

**Supplemental Report: Investigation of *Ceratomyxa Shasta*
In The Klamath River: Keno Reservoir To The Confluence
Of Beaver Creek**

**Klamath Hydroelectric Project
FERC Project No. 2082**

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SUPPLEMENTAL REPORT: INVESTIGATION OF *CERATOMYXA SHASTA* IN THE KLAMATH RIVER: KENO RESERVOIR TO THE CONFLUENCE OF BEAVER CREEK

DESCRIPTION AND PURPOSE

This section supplements the February 2004 Technical Report (PacifiCorp 2004) of investigations on *Ceratomyxa shasta* (*C. shasta*), and its polychaete host *Manayunkia speciosa* (*M. speciosa*) between Keno reservoir and the confluence of Beaver Creek. Supplemental information includes final reports of temporal and spatial distribution of *C. shasta*, polychaete habitat studies, and development of a Quantitative Polymerase Chain Reaction (QPCR) assay for measuring *C. shasta* concentrations in water. The purpose of this investigation was to address concerns that the Klamath Hydroelectric Project (Project) may be influencing the distribution and concentrations of *C. shasta* and *M. speciosa*.

OBJECTIVES

The supplemental information presented here addresses the following research objectives:

- 1) Relative infection rates at selected river /reservoir locations during the months of September and November
- 2) Habitat preference/abundance by habitat of *M. speciosa*
- 3) Relative abundance of the parasite at selected river/reservoir locations as determined using a Quantitative PCR assay.

As clarified in the February 2004 Technical Report (PacifiCorp 2004), a limitation of this work is the known temporal variance of *C. shasta* infection rates between seasons and from year to year. The data collected and related interpretations provided a summary based on specific conditions during the sampling period. Data collected in this study will provide a basis for determining spatial and temporal infectivity only for the area of study. It must be recognized that additional contributions of the parasite occur in the river downstream of the geographic scope of this work.

RELICENSING RELEVANCE AND USE IN DECISIONMAKING

Results of this *C. shasta* investigation may provide important information for use in interpreting how habitat and water quality conditions, including those affected by the Project, influence the health and wellbeing of the existing fish populations and how proposed protection, mitigation, and enhancement measures might alter these populations in the future.

METHODS AND GEOGRAPHIC SCOPE

The geographic scope of this study included the Klamath River from Keno reservoir to upstream of the confluence of Beaver Creek. Reported here are study objectives that began in September 2003 and were completed in August 2004. Sentinel study sites are indicated in Table 1 and in Figure 1; the sites where

intensive sampling was conducted are shown in Figure 2; sites where samples were collected for QPCR are indicated in Figure 3.

Table 1. Description of study area and of the locations for sentinel fish exposures for the detection of *Ceratomyxa shasta* in the Klamath River during September and November, 2003.

River Area	Description	Sentinel Site Location
Keno Reservoir (KRes)	Keno reservoir is the widened part of the head of the Klamath River. It extends from the upper end of Lake Ewauna at RM 253.1 to Keno Dam at RM 233.0.	Keno dam; RM 233.0 Suspended in dam forebay
Keno Reach (KR)	Keno reach is approximately 4.7 miles long, extending from Keno dam at RM 233.0 to J.C. Boyle reservoir at RM 220.4	RM 229.0 Held in eddy below riffle at Sportsman's Club
J.C. Boyle Reservoir (BRes)	The J.C. Boyle Reservoir is wide and shallow, deepening below the Hwy 66 bridge where the canyon narrows.	J. C. Boyle Dam; RM 224.7 Suspended in dam forebay on south bank
J.C. Boyle Bypass Reach (BBR)	The J.C. Boyle bypass reach is approximately 4.3 miles long. It extends from the J.C. Boyle dam at RM 224.7 to the discharge of the powerhouse at RM 220.4	RM 220.3 Directly upriver of powerhouse discharge
J.C. Boyle Peaking Reach (BPR)	The J.C. Boyle peaking reach is approximately 17.3 miles long and extends from the powerhouse at RM 220.4 to the upper end of Copco reservoir at RM 203.1. Shovel Creek, a Klamath River tributary, is located in California.	Shovel Creek; RM 206.5 Suspended from bank at bridge upstream of Shovel Creek
Copco Reservoir (CRes)	Copco reservoir is deeper than the upstream reservoirs, being located in a relatively steep canyon.	RM 199.4 Suspended from buoy cable in dam forebay
Copco Bypass Reach (CBR)	The Copco bypass reach is approximately 1.4 miles long and extends from Copco No. 2 dam at RM 198.3 to Copco No. 2 powerhouse at RM 196.9.	RM 197.3 Off river bank at Copco Village II
Beaver Creek (BC)	Beaver Creek is a tributary of the Klamath River at RM 161.0.	Beaver Creek; RM 161.0 Suspended from dike bank upstream of Beaver Creek

Methods

Data collected for this study incorporated three main investigative techniques: fish live box exposures (sentinel studies), substrate sampling for detection of polychaetes and water sampling for detection of parasites using a QPCR assay. Although the initial phases of this study (April through July 2003) were a collaborative effort between Oregon State University (OSU) and the U.S. Fish and Wildlife Service (USFWS), studies reported here were conducted by OSU.

Sentinel Studies

Protocols for sentinel studies were described in the February 2004 Technical Report (PacifiCorp 2004). Live cages were placed at each of the exposure locations and sentinel fish were held at each site for 3-5 days during the periods of September 22-26 and Oct 30-Nov 4. Rainbow trout used for the exposures were acquired from the Oregon Department of Fish and Wildlife (ODFW) and approval for their use was granted by the California Department of Fish and Game. For each exposure, 50 rainbow trout of a stock known to be susceptible to *C. shasta* were held to determine parasite presence. Water temperatures during the exposure period were monitored by thermistors attached to the live box.

After exposure, the fish were transported to holding facilities at the Salmon Disease Laboratory at Oregon State University. Control fish were unexposed, but otherwise were treated identical to the experimental groups. Following transfer, all fish were treated to prevent losses to bacterial pathogens, external parasites and fungi that they may have encountered during exposure. Fish were maintained for at least 70 days at 15°C, during which time they were fed daily and observed for clinical disease signs. Any fish that died during holding was necropsied and visually examined. If *C. shasta* spores were not identified, samples of intestinal tract were tested by PCR assay. Procedures for examining control fish were identical. At termination, 10 fish from each group were visually examined for spores. If any fish showed signs of infection, a total of 25 fish from that group (or all remaining fish if less than 25) were examined. Infection prevalence was calculated as the percent of fish examined that were positive for *C. shasta* either by visual examination or PCR assay.

Collection and Analysis of Substrate Samples to Identify Polychaete Habitat

Two sites were selected for intensive sampling in November: an eddy located in Keno Reach (20 samples) and J.C. Boyle Reservoir (25 samples)(Figure 2). Substrate samples for the identification of polychaete habitat were collected using semi-quantitative methods for aquatic oligochaete sampling (Aquatic Resources Center, 1999). Shallow, wadable areas in the Keno eddy were sampled using a kicknet, with manual collection of larger substrate; nonwadable habitats in the reservoir were sampled using a Ponar sampler. A hand-held GPS was used to record location of the described habitats. Data on location, stream characteristics (substrate, depth, water velocity), physical parameters (temperature, Dissolved Oxygen [DO], pH, conductivity, total dissolved solids [TDS]) and habitat type were recorded for each sample. A student's T-test was performed to determine if any physical parameters correlated with polychaete abundance.

Material collected from each sampling effort was placed in a plastic Ziploc™ bag, labeled, and placed on ice in a cooler. Samples were fixed in 100% ethanol, transported to the laboratory and individually sorted using a dissecting microscope. Standard measures for particle size distribution were performed to classify each sample. All polychaetes were removed from the sample, counted, and preserved for PCR analysis. From the Keno eddy site, 100 polychaetes, pooled in groups of 10, were tested by PCR to determine infection prevalence.

Development of a real-time PCR assay for the detection of Ceratomyxa shasta in environmental water samples

The primers and probe for use in the QPCR assay were selected using Primer Express v.2.0.0 software (Applied Biosystems). A combination specific to the 18S rDNA sequence of *C. shasta* was designed after reference to the host/parasite alignment of 18S rDNA sequences of Bartholomew et al. (1997), as well as additional myxozoan sequences available in GenBank. A forward primer (Cs-1034F), TaqMan TAMRA probe (CsProbe-1058T) and reverse primer (Cs-1104R) were designed to produce a 71bp amplicon. A BLAST search in GenBank using each oligonucleotide sequence indicated no identity with any other myxozoan or potential host group.

Collection and processing of environmental water samples

In June 2004, river samples were taken concurrently with sentinel fish exposures at 19 sites in the mainstem of the Klamath River, including lakes and reservoirs, as well as the Williamson River, Spencer Creek, Salmon River, Trinity River and Hunter Creek tributaries, to assess spatial prevalence of *C. shasta* (Figure 3). At two sites, J.C. Boyle Bypass Reach and Beaver Creek, samples were taken at 2-hourly intervals throughout the day (6 samples on 19 June and 9 samples on 21 June, respectively) to assess temporal variation in parasite prevalence. Temperature and dissolved oxygen were recorded and relative water level was noted.

The protocol for collection and processing of environmental water samples was as follows: one sample per site taken from approximately 30cm below the water surface was collected in a 2L clear plastic bottle and kept cool until processing (within 24 hours of collection). Samples were divided into 500 mL or 1L aliquots (depending on amount of suspended material) and filtered through a Millipore membrane filter supported by a glass holder using a vacuum pump. The filter membrane was then folded in half three times using forceps, placed inside a 2.0µL microfuge tube and frozen. Prior to lysis the filter membrane was cut into strips to promote contact with the buffer.

In addition to the water samples, infected fish tissue and counted purified *C. shasta* spores were included as positive controls and to provide a standard curve. Samples were placed in microcentrifuge tubes and frozen prior to DNA extraction (QIAGEN DNeasy protocol). The environmental water samples were lysed in 360µL buffer to immerse the entire filter paper. Pairs of 500mL sub-samples were combined upon application to the spin column such that each site was represented by 2 x 1L sub-samples. Purified DNA was eluted through the columns then stored at -20°C.

The optimal combination of primer, probe concentrations and template (sample) DNA was determined according to the TaqMan Universal PCR Master Mix protocol guidelines (Applied Biosystems). A 20µL reaction volume was used, and comprised 800µM of each primer, 200µM probe, 10µL TaqMan Universal PCR Master Mix (contains polymerase, AmpErase UNG, dNTPs with dUTP, passive reference and optimised buffer components), and DNA template (sample). Reactions were performed in an ABI PRISM 7000 Sequence Detection System with the following thermal cycling conditions: initial 2 min 50°C step; 10 min 95°C step followed by 40–50 cycles of denaturation at 95°C for 15 secs and annealing/extension at 60°C for 1 min. Reactions with environmental water included 10mg/mL BSA (final concentration) to reduce inhibition. A positive control (parasite DNA) and a negative control (purified water) were included in each reaction run. For environmental water assays, 1µL of the DNA template was used.

Figure 1. Map of sentinel exposure sites. Site abbreviations listed in Table 1.

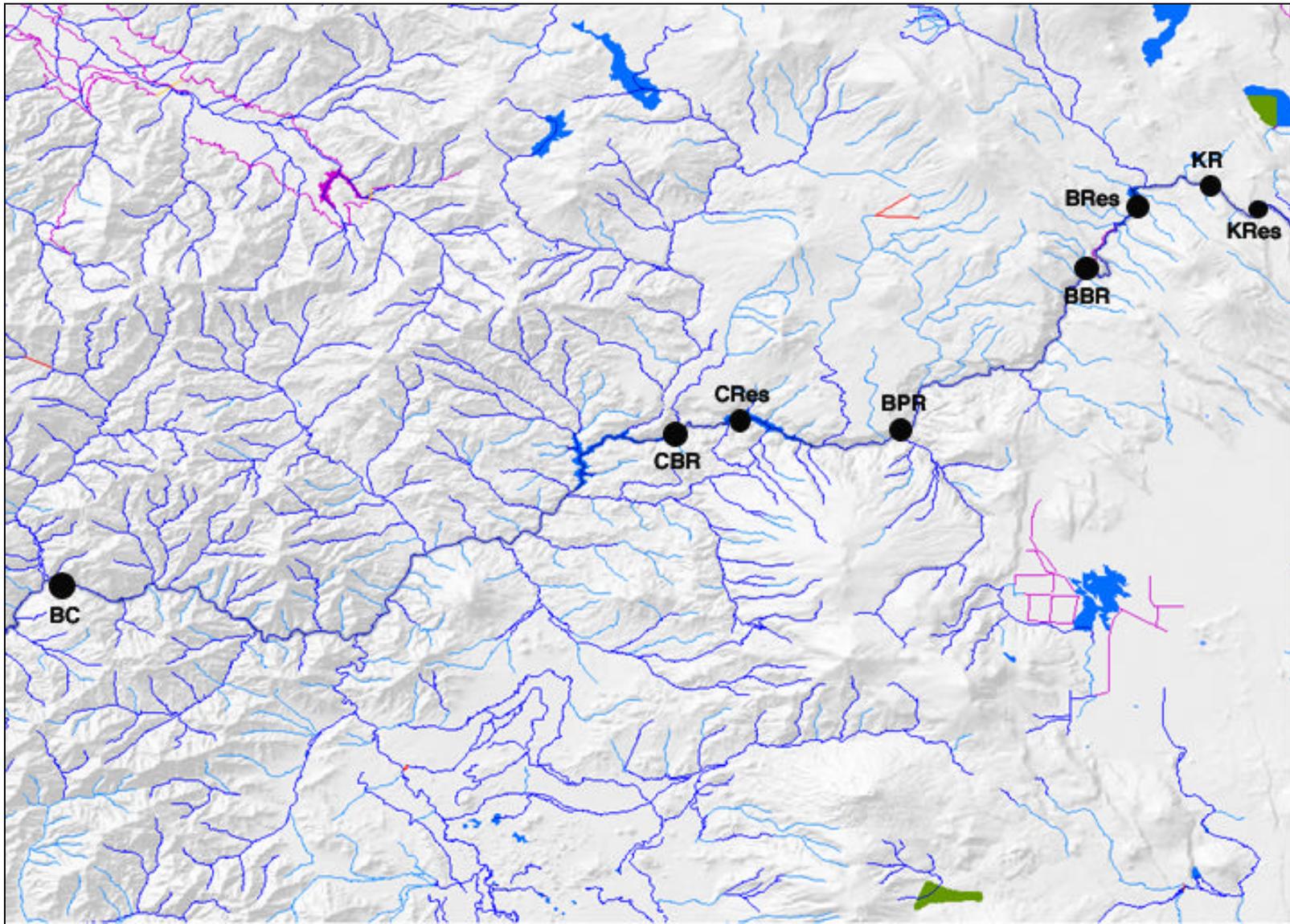
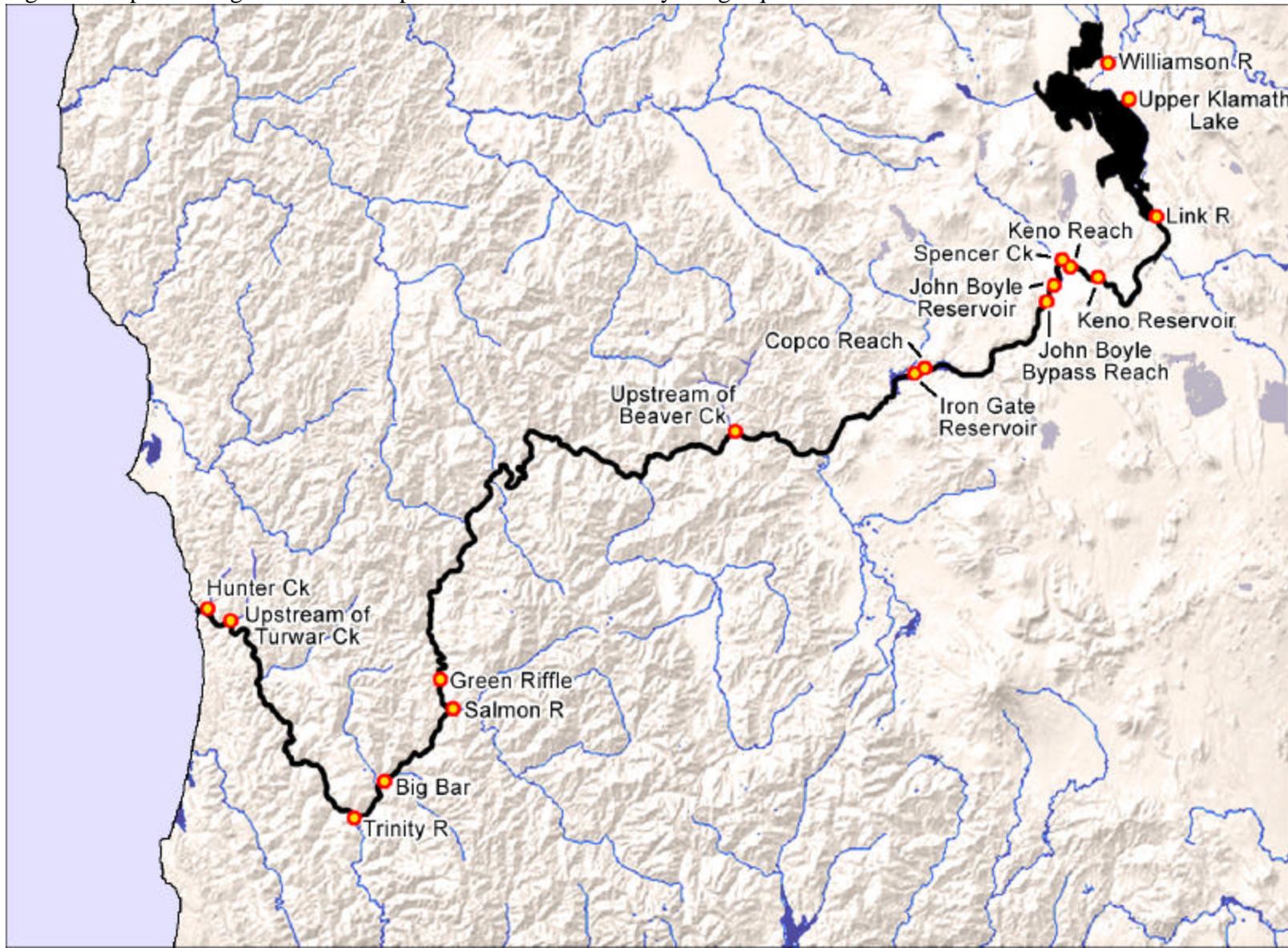


Figure 2. Photographs of sites where intensive sampling for polychaete habitat was conducted: the transition zone of Boyle Reservoir (top photo) and an eddy located in the Keno reach section.



Figure 3. Map indicating sites where samples were collected for assay using a quantitative PCR.



RESULTS

Sentinel Study

Data on infection prevalence, as determined by visual or molecular diagnostics (PCR), mortality from *C. shasta*, and average water temperatures during exposure are presented for the rainbow trout exposed at the sentinel locations in Figure 4 and Table 2. The table also includes exposure data from April – July for comparison. Details for each exposure period are presented below. A compiled chart of mortality versus water temperature for all sentinel samples during the project is included as Appendix A.

September Exposure

In September, the prevalence of *C. shasta* infection was >90% from all sites but Keno reservoir, where infection was not detected. Mortality as a result of the infection occurred in groups held at three sites upstream of Iron Gate dam: the J.C. Boyle bypass and peaking reaches and Keno reach. At these sites, mortality related to the infection remained below 25%. Loss from *C. shasta* in fish held downstream of Iron Gate dam, near Beaver Creek, was 100%. Data for September is presented in Figure 4A.

November Exposure

In November, fish held at all sites continued to have high infection prevalence except for those exposed at Copco and Keno reservoirs where infection was not detected. Mortality occurred only from fish exposed at Beaver Creek, and here it was only slightly decreased (86%) from September levels. Data for November is presented in Figure 4B.

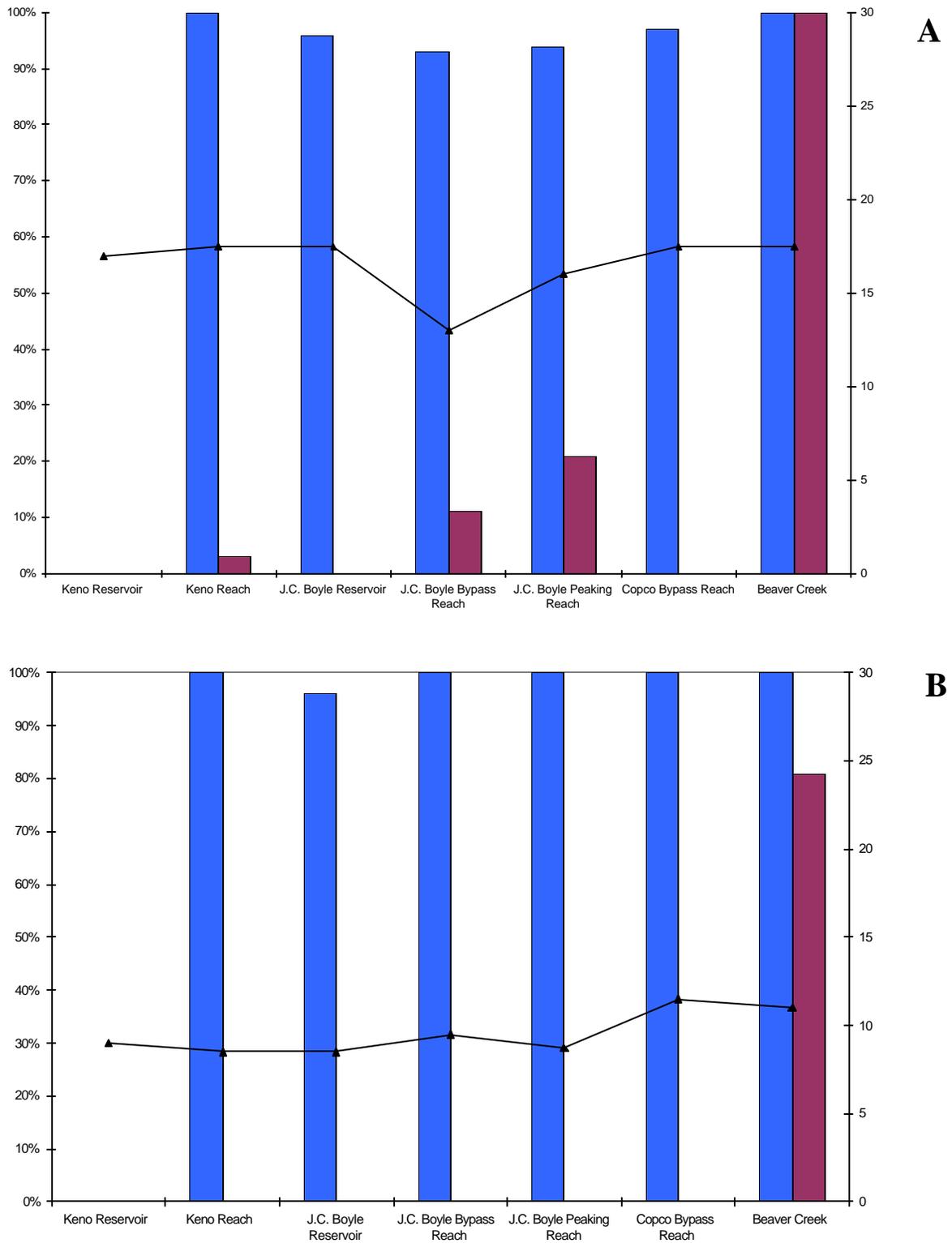


Figure 4. Prevalence of infection (■), percent mortality (■) and average water temperature (—) in groups of fish held at sentinel sites during September (A) and November (B).

Table 2. Results of sentinel exposure of rainbow trout at sites in the Klamath River for which exposures were conducted in September and November 2003.

Exposure Location	Exposure	Average Water Temperature	Infection Prevalence (PCR, visual) ¹	Percent Mortality with <i>C. shasta</i>
Keno Reservoir	April	9	0 (n=15)	0
	June ²	23	-	-
	July ³	27	-	-
	September	17	0 (n=10)	0
	November	9	0 (n=10)	0
Keno Reach	April	9	100 (n=15)	0
	June	22.5	100 (n=19)	0
	July ^c	29	-	-
	September	17.5	100 (n=25)	3
	November	8.5	100 (n=25)	0
J.C. Boyle Reservoir	April	9	67 (n=12)	0
	June	23	100 (n=17)	0
	July ³	27	-	-
	September	17.5	96 (n=25)	0
	November	8.5	96 (n=25)	0
J.C. Boyle Bypass Reach	April	10.5	53 (n=15)	0
	June	15	100 (n=11)	14
	July	16.5	100 (n=4)	59
	September	13	93 (n=25)	11
	November	9.5	100 (n=25)	0
J.C. Boyle Peaking Reach	April	10	60 (n=15)	5
	June	21.5	92 (n=25)	0
	July	22.5	100 (n=13)	21
	September	16	94 (n=25)	21
	November	8.7	100 (n=25)	0
Copco Bypass Reach	April	10	33 (n=15)	0
	June	20.5	100 (n=25)	2.1
	July	22	100 (n=10)	4.5
	September	17.5	97 (n=25)	0
	November	11.5	100 (n=25)	0
Beaver Creek	April	5.5	25 (n=4)	8
	June	20.4	ND	75
	July ⁴	20.4	100 (n=4)	90

Table 2. Results of sentinel exposure of rainbow trout at sites in the Klamath River for which exposures were conducted in September and November 2003.

Exposure Location	Exposure	Average Water Temperature	Infection Prevalence (PCR, visual) ¹	Percent Mortality with <i>C. shasta</i>
	September	17.5	100	100
	November	11	100	81

¹ Infection prevalence at termination of study.

² Fish lost because of columnaris infection during exposure.

³ No exposure conducted.

ND -- Exposure conducted but no data for this parameter.

⁴ Location of exposure site moved to Beaver Creek thermal refugia because of high temperatures

General Conclusions: Sentinel studies

Sites where exposure groups suffered mortality by *C. shasta* during this study were J.C. Boyle bypass and peaking reaches, Keno reach, Copco bypass reach and downstream of the hydroelectric projects in the mainstem of the Klamath River near the confluence of Beaver Creek. The parasite was detected at a high prevalence in the fish held in J.C. Boyle Reservoir using PCR, yet there was no mortality in any exposure group held in this, or any other reservoir. Infection was not detected from any group exposed in the Keno Reservoir. Infectivity in Iron Gate and Copco reservoirs was also limited, with only a single fish positive by PCR from the Iron Gate Reservoir cove site in July (Foott et al. 2004). It must be pointed out that fish exposed in the latter reservoirs were held for only 25 d, compared with at least 70 d for groups exposed at the sites reported here. In addition, high water temperatures precluded exposure at most reservoir sites during late July when infection levels were high at Beaver Creek, which served as a reference site. However, the report by the USFWS points out that the low infection rate detected from fish held in Iron Gate Reservoir is supported by the absence of ceratomyxosis at Iron Gate Hatchery where fish receive continual exposure to that water source.

For exposure sites above Iron Gate dam, mortality was highest (59%) in the group held in the J.C. Boyle bypass reach in July. Mortality at this site decreased in September (11%) and no mortality occurred in the November exposure group although infection prevalence remained greater than 90%. In exposure groups held in the J.C. Boyle peaking reach, mortality in September (23%) was similar to levels in July (21%). Mortality did not occur in groups held in the Keno reach in April or June despite high infection prevalence, and was only 3% in the September exposure group. Mortality in groups held in the Copco bypass reach was low in June and July and no fish died following the exposures conducted in the fall.

Although *C. shasta* was detected at a high prevalence by both visual and molecular methods from most upriver sites, mortality in these groups was generally low. This was unexpected as a high prevalence of infection is generally accompanied by high mortality in susceptible fish, especially when parasites are visually detected. The slow rate of disease development has also been surprising, as rainbow trout held at 16°C generally show disease signs by 30 days with mortality occurring by 40 days.

The low mortality in groups exposed above Iron Gate dam is contrasted by the high mortality in groups exposed in the lower Klamath River near Beaver Creek. A high level of infectivity was reported here in previous studies by the USFWS, and results of their exposures between April and July 2003 support the supposition that infectivity was decreased during that period in contrast with previous years. However, by fall, infectivity was high, possibly reflecting typical exposure levels.

One hypothesis for the high infectivity at Beaver Creek and other locations in the lower Klamath River is that it results from the combined effects of abundant populations of polychaetes and a higher infection rate in those populations. Absence of infection in the reservoirs is likely a result of lack of suitable polychaete habitat and dilution of parasites entering the reservoir. The actinospore stage of *C. shasta* is short-lived and may not survive passage through a slow-flowing reservoir.

Water temperature was not a good predictor of infection. The most severe infections were detected at the Beaver Creek site in July and September when temperatures averaged 20.4 and 17.5°C, respectively. However, mortality did not decrease significantly in November when temperatures at this site dropped to 11°C. Mortality levels were also high from the J.C. Boyle bypass reach, where water temperatures were consistently lower than all other sites.

Polychaete Habitat Studies

Polychaetes were identified from 19 of 20 samples collected from the Keno eddy site, with estimated abundances from 10 – 1,227/g material. The microhabitat that occurred most frequently and was associated with the highest concentration of the polychaete was a periphytic algae (*Cladophora* sp.). Another periphytic microhabitat that was encountered less frequently but also supported high concentrations of polychaetes was a loose, flocculent matrix of diatoms. Polychaetes were not detected from coarse organic material,

From J.C. Boyle Reservoir, polychaetes were detected from 12 of the 24 samples collected. The most abundant habitat here was a coarse organic matrix that only supported small numbers of polychaetes, as did a dense matrix of diatoms. Although less abundant, freshwater sponges supplied abundant habitat, with estimated densities of 8 – 3,054 polychaetes/g material. As had been observed in previous sampling, polychaetes were not present in samples consisting primarily of mud and silt.

No correlation was found between the physical parameters measured (TDS, pH, conductivity, DO, temperature) and polychaete abundance. These data are summarized in Figure 5. Detailed information on each sample is provided in Appendix B.

PCR assay of 100 polychaetes from the Keno eddy did not detect infection.

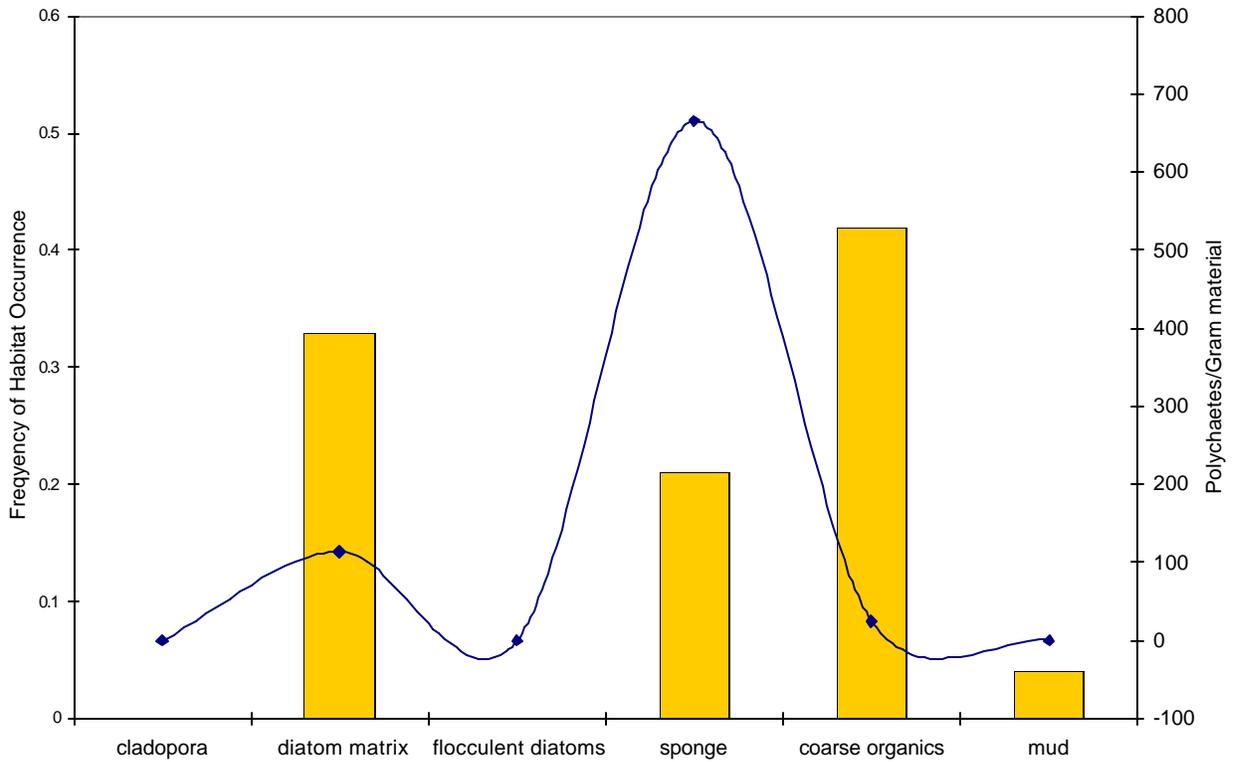
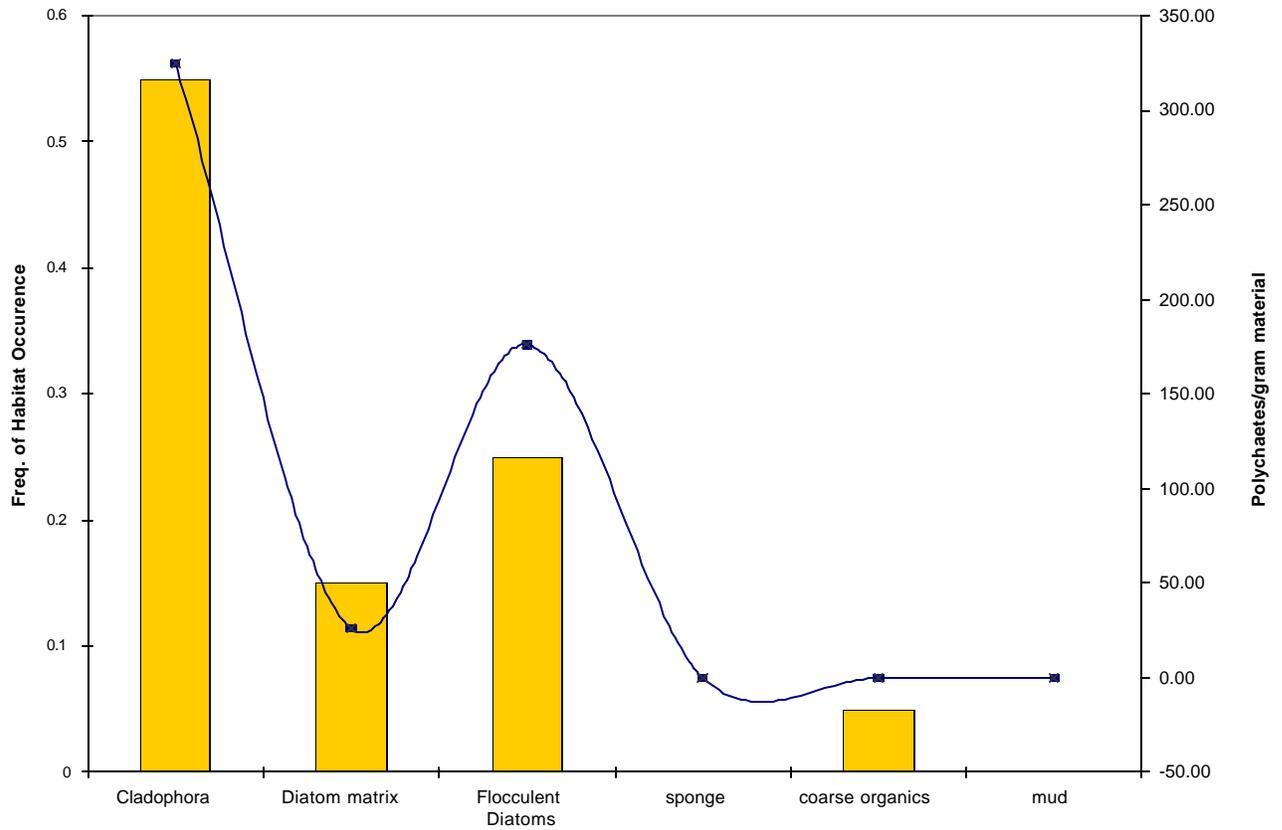


Figure 5. Frequency of microhabitat occurrence (■) versus polychaete density (—) in samples collected from the Keno eddy (top) and J.C. Boyle Reservoir (bottom).

General Conclusions: Polychaete Habitat

Polychaetes were most abundant in the riverine sections of the river and reservoirs. They were not associated with the very fine organic silt/mud substrate that occurs in reservoirs, especially behind the dams. This may explain the lack of infection detected in the sentinel fish held at these sites.

Polychaetes require fine particulate organic material for construction of their living tubes. This material may be trapped in periphytic *Cladophora* sp. and freshwater sponges, which provide a protected substrate for tube construction and can support high polychaete densities. Diatoms are found consistently in the gut, suggesting this may constitute a major portion of their diet.

Polychaetes were generally found in slower moving water (velocity between 0.02 m/s - 0.45 m/s), particularly in eddies and pools. In slow flowing water they could be found in the flocculent detritus that collects on the surface of fine sand/silt. However, measurements of water velocity were only taken at the time of sampling and may not reflect daily or seasonal fluctuations.

No polychaetes were found in the lucustrine zones of Keno and J.C. Boyle reservoirs. This sediment was primarily mud and organic material with very low dissolved oxygen levels. Polychaetes were not detected in the bank edge-water areas of either reservoir. This may be attributable to the heavy macrophytic growth along the banks, which may impede establishment.

The inability to detect infection among 100 polychaetes tested from the Keno eddy is not surprising given the results of sentinel fish exposures at that site. Although infection prevalence was high, the low mortality suggested that the infectious dose was low. Infection rates in natural populations of polychaetes are likely less than 1% (based on our previous findings in the Willamette River). Larger numbers of polychaetes from this and other locations will need to be tested to obtain a true measure of how infection varies in populations above and below the Project.

Quantitative PCR Assay

The QPCR assay is capable of detecting 1/1000th of a *C. shasta* spore in serial dilution trials, which indicates that each spore must have greater than 1000 copies of 18S rDNA. Thus, although river water contained compounds that could inhibit the assay, the QPCR is sufficiently sensitive to detect very low numbers of parasites. It should be noted here that this assay does not differentiate between the myxospore of *C. shasta* released from fish and the actinospore stage released from the polychaete.

Spatial prevalence

The majority of the 19 sites sampled along the Klamath River tested positive for *C. shasta* (Table 3). The number of amplification cycles necessary for detecting signal (Ct value) was generally consistent between the 2 x 1L sub-samples from each site. Parasite levels fluctuated along the river mainstem. All mainstem sites, with the exception of Keno Reservoir, were positive. Only 1 of the 2 sub-samples from Upper Klamath Lake was positive and the Klamath River upstream of Spencer Creek showed very low parasite levels. The “hottest” site was the Klamath mainstem upstream of Beaver Creek, closely followed by Williamson River. Comparison of the Ct of these sites with that of known starting numbers

of spores (processed similarly) indicates that these river samples contained in excess of 10 spores per litre.

Two tributaries, Williamson River and Hunter Creek, contributed parasites to the main river. Trinity River had very low numbers of parasite present (only 1 of the 2 samples from Trinity River had a detectable Ct value) and both Spencer Creek and Salmon River were negative for *C. shasta*.

Temporal prevalence

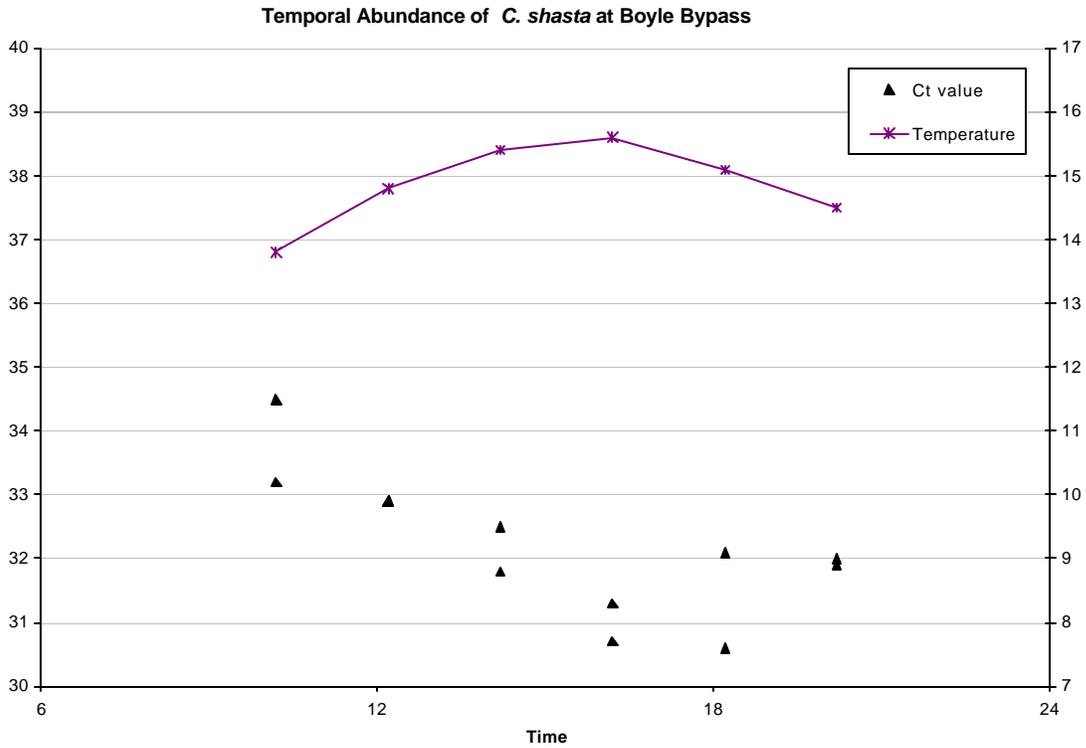
Six water samples were taken 2-hourly over a 10 hour period from 10:10 until 20:10 on a single day at J.C. Boyle bypass reach sampling site. A temporal pattern was evident, even over this partial time period (Figure 5, top). Spore quantity increased by over 10 fold over the 6 hour period from 10:10 until 16:10, then increased until 20:10. This directly corresponded with water temperature which also increased from start of sampling (13.8°C) until 16:10 (15.6°C) then decreased thereafter. The highest spore count corresponded with the highest temperature recorded as well as with the first distinct drop in river water level (~30cm) at 16:10. Although the water level continued to drop by another ~30cm over the next 2 hours, spore abundance began to decline. Flow in the J.C. Boyle bypass reach is controlled by Project operations and is typically stable (combination of FERC minimum flow requirement and spring accretion). Since this sampling site was directly above the J.C. Boyle powerhouse, the water temperature and flow fluctuations noted are probably the result of a small “backwater” effect caused by the powerhouse discharge from J.C. Boyle Reservoir.

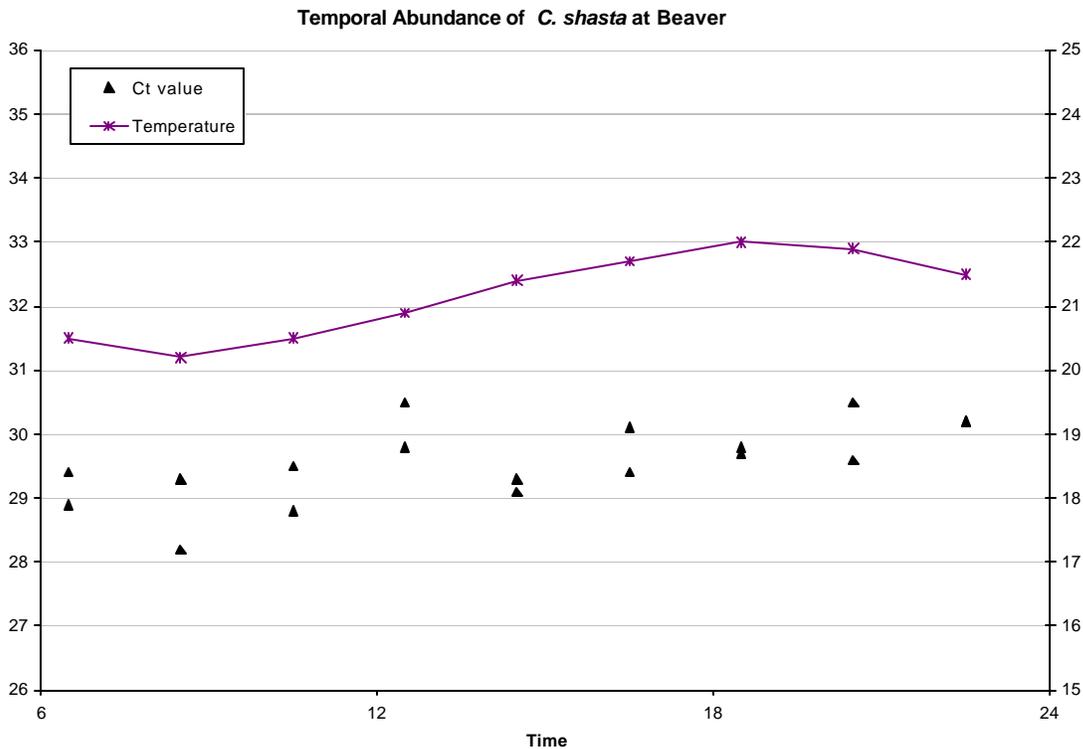
In contrast, the 9 samples taken at 2-hour intervals over a 16 hour period from 06:30 until 22.30 upstream of Beaver Creek presented no temporal relationship and exhibited less variation in abundance (<10 fold difference) (Figure 5, bottom). The temperature difference recorded over the sampling period was equivalent for the two collection sites (1.8°C).

Table 3. Relative abundance of *Ceratomyxa shasta* at 19 sites along the Klamath River and its tributaries (*). Ct values are inversely proportional to spore prevalence. Sites are listed upstream to downstream. Two 1L samples were taken at each location. UD = undetermined i.e. no Ct value registered within 50 cycles. Numbers in parentheses indicate re-purified value for samples that showed inhibition.

Sample site	Ct value 1L A	Ct value 1L B
*Williamson River	30.8	30.7
Upper Klamath Lake	39.0	UD
Link River	37.4	41.0
Keno Reservoir	UD	UD
Keno Reach (above eddy)	37.7 (34.4)	36.5
Keno Reach (eddy)	38.6 (35.1)	38.8
John Boyle Reservoir	48.6 (38)	43.1
John Boyle Bypass Reach	32.5	33.3
Upstream of Spencer Creek	48.8 (41.7)	47.6
*Spencer Creek	UD (UD)	UD
Copco Reach	39.2	38.4
Irongate Reservoir	39.4	37.8
Upstream of Beaver Creek	29.6	29.2
Upstream of Salmon River (Green Riffle)	31.9	33.0
*Salmon River	UD	UD
Upstream of Trinity River (Big Bar)	34.0	33.2
*Trinity River	45.0	UD
Upstream of Turwar Creek	35.7	36.6
*Hunter Creek	35.7	34.2

Figure 6. Graphs showing Ct value (inversely proportional to spore prevalence) and temperature plotted for each sample collected over a 10 hour period at J.C. Boyle bypass reach (top) and over a 16 h period upstream of Beaver Creek (bottom).





General Conclusions: QPCR

Spatial abundance: The QPCR results indicate that *C. shasta* is present throughout the Klamath River, but its abundance fluctuates. This is consistent with sentinel fish studies conducted in 2003 (Appendix A) and the preliminary results of the sentinel exposures conducted simultaneously with water sample collection in 2004 (data not complete). Results of the QPCR indicate that a large spore load is contributed near the source of the Klamath River, the Williamson River. This site registered the second highest prevalence of spores of the 19 sites. This initial high input (potentially >10 spores/L) is apparently diluted in the large Upper Klamath Lake as indicated by the low parasite numbers in our sample. One hundred river miles downstream, the site upstream of Beaver Creek registered the highest parasite load (>10 spores/L), indicating this is downstream of another major source of spores. Parasite levels in the mainstem of the lower Klamath River were consistently higher than any locations in the upper Klamath River, with the exception of the Williamson River and J.C. Boyle bypass reach sites. In the mainstem Klamath River, the highest Ct levels, correlating to lowest parasite levels, were from the reservoir samples. This correlates well with data from the 2003 sentinel exposures, particularly the inability to detect parasites by QPCR from Keno Reservoir, where we were similarly unable to infect fish.

Absence of parasites in the Spencer Creek and Salmon River tributaries was expected, as establishment of the life cycle has as yet only been demonstrated in the mainstem Klamath River (Hendrickson et al. 1989). However, two tributaries, the Trinity River and Hunter Creek, both showed parasite presence. In the Trinity River parasite levels in the sample were very low, and one of the replicate samples was negative. However samples from Hunter Creek showed a

parasite load intermediate to those found in the lower versus upper Klamath River. There are two explanations for this: one, that the parasite life cycle is established in Hunter Creek; two, that parasite detection is a result of myxospores released from infected fish rather than the stage released from the polychaete. Researchers expect to resolve this question when results of the 2004 sentinel survey are complete.

Parasite abundance at J.C. Boyle bypass reach directly corresponded to water temperature; the highest number of spores was recorded when water temperature was highest. However, there was no overall temperature/spore correlation at the Beaver Creek site. The water temperature in the mainstem site upstream of Beaver Creek was 6.4-8.2° higher, but the daily range was equivalent (1.8°). The only other obvious difference between the two locations was water level. This varied considerably (~1m) at the first site but was constant at Beaver Creek. The relationship between water level and parasite abundance at the J.C. Boyle bypass reach site probably relates to the source of water in the bypass reach. When water levels were high, J.C. Boyle powerhouse was operating and water from J.C. Boyle reservoir (lower parasite abundance) dominated this site. As water levels decreased with decreasing powerhouse operations, the bypass reach water (higher parasite abundance) became more dominant. Because of the “backwater effect” in the lower bypass site, flow levels may have a local impact on parasite density.

Conclusions to Date

In general, infections by *C. shasta* were less severe in fish held at sites above Iron Gate dam than at the control site located below the Project near the confluence of Beaver Creek. Based on results from the Beaver Creek site, for which there is multiple year sentinel exposure records for comparison (Foott et al. 2002, 2003, 2004), sentinel fish exposed at all sites prior to July received a low level of *C. shasta* exposure. This is supported by the predominance of sub-clinical infections in the exposure groups. Because parasite levels are typically high during April through June, it is difficult to draw conclusions about Project impacts during this period. However, with the exception of the fish held in the J.C. Boyle bypass reach in July, infectivity remained low at all upriver sites despite severe infections at the Beaver Creek site in July through November. Whether these results would be consistent on an annual basis is unknown. Pathogen surveys are generally conducted over several field seasons to obtain data that would reflect the effects of variable temperature and flow patterns.

Data from polychaete habitat surveys indicate that habitat is available in areas of the Project, primarily in free-flowing stretches of the river and riverine segments of the reservoirs. Given that polychaetes are abundant both above and below the Project it may be a combination of habitat and infection rate in the polychaete populations that ultimately dictates infection levels in the fish.

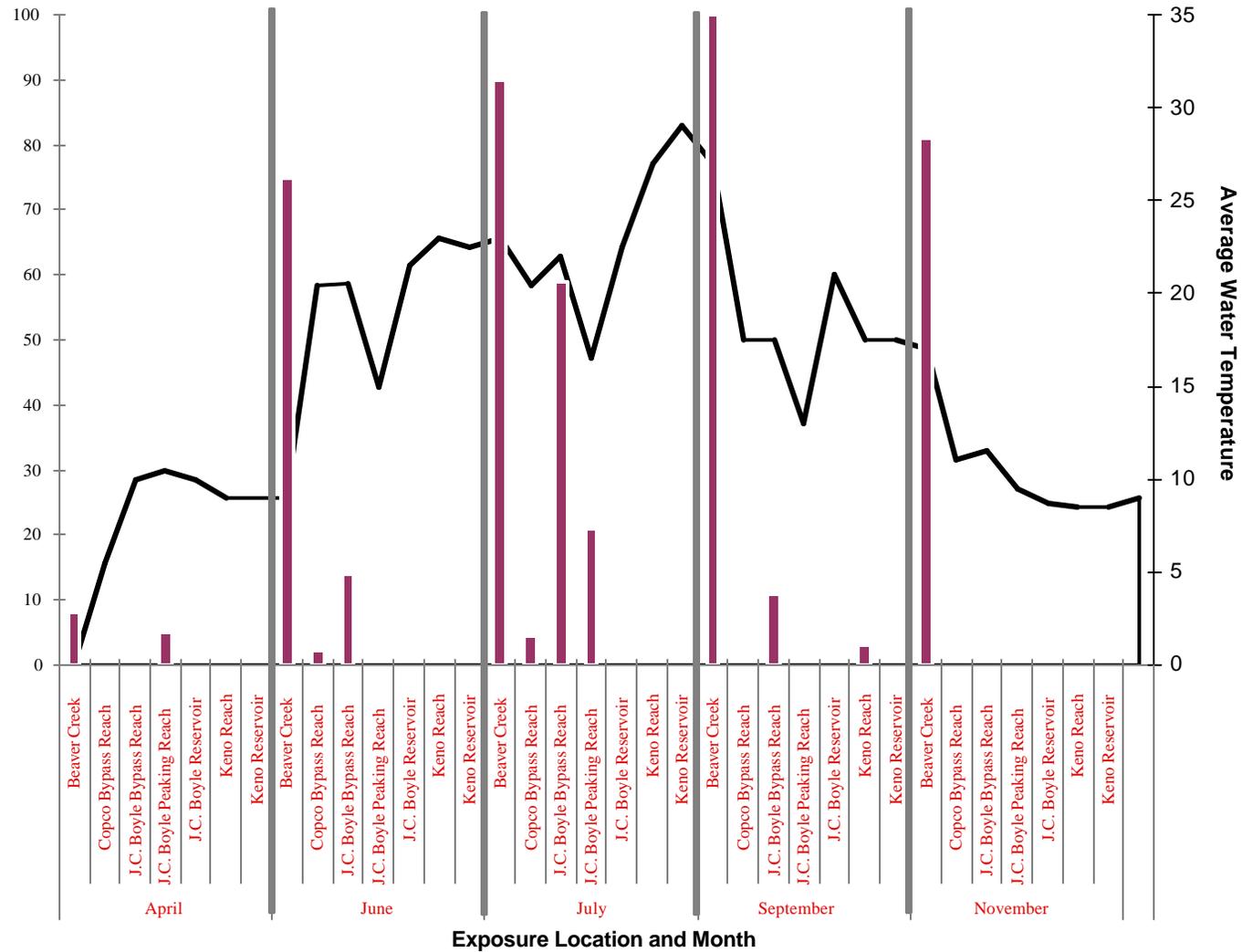
Results of the quantitative PCR assay support the findings above, demonstrating a greater abundance of parasites in samples collected below Iron Gate dam. Although researchers are not yet able to draw conclusions about infectivity at upriver sites based on sentinel fish data, the findings suggest that

collection of water samples may allow a rapid means of assessing infectivity both spatially and temporally. This could be an invaluable tool in monitoring the potential effects of flow management.

REFERENCES

- Aquatic Resources Center. 1999. Standard Operating Procedures: Aquatic oligochaete sampling and processing, including habitat assessments. Aquatic Resources Center, College Grove, TN
- Bartholomew, J. L., M. J. Whipple, D. G. Stevens and J. L. Fryer. 1997. The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. *American Journal of Parasitology*. 83:859-868.
- Foott, J. S., T. Martinez, R. Harmon, K. True, B. McCasland, C. Glase and R. Engle. 2002. FY2001 Investigational Report: Juvenile Chinook Health Monitoring in the Trinity River, Klamath River and Estuary. June-August 2001. USFWS, California-Nevada Fish Health Center, Anderson, CA.
- Foott, J. S., R. Harmon and R. Stone. 2003. FY2002 Investigational Report: Ceratomyxosis resistance in juvenile Chinook salmon and steelhead trout from the Klamath River. USFWS, California-Nevada Fish Health Center, Anderson, CA.
- Foott, J. S., R. Harmon and R. Stone. 2004. FY2003 Investigational Report: Abundance of *Ceratomyxa shasta* in Iron Gate and Copco reservoirs. USFWS, California-Nevada Fish Health Center, Anderson, CA.
- Hendrickson, G. L., A. Carlton and D. Manzer. 1989. Geographic and seasonal distribution of the infective stage of *Ceratomyxa shasta* (Myxozoa) in Northern California. *Diseases of Aquatic Organisms* 7:165-169.
- PacifiCorp. 2004. Final License Application, Klamath Hydroelectric Project (FERC No. 2082). PacifiCorp, Portland, Oregon. February 2004

Appendix A. Summary of *Ceratomyxa shasta* mortality for all sites where exposures were conducted in April, June, July, September and November. Average water temperature at each site for that exposure period is graphed.



Appendix B. Habitat: Contains data for sites in J.C. Boyle Reservoir and Keno reach where samples were collected for detection of *Manayunkia speciosa*.

Site	Depth (m)	Substrate type	Polychaete: total Annelid Ratio	Material collected	Dry Weight (gm)	Number Polychaetes	Est. Poly/ (g) material
Boyles Res	7.32	Rock	0.22	Diatom matrix	0.10	8	79
Boyles Res		Mud	0.00	Diatom matrix	0.51	0	0
Boyles Res		Mud	0.00	Diatom matrix	0.17	0	0
Boyles Res	6.76	Mud	0.00	Diatom matrix	0.32	0	0
Boyles Res	7.87	Mud	0.00	Diatom matrix	0.21	0	0
Boyles Res	9.37	Mud	0.00	Diatom matrix	0.40	0	0
Boyles Res	6.38	Mud	1.26	Diatom matrix	0.04	24	609
Boyles Res	8.19	Mud	0.42	Diatom matrix	0.05	11	223
Boyles Res	5.36	Rock	0.04	Sponge	0.17	3	18
Boyles Res	4.98	Rock	0.02	Sponge	0.40	2	5
Boyles Res	6.30	Rock	11.23	Sponge	0.05	146	3054
Boyles Res	5.06	Rock	1.29	Sponge	0.15	22	146
Boyles Res		Rock	1.00	Sponge	0.16	16	103
Boyles Res	6.12	Rock	0.81	Coarse organics	0.13	22	168
Boyles Res		Mud	0.00	Coarse organics	0.16	0	0
Boyles Res		Mud	0.00	Coarse organics	0.06	0	0
Boyles Res	6.35	Mud	0.00	Coarse organics	0.02	0	0
Boyles Res	6.96	Mud	0.83	Coarse organics	0.43	25	58
Boyles Res	8.41	Mud	0.03	Coarse organics	0.16	1	6
Boyles Res	5.97	Mud	0.00	Coarse organics	0.21	0	0
Boyles Res	9.37	Mud	0.05	Coarse organics	0.33	3	9
Boyles Res	7.32	Mud	0.00	Coarse organics	0.18	0	0
Boyles Res	7.32	Mud	0.00	Coarse organics	0.45	0	0
Boyles Res	6.76	Mud	0.00	Mud	0.16	0	0
Keno eddy	0.75	Bld	5.29	Cladophora	0.06	74	1140
Keno eddy	1.10	Cob	1.84	Cladophora	0.30	256	849
Keno eddy	1.25	Cob	2.48	Cladophora	0.10	99	983
Keno eddy	0.75	Cob	2.18	Cladophora	0.13	131	1030
Keno eddy	0.75	Bld	0.20	Cladophora	0.20	2	10
Keno eddy	1.00	Bld	1.13	Cladophora	0.19	69	357
Keno eddy	1.10	Bld	3.42	Cladophora	0.14	164	1153
Keno eddy	0.60	Bld	0.82	Cladophora	0.18	60	342
Keno eddy	1.10	Bld	1.66	Cladophora	0.23	146	647
Keno eddy	0.50	Bld	2.15	Cladophora	0.19	103	538
Keno eddy	1.00	Bld	1.79	Cladophora	0.14	50	362
Keno eddy	0.80	Bld	2.22	Diatom matrix	0.61	180	297
Keno eddy	1.10	Bld	3.50	Diatom matrix	0.41	318	778
Keno eddy	1.10	Bld	10.14	Diatom matrix	0.09	71	771
Keno eddy	1.25	Cob	4.42	Flocculent diatoms	0.20	115	584
Keno eddy	2.00	Bld	9.88	Flocculent diatoms	0.16	168	1054
Keno eddy	0.85	Bld	7.05	Flocculent diatoms	0.12	148	1227
Keno eddy	1.15	Bld	4.03	Flocculent diatoms	0.11	117	1056
Keno eddy	1.25	Bld	2.61	Flocculent diatoms	0.18	120	686
Keno eddy	0.75	Cob	0.00	Coarse organics	0.24	0	0