United States Department of the Interior

FISH AND WILDLIFE SERVICE

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In Reply Refer To:
AFWO

Technical Memorandum

TO: Dave Hillemeier, Yurok Tribal Fisheries, and

Craig Tucker, Karuk Department of Natural Resources

FROM: Nicholas A. Som and Nicholas J. Hetrick, Arcata Fish and Wildlife Office

SUBJECT: Response to Request for Technical Assistance – *Ceratonova shasta* Waterborne Spore Stages

DATE: September 23, 2016

Purpose. The Arcata Fish and Wildlife Office (AFWO) Fisheries Program is working with its scientific co-investigators to develop a series of four technical memorandums that summarize recent findings of studies that contribute to our current understanding of *Ceratanova shasta* (syn *Ceratomyxa shasta)* infections in the Klamath River, in response to requests for technical assistance from the Yurok and Karuk tribes. Each of the topics addressed in the four technical memorandums: 1) geomorphic channel conditions and flow, 2) polychaete distribution and infections, 3) actinospore and myxospore concentrations, and 4) prevalence of *C. shasta* infections in juvenile and adult salmonids, are identified in a conceptual model diagram (Figure 1) taken from Foott et al. (2011). The intent of the technical memorandums is to provide managers with a contemporary understanding of the state of the science with regard to the *C. shasta* in the Klamath River, and to provide a scientific basis to inform and support resource management decisions. In this technical memorandum, we summarize the state of the science regarding the waterborne spore stages of the parasite and how they infect the salmonid (via actinospores) and benthic invertebrate (via myxospores) hosts in the Klamath River.

Background. High infection rates by the myxozoan parasite *C. shasta* have been documented in emigrating juvenile salmon populations during spring and early summer in the Klamath River (Foott et al. 1999; Nichols and Foott 2006; True et al. 2016; among others), which have been linked to population declines in fall Chinook Salmon (Fujiwara et al. 2011, True et al. 2013). While native salmonids exposed to low doses of the parasite exhibit some degree of resistance (Ching and Munday 1984; Bartholomew et al. 2001), they can become overwhelmed by high infectious doses that result in a diseased state and cause mortality (Ratliff 1981; Ching and Munday 1984; Bartholomew 1998; Stone et al. 2008). Fish that display clinical signs of *C. shasta* infection are also likely to be more prone to mortality because of increased susceptibility to other pathogens such as *Parvicapsula minibicornis* (Figure 2*)*, to predation, and as a result of a compromised osmoregulatory system that is essential for successful ocean entry (S. Foott personal communication).

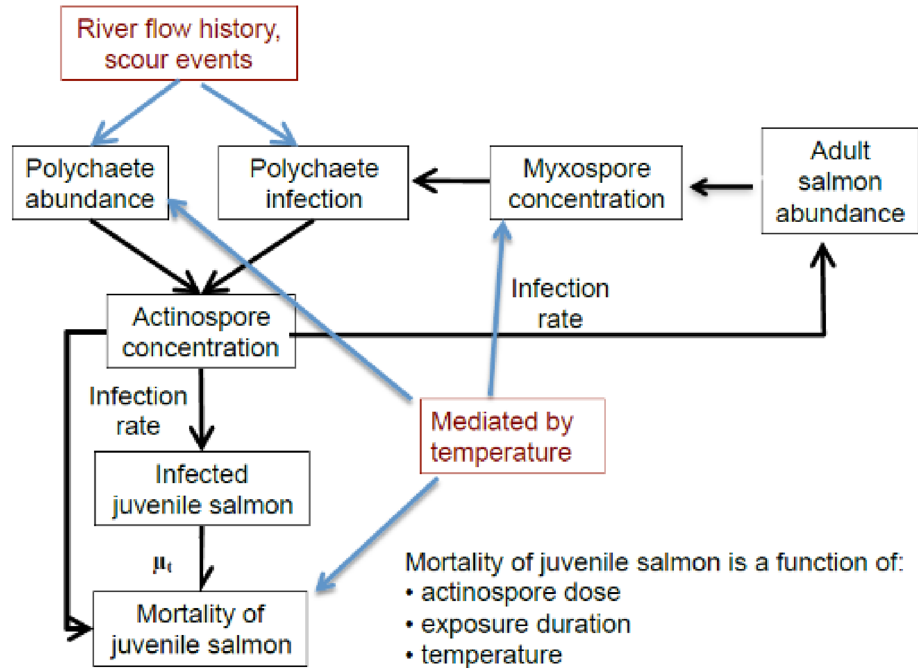

Figure 1. Conceptual model for variables that influence infection and mortality of juvenile Chinook Salmon. with μ_t being the mortality rate of infected juvenile salmon, estimated from weekly actinospore concentrations in water samples. (taken from Foot et al. 2011).

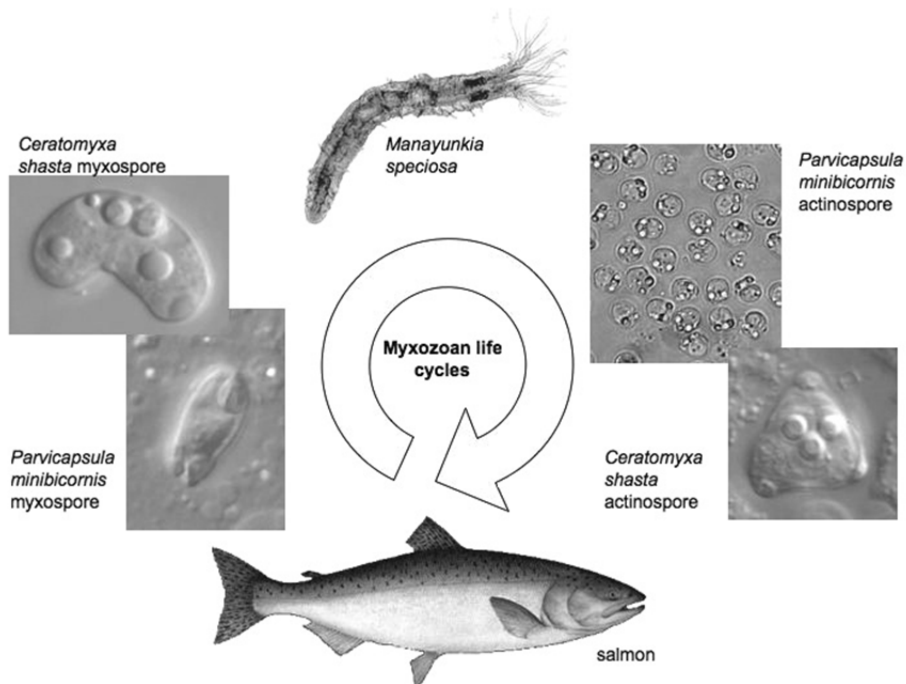

Figure 2. The life cycle of *Ceratomyxa shasta* and *Parvicapsula minibicornis* (graphic provided with permission from J. Bartholomew, Oregon State University). *Manayunkia speciosa* is a small freshwater polychaete worm (3-5 mm in length) and intermediate host of both parasites.

The parasite *C. shasta* is endemic to the Klamath Basin and is assumed to have co-evolved with the different species of salmonids it infects. Coevolution results in parasites that are in dynamic equilibrium with their hosts and low virulence, assuming continued environmental variation under which this equilibrium evolved (Toft and Aeschilimann 1991; Esch and Fernandez 1993). When environmental conditions are significantly altered, however, the change will most often favor the parasite because of its shorter generation time and greater genetic variation as compared to the host (Webster et al. 2007). In general, the parasite adapts more quickly to environmental change than the host, causing the parasite-host equilibrium to shift out of balance (Thompson 1994). This imbalance can be expressed as an elevated prevalence of host infections over naturally-occurring background or equilibrium levels, which is consistent with the abnormally high infection levels observed in juvenile salmon in the Klamath River during some years.

The life cycle of *C. shasta* is complicated and involves salmonids and a freshwater polychaete *Manayunkia speciosa* as alternate hosts, and two microscopic waterborne spore stages (Bartholomew et al. 1997, Meaders and Hendrickson 2009, Figure 2). Actinospores develop within infected polychaete worms that are later released into the water column where they may encounter and infect adult and juvenile salmonids. Clinical signs of the disease state exhibited by infected salmonids include necrosis of intestinal tissue that can be accompanied by a severe inflammatory reaction (enteronecrosis) and subsequent death (Bartholomew et al. 1989). The polychaete invertebrate host is necessary for completion of the life cycle and neither horizontal (fish to fish), or vertical (fish to egg) transmissions have been documented under laboratory conditions. Myxospores develop within infected salmonids and are released into the environment. After release, myxospores may be consumed by and infect polychaete worms, thus completing the life cycle.

The complexity of the *C. shasta* life cycle may lend itself to a variety of management approaches because actions can be tailored to target the different hosts or parasite spore stages, thus arresting the life cycle. Of particular interest, are aspects of the *C. shasta* life cycle that are susceptible to alteration via management alternatives (Figure 1). Given the nature of the parasite's life cycle, disruption of even a single element of the cycle could have profound impacts on survival of juvenile salmonids in the Klamath River.

Environmental Detection. The waterborne spore stages of the parasite alternatively infect the salmonid (via actinospores) and benthic invertebrate (via myxospores) hosts. Prior to the work of Hallett and Bartholomew (2006), detection of infectious actinospores in water relied on the fates of exposed sentinel fish. Hallett and Bartholomew (2006) developed a DNA-based method for water samples that quantifies abundance of *C. shasta* by a quantitative polymerase chain reaction (qPCR) assay. This qPCR assay provides evidence of waterborne *C. shasta* spores much more quickly than exposed sentinel fish. Although this method cannot distinguish between the actinospore or myxospore stages, or between viable and non-viable (dead or partial) spores, DNA collected in water samples can be further processed to quantify the different genotypes of spores present in the Klamath River (Atkinson and Bartholomew 2010). Of most management concern in the Klamath Basin are Type I, which is associated with mortality in Chinook Salmon, and Type II, which is associated with mortality in Coho Salmon. The detection assay has been used to monitor the temporal and spatial distribution of spores in the Klamath River, and help evaluate the effectiveness of naturally occurring or prescribed experimental flows aimed to reduce the concentration of spores in water during periods of juvenile salmonid outmigration.

The J. L. Bartholomew Laboratory began monitoring spore concentrations in 2005 and added long-term monitoring index sites in 2006. In 2007, the sampling calendar was modified to begin

earlier in the water year to capture the initial rise of spore concentrations associated with warming water temperatures. Over time, the sampling calendar and spatial extent of sampling have increased to address various research and monitoring needs. Currently, weekly water samples are collected and processed at five sites during the months of April through October, and year-round at two of those sites. The sampling effort at each site consists of four 1-liter samples extracted from an automatic sampler that pulls 1-liter riverine samples every 2 hours over the prior 24-hour period. The collection and filtration of water samples is coordinated with the Karuk Natural Resources Department and Yurok Tribal Fisheries Program.

Temporal and Spatial Distribution. From late winter to early summer, which encapsulates a single outmigration period, actinospore concentrations (confirmed via sentinel exposures) increase with increasing water temperatures, and then decrease forming a curvilinear pattern (Hallett et al. 2012, Hurst et al. 2012). The timing of spore release (i.e., when spore levels increase to the point of detectability in water samples) usually coincides with the descending limb of the spring hydrograph, and is likely dependent on accumulating thermal units. Meaders and Hendrickson (2009) infected polychaetes with Type II myxospores and tracked actinospore development within a laboratory. Actinospores were generated after 49 days averaging 17.3°C (approximately 850 degree days). Recent laboratory experiments suggest that Type I spores may develop more quickly (approximately 735 degree days) in polychaetes, with actinospores generated after 35 days averaging 21°C (J. Alexander, personal communication).

Actinospore and myxospore viability are both affected by water temperatures, but myxospores are more resilient to higher water temperatures (Chiaramonte 2013). At temperatures near 20°C in a controlled laboratory setting, 50% survival was observed after approximately 25 days, and the hardiest myxospores survived approximately 50 days (Chiaramonte 2013). At 15°C, 50% survival was observed as late as 100 days and the hardiest myxospores lasted 150 days. This is in stark contrast to laboratory-monitored actinospores held in 20°C water, where only 20% survival was observed after 3 days, the most robust of which lasted only 9 days (Bjork 2010). Actinospore survival also related to temperature, and at temperatures near 12°C approximately 50% survival was observed at 3 days and the hardiest spores survived 15 days.

The current hypothesis is that myxospores released from adult salmon carcasses contribute the bulk of myxospore to the system (Foott et al. 2016). The release of myxospores from adults likely occurs within several weeks of pre- or post-spawn mortality. Hence, the timing of myxospore contribution closely aligns with the timing of salmon spawning, which in recent years coincides with a stable hydraulic period below Iron Gate Dam (Figure 3). Recent laboratory studies estimate the settling rate of *C. shasta* myxospores at 0.35—0.45 m/day (Miao and Deas 2015). This rate suggests that suspended myxospores could settle in riverine locations quite distant from entry location under turbulent high-flow conditions. The settling characteristics and relative resiliency of myxospores suggests that they could be prone to redistribution and potential infection of polychaetes at spatial locations and time periods distant from their initial release time and location.

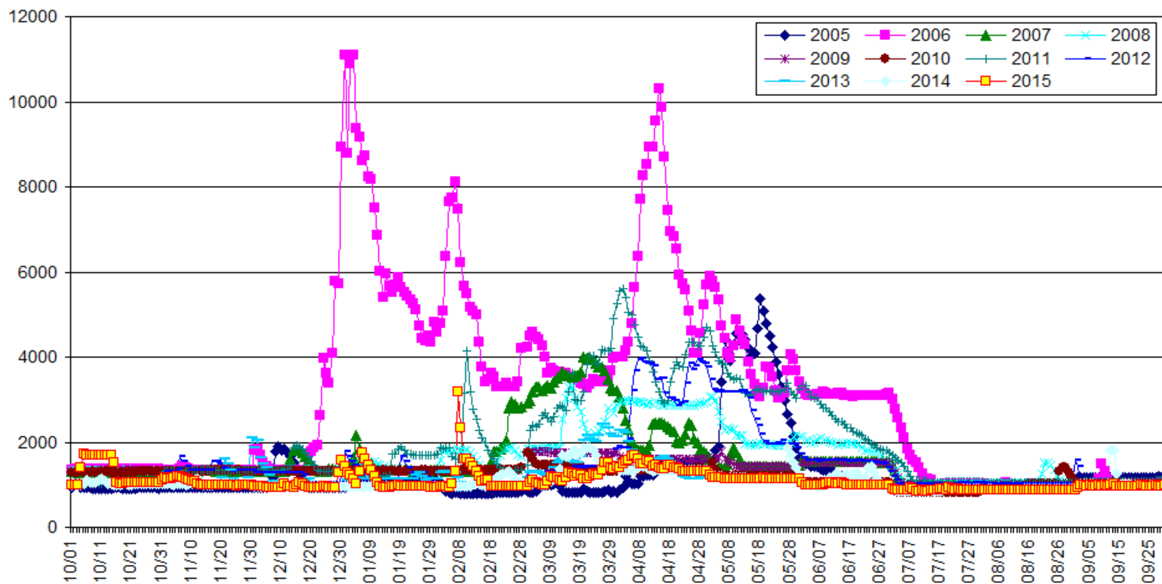

Figure 3. Daily discharge from Iron Gate Dam as measured by U.S. Geological Survey gauge 11516530 for water years 2005 – 2015. Reported discharge values are in units cubic feet per second. http://waterdata.usgs.gov/ca/nwis/uv?format=gif&period=10&site_no=11516530

The concentration of spores varies spatially within the mainstem river, with the highest spore concentrations typically detected near the confluence of Beaver Creek (Hallett et al. 2012). However, the spatial peak has occurred downstream of Beaver Creek (e.g., near confluences of Seiad Creek or Tully Creek) in some years (Bartholomew 2010, 2011, Table 1). In 2016, the index water sampling sites near Orleans and Tully Creek had much higher peak spore concentrations than any of the upstream locations, which has not previously been observed (http://microbiology.science.oregonstate.edu/content/monitoring-studies). Further, the difference between the peak spore concentrations at the Orleans index site and the upriver sites was of a much higher magnitude than observed in previous years. One hypothesis for the unique spatial pattern of spore concentrations observed in 2016 relates to the 11,200 cfs Iron Gate Dam discharge event occurring March 2016. This event could have dislodged and moved high numbers of polychaete worms downstream in the drift and these redistributed worms, if infected, may have contributed to the relatively high spore concentrations observed in the lower river (J. Alexander, pers. comm).

The annual peak concentration of actinospores varies considerably, with observed differences of several orders of magnitude among years. (Table 1). Predicting annual peak actinospore concentration levels remains elusive. The difficulty in prediction is likely attributable to the currently unavailable population-level estimates of both myxospores released by adult salmon, and abundances of infected polychaetes in each year. Further, knowledge of the exact timing or dynamics of infection and parasite transmission to polychaetes is still not well understood under riverine conditions.

Table 1. Annual maximum actinospore concentrations recorded at the Beaver Creek ("BC Peak") water monitoring index site during the Chinook Salmon outmigration period (spring and early summer). Values provided are approximations of spores per liter based on a standard curve transformation of detection assay readings, and include the totals pooling over all genotypes. In years where the system maximum value occurred at another site, the location and maximum value are indicated ("Alt. Peak"), where KTC represents Tully Creek, KSV represents Seiad Valley, and KOR represents the Orleans water monitoring index sites. Data provided by the J. L. Bartholomew Laboratory, Oregon State University.

Year	BC Peak	Alt. Peak
2005	75	
2006	8	
2007	250	
2008	300	
2009	150	
2010	20	KTC: 100
2011	10	
2012	1	KSV: 5
2013	5	KTC: 8
2014	100	
2015	1200	
2016	50	KOR: 250

Effects of Discharge on Spore Concentrations. Due to water management concerns impacting the entire Klamath Basin, experimental discharge increases have not generally been available to assess the effects of within-year discharge changes on spore concentrations. There have been several years where elevated discharges from Iron Gate Dam have occurred between April and June, the time of year when actinospore levels are rising or at their highest. During high flows events occurring in spring 2006 (10,300 cfs) and 2011 (5,700 cfs), spore concentrations were below the detection limit prior to the increases in discharge. Hence, it is not possible to assess the dilution potential of elevating discharge in those years. Some insight into the dilution potential of elevated flows may be gained from two additional events that occurred over the last decade. The first event was an unplanned discharge increase occurring in May of 2005, and associated fish-health and water monitoring data may demonstrate an effect of discharge on spore concentrations. A planned pulse event occurred in 2014, and this event was well monitored. Both are described below.

By 2005, the impacts of *C. shasta* on salmonids in the Klamath Basin had risen to the point that fish health and water monitoring programs were being implemented throughout the basin. Weekly-stratified fish-health surveys began in March of 2005. Water monitoring assays adept at quantifying spore concentrations in water samples had just been developed (Hallett and Bartholomew 2006), and water sampling began in May of 2005.

In April, the weekly-stratified prevalence of infection estimates began to quickly rise, and eventually reached 100% of sampled fish at the beginning of May (Figure 4). Despite no water sampling occurring during this time of year in 2005, the estimated prevalence of fish infections undoubtedly demonstrates the presence of actinospores in the water. Further, given the relatively

high estimated prevalence of fish infections, once could safely assume concentrations well above 10 spores/L threshold reported by Hallett et al. (2012) for infectioning Chinook Salmon.

Towards the end of April, releases from Iron Gate Dam increased slightly, but in early May discharge spiked sharply and remained elevated for nearly a month (Figure 4). Soon after the spike in discharge, weekly prevalence of infection values began to decrease. One explanation for a decrease in the weekly prevalence of infection estimates could be the addition of hatchery-released fish that were not in the river long enough to become infected. This influx of uninfected fish would dilute the weekly samples, resulting in lower prevalence of infection estimates. However, the first Iron Gate Hatchery release in 2005 occurred on May 15, several weeks after the observed decrease in weekly prevalence of infection estimates. Additionally, the first water monitoring sample was collected when discharge was elevated and resulted in a non-detection (equivalent to an estimate of ~ zero spores/L). After flows had receded to base levels, subsequent water monitoring samples revealed detectable levels of spore concentrations (Figure 4).

In 2014, water monitoring samples indicated that spore densities had reached levels of concern for Coho Salmon. To prevent or reduce a disease outbreak in juvenile fish, federal agencies and partners agreed to increase discharge as a possible solution to dilute spore concentrations or otherwise disrupt the *C. shasta* life cycle. For this event, discharge was increased to approximately 1,900 cfs on May 27, and held for 24 hours. Flows were reduced approximately 200 cfs each subsequent day until a base discharge of 1,000 cfs was reached on June 2 (Figure 5).

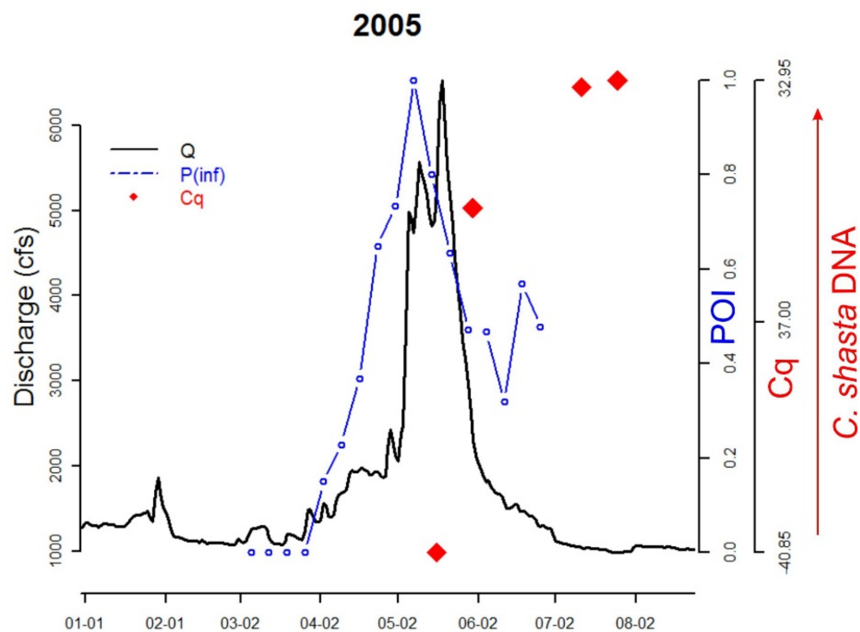

Figure 4. Daily river discharge (solid black line), weekly-stratified prevalence of *C. shasta* infection among sampled Chinook Salmon (open blue circles connected by blue lines), and Cq scores for water monitoring samples (solid red diamonds), all estimated for an area of the mainstem Klamath River between the Shasta and Scott confluences. The inset right axis represents the range of prevalence of infection values in fish, and the outset right axis represents Cq values that reflect quantities of *C. shasta* DNA; these are scaled so that increasing values correspond to increases in spore concentrations.

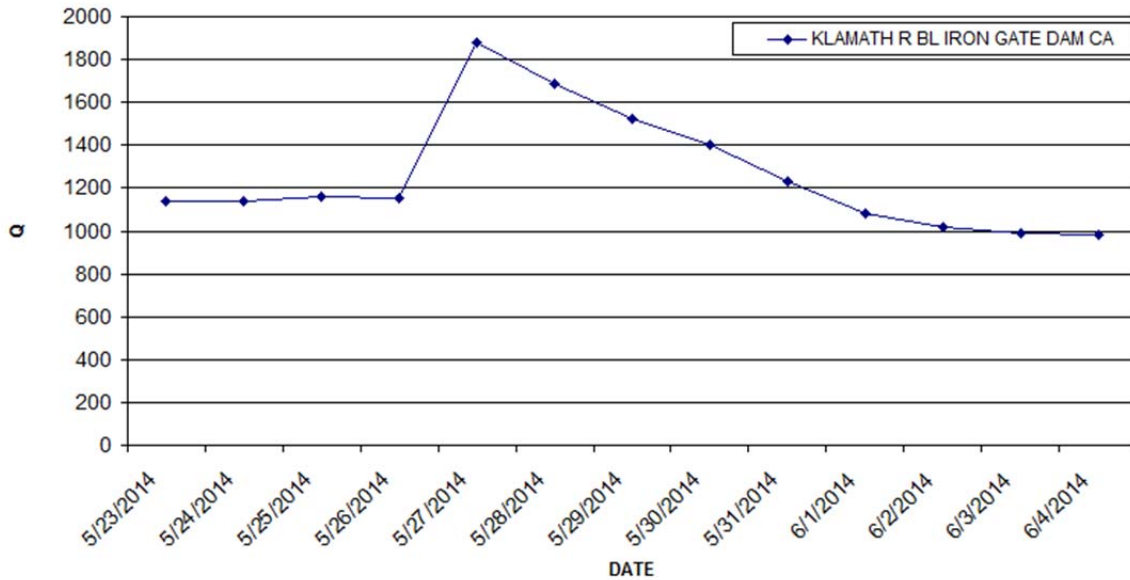

Figure 5. Daily discharge (Q) from Iron Gate Dam as measured by U.S. Geological Survey gauge 11516530 for May 23, 2005 – June 4, 2005. Reported discharge values are in units cubic feet per second.
http://waterdata.usgs.gov/ca/nwis/uv?format=gif&period=10&site_no=11516530

As a planned event, monitoring to evaluate the effectiveness of the increased discharge was coordinated. In particular, Oregon State University monitored spore concentrations at two locations. Water samples were collected daily at Beaver Creek and Seiad Valley index sites, beginning three days prior to the event and ending on May 30. Samples were collected every 2 hours using automated samplers, and pooled to make a 6-hour composite sample that was assayed using a *C. shasta*-specific qPCR. Data were more complete for Seiad Valley (Figure 6). At both sites there was a noticeable decrease in spore concentrations immediately following the increase in discharge. This reduction in spore concentrations, however, did not persist, and spore concentrations increased with decreasing discharge (Figures 5 and 6). The continued rise in spore concentrations may have been amplified by increasing temperatures.

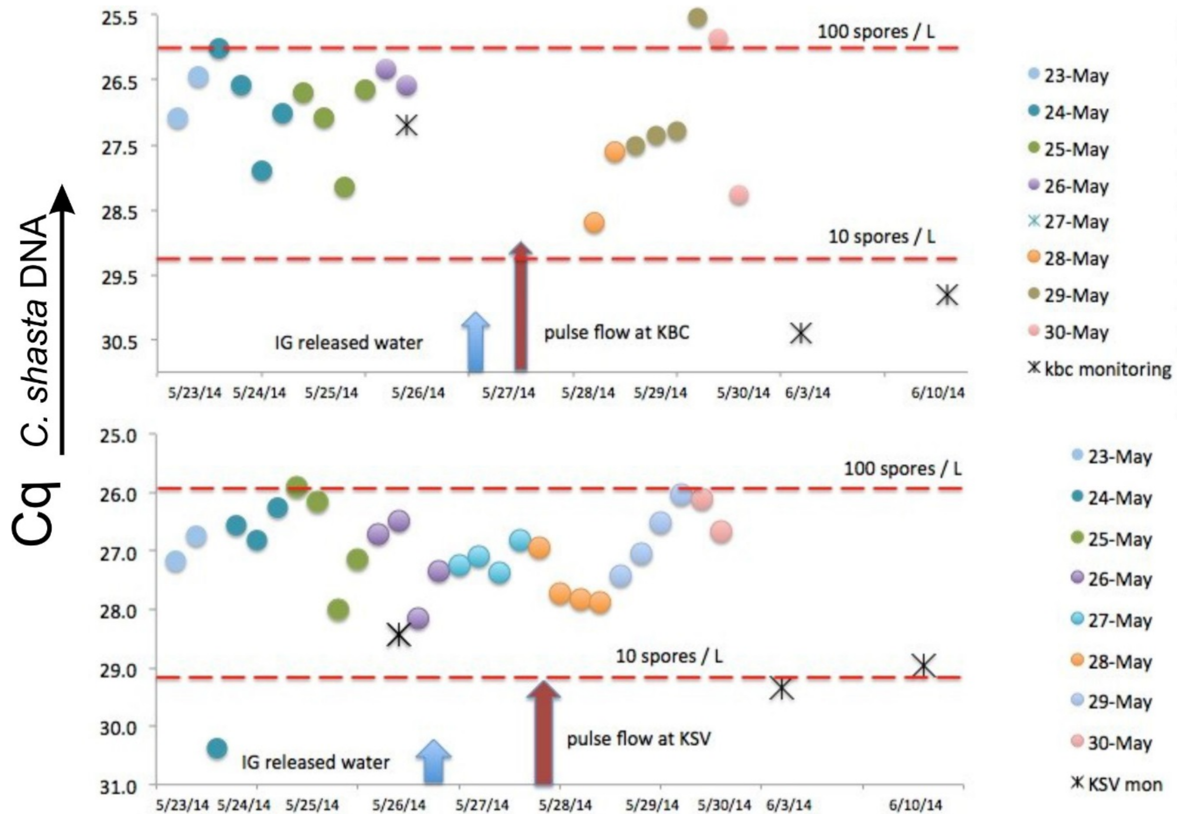

Figure 6. Quantitative PCR results, also expressed as *C. shasta* spores/L, at the Beaver Creek (top) and Seiad Valley (bottom) index water monitoring locations. Each data point is the average Cq of 3 x 1L water samples (6h composite). A lower Cq value indicates more parasite is present. Blue arrows mark the date of increasing discharge from Iron Gate Dam, and red arrows mark the arrival of the increased discharge at each index location. Figure provided by the J. L. Bartholomew Laboratory, Oregon State University.

Summary Guidelines.

- A DNA assay allows for the quantification of spore concentrations in water samples.
- Spore genotypes have been shown to associate with salmonid species-specific mortality.
- Myxospores are hardier than actinospores, and likely survive for a longer period after release from their host organism.
- The majority of myxospore load to the system is likely via adult salmon carcasses in the fall.
- Generally, actinospore spore concentrations increase with increasing water temperatures in the spring and then decrease as water temperatures further increase during summer.
- The location of peak actinospore concentrations varies among years, but most frequently occurs near the confluence of Beaver Creek.
- It is not uncommon for actinospore concentrations to peak as far downriver as the Tully Creek confluence. However, the magnitude of the difference between the peak spore

concentrations downriver and at Beaver Creek was much higher in 2016 than for any other year since water monitoring began.

- Annual peak actinospore concentrations vary by several orders of magnitude.
- Actinospore development within polychaetes is likely a function of accumulating thermal units, and likely takes between 100 and 115 days.
- Though managed discharge events have not produced dramatic reductions in spore concentrations, the planned discharge increases were likely too small to be biologically effective. An unplanned discharge increase in 2005 likely demonstrates the potential for larger discharges to effectively reduce spore concentrations.

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