Klamath River Fish Health Studies: Salmon Disease Monitoring and Research

Oregon State University, BOR/USGS Interagency Agreement #R15PG00065

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ANNUAL REPORT

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Four aspects of OSU's Klamath River research – the parasite, host habitat, polychaete and fish hosts.

Summary

The myxozoan parasite *Ceratonova shasta* infects the intestine of salmonid fishes, which can lead to enteronecrosis and mortality. The parasite is endemic to the Pacific Northwest of North America and has been responsible for high mortality in juvenile salmon in the Klamath River basin. *Ceratonova shasta* cycles between two hosts and two spore stages: waterborne actinospores released from freshwater polychaete worms infect salmonids and develop into myxospores, which are then infectious to polychaetes. The Bartholomew Lab at Oregon State University has been monitoring the spatial and temporal abundance of the parasite in the Klamath River basin since 2006 using sentinel fish exposures, river water sampling, and polychaete sampling. This report describes monitoring studies conducted in 2016. Those data are informing several models being developed to better predict disease effects under various temperature and flow conditions.

Sentinel fish exposures commenced later in the month in April 2016 than in 2015 because lower levels of *C. shasta* parasites were observed in river water samples (which was in line with most previous years). April and May sentinel exposures resulted in low loss of Chinook with *C. shasta*-infections at index sites below Iron Gate Dam. In April, only 2.6% of the Chinook exposed near Beaver Creek died from *C. shasta* and none at Seiad Valley. For the May exposures, the loss of Chinook with *C. shasta*-infections was 2.5% at I5 Bridge, 0% near Beaver Creek, 2.4% at Seiad Valley and 10% at Orleans. The June sentinel exposures of Chinook at the four index sites below Iron Gate Dam demonstrated an expanded infectious zone similar to 2015 with moderate levels of *C. shasta* at I5 Bridge (26%) and Beaver Creek (30%), higher severity at Seiad Valley (60%) and the highest at the lowermost site tested, Orleans (81%). The 2016 infectious zone was the most extensive since we began sentinel exposures (2006), but with the greatest loss at the lowest site tested (Orleans). Sentinel exposures of Chinook in September resulted in no loss associated with *C. shasta* for those exposed near Beaver Creek, 9.4% for those at Seiad Valley and 39% of those from Orleans; thus similar to May and June, in September the Chinook exposed at the lowermost site (Orleans) had the highest loss associated with *C. shasta*. No Chinook exposed in the upper river in May and June died from *C. shasta*.

No Iron Gate Hatchery coho were available for exposures this year, but we were able to obtain Trinity River Hatchery juvenile coho, which we exposed near Beaver Creek and Seiad Valley in June; no *C. shasta*-mortalities occurred. The absence of any mortal infection in coho may have been the result of a very low level of the parasite in the river in June or perhaps Trinity River Hatchery coho are more resistant to *C. shasta;* we have never compared the relative susceptibility of coho from these two hatcheries.

For the Williamson River of the upper Klamath basin, we had predicted that changes in stocking practices of susceptible rainbow trout would result in decreasing levels of the parasite (genotype II). However, sentinel exposures of susceptible rainbow trout in the Williamson River resulted in high mortality, as in previous years. At Keno Eddy, exposed susceptible rainbow trout in May and June became infected with *C. shasta* genotype 0 resulting in development of myxospores in the intestine but the fish did not develop any clinical disease signs. During our May, June and September exposures of rainbow trout near Beaver Creek, in all previous years we observed 95-100% mortality but in 2016 the loss was much reduced: May 17%, June 26% and September 36%.

Molecular analysis of water samples detected *C. shasta* throughout the lower basin. Density of *C. shasta* at the original 'hot spot', Beaver Creek index site, was lower in 2016 than 2015 but higher than 2014. However, spatial distribution of the parasite differed this year with the highest levels recorded at Orleans followed by Tully Creek. Peak levels at Orleans were more than three times as high as those at Beaver Creek (>300 spores/L versus <100 spores/L). Levels detected in water samples were consistent with the sentinel fish exposure outcomes. In 2016, as in all years, the majority of spores at all time points during salmonid outmigration at Beaver Creek were genotype I (Chinook). Only low levels (none to trace) of type II (coho) were detected throughout the sampling period except on one day, June 13, when the average reached 5 spores/L. Type 0 (Steelhead) was detected also.

We assembled and annotated a higher quality *C. shasta* transcriptome, and used this to train *de novo* gene prediction models to apply to our draft genome assembly. Using the genome and transcriptome we discovered

that *C. shasta* has five protein-coding mitochondrial genes (*cox1*, *cox2*, *cob*, *nad1* and *nad5*). We designed specific primer pairs to amplify *cox1* from different *C. shasta* samples that we had already ITS-1 genotyped from fish tissue, including coho salmon and rainbow trout (presumptive biotypes IIC and IIR, respectively). Initial multi-sequence alignments showed that the *cox-1* locus has higher resolution than ITS-1 for genotyping fish isolates: We have identified several genetic markers (SNPs) that distinguish biotypes IIC and IIR at the genetic level. Interestingly, these first data suggest that the two biotypes represent upper Klamath basin versus lower Klamath basin *C. shasta* strains, rather than coho-specific versus rainbow-specific types. We are on track to publish the genome/transcriptome summary paper in 2017, once *de novo* gene prediction is complete. We have completed the penultimate version of our manuscript that reports the finding that genotypes "II" and "III" are actually just genetic variants of the same strain of *C. shasta* (and should therefore no longer be regarded as distinct genotypes).

Results from polychaete density and infection assays completed in 2016 were remarkably different from those obtained in previous years: Densities decreased at all monitoring sites following the high magnitude flow event in March 2016. Infection prevalence was generally low in 2016 (<1%) which is in contrast to levels observed in 2015 (>1%). However, by late spring (June), densities had begun to increase at river sites downstream IGD including the Seiad Valley and Orleans sites, which are not normally characterized by elevated densities prior to late summer. However, prevalence of infection was high in polychaetes at the Orleans site, resulting in estimates of 5,000-35,000 infected polychaetes m^{-2}. We suggest that polychaetes displaced from reaches below Iron Gate dam during the high magnitude but short duration flood in March settled out at KOR resulting in the relatively high densities detected at this site.

We refined the three-dimensional hydrodynamic model coupled with CE_QUAL_W2 temperature model and Lagrangian particle tracking model to 1) estimate water age, 2) model dispersion of waterborne parasite spore along the river, and 3) predict water temperature. The manuscript describing this approach has been submitted to the *Journal of Hydrology* and data outputs from the particle model are being used to develop models for predicting polychaete infection risk at our index sites.

We completed and validated models that predict distribution of *M. speciosa* under alternate flow scenarios, 1,200 cfs and 7,950 cfs, to simulate dry and wet water years, and actual peak flows from 2012-2014, and 2016. We built predictive models for density and distribution of infected polychaetes and are in the process of testing these models.

Highlights:

- the infectious zone in the lower Klamath River was more extensive in 2016, extending downstream beyond Seaid Valley (KSV) to Orleans (KOR); reflected in sentinel fish exposures as well as water samples

- the highest Chinook mortality occurred at the Orleans index site in all months tested (May, June and September); in June, Chinook exposed at Orleans succumbed to disease quickly (began dying on day 13 post-exposure and most fish had died before 25 days post-exposure)

- no Trinity River Hatchery coho exposed at either Beaver Creek or Seiad Valley in June died from *C. shasta*-infection

- high mortality continued in susceptible rainbow trout exposed in the upper Klamath basin despite the change in stocking practices

- ITS-1 genotype 0 produced myxospores in susceptible rainbow trout exposed at Keno Eddy and infection was not fatal

- rainbow trout mortality at Beaver Creek was low; the highest being 36% compared with 95-100% in previous

years (2016 was the first year in which no rainbow trout died with *C. shasta* infection when exposed near Beaver Creek in April; consistent with no type II detected in water samples during April)

- ITS-1 genotype I dominated the water samples; none to <5 spores/L of type II were detected during salmonid outmigration; type 0 was also detected

- polychaete densities decreased at all index sites following the high magnitude peak flow event in March 2016 and densities were high at Orleans compared to previous years, which supports results from water sampling and sentinel exposures that demonstrate high parasite levels at this site

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Research outcomes

<u>Objective 1.</u> Develop a long-term dataset on disease severity for Chinook and coho salmon that encompasses years differing in the magnitude and timing of flows, temperatures during spring and summer, and adult returns.

Task 1. The following metrics will be measured at established index locations in the upper and lower Klamath River during each study year:

(a) Infection and disease severity in sentinel Chinook and coho salmon
(b) Parasite density in water samples
(c) Density and infection of the invertebrate (polychaete) host

<u>Task 1.1. Determine infection and disease severity in sentinel Chinook and coho salmon, according to the following site schedule.</u>

Sentinel fish exposures will occur at the following sites:

(1) Lonesome Duck - RKM 14.4 (Williamson River)
(2) Williamson River – RKM 441
(3) Keno Eddy - RKM 369
(4) I5 Bridge Fish Trap - RKM 287
(5) above Beaver Creek – RKM 258
(6) Seiad Valley – RKM 207
(7) Orleans – RKM 90
(8) Tully Creek – RKM 62

Sentinel fish exposures will occur according to the following schedule:

(1) late April - above Beaver Creek and near Seiad Valley
(2) mid-May – six mainstem sites
(3) mid-June – seven mainstem sites
(4) July – possible exposure above Beaver Creek
(5) mid-September - above Beaver Creek, near Seiad Valley and Orleans

<u>Task 1.1. Methods</u>

Sentinel fish exposures were conducted according to the sites and schedule above. In 2016, there were four exposures for 72 h each at two to seven index sites (Figure 1.1.1) in the lower and upper Klamath River mainstem during the following dates: April 21-24, May 16-19, June 21-24 and September 17-20. As in previous years, known *C. shasta*-susceptible triploid rainbow trout stock from Roaring River Hatchery (Oregon Department of Fish and Wildlife) was held at most sites. Klamath River fall Chinook juveniles from Iron Gate Hatchery (IGH) (California Department of Fish and Wildlife (CDFW)) were held at all sites except for one location, the Lonesome Duck Resort on the Williamson River. As in 2015, juvenile coho salmon were not available in 2016 from IGH because they did not have sufficient fish to meet their production goals. However, a limited number of juvenile coho salmon from Trinity River Hatchery (TRH) (CDFW) were available for sentinel exposures near Beaver Creek and Seiad Valley in June. Generally, the number of each fish species held in live cages other than when noted was 40 rainbow trout, 40 IGH fall Chinook salmon and 30 coho salmon. In April, the sentinel juvenile fish were approximately 0.9-1.6 g, in May 2-3 g, June 3-7 g and in September 14 – 20 g. The TRH coho salmon were about 5-6 g during the June exposures.

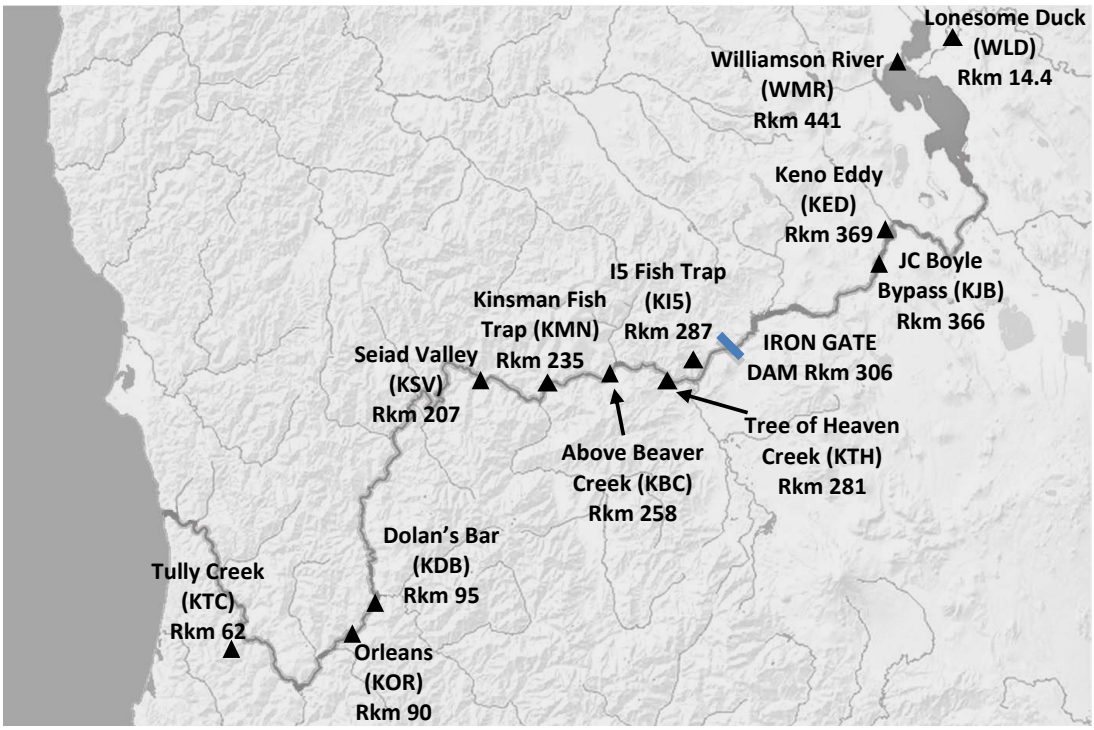

FIGURE 1.1.1. Klamath River index sites for 2016 with site abbreviations and river kilometers (Rkm).

Following the river exposure, the fishes were transported to the OSU John L. Fryer Aquatic Animal Health Laboratory (AAHL), Corvallis, Oregon and held in well water at a water temperature similar to the river water temperature during the 72-h exposure. However, even if river water temperatures averaged greater than 18°C, fish were maintained in no greater than 18°C water post-exposure because attempting to hold fish at higher water temperatures such as 20-22°C made infections of *Flavobacterium columnare,* the cause of columnaris disease, difficult to prevent. During the last hour of transport, the fish were given a 1-2 µg/mL Furanase or Furan 2 bath in their transport containers to prevent columnaris disease. Also, within one to two weeks of their arrival at the AAHL, all fishes were treated with formalin baths and oxytetracycline (TM 200) medicated food to prevent external parasites and bacterial infections. Control groups of each fish stock not exposed at the Klamath River sites were included for each monthly exposure and given the same preventative treatments as the river exposed fish. All groups of fish were monitored daily for *C. shasta* clinical disease signs for two months. Moribund fishes were euthanized and examined microscopically for *C. shasta*-infection by observing wet mounts of lower gut material; if no myxospores were observed then intestinal samples were collected for *C. shasta*-PCR testing. A subsample of moribund fish from each group was also necropsied for other parasite and bacterial infections to eliminate those as causes of loss. Mortality percentages given in the results section below represent total fish loss with *C. shasta*-infections determined microscopically or by PCR testing from fish that died later than five days after they were brought to the laboratory.

Methods specific to each of the exposures are listed as follows:

April 21-24 exposures: In 2015, the April sentinel exposures were conducted earlier than in previous years (usually the third or fourth week of April) because ongoing river water sampling for presence of *C. shasta* indicated the parasite level was increasing possibly due to the drought conditions in the Klamath River watershed. However, in 2016 we reverted to conducting the exposures during the third week of April because parasite levels found in the river in early April were low. Susceptible rainbow trout and IGH fall Chinook salmon were exposed in the Klamath River at the lower mainstem site upstream of the Beaver Creek confluence (KBC, 40 of each species) and only IGH Chinook salmon near Seiad Valley (KSV, 40 fish/cage). The river water temperatures during exposure ranged from 11-14°C so all fishes were held at 13°C upon return to the laboratory during the post-exposure rearing.

May 16-19 exposures: The May sentinel exposures were done at six sites including two in the upper basin, the lower Williamson River (WMR) and Keno Eddy (KED), and four sites below Iron Gate Dam including near the I5-bridge (KI5), near Beaver Creek, Seiad Valley and Orleans (KOR). Susceptible rainbow trout (40 fish/ cage) were exposed at four sites, two in the upper river, WMR and KED and two sites below Iron Gate Dam, KI5 and KBC. In the previous four years, mortality of susceptible rainbow trout after the May exposure was near 100% at the three lowermost sites (KBC, KSV and KOR). Anticipating the same results for the lower river sites in 2016, rainbow trout were exposed only at KBC, as a representative of the three sites, and at KI5 where losses have varied from year to year. Iron Gate Hatchery Chinook (40 fish/cage) were held at all six sites. No juvenile coho from IGH were available for the sentinel exposures in May. The river water temperature during the exposure averaged 15-17°C in the upper river and 16-18°C at the lower river sites. Upon return to the AAHL, groups were reared at 18°C water temperature.

June 21-24 exposures: This month, fish were placed at seven sites including two locations on the lower Williamson River (Nature Conservancy at the mouth of the river, WMR and Lonesome Duck Resort, a few km upriver, WLD) and Keno Eddy in the upper river. In the lower River, fish were held below Iron Gate Dam near the I5 Bridge, near Beaver Creek, Seiad Valley and Orleans. Juvenile Chinook (40 fish/cage) were exposed at six sites but only rainbow trout (40 fish/cage) were held at Lonesome Duck Resort on the lower Williamson River. Susceptible rainbow trout (40/cage) were exposed at the three sites in the upper river (WMR, WLD and KED) and only at KI5 and KBC for the same reason these two sites were chosen in May (see above). Trinity River Hatchery juvenile coho (30 fish/cage) were held in the river only near Beaver Creek and Seiad Valley. In the Williamson River and at Keno Eddy, the average water temperature during the June sentinel exposures was 17-18°C but at sites below Iron Gate Dam the water temperature was higher, averaging 20-21°C. After the 72-h exposure, all groups were transported to the AAHL, reared in 18°C well water and monitored for loss from *C. shasta*.

September 17 - 20 exposure: In September, IGH fall Chinook salmon and the Roaring River Hatchery *C. shasta*-susceptible triploid rainbow trout (40 fish of each species) were exposed at three sites in the Klamath River, near Beaver Creek, near Seiad Valley and at Orleans. The Orleans site was added September this year because exposure results for June showed greater loss in Chinook salmon with *C. shasta* infections at the two lower river sites, Orleans and Seiad Valley. The river water temperatures during exposure averaged 19-20°C. After the 3-day exposure, fishes were reared in well water at 18°C at the AAHL and monitored for enteronecrosis.

Task 1.1. Results and Discussion

Average water temperatures during the 72-h exposures at all sites and the laboratory post-exposure rearing temperature are shown in Table 1.1.1. Average water temperatures ranged from11-14°C during the exposure in April to 19-20°C in September. The maximum laboratory rearing water temperature of 18°C was chosen to avoid loss from *F. columnare*. For comparison, Figure 1.1.2 shows the average daily water temperature during the months of March to September near Beaver Creek for 2011 - 2016. Water temperatures in April and May of 2016 were somewhat in the middle range compared to the much cooler spring of 2011 and the higher temperature peaks of May 2015. However, in June 2016 water temperatures were cooler than the previous five years.

TABLE 1.1.1. Average Klamath River water temperature (°C) at sentinel sites during the 72-h fish exposures in 2016.

Site	April 21-24	May 16-19	June 21-24	Sept 17 - 20
Williamson R-WMR		15.3	17.9	
Williamson R-WLD			17.1	
Keno Eddy-KED		17.1	18.5	
Klamath R I5-KI5		17.3	20.0	
Beaver Creek-KBC	13.9	17.8	20.9	19.2
Seiad Valley-KSV	11.1	16.3	20.7	19.5
Orleans-KOR		15.9	20.1	19.8
Lab rearing	13.8	18.0	18.0	18.0

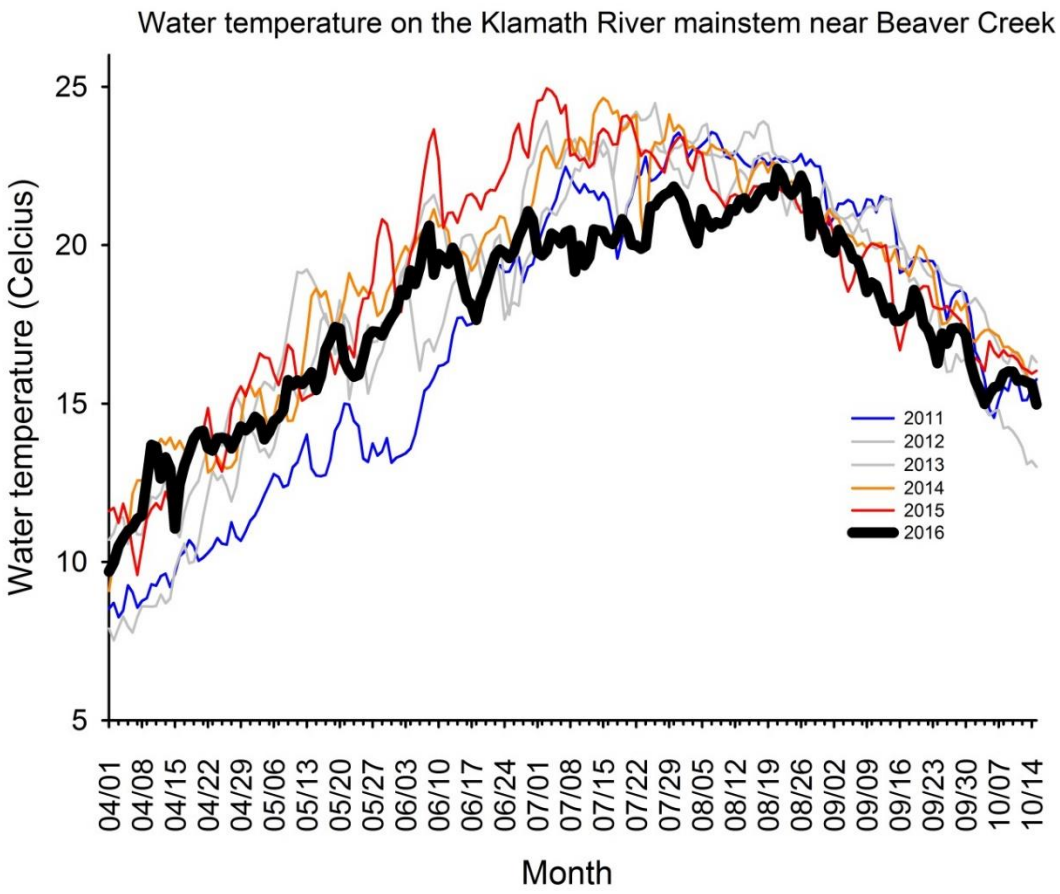

FIGURE 1.1.2. Average daily water temperatures during the months of March-September during the years 2011-2016 at the sentinel site near Beaver Creek (KBC) on the Klamath River mainstem.

Results of the sentinel exposures in April, May, June, and September are summarized in Table 1.1.2 for all exposures in 2016 and are shown for each month in Figures 1.1.2-1.1.15. The percent loss represents fish that were moribund or dead and were removed from the tanks during the post-exposure rearing, not including any loss that occurred during the first five days. These fish were found to be positive for infections of *C. shasta* either by microscopic observation for myxospores in intestinal wet mounts or polymerase chain reaction (PCR) testing of intestinal tissue. The results for each exposure are discussed below after each figure along with a comparison with previous year's results.

TABLE 1.1.2. Percent loss associated with infection by *C. shasta* by site and fish species in 2016 following a three-day river exposure. Fishes were held at ambient Klamath River temperature at the Aquatic Animal Health Laboratory and monitored for disease signs for 62-65 days post-exposure. Numbers represent total loss after the initial 5 days of rearing when the fish were brought to the laboratory and are based on the observation of myxospores in wet mounts and include PCR testing on all microscopically negative fish.

Exposure dates	Exposure site and water temperature	Fish species		
		IGH Chinook salmon	TRH coho	Rainbow trout
April 21-24	KBC 14°C	3		0
	KSV 11°C	0		
May 16-19	WMR 15°C	0		100
	KED 17°C	0		2
	KI5 17°C	3		7
	KBC 18°C	0		17
	KSV 16°C	2		
	KOR 16°C	10		
June 21-24	WMR 18°C	0		90
	WLD 17°C			90
	KED 18°C	0		0
	KI5 20°C	26		7
	KBC 21°C	30	0	26
	KSV 22°C	60	0	
	KOR 20°C	81		
September 17 - 20	KBC 19°C	0		36
	KSV 19°C	9		81
	KOR 20°C	39		100

April 21-24 exposure (Figure 1.1.2): By termination, after 65 days of rearing, only 2.6% (1 fish) of the Chinook salmon exposed near Beaver Creek and no fish at Seiad Valley had become moribund. For the susceptible rainbow trout, no loss occurred of those exposed near Beaver Creek. One control rainbow trout (unexposed) died during the holding and was found to have opportunistic bacteria in the kidney but no infection of *C. shasta*. Fish that died were subject to microscopic examination to determine if myxospores of *C. shasta* were present in lower gut tissue. Negative samples were tested by PCR for *C. shasta*. The one Chinook that became moribund of those exposed near Beaver Creek was positive for *C. shasta*. The late April 72 hour exposures resulted in a very low mortality of Chinook (2.6%) and no loss with *C. shasta* infection of the rainbow trout near Beaver Creek and no loss of Chinook at Seiad Valley.

C. shasta loss of IGH Chinook salmon exposed in late April during 2016 was compared with previous years since 2009 when Chinook salmon were exposed during the third or fourth week of the month or in 2015 in early April, (Figure 1.1.3) at Beaver Creek. No Chinook salmon died in the April exposures from 2010-2013, but in 2014, 7% died, 10% in 2015 and 2.6% in 2016. Of eight years of sentinel exposures in April, half of the years resulted in a low loss of Chinook salmon with *C. shasta* infections. Coho juveniles were exposed only in 2009 and 2010 in April and no loss with *C. shasta* infections occurred. Rainbow trout exposed in April near Beaver Creek have experienced variable percent loss with *C. shasta* infections from very high (April 2009 and 2014, just under 100%, and about 80% in 2010) to very low (less than 20% in 2011, 2013 and 2015) but 2016 was the first year in which no rainbow trout died with *C. shasta* infection when exposed near Beaver Creek in April.

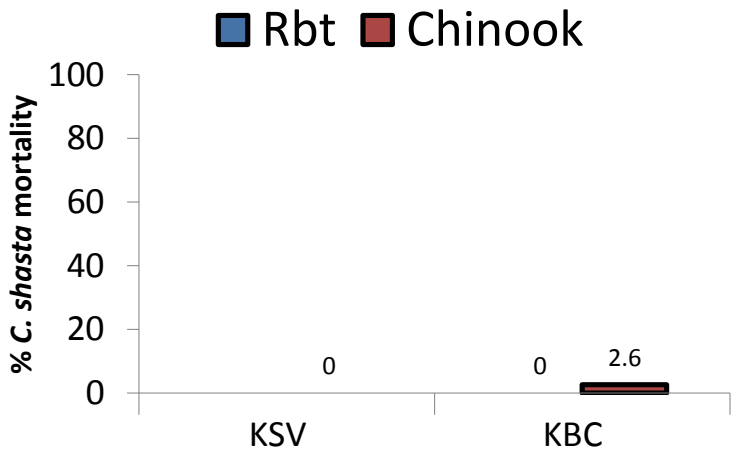

FIGURE 1.1.2. Percent mortality with *C. shasta* infections of rainbow trout (Rbt) exposed near Beaver Creek (KBC) and IGH fall Chinook salmon exposed at both Beaver Creek and Seiad Valley index sites in the lower Klamath River during April 21-24, 2016 and held for 65 days post-exposure at 13°C.

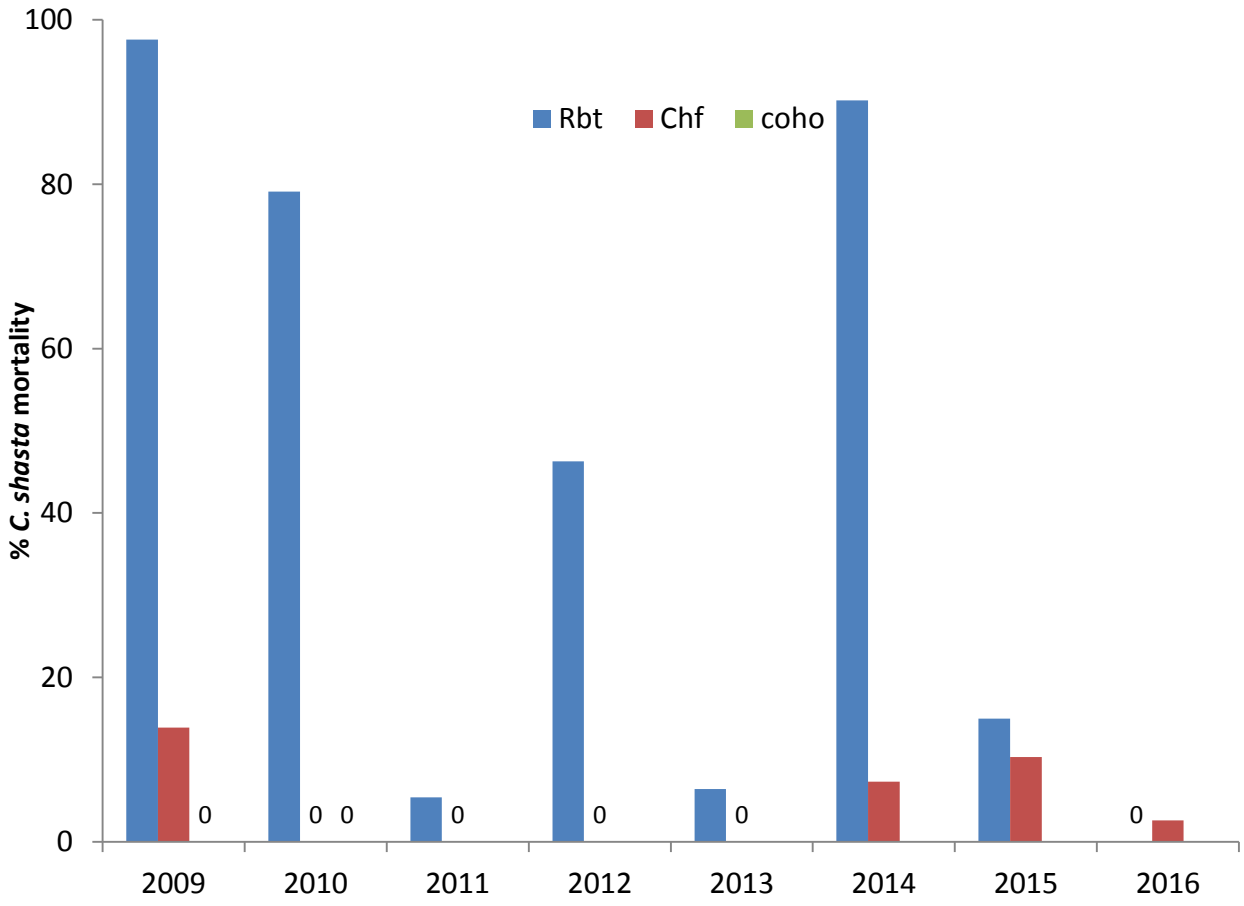

FIGURE 1.1.3. Comparison of percent loss from *C. shasta* infections in rainbow trout (Rbt) and IGH Chinook salmon (Chf) exposed in 72 h sentinel studies near Beaver Creek during April 2009-2016.
Coho (IGH) were only exposed in 2009 and 2010.

May 16-19 exposure (Figures 1.1.4-8): After 64 days of rearing, all groups except rainbow trout exposed at Keno Eddy were euthanized with MS222 and enumerated. A subset of the rainbow trout exposed at Keno Eddy were enumerated after the 64 days of rearing and the remainder held for an extended rearing to further monitor for *C. shasta*. Results from previous year's exposure of rainbow trout at Keno Eddy showed that these fish often would become infected with *C. shasta* in the river but would not succumb to the infection within the regular monitoring period. The May 2016 sentinel exposures, except for high loss of rainbow trout in the lower Williamson River, resulted in either no loss or very low loss with *C. shasta*-associated infections in both juvenile Chinook salmon and rainbow trout (Figure 1.1.4). Juvenile Chinook salmon exposed at the Nature Conservancy site on the Williamson River and at Keno Eddy in the upper river experienced no loss with *C. shasta* infections during the post-exposure rearing. At the I5-bridge site in the lower river, Chinook salmon had a low loss of 2.5% (1 fish, in which myxospores had developed). Chinook exposed near Beaver Creek incurred a very low loss of 2.4% (1 fish) that was negative for *C. shasta* compared to 2.4% loss (1 fish) that was *C. shasta*-positive at Seiad Valley. Chinook salmon exposed in the river near Orleans had a 12.5% loss and 10% (four fish) were positive for *C. shasta*. *Ceratonova shasta*-infections were detected in 2.4-100% of the dead susceptible rainbow trout depending on site at the four locations tested in May of 2016. All of the rainbow trout exposed in the lower Williamson River died with *C. shasta* infection but only one fish (2.4%) died with *C. shasta* infection at Keno

Eddy, 6.7% (three fish) from I5-Bridge and 17.1% (seven fish) near Beaver Creek in May 2016. The extended-held rainbow trout exposed at Keno Eddy were sampled after 75 days post-exposure when 11 fish were euthanized and their lower intestine examined microscopically for spores of *C. shasta*. Seven of the 11 fish were infected with *C. shasta*. The spores were genotyped and the Keno Eddy rainbow trout were infected with ITS-1 genotype 0.

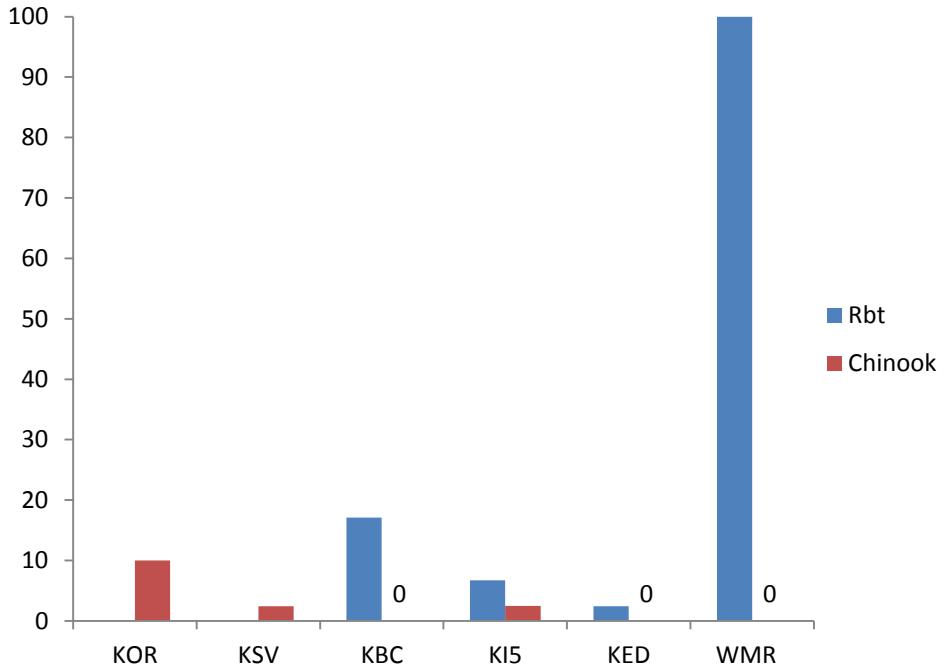

FIGURE 1.1.4. Percent *C. shasta*-associated mortality of sentinel Iron Gate Hatchery juvenile Chinook salmon and Roaring River Hatchery susceptible Rainbow trout (Rbt) exposed in the upper River in the lower Williamson River (WMR) and at Keno Eddy (KED), below Iron Gate Dam near the I5 Bridge (KI5), near Beaver Creek (KBC) in the Klamath River. Only juvenile Chinook salmon were exposed at Seiad Valley (KSV) and Orleans (KOR). Sentinel fish at all sites were exposed for 72 hours May 16-19, 2016. Fishes were held at the OSU Aquatic Animal Health Laboratory and monitored for 64 days post-exposure. Fish in which no myxospores were observed had an intestinal sample PCR-tested.

Cumulative mortality curves display disease progression as well as total mortality. Following the May exposure, more Chinook salmon exposed at Orleans died with *C. shasta* infections (10%) than those exposed at Seiad Valley (2.4%) or near Beaver Creek (0%) (Figure 1.1.5). Furthermore, the first Chinook with *C. shasta* infection from Orleans died on post-exposure day 22 and the last one on day 28, whereas at Seiad Valley, the one Chinook died on day 33 post-exposure. Rainbow trout exposed at two locations in the upper river (lower Williamson River and Keno Eddy) and at two locations in the lower river (I5 Bridge and near Beaver Creek) in May 2016 became infected with *C. shasta*. However, the fish exposed at the lower Williamson River died with *C. shasta* infections most rapidly of all four sites tested; most had died by day 22 and all fish were dead by day 26 post-exposure (Figure 1.1.6). The one fish that died at Keno Eddy died on day 31 post-exposure. At KI5, the first positive *C. shasta* rainbow trout died on day 39 and the last on day 48. And a Beaver Creek, the first fish died on day 26 post-exposure and the last positive fish died on day 49.

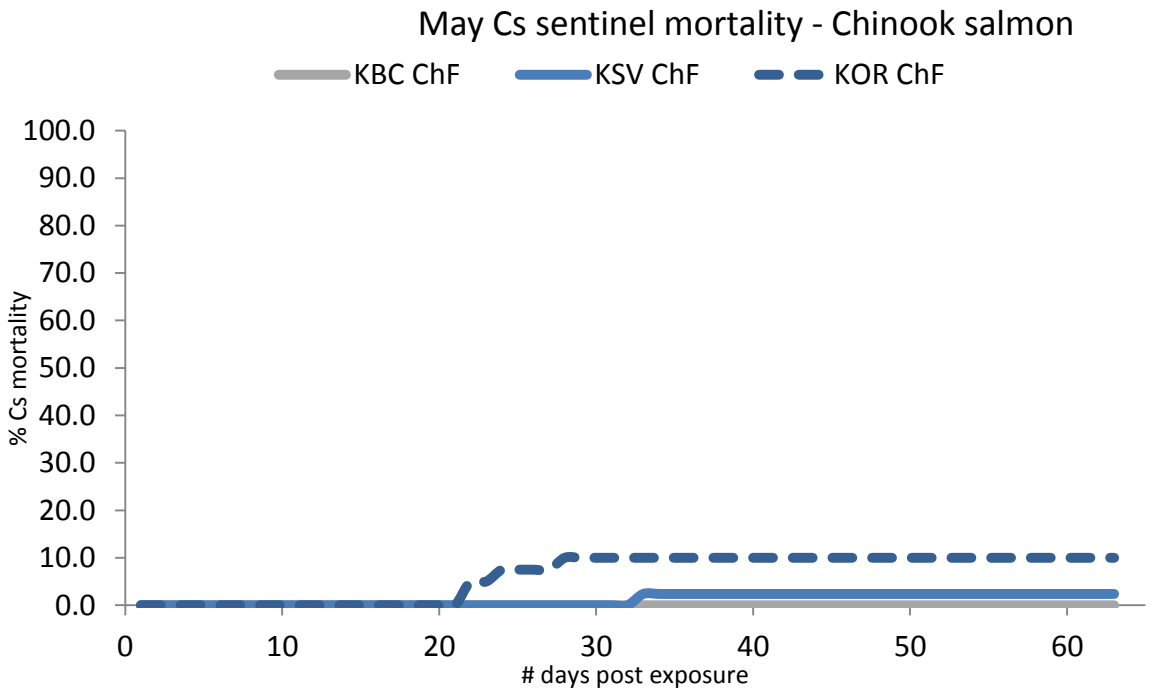

FIGURE 1.1.5. Cumulative percent loss with *C. shasta* infections of IGH Chinook salmon exposed in sentinel cages for 72 h in May 2016 near Beaver Creek (KBC), Seiad Valley (KSV) and Orleans (KOR) in the Klamath River.

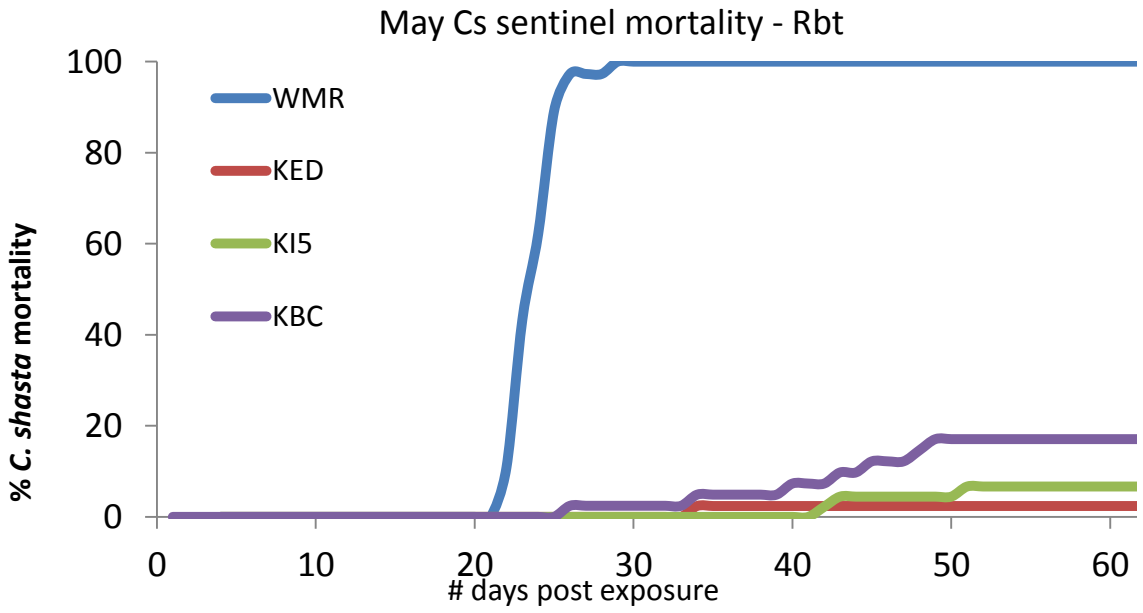

FIGURE 1.1.6 Cumulative percent loss associated with *C. shasta* in rainbow trout at four sentinel sites in May 2016.

When comparing the May exposure loss associated with *C. shasta* infections in IGH Chinook salmon at the upper Klamath River sites from 2007 to 2016, consistently no mortalities occurred in the Williamson River-exposed fish (WMR) and only low mortality associated with *C. shasta*-infection was detected in one year (2010) at Keno Eddy (Figure 1.1.7). Below Iron Gate Dam before 2015, the greatest loss of Chinook occurred in 2008 and 2009 at Beaver Creek and Seaid Valley and both locations are considered the "hot zone" where more fish typically become infected than elsewhere in the lower river. In May 2010 - 2013, Chinook salmon exposed for 72 h had losses with *C. shasta* that were very low, or none died. In 2014, juvenile Chinook died at a higher level than the previous four years and they were infected with *C. shasta* (40.7% at Beaver Creek and 32.5% near Seiad Valley). In May 2015, Chinook suffered an extremely high loss with *C. shasta* infections of 59% near Beaver Creek and 90.5% at Seiad Valley. In May 2016, similar to the May 2010 - 2013 exposure results, Chinook salmon exposed for 72 h had very low losses with *C. shasta* infections or none died. Sentinel exposures of the Chinook in the lower river in May 2016 resulted in much lower losses compared to those in May 2014 and 2015.

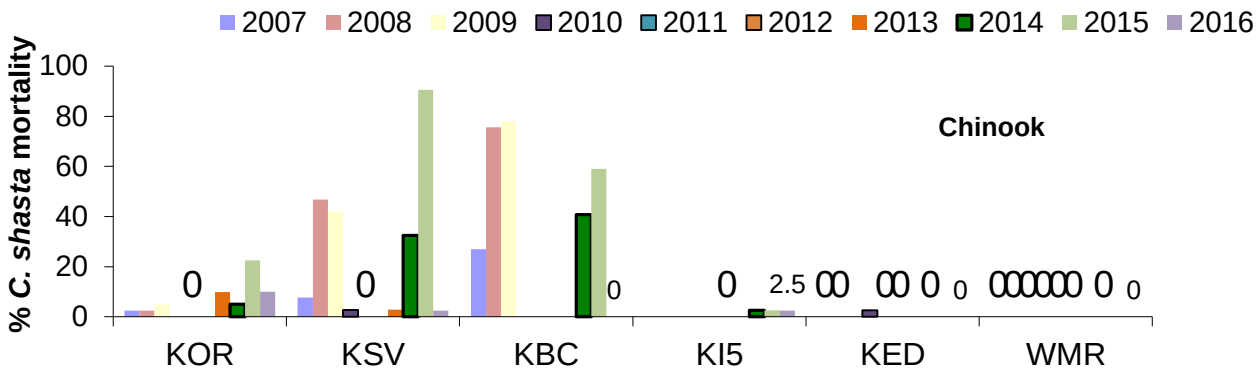

FIGURE 1.1.7. Comparison of percent loss from *C. shasta* of juvenile IGH Chinook salmon at six index sites in May of 2007 - 2016. The Chinook salmon were exposed at most sites and most years, zeros indicate exposure but no loss.

The juvenile rainbow trout sentinel fish percent loss with *C. shasta* infection for seven sites in May 2007 - 2016 in the Klamath River watershed (Figure 1.1.8) has generally been very high for most sites. Two exceptions to this were the Klamathon (which was shifted to near the Interstate 5 Bridge (KI5) in 2013) and Keno Eddy sites where percent loss levels varied from low to high depending on the year. In 2015, rainbow trout loss with *C. shasta* was 78.9% at KI5 and 0% at Keno Eddy and near 100% at all other sites. In 2016, rainbow trout loss with *C. shasta* at the lower Williamson River continued to be very high (100%) but was greatly reduced at all of the other three sites tested. At I5 Bridge, rainbow trout loss with *C. shasta* was very low, 6.7% similar to May exposures in 2010 - 2012. At Beaver Creek, a site where rainbow trout exposed in May in previous years always incurred high losses from *C. shasta,* losses were only 17% in 2016.

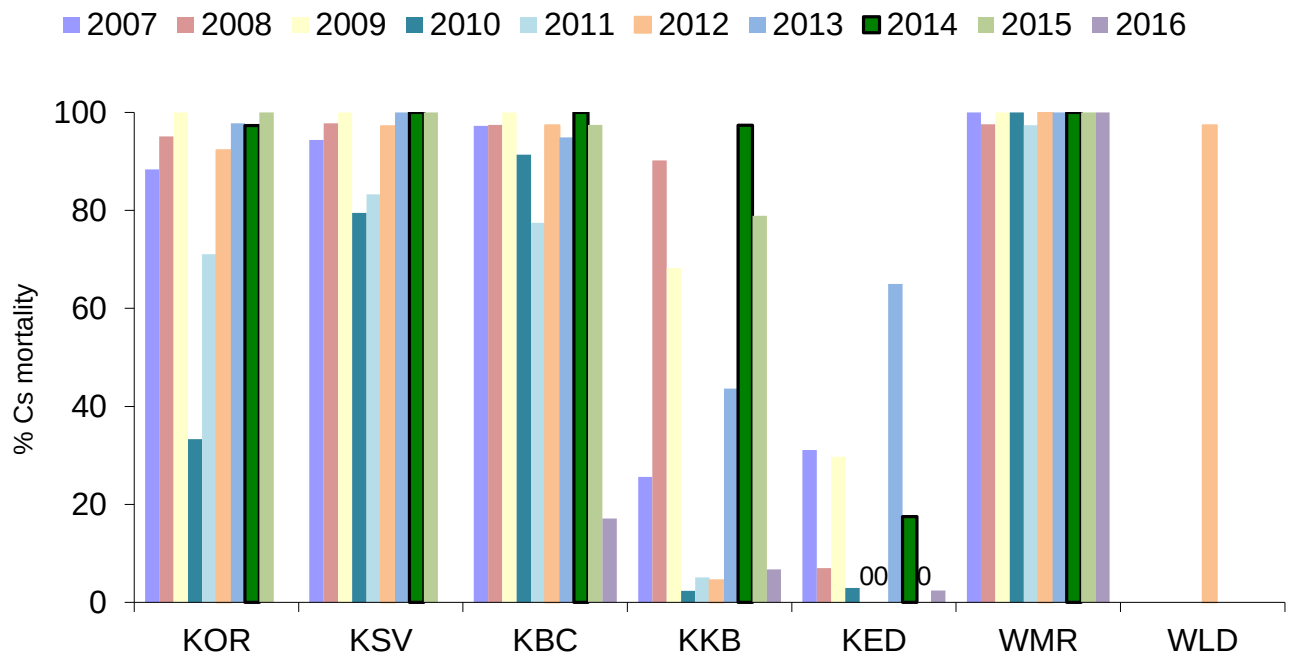

FIGURE 1.1.8. Comparison of percent loss from *C. shasta* in juvenile rainbow trout exposed for 72 h during May 2007 - 2016 at seven different Klamath River sentinel index sites and held for more than 60 days.

June 21-24 exposure (Figures 1.1.9-13): During the June 2016 sentinel exposures, river water temperatures averaged 17-18°C in the three upper river sites and 20-21°C at exposure sites below Iron Gate Dam. A few fish from several sites died of columnaris disease during the exposure in the cages or soon after they were brought to the laboratory. Three each of IGH Chinook exposed in the river at Seiad Valley and Orleans succumbed to *F. columnare* infections. Also, at Beaver Creek and Seiad Valley, when Trinity River Hatchery coho salmon were exposed, four fish from each exposure died either in the cage or within four days after transport to the AAHL from *F. columnare* infections. On August 25, after 62 days of rearing of the June exposure groups at the AAHL, all groups except the Keno Eddy rainbow trout were euthanized with MS222 and enumerated. Similar to the May exposures, the Keno Eddy-exposed rainbow trout were enumerated then held for extended rearing and observation of *C. shasta* infections.

None of the juvenile Chinook salmon exposed in the upper Klamath River watershed at the Nature Conservancy site on the Williamson River or at Keno Eddy died with *C. shasta* infections. Of those fishes that were exposed at several lower river sites below Iron Gate Dam, the Chinook experienced greater loss with *C. shasta* infection compared to May, increasing at each site down river with the most at Orleans, the lowermost site where Chinook were exposed (Figure 1.1.9). The June exposure of IGH Chinook resulted in the greatest loss with *C. shasta* infections with the two lower river sites of Seiad Valley and Orleans. Chinook exposed near I5-Bridge had a 28.2% loss and 25.6% were *C. shasta* positive, Beaver Creek, 31.8% died and 29.5% were positive compared to 60% loss and all that died were positive at Seiad Valley. Chinook exposed in the river near Orleans had an 83.8% loss and 81.1% of the fish exposed died with *C. shasta* infections. When the juvenile Trinity River Hatchery coho were exposed near Beaver Creek and Seiad Valley four fish died at each site from *F. columnare* infections but no *C. shasta* infections were observed during the post-exposure rearing. Apparently, the genotype of *C. shasta* was much lower in the river in June 2016 for coho than Chinook salmon, or Trinity River

Hatchery coho have a greater resistance to *C. shasta* infections than IGH coho used in previous years.

The susceptible rainbow trout were exposed at three upper river sites WLD, WMR and KED and at two sites below Iron Gate Dam, KI5 and KBC. Rainbow trout exposed at the two Williamson River sites experienced losses of 90% at WMR and 90.2% at WLD and no loss at KED. The extended held rainbow trout from KED were sampled on day 75 and 9 of 10 rainbow trout had myxospores of *C. shasta* in the intestine and all were PCR positive for *C. shasta*. The myxospores were genotype 0. The rainbow trout exposed near I5 Bridge had only an 8.8% loss and 6.7% had *C. shasta* infections. Rainbow trout exposed near Beaver Creek had a loss of 25.6% and all were positive for *C. shasta*.

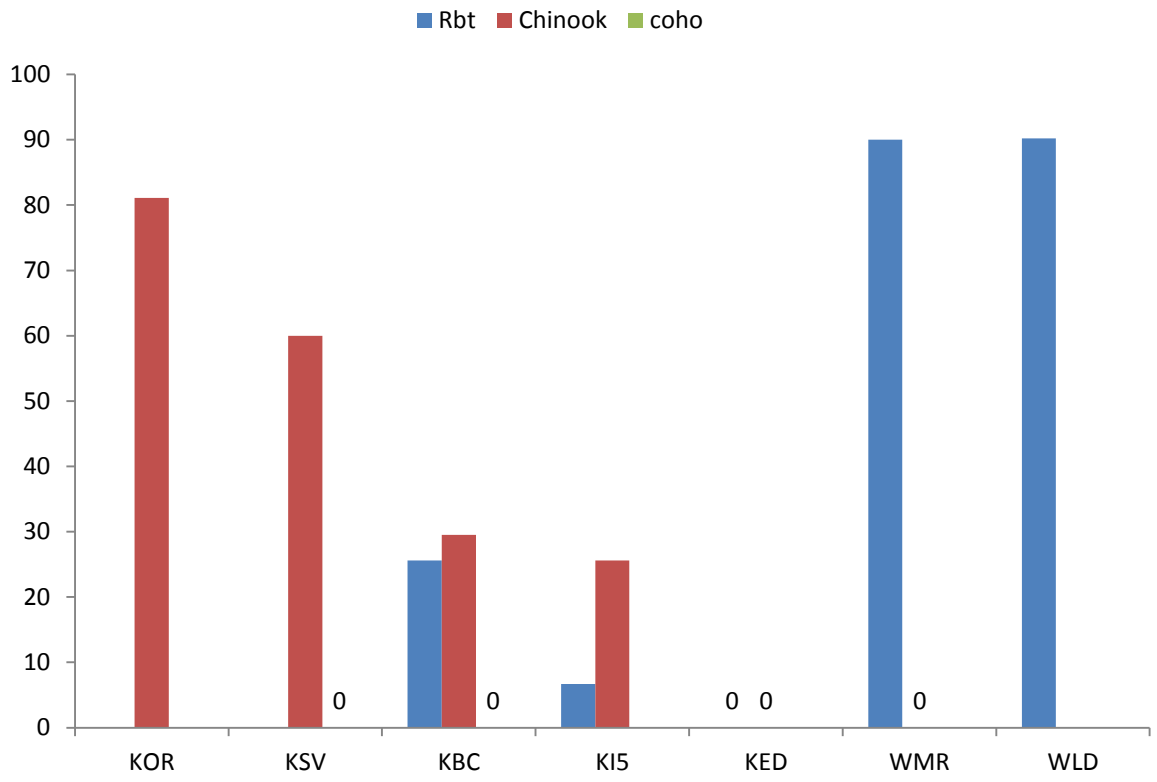

FIGURE 1.1.9. Percent *C. shasta*-associated mortality of sentinel Iron Gate Hatchery juvenile Chinook salmon and Roaring River Hatchery susceptible Rainbow trout (Rbt) exposed in the upper River in the lower Williamson River (WMR) and at Keno Eddy (KED), below Iron Gate Dam near the I5 Bridge (KI5), near Beaver Creek (KBC), Seiad Valley (KSV) and Orleans (KOR) in the Klamath River for 72 hours June 21-24, 2016. Also, coho from Trinity River Hatchery were held during the same time near Beaver Creek and Seiad Valley. Only rainbow trout were exposed at the Williamson River Lonesome Duck Resort site. Fishes were held at the OSU Aquatic Animal Health Laboratory and monitored for 62 days post-exposure. Fish in which no myxospores were observed had an intestinal sample PCR-tested.

The cumulative loss of IGH Chinook salmon exposed for 72 h in June for the four exposure sites below Iron Gate Dam is shown in Figure 1.1.10. The Chinook began dying with *C. shasta* infections at all four lower river sites before day 20. The Chinook exposed at Orleans began dying on day 13 post-exposure and most fish had died before 25 days post-exposure. The farther down river the exposure sites the more severe the loss and higher infection levels of *C. shasta*. For Chinook, the infectious zone extended from I5 Bridge and KBC of 25%, 60% at KSV and down to Orleans (81.1%).

The cumulative loss of rainbow trout exposed at five Klamath River sentinel sites in June 2016 show the most rapid loss occurring at the two Williamson River sites, lower Williamson River Nature Conservancy and Lonesome Duck Resort, followed by Beaver Creek, and I5 Bridge (Figure 1.1.12). The rainbow trout exposed in the lower Williamson River had begun to die about 20-21 days after exposure and most mortalities occurred by the 30th day post-exposure. The rainbow trout loss with *C. shasta* infections at I5 Bridge was low (6.7%) and unusually low near Beaver Creek (25.6%) similar to the May exposures. This is the lowest loss of rainbow trout exposed near Beaver Creek in June of all the years of our sentinel exposures at this site.

The Oregon Department of Fish and Wildlife stopped stocking *C. shasta* susceptible rainbow trout into Spring Creek, a tributary in the Williamson River watershed, in 2011. However, a reduction in *C. shasta* infection in susceptible rainbow trout when exposed for 72 hours in either May or June has not been detected in the sentinel fish exposures at the Nature Conservancy and Lonesome Duck Resort sentinel sites tested in 2012 - 2016.

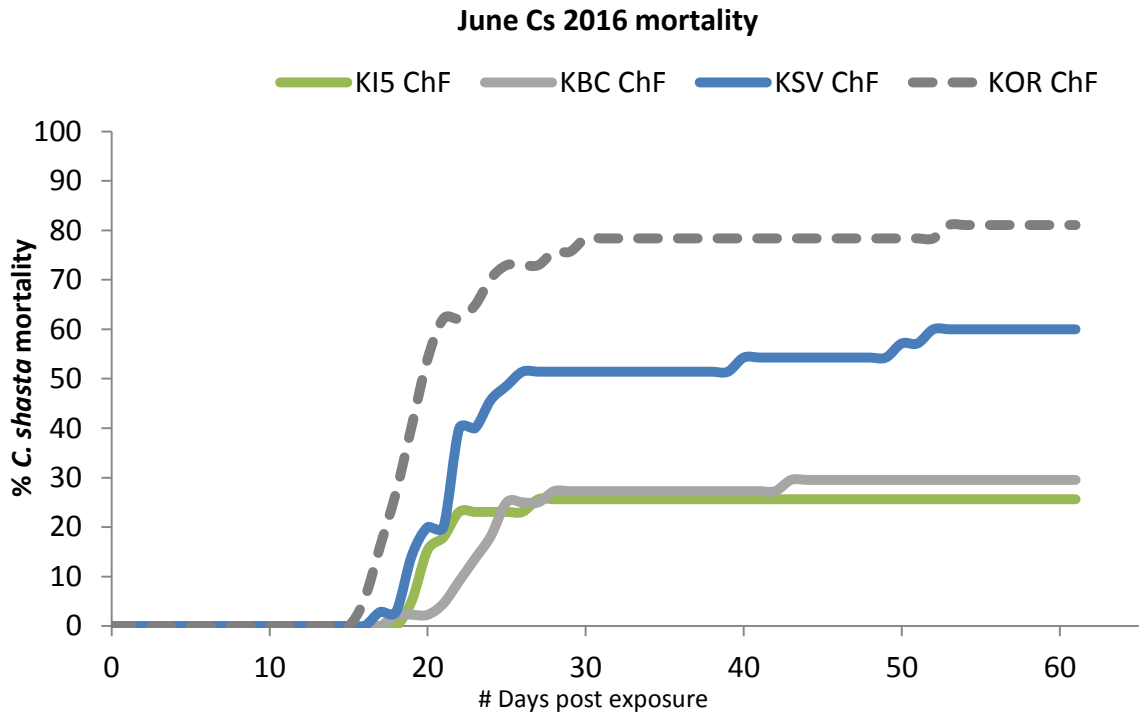

FIGURE 1.1.10. Cumulative loss associated with *C .shasta* in IGH Chinook salmon exposed for 72 h near I5 Bridge (KI5), near Beaver Creek (KBC), Seiad Valley (KSV), and Orleans (KOR) in June 2016.

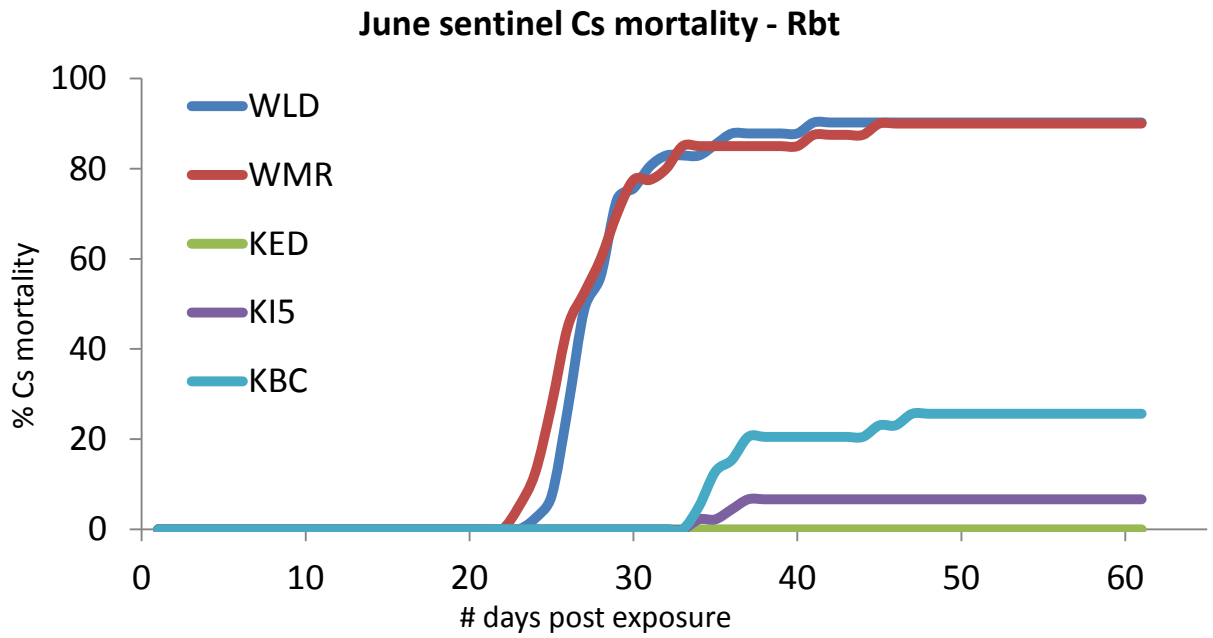

FIGURE 1.1.11. Cumulative percent loss associated with *C. shasta* in rainbow trout following exposure at five sentinel sites in June 2016.

During the June sentinel exposures, the greatest loss of Chinook salmon occurred in 2007, 2008 and 2009 at KBC and KSV and both locations are considered the "hot zone" where more fish become infected than elsewhere in the lower river (Figure 1.1.12). In June 2010 - 2013, Chinook salmon exposed for 72 h incurred low losses from *C. shasta*, i.e. less than 20%. In 2014, Chinook loss was greater than 40% at Beaver Creek and Seiad Valley. In 2015, Chinook loss was near 40% at I5 Bridge, near Beaver Creek and Orleans. Interestingly, even though water temperatures were very high at Seiad Valley, only 7.7% Chinook loss occurred. This is the first year that Chinook loss in June reached 40% at I5 Bridge, and thus expanded the infectious zone from I5 Bridge to Orleans, with the exception of Seiad Valley. For the June exposure in 2016, loss of Chinook with *C. shasta* infections similar to 2015 occurred from I5 Bridge down to Orleans showing the same expansion of the infectious zone however, percent mortality increased substantially the further downriver with the highest loss at Orleans (81.1%). The 2016 June exposure at the lowermost test site, Orleans, resulted in the highest *C. shasta* loss for this site seen in all the test years since 2007.

For coho salmon, the June exposures resulted in the greatest percent infections at KBC and KSV in 2007, 2008, 2011 and 2013. In June 2013, coho salmon had a 28.6% *C. shasta* infection at Beaver Creek and 44.8% at Seiad Valley that was greater than coho exposed in June 2010 and 2012. In June, 2014 the coho loss was severe at both Beaver Creek and Seiad Valley and was 32.1% at Orleans. The sentinel coho were more affected in June 2014 than the Chinook salmon. In June 2015, the yearling coho loss at Beaver Creek and Seiad Valley was much lower, 11.1 and 25%; respectively but 57% of the surviving coho exposed at KBC had eye lesions that were likely *C. shasta* infections. In June 2016, Trinity River Hatchery juvenile coho were exposed near Beaver Creek and Seiad Valley and no mortal infections of *C. shasta* were observed in these fish. Since this was the first year we used TRH coho in sentinel studies instead of IGH coho either the genotype of *C. shasta* that infects coho

was present at a very low level in the river water or TRH coho have greater resistance to *C. shasta* or both may be the case.

The rainbow trout sentinel loss for seven sites in June 2007 - 2015 in the Klamath River watershed (Figure 1.1.13) show greater than 90% loss at most locations. Rainbow trout exposed at Keno Eddy and KI5 show in some years there is moderate *C. shasta* loss and other years very low. In 2016, for the five sites tested, in the Williamson River continued high level of loss or slightly reduced to 90% instead of the usual 100%, no loss at KED and low loss at I5 Bridge and near Beaver Creek. For June 2016, near Beaver Creek, this was an unusual year with much lower infections of the susceptible rainbow trout. The genotype affecting this stock of rainbow trout must have been very low in the river. Most previous years have shown 95-100% loss of rainbow trout at Beaver Creek.

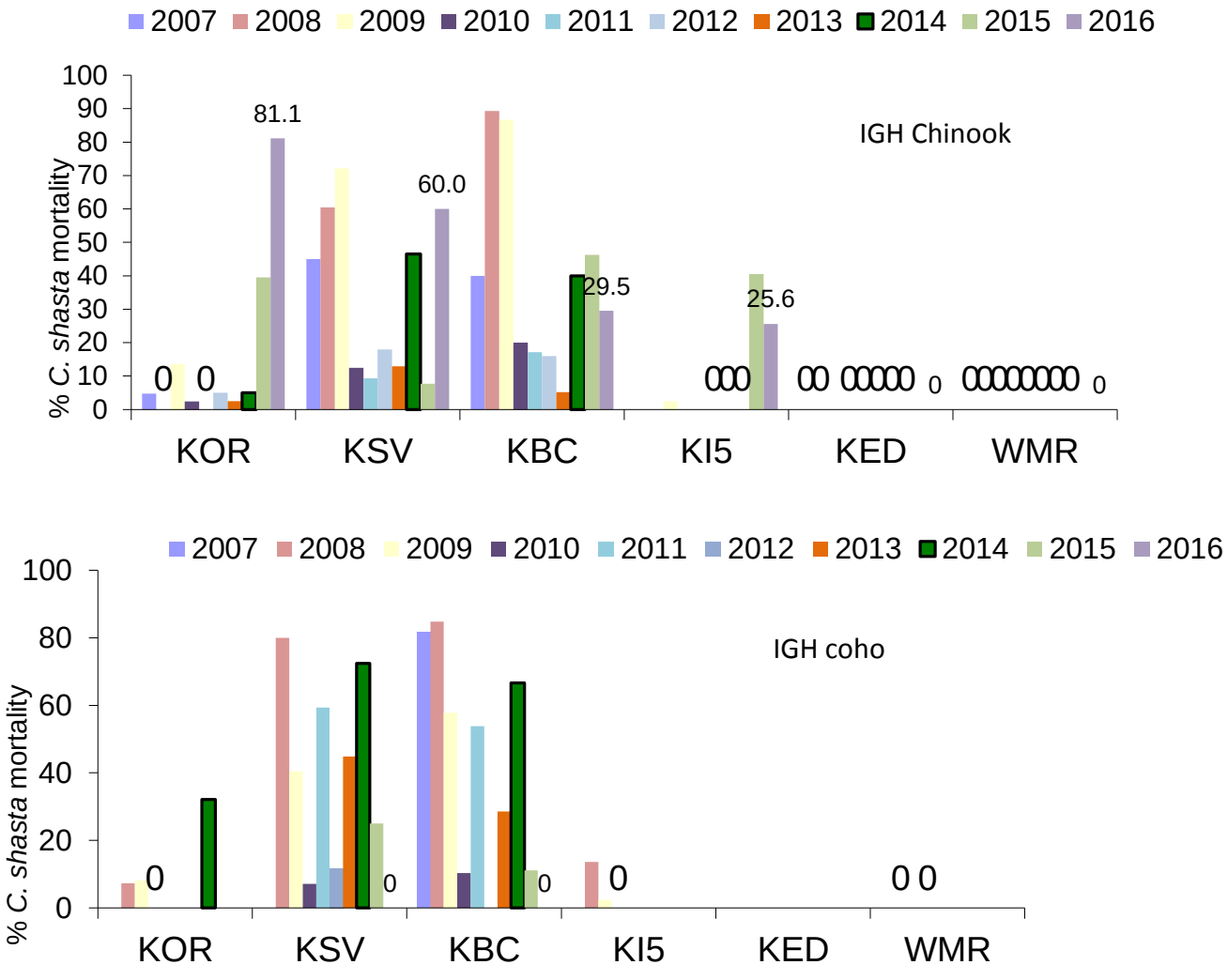

FIGURE 1.1.12. Comparison of percent loss from *C. shasta* of juvenile IGH Chinook salmon (upper figure) and coho salmon (lower figure) at six index sites exposed in June of 2007 - 2016. The Chinook salmon were exposed at most sites and most years, zeros indicate exposure but no loss. The coho salmon were not exposed at all locations each year and no exposures at KED have ever been done.

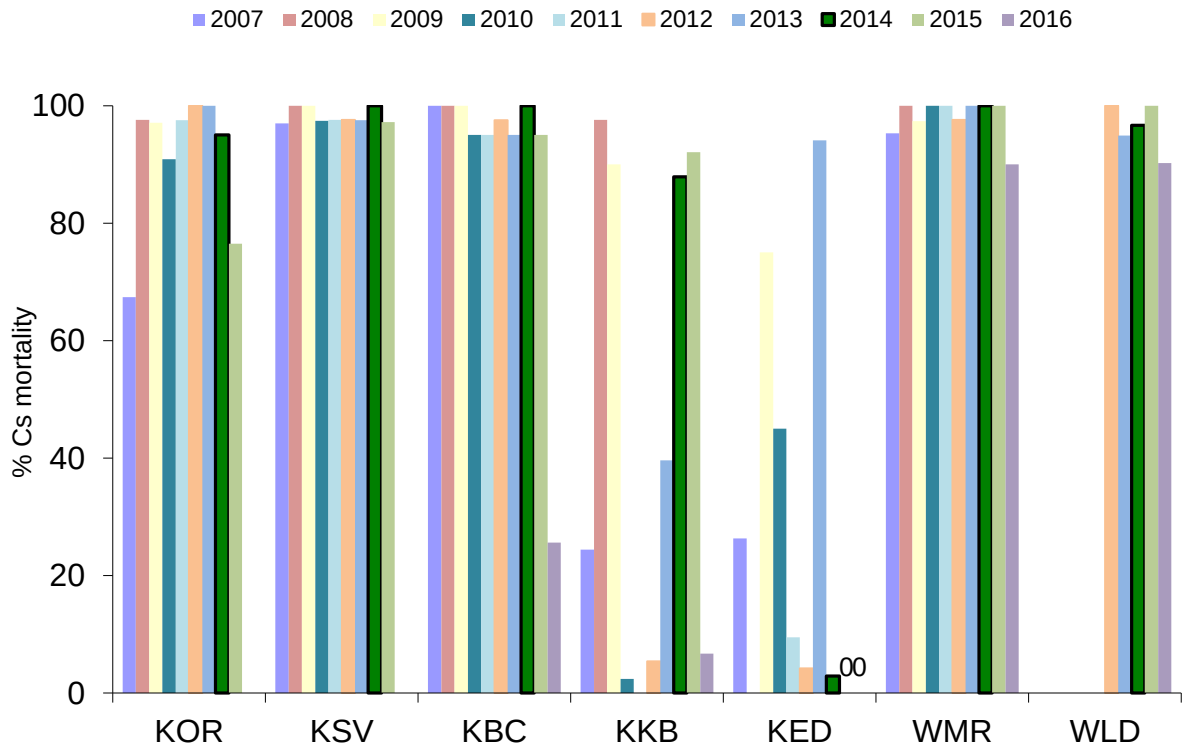

FIGURE 1.1.13. Comparison of percent *C. shasta* mortality for rainbow trout exposed in June 2007 - 2016 at seven index sites of the Klamath River basin. The Klamathon site (KKB) was exchanged for a site downstream a small distance to near the Interstate 5 Bridge in 2013.

September 17 - 20 exposure (Figures 1.1.14-15). The September exposure of Iron Gate Hatchery Chinook and susceptible rainbow trout occurred at sites near Beaver Creek, Seiad Valley and Orleans. The lower river Orleans site was added for the September exposure because our June exposures in the lower river resulted in a higher loss of IGH Chinook at the lowest site (Orleans). After the 72-hour exposure, the groups of fish were held for 62 days at 18°C. The water temperature during exposure averaged 19 - 20°C and *F. columnare* caused loss in Chinook in the first five days post-exposure at Beaver Creek (3 dead), Seiad Valley (8 dead) and Orleans (6 dead). No rainbow trout died from *F. columnare* during or after the exposure. The percent loss with C. *shasta* for both Chinook and rainbow trout became progressively higher at each site going down river. For sentinel exposures at Beaver Creek, no Chinook that died during post-exposure rearing were found to be infected with *C. shasta*. At Seiad Valley, three (9.4%) of the Chinook died and were positive and at Orleans 81.8% died during holding and 39.4% died with severe disease signs including hemorrhaged lower intestines or vent typical for *C. shasta*. The Chinook exposed at Orleans when water temperatures were 20°C in September were likely exposed to high levels of *Ichthyophthirius* in the river. Even though all sentinel groups were given prophylactic formalin baths to prevent *Ichthyophthirius* infections once they were brought back to the AAHL, on day 31 of the rearing, the Orleans Chinook began to die of *Ichthyophthirius* infestations with great numbers of the parasite present on the gills and skin. Formalin baths were administered daily to control this parasite but 13 fish died during days 31-35 of which five were also positive for *C. shasta* by PCR testing. These five fish did not have disease signs typical of *C. shasta* and we consider it unlikely that they would have died from *C.*

shasta infection if they had not died from *Ichthyophthirius*; therefore, we did not include those five Chinook in the total loss associated with *C. shasta*. The September sentinel results were similar to June exposures in that there was a shift down river of the infectious zone, with no Chinook loss at Beaver Creek, 9.4% at Seiad Valley and 39.4% at Orleans. Also, the rainbow trout loss with *C. shasta* infection showed a greater severity the further down river, i.e. 35.9% at Beaver Creek, 80.5% at Seiad Valley and 100% at Orleans.

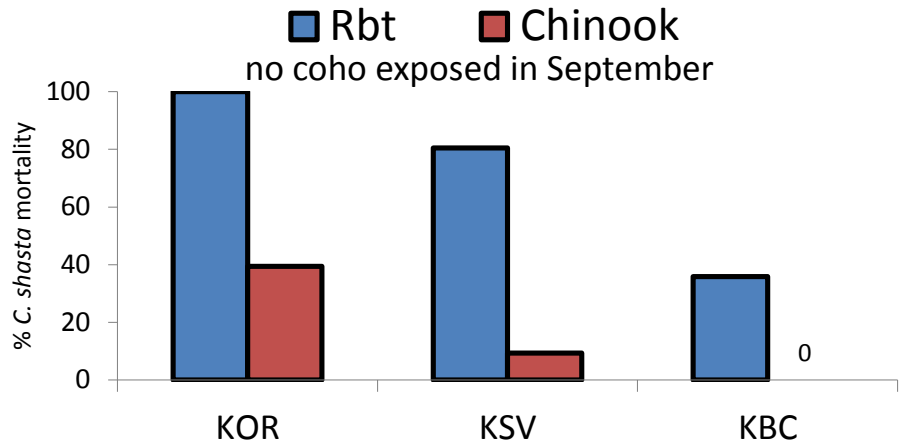

FIGURE 1.1.14. Percent mortality of rainbow trout (Rbt) and IGH fall Chinook salmon exposed September 17 - 20, 2016 at three Klamath River sentinel index sites, near Beaver Creek (KBC), Seiad Valley (KSV) and Orleans (KOR) then held for 62 days post-exposure at 18°C.

The cumulative loss of juvenile Chinook salmon exposed in September 2016 at the three lower river sites is shown in Figure 1.1.15. At Orleans, the first Chinook to die with *C. shasta* infection was on day 14 and loss continued until day 23, then on day 31 the *Ichthyophthirius* outbreak occurred and 15% more died in a five day period. The Seiad Valley fish began to die on day 18 then day 28 and day 33.

Comparison of percent *C. shasta* infections in Chinook salmon exposed in September of 2007 - 2016 at selected sites in the Klamath River basin shows that generally *C. shasta* infections in this month are very low (Figure 1.1.16). In half of the last 10 years when exposures were conducted in September, in 2007, 2008, 2014, 2015 and 2016 sentinel Chinook became infected, and mostly with a low level of mortality. Exposures in other years were negative for *C. shasta* infections. However, in 2016 the greatest loss with *C. shasta* was at Orleans and the prevalence was much higher at this site than in any previous September exposures. September exposures at Orleans had only previously been conducted in 2007 - 2009.

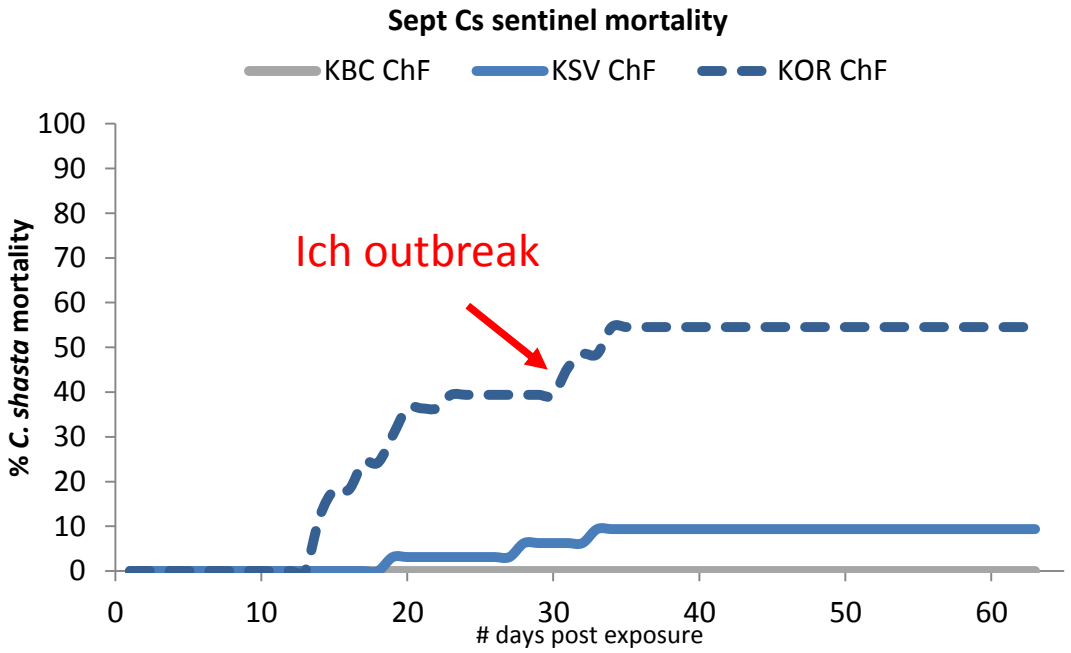

Figure 1.1.16 Cumulative percent loss associated with *C. shasta*

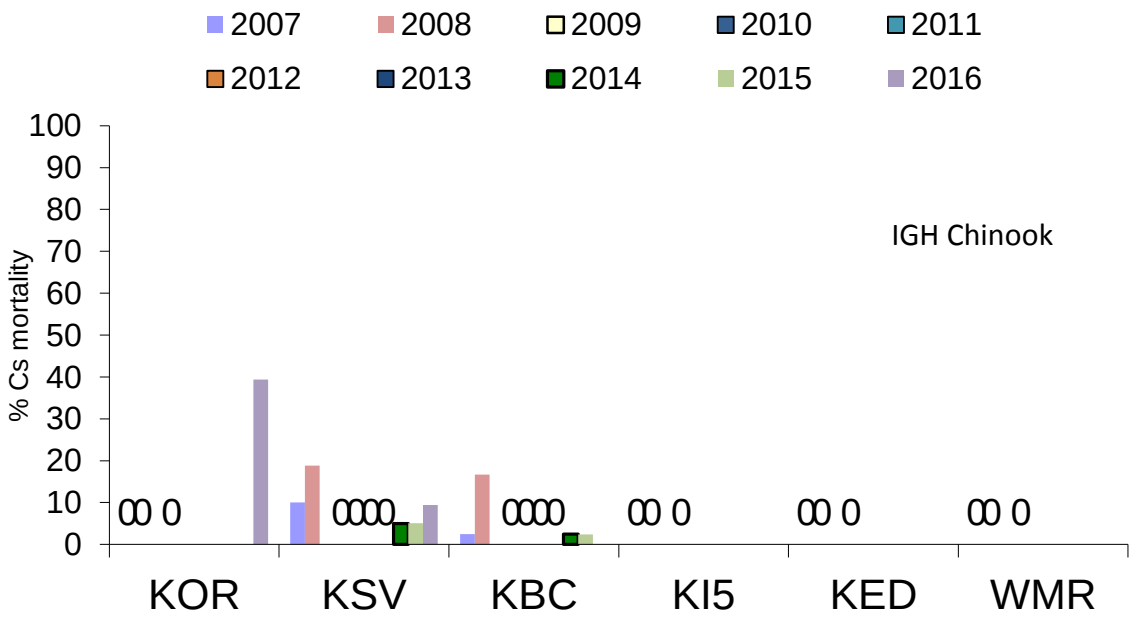

FIGURE 1.1.17. Comparison of percent loss from *C. shasta* of juvenile IGH Chinook salmon at six index sites exposed in September of 2007 - 2016. The Chinook salmon were exposed at most sites in September in 2007,2008 and 2009 but only near Beaver Creek and Seiad Valley in 2011, 2012, 2013, 2014, 2015 and Orleans was added in 2016. Zeros indicate exposure but no loss.

Comparison of sentinel results for the juvenile IGH Chinook and juvenile or yearling coho salmon exposed at Beaver Creek in 2007 - 2016 indicate a shift toward more severe effects of *C. shasta* on the Chinook than coho from 2007 to 2009 (Figure 1.1.18). In 2007, the loss of juvenile coho was very high while the Chinook loss was lower. In 2008, both species suffered high loss in May and June. In 2009, the greatest loss occurred in May and June in the fall Chinook. In general, however, losses for both species due to *C. shasta* have been high in May and June of 2007 - 2009. In contrast, for 2010 - 2013, Chinook suffered decreased infection and mortality from *C. shasta*. In 2011 and 2013, the coho loss was much higher near Beaver Creek. In 2014, loss of both Chinook and coho near Beaver Creek was much greater than 2010 - 2013 with the exception of coho loss in June 2011. The loss in 2014 is similar to the high loss years of 2007 - 2009. In 2014, the greatest infection level was observed in the coho. But in 2015, the Chinook had the greatest infection level and loss and yearling coho salmon much lower. In 2016, Chinook loss was none or low loss in all months except June (29.5%) and coho were exposed only in June with no loss occurring with *C. shasta* infection.

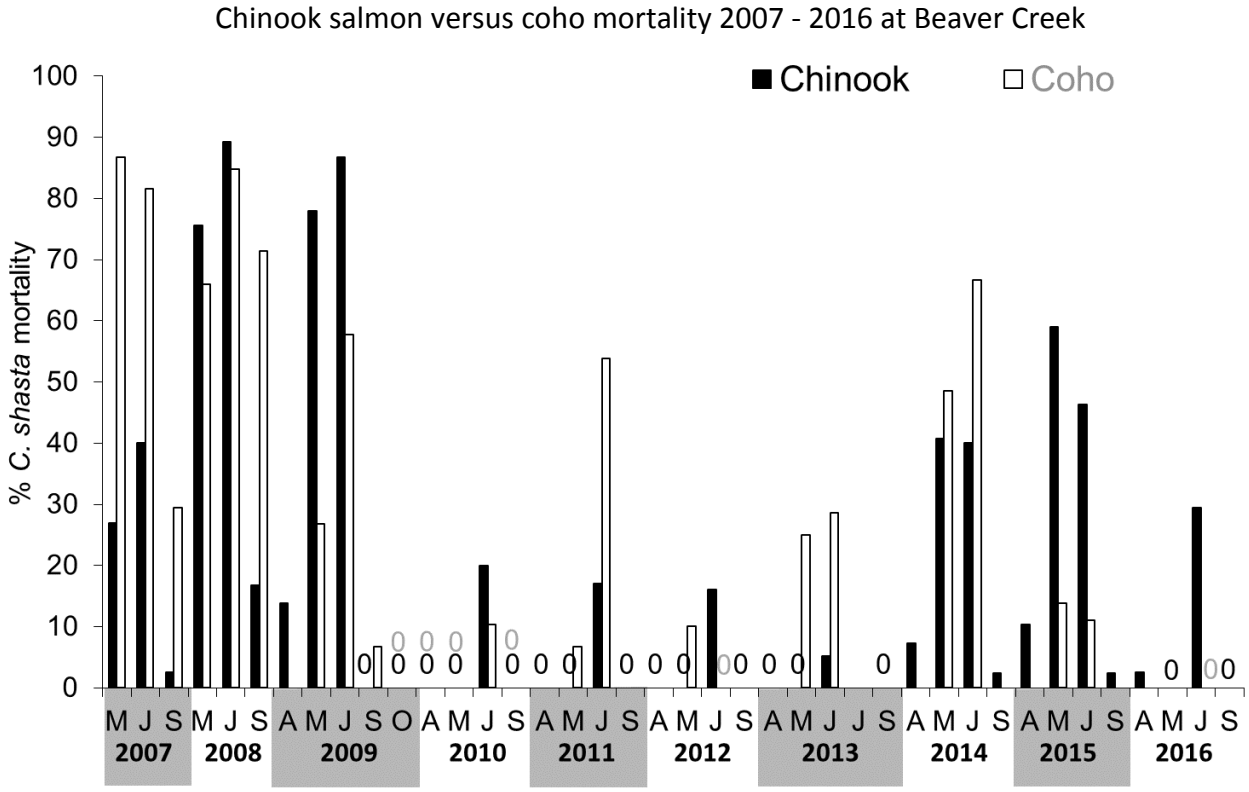

FIGURE 1.1.18. Comparison of *C. shasta* mortality of Juvenile IGH fall Chinook and coho salmon exposed in the Klamath River near Beaver Creek for 72 h in April, May, June and September (when conducted) in years 2007 - 2016.

The comparison of *C. shasta* mortality of juvenile IGH fall Chinook salmon and juvenile or yearling coho salmon exposed at Seiad Valley for 72 h in May and June of years 2007 - 2016 show similar results as the comparison for the same years near Beaver Creek (Figure 1.1.19). No comparison can be made for Chinook and coho in 2007 since no coho salmon were exposed at Seiad Valley in that year. The coho salmon loss from *C. shasta* in 2011 and 2013 was much higher than the Chinook salmon. Also, in 2013 and 2014 there appears to be a slight

downstream shift of greater *C. shasta* rate at Seiad Valley compared to near Beaver Creek. In 2014, the coho loss associated with *C. shasta* was the most severe observed since we began sentinel studies in the Klamath River. In contrast in 2015, the juvenile Chinook loss associated with *C. shasta* infections was the greatest we have observed at least in the month of April since we began sentinel studies and in May since 2009. In 2016, coho were exposed only in June and no fish died with *C. shasta* infections while 60% of the Chinook exposed at Seiad Valley in June died with *C. shasta* infections.

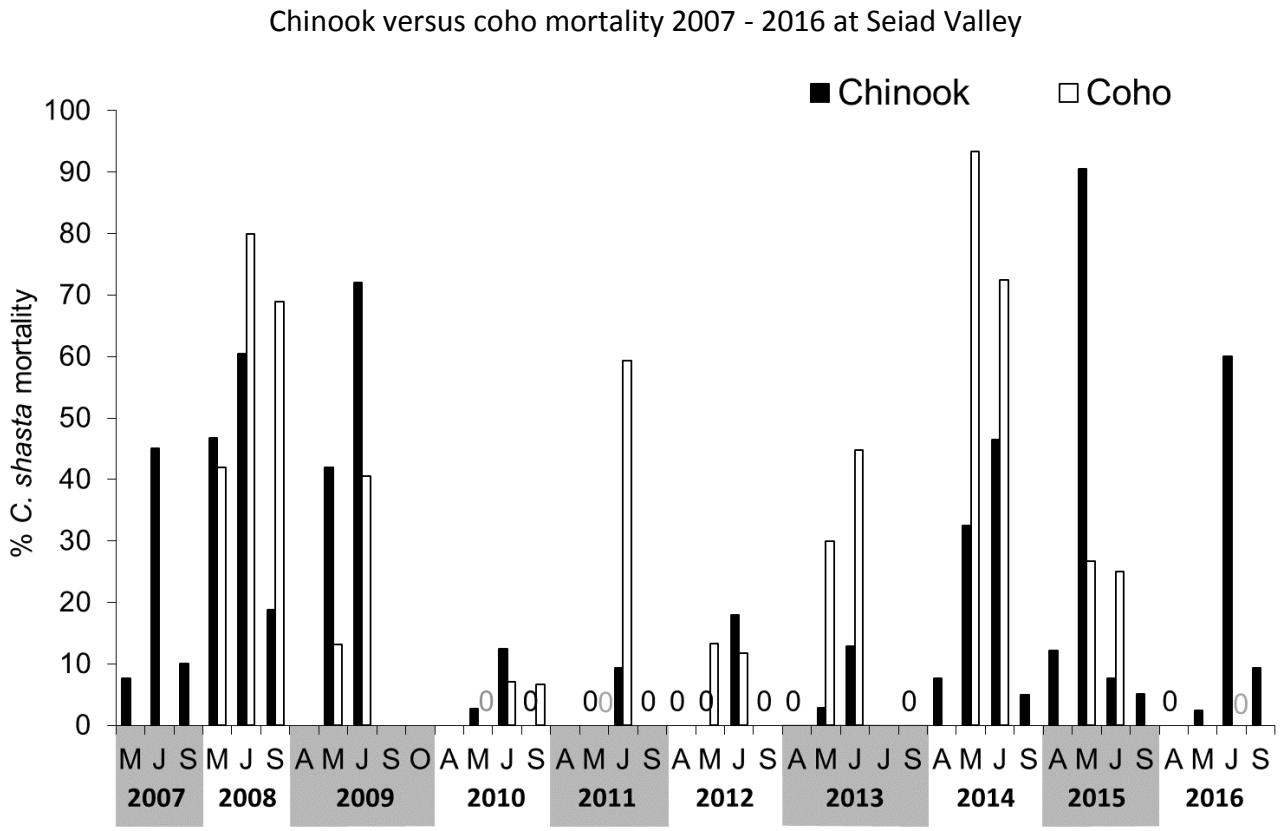

FIGURE 1.1.19. Comparison of *C. shasta* mortality of Juvenile IGH fall Chinook and coho salmon exposed in the Klamath River near Seiad Valley for 72 h in April, May, June and September (when conducted) in years 2007 - 2016

Pulse-Flow exposures: None conducted in 2016.

Task 1.2. Determine parasite density in water samples to include data collection during spring out-migration and fall in-migration. Water collection will occur at the following sites according to the following schedule:

(1) All of the fish exposure sites during exposures
(2) Weekly all year at Beaver Creek and Seiad Valley (to encompass previous and current spring parasite 'hot spots')
(3) Weekly from March through October at I5 Fish Trap, Orleans and Tully Creek
(4) March through mid-June at Kinsman Fish Trap

Task 1.2. Methods

Water samples were collected weekly by ISCOs (automatic samplers) at three Klamath River mainstem sites, the I5 fish trap (KI5), Orleans (KOR) and Tully Creek (KTC), from March 28 through November 15 and throughout the year at two other mainstem sites: upstream of the confluence with Beaver Creek (KBC) and Seiad Valley (KSV) (Figure 1.1.1). An additional ISCO collected water samples weekly at the Kinsman fish trap (KMN) during the outmigration period, March 28 to May 31. Temperature loggers (Hobos) attached to each ISCO recorded river temperature every 15 minutes. Project funds this year enabled the acquisition of a flow meter for the Beaver Creek and Seaid Valley sites. The use of automated water samplers and the field assistance provided by the Karuk and Yurok tribal biologists allowed weekly collections of 24-h composite water samples. The ISCOs were programmed to begin sampling at 8 am and 1 L was collected from the river every 2 h for 24 h, then the total sample was mixed manually and 4 x 1 L samples taken. All samples were chilled until filtered, within 24 h of collection, then shipped overnight to OSU for molecular analysis.

Water samples (four 1 L samples) were also collected manually at the start and end of each sentinel fish exposure. In April this occurred at two sites, in May at six sites, June at seven sites and in September samples were collected at three sites.

DNA was extracted from three of the four replicate filtered 1 L samples collected at each site and time point using a commercial kit (Hallett *et al.* 2012). A duplex *C. shasta*/IPC assay enables simultaneous detection of *C. shasta*-DNA and assessment of inhibition using the ABI Internal Positive Control. Each sample was run in duplicate and sample pairs with values differing by more than 1.5 Cq were rerun. Positive (tissue or artificial template) and negative (molecular grade water) controls were included in each qPCR run. Reference dilution series of the target DNA were included for standard curve calibration and assay efficiency assessment on each plate. Samples with inhibition less than 1.5 Cq had their final Cq value adjusted by this level of inhibition whereas samples with inhibition greater than 1.5 Cq were diluted and rerun.

Task 1.2. Results and discussion

Relatively high levels of waterborne *C. shasta* were detected throughout the lower Klamath River in 2016 (Figure 1.2.1). Detectable levels began increasing in April and fluctuated throughout Spring, Summer and Fall. At the original 'hot spot', Beaver Creek index site, levels were lower in 2016 than 2015 and 2009, but higher than 2014 (Figure 1.2.2-3). Spatial distribution of the parasite differed this year with highest levels recorded at Orleans followed by Tully Creek (Figure 1.2.1). Spore levels increased one week sooner at Orleans than at Beaver Creek (first week of April)(Figure 1.2.4). Spore levels at Orleans peaked late June/early July at >300 spores/L whereas the highest levels measured at Beaver Creek in 2016 were <100 spores/L. Spore levels decreased at both sites early August but a late peak was observed at both sites mid-September (Figure 1.2.4).

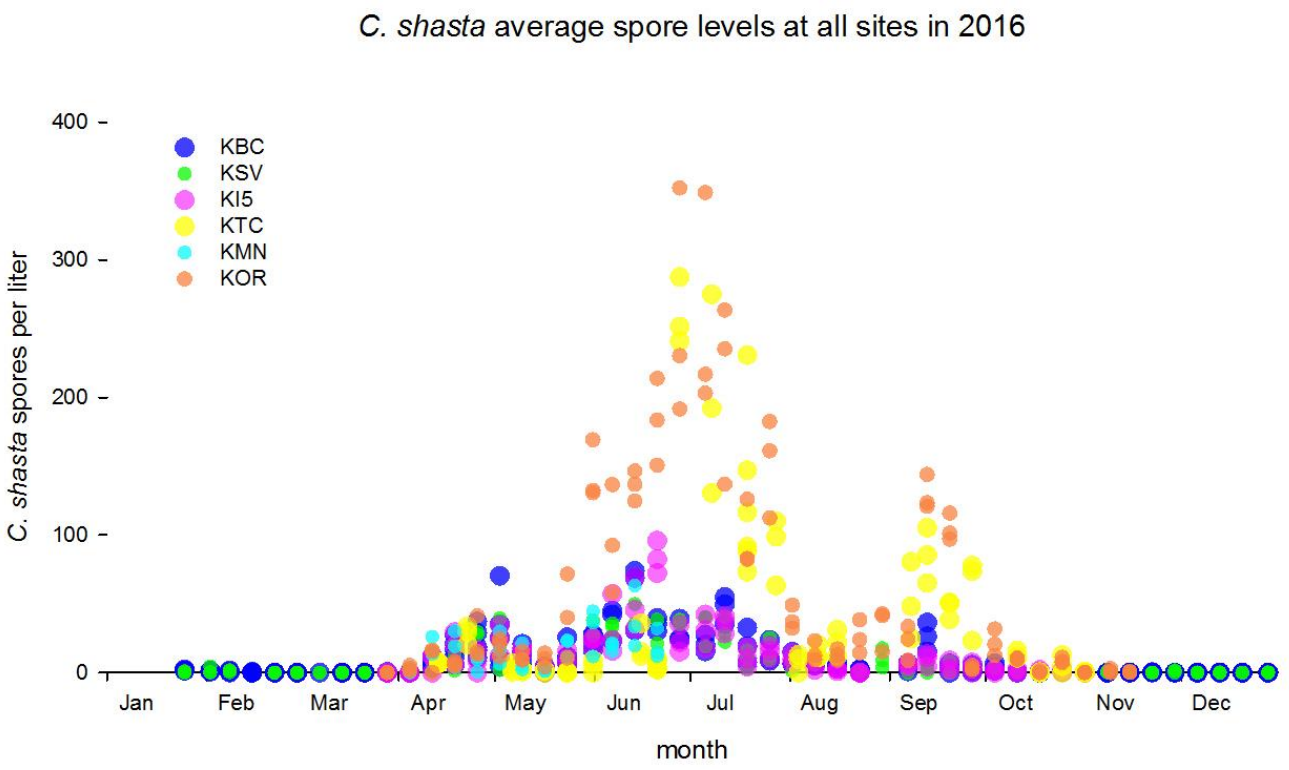

FIGURE 1.2.1. Spatial and temporal density of *Ceratonova shasta* in water samples collected at Klamath River mainstem index sites in 2016. Each data point is a 1L water sample (3 replicates/site/time).

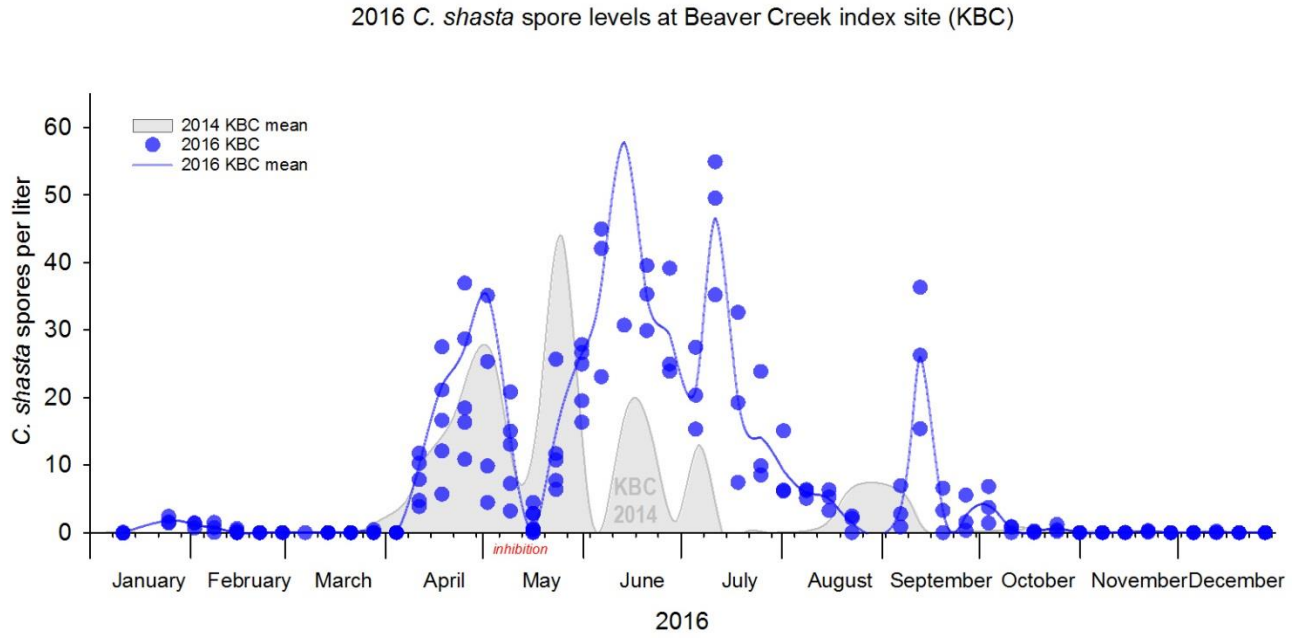

FIGURE 1.2.2. Density of *Ceratonova shasta* in water samples collected at the Beaver Creek index site in 2014 and 2016. Each data point represents the number of spores in 1L river water (3 replicates/site/time).

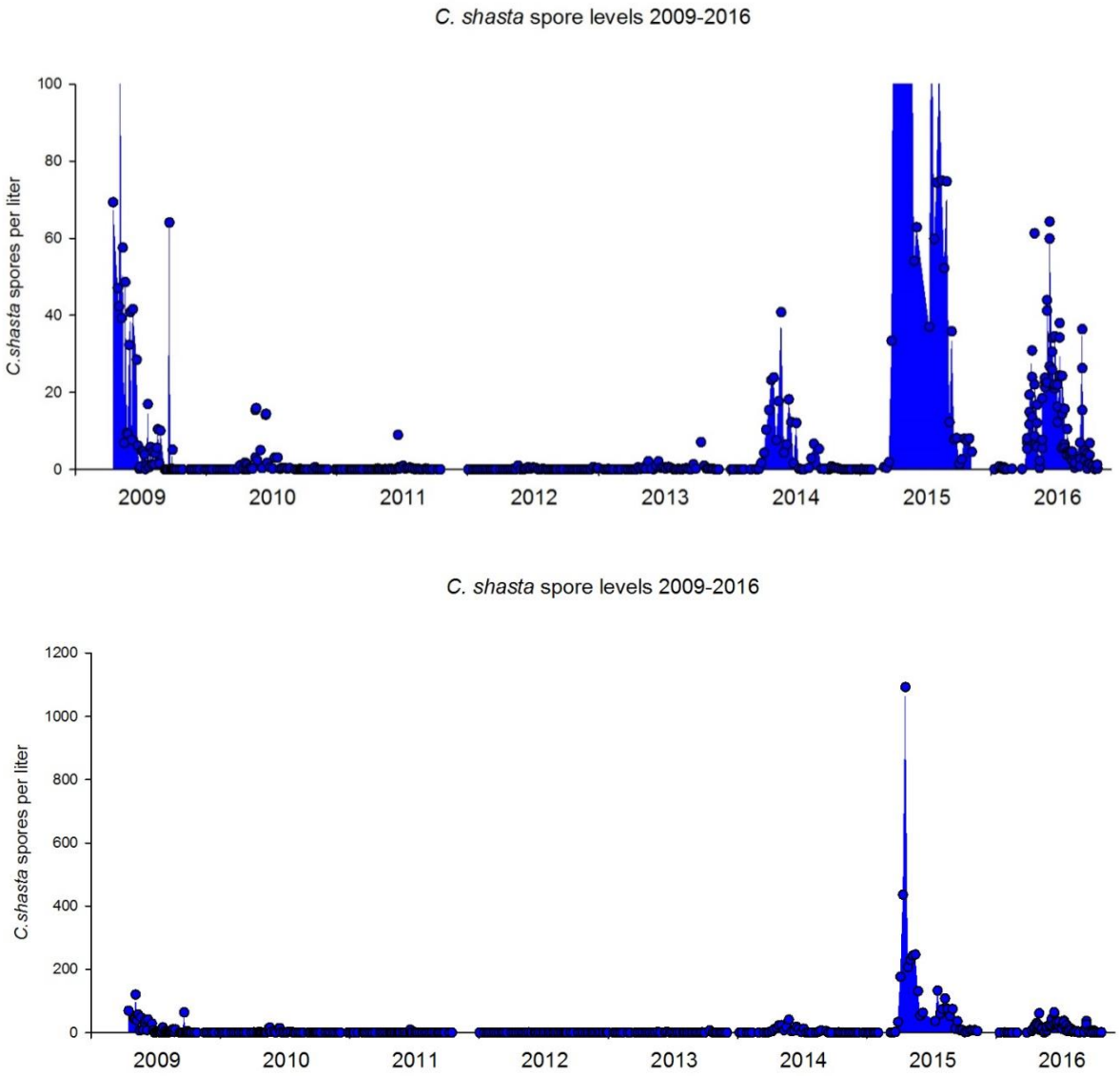

FIGURE 1.2.3. Density of *Ceratonova shasta* in water samples collected at the Beaver Creek index site from 2009 - 2016. Each data point is the average of 3 x 1L water samples. The upper graph is scaled to show the relatively lower spore levels recorded in most years.

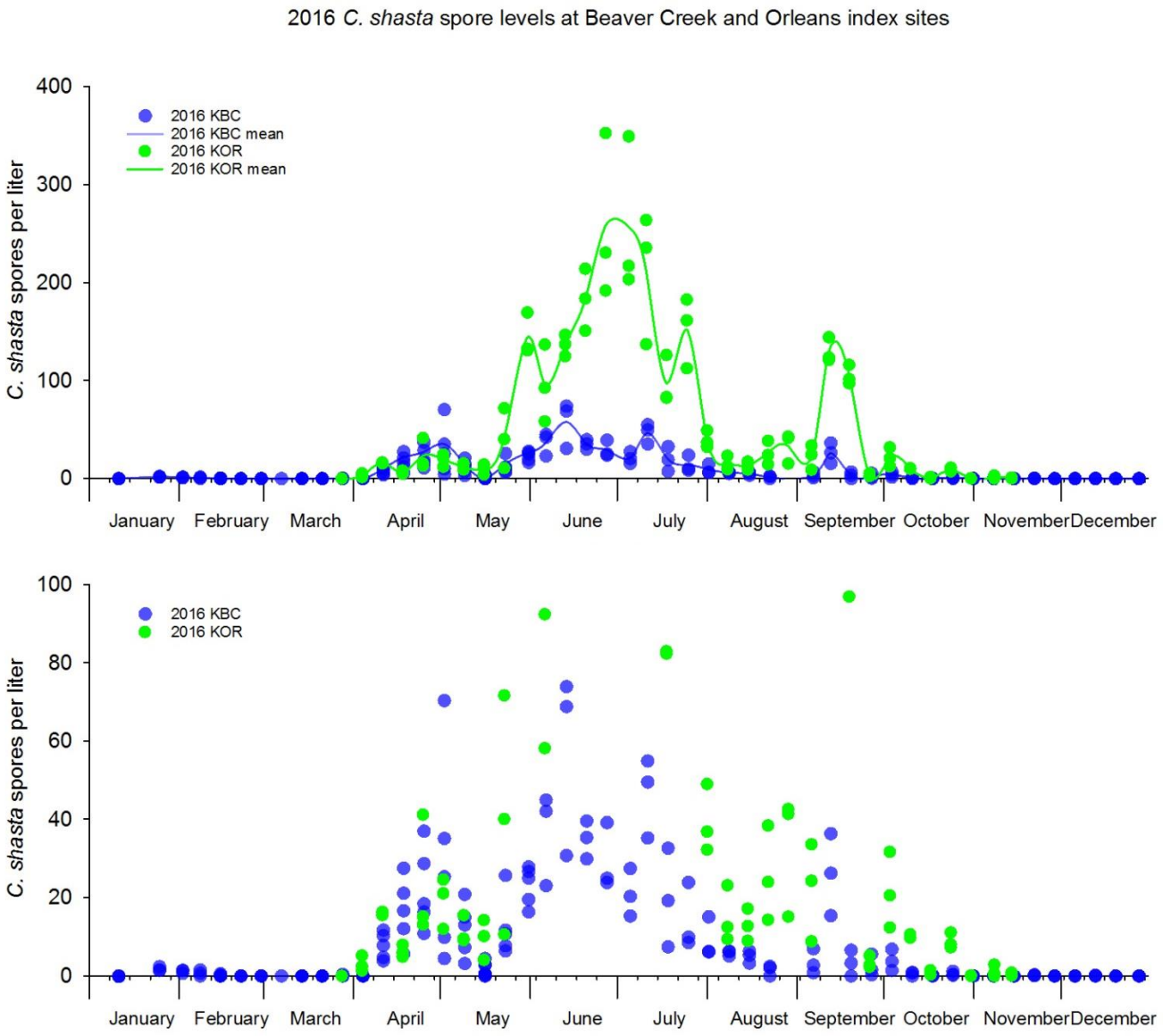

FIGURE 1.2.4. Spatial and temporal density of *Ceratonova shasta* in water samples collected at two Klamath River mainstem index sites in 2016 – Beaver Creek (KBC) and Orleans (KOR). Each data point is a 1L water sample. The lower graph is scaled to show lower spore values.

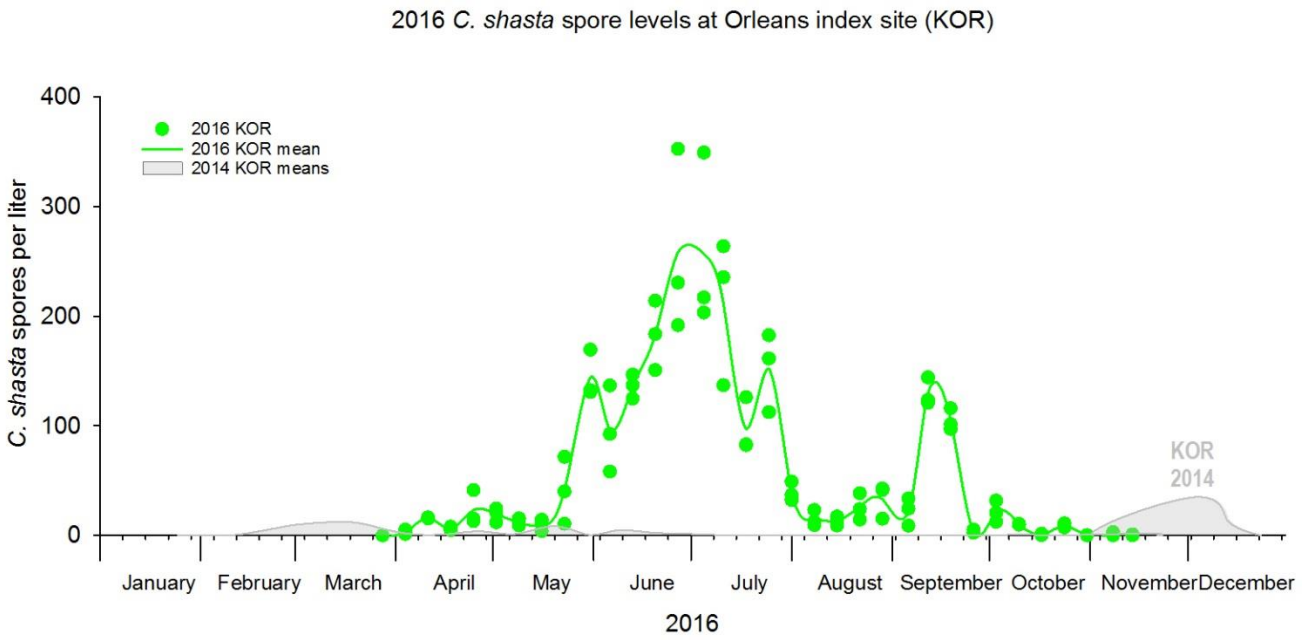

FIGURE 1.2.5. Density of *Ceratonova shasta* in water samples collected at the Orleans index site in 2014 and 2016. Each data point represents the number of spores in 1L of river water.

Previous research by the Bartholomew Lab (Atkinson and Bartholomew 2010a, b) revealed that there are multiple ITS-1 genotypes of *C. shasta* simultaneously present in the Klamath River. These genotypes differentially cause disease in the various salmonid species: Type I causes mortality in Chinook salmon whereas Type II can be fatal for coho salmon. Also, a 40% mortality threshold is reached for coho with Type II at 5 spores/L and for Chinook with Type I at 10 spores/L (Hallett *et al.* 2012).

In 2016, as in all years, the majority of spores at all time points during salmonid outmigration at Beaver Creek were genotype I (Chinook). Although Type I levels surpassed the 10 spore/L 40% mortality threshold for Chinook in early April and remained above that in all except one week (May 16), only low levels (none to trace) of type II were detected throughout the sampling period except on one day, June 13, when the average (of 3 samples) was near the 5 spores/L threshold for coho (one bottle had 7 spores/L), but this genotype was not detected again the following week at KBC. Type 0 (Steelhead) was detected in 4 of the 12 weeks.

TABLE 1.2.1. ITS-1 genotype of *Ceratonova shasta* in 1 L water samples collected during outmigration at the Beaver Creek index site.

Date	Site	Average (range) spores/L in 3x1L river water samples	ITS-1 genotypes
4/4/2016	KBC	0 (0 - 0)	insufficient to genotype
4/11/2016	KBC	10 (8 - 12)	100% I
4/18/2016	KBC	22 (17 - 28)	100% I
4/25/2016	KBC	27 (16 - 37)	100% I
5/2/2016	KBC	35 (10 - 70)	93% I, 7% O

5/9/2016	KBC	14 (7 - 21)	50-78% I, 22-50% O, trace II
5/16/2016	KBC	1 (<1 - 3)	insufficient to genotype
5/23/2016	KBC	15 (8 - 26)	75-95% I, 5-25% O, trace II
5/31/2016	KBC	27 (25 - 28)	>95% I, trace II
6/6/2016	KBC	37 (23 - 45)	>98% I
6/13/2016	KBC	58 (31 - 74)	89-94% I, 6-11% II (3, 4, 7 spores/L)
6/20/2016	KBC	35 (30 - 40)	90% I, <10% O

Water samples (four 1 L samples) were also collected manually at the start and end of each sentinel fish exposure. These provide an indication of spore levels at one point in time, rather than over a 24-h period as for the ISCO-collected samples.

TABLE 1.2.2. Density of *Ceratonova shasta* in 1 L water samples (average of 3 x 1L) collected at the start and end of sentinel fish exposures. Red numbers indicate high levels of inhibition even when re-run diluted, hence parasite levels are probably higher.

	APRIL		MAY		JUNE		September	
SITE	FISH IN April 21	FISH OUT April 24	FISH IN May 16	FISH OUT May 19	FISH IN June 21	FISH OUT June 24	FISH IN Sept 17	FISH OUT Sept 20
WMR			46	180	22	17		
WLD					24	25		
KED			105	105	9	5		
KI5			14	41	86	240		
KBC	3	38	17	60	61	53	6	13
KSV	11	18	86	17	29	68	18	18
KOR			71	120	250	360	59	39

Task 1.3. Determine density and infection of the invertebrate (polychaete) host. Sampling will occur quarterly at the following polychaete index sites:

(1) Keno Eddy - RKM 369
(2) JC Boyle - RKM 366
(3) I5 Bridge Fish Trap - RKM 287
(4) Tree of Heaven – RKM 281
(5) Beaver Creek – RKM 258
(6) Seiad Valley – RKM 207
(7) Orleans Dolan's Bar River Access– RKM 90

Polychaete collections will occur according to the following schedule:

(1) Winter- prior to peak flow, typically March
(2) Spring– after peak flow, typically June
(3) Summer– during peak period of polychaete density, July/August
(4) Fall– after beginning of adult returns, October/November

Task 1.3. Overview

The aim of this task is to describe demography and prevalence of *C. shasta* infection in polychaete populations in the Klamath River during each season of the year. Monitoring populations in the spring and fall is important because they overlap with peak juvenile salmon outmigration (spring) and adult salmon returns (fall), winter is important for understanding the dynamics of *C. shasta* infection in this host and summer is important for understanding polychaete host population dynamics. Our specific objectives are to describe the density of *M. speciosa* populations, to survey populations for prevalence of *C. shasta* infection, and to examine relationships among these factors and the environments of seven sites on the Klamath River.

Task 1.3. Methods

Polychaetes were collected four times per year in winter (mid-March), spring (June), summer (July), and fall (October). We added an additional sample period in late March following a high magnitude flood event because we were unable to sample all index during the first March sampling period due to high flows. Polychaete samples were collected by targeting previously identified polychaete assemblages at seven sites; from upstream to downstream these include Keno (KED), the Boyle bypass reach (KJB), I-5 bridge (KI5), Tree of Heaven Campground (KTH), Fisher's RV park near Beaver Creek (KBC), Seiad Valley (KSV), and Dolan's Bar near Orleans (KDB) (Figure 3.1.3.1). Three samples were collected at each site with a modified Hess sampler (a T section of PVC pipe with a base opening of 229cm^2, fitted with an 84µm collection net) and a scraping device. Samples were preserved in 70% ETOH in the field and returned to the laboratory (J.L. Fryer Salmon Disease Laboratory, Oregon State University, Corvallis, OR) for processing. All samples were subsampled by placing the entire sample into a sorting tray (20cm x 28cm, Wildco, FL) and randomly selecting three 25cm x 25cm subsamples. Subsamples were stained (20% Rose Bengal, Fisher Scientific) and all polychaetes in each subsample were counted using a dissecting microscope (20-50X magnification).

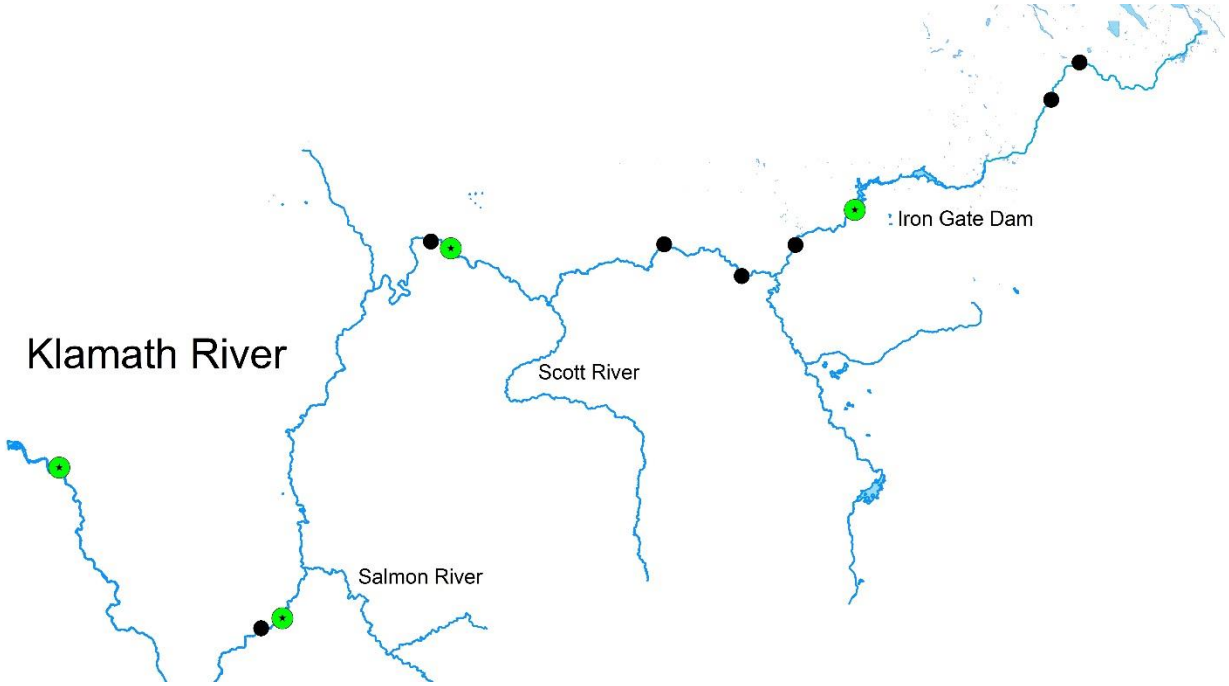

FIGURE 1.3.1. Locations of monitoring sites from upstream (KED) to downstream (KOR) shown by black circles, USGS discharge gages (green circles). Sites, in order from upstream to downstream, include KED, KJB in the JC Boyle bypass

reach, KI5 near the I5 overpass, KTH near the Tree of Heaven Campground, KBC near the confluence of Beaver Creek and the mainstem river (Fisher's RV park), KSV near Seiad Valley, and KOR at Dolan's Bar fishing access near Orleans CA.

Polychaete density: Subsample counts were adjusted to account for misidentified specimens and missed (progeny and immature) polychaetes that were observed in the samples. Adjusted polychaete density was calculated as [(adjusted count/# subsamples)/(grid cell area)x(tray area)/Hess area] and expressed per m^2 for each sample.

Prevalence of infection and estimated densities of infected polychaetes: Prevalence of *C. shasta* infection in polychaetes was determined using polychaetes collected for density estimates (see above). Up to 200 polychaetes per sample, or as many as were available if fewer than 200, were prepared for DNA extraction and tested for *C. shasta* infection by qPCR (Hallett and Bartholomew 2006).

Task 1.3.1 Results and Discussion

Polychaete density and prevalence of infection (POI): Assays have been completed for winter, spring, summer and fall sampling collections.

Preliminary results of density assays (excludes summer 2016): population dynamics differed among river sections and seasons in 2016. Densities decreased at all index sites following the high magnitude discharge event in late March 2016 (Figure 1.3.2). In contrast to 2015, when overall densities were highest at sites in the upper river section (KJB and KED), in 2016, densities were highest at lower river sites (KSV and KOR, particularly at KOR). We hypothesize that polychaetes displaced from mid river sites (e.g., KTH and KBC) may have settled out in the lower river because of the short duration of the March 2016 flow event.

Preliminary results of infection assays (summer excluded, Table 1.3.1): Prevalence of infection was highest at KI5 in winter 2016 prior to the flood event in late March. In June, prevalence was highest in polychaetes collected from KTH, and in October, prevalence was highest in polychaetes collected from KOR.

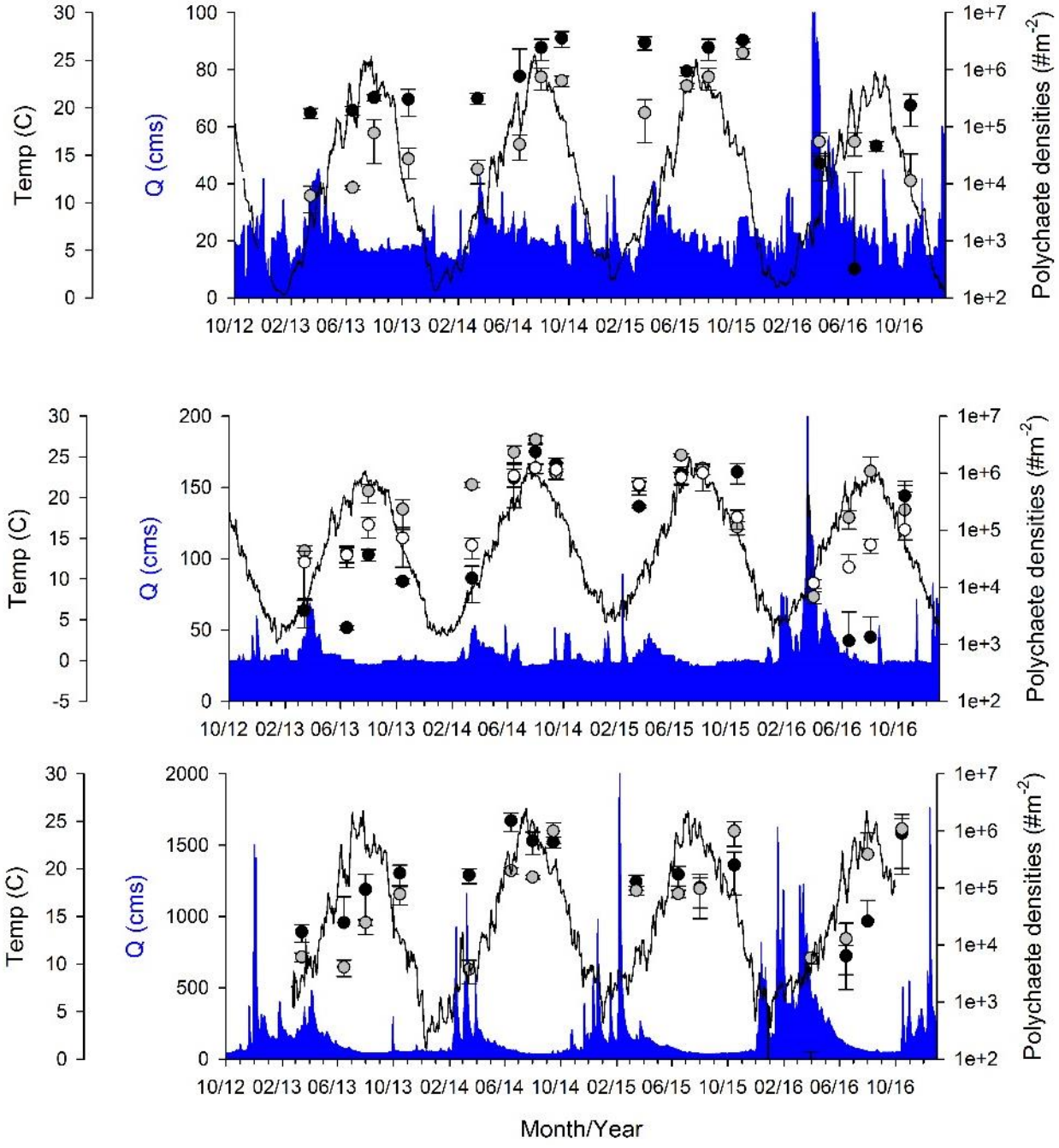

FIGURE 1.3.2. Densities of *Manayunkia speciosa* (circles), the polychaete host of *C. shasta* at 7 monitoring sites in 2013 - 2016, including discharge (blue), and water temperature (black lines), from top to bottom monitoring sites include KED (grey circles) and KJB (black circles) in the upper basin (top plot), KI5 (black circles), KTH (grey circles) and KBC (white circles) in the mid basin (middle plot), and KSV (black circles) and KOR (grey circles) in the lower basin (lower plot). Discharge (right plots, denoted in blue) and water temperature (denoted by grey solid line) were obtained from USGS gaging stations and OSU temperature loggers deployed near the sampling sites.

TABLE 1.3.1. Prevalence of *C. shasta* in *M. speciosa* at 7 monitoring sites in 2016. NS=not sampled due to unsafe flows.

Site		Prevalence (%) of *C. shasta* infection in polychaetes by site and month			
	Early March	Late March	Early/Mid-June	Late July/early August	Mid October
KOR	NS	0	1.4	1.3	3.3
KSV	NS	0	0.7	0.45	1.9
KBC	1.0	0	0.3	0	0.3
KTH	1.5	0.3	0.7	0.33	0.3
KI5	6.0	0.5	1.9	0.8	0.7
KJB	NS	0	0	0	0.6
KED	NS	0	0	0	0

Density, prevalence of infection and the environments of monitoring sites: In contrast to 2015, when infected polychaetes were detected at all but three site sampling period combinations [including KOR (June), KSV (late July), and KED (Feb)], our molecular assays show we detected infected polychaetes less frequently in 2016 (Table 1.3.1). In 2016, prevalence of infection was generally detected at levels <1.0%, which also contrasts with results in 2015, when infection prevalence was generally >1.0%. The 2016 results are similar to several previous years when disease risk to fish was lower. For example, from 2010 - 2013 the distribution of infected polychaetes was limited and infected polychaetes were detected at few sites and were characterized by low infection prevalence. In contrast, in 2014 - 2015, when disease risk to fish was high, infected polychaetes were detected at most sites and in most sampling months. Densities of infected polychaetes varied among sites and sampling periods. Prior to the high flow event in March ("late winter"), densities of infected polychaetes were highest at KI5 (mean of 31,539 individuals m^{-2}, Figure 1.3.3). Following the flow event, densities of infected polychaetes were low at all sites until spring (June), when the highest densities of infected polychaetes were detected at KTH and KOR (mean densities ranged from 180-226 individuals m^{-2}. The highest densities of infected polychaetes were thereafter detected at KOR (over 5,000 individuals m^{-2} in summer and >35,000 individuals m^{-2} in fall). This result indicates a significant shift in the distribution of infected polychaetes compared to data collected in previous years. In addition, the result corroborates water sampling data from 2016, which demonstrated high levels of *C. shasta* DNA proximal to Orleans (KOR). Analyses examining relationships among water year, fish disease risk, and polychaete data (density, prevalence of infection, and density of infected polychaetes) from 2010 - 2016 are in progress. We have built preliminary predictive models with data collected from 2010 - 2015, and plan to test the models using data we collected from 2006 - 2009 when possible, and 2016 - 2018.

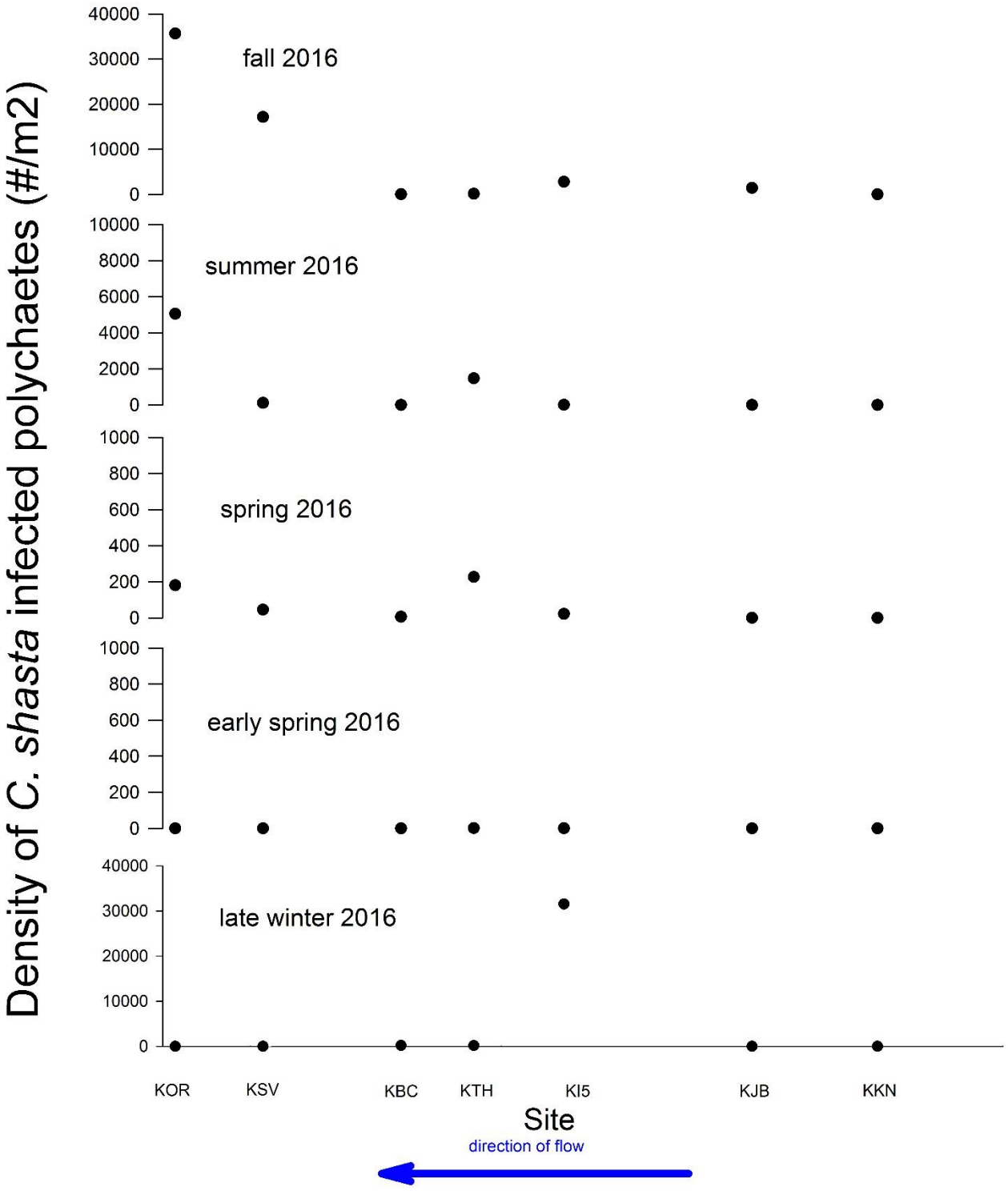

FIGURE 1.3.3. Densities of *Manayunkia speciosa* (circles) infected with *C. shasta* at 7 monitoring sites in 2016 shown by sampling period (progression in time from bottom to top) and sampling site (shown along x-axis to demonstrate spatial distribution of infected polychaete hosts).

Objective 2. Comply with 2013 Biological Opinion for Reclamation's operation of the Klamath Project to ensure weekly monitoring of actinospore genotype II concentrations in the mainstem Klamath River immediately upstream of Beaver Creek mid-April to June, and expedite analysis and data dissemination.

Task 2.1. Genotype water samples collected weekly for 7 weeks from April 14 or a date within 6 days prior to April 14 through the first full week of June.

Water samples from both Beaver Creek and Seiad Valley were processed rapidly. Data are shared under Task 1.2.

Objective 3. Determine whether a pulse flow can affect *C. shasta* densities and infection risk in fishes.

Task 3. Monitor *C. shasta* in association with pulse flow events - before, during and after an event using the following metrics: infection prevalence and severity in sentinel fish, density of waterborne parasite, infection prevalence in polychaetes and density of the polychaete host.

Task 3.1. Collect water samples daily at Beaver Creek and Seiad Valley index sites, beginning three days prior to the event and ending ~ three days after the event. Samples will be collected every 2h using automated samplers, and pooled to make a 6h composite sample that is assayed using a *C. shasta*-specific qPCR.

Task 3.2. Expose ~30 sentinel IGH Chinook and coho salmon juveniles at Beaver Creek and Seiad Valley index sites for 72 h before and during an event. After exposures, fish will be transported to the Salmon Disease Laboratory, reared at 18°C water temperature and monitored for *C. shasta* infections for 60 days as described in Task 3.1.1., above.

Task 3.3. Collect polychaetes from 4 sites before and after a pulse flow. Three Hess samples each will be collected from a reference site (KN) upstream of Iron Gate Dam, and from both fine and coarse sediments at long term monitoring sites, Trees of Heaven, Beaver Creek and The Grange. Samples will be preserved and returned to the laboratory for density and infection assays, as described in Task 1.3., above.

No pulse flow event occurred in 2016.

Objective 4. Validate the index for predicting disease severity for Chinook and coho salmon by correlating data on infection prevalence and disease severity in each fish species with genotype-specific spore densities in water collected at each site.

Task 4.1. Develop a method for high throughput genotyping of *C. shasta* by identifying a genetic locus for distinguishing among the 4 *C. shasta* ITS genotypes and between the 2 genotype II biotypes of *C. shasta*.

and

Task 4.2. Use these loci to develop a method for high throughput genotyping of *C. shasta*.

Tasks 4.1 and 4.2 Methods

To identify a new assay locus we need to compare a wide range of different genes from the different *C. shasta* genotypes. For this, we have been working to fully annotate our high quality genome (from 2015) and sequence and annotate a better transcriptome (the first pass transcriptomes from 2013 - 2014 were highly contaminated with host fish). We received the new sequence data in early 2016, and we determined that they had only a low level of fish contamination. We assembled and annotated this new transcriptome, which is now our core reference for *C. shasta* type II. We used the transcriptome to train gene prediction models to apply to the genome assembly. We completed a first-pass *de novo* gene prediction, which estimated *C. shasta* has 30,000 coding regions – this is probably an over-estimation, as most animals have 15,000-20,000. At the end of 2016 we were in the process of refining these predictions. Using the genome and transcriptome we discovered that *C. shasta* has five protein-coding mitochondrial genes (*cox1*, *cox2*, *cob*, *nad1* and *nad5*). These genes were highly divergent from those in free-living Cnidaria, which explains why we were unable to find them using universal PCR primers. We designed specific primer pairs to amplify *cox1* from different *C. shasta* samples that we had already ITS-1 genotyped from fish tissue, including coho salmon and rainbow trout (presumptive biotypes IIC and IIR, respectively). As with our original ITS-1 genotyping, the *cox-1* genotyping identified different sequences in different host salmon and trout – this was an important validation of the entire genotyping approach. Initial multi-sequence alignments showed that the *cox-1* locus has higher resolution than ITS-1 for genotyping fish isolates: We have identified several genetic markers (SNPs) that distinguish biotypes IIC and IIR at the genetic level. Interestingly, these first data suggest that the two biotypes represent upper Klamath basin versus lower Klamath basin *C. shasta* strains, rather than coho-specific versus rainbow-specific types. Thus *cox-1* is a promising genetic marker for developing a more specific quantitative genotyping assay in 2017. We are now testing water samples to confirm that *cox-1* genotyping works with waterborne spores; it is important to check this as our genome sequence data suggests that spore stages might be enigmatically low in mitochondrial genes, compared with stages in fish tissue. If cox-1 is detectable with suitable sensitivity in water samples, we will move forward with using it to design a high-throughput qPCR genotyping assay.

Publication update: We are on track to publish the genome/transcriptome summary paper in 2017, once *de novo* gene prediction is complete. We have completed the penultimate version of our manuscript that reports the finding that genotypes "II" and "III" are actually just genetic variants of the same strain of *C. shasta* (and should therefore no longer be regarded as distinct genotypes).

Task 4.3. Correlate genotype-specific parasite density with infection prevalence and severity in Chinook and coho salmon.

This analysis is underway.

Objective 5. Validate and refine the epidemiological model to identify sensitive parameters in the host-parasite life cycle, simulate the effect of potential management strategies on the different stages of the life cycle, and predict disease severity in juvenile salmonid populations under different parasite densities, water temperatures and flows.

Task 5.1. Investigate data gaps that are defined as the model is further developed.

Task 5.1.1. Epidemiological model development and progress

We continued work on the epidemiological model that was initially developed in 2013. In parallel, we completed models that predict effects of discharge on parasite spores (5.1.2 particle transport and water temperature models) based on data gaps highlighted by the epidemiological model. Results from these modeling efforts have been used to modify the epidemiological model. We began work to further develop the model to incorporate polychaete host demography parameters and have begun developing a model that includes terms for parasite genotype.

Task 5.1.2. Spore transport and water temperature models

Flow modification has the potential to affect *C. shasta* because of impacts on discharge and temperature. The parasite is fully aquatic, and has non-motile, waterborne infectious stages (myxospores and actinospores) that alternate between an invertebrate host (polychaete) and salmonids. Annual prevalence of *C. shasta*-infection in out-migrating juvenile Chinook salmon has been estimated at up to 91 percent in some years (True et al., 2016). High mortality in juvenile salmonids has been observed following low magnitude peak winter and spring flows when river temperatures reach 15-18°C in late spring (Bartholomew and Foott, 2010; Hallett *et al*., 2012). In contrast, reduced *C. shasta* infection and related mortality in salmonids have been observed following high magnitude winter and spring discharge (True *et al*., 2011; Hallett *et al.*, 2012). The addition of cool, parasite-free water from Iron Gate Dam (the lowermost dam) to the mainstem Klamath River may be an effective management action when disease risk to fish is high. Water temperature is correlated with disease progression and mortality in infected fish hosts (Ray et al., 2012). Parasite density is also correlated with mortality; 5 and 10 spores per L of river water cause >40% mortality in Klamath River Coho and Chinook, respectively (Hallett *et al*., 2012). Thus, managed water releases could reduce disease risk to fish by reducing downstream river water temperature or by diluting waterborne parasite stages.

Knowledge of water age, or the travel time required for a parcel of water released from the dam to reach specific location, is necessary for predicting how the timing and volume of water released will affect parasite density and water temperature within high risk for fish zones located downstream. In addition, water age is relevant for (i) determining timing and volume of water to be released to achieve decreases in parasite density or water temperature, and (ii) designing monitoring plans to measure effects on disease risk for fish (e.g. measure parasite density before, during and after a managed water release event). Thus, a model predicting water temperature and age would be useful for managers considering scenarios of flow manipulation strategies.

We built a three-dimensional hydrodynamic model coupled with CE_QUAL_W2 temperature model and Lagrangian particle tracking model to 1) estimate water age, 2) model dispersion of waterborne parasite spore along the river, and 3) predict the water temperature of river. These objectives have important potential uses and implications for managing *C. shasta* in this system. First, estimating water age is applicable in the context of conducting cost-benefit analyses for managed flow events. Timed 'pulsed flow' events have been conducted in the Klamath River as a method of to the exposure of fish to waterborne actinospore stages. However, monitoring data have been constrained by logistics related to the timing of the peak of these flow events. Having a model that predicts the arrival of the peak is imperative to cost effective monitoring. Second, modeling the dispersion of waterborne parasite spores is important for understanding parasite dynamics. Although both parasite stages are waterborne, actinospores are neutrally buoyant, whereas myxospores are negatively buoyant. Identification of hot spots of parasite spores may be useful for designing monitoring and management approaches. Finally, the ability to predict water temperature at x, y locations within the river

under different management scenarios will be useful for assessing relative risk of infection for fish and mortality and production of parasite spores. Different dam release scenarios were tested to assist decision makers in identifying management actions that could decrease disease effects in salmonids.

We found that flow rate and bottom roughness were the two most important parameters that influence water age. Water temperature was more sensitive to inflow temperature, air temperature, solar radiation, wind speed, flow rate, and wet bulb temperature respectively. Specific findings include i) the concentration of waterborne spores is predicted to decrease by 60% within the infectious zone following the release of 170 cms from Iron Gate Dam, however, ii) the concentration of myxospores is also predicted to increase because the higher velocity moves the spores downstream, iii) releasing cool water from the dam could be an effective method to decrease the water temperature because results show that by releasing 55-85 cms from Iron Gate dam, water temperature at Seiad Valley can be decreased from 1-4°C dependent on the reservoir temperature. Our results are relevant for managers because they provide a framework for predicting how water within 'high infection risk' sections of the river will respond to dam water (low infection risk) input. Moreover, these data will be useful for prioritizing the use of water age (dilution) versus temperature (spore viability) under certain contexts when considering flow manipulation as a method to reduce risk of infection and disease in Klamath River salmon.

The manuscript has been submitted to the *Journal of Hydrology* (Feb 2017).

Objective 6. Investigate the occurrence of *C. shasta* below the Trinity River confluence and ascertain spore type of waterborne stages. Tribal biologists will assist with the following tasks.

Task 6.1. Conduct sentinel fish exposures when parasite abundance exceeds 10 spores/L but temperatures are not lethal for salmon.

Water temperature was too high for sentinel fish exposures.

Task 6.2. Quantify parasite levels in water samples.

Details of this task were shared earlier, under Task 1.2.

Task 6.3. Characterize density and infection of the invertebrate (polychaete) host in the mainstem downstream from the Trinity River confluence.

This aspect of the research requires that we collaborate with the Yurok tribe for access to relevant river sections. We planned this research for August 2016, but high water temperatures resulting in an Ich outbreak prevented the Yuroks from assisting during this period. We are working with the Yurok tribal biologists to determine an appropriate time to sample this reach for polychaetes in 2017 and 2018 to ensure it occurs on schedule.

Objective 7. Develop and validate predictive models for polychaete hosts including distribution, density, infection, and recolonization rates under different peak discharge, water years, and dam removal scenarios.

Task 7.1. Validate and refine the polychaete distribution model for predicting distribution under different peak discharge, water years, and dam removal scenarios.

and

Task 7.2. Add polychaete density and infection prevalence data to the predictive model to examine how flow regimes will affect density and infection in this host.

We developed and published (Alexander *et al.* 2016, *Freshwater Science*) a tandem modeling approach to predict the distribution of *M. speciosa* and evaluate the effects of discharge scenarios in sections of the Klamath River. Two-dimensional hydraulic models (2DHM) were built for three river sections using topographic survey data, water surface elevation profiles, stage-discharge relationships, and spatial maps of substrate (Wright 2014). The 2DHMs were used to describe hydraulic variation and stratify sampling locations across depth velocity gradients within substrate classes. Benthic samples were collected in July 2012, July 2013, July 2014 and July 2016. Samples collected in 2012 were used to build a statistical model estimating the relationship between physical habitat characteristics and the distribution of *M. speciosa*. Samples collected in other years are used for model validation. We predicted the distribution of *M. speciosa* under alternate flow scenarios, 1,200cfs and 7,950 cfs, to simulate dry and wet water years, respectively. The values were selected to fall within the range of discharge possible for future management solutions. Preliminary results suggest that manipulating the hydrograph could influence distribution of polychaete hosts (Figure 7.1.1). Validation of the model predictions using real data collected at both the low and high peak discharge scenarios are in progress, and are needed before we can evaluate whether manipulation of the hydrograph may in turn influence prevalence of *C. shasta* and disease in salmonids, but the preliminary results are very exciting. We ran simulations of the peak flood event that occurred in March 2016 (>10,000 cfs from IGD) and examined predictions against empirical data. Although the 2D models had difficulty running at discharge >10,000, the predictive model performed well, predicting within 90% accuracy among sites.

We built preliminary predictive models for density and prevalence of infection using data collected 2012 - 2014. Models will be refined in 2017, and submitted for publication.

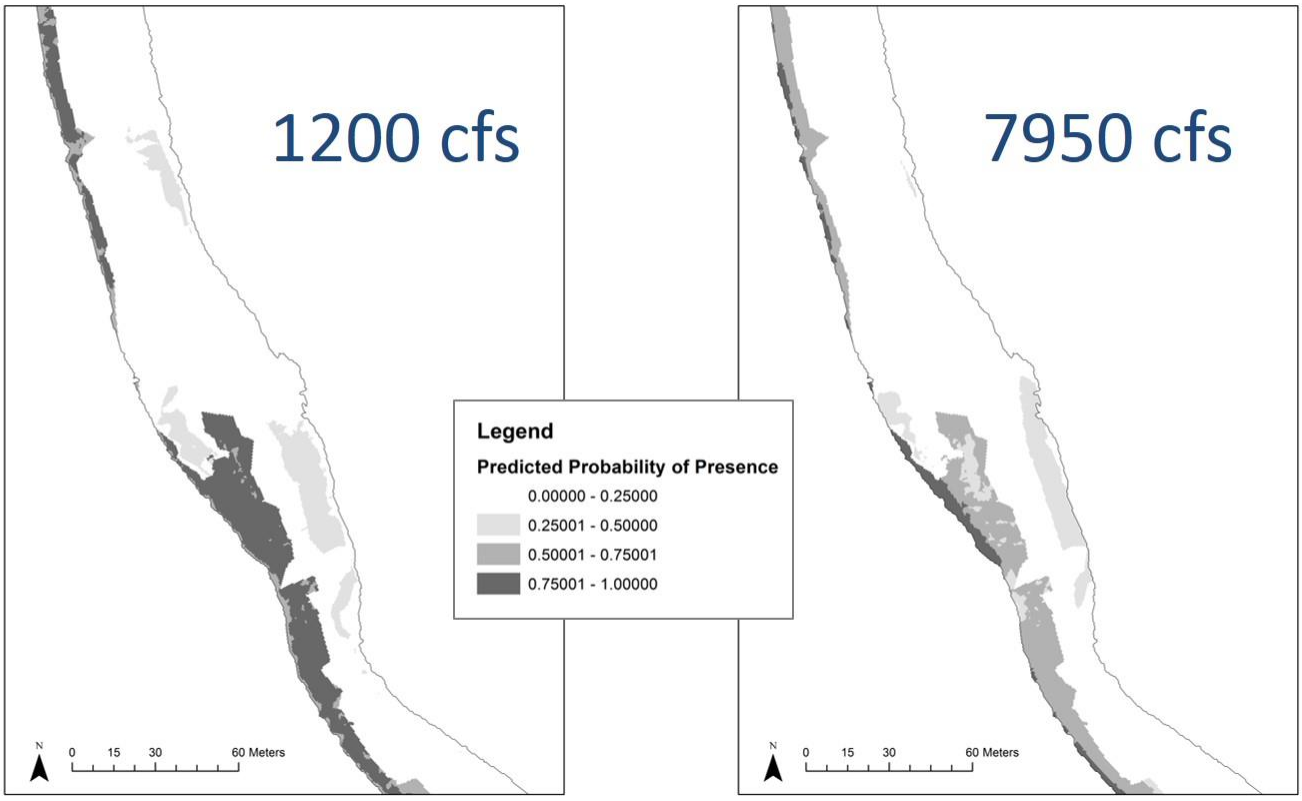

FIGURE 7.1.1. Modeled effects of peak discharge on the probability of polychaete presence at x, y locations in the Tree of Heaven Study reach. Predicted polychaete distributions under two modeled peak discharge scenarios including a dry water year having a peak discharge of 1,200 cfs out of Iron Gate Dam (left) and a wet water year having a peak discharge of 7,950 cfs (right).

Task 7.3. Estimate polychaete recolonization rates. Use the physical models to characterize hydraulic conditions before and after disturbance. Predict polychaete distribution using the refined distribution model. Validate with empirical data where available.

The polychaete distribution model developed and validated for predicting distribution and density (7.1 and 7.2) and four locations we have monitored for recolonization since 2010 ("recolonization monitoring sites") are providing data for this task. We completed data collection in 2016 and found model predictions to be highly accurate (Figure 7.3.1), and plan to collect data again in 2017 to assess the effects of longer duration but similar flood magnitude (peaks similar but duration much shorter in 2016 compared to 2017). Polychaete tubes were observed at the reach 1 pool and eddy sites and the reach 2 pool and eddy sites in 2015 but not in 2016. These habitats will continue to be monitored in 2017-2018 to determine the impacts of the March 2016 flood event on recolonization.

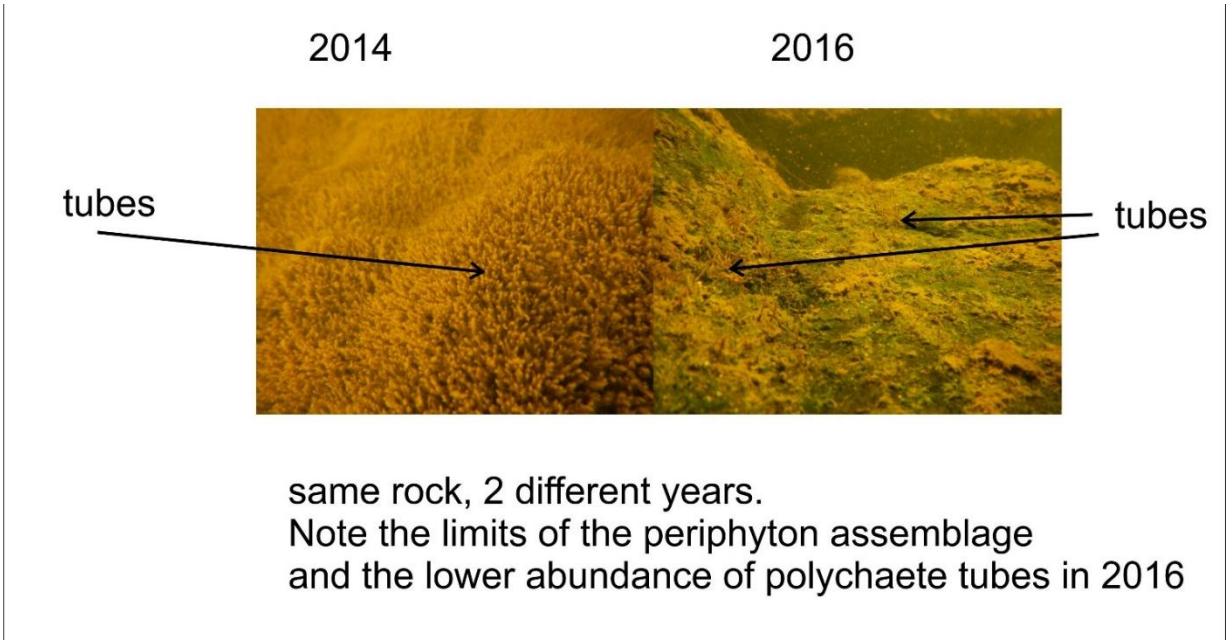

FIGURE 7.3.1. Observations of polychaete assemblages in 2014 (low peak discharge) and 2016 (high peak discharge) on a georeferenced sampling location at the Tree of Heaven Study reach. The polychaete distribution model predicted low probability of polychaete occurrence at this location in 2016 given the hydraulic conditions during peak discharge, and our observations support the model predictions.

Objective 8. Develop and synthesize a dataset, encompassing environmental risk factors and their relationship with polychaete host ecology, to facilitate predictions about how polychaete densities and infection levels may change under future climate and temperature regimes.

Task 8.1. Synthesize an "environmental risk factor" dataset comprised of water quality data and future predictions for water temperature and discharge for examining correlations with polychaetes.

Analyses examining correlations between water temperature and discharge have been completed and we have developed preliminary models to examine relationships between polychaetes and temperature and discharge. The results are being used as inputs for the epidemiological model and to guide laboratory experiments to address outstanding questions relevant to polychaetes.

Task 8.2. Examine correlations between environmental risk factors and polychaete host data including density, population structure, and infection prevalence.

Preliminary correlations have been completed for 2010-2015. We plan to add data from 2016 once complete and then validate these relationships using data collected 2006-2009. We chose to include 2016 to increase leverage within the dataset because it extends the range of peak discharge values we can include.

Task 8.3. Construct models for generating predictions about how polychaete densities and infection levels may change under future climate and temperature regimes, and how these changes in turn may affect disease risk in salmon hosts.

We have linked a series of Klamath River models including i) a fine-scale climate change model to predict future stream temperatures and discharge (Perry *et al.* 2011), ii) a 2-D hydraulic model coupled with a statistical model to predict changes in polychaete populations under different river discharge scenarios (Task 7, above), iii) a degree-day model to predict the potential number of generations per year under different thermal regimes (Chiaramonte 2013), and iv) the epidemiological model to quantify the risk of disease in the salmon host under the different climate scenarios (Ray 2013, Task 5).

The models and their outputs predict changes in disease severity in salmon as a result of *C. shasta* infection under different climate scenarios. We are examining predictions using past years (hindcasting) and long-term data for on the intensity and distribution of *C. shasta* infections in juvenile salmon. The next steps will include using future climate predictions to predict the effects of climate change on temperature and precipitation on the phases of the *C. shasta* life cycle involving *M. speciosa*.

Objective 9. Regularly disseminate research findings to provide stakeholders, managers, researchers and the general public ready access to current information and historical datasets pertinent to *C. shasta* in the Klamath River.

(a) Preliminary Result Summaries: The contractor will provide brief preliminary summary information to Reclamation on a monthly basis each field season or as-requested by Reclamation. Additionally, preliminary findings may be available in the form of a professional presentation at a meeting with Reclamation and other state, federal, and tribal agencies.
(b) Annual Reports: The contractor will provide Reclamation an annual report of research for this study, due March 31 each contract year. This report will include a description of the study questions, methods of data collection and analyses, results of data analyses, and a discussion of the significance of the data. Draft copies of the annual report of research will be distributed to Reclamation and other interested parties for review before the report is finalized.
(c) Website: maintained by the contractor for dissemination of results and project information to the public.
(d) Annual Klamath River Fish Health Workshop: public forum to review results of disease research, and will be coordinated by the contractor.
(e) Annual project coordination meeting: with project collaborators; coordinated by the contractor.
(f) Publications: submit findings for publication in peer-reviewed scientific journals.
(g) Final report: the final report that summarizes and synthesizes the multi-year findings will be submitted by Dec 31 2019.

Research summaries were provided as requested, at professional meetings, conference calls and online. This document serves as an annual report. Data are posted on the website regularly during salmonid outmigration. OSU organized the annual Klamath River Fish Health Workshop and management meeting; both were held March 2017 in Ashland, Oregon.

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