes allowing for specific Weeks at Large calculations for each CWT (062240 code occurred in each release and was assigned May15 as its release date), these fish were trucked downriver and released below Iron Gate dam. Total smolt release (5/15 and 5/22) was 1.6 million at a 25% mark rate. For more information and the definition of Weeks at Large, refer to Appendix A. Intestine sampling and DNA extraction were modified from Voss et al., 2020. *Ceratonova shasta* was assayed by quantitative PCR. Refer to Appendix B for detailed methodology on quantitative PCR. To provide context as to whether fish are in a disease state, DNA copy number (amount of parasite DNA in the fish tissue) was used to establish severity. A rating of CS1 indicates a positive sample but less than 3 logs DNA, low severity. A rating of CS2 is ≥ 3 logs DNA, severe infection likely associated with mortality (True et al. 2012, Voss et al. 2022). Refer to Appendix A for further detail.

Table 1. Reach locations, rotary screw trap locations, distances, and cooperating agencies performing fish collection on the main-stem Klamath River.

Collection Reach Klamath River main stem	Reach Code	River miles (Upstream – Downstream)	Collector
Iron Gate Dam to Shasta R.	K5	190-177	USFWS
I-5 rotary screw trap[1]		~183	USFWS
Shasta R. to Scott R.	K4	177-144	USFWS
Kinsman rotary screw trap[1]		~ 147	USFWS/Karuk Tribe
Scott R. to Salmon R.	K3	144-66	Karuk Tribe
Salmon R. to Trinity R.	K2	66-44	Karuk Tribe
Weitchpec rotary screw trap[1]		~ 44.2	USFWS/Yurok Tribe
Trinity R. to Blue Creek	K1	44-16	Yurok Tribe
Blue Creek to Estuary	K0	16-0	Yurok Tribe

[1] Primary collection site within that reach

Results and Discussion

In 2024, we tested a total of 1197 juvenile Chinook Salmon collected from the main-stem Klamath River (Natural fish 43% (n =518), fish of unknown origin 41% (n =490), and Fall creek hatchery CWT fish 16% (n = 189) (Fig 1). Along with fall-creek CWT salmon, 165 Trinity Hatchery CWT salmon were collected in K1 and K0. The *C. shasta* infection status of these TRH fish is not reported. The number of fish collected and tested weekly is presented in Appendix C.

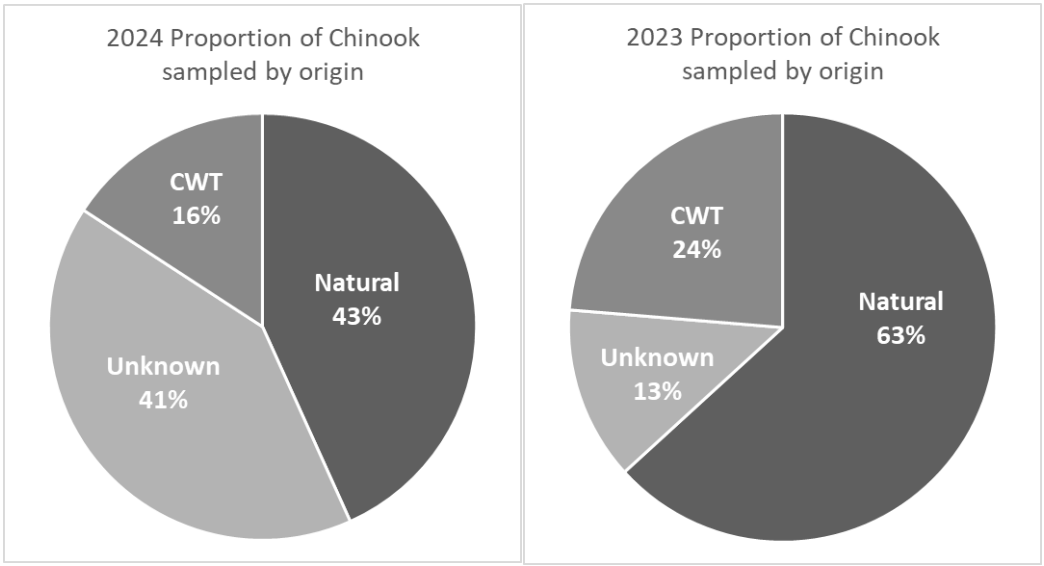


Figure 1. Proportion and origin of Chinook samples used for prevalence of infection analysis in 2024 and 2023.

Prevalence of Infection

The prevalence of *C. shasta* infection in all juvenile Chinook Salmon intestinal tissue was 45% (536 / 1197, confidence interval [ci] = 42-48%). Prevalence tended to increase downriver and ranged from 22 – 89% (Fig. 2). There were two general groupings of fish size, smaller fish were observed in reaches K5-K3 compared to larger fish observed in K2-K0 (Table 2). Both POI and fork length reach data is confounded by the differences in collection timing and proportion of hatchery smolts collected (Appendix C).

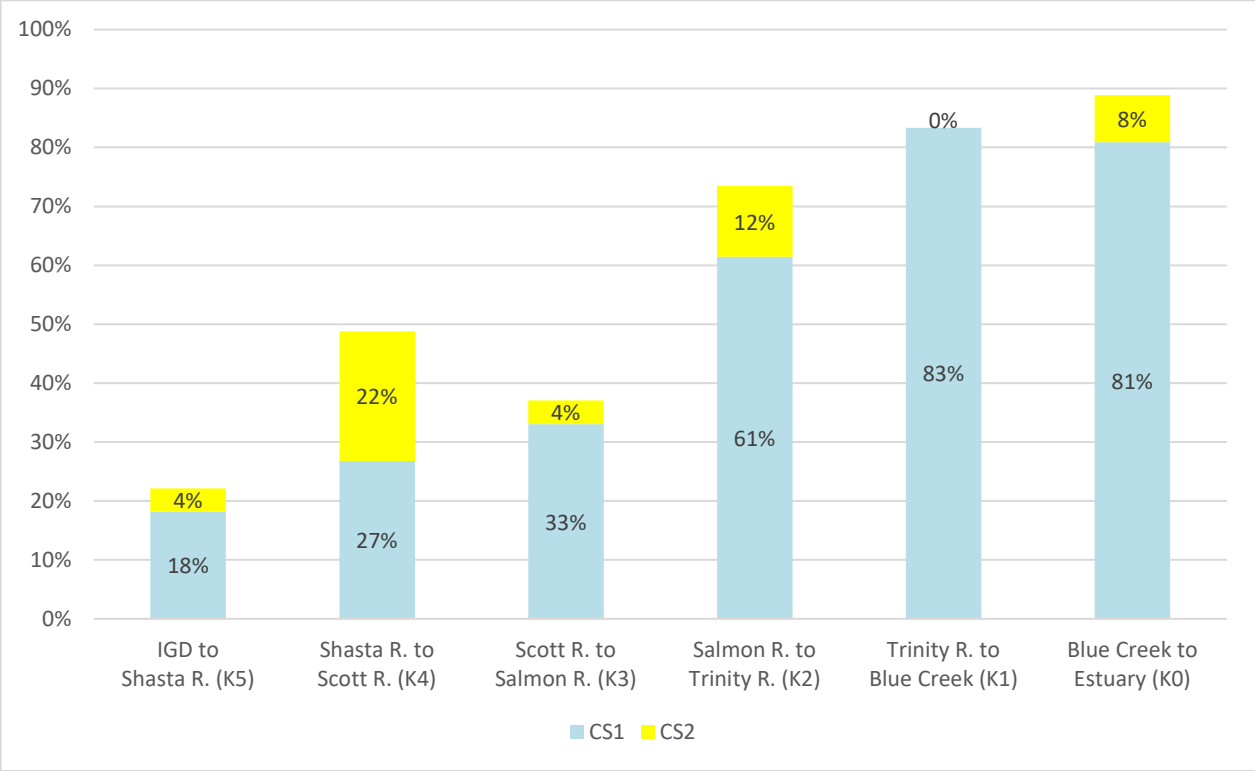


Figure 2. Prevalence of *Ceratonova shasta* infection severity (CS1 positive but less than 3 logs DNA, CS2 ≥ 3 logs DNA) by reach in juvenile Klamath River Chinook Salmon tested by QPCR in 2024; Iron Gate Dam to Shasta River (K5), Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to Blue Creek (K1), and Blue Creek to Klamath River Estuary (K0).

Table 2. Average (minimum – maximum) fork length (mm) of salmon collected in each reach.

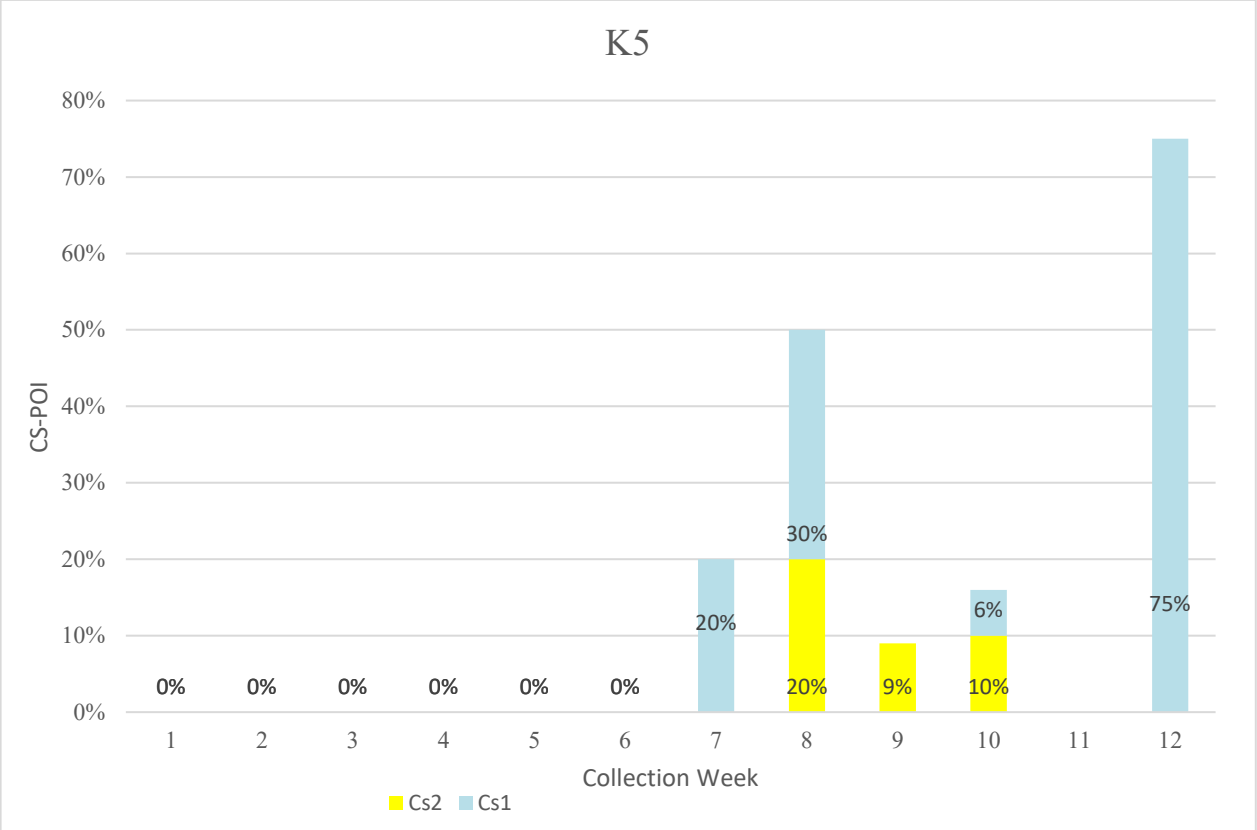
Reach	Mean (range)
K5	66 (32 – 90)
K4	56 (32 – 90)
K3	57 (35 - 94)
K2	86 (61 – 125)
K1	80 (62 -95)
K0	91 (73 – 100)

Natural and unknown origin Chinook Salmon

Initial *C. shasta* detection occurred on April 23 at the Kinsman trap site which was one week prior to initial detection at the up-river I-5 trap site (Fig. 3). Collection numbers were considered low (< 20) for most weeks at the I-5 site. Peak disease severity (CS2 rating) occurred in early and mid-May at the above trap sites. Severity (CS2 rating) was much higher at the Kinsman site (K4) than the downriver K3 and K2 sample groups (Fig. 3). It would appear that "diseased" K4 fish were not captured downriver and may have died prior to these reaches.

Oregon State University (OSU) monitored waterborne spore stages of *C. shasta* in 2024 March 18 through July 1 (Appendix E). This data showed that ≥ 10 spores/L threshold occurred beginning in mid-April from sample sites at or above the Kinsman RST. It then declined below this threshold value in June. Peak spore concentration (~ 58 spore/L) occurred in April at the Beaver Creek site. This data indicates that infectivity in K4 occurred weeks earlier than in previous years and could be related to the higher severity of infection (21% CS2 or ≥ 3 logs DNA) observed in this reach. Water temperatures in March and April 2024 were 2 to 3 ºC higher in these reaches than 2023 and likely influenced the earlier release of *C. shasta* actinospores (Appendix E).

K1 and K0 collections of unknown origin salmon were limited in number (47) and time (mid-June and July) (Table 3). Prevalence of CS1 rated infections ranged from 65 – 100% (Table 3). No samples contained > 3 logs of *C.shasta* DNA (CS2 rating).


K5
80%
70%
60%
50%
40%
30%
20%
10%
0%
CS-POI
0%
0%
0%
0%
0%
0%
20%
30%
20%
9%
6%
10%
75%
1
2
3
4
5
6
7
8
9
10
11
12
Collection Week
Cs2
Cs1

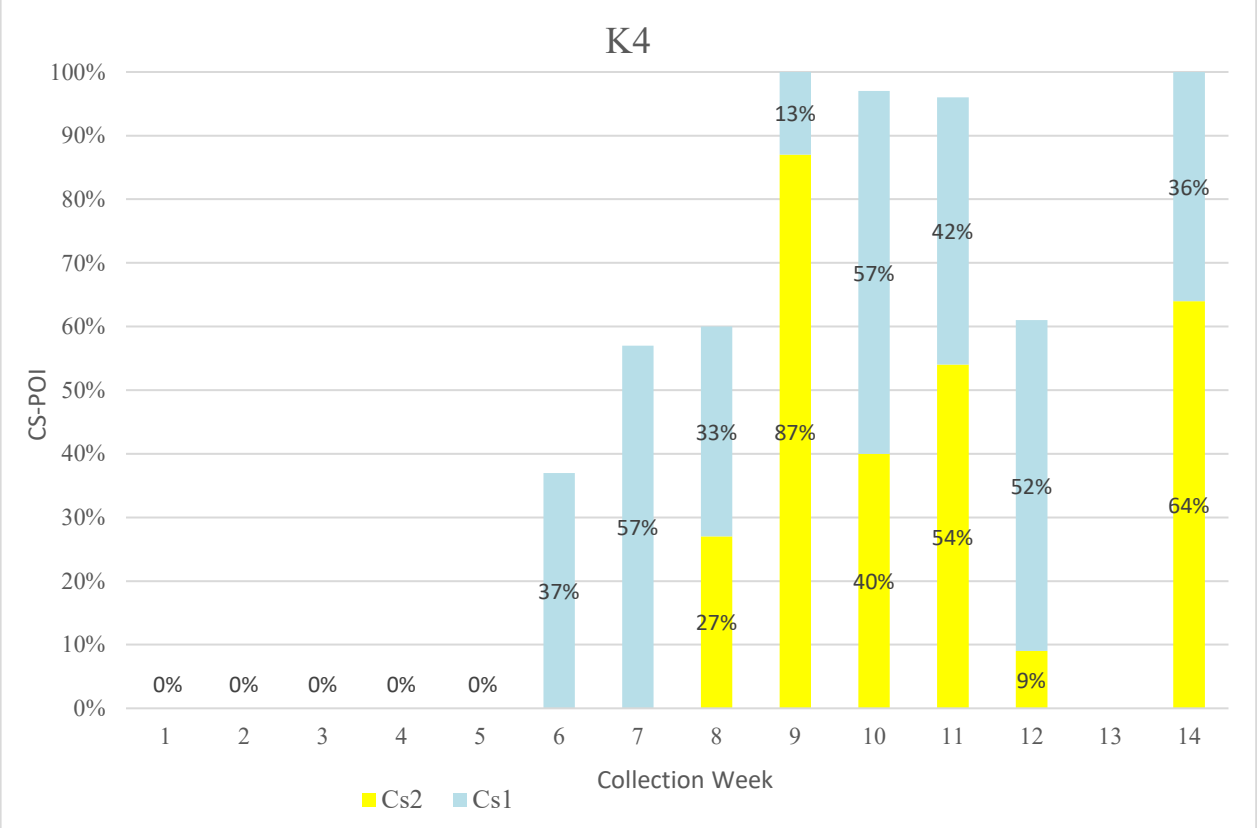

K4
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
CS-POI
0%
0%
0%
0%
0%
37%
57%
33%
27%
13%
87%
57%
40%
42%
54%
52%
9%
36%
64%
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Collection Week
Cs2
Cs1

Figure 3. Prevalence of CS1 and CS2 rated infections for each weekly collection in reaches K5 to K2.

Table 3. Prevalence of CS1 rated infections in unknown origin salmon from K1 and K0.

Reach	Week	CS1-POI
K1	14 15	5 / 6 (83%) 10 / 12 (83%)
K0	14 15 19	15 / 23 (65%) 4 /4 (100%) 2 / 2 (100%)

Fall Creek Hatchery CWT Weeks At Large and Pre-liberation sample

No *C. shasta* DNA was detected in the 58 pre-liberation Fall Creek hatchery samples tested. Hatchery smolts showed rapid infection with 40% CS-POI within 1 week of release (Fig. 4). Overall CS-POI for CWT salmon was 73% (138 / 189). The CS-POI was 94 -100% after 4 Weeks at Large (WAL). In weeks with sufficient sample numbers (1 – 8 WAL), there were two peaks in mean copy number approaching the 3 log "disease" levels during weeks 4 and 8 (Fig. 4). It is unclear why severity declined in 5 and 6 WAL. With the exception of mid-May Shasta River and Kinsman samples, spore concentrations were < 10 spores/L during the hatchery smolt out-migration period in late May and June (Appendix E). Despite an earlier release timing compared to previous Iron Gate Hatchery operations, Fall Creek smolts appeared to incur a significant infectious challenge with likely reduced survival. For more information and the definition of Weeks at Large, refer to Appendix A.

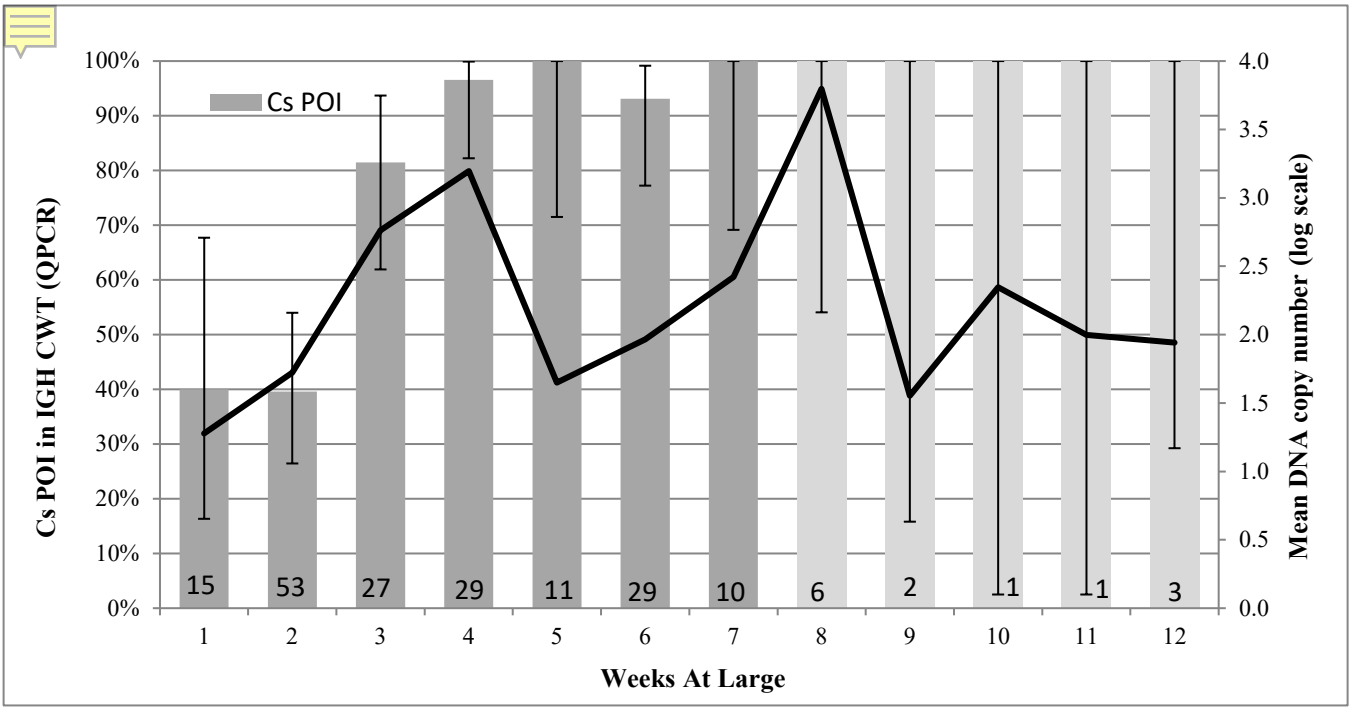


Figure 4. *Ceratonova shasta* prevalence of infection in Fall Creek hatchery CWT intestinal tissue by Weeks At Large (WAL) post hatchery release. The bar graph is prevalence of infection (%) on the primary y-axis and the line graph is the mean *C. shasta* DNA copy number (log scale) on the secondary y-axis. The number of fish collected is listed inside the base of each bar.

Gill and intestine testing comparison

Gill and intestine samples were compared in 238 salmon from K4, weeks 4, 6-12. Initial detection (April 23) occurred in both sample types. Overall prevalence of infection was lower in gill (49%, 114 / 235) than intestine (62%, 148 / 238). Parasite DNA quantity (copy number) tended to be lower and more uniform in gill samples than intestine (81% of gill samples ≤ 400 copies compared to 49% for intestine). This data suggests that there is little prognostic value in testing gill tissue.

Water sampling conducted by OSU began March 25 at the Kinsman rotary screw trap (RST) index site (KMN). *Ceratanova shasta* was detected for the first time in water samples on April 15 at 5 spores/L and increased quickly to a peak of 36 spores/L April 29. Spores per liter ranged from 2-18 throughout the month of May and stayed at 10 spores/L or higher May 13 – 28 (10-18 spores/L). There were 11 spores/L the first week of June (Oregon State University, 2024). The prevalence of infection in fish tissues was overlaid with spore density data from OSU (Figure 5). Spore per liter data can be seen graphically in Appendix E.

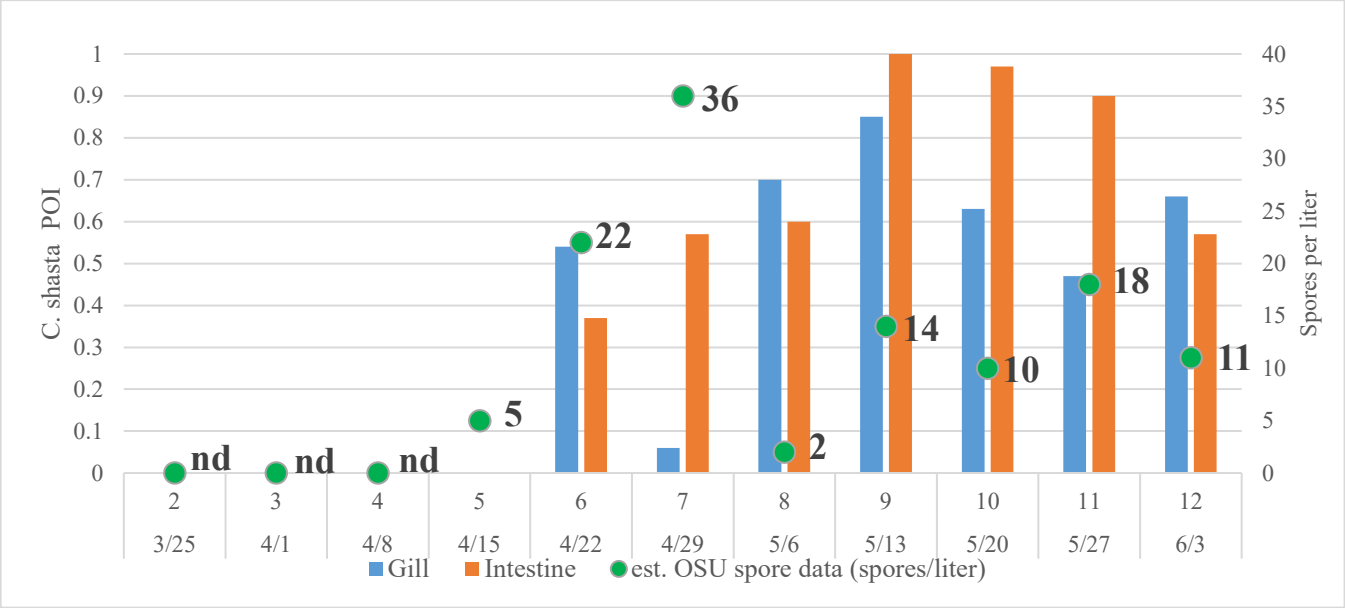


Figure 5. Weekly QPCR results for fish collected at Kinsman RST during real-time monitoring. *Ceratonova shasta* POI by QPCR is shown in bar graph for gill tissue (blue) and intestinal tissue (orange). Weekly water sampling results from Oregon State University is shown with a green dot and the number of spores/L. nd = water samples tested but *C. shasta* was not detected.

Historical Comparison

Prevalence of *C. shasta* infection during peak out-migration May 1 – July 31 by QPCR increased slightly in 2024 compared to 2023 (Table 4). POI during this period in 2024 was 64% (464/729, ci = 60-67%). In 2023, POI was 62% (665/1,066, ci = 59-65%). It was also greater than the 15-year average of 51% (2009-2023).

Year	QPCR	(% Positive)
2009	47%	(264/561)
2010	17%	(128/774)
2011	17%	(62/374)
2012	30%	(160/526)
2013	46%	(234/508)
2014	81%	(467/576)
2015	91%	(437/482)
2016	48%	(243/504)
2017	26%	(153/600)
2018	20%	(114/570)
2019	68%	(395/581)
2020	73%	(433/593)
2021	82%	(368/447)
2022	53%	(472/896)
2023	62%	(665/1066)
2024	64%	(464/729)
Mean	**52%**	**5059/9787**

Table 4. Historical annual *Ceratonova shasta* POI (QPCR only) in all juvenile Chinook Salmon (any origin) collected from the main-stem Klamath River between Iron Gate Dam and Trinity River confluence during the peak out-migration period of May through July 2009-2024. Percent positive by assay is reported, as well as the number positive/number tested in parenthesis.

Environmental Conditions

California's Water Year 2024 was considered a normal water year and saw statewide precipitation within the 30-year average however river conditions such as flow, turbidity and temperature were altered by the Klamath dam removal efforts. Detailed figures of water temperature, turbidity and discharge are provided in Appendix D.

Acknowledgments

We wish to acknowledge significant contributions by biologists and technicians with the USFWS Arcata Fish and Wildlife Office, Yurok Tribe, and Karuk Tribe for fish health monitoring in the field and sample collection.

Cover Photo: Iron Gate dam removal October 2024, SFgate.com

Author Roles

The contributions of each author have been summarized below.

- Ron Stone – Project lead and real-time data analysis, sampling coordination, QPCR assays, data entry, data management and quality control, pivot tables, and primary author for 2024 annual report.
- Scott Freund – Sample pick up, necropsy assistance, CWT extraction and reading, and data entry.
- Scott Foott – Sample pick up, necropsy assistance, DNA extraction, and report writing

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Appendix A - Definitions and Data Analysis

Testing

In 2024, tissues were tested for *C. shasta* by QPCR only. The six collection reaches are shown in figure A1.

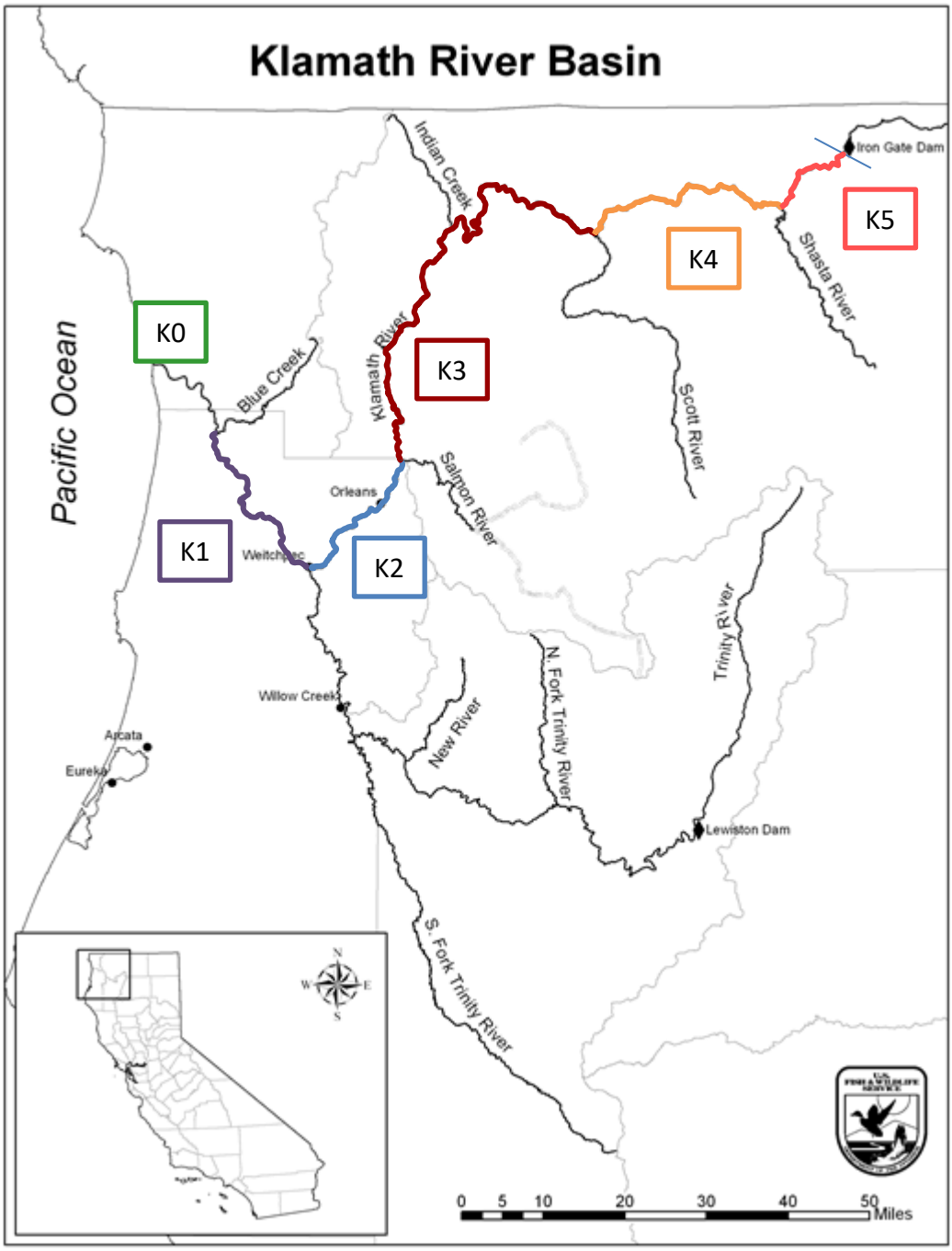

Figure A1. Klamath River watershed, major tributaries, and sample reaches: Iron Gate Dam to Shasta River (K5), Shasta River to Scott River (K4), Scott River to Salmon River (K3), Salmon River to Trinity River confluence (K2), Trinity River to upper Estuary (K1), and Klamath River Estuary (K0). Map provided by the Arcata Fish and Wildlife Office.

Prevalence of Infection and Copy Number

Point prevalence of infection and annual prevalence (defined by Durfee, 1978; USFWS, 2022) for *C. shasta* is reported with 95% confidence intervals (denoted ci) due to sample size. The larger the sample size, the smaller the confidence interval. Prevalence of infection (POI) is used to describe the proportion of infected Chinook Salmon (numerator) in the sample (number of animals examined) for a particular calendar week. Annual prevalence is used to describe the overall prevalence of infection in the sampled population during the entire sampling period that year. Annual prevalence estimates are not estimates of the annual proportion of the population that is infected, because weekly estimates are not weighted by abundance values. To assess *C. shasta* disease in out-migrating juvenile fish, it is important to look at both prevalence of infection, as well as DNA copy number. As stated above, POI is the proportion of fish infected; however, POI alone does not provide the entire picture of infection status. For example, a high weekly POI suggests that a large proportion of fish are infected, but provides no context as to whether fish are in a disease state. It is informative to also consider DNA copy number (how much parasite DNA is in the fish tissue) to get a more complete picture of infection.

DNA copy number is a term used to describe the quantity of parasite DNA within fish tissue. The QPCR assay quantitates unknown samples based on a known quantity from a standard curve. DNA copy number is reported in log scale. The log value is calculated by transforming the DNA copy number for an individual fish to log scale first and then taking the mean of the log values for that group of fish. Unlike water samples that relate DNA concentration to actinospore number, tissue samples contain various multinucleated parasite developmental forms (trophozoites, pansporoblast) that have not been empirically correlated to DNA concentration. Three logs or greater of *C. shasta* DNA is the threshold for describing *C. shasta* infections likely to lead to mortality under spring-summer temperatures. This threshold was based on a sentinel exposure study conducted in 2008 (True et al. 2012) and additional samples collected in 2020 and 2021 (Voss et al. 2022). The relationship between copy number and histological rating of tissue for *C. shasta* infection in the intestine was determined by dividing tissues from a single fish and testing by both QPCR and histology. There was good agreement between histological signs of disease and QPCR values 3 logs or greater.

Weeks At Large Analysis

In the monitoring program, temporal data is derived from Fall Creek CWT codes obtained from juvenile Chinook Salmon with known exposure periods (hatchery release [May 15 or 22] to in-river recapture date). The period of how long fish reside in the Klamath River post-hatchery release is termed Weeks At Large (WAL). For example, fish captured six days or less after hatchery release would be classified as <1 WAL. Fish captured between 7 and 13 days would be classified as 1 WAL, fish captured between 14 and 20 days would be 2 WAL, etc.

Historical Comparison

Prevalence of infection by QPCR is the metric that has been used for historical comparisons of *C. shasta* prevalence in the monitoring program since 2009. Data are confined to the peak migration timing of May 1 to July 31 (USFWS 1997) and fish of any origin collected above the Trinity confluence.

Appendix B – Methods for *Ceratonova shasta* Quantitative PCR Assay

A *Ceratonova shasta* quantitative PCR assay targeting the 18S ribosomal DNA sequence was used to assay DNA extracted from fish tissues. Forward primer (Cs-1034F 5' CCA GCT TGA GAT TAG CTC GGT AA), reverse primer (Cs-1104R CCC CGG AAC CCG AAA G), and probe (CsProbe-1058T 6FAM CGA GCC AAG TTG GTC TCT CCG TGA AAA C TAMRA) sequences were used from Hallett et al., 2006.

All reactions (30µL) were conducted in a 96-well optical reaction plate using 0.9 µM of both primers, 0.25 µM of probe, 15 µL of TaqMan Universal PCR Master Mix, and 5uL of DNA template. Samples were not assayed in replicates. Reactions were assayed using a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems) with the following cycling conditions: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Each assay plate included a standard curve with three concentrations of reference standards (two replicates each) at known DNA copy number and two negative control wells.

The *C. shasta* reference standard curve was obtained using synthesized DNA (gBlock Gene Fragments, Integrated DNA Technology, Coralville Iowa) containing the 18S ribosomal DNA target sequence. Specifically, 1 ng of DNA, corresponding to 6.83×10^9 copies of *C. shasta* DNA was serially diluted over ten orders of magnitude in Tris-EDTA (ethylenediamine tetraacetic acid) buffer. Using QPCR analysis software, the cycle threshold (C_T) values for each standard concentration were calculated (QuantStudio 6 software, v 1.7.2, Applied Biosystems). The standard curve was used to evaluate PCR amplification efficiency (slope of the standard curve, efficiency was 100.7%), fit to the curve (R^2 value = 0.999), and the y-intercept (38.7) which is the theoretical C_T value for a single copy of parasite DNA when assays are 100% efficient (Applied Biosystems, 2014).

Each assay was evaluated for expected C_T values of the reference standards and assay efficiency. At the end of the season, any plates with more than a 3% decrease in assay efficiency from the mean were retested and reevaluated. Based on this criteria no plates require retesting in 2024.

Our criteria for a positive test result required samples to produce a change in normalized fluorescent signal (Δ Rn) greater than or equal to 100,000 florescent units, verifying significant amplification above background levels of the instrument. Samples were also required to have a quantity (DNA copy number) greater than or equal to five copies, as very low copy numbers are not reliable given the limitations of the Poisson distribution (Applied Biosystems, 2016).

In 2022, it was decided in that auto-threshold and auto-baseline settings would be used for analysis on the QuantStudio 6 instrument software. These auto settings will be used as long as the reaction efficiencies are similar between experiments (within 3%) and efficiencies are between 90% and 110%. These analysis settings will also be used in future years of *C. shasta* testing on the QuantStudio 6 instrument.

Appendix C – QPCR Results

Table C-1. *Ceratonova shasta* infection by QPCR in juvenile Chinook Salmon sampled from six reaches within the Klamath River during 2024. The prevalence [% (number positive/number tested)] is presented for each sample reach by collection week and sample date (Sunday of each week).

Week	Sample Date	IGD to Shasta R. (K5)	Shasta R. to Scott R. (K4)	Scott R. to Salmon R. (K3)	Salmon R. to Trinity R. (K2)	Trinity R. to Estuary (K1)	Estuary (K0)
1	18-Mar	0% (0/2)	0% (0/28)				
2	25-Mar	0% (0/3)	0% (0/24)				
3	1-Apr	0% (0/2)	0% (0/28)	0% (0/30)			
4	8-Apr	0% (0/4)	0% (0/30)	0% (0/30)			
5	15-Apr	0% (0/20)	0% (0/2)	0% (0/30)			
6	22-Apr	0% (0/6)	37% (11/30)	0% (0/30)			
7	29-Apr	20% (2/10)	57% (17/30)	10% (3/30)			
8	6-May	50% (5/10)	60% (18/30)	7% (2/30)			
9	13-May	8% (1/12)[B]	100% (30/30)	34% (10/29)	43% (13/30)		
10	20-May	16% (5/31)[C]	97% (29/30)[C]	80% (24/30)[C]	77% (23/30)[B]		
11	27-May	29% (8/28)[A]	90% (27/30)[B]	57% (17/30)[C]	85% (17/20)[C]		
12	3-Jun	47% (14/30)[B]	57% (16/28)[B]	62% (18/29)[B]	47% (14/30)[B]		
13	10-Jun			93% (27/29)[C]	100% (28/28)[B]		
14	17-Jun		94% (17/18)[B]	55% (16/29)[B]	100% (12/12)[A]	83% (5/6)[C]	67% (12/18)[C]
15	24-Jun			87% (26/30)[C]	72% (21/29)[B]	83% (10/12)[C]	
16	1-Jul				86% (24/28)[B]		94% (15/16)[A]
17	8-Jul						100% (8/8)[A]
18	15-Jul						92% (35/38)[B]
19	22-Jul						100% (3/3)[A]
20	29-Jul						100% (1/1)[A]
21	5-Aug						
22	12-Aug						100% (6/6)[B]
		K5 Total **22% (35/158)**	K4 Total **49% (165/338)**	K3 Total **37% (143/386)**	K2 Total **73% (152/207)**	K1 Total **83% (15/18)**	K0 Total **89% (80/90)**

[A] Weekly sample contains CWTs only [B] Weekly sample contains unknown and CWTs [C] Weekly sample contains unknown fish only

Appendix D – Environmental Conditions

River Water

Mean daily temperature at the Iron Gate gauge was approximately 3 ºC warmer in March and April 2024 than during the same months in 2023 (median difference for mean daily temperature 3.09 and 3.23 ºC) (Fig. D1). Similar to the Iron Gate data, Seiad Valley gauge temperatures were 2 ºC warmer in March and April 2024 than during the same months in 2023 (median difference for mean daily temperature 1.9 and 1.6 ºC)(Fig. D2). Dam removal operations resulted in high turbidity during the spring of 2024 compared to previous years. Turbidity data collected at Iron Gate (waterquality.karuk.us) showed elevated and variable Formazin Nephelometric Units (FNU) in March ranging from 38 – 441 FNU (Fig. D3). The April – May period averaged 28 FNU that was approximately 7X higher than 2023 (average 4 FNU). Mean daily discharge (cfs) at the Iron Gate Gauge (USGS gauging station 11516530) ranged from 1020 - 1740 during March and April 2024 (waterdata.usgs.gov, Fig D4). This was similar to 2023 except for increase flows in late April 2023. The Seiad Valley gauge (USGS gauging station 11520500) discharge ranged 3140 – 4880 cfs during the same period from during March and April 2024 (waterdata.usgs.gov, Fig. D5).

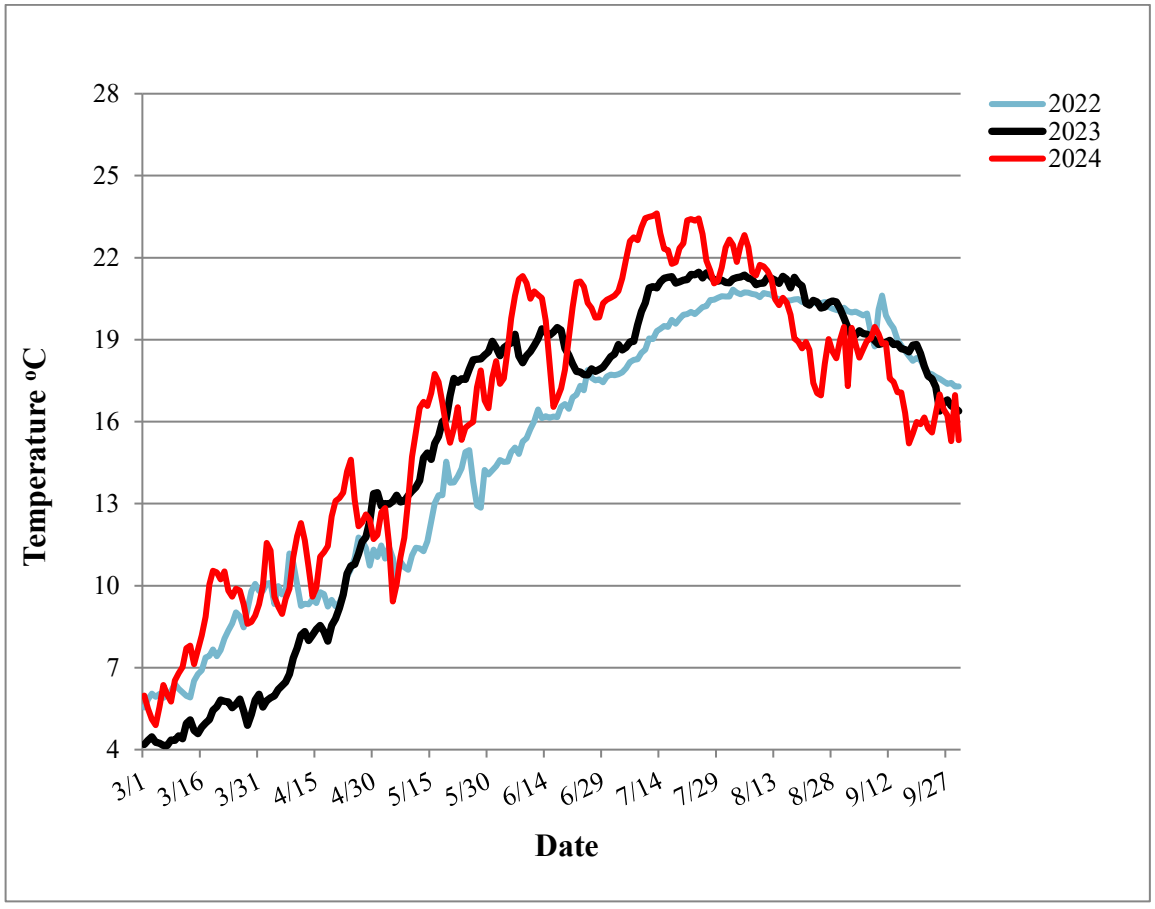

Figure D-1. Mean daily Klamath River water temperature below Iron Gate Dam from March through September 2022-2024. Temperature data was acquired from the Karuk Tribe.

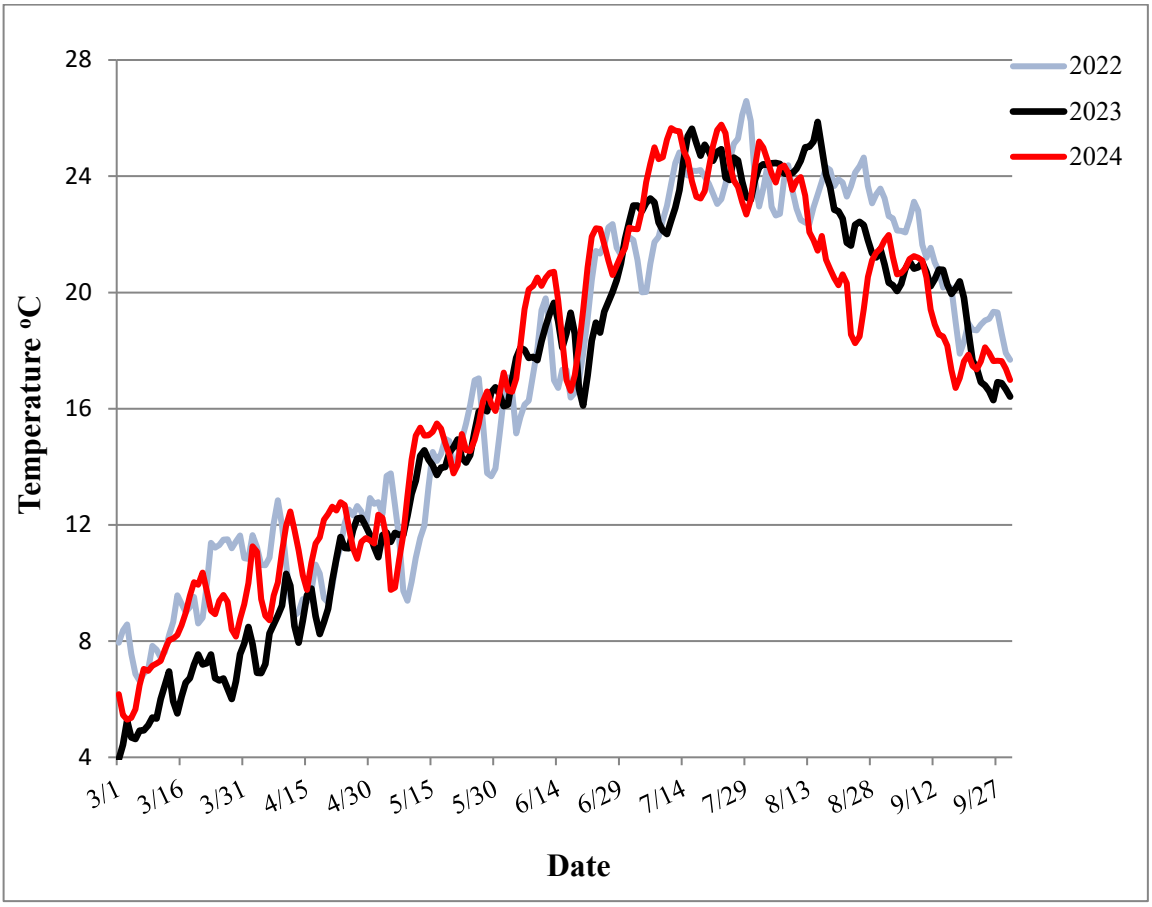

Figure D-2. Mean daily Klamath River temperature at Seiad Valley from March through September 2022-2024. Temperature data was acquired from the Karuk Tribe.

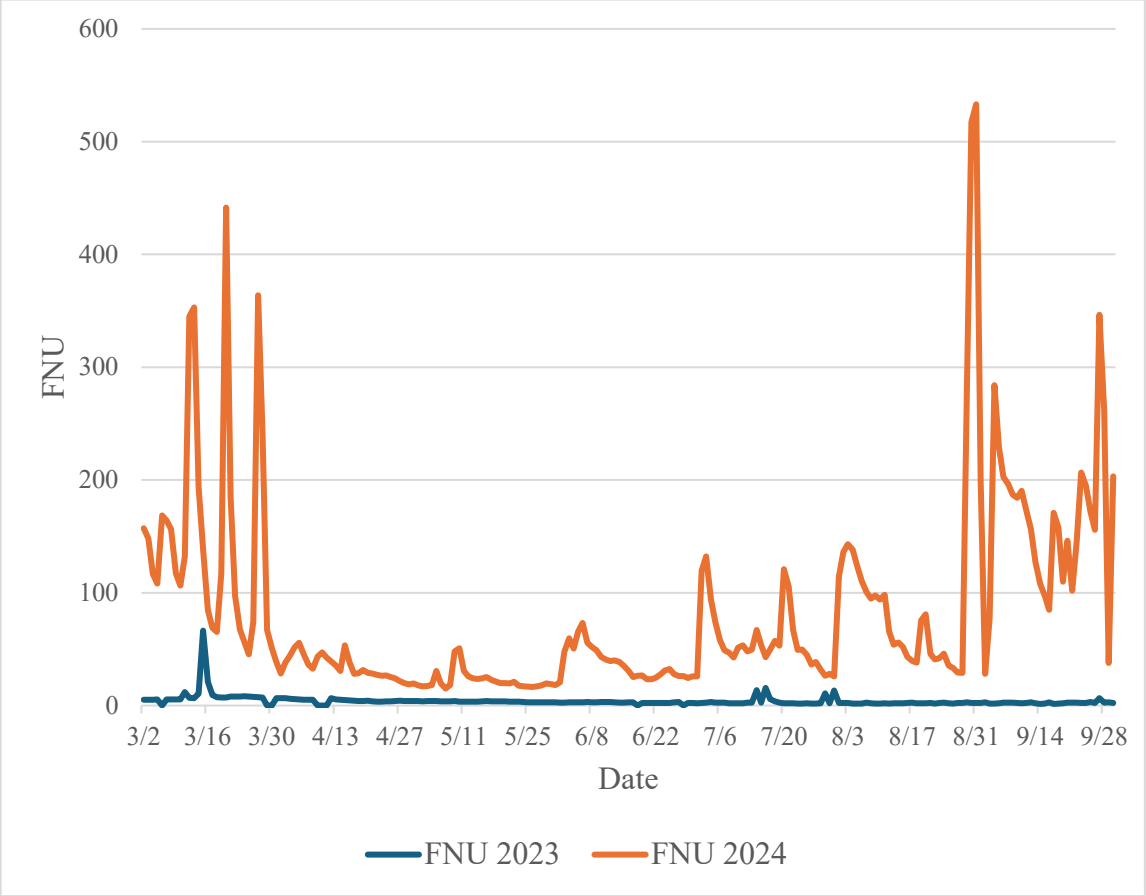

Figure D-3. Turbidity measures at Iron Gate (Formazin Nephelometric Units (FNU)) in 2023 and 2024.

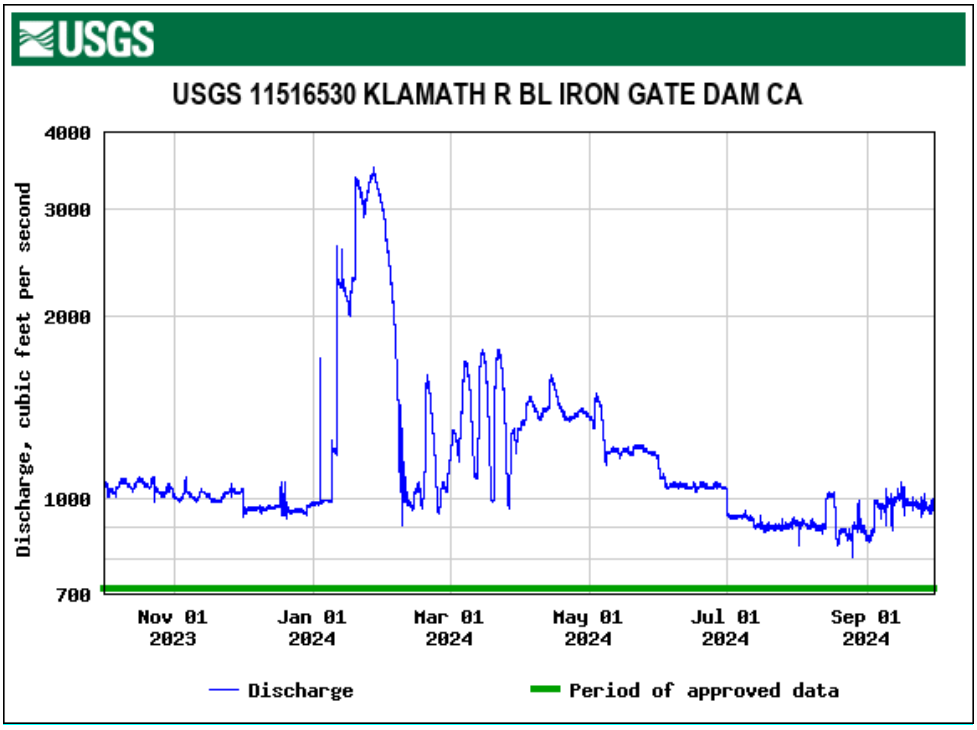

Figure D-4. Daily mean discharge (cfs) below Iron Gate Dam for Water Year 2024 (October 1, 2023 through September 30, 2024. Data was collected from USGS gaging station 11516530 at waterdata.usgs.gov.

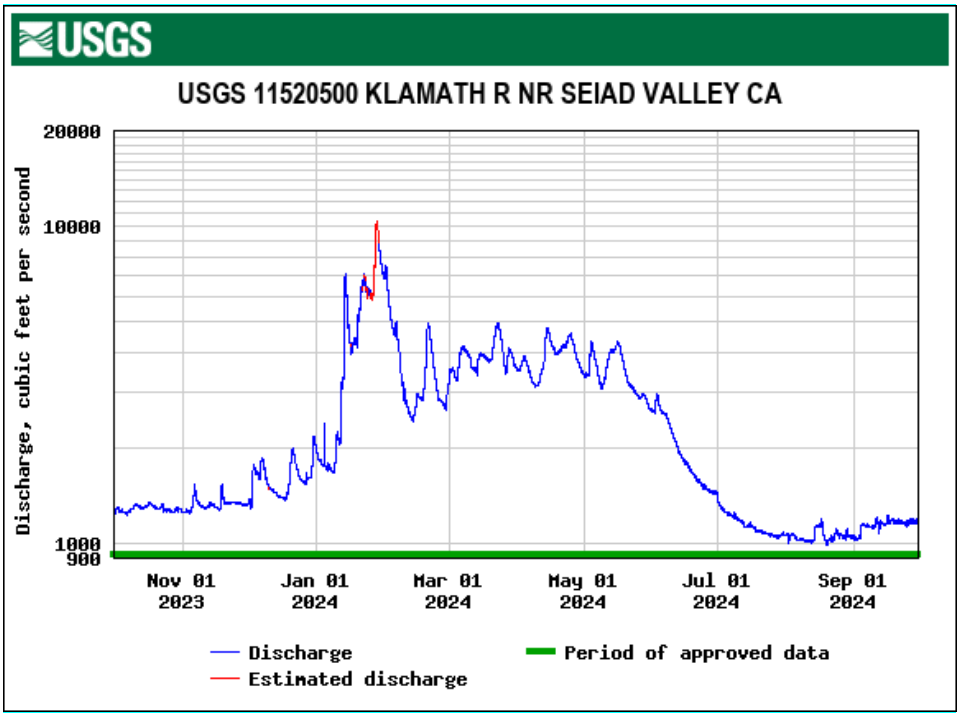

Figure D-5. Daily mean discharge (cfs) near Seiad Valley for Water Year 2024 (October 1, 2023 through September 30, 2024. Data was collected from USGS gaging station 11520500 at waterdata.usgs.gov

Appendix E – Spore Density

Dr. Sascha Hallett from Oregon State University (OSU) monitors and quantifies the waterborne stages of *C. shasta* from Klamath River water samples. Below is a figure from OSU showing the *C. shasta* average spore density in water samples during 2024. More information can be found on the OSU monitoring website: https://microbiology.oregonstate.edu/research/aquatic-microbiology-ecology/monitoring-studies

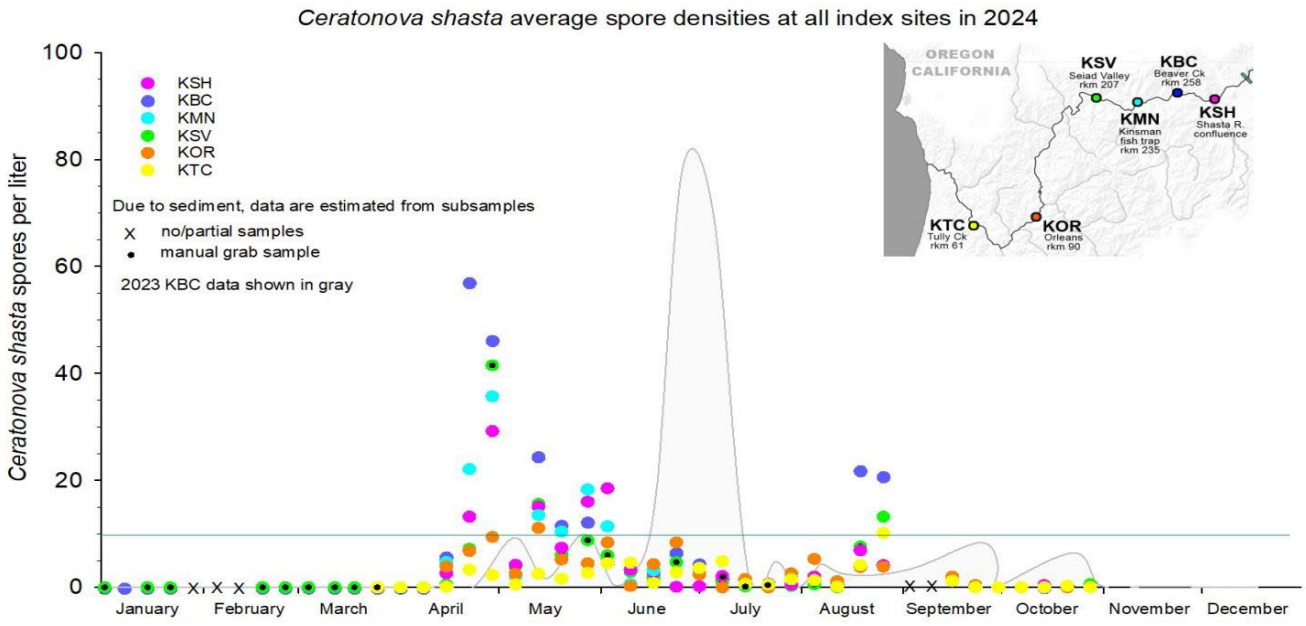

Figure E-1. Density (average spores per liter) of *Ceratonova shasta* in 24-hour composite water samples collected at the mainstem index sites in 2024. Note that KMN is sampled only during salmonid outmigration, KBC and KSV year round and remaining sites April through October. KBC = near Beaver Creek, KSV = Seiad Valley, KSH = near Shasta River, KTC = Tully Creek, KMN = Kinsman Fish Trap, KOR = Orleans. The line denotes 10 spores per liter which corresponds with 40% mortality threshold in Chinook Salmon.