California Nevada Fish Health Center
FY2008 Investigational Report:

Ceratomyxosis in radio-tagged yearling coho sentinels exposed in the Klamath River during May 2008.

J.Scott Foott*, R. Stone, Greg Stutzer,and R. Fogerty.**
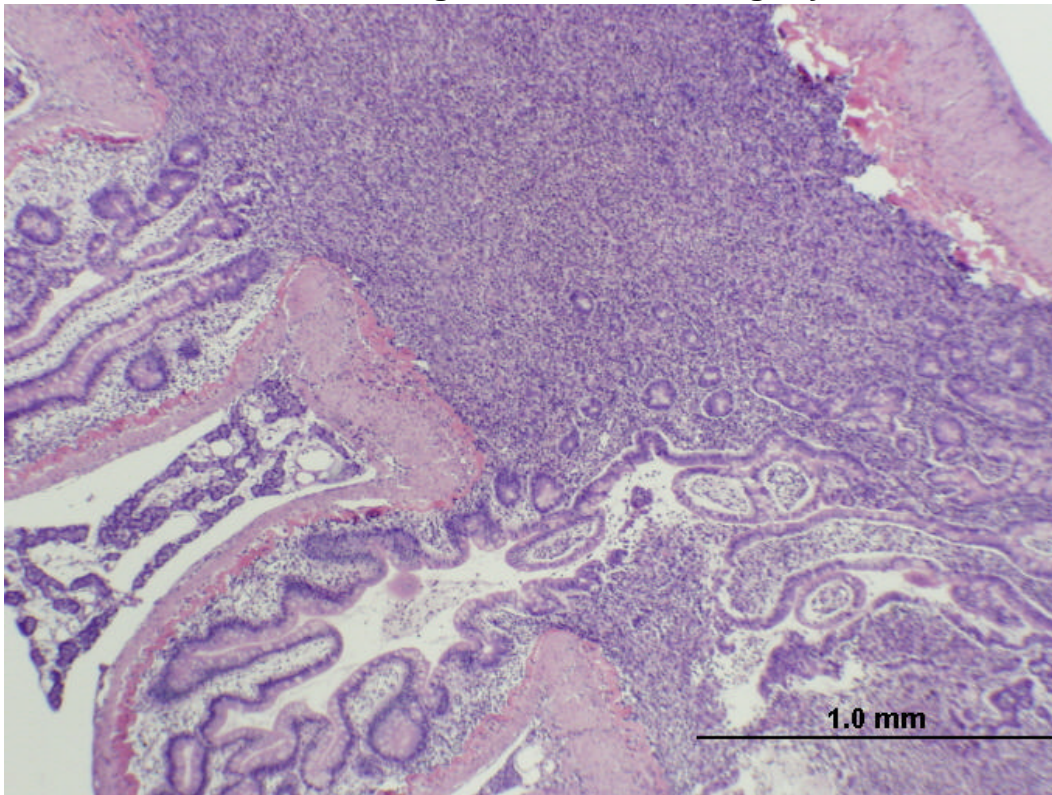
1.0 mm

*   direct correspondence
**   USFWS, Arcata Fish & Wildlife Office, Arcata, CA

Summary: Yearling coho salmon (mean fork length 140mm) were exposed to the Klamath River at two sites (adjacent to Iron Gate Hatchery (rkm 309) and at the Tree of Heaven campground (rkm 280) between 16 May and 23 May, 2008. Half of each group was implanted with radio transmitters. The sites and tag implantation procedures were chosen to replicate a 2007 radiotelemetry study in which lower survival occurred among salmon released from 10 April to 18 May near Tree of Heaven in comparison to cohorts released at Iron Gate Hatchery. In the present study, the Tree of Heaven group had an 85% cumulative mortality due to ceratomyxosis. No mortality associated with *Ceratomya shasta* infection occurred in the Iron Gate Hatchery exposure group however there was a 44% incidence of infection in fish sampled 34 days post exposure. *Parvicapsula minibicornis* was observed in the kidneys of all exposed salmon but did not induce significant kidney lesions. Implantation did not influence the incidence of *C.shasta* infection or severity of disease. While it is impossible to reconstruct the effect of *C.shasta* infection in 2007, the current study provides evidence that ceratomyxosis could have contributed to the low survival of some Tree of Heaven release groups. This data also adds to the body of knowledge demonstrating the significant effect of ceratomyxosis on juvenile coho survival in the Klamath River.

Background - *Ceratomyxa shasta* and *Parvicapsula minibicornis* are myxosporean parasites found in a number of Pacific Northwest watersheds including the Klamath River (Hoffmaster et al. 1988; Bartholomew et al. 1989; St.-Hilaire et al. 2002; Jones et al. 2004; Bartholomew et al. 2006, Bartholomew et al. 2007). The lifecycles of both parasites include the polychaete host, *Manayunkia speciosa*, and salmonids (Bartholomew et al. 1997; Bartholomew et al. 2006). *Ceratomyxa shasta* infection can occur from spring through fall at water temperatures > 4°C (Ching and Munday 1984; Hendrickson et al. 1989; Bartholomew et al. 1989). Nichols and True (2007) report that both parasites infect Klamath River juvenile coho salmon (*Oncorhynchus kisutch*) and that these infections can be severe (Stone et al. 2008). *Ceratomyxa shasta* infection in Klamath River juvenile salmon tends to begin in late April – early May with severe infections (disease state) occurring in late May through August. *C.shasta* infectivity, as related to the concentration of infectious actinospores in the river, appears to be highest in the I-5 bridge to Seiad Valley reach of the lower Klamath River (Hallett and Bartholomew 2006, Stocking et al. 2006).

In April and May of 2007, a joint US Fish and Wildlife Service and US Geological Survey (Biological Resource Division) study of survival and migration rate of radio-tagged yearling coho salmon observed a lower than expected survival rate in salmon released from the Tree of Heaven (T of H) campground in comparison to cohorts released up-river near the California Department of Fish and Game (IGH) Iron Gate Hatchery (Beeman et al. in press). The survival between T of H campground (rkm 280) and rkm 33 was 0.700 (SE 0.058) for fish released near IGH and 0.301 (SE 0.041) for those released near the campground. The largest difference between the groups was in the reach from the campground to the Scott River, in which the survivals were 0.814 (SE 0.044) and 0.589 (SE 0.045). The point estimates of survival for coho released near IGH were higher than T of H cohorts in every reach. The fish released near the campground had longer travel times through most reaches than those released near IGH, but the authors could not determine if this was the cause of the survival differences. The Klamath River near the campground has been shown to be highly infectious for

known to *C. shasta* and *P. minibicornis* (Hallett and Bartholomew 2006, Bartholomew et al. 2007).

The objectives of this 2008 study were: 1) determine if incidence of parasitic infection, disease, and mortality was higher among yearling coho salmon held at the T of H site than for fish held near IGH, and 2) determine if incidence of parasitic infection, disease, and mortality was greater among yearling coho with radio transmitters surgically implanted compared to control fish.

Methods: Forty IGH yearling coho salmon were alternately allocated into control (handling and anesthesia only; *n*= 10 per site) and implanted (intraperitoneal placement of a dummy radio-transmitter; *n* =10 per site) treatment groups. Dummy transmitters (NTC-M-2; Lotek Wireless Inc, Newmarket Ontario Canada) were identical in terms of size, weight, antenna material and coating type to functional transmitters used in previous survival studies (Beeman et al 2007, Beeman et al.in press). Procedures for surgical implantation of radio transmitters were similar to those described by Adams et al. (1998). Prior to insertion, transmitters were disinfected using a 0.5% disinfectant solution of chlorohexidine diacetate (Nolvasan® Fort Dodge Animal Health, Fort Dodge, Iowa). Transmitters were rinsed twice in sterile water and placed on the sterile portion of a surgical glove wrapper along with the surgical instruments immediately before surgery. Two sets of surgical instruments were alternately employed, enabling one set to be disinfected by soaking in 100 percent ethanol while the other set of instruments was being used in surgery. To implant the transmitter, a 7-mm (approximate) incision was made about 5 mm anterior to the pelvic girdle and about 3 mm away from and parallel to the mid ventral line. The incision made was only deep enough to penetrate the peritoneum (Summerfelt and Smith 1990). The shielded-needle technique described by Ross and Kleiner (1982) was used to provide an outlet through the body wall for the transmitter antenna. The transmitter was positioned to lie slightly posterior to the incision by gently pulling on the antenna. A single simple interrupted suture (Ethicon coated vicryl braided, 5-0 reverse cutting P-3 needle) closed the incision. Only fish weighing

8.6 g or greater were tagged to ensure the transmitter weight did not exceed 5 % of the individual's body weight (Adams et al. 1998).

On 16 May 2008, fish were transported to the T of H campground (rkm 280) and a site directly below IGH (rkm 309), transferred to a floating net pen (1.2 x 0.6 x 0.6 m, lined with 5 x 5 mm bar mesh) and held for 7d in the Klamath River before being transported to the California Nevada Fish Health Center. All fish were transported within a 115 L tank with a battery powered re-circulating pump. Stress Coat® (Aquarium Pharmaceuticals Inc., Chalfont, Pennsylvania) was added to the tank (1 ml/10 L) prior to transport to reduce electrolyte loss and damage to skin tissue.   Prior to and during transport dissolved oxygen in the transport tank was maintained at a minimum level of 80% saturation using oxygen supplied through an air stone at 10 psi. Water temperature was maintained within 2°C of the collection source temperature during transport using dechlorinated ice.

Both groups (19 from IGH cage and 20 from T of H) were transported to the California Nevada Fish Health Center wet lab on 23May and held within a 363 L tank divided into 2 sections by a screen. Inflow of aerated water was 29 L/min and the tank was covered by shade screen. The lab receives ozonated Coleman National Fish Hatchery water and there is no history of either *C. shasta* or *P. minibicornis* infection at this facility. In order to reduce the occurrence of columnaris disease (infection by *Flavobacterium columnare*), 10 minute prophylactic baths of 1 mg L^{-1} furanase were administered for three consecutive days post arrival. River temperature at the 2 sites on 23May was 13-14°C and the fish were held at this temperature for 4 d. In order to simulated Klamath River conditions, water temperature was increased over the remaining 23d to a target temperature of 18°C. Fish were offered 20g of salmon diet daily in 2 feedings. The following samples and data were obtained from each mortality or sampled fish: fork length, weight, presence of tag, external condition of gill (columnaris lesion, anemia) and skin (hemorrhage, fungal infection), presence of blood or catarrhal exudate within the intestine, kidney, intestinal content, and spleen imprints, histological sample of intestine and kidney, and subsample of

these same tissues archived for PCR analysis. Imprints were fixed in methanol, stained with Diff-Quick stain for microscopic evaluation of *C.shasta* trophozoites, gram stained for bacterial infection, or stained with labeled antibody to *Renibacterium salmoninarum* (Direct FAT). The kidney was inoculated onto Brain Heart Infusion agar from a subsample of moribund fish for bacterial isolation. Histological samples were placed in Davidson's fixative, processed for 5 μm paraffin sections, stained with hematoxylin and eosin, and examined for the presence of *C.shasta* (intestine) and *P. minibicornis* (kidney). The severity and distribution of parasite-associated lesions in the tissues was rated as 0 = none, 1= < 33% of section, and 2 = > 33% of section.

Results and Discussion:

General observations- Mean fork length of the exposed coho was 140 mm (SD 14) with average weight of sampled live and dead fish being 31 g. The two groups fed poorly throughout the 27 rearing day period. Survivors were sampled and the study ended on 19 June after a total of 34 dpe.

Tree of Heaven group - The cumulative mortality of the T of H group was 85% (17 of 20) with the first mortality occurring at 16 dpe (Fig. 1). This group experienced a daily mortality rate of 1 or 2 fish per day after 16 dpe. *C.shasta* trophozoites were observed in 89% (17 or 19) of the histological sections or smears taken from this group. Additionally, *C.shasta* myxospores were seen in 11 of the 17 positive fish (65%). Severe erosion of the intestinal epithelium and associated hemorrhages were seen in 16 of the 17 positive samples (see micrograph on title page). *P.minibicornis* was observed in the glomeruli and tubules of all 14 kidney sections examined. Signs of glomerulonephritis (swollen glomeruli with inflammatory cells and protein casts within tubules) were seen in 4 of 14 kidney sections. Those fish with glomerulonephritis were sampled between 32 and 34 dpe. Gram- negative bacteria were either cultured or observed in the spleen or kidney from six T of H mortalities. The bacteria were presumptively identified as aeromonids.

Although sample sizes were small, differences in the time to mortality and incidence of *C.shasta* infection between treatment groups at the T of H site (tagged vs. control) were small and indicate that the surgical procedure and presence of a radio transmitter did not confer an increased susceptibility to disease from *C. shasta*. The mean (±1SD) time to mortality (dpe) for tag and control fish was 28.3 (6.33) and 24.2 (5.87), respectively, and was not significantly different ($t_{0.05\,(1),18}$ = -1.50; P = 0.075; power = 0.30). Similarly, the null hypothesis stating that tagged fish would display less than or equal incidence of clinical disease (parasite present and inflammatory tissue in > 33% of the intestine) as control fish was supported by the data (Cs 2 lesion score among 80% of tagged fish vs. 100% for control fish).

Iron Gate Hatchery group - The IGH group had a 16% cumulative mortality (3 of 19) with all 3 mortalities occurring between 7 and 10 dpe to fish with external fungus infections. An additional fish died 1 day after exposure to the river when it became impinged on the rear screen of the net pen by water flow. We presume the fungal infections were a result of skin damage that occurred in the live cages at IGH where river velocities in the pens were high. Neither *C.shasta* nor *P.minibicornis* was observed in histological sections or smears from these 3 "early" IGH mortalities. *C.shasta* trophozoites were observed in 7 of the 16 IGH salmon (44%) that were sampled at 34 dpe. Three of the 7 infected fish showed signs of severe ceratomyxosis. *P.minibicornis* was observed in the glomeruli and tubules of all 16 kidney samples with 6 infected fish showing signs of mild glomerulonephritis. The null hypothesis stating that tagged fish would display less than or equal incidence of clinical infection rates (parasite present and inflammatory tissue in 33% of the intestine) as control fish was supported by the data (Cs 2 lesion score among 25% of tagged fish vs. 25% for control fish).

No cells resembling *Renibacterium salmoninarum* (agent of bacterial kidney disease) were observed in any of the 39 kidney imprints. *Aeromonas hydrophilia* was isolated from one of the early mortality fish with external fungal infection. No PCR analysis for *C.shasta* was performed due to high incidence of parasite infection detected in histological and smear samples.
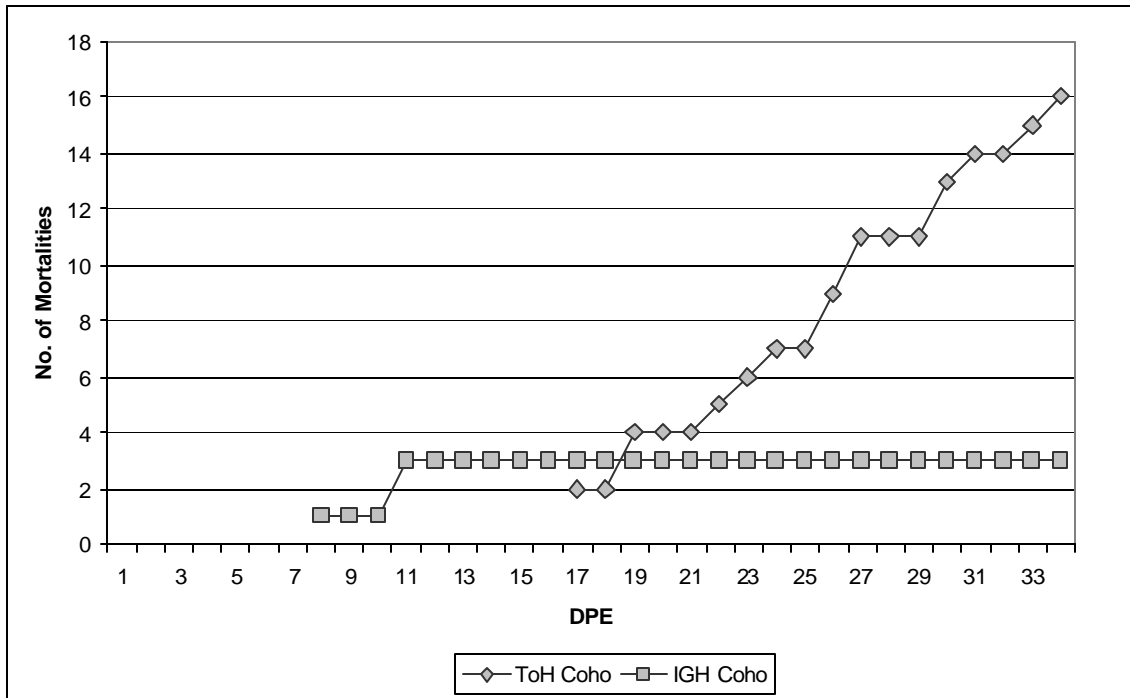
Figure 1. Cumulative mortality curve for yearling coho sentinels exposed at Trees of Heaven (ToH) and directly below Iron Gate Hatchery (IGH) for 7d and held at the wet lab for up to 34 days post-exposure (DPE).

It is apparent that ceratomyxosis resulted in the high T of H group mortality in the May 2008 exposure. This data highlights the negative effect of myxozoan parasites on listed coho salmon survival in the Klamath River and may help explain some of the mortality seen in 2007 radio-tagged coho yearlings. Extrapolating the 2008 finding to all T of H coho released during April and May of 2007 may not be entirely accurate as early outmigrants should have experienced lower actinospore levels and be at a lower risk for lethal disease than later release groups. The available data on 2007 *C.shasta* infectivity indicates that actinospore levels were not considered "high" until May. Nichols and True (2008) report that *C.shasta* DNA was first detected by QPCR in juvenile Chinook captured in the Shasta to Scott R. reach during the last week of April (23%) and that the incidence rose to 37% by mid-May. The potential negative effect of *C.shasta* infection during May on juvenile coho survival is demonstrated by a 13 May young-of-year coho sample with 85% (11 of 16 fish) incidence of *C.shasta* infection and the >80% lethal ceratomyxosis observed in juvenile coho sentinels held for 3 days near the mouth of Beaver Creek in mid-May 2007 (Nichols and

8

True 2008, Bartholomew 2008). In contrast, no infection was detected by QPCR in yearling coho sampled between April and mid-May.

An alternate hypothesis to explain elevated mortality among the 2007 T of H radio-tagged group released prior to May could be that ceratomyxosis can occur in coho that are challenged by lower actinospore levels but for longer periods (weeks). Support for this hypothesis can be gleaned from the migration behavior of radio-tagged juvenile coho in the Klamath River since 2005 (Stutzer et al. 2006; Beeman et al. 2007; Beeman et al. in press). Fish tended to rear near their release site (for up to 43 days in 2007) and would then rapidly move downriver (Beeman et al. in press). In 2007, the average residence time for IGH releases was 11.6 d (range 0.2 – 43.2 d) while those at T of H had an average of 7.2d (range 0.3 – 37.0d). The median travel time decreased for both groups as the season progressed and this is consistent with previous observations. These results illustrate that although the majority of fish in both groups reared in the vicinity of their release site prior to emigration, there was a considerable difference in the amount of time IGH release groups spent in the T of H to Scott River reach compared to the T of H cohorts; an area known to have higher levels of pathogen concentration and infectivity among sentinel trial fish (Stocking et al. 2006).

Ceratomyxosis has been demonstrated to exert a strong influence on juvenile coho survival in the Klamath River. An important research question is to determine the challenge threshold (actinospore concentration and duration) that results in lethal ceratomyxosis of juvenile coho salmon. This data would link seasonal monitoring of actinospore levels in the river to survival models.

References:

Adams, N. S., D. W. Rondorf, S. D. Evans, J. E. Kelly, and R. W. Perry. 1998. Effects of Surgical and Gastrically Implanted Radio Transmitters on Swimming Performance and Predator Avoidance of Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences **55**:781-787.

Bartholomew JL. 2008. *CERATOMYXA SHASTA* 2007 STUDY SUMMARY, OREGON STATE UNIVERSITY FINAL REPORT TO BUREAU OF RECLAMAITON.
HTTP://WWW.FWS.GOV/ARCATA/FISHERIES/PROJECTUPDATES.HTML

Bartholomew JL, SD Atkinson, SL Hallett, CM Zielinski and JS Foott. 2007. Distribution and abundance of the salmonid parasite *Parvicapsula minibicornis* (Myxozoa) in the Klamath River Basin (Oregon-California, USA). Diseases of Aquatic Organisms 78(2):137-146.

Bartholomew JL, SD Atkinson, and SL Hallett. 2006. Involvement of *Manayunkia speciosa* (Annelida: Polychaeta: Sabellidae) in the life cycle of *Parvicapsula minibicornis*, a myxozoan parasite of Pacific salmon. Journal of Parasitology 92:742-748.

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.

Bartholomew JL, JS Rohovec and JL Fryer. 1989. *Ceratomyxa shasta*, a myxosporean parasite of salmonids. US Fish and Wildlife Service. Fish Disease Leaflet 80. Available online: http://www.lsc.usgs.gov/FHB/leaflets/index.asp.

Beeman, J. W., S. D. Juhnke, G. M. Stutzer , and N. J. Hetrick. In press. Survival and migration behavior of juvenile coho salmon in the Klamath River relative to discharge at Iron Gate Dam, 2007. U. S. Geological Survey Open-file report.

Beeman, J. W., G. M. Stutzer, S. D. Juhnke, N. J. Hetrick. 2007. Survival and migration behavior of juvenile coho salmon in the Klamath River relative to discharge at Iron Gate Dam, 2006. Final report prepared by U. S. Geological Survey, Cook, Washington and U. S. Fish and Wildlife Service, Arcata, California for the U. S. Bureau of Reclamation, Mid-Pacific Region, Klamath Basin Area Office, 06AA204092 and 07AA200181, Klamath Falls, Oregon.

Ching HL and DR Munday. 1984. Geographic and seasonal distribution of the infectious stage of *Ceratomyxa shasta* Noble, 1950, a myxozoan salmonid pathogen in the Fraser River system. Canadian Journal of Zoology 62:1075-1080.

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

Henderickson GL, A Carleton, 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.

Hoffmaster JL, JE Sanders, JS Rohovec, JL Fyer, and DG Stevens. 1988. Geographic distribution of the myxosporean parasite, *Ceratomyxa shasta* Noble, 1950, in the Columbia River basin, USA. Journal of Fish Diseases. 11:97-100.

Jones S, G Prosperi-Porta, S Dawe, K Taylor and B Goh. 2004. *Parvicapsula minibicornis* in anadromous Sockeye (*Oncorhynchus nerka*) and Coho (*Oncorhynchus kisutch*) Salmon from tributaries of the Columbia River. Journal of Parasitology 90(4): 822-885.

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 by August 2008: http://www.fws.gov/canvfhc/reports.asp.

Nichols K and K True. 2007. FY 2006 Investigational Report: Monitoring incidence and severity of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infections in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and Coho salmon (*Oncorhynchus kisutch*) in the Klamath River, 2006. US Fish and Wildlife Service California-Nevada Fish Health Center. Anderson, CA. Available online: http://www.fws.gov/canvfhc/reports.asp.

Ross, M. R. and C. F. Kleiner. 1982. Shielded-needle technique for surgically implanting radio-frequency transmitters in fish. The Progressive Fish Culturist 44:41-43.

St-Hilaire S, M Boichuk, D Barnes, M Higgins, R Devlin, R Withler, J Khattra, S Jones and D Kieser. 2002. Epizootiology of *Parvicapsula minibicornis* in Fraser River sockeye salmon, *Oncorhynchus nerka* (Walbaum). Journal of Fish Diseases 25:107-120.

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.

Stone R, JS Foott, and R. Fogerty. 2008. FY 2006 Investigational Report: Comparative susceptibility to infection and disease from *Ceratomyxa shasta* and *Parvicapsula minibicornis* in Klamath River basin juvenile Chinook, Coho and Steelhead populations. US Fish and Wildlife Service California-Nevada Fish Health Center. Anderson, CA. Available online: http://www.fws.gov/canvfhc/reports.asp

Stutzer, G. M., J. Ogawa, N. J. Hetrick, and T. Shaw. 2006. An initial assessment of radio telemetry for estimating juvenile coho salmon survival, migration behavior, and habitat use in response to Iron Gate Dam discharge on the Klamath River, California. U. S. Fish and Wildlife Service, Arcata Fish and Wildlife Office, Arcata Fisheries Technical Report Number TR2006-05, Arcata, California.

Summerfelt, R. C. and L. S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 218-272 *in* C. B. Schreck and P. B. Moyle, editors. Methods for Fish Biology. American Fisheries Society, Bethesda.