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

8.1 DESCRIPTION AND PURPOSE

This section presents the findings of an investigation of *Ceratomyxa shasta (C. shasta)*, and its polychaete host *Manayunkia speciosa (M. speciosa)*. Temporal and spatial distribution and concentrations in the Klamath River from Keno reservoir to the confluence of Beaver Creek were addressed. 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*.

Previous studies on the distribution of *C. shasta* (Hendrickson et al. 1989; Buchanan et al. 1989) demonstrated its presence throughout the Klamath Basin (Table 8.1-1). More recent studies monitoring the prevalence of selected fish pathogens in smolts sampled during outmigration implicated *C. shasta* directly as a cause of extensive losses in juvenile Chinook salmon (*Oncorhynchus tshawytscha*)(Foott et al. 1999, 2002, 2003; Williamson and Foott, 1998). Subsequently, sentinel studies demonstrated infection rates of 100 percent in juvenile Chinook salmon exposed for 4 days in the mainstem upper Klamath River near Beaver Creek, with associated mortality rates exceeding 83 percent within 17 days of exposure (Foott et al. 2003). Understanding the distribution of the alternate host species, its related habitat, and the temporal variance in abundance of the infectious stage of *C. shasta* (actinosporean) may provide practical management information to enhance outmigrant survival and increase overall production of salmonids in the basin. Currently it is not known what role, if any, the Project has on the distribution and abundance of *C. shasta* or its intermediate host.

Table 8.1-1. Distribution of the infectious stage of *C. shasta* in the Klamath River

Exposure Location	River Mile	Result
Upper Klamath Lake Tributaries		
Lower Williamson River	**NA**	Positive[2]
Sprague River	**NA**	Positive[2]
Mainstem Klamath River		
Klamath Glen	7	Positive[1]
Weitchpec	43.5	Positive[1]
Above Salmon River	71	Positive[1]
Beaver Creek	161	Positive[1]
Above Scott Creek	144	Positive[1]
Iron Gate Fish Hatchery	189.5	Positive[1]
Copco Lake	202	Positive[1]
Klamath Lake	~254	Positive[1]
Klamath River Tributaries		
Bogus Creek	190.4	Negative[1]
Shasta River	177	Negative[1]
Humbug Creek	172	Negative[1]

Table 8.1-1. Distribution of the infectious stage of *C. shasta* in the Klamath River

Exposure Location	River Mile	Result
Scott Creek	143	Negative[1]
Salmon River	66	Negative[1]
Trinity River	44	Negative[1]

[1] Hendrickson et al. 1989

[2] Buchanan et al. 1989

C. shasta, like most myxozoan parasites, has a complex life cycle that requires development in an alternate host. Although the annelid host for *C. shasta* was identified as being the freshwater polychaete *M. speciosa* (Bartholomew et al. 1997), information in the literature concerning the habitat requirements of this organism is limited. Most studies on the distribution of *C. shasta* were conducted prior to the determination of the life cycle, but each concluded that the infectious stage of the parasite has a variable distribution consistent with the hypothesis of an intermediate host with specific ecological requirements (Ratliff 1983; Bartholomew et al. 1989; Hendrickson et al. 1989). These habitat requirements influence both the distribution and abundance of the parasite and consequently the presence and severity of infection in fish. Detailing potential Project-related impacts on the distribution of this host species can only be determined by understanding the habitat preferences and water quality requirements for this species. Observational data from other systems have associated the polychaete with different habitat types. For example, in the Willamette River polychaete habitat was characterized by dense periphyton on rocks and freshwater mussels in both the mainstem river and backwaters (Bartholomew, unpublished observations). In the Cowlitz River in Washington State, the polychaetes have been identified as directly inhabiting fine sediments (Elysa Ray, WDFW, pers. comm., June 5, 2003). However, the relationship between these habitats is not understood and there have been no studies directed at identifying polychaete habitat requirements.

Research has been performed to determine the infectivity and related mortality of the infectious stage (actinosporean) of *C. shasta* in relation to water quality parameters, most notably temperature (Udey et al. 1975; Foott, 2003). By holding naturally exposed fish at a range of temperatures following exposure, Udey et al. (1975) demonstrated that as water temperatures increased from 39˚F (3.9˚C) to 74˚F (23.3˚C), percent mortality increased and mean time to death decreased. This suggests that once the fish is infected, high water temperatures will increase the severity of the infection. There is less known about the relationship between temperature and the alternate host or life stages that exist outside the fish. Foott et al. (2003) compared mortality among Chinook salmon exposed at Beaver Creek during June and July, 2002, and found that as water temperatures increased, the infection prevalence decreased. Although the data is limited, this suggests a theory of a bimodal infectivity period occurring in spring and fall in the Klamath River (Foott, 2003). This bimodal period of infectivity may be related to effects of high water temperature compromising the infection ability of the actinospore as stated above, or may be related directly to water quality effects on *M. speciosa* preventing the maturation and release of the infectious spore.

8.2 OBJECTIVES

There were two main objectives of the study that relate to *C. shasta* and how the Project is associated with the parasite: One aspect of the study related to *C. shasta*, the parasite itself. The second involved determining basic habitat requirements of the intermediate host *M. speciosa* and how that habitat relates to the Project.

A general limitation of this work revolved around the known temporal variance of *C. shasta* infection rates that exists by season and from year to year. The data collected and related interpretations provided a summary based on specific conditions during the sampling period. Potential areas of infection exist through the Lower Klamath River Basin, as indicated in Table 8.1-1. 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 infectious spore occur in the river downstream of the proposed geographic scope of this work.

The study was designed to address the following:

- Relative infection rates at selected river /reservoir locations, including identification of "hot spots" of infection
- Relative abundance of the infectious actinospore at selected river/reservoir locations
- Habitat preference/abundance by habitat of *M. speciosa*

8.3 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.

8.4 METHODS AND GEOGRAPHIC SCOPE

The geographic scope of this study included the Klamath River from Keno reservoir to the confluence of Beaver Creek. The study began in April 2003 and data were collected through July 2003. Table 8.4-1 lists and describes the river reaches and reservoirs encompassed in this study. Sentinel study sites also are indicated in Table 8.4-1 and in Figure 8.4-1; habitat sampling locations are shown in Figure 8.4-2 (6 pages). Keno reservoir represents the upper bounds of the study area and study sites extend downstream to the confluence of Beaver Creek, which is a positive control site for presence of *C. shasta*.

Table 8.4-1. Description of study area and of the locations for sentinel fish exposures for the detection of *Ceratomyxa shasta* in the Klamath River.

River Area	Task	Description	Sentinel Site Location
Keno Reservoir	OSU	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	OSU	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 off-channel pool below riffle at Sportsman's Club
J.C. Boyle Reservoir	OSU	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	OSU	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	OSU	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 (surface and bottom)	USFWS	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	OSU	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 2 Village
Iron Gate reservoir cove	USFWS	Iron Gate reservoir is similar to Copco reservoir in that it is a deep and relatively steep canyon. It extends from the Copco No. 2 powerhouse at RM 196.9 to Iron Gate dam at RM 190.1	RM 195 South shore at 25 ft depth
Iron Gate reservoir (surface and bottom)	USFWS	Iron Gate reservoir ends at Iron Gate dam (RM 190.1)	RM 190.2 Exposures cages held at surface and at 50 ft depth
Klamath River above Iron Gate fish hatchery	USFWS	Iron Gate fish hatchery is located directly downstream of Iron Gate dam (RM 190.1)	RM 189.5 Upstream of Iron Gate fish hatchery
Klamath River at Beaver Creek	USWFS	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

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Figure

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8.4.1 Methods

Data collected for this study incorporated three main investigative techniques: fish live box exposures (sentinel studies), analysis of water, and substrate sampling. The initial phase of this study was a collaborative effort between Oregon State University (OSU) and the U.S. Fish and Wildlife Service (USFWS). The latter agency conducted sentinel exposures at locations in Copco reservoir, Iron Gate reservoir, above Iron Gate fish hatchery, and at Beaver Creek during the period from May through July 2003. OSU conducted sentinel exposures at locations in Keno reservoir, Keno reach, J.C. Boyle reservoir and the downstream peaking and bypass reaches, and at the inflow of Iron Gate Reservoir during that period. All research methods for this portion of the study were developed collaboratively to enable comparison of the resulting data. The USFWS performed histological analysis on a subsample of all exposure groups and OSU performed the molecular diagnostics for all fish sampled.

Chinook salmon were acquired from the Iron Gate hatchery, while the Oregon Department of Fish and Wildlife (ODFW) supplied rainbow trout for use in this study. Approval for the use of these fish was granted by the California Department of Fish and Game (CDFG) and the ODFW.

Responsibilities of OSU also included the collection and analysis of sediment and water samples.

8.4.1.1 Sentinel Studies

Protocols for sentinel studies are well established (Bartholomew, 1998; Udey et al. 1975) and data provided by these exposures allowed comparison of relative infectivity through this section of the river and aided in identifying habitats that support the alternate host. However, because the longevity of the actinospore stage is unknown, it was not possible to determine where the parasite originated.

Live cages were placed at each of the exposure locations and sentinel fish were held at each site for 4 days (Table 8.4-2, 8.4-3; Figure 8.4-3). The July exposure was 3 days because of elevated water temperatures. For each exposure, 50 rainbow trout of a stock known to be susceptible to *C. shasta* were held to determine parasite presence. During the June and July sample period, an additional cage was placed at each site for exposure of 50 juvenile Chinook salmon from Iron Gate fish hatchery. The purpose of using this species was to determine if *C. shasta* levels are sufficient to cause infection and to determine the relative parasite dose required to cause disease in both stocks. Water temperatures encountered during the exposure period were monitored by thermistors attached to one live box at each site.

Table 8.4-2 Dates sentinel exposures were conducted and experimental species exposed.

Sentinel Exposure Dates	Species Exposed
April 24 – 28, 2003	Rainbow trout
June 5 – 9, 2003	Rainbow trout, Chinook salmon
July 21 – 24, 2003	Rainbow trout, Chinook salmon

After 4 days, the fish were transported to holding facilities at either the USFWS laboratory or the Salmon Disease Laboratory at OSU. Unexposed control fish of each species were held at each facility and treated identically 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. In both laboratories, fish were maintained at 16˚C to speed the progress of infection, during which time they were fed daily and observed for clinical disease signs. At 10-11 days, 10 fish were sampled from each exposure group. Samples of the intestinal tract were removed and processed for PCR (Polymerase Chain Reaction) analysis (Palenzuela and Bartholomew, 2002). At 18-20 days, 15 fish were collected from each exposure group and sampled as above for PCR. In addition, intestinal tissue from five fish was sampled for histological analysis. For the USFWS exposures, 18 days was the final sample period and all fish were euthanized. For OSU exposure sites the remaining fish were held up to 90 days post-exposure to assess mortality. In both laboratories, any fish that died during holding was necropsied and visually examined. If *C. shasta* spores were not identified, samples of intestinal tract were examined by PCR assay. Procedures for examining control fish were identical.

8.4.1.2 Analysis of Substrate Samples to Identify Polychaete Habitat

The objective of this task is to identify habitats most commonly utilized by the polychaete *M. speciosa*. Assay of samples using the Q-PCR (to be done early in 2004) will provide information on relative numbers of parasites in polychaetes from different habitats.

Substrate samples for the identification of polychaete habitat were collected using semi-quantitative methods for aquatic oligochaete sampling (Aquatic Resources Center, 1999). Because *M. speciosa* has been observed both on rocks and in fine sediment, a variety of sampling methods were tested. Shallow, wadable areas were sampled using a combination of kicknet, with manual collection of larger substrate; nonwadable habitats were sampled using a Ponar sampler from a boat or off the face of the dams.

Determination of sampling locations was made using existing data on habitat mapping and included existing sentinel site locations. Sites selected were highly influenced by access, but an attempt was made to include all habitats, with multiple samples collected per substrate type. 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, DO, pH, conductivity, total dissolved solids [TDS]) and habitat type were recorded for each sample. Sampling of substrates in free-flowing reaches of the river necessarily differed from sampling of the reservoir. An attempt was made to sample both longitudinally and across the river when possible. For reservoir sampling, a boat and Ponar grab were used for sampling depths, but sampling was also conducted in wadable areas with representative sites above and below minimum lake elevations.

Material collected from each sampling effort was placed in a plastic Ziploc™ bags, labeled, and placed on ice in a cooler. Samples were transported to the laboratory and individually sorted using a dissecting microscope, if possible within 24 hours. Standard measures for particle size distribution were performed to classify each sample. All *M. speciosa* were removed from the sample, counted, and preserved for PCR analysis.

8.5 RELATIONSHIP TO REGULATORY REQUIREMENTS AND PLANS

This investigation is intended to provide information on *C. shasta* that, together with environmental data and results of other past and ongoing studies, can be used to assess effects of Project operations on fish resources and to help formulate recommendations for protection, mitigation, and enhancement measures consistent with agency and tribal management goals.

8.6 TECHNICAL WORK GROUP COLLABORATION

PacifiCorp worked with stakeholders to establish a collaborative process for planning and conducting studies needed to support Project relicensing documentation. Beginning in early 2002 the stakeholders and PacifiCorp developed a Process Protocol to guide the collaborative effort. The structure is comprised of a Plenary group (all interested stakeholders) and a number of technical working groups. As part of this structure, an Aquatics Work Group (AWG) was established to address most of the fisheries studies. The AWG added specific sampling sites and fall sampling before approving this study plan.

8.7 RESULTS AND DISCUSSION

8.7.1 Sentinel Study

Data on infection prevalence, as determined by 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 Table 8.7-1 (See Figure 8.4-1 for sentinel fish locations.). Details for each exposure period are presented below. Additional data on the PCR infection prevalence among fish exposed at the OSU sites for each sample period are presented in the PCR appendix (Appendix 8A). Sentinel fish from USFWS exposure locations were held for 18 days following exposure; therefore, mortality data as a result of *C. shasta* infection are not available for these sites. Infection prevalence in these groups was determined by PCR assay of a subsample of the fish that survived to 18 days.

8.7.1.1 April Exposure

Rainbow trout were recovered from all OSU exposure sites. At termination (70 days), *C. shasta* was detected by PCR in fish from the following exposure sites: Keno reach (100 percent), J.C. Boyle reservoir (67 percent), J.C. Boyle bypass reach (53 percent), J.C. Boyle peaking reach (60 percent) and Copco bypass reach (33 percent). Infection was not detected from fish held in Keno reservoir. There was a single mortality during the 70-day holding period, from the Shovel Creek site (J.C. Boyle peaking reach), and this fish was microscopically positive for *C. shasta* spores.

From the USFWS sites, fish were recovered from all sites where fish were exposed, although mortality was high in the group exposed at the Copco reservoir site. At 18 days post-exposure, all groups were terminated and samples taken for PCR and histology to detect infection. Infection prevalence (at 18 days) by PCR was 25 percent (One of four fish) at Beaver Creek and no infections were detected at other sites. Histology confirmed infection at Beaver Creek in one of ten fish examined.

8.7.1.2 June Exposure

For the OSU exposure sites, rainbow trout held at the Keno reservoir site were lost as a result of columnaris infection, but fish were retrieved from all other sites. Infection prevalence, as determined by PCR, from fish sampled at termination demonstrated infected rainbow trout from all sites from which they were recovered. Infection prevalence at J.C. Boyle reservoir was 60 percent, and 100 percent for all other sites. Mortality caused by *C. shasta* in rainbow trout exposure groups was low and occurred only from groups held at the J.C. Boyle bypass reach (5 percent) and Copco bypass reach (1.5 percent). Infection was not detected by PCR in Chinook salmon held at any site and clinical disease was not evident in any group.

Among the USFWS exposure groups, recovery following exposure was low among both rainbow trout and Chinook salmon exposed at Beaver Creek. There were insufficient numbers of rainbow trout remaining to determine infection prevalence at 18 days, but *C. shasta* was detected in one of five remaining Chinook salmon at 11 days post-exposure. Infection (at 18 days) was not detected either by PCR or histology from fish exposed at any other site.

8.7.1.3 July Exposure

Because of lethal water temperatures (greater than 27˚C), fish were not placed in J.C. Boyle reservoir, Keno reservoir and Keno reach. Fish were recovered from the remaining sites, although only rainbow trout survived in the J.C. Boyle peaking reach location (Shovel Creek). Chinook salmon at that site were lost as a result of vandalism. Mortality was high in both species held in the Copco bypass reach because of the elevated water temperature (22˚C). As a result of the low recovery, samples for PCR and histology were not collected 10 days post-exposure. Mortality caused by *C. shasta* occurred in rainbow trout held at sites in the J.C. Boyle bypass reach (59 percent), the J.C. Boyle peaking reach (21 percent) and the Copco bypass reach (4.5 percent). Infection was not detected by PCR in Chinook salmon held at any site and clinical disease was not evident by group.

Among the USFWS exposure groups, mortality was high in rainbow trout held at Beaver Creek, above Iron Gate fish hatchery, and in Copco reservoir (bottom). At 18 days, the infection prevalence among the surviving fish from the Beaver Creek site was 100 percent, 67 percent from above Iron Gate fish hatchery, and no infection was detected at the remaining sites.

8.7.1.4 General Conclusions

The only sites where the exposure groups suffered mortality by *C. shasta* were the J.C. Boyle bypass and peaking reaches and the Copco bypass reach. The parasite was detected from fish held in Keno reach and J.C. Boyle reservoir during April and June using PCR, but high water temperatures at these sites precluded holding fish at these locations during July, when infection levels were highest. The only site from which infection was not detected was Keno reservoir, and here the only exposure conducted successfully was in April.

Although *C. shasta* was detected using molecular techniques from most upriver sites (OSU sites), the low level of mortality in these groups suggests that parasite levels were low throughout the project area during the April and June exposures. This was unexpected as the high prevalence of infection detected by PCR is generally accompanied by high mortality in

susceptible fish. 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. In the Klamath and other enzootic rivers, lethal exposure to *C. shasta* generally occurs as early as April, but we were similarly unable to infect fish in the Willamette River until late June.

Table 8.7-1. Results of sentinel exposure of rainbow trout at sites in the Klamath River tested by Oregon State University. (See also Appendix 8A.)

Exposure Location	Exposure	Average Water Temperature	Infection Prevalence (PCR)[1]	% Mortality with *C. shasta*
Keno Reservoir	April	9	0 (n=15)	0
	June[2]	23	--	-
	July[3]	27	--	-
Keno Reach	April	9	100 (n=15)	0
	June	22.5	100 (n=19)	0
	July[3]	29	--	--
J.C. Boyle Reservoir	April	9	67 (n=12)	0
	June	23	100 (n=17)	0
	July[3]	27	--	--
J.C. Boyle Bypass Reach	April	10.5	53 (n=15)	0
	June	15	100 (n=11)	5
	July	16.5	100 (n=4)	59
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
Copco Reservoir (surface)	April	10.0	ND	ND
	June	21.6	0 (n=7)	ND
	July[3]	21.8	--	--
Copco Reservoir (bottom)	April[3]	ND	--	--
	June	ND	0 (n=7)	ND
	July	ND	0 (n=5)	ND
Copco Bypass Reach	April	10	33 (n=15)	0
	June	20.5	100 (n=25)	1.5
	July	22	100 (n=10)	4.5
Iron Gate Reservoir (cove)	April	12.5	ND	ND
	June	20.5	0 (n=12)	ND
	July	20.8	0 (n=16)	ND
Iron Gate Reservoir (surface)	April	10.7	0 (n=5)	ND
	June	20.5	0 (n=9)	ND
	July[3]	21.2	--	--
Iron Gate Reservoir (bottom)	April	ND	0 (n=4)	ND
	June	20.5	0 (n=9)	ND
	July	ND	0 (n=2)	ND

Table 8.7-1. Results of sentinel exposure of rainbow trout at sites in the Klamath River tested by Oregon State University. (See also Appendix 8A.)

Exposure Location	Exposure	Average Water Temperature	Infection Prevalence (PCR)[1]	% Mortality with *C. shasta*
Iron Gate Hatchery (Upstream)	April	10.5	0 (n=1)	ND
	June	19.2	0 (n=5)	ND
	July	22.2	67 (n=6)	ND
Beaver Creek	April	5.5	25 (n=4)	ND
	June	20.4	ND	ND
	July	ND	100 (n=4)	ND

[1] Infection prevalence at termination of study: 70 days for OSU sites; 18 days for USFWS sites.

[2] Fish lost because of columnaris infection during exposure.

[3] No exposure conducted.

ND—Exposure conducted but no data for this parameter.

Results of studies by the USFWS in the lower river during previous years also indicate that infection was delayed this year and levels appear to be decreased. From the lower river sites, infections were detected only by PCR and histology. This was likely a result of the short holding period and the slow development of the infection. The inability to hold fish exposed at the USFWS sites until development of clinical disease makes it difficult to compare the prevalence and severity of ceratomyxosis between exposure groups from the upper and lower river. Similarly, PCR data from fish held 70 days post-exposure (OSU sites) is difficult to compare with data from fish held 18 days (USFWS sites). Exposures at the sites administered by the USFWS were also complicated by lethal water temperatures and columnaris infection. However, the only lower river sites (USFWS) where infection was detected was at Beaver Creek (April, July) and Iron Gate fish hatchery (July); infection was not detected from fish held at any sites in Copco or Iron Gate reservoirs.

8.7.2 Polychaete Habitat Studies

Eighty-eight samples were collected during three sampling efforts in early and late July and late August. Locations of sample sites are shown in Figure 8.4-2. In the reservoirs, samples were collected by boat using a ponar grab, and in the free-flowing reaches samples were collected by kicknet where access permitted. Sampling of the lower river sites was conducted during the last week of July. The efficiency of sampling by kicknet was assessed by collecting samples manually from the same site. Manual collection yielded higher numbers of polychaetes because it included cobble-sized rocks with algal growth, which appears to be a habitat for the worm.

Polychaetes were identified from 13 sites (Figure 8.4-2) with estimated abundances from 1 – 168/m^2. These data are summarized in Table 8.7-2. Detailed habitat information on each sample site is provided in the appendix (Appendix 8B). Statistical analysis has not yet been performed to determine the physical parameters correlated with polychaete presence

8.7.2.1 General Observations

Because the study is not completed it is difficult to draw firm conclusions, but we can make some general observations. Polychaetes appear to be most abundant in the riverine sections of the river and reservoirs. They have not been associated with the very fine organic silt/mud substrate, but require fine particulate organic material for construction of their living tubes. This material may be trapped in periphytic *Cladophera* 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 generally were found in slower moving water (velocity between 0.02 m/s - 0.45 m/s), particularly in eddies and pools. However, measurements of water velocity were taken only 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 and was undergoing strong methanogenesis; in addition, DO levels were very low. Polychaetes were not detected in the bank edge-water areas of either reservoir. This may be attributed to the heavy macrophytic growth along the banks, which may impede establishment.

Table 8.7-2. Summary of sampling efforts for the polychaete, *Manayunkia speciosa* in the Klamath River

River Section	No. sites/method	Detection of Polychaetes	Notes
Keno Reservoir	17/ponar 1/kicknet	None detected	Thick, dark, fine organic sediments did not appear to support polychaetes. Possible polychaete tubes were detected from riverine sections and additional sampling in these sections and along the banks may be more productive
Keno Reach	1/kicknet 2/manual	Polychaete density high	Limited access. Sample site was an off-channel area with moderate flow, cobble substrate, at depths of at least 0.5 m. Associated fauna, short dense periphytic cladophera
J.C. Boyle Reservoir	11/ponar (depth) 5/manual 5/kicknet	Polychaete density moderate at some sites	No polychaetes detected from the fine organic sediment near the dam, as in Keno Reservoir. Polychaetes detected in samples from depths of ~ 20 ft from ponar samples that scraped rocks and contained fine particulate organic matter. No polychaetes detected in samples along banks although possible polychaete tubes.
J.C. Boyle Bypass Reach	1/kicknet	None detected	Limited access. Sample site located above sentinel site.
J.C. Boyle Peaking Reach (Shovel Creek)	5/kicknet 6/manual	Polychaete density low	Only a few polychaetes collected: one from a mussel shell from a slow flow pool with silt, sand, and pebbles; one from an area with boulder/cobble substrate and attached Cladophera.
Copco Bypass Reach (Copco Bridge)	2/kicknet	Polychaete density moderate at one site	Sites where Cladophera was present on rocks yielded polychaetes.
Below Iron Gate	31/kicknet	Polychaete density moderate at some sites.	Detected in eddies, deep pools with low water flow.

8.7.3 Conclusions to Date

Prior to the July exposure, sentinel fish received a low level of exposure to *C. shasta*, as evidenced by the predominance of sub-clinical infections in the exposure groups. Because this is a period when parasite levels are typically high, it is difficult to draw conclusions about project impacts during this period. This conclusion is based on the low parasite abundance at sites lower in the river where there is multiple year sentinel exposure data for comparison. However, there is no previous data on *C. shasta* exposure levels above Iron Gate dam to compare with the current study. It is possible that the decreased exposure in April-June was a result of high water flows or low water temperatures at a time that affected the replication of the polychaete host. If so, then it is likely that our habitat survey information will similarly reflect a decreased abundance from what is normal and reflect a more limited habitat distribution.

Pathogen surveys are generally conducted over several field seasons to obtain data that would reflect the effects of variable temperature and flow patterns. For fall 2003 studies, exposures were conducted at the same study sites and in addition, fish were held at several sites used in the USFWS survey.

We have also collected samples at two sites that represent divergent habitats where polychaetes were present. To determine if the samples collected will allow us to draw any conclusions regarding the effects of project operations on the alternate host, data will be analyzed by correlating physical parameters with relative polychaete abundance. Further development of the PCR as a quantitative assay will provide some estimation of relative parasite abundance among populations of polychaetes from these different habitats.

8.7.4 Ongoing Work

During the collaborative Klamath relicensing meetings, PacifiCorp committed to including fall sampling under this study plan. Additional data (sentinel studies and polychaete sampling) were collected in the Fall 2003. The fall sampling will be analyzed and made available in early 2004.