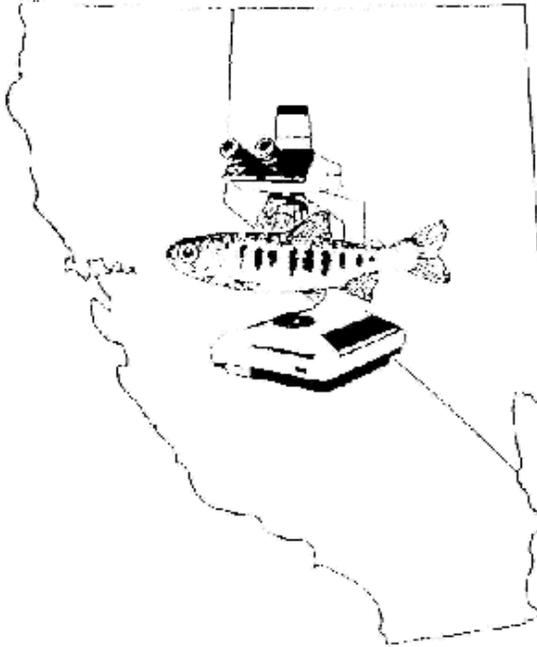


FY2003 Investigational Report:
Abundance of *Ceratomyxa shasta* in Iron Gate and Copco reservoirs.



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Summary:

Juvenile rainbow trout and Chinook salmon sentinel groups were exposed for four days at sites in the Klamath river below Iron Gate dam, Iron Gate reservoir and Copco reservoir in April through July 2003. Simultaneous sentinel exposures were also conducted by an Oregon State University group at sites along the upper Klamath river from Keno reservoir (rm 233) to Copco reservoir bypass (rm 197). This group reported infections of fish exposed in Keno reach, Boyle reservoir and bypass reaches, as well as the Copco bypass reach during April, June, and July. In our study, *Ceratomyxa shasta* was detected by Polymerase Chain Reaction (PCR) assay in only 1 of 318 samples of fish held in Iron Gate reservoir and none of the 140 samples collected from Copco reservoir exposures. In the Klamath River below Iron Gate dam, infections occurred in sentinels held near Beaver creek (rm 161) throughout the study and during late June and July at a site adjacent to Iron Gate Hatchery (rm 190). The absence of clinical signs and mortality, due to ceratomyxosis, among infected sentinel groups indicates that the infective stage was only moderately abundant in the river below Iron Gate dam during 2003. Elevated water temperatures (>20°C) during June and July were associated with high in-situ mortality of sentinel groups held in the reservoirs and at the Beaver creek site. A pilot study, to assay water samples for *C. shasta* 18S rDNA by quantitative PCR, did not detect the parasite in Beaver Creek samples. In general, infection by *C. shasta* tended to occur in the upper river above Copco reservoir and below Iron Gate dam. Adverse water quality and inadequate habitat for the polychaete alternate host in both Iron Gate and Copco reservoir may limit *C. shasta* infectivity in these systems. Conclusive data on parasite abundance will require multiple year surveys. Direct methods to assay water for actinospore concentration are needed to help understand the biology of ceratomyxosis in the Klamath River basin.

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Introduction:

Ceratomyxa shasta is a myxosporean parasite of salmonid fishes that occurs in a number of watersheds of the Pacific Northwest and whose lifecycle includes the polychaete, *Manayunkia speciosa*, as its alternate host (Hoffmaster et al. 1988, Bartholomew et al. 1997). Infection can occur from spring through fall at water temperatures $\geq 7^{\circ}\text{C}$ (Ching & Munday 1984, Hendrickson et al. 1989). Hendrickson et al. (1989) describe the parasite's distribution in California to include the San Joaquin, Sacramento, Pit, and Klamath River systems. In the Klamath basin, *C. shasta* infection has been detected in salmonids from the mouth of the Klamath River to Iron Gate dam (rm 190), Copco reservoir, both Klamath and Agency Lakes, and the lower reaches of the Williamson and Sprague Rivers (Hendrickson et al. 1989, J. Bartholomew, Oregon State University, personal communication of Oregon Department of Fish and Wildlife information). Other Klamath River tributaries appear to be free of the infective actinospore stage. No infected juvenile Chinook smolts have been detected in health monitoring work in the Scott, Shasta, and Trinity Rivers (Foott et al. 2001, Nichols et al. 2003). In the spring of 2000, sixty juvenile Chinook salmon were collected from both the Scott and Shasta Rivers. No *C. shasta* was detected in histological sections of intestine. Infectivity data on Iron Gate Reservoir is lacking however ceratomyxosis has not occurred at the California Department of Fish and Game's Iron Gate Hatchery (IGH) that uses reservoir water. Infections have also not been detected by histological examination of IGH Chinook salmon sampled prior to their June release in 1992 – 1995 (Walker and Foott, 1992, Foott et al. 1999).

High mortality due to ceratomyxosis has been observed in juvenile Chinook salmon out-migrants in the Klamath R. basin (Foott et al. 1999 and 2002, Nichols et al. 2003). Unlike Chinook salmon, Klamath R. steelhead have been shown to be resistant to ceratomyxosis (Foott et al. 2003). Elevated water temperatures in the Klamath system, commonly in excess of 20°C in the late spring and summer, act to accelerate the disease process within infected fish (Udey et al. 1975). Our study examines the presence of the actinospore infective stage of *Ceratomyxa shasta* in waters of Copco and Iron Gate reservoirs as well as two river sites below Iron Gate dam. We addressed 3 questions: 1) Do the reservoirs harbor the infectious actinospore stage and contribute to the infectivity of the mainstem Klamath River, 2) Is there a seasonality to such infectivity, and 3) What is the incidence of *C. shasta* infection in returning adult salmon at Iron Gate hatchery. A preliminary study on the application of Quantitative Polymerase Chain Reaction assay on detecting *Ceratomyxa shasta* in water samples was also attempted during the study.

Methods:

Fish handling – Two thousand juvenile rainbow trout (*Oncorhynchus mykiss*, Oak Spring strain), obtained from Oregon Department of Fish and Wildlife's Wizard Falls Hatchery in April 2003, were held at the California Nevada Fish Health Center's Wet laboratory and used for the entire study. Approval for the use of these trout was granted by the California Department of Fish and Game. The trout had a mean fork length of 64 mm during the April exposure and 80 mm for the final exposure in late July. Juvenile Chinook (*O. tshawytscha*) and coho (*O. kisutch*) salmon, Klamath River stock, were obtained from Iron Gate Hatchery on the day of the exposure. Mean fork length of Chinook used in the exposure was 62 mm and 63 mm for the coho. Twenty-five to forty fish were held for 4 days (d) in 0.017 m³ cages at each exposure site and then transported back to the wet laboratory. In order to examine the influence of the thermocline on infectivity, separate cages were held at the surface (5ft, 1.5meter) and below the thermocline (50ft, 15meters) in both Iron Gate and Copco reservoirs. Exposures were conducted at 5 sites (Table 1 and Figure 1). The dates of the five exposures were:

1. April 23 – 28
2. May 16 – 20
3. June 6 – 10
4. June 27 – July 1
5. July 18 – 22, 2003.

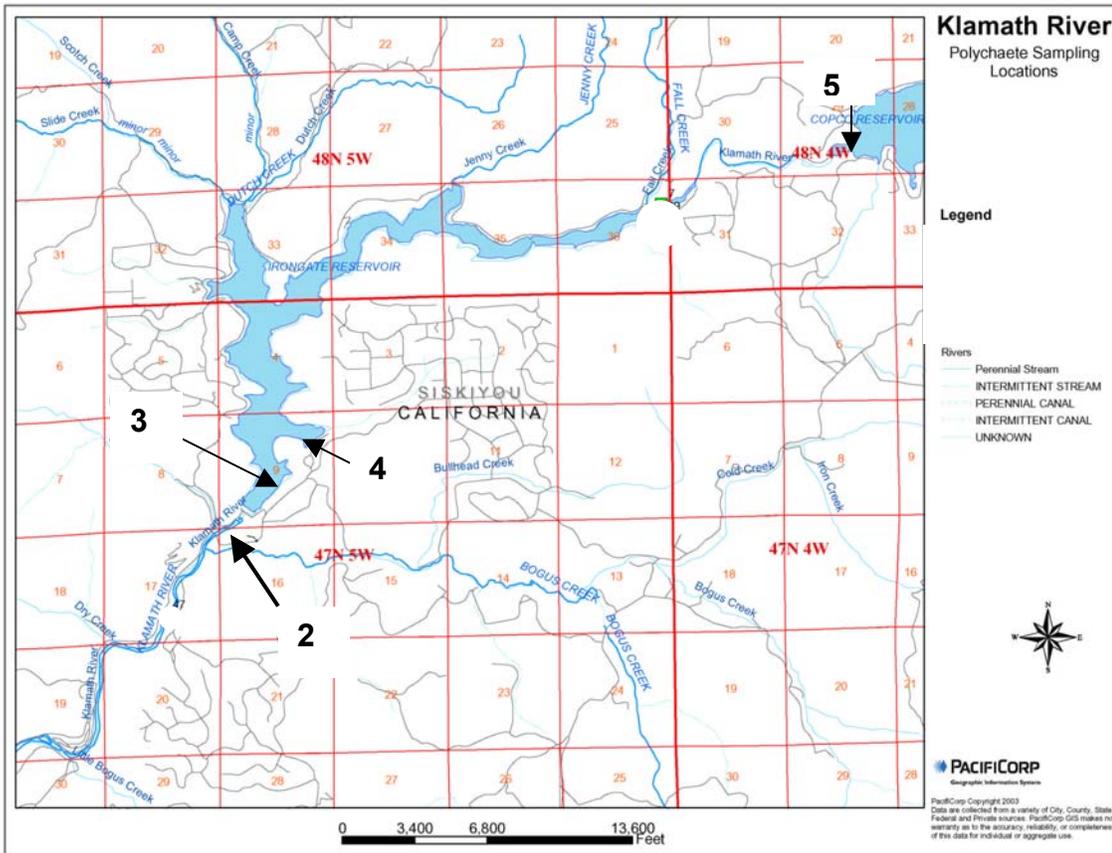
In order to reduce the occurrence of columnaris disease, each exposure group received a 10 min., prophylactic bath of 1 ppm furanase upon arrival at the laboratory. Each exposure group was reared in 17 L, flow-through aquaria for 18 days post-exposure. The water supply was ozone-treated and varied in temperature from 12 - 17 °C (Appendices 1 – 5). The laboratory effluent was treated with 5 mg / L chlorine for 50 min, dechlorinated through activated charcoal filters, and discharged into a 1.3 ha abatement pond. Water temperature, at both the exposure site and wet lab, was monitored every 2 hrs with Onset™ Stowaway temperature loggers. The mean daily temperature (°C) was summed to calculate temperature units (TU) for each exposure group. Temperature units were monitored to identify exposure site temperature effect on disease progression. A commercial salmon diet was offered twice a day. Dissolved oxygen was measured with an YSI 95 meter at the start and finish of each exposure as well as twice weekly at the wet lab. At exposure sites with cages deeper than 5 ft (1.5 m), a water sample was collected at 50ft (15m) with a 1 liter horizontal bottle (Aquatic Ecosystems Inc, Apopka FL). An aliquot of the sample was tested for dissolved oxygen content using the Winkler titration method.

Adult samples – Spawned adult fall-run Chinook salmon were sampled for intestine and gill tissues at Iron Gate Hatchery on 9 October and 21 October 2002. Lower intestine was fixed for histological sections and gills were examined fresh for the presence of *Ichthyophthirius multifiliis*.

Table 1. Exposure locations in the Klamath River below Iron Gate Dam, Iron Gate reservoir, and Copco reservoir.

Map No.	Site	Abbreviation	GPS Coordinates	Box Depth
	2 mi upstream of Beaver Creek mouth, Klamath R. Exposures 1 - 4		N 41° 52.165' W 122° 48.631'	3 ft. 1 m
	Mouth of Beaver Creek (Refugia) Klamath R. exposure 5		N 41° 52.165' W122° 48.984'	3 ft 1 m
2	Adjacent to Iron Gate Fish Hatchery 0.5 mi. below Iron Gate dam (IGD) Klamath R.	IGH	N 41° 56.006' W122° 26.211'	2 ft. 0.6 m
3	Iron Gate Reservoir 0.1 mi upstream of IGD attached to debris boom surface	IGR (surface)	N 41° 56.071' W122° 26.083'	5ft. 1.5 m
3	Iron Gate Reservoir 0.1 mi upstream of IGD attached to debris boom bottom	IGR (bottom)	N 41° 56.071' W122° 26.083'	50ft. 15 m
4	Iron Gate Reservoir Cove South shore, rm 195	IGR (cove)	N 41° 56.664' W122° 25.689'	25ft. 7.6 m
5	Copco Lake surface Attach to buoy line upstream of dam, rm 199	CC (surface)	N 41° 58.803' W 122° 19.967'	5ft. 1.5m
5	Copco Lake bottom Attach to buoy line upstream of dam at rm 199	CC (bottom)	N 41° 58.803' W 122° 19.967'	50ft. 15 m

Figure 1. USFWS sentinel exposure sites: 2) Klamath River adjacent to Iron Gate Hatchery, 3) Iron Gate Reservoir, 4) Iron Gate Reservoir cove, and 5) Copco Reservoir. Beaver Creek (not shown) site is located approximately 28 mi downriver of the #2 IGH site. Map courtesy of J. Bartholomew.



Necropsy – Sentinel fish were euthanized by an overdose of MS222, measured for fork length and weight, and examined for pale gill (anemia) and any external abnormality such as swollen abdomen or hemorrhagic vent. Upon dissection, internal abnormalities such as intestinal hemorrhage were noted and the intestinal tract and kidney was fixed for histological examination. Prior to the fixation step, a 1- 2 mm section of lower intestine was removed by DNA-free tools (bleached and 2X washed scalpels and disposable sticks) for storage at -70°C as a PCR archive sample. Intestinal samples were tested for *C. shasta* 18S rDNA by the Polymerase Chain Reaction (PCR) method of Bartholomew (2001).

Histology – Intestinal tract (including the caeca) and kidney was placed in Davidson’s fixative, processed for 5 µm paraffin sections and stained with either hematoxylin and eosin, or giemsa stains (Humason 1979). All tissues for a given fish were placed on one slide and identified by a unique code number. Each slide was examined at both low (40X) and high magnification (400X) without knowledge of the sample group.

Water and polychaete samples- Three liter samples of water were collected at the Beaver creek site on 20 May, 10 June, and 1 July 2003. Four 150 mL aliquots were centrifuged at 2,000x g for 20 min and the pellet removed for storage at -70°C. These pellets were later processed for Quantitative Polymerase Chain Reaction (QPCR) analysis at the Fish Health Center. An additional PCR sample from the 10 June and 1 July collection was prepared by passing 100 mL through a 0.45µm polycarbonate filter and cutting the filter into 4 equal parts for later QPCR analysis. Quantitative Polymerase Chain Reaction utilizes a unique fluorogenic probe with a reporter molecule and quencher dye, commonly called the TaqMan probe. This probe, along with specific primers against *Ceratomyxa shasta* 18S rDNA, was used to detect and quantify *C. shasta* in real-time using a sophisticated fluorometer and specialized software (Applied Biosystem, Foster City, CA). During the assay, samples containing specific target DNA undergo amplification, increasing the quantity of DNA, and this is detected by fluorescent signals that surpasses normal background levels.

On 15 July, benthic samples were collected at 10 locations in the Klamath River between the Forest Service launch sites of *Trees of Heaven* (N41° 49.523' W122° 39.493', approximately 1/4 mi. upstream of the mouth of Humbug creek (rm 171) to *Skeahan Bar* (N41°51.449' W122°42.281') with a kicknet and sorted for polychaetes. The collections occurred in eddy pools, stagnant backwaters, and between run reaches.

Coordination: In April, June, and July 2003, Dr. Jerri Bartholomew (Oregon State University) led a research team that conducted sentinel exposures at 6 sites in the upper Klamath basin from Keno reservoir (rm 233) to Copco bypass reach (rm 197.3). Benthic samples, to determine spatial and environmental parameters associated with polychaete habitat, were also collected by this team throughout the summer. The Fish Health Center (USFWS) and Dr. Bartholomew (OSU) closely coordinated their studies to use identical sentinel fish (Oak springs rainbow), exposure duration (4 day), and date of exposure in April and June. In addition, OSU performed all PCR analysis of fish tissues while the USFWS conducted the histological aspects for both studies.

Results:

Adult Chinook salmon infections - *Ceratomyxa shasta* trophozoites were observed in 14 of 46 (30%) intestinal sections with 5 fish demonstrating severe lesions. Cross – sections of an immature cestode was seen in 20 of the 46 (43%) of the samples. The cestode was not identified but may be in the genus *Phyllobothrium* based on the 5 suckers seen in the scolex of several specimens. Light infestation of *Ichthyophthirius multifiliis* trophozoites were observed in 15 of 40 (38%) gills. In September 2003, *Ichthyophthirius multifiliis* infection was associated with a significant salmon die-off in the lower Klamath River. These results do not indicate a significant health problem associated with parasitic infections for the adults selected for spawning in the fall of 2002.

Sentinel exposure data - A total of 643 fish survived the exposures and were later sampled for either PCR or histological analysis. In the April exposure, oxygen was bubbled into the transport tank. This action inadvertently killed 40% of the fish inside the Beaver creek and Copco reservoir cages located near the supersaturated water surface (Table 2). Agitation was later used for transport tank aeration. Extensive in-situ mortality occurred in groups exposed at Beaver creek in June and July (exposure #3-5). All Beaver creek sentinels died in the cages during the late June exposure (#4). In-situ mortality was also high for sentinels held in the river near Iron Gate Hatchery and in the surface waters of both Iron Gate and Copco reservoirs in late June (Table 5). High water temperatures ($\geq 20^{\circ}\text{C}$) were associated with these elevated in-situ mortality events (Appendices 1 – 5). Maximum water temperatures of 23°C or greater were measured at Iron Gate reservoir and Beaver creek beginning in June. No thermocline effect was detected in either reservoir during the July exposures with mean daily temperatures of $\geq 18^{\circ}\text{C}$. Despite prophylactic treatment, columnaris occasionally occurred in the sentinel fish during the 18 d rearing period and caused mortality. No consistent association with high in-situ mortality and low dissolved oxygen concentrations was observed in the data. For example, the late June Copco reservoir cove bottom cage experienced only a 13% in-situ mortality despite a 1.7 ppm oxygen measurement taken at the time of retrieval. Low concentrations (<4 ppm) were measured at both Iron Gate reservoir sites in July and the Copco bottom cage in late June (Table 3). Water quality during the wet laboratory rearing portion of the study was optimal for salmonids (dissolved oxygen 5.7 – 9.6 mg /L, pH from 7.5 – 8.0, and unionized ammonia below 0.05 mg / L).

Ceratomyxa shasta infections were diagnosed in fish exposed at Beaver creek from April – July (exposures #1,2,3, and 5), adjacent to Iron Gate hatchery in late June and July (exposures #4 and 5), and one fish exposed in Iron Gate reservoir cove during late June (Tables 2,4 - 7). Diagnosis of the positive Iron Gate reservoir cove sample was based on PCR with no parasites observed in the corresponding histological section. Overall, there was a 46% (11 of 24) agreement between PCR positive samples and *C. shasta* observations in corresponding histological sections. No obvious clinical sign of ceratomyxosis was observed in sentinel fish at the time of necropsy however, lesions were observed in 13 of 22 (59 %) intestine histological sections that contained *C.shasta*. Temperature units (TU) over 22 d (4d exposure + 18d rearing) ranged from 246 – 352. No TU trend with disease severity was observed in the infected fish. A myxosporean parasite, not resembling *C.shasta*, was observed in kidney sections of Chinook salmon held at Beaver creek but not Iron Gate reservoir. This parasite was not observed in rainbow trout regardless of sentinel site. Parasite infections were not detected, by either diagnostic method, in the control trout or salmon groups (data not shown).

Water and polychaete samples – *Ceratomyxa shasta* DNA was not detected by QPCR in water samples from Beaver creek. Low numbers of the freshwater polychaete, *Manayunkia speciosa*, were collected on 15 July from several sites in the Klamath River. The highest concentration of worms was collected from an eddy pool near the *Trees of Heaven* campground and was associated with sand / organic material substrate that was also inhabited by mussels. Worms were infrequently found on the algal-covered rocks in reaches with moderate flows. No worms were encountered in quiescent backwater sites. Ten polychaetes were maintained in river water at 15°C for up to 12 days post-capture. No actinospores were seen in microscopic examination of the culture wells.

Table 2. Exposure 1 infection and mortality data, Rainbow trout were exposed at the following Klamath river sites: Beaver creek, adjacent to Iron Gate Hatchery (IGH), Iron Gate Reservoir (IGR) surface and bottom, Iron Gate reservoir cove (IGR cove), and Copco reservoir cove (CC) surface during the 4 day period of April 24 – 28, 2003. Incidence of infection data of intestinal samples collected at 11 and 18 day (d) post-exposure (PE) and assayed by both PCR and histological examination is recorded as (number positive / total sample {%}). Percent mortality of the sentinel fish at the end of the exposure and the number of survivors held at the wet laboratory is also recorded.

	4 d PE	4d PE	Cshasta infection11d PE		Cshasta infection18d PE	
	%mortality	survivors	PCR	Histology	PCR	Histology
Beaver Creek	40% *	14	ND	ND	1 / 4 (25%)	1 / 10 ** (10%)
IGH	0%	25	0 / 1 (0%)	ND	0 / 1 (0%)	0 / 2 (0%)
IGR surface	0%	25	0 / 5 (0%)	ND	0 / 4 (0%)	0 / 8 (0%)
IGR bottom	4%	24	0 / 5 (0%)	ND	0 / 4 (0%)	0 / 9 (0%)
IGR cove	10%	14	ND	ND	ND	0 / 8 (0%)
CC surface	40% *	10	ND	ND	ND	0 / 6 (0%)

ND not done

* suspected gas supersaturation incident during transport

** Positive PCR results and histology observation in different fish.

Table 3. Dissolved oxygen concentration (mg /L) measured at the time of cage retrieval.

	Exposure 1	Exposure2	Exposure 3	Exposure 4	Exposure 5
Beaver Crk	9.3	9.9	9.1	9.0	na
Mouth Beaver crk	na	na	na	na	8.4
IGH	8.6	8.9	10.3	8.2	8.0
IGR surface	8.6	9.1	8.6	9.5	ND
IGR bottom	ND	ND	8.0	7.5	3.7
IGR cove	8.8	9.3	9.1	8.9	3.3
CC surface	9.3	8.3	7.5	8.4	ND
CC bottom	na	ND	5.0	1.7	3.4

na not applicable, no exposure performed

ND not done for exposure

Table 4. Exposure 2 infection and mortality data, Rainbow trout were exposed at the following Klamath river sites: Beaver creek, adjacent to Iron Gate Hatchery (IGH), Iron Gate Reservoir (IGR) surface and bottom, Iron Gate reservoir cove (IGR cove), and Copco reservoir cove (CC) surface and bottom during the 4 day period of May 16 - 20, 2003. Juvenile Chinook salmon (CHK) were also exposed at Beaver Creek. Incidence of infection data of intestinal samples collected at 11 and 18 day (d) post-exposure (PE) and assayed by both PCR and histological examination is recorded as (number positive / total sample {%}). Percent mortality of the sentinel fish at the end of the exposure and the number of survivors held at the wet laboratory is also recorded.

	4 d PE	4d PE	Cshasta infection11d PE		Cshasta infection18d PE	
	%mortality	survivors	PCR	Histology	PCR	Histology
Beaver Creek (RBT)	0%	25	0 / 4 (0%)	ND	4 / 4 (100%)	4 / 12 * (33%)
Beaver Creek (CHK)	0%	25	5 / 5 (100%)	ND	4 / 4 (100%)	12 / 14 * (86%)
IG Hatchery	0%	25	0 / 5 (0%)	ND	0 / 4 (0%)	0 / 8 (0%)
IGR surface	0%	25	0 / 5 (0%)	ND	0 / 4 (0%)	0 / 8 (0%)
IGR bottom	0%	25	0 / 5 (0%)	ND	0 / 4 (0%)	0 / 8 (0%)
IGR cove	0%	27	0 / 5 (0%)	ND	0 / 4 (0%)	0 / 8 (0%)
CC surface	0%	25	0 / 5 (0%)	ND	0 / 4 (0%)	0 / 8 (0%)
CC bottom	4%	24	0 / 4 (0%)	ND	0 / 4 (0%)	0 / 8 (0%)

ND not done

* *C.shasta* positive PCR and histology results matched in 3 of 4 paired samples.

Table 5. Exposure 3 infection and mortality data. Rainbow trout (RBT), Chinook (CHK), and coho salmon were exposed at one of the following Klamath river sites: Beaver creek, adjacent to Iron Gate Hatchery (IGH), Iron Gate Reservoir (IGR) surface and bottom, Iron Gate reservoir cove (IGR cove), and Copco reservoir cove (CC) surface and bottom during the 4 day period of June 6 - 10, 2003. Incidence of infection data of intestinal samples collected at 11 and 18 day (d) post-exposure (PE) and assayed by both PCR and histological examination is recorded as (number positive / total sample {%}). Percent mortality of the sentinel fish at the end of the exposure and the number of survivors held at the wet laboratory is also recorded.

	4 d PE	4d PE	Cshasta infection11d PE		Cshasta infection18d PE	
	%mortality	survivors	PCR	Histology	PCR	Histology
Beaver Creek (RBT)	92% *	2	0 / 1 (0%)	0 / 1 (0%)	ND	ND
Beaver Creek (CHK)	80%*	5	1 / 1 (100%)	1 / 1 (100%)	ND	ND
Beaver Creek (Coho)	60%*	10	ND	ND	ND	0 / 1* (0%)
IGH (RBT)	18%	14	0 / 5 (0%)	0 / 6 (0%)	0 / 5 (0%)	0 / 2 (0%)
IGR surface (RBT)	0%	25	0 / 7 (0%)	0 / 2 (0%)	0 / 9 (0%)	0 / 7 (0%)
IGR bottom (RBT)	0%	25	0 / 5 (0%)	0 / 4 (0%)	0 / 9 (0%)	0 / 8 (0%)
IGR bottom (CHK)	25%	20	0 / 5 (0%)	ND	0 / 10 (0%)	0 / 8 (0%)
IGR cove (RBT)	8%	24	0 / 5 (0%)	0 / 3 (0%)	0 / 12 (0%)	0 / 7 (0%)
CC surface (RBT)	12%	23	0 / 4 (0%)	0 / 3 (0%)	0 / 7 (0%)	0 / 7 (0%)
CC bottom (RBT)	16%	21	0 / 6 (0%)	0 / 6 (0%)	0 / 7 (0%)	0 / 4 (0%)

ND not done

* mean water temperature of 20°C, columnaris infections

Table 6. Exposure 4 infection and mortality data. Rainbow trout (RBT), Chinook (CHK), and coho salmon were exposed at one of the following Klamath river sites: Beaver creek, adjacent to Iron Gate Hatchery (IGH), Iron Gate Reservoir (IGR) surface and bottom, Iron Gate reservoir cove (IGR cove), and Copco reservoir cove (CC) surface and bottom during the 4 day period of June 27 – July 1, 2003. Incidence of infection data of intestinal samples collected at 11 and 18 day (d) post-exposure (PE) and assayed by both PCR and histological examination is recorded as (number positive / total sample {%}). Percent mortality of the sentinel fish at the end of the exposure and the number of survivors held at the wet laboratory is also recorded.

	4 d PE	4d PE	Cshasta infection11d PE		Cshasta infection18d PE	
	%mortality	survivors	PCR	Histology	PCR	Histology
Beaver Creek (CHK)	100%*	0	ND	ND	ND	ND
Beaver Creek (Coho)	100%*	0	ND	ND	ND	ND
IGH (RBT)	20%	24	0 / 5 (0%)	0 / 4 (0%)	4 / 12 + (33%)	0 / 13 (0%)
IGR surface (RBT)	57%	13	0 / 2 (0%)	0 / 2 (0%)	0 / 6 (0%)	0 / 6 (0%)
IGR bottom (RBT)	23%	23	ND	0 / 2 (0%)	0 / 5 (0%)	0 / 7 (0%)
IGR cove (RBT)	6%	28	0 / 5 (0%)	0 / 8 (0%)	1 / 19** (5%)	0 / 9 (0%)
CC surface (RBT)	100%	0	ND	ND	ND	ND
CC bottom (RBT)	13%	26	0 / 4 (0%)	0 / 4 (0%)	ND	0 / 8 (0%)

ND not done

* mean water temperatures 23°C.

** histological section of PCR + fish was negative

+ no match of PCR+ sample and histological section

Table 7. Exposure 5 infection and mortality data. Rainbow trout were exposed at Klamath river sites: mouth of Beaver creek, adjacent to Iron Gate Hatchery (IGH), Iron Gate Reservoir (IGR) 25 ft thermocline, Iron Gate reservoir cove (IGR cove), and Copco reservoir cove (CC) 25 ft thermocline during the 4 day period of July 18 – 22, 2003. Incidence of infection data for intestinal samples collected at 11 and 18 day (d) post-exposure (PE) and assayed by both PCR and histological examination is recorded as (number positive / total sample {%}). Percent mortality of the sentinel fish at the end of the exposure and the number of survivors held at the wet laboratory is also recorded.

	4 d PE	4d PE	Cshasta infection11d PE		Cshasta infection18d PE	
	%mortality	survivors	PCR	Histology	PCR	Histology
Beaver Creek (mouth)	90%*	4	ND	ND	4 / 4 (100%)	2 / 4 (50%)
IGH	70%	12	0 / 3 (0%)	0 / 3 (0%)	4 / 6 (67%)	2 / 6 (33%)
IGR bottom	8%	37	0 / 5 (0%)	0 / 9 (0%)	0 / 21 (0%)	0 / 8 (0%)
IGR cove	43%	23	0 / 5 (0%)	0 / 4 (0%)	0 / 16 (0%)	0 / 9 (0%)
CC bottom	73%	11	0 / 3 (0%)	0 / 3 (0%)	0 / 5 (0%)	0 / 5 (0%)

ND not done

* mean water temperature > 20°C.

Discussion:

Ceratomyxosis continues to be a significant cause of mortality in juvenile Chinook salmon rearing and migrating through the Klamath River. Prevalence of infection of 40% or more has been documented in juvenile Chinook collected in the spring and summer months with many of these infected fish demonstrating signs of clinical infection (Foott et al. 1999 and 2002, Nichols et al. 2003). It is expected that few if any of these infected salmon would survive (Foott et al. 2003). This disease may be a limiting factor in Chinook salmon survival in the basin.

The effect of ceratomyxosis on adult pre-spawn mortality in the basin is not well understood however, limited diagnostic data obtained from moribund adults in the lower Klamath and Trinity River since 2001, has not shown that ceratomyxosis is a major health issue (Fish Health Center case records 2001 – 2003). External infection with *Flavobacterium columnare* and *Ichthyophthirius multifiliis*, along with trauma (hook damage, seal bites, net scars, lamprey bite) are the typical cause of death. Relatively mild *C.shasta* infections were observed in 30% of the Fall-run Chinook broodstock sampled at IGH in October 2002 with many of these samples containing developed spores. Declining water temperatures in the autumn would decrease the rate of parasite multiplication and thereby limit the acute disease situation observed in juveniles during the summer month (Udey et al 1975). An infection of longer duration would also increase the number of spores that developed in the infected host. Release of these spores from infected adults will continue the life cycle of *C.shasta* by infecting the polychaete alternate host, *Manayunkia speciosa* (Bartholomew et al. 1997).

Previous studies on *C. shasta* infectivity of specific waters have relied on sentinel fish exposure (Ratliff 1983, Hendrickson et al 1989, Ching and Munday 1984). Ratliff (1983) used the Oak Springs strain of rainbow trout employed in this study. Our detection of *C. shasta* infection, by both PCR and histology, in salmon and trout sentinels exposed at Beaver creek indicate that negative site results were due to an absence of the infective actinospore stage. The sentinel fish approach to *C. shasta* research in the Klamath basin is limited by in-situ mortality associated with summer water temperatures. A direct method to determine actinospores concentrations in the river will be invaluable in answering questions about seasonality of the disease, habitat and environmental preferences of the alternative polychaete host, and challenge levels that lead to disease. Our inability to detect *C. shasta* DNA in Beaver creek water samples by QPCR does not allow for any conclusions on actinospore numbers. Water filtration and assay techniques will require considerable experimentation to accurately determine actinospore concentrations in the river.

Infectivity in Iron Gate and Copco reservoirs was quite limited in the 2003 sampling period. Only 1 trout out of the 318 sentinels exposed at the Iron Gate reservoir cove site was PCR positive for *C. shasta* infection however this asymptomatic infection was not detected in the corresponding histological section. No infections in Iron Gate reservoir sentinels were detected in the following July exposure. Bartholomew (2003) reported PCR positive samples from sentinel trout held in Keno reach, Bolye reservoir, Bolye bypass and peaking reaches, and Copco bypass reach in April, June, and July 2003. Curiously, no infection was detected in any of the 140 Copco reservoir sentinels in our study. The Copco bypass reach is the river between Copco dam and Copco powerhouse at 197.3 m while our Copco sentinels were exposed just upstream of the dam. It is possible that the bypass reach contained infected polychaetes resulting in a localized zone of infection. The low post-exposure mortality and general absence of clinical signs of disease in the sample groups indicates that infected fish received relatively moderate parasite challenges. Given past sentinel work at Beaver Creek, an 18 day rearing period and 16°C water temperature should have resulted in severe ceratomyxosis. The OSU sentinel groups were held for up to 70 days yet little mortality was reported (Bartholomew 2003). Actinospore levels may have been reduced in 2003 in comparison to previous years. Ratliff (1983) reported that infectivity in Lake Simtustus (Deschutes River, Oregon) only occurred below the epilimnion in the summer months. No such difference was observed between the surface or bottom sentinel groups in Iron Gate or Copco reservoir. The low infectivity of Iron Gate reservoir is also supported by the absence of ceratomyxosis at Iron Gate Hatchery (M. Willis, CDFG pathologist retired, personal communication). The detection of *C. shasta* infection in the river approximately 0.5 mile below Iron Gate dam (IGH site) during late June and July exposures poses a question on the source of infectivity. As it appears that Iron Gate Reservoir does not produce high levels of actinospores, infectivity at this site may be due to local concentrations of infected polychaetes. Benthic sampling was not performed at this site. The myxosporean parasite observed in kidney sections of Chinook exposed at Beaver creek has recently been identified by PCR analysis as *Parvicapsula minibicornis* (S. Jones, Pacific Biological Station, Nanaimo British Columbia, personal communication). Dual infection by *C. shasta* and *P. minibicornis* is a common condition for juvenile Chinook salmon migrants in the Klamath R. basin. The incidence of *P. minibicornis* infection in smolts collected from the Klamath River and estuary has ranged from 80 – 100% (Foott et al. 1999 & 2002).

The high degree of *C. shasta* infectivity found at the Beaver creek site is probably due to abundant populations of the polychaete alternate host above the site. A relatively stable flow regime may be an important factor in ceratomyxosis. Reaches of the river, that do not undergo scouring events and also

maintain a consistent wetted surface area in the summer, could maintain larger populations of polychaetes. The highest concentration of worms collected on 15 July was within a mussel bed at the *Tree of Heaven* site suggesting a preference for particulate organic / sand substrate areas of moderate flow. We also collected this tube-dwelling filter feeder attached to algal covered rocks. In general, infection by *C. shasta* tended to occur in the upper river above Copco reservoir and below Iron Gate dam. Adverse water quality and inadequate habitat for the polychaete alternate host in both Iron Gate and Copco reservoir may limit *C. shasta* infectivity in these systems. Conclusive data on parasite abundance will require multiple year surveys. Direct methods to determine actinospore concentration in water are needed to help understand the biology of ceratomyxosis in the Klamath River basin.

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Appendix 1.

Exposure 1 (4/23 – 28) temperature (°C) summary. Data includes daily temperature units (TU= sum of MDTs) and mean daily temperature (MDT) for 4 day exposure period, 18 day laboratory rearing period, and 22 day combined total.

Exposure 1

	In Live Box							
	TU	MDT 1d	MDT 2d	MDT 3d	MDT 4d	StdDev	Max	Min
Copco Cove	39.34	10.15	9.77	9.68	9.74	0.21	10.97	9.17
IGH	39.76	9.97	9.91	9.94	9.94	0.02	11.26	9.46
IGR Surface	40.86	10.20	10.14	10.29	10.23	0.06	10.56	9.84
IGR Bottom	38.37	9.76	9.45	9.59	9.58	0.13	9.94	9.01
IGR Cove	40.77	10.14	10.08	10.29	10.26	0.10	10.56	9.84

	18d Lab Rearing				
	TU	MDT	StdDev	Max	Min
Beaver Creek	207.29	12.96	2.09	20.05	9.84
Copco Cove	207.45	12.97	2.03	20.12	9.89
IGH	211.59	13.22	2.09	20.39	10.18
IGR Surface	217.42	13.59	1.90	20.40	10.20
IGR Bottom	214.97	13.44	2.01	20.50	10.26
IGR Cove	209.86	13.12	2.04	20.41	9.84

	Total					hrs
	TU	MDT	StdDev	Max	Min	>21C
Beaver Creek	246.82	12.34	2.24	20.05	8.76	0.00
Copco Cove	246.79	12.34	2.22	20.12	9.17	0.00
IGH	251.35	12.57	2.29	20.39	9.46	0.00
IGR Surface	258.28	12.91	2.18	20.40	9.84	0.00
IGR Bottom	253.34	12.67	2.39	20.50	9.01	0.00
IGR Cove	250.63	12.53	2.17	20.41	9.84	0.00

Appendix 2.

Exposure 2 (5/16 – 20) temperature (°C) summary. Data includes daily temperature units (TU= sum of MDTs) and mean daily temperature (MDT) for 4 day exposure period, 18 day laboratory rearing period, and 22 day combined total.

Exposure 2

	In Live Box TU	MDT 1d	MDT 2d	MDT 3d	MDT 4d	StdDev	Max	Min	HRS >21C
Beaver Creek	53.58	13.28	13.04	13.10	14.16	0.52	15.51	11.27	0.00
Copco Cove Surface	55.75	13.87	13.60	13.96	14.33	0.30	14.86	12.74	0.00
Copco Cove Bottom	44.75	10.83	10.85	10.88	12.19	0.67	15.50	10.68	0.00
IGH	51.06	12.85	12.77	12.56	12.88	0.15	15.49	11.97	0.00
IGR Surface	54.70	13.70	13.23	13.40	14.37	0.50	15.51	12.69	0.00
IGR Bottom	31.84	7.41	7.50	7.45	9.49	1.02	15.54	7.17	0.00
IGR Cove	42.25	10.29	10.20	10.38	11.38	0.55	15.16	9.48	0.00

	18d lab rearing TU	MDT WL	StdDev	Max	Min	HRS >21C
Beaver Creek	226.20	16.16	0.69	19.00	14.46	0.00
Copco Cove Surface	225.59	16.11	0.73	19.07	14.51	0.00
Copco Cove Bottom	231.82	16.56	0.75	19.66	14.87	0.00
IGH	228.59	16.33	0.76	19.34	14.79	0.00
IGR Surface	228.75	16.34	0.75	19.36	14.81	0.00
IGR Bottom	231.94	16.57	0.72	19.53	14.90	0.00
IGR Cove	226.91	16.21	0.73	19.01	14.46	0.00

	Total TU	MDT	StdDev	Max	Min	HRS >21C
Beaver Creek	279.78	15.54	1.34	19.00	11.27	0.00
Copco Cove Surface	281.34	15.63	1.13	19.07	12.74	0.00
Copco Cove Bottom	276.57	15.36	2.41	19.66	10.68	0.00
IGH	279.64	15.54	1.66	19.34	11.97	0.00
IGR Surface	283.45	15.75	1.33	19.36	12.69	0.00
IGR Bottom	263.79	14.65	3.76	19.53	7.17	0.00
IGR Cove	269.16	14.95	2.51	19.01	9.48	0.00

Appendix 3.

Exposure 3 (6/6 – 10) temperature (°C) summary. Data includes daily temperature units (TU= sum of MDTs) and mean daily temperature (MDT) for 4 day exposure period, 18 day laboratory rearing period, and 22 day combined total.

	In Live Box TU	MDT 1d	MDT 2d	MDT 3d	MDT 4d	StdDev	Max	Min	>21C	hrs
Beaver Creek	83.26	20.63	21.15	20.86	20.63	0.25	22.13	19.00	24.00	
Copco Cove Surf	82.78	20.49	20.70	21.10	20.49	0.29	21.86	11.32	26.00	
Copco Cove Bot	44.65	11.14	11.14	11.18	11.19	0.03	11.30	11.14	0.00	
IGH	77.11	19.19	19.84	20.18	17.90	1.01	21.43	10.18	3.00	
IGR Surface	86.85	21.04	22.59	22.53	20.69	0.99	23.67	10.88	40.00	
IGR Bottom	34.05	8.42	8.42	8.42	8.78	0.18	10.92	8.03	0.00	
IGR Cove	TEMP LOGGER FAILURE NO DATA	Assume similar to IGR bottom								
	18d lab rearing TU	MDT WL	StdDev	Max	Min	>21C				
Beaver Creek	244.91	17.49	0.78	20.05	11.62	0.00				
Copco Cove Surf	242.57	17.33	0.75	19.77	11.68	0.00				
Copco Cove Bot	249.03	17.79	0.75	20.31	11.76	0.00				
IGH	246.81	17.63	0.79	20.04	11.61	0.00				
IGR Surface	251.07	17.93	0.79	20.50	11.80	0.00				
IGR Bottom	246.05	17.57	0.85	20.40	11.63	0.00				
IGR Cove	TEMP LOGGER FAILURE NO DATA									
	Total TU	MDT	SD	Max	Min	Hrs >21C				
Beaver Creek	328.17	18.23	1.58	22.13	11.62	24.00				
Copco Cove Surf	325.35	18.07	1.59	21.86	11.32	26.00				
Copco Cove Bot	293.69	16.32	2.91	20.31	11.14	0.00				
IGH	323.92	18.00	1.08	21.43	10.18	3.00				
IGR Surface	337.92	18.77	1.81	23.67	10.88	40.00				
IGR Bottom	280.10	15.56	3.95	20.40	8.03	0.00				
IGR Cove	TEMP LOGGER FAILURE	Assume similar to IGR bottom								

Appendix 4.

Exposure 4 (6/27 – 7/1) temperature (°C) summary. Data includes daily temperature units (TU= sum of MDTs) and mean daily temperature (MDT) for 4 day exposure period, 18 day laboratory rearing period, and 22 day combined total.

Exposure 4

	In Live Box						hrs		
	TU	MDT 1d	MDT 2d	MDT 3d	MDT 4d	StDev	Max	Min	>21
Beaver Creek	89.40	22.61	23.10	22.14	21.55	0.66	23.63	20.79	42
Copco Cove Surface	83.01	20.39	21.33	20.65	20.63	0.40	21.73	12.19	27
Copco Cove Bottom	43.64	10.91	10.91	10.91	10.91	0.00	10.91	10.91	0.00
IGR Surface	90.05	23.05	22.99	21.51	22.49	0.72	24.32	20.12	44.00
IGR Bottom	35.64	8.85	8.85	8.91	9.03	0.09	9.48	8.39	0.00
IGR Cove	43.84	10.75	10.90	10.96	11.23	0.20	13.03	10.18	0.00

IGH **TEMP LOGGER FAILURE NO DATA**

	18d lab					hrs
	TU	MDT	StDev	Max	Min	>21
Beaver Creek	230.96	15.31	1.87	21.79	11.88	4.00
Copco Cove Surface	211.53	14.10	1.75	17.36	11.88	0.00
Copco Cove Bottom	224.36	14.96	1.79	17.36	11.88	0.00
IGR Surface	219.55	14.64	1.76	16.97	11.32	0.00
IGR Bottom	223.23	14.88	1.76	17.26	11.63	0.00
IGR Cove	222.34	13.90	1.76	17.24	11.61	0.00

IGH **TEMP LOGGER FAILURE NO DATA**

	Total					hrs
	TU	MDT	StDev	Max	Min	>21
Beaver Creek	320.36	16.80	3.39	23.63	11.88	46.00
Copco Cove Surface	294.53	15.50	2.86	21.73	11.88	27.00
Copco Cove Bottom	268.00	14.11	2.32	17.36	10.91	0.00
IGR Surface	309.60	16.29	3.66	24.32	11.32	44.00
IGR Bottom	258.87	13.62	2.94	17.26	8.39	0.00
IGR Cove	266.17	13.31	2.24	17.24	10.18	0.00

IGH **TEMP LOGGER FAILURE NO DATA** assume similar to Beaver Creek

Appendix 5.

Exposure 5 (7/18 – 22) temperature (°C) summary. Data includes daily temperature units (TU= sum of MDTs) and mean daily temperature (MDT) for 4 day exposure period, 18 day laboratory rearing period, and 22 day combined total.

Exposure 5

	In Live Box						hrs		
	TU	MDT 1d	MDT 2d	MDT 3d	MDT 4d	StDev	Max	Min	>21
Beaver Creek	88.59	22.58	22.78	22.86	20.37	1.19	23.63	12.07	45.00
IGR bottom	80.33	19.85	20.55	20.61	19.32	0.61	21.16	12.03	4.00
IGR Cove	78.37	18.71	19.77	19.94	19.95	0.59	26.77	12.34	2.00
Copco bottom	75.93	18.75	19.45	19.51	18.22	0.61	20.06	10.93	0.00
IGH (probe lost)	90.91	22.47	22.76	22.85	22.83	0.18	23.78	21.84	48.00
Data from nearby FWS trap									
	18d lab					hrs			
	TU	MDT	StDev	Max	Min	>21			
Beaver Creek	260.71	17.38	2.28	22.29	10.68	11.00			
IGR bottom	253.96	16.93	2.31	21.86	10.25	7.00			
IGR Cove	257.43	17.16	2.32	22.14	10.56	11.00			
Copco bottom	249.50	16.63	2.36	21.65	9.93	5.00			
IGH	260.71	17.38	2.29	22.29	10.68	11.00			
	Total					hrs			
	TU	MDT	StDev	Max	Min	>21			
Beaver Creek	349.30	18.38	2.88	23.63	10.68	56			
IGR bottom	334.29	17.59	2.44	21.86	10.25	11.00			
IGR Cove	335.80	17.67	2.29	26.77	10.56	13.00			
Copco bottom	325.43	17.13	2.31	21.65	9.93	5.00			
IGH	351.62	18.51	3.01	23.78	10.68	59.00			