

US Fish and Wildlife Service

California Nevada Fish Health Center

FY2007 Technical Report:

***Ceratomyxa shasta* myxospore survey of adult Rainbow trout / Steelhead, Chinook and Coho salmon in the Klamath River basin in 2007-2008: Cooperative Humboldt State University -Yurok Fisheries-CA-NV FHC project.**

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Summary: Intestine and kidney tissues were collected from 567 adult salmonids (Chinook, coho, and rainbow / steelhead) in the Klamath River basin between 30Aug2007 and 17Jan2008. *Ceratomyxa shasta* myxospores were seen primarily in Chinook salmon carcasses and not from live fish (spawn or captured). Conversely, both coho (36%) and rainbow/steelhead (29%) live fish samples had myxospores. Histological data indicates that spawned salmon at Iron Gate and Trinity R. hatcheries had high levels of infection by the pre-sporogonic stages of *C. shasta*. Adult Chinook collected from the estuary and lower Klamath R. (Weitchpec) appeared to have early stage *C.shasta* infections that were only detected by quantitative polymerase reaction assays. This observation indicates adults become infected soon after entering the river but require a number of weeks to produce myxospores. *Parvicapsula minibicornis* was observed in 96% of Chinook and 23% of the coho kidney sections from spawned fish. *Parvicapsula minibicornis* myxospores were seen in the kidney tubules of 18 - 22% of the Chinook and 15% of the coho. Holding intestines for 3-7 days at 4-10°C did not result in myxospore formation (no increased detection). It is likely that *C.shasta* requires specific temporal and microenviromental conditions within the decomposing intestine (low pH, lytic enzymes, low oxygen) for sporogenesis. Pilot efforts to reduce myxospore input should focus on carcasses and not spawned fish.

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Notice

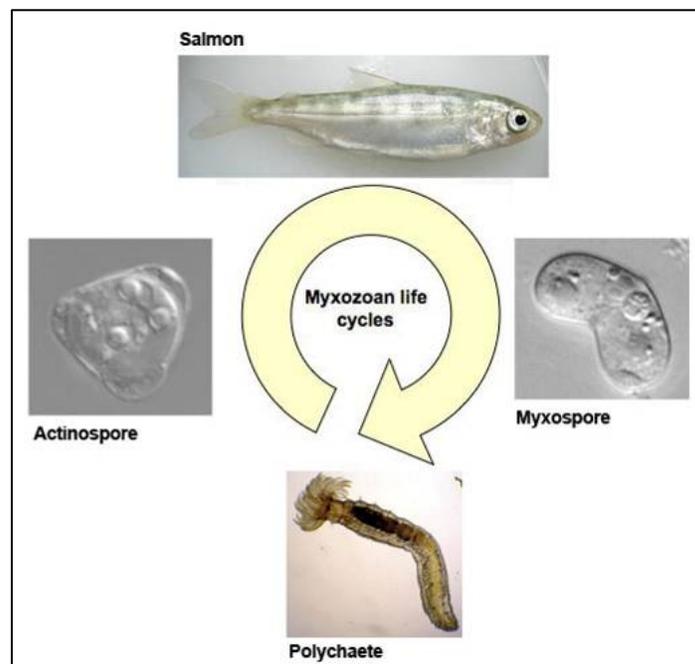
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Introduction:

Severe infection, of juvenile Klamath River Chinook salmon and coho salmon, by the myxozoan parasite *Ceratomyxa shasta* may be a contributing factor in declining adult returns in the basin. The incidence of *C. shasta* infection, observed in histological sections of juvenile Chinook collected in the Klamath River above the confluence of the Trinity River between May and July, has ranged from 21 – 35% (Nichols et al. 2008). This incidence is 10 – 27% higher in samples assayed by the more sensitive quantitative polymerase chain reaction assay (QPCR). Approximately 70% of the histological positive samples demonstrated pathology due to the infection. The high prevalence and severity of infection, in native fish that should have high resistance to the disease, indicates this parasite is a key factor limiting salmon recovery in Klamath River.

Ceratomyxa shasta has a complex life cycle, involving an invertebrate (polychaete worm) host as well as salmon (Bartholomew et al. 1997, see diagram below). A section of the lower Klamath River has been identified to be highly infectious to salmon (Stocking et al. 2006) and should be a focus for management actions to disrupt the parasite's life cycle (Fig. 1). One proposed management action is the removal of adult salmon carcasses to reduce their input of myxospores released back into the system. The hypothesized effect of this action would be reduced infection of polychaete populations and thus a reduction of actinospores released to infect juvenile fish the following spring. This survey effort in 2007-2008 was focused on describing the occurrence of *C. shasta* myxospores in different Klamath R. basin adult salmonid populations. A secondary objective was to survey for *Parvicapsula minibicornis* kidney infections.

Figure 1. Lifecycle of *Ceratomyxa shasta* (J. Bartholomew).



Participate Roles:

1. Ryan Slezak (Humboldt State University {HSU}): Survey adult Fall-run Chinook salmon for *C.shasta* and *P. minibicornis* infection for Master's research project. Additional analysis of this sample set will be published in thesis.

2. Yurok Tribal Fisheries (YTF): Collect intestine and kidney samples from adult Fall-run Chinook (Weitchpec net harvest), coho (Iron Gate Hatchery), and Steelhead / rainbow trout (IGH and Klamath R.). Examine intestinal scrapings for *C.shasta* myxospores.

3. CA-NV Fish Health Center (FHC): Provide initial laboratory and collection training (8/22/07), histological support (HSU- training, access to equipment and supplies, quality control review; YTF – processing and examination of coho and steelhead samples), quality control review of scraping samples, QPCR analysis, production of summary report.

Methods:

Carcass myxospore – Intestinal tract (rectum to small intestine / pyloric ceca junction) and 5 cm section of kidney were dissected from live (spawned or recently captured) fish. Only intestine was collected from hatchery mortalities or carcasses. A 2- 5 mm section of rectum and 25 mg section of kidney was placed into 95% ETOH vial for QPCR archive and similar size samples placed into Davidson's fixative for histological examination. Samples were stored at room temperature. Intestine samples from freshly killed samples were stored in the refrigerator for 3-7 d prior to processing or frozen storage. This step was done to provide time for pre-sporogonic stages to complete spore formation. An intestinal tract content sample (scraping) was obtained by grasping the end of the section with forceps and pushing the backside of a #21 scalpel blade, held at 45° angle, along the outside of the intestine. This process was repeated several times until only the serosa to stratum compactum layers remained. All material scraped from the intestine was mixed vigorously in 5 mL of PBS and poured through a single layer of cheese-cloth to large fibers and cestodes. The cloth was washed once with an additional 5 mL PBS and the 10mL suspension centrifuged at 3200xgx 15 min. The supernatant was carefully removed with a pipette until approximately 2 mL remained above the pellet. The pellet was mixed with a disposable pipette and a 10µL drop placed onto 4 replicate coverslips (30x 40mm #1). Wet mounts were examined for the characteristic *C.shasta* myxospore at both 20x and 40x phase for a total of 100 fields / slide. The total number of *C.shasta* myxospores seen in each coverslip preparation was recorded. Any samples positive for myxospores were frozen for later confirmation by FHC. Quantitative PCR was performed on a subset of scrapings from estuary samples using methods described in Nichols et al. 2008. Select histological samples were processed for 5µm paraffin sections and stained with either Giemsa or hematoxylin and eosin, and examined using bright field microscopy at 40x and 400x magnification. Records maintained by HSU and YTF included sample date, location, fish species and condition (spawn, mortality, carcass),

unique subsample identity, and wet mount examination results of intestinal scraping.

Results and Discussion:

Fall-run Chinook adults - Five hundred and nine adults were sampled between 30Aug and 20Nov (Table 1). *Ceratomyxa shasta* myxospores were found in intestinal wet mounts from 44% (28 of 64) of the carcasses collected from Bogus creek, Klamath R. and Shasta R. In contrast, myxospores were seen in 4 -6% of the wet mounts from adults collected after being spawned at Iron Gate Hatchery (IGH) and Trinity River Hatchery (TRH). As demonstrated by histological examination, these fish showed high levels (75 % and 86%) of infection by the pre-sporogonic stages of *C. shasta* (Table 2). Similar to the wet mount data, only 7% of the intestine sections contain the myxospore stage. *Parvicapsula minibicornis* was observed in kidney sections from 96% of hatchery samples with 18 – 22% of the samples having the myxospore stage within the kidney tubules.

Ceratomyxa shasta myxospores were not detected in either intestinal wet mounts or histological sections from adults collected in either the estuary or the Klamath R. (Weitchpec) net harvest (Table 1 and 2). While pre-sporogonic stages were not observed in 27 intestine sections from the estuary collection, *C. shasta* DNA was detected by QPCR in 6 of 24 (25%) intestinal scraping samples from this collection group. Low DNA target copy number (CT= 39) was observed in 4 additional samples. This data suggests that returning adults become infected with *C.shasta* soon after entering the lower Klamath R. but these infections do not produce myxospores for weeks. It is generally assumed that QPCR is more sensitive than histology and would likely detect early infections.

A review of IGH Fall-run adult *C.shasta* data, collected in 2005 – 2006, indicates that incidence of *C.shasta* infection is high however the myxospore stage is primarily in carcasses and not live (spawned) fish. In 2005, histological sections from spawned adults showed an 80% incidence of infection by the pre-sporogonic stages (16 of 20 sections, CA-NV FHC unpublished data). In 2006, *C.shasta* myxospores were observed in only 1 of 60 intestinal scraping samples collected from spawned adults at IGH. *Cshasta* DNA was detected by QPCR in 12 of 20 (60%) of these intestinal scrapings and trophozoite stages were observed in one of 20 kidney sections from the same fish (CA-NV FHC unpublished data). A similar effort, by Oregon State University and California Department of Fish and Game, to survey IGH Fall-run Chinook spawners by QPCR yielded 70% and 85% *C.shasta* DNA detection rates in 2005 and 2006, respectively (J. Bartholomew, OSU pers. comm.).

Table 1. Incidence of *Ceratomyxa shasta* myxospore detection (number positive / total sample number {%}) in intestinal scraping from adult Fall-run Chinook salmon collected by Ryan Slezak (Humboldt State University) or Yurok Tribal Fisheries in 2007. Period of sample collection is listed for the sample locations include the Klamath estuary, Klamath River at Weitchpec, Trinity River Hatchery (TRH), Iron Gate Dam to Klamathon reach of Klamath R., Iron Gate Hatchery (IGH), Bogus creek weir, and Shasta River weir.

	R Slezak (HSU)	Yurok Tribal Fish.
Estuary	0 / 61 30Aug-12Sep	nd
Weitchpec (KR)	Nd	0 / 93 04Sep – 12Oct
TRH	8 / 125 (6%) 20Sep – 14Dec	nd
Klamath R. IGD-Klamathon	4 / 8 (50%) ** 4-5Dec	nd
IGH	6 / 166 (4%) 12Oct-21Nov	nd
Bogus C. weir	9 / 32 (28%) ** 9Nov – 20Nov	nd
Shasta R. weir	15 / 24 (63%) ** 9Nov – 20Nov	nd

nd not done

** carcass sample

Table 2. Incidence of infection data (positive / total samples {%}) from histological sections of adult Fall-run Chinook lower intestine and kidney. Samples collected from spawned fish at Trinity R. Hatchery (TRH) and Iron Gate Hatchery (IGH) as well as from net harvest in the Klamath Estuary. Incidence of trophozoites stages of *Ceratomyxa shasta* and *Parvicapsula minibicornis* as well as their myxospore stages.

	Lower intestine		Kidney	
	<i>C.shasta</i>	myxospore	<i>P. minibicornis</i>	myxospore
Estuary	0 / 27 (0%)	0 / 27 (0%)	0 / 23 (0%)	0 / 23 (0%)
TRH	50 / 58 (86%)	4 / 58 (7%)	59 / 60 (98%)	13 / 60 (22%)
IGH	76 / 101 (75%)	7 / 101 (7%)	95 / 97 (98%)	17 / 97 (18%)

Coho adults- Kidney and intestine was collected by YTF from adult coho salmon spawned at IGH between 06Nov and 12Dec 2007. The incidence of *C.shasta* myxospore detection in intestinal scraping samples was 36% (16 of 44 fish) with the highest spore counts of 546 – 2439 / 10uL wet mount occurring in a pre-spawn mortality collected 13Nov. Histological sections were processed from 18 of these coho. The incidence of *C shasta* infection (pre-sporogonid stages) observed within lower intestine cross-sections was 61% (11 of 18 fish). Myxospores were seen in 2 of the 11 infected fish. *Parvicapsula minibicornis* was observed in the kidney tubules from 3 of 13 (23%) kidney sections (2 fish had spore stages). *P. minibicornis* was not seen in the kidney glomeruli and no pathology was associated with the parasites. Low numbers of metacercaria (trematode) were seen in 7 of the 13 (54%) kidney sections.

Rainbow trout / Steelhead adults – The incidence of *C.shasta* myxospore detection in intestinal scraping samples was 29% (4 of 14 fish). Eleven of these adults were spawned or mortalities from IGH that were collected between 30Nov07 and 17Jan08. Three fish were collected from the Klamath R (IGD – Klamathon reach) between 17Oct and 31Oct. Neither *C.shasta* nor *P. minibicornis* was observed in sections of intestine and kidney from 2 fish collected in the Klamath R. on 31Oct.

A group of histological samples from Steelhead and coho could not be evaluated due to extensive post-mortem degradation (case 08-52).

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Reference:

Bartholomew JL, MJ Whipple, DG Stevens, and JL Fryer. 1997. The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternative host. *Journal of Parasitology* 83(5):859-868.

Hallett, S. L. and J. L. Bartholomew. 2006. Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in water samples. *Diseases of Aquatic Organisms* 71:109-118.

Nichols K, K True, R Fogerty and L Ratcliff. 2008. FY 2007 Investigational Report: Klamath River Juvenile Salmonid Health Monitoring, April-August 2007. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA. Available online: <http://www.fws.gov/canvfhc/reports.asp>.

Stocking RW, RA Holt, JS Foott, and JL Bartholomew. 2006. Spatial and temporal occurrence of the salmonid parasite *Ceratomyxa shasta* in the Oregon-California Klamath River Basin. *Journal of Aquatic Animal Health* 18:194 – 202.