Klamath River Fish Health: Disease Monitoring and Study
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ANNUAL REPORT

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Confluence of the Trinity River and the Klamath River mainstem.
Summary

The myxozoan parasite *Ceratonova shasta* infects the intestine of salmonid fish which can cause enteronecrosis and mortality. The parasite is endemic to the Pacific North West of North America but has been responsible for high mortality in juvenile salmon in the Klamath River basin. To propagate, *Ceratonova shasta* cycles between two hosts and two spore stages; waterborne actinospores released from freshwater polychaete worms infect salmonids and develop into myxosspores which are then infectious to polychaetes (refer to diagram on Page 1). The Bartholomew Lab at Oregon State University has been monitoring the spatial and temporal abundance of the parasite in that basin since 2006 using sentinel fish exposures, river water sampling and polychaete sampling. This report describes monitoring studies conducted in 2015. Those data are informing several models that are being developed to better predict disease effects under various conditions. Unprecedented levels of parasite and associated infection were observed in 2015.

The sentinel fish exposures were conducted earlier in 2015, in early April instead of during the third or fourth week because water sample analysis indicated rapidly increasing levels of *C.shasta*. Low mortality (2.4% - 12.2%) was observed in sentinel Chinook salmon held at Seiad Valley and Beaver Creek during April and September 2015. However, *C. shasta*-associated loss during May near Beaver Creek and Seiad Valley, the demonstrated infectious zone, was severe in the Chinook salmon reaching the highest ever observed of 90.5% at Seiad Valley. In June, the infectious zone appeared to expand to include in the Klamath River near I5 Bridge, Beaver Creek and Orleans where Chinook loss with associated *C. shasta* infections was 40.5, 46.3 and 39.5%, respectively. In contrast, at Seiad Valley, where river water temperatures were the highest during the exposures, Chinook loss was low (7.7%). No infections were detected in Chinook exposed at sites in the upper Klamath River. Yearling coho losses with associated *C. shasta*-infection were much lower near Beaver Creek and Seiad Valley in May and June in 2015 ranging from 11.1-26.7% compared to 48.5-93.3% in 2014. However, more than 50% of the surviving coho after the post-exposure holding had eye lesions suspected to be caused by *C. shasta*. For exposures in the Williamson River, in the upper Klamath basin, we had predicted that changes in stocking practices would result in decreasing levels of the parasite. However, sentinel exposures of susceptible rainbow trout in the Williamson River resulted in high mortality, as in previous years.

Molecular analysis of water samples detected waterborne *C. shasta* throughout the lower basin. Density of *C. shasta* was higher in 2015 than in any other year since monitoring began in 2006; in 2015, levels were up to an order of magnitude higher than the previous highest level of ~100 spores/L in 2007-9 at Beaver Creek. Additionally, levels peaked sooner in 2015 than usual, in April rather than May/June. Furthermore, until 2015, only low levels of parasite had been detected at our uppermost index site, I5, whereas in 2015 >100 spores/L were detected during Spring (April-June). We used an optimized regular PCR assay to determine genotype-specific spore densities in water samples, particularly to distinguish between genotypes I (Chinook) and II (coho). This approach was impeded this year by extraordinarily high levels of genotype I in March and April, relative to low levels of genotype II (the limit of resolution of this assay is about 5% (5 spores in 100), so when genotype I is above 100 spores per liter, we will be unable to detect if genotype II is present at 5 spores per liter – only at higher levels). As an interim solution, we are investigating a genotype-II specific Taqman qPCR probe, to quantify genotype II irrespective of the genotype I level. In parallel, we are continuing to search for a different assay locus and have sequenced additional, higher quality *C. shasta* samples to overcome contamination problems in the initial transcriptome datasets. Also, by genotyping individual parasite spores we discovered that genotype II and III are actually genetic variants of only a single strain of *C. shasta* – this discovery simplifies the task of developing an assay to distinguish between the genotypes.

Polychaete population dynamics differed among river sections but densities were lowest in winter (all sites) and peaked either in spring or summer (most sites in the middle and upper river) or fall (sites in the lower river and one site in the middle river). We detected high prevalence of infection (5%) in winter (February) polychaete samples at the I5 site that persisted through spring (June) that explain the high infection risk for salmon in 2015. Although prevalence of infection was lower (~1%) at I5 in fall (October) densities remained high, and densities of infected polychaetes were higher in 2014-2015 than we have previously observed.
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 work on the epidemiological model that was piloted in 2013. Data outputs from these models will be used to inform monitoring efforts for future pulse flow events and to parameterize data gaps highlighted by the epidemiological model.

We have built and validated models that predicted the distribution of *M. speciosa* under alternate flow scenarios, 1,200 cfs and 7,950 cfs, to simulate dry and wet water years, respectively. We are in the process of building predictive models for density and prevalence of infection to examine how flow regimes may affect these factors.
Objective 1 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:

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

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:

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

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.

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.

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. .................................................................42

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.42
Task 4.2. Use these loci to develop a method for high throughput genotyping of C. shasta ................. 42
Task 4.3. Correlate genotype-specific parasite density with infection prevalence and severity in Chinook and coho salmon. ................................................................................................................. 43

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. ..................................................................................................................43

Task 5.1. Investigate data gaps that are defined as the model is further developed. ......................... 43

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. ...............................................................51

Task 6.1. Conduct sentinel fish exposures when parasite abundance exceeds 10 spores/L but temperatures are not lethal for salmon. ...................................................................................................................... 51
Task 6.2. Quantify parasite levels in water samples. ........................................................................... 51
Task 6.3. Characterize density and infection of the invertebrate (polychaete) host in the mainstem downstream from the Trinity River confluence. ......................................................................................... 51

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. .51

Task 7.1. Validate and refine the polychaete distribution model for predicting distribution under different peak discharge, water years, and dam removal scenarios.............................................................. 51
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................................................................. 52
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......................................................... 53

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. .................................................................53

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......... 53
Task 8.2. Examine correlations between environmental risk factors and polychaete host data including density, population structure, and infection prevalence............................................................... 53
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. ................................................................................................................. 53
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..........................................................................................................................54

List of Figures and Tables
FIGURE 1.1.1. Klamath River index sites for 2015 with site abbreviations and river kilometers (Rkm). .................................................11
TABLE 1.1.1. Average Klamath River water temperature (°C) at sentinel sites during the 72 hr fish exposures in 2015. ......13
FIGURE 1.1.2. Average daily water temperatures during the months of March-September during the years 2008-2015 at the sentinel site near Beaver Creek on the Klamath River mainstream. .................................................................13
TABLE 1.1.2. Percent loss attributable to infection by C. *shasta* by site and fish species in 2015 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 63-68 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. ...........................................................................................................14
FIGURE 1.1.2. Per cent mortality with *C. shasta* infections of rainbow trout (Rbt) and IGH fall Chinook salmon exposed April 6-9 2015 at two index sites in the lower Klamath River and held for 64 days post-exposure at 13°C.................................................................15
FIGURE 1.1.3. Comparison of percent loss from *C. shasta* infections in rainbow trout (Rbt) and IGH Chinook (Chf) exposed in 72 hr sentinel studies near Beaver Creek during April 2009-2015. .........................................................................................16
FIGURE 1.1.4. Percent *C. shasta*-associated mortality of sentinel Iron Gate Hatchery juvenile Chinook 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 (KIS), near Beaver Creek (KBC), Seiad Valley (KSV) and Orleans (KOR) in the Klamath River for 72 hours May 11-14, 2015. Also, coho from Iron Gate Hatchery were held during the same time near Beaver Creek and Seiad Valley. Fishes were held at the OSU Aquatic Animal Health Laboratory and monitored for 68 days post-exposure. Fish in which no myxospores were observed had an intestinal sample PCR-tested.................................................17
FIGURE 1.1.5. Eye lesion in yearling coho exposed May 11-14 near Beaver Creek and held for 68 days at the Aquatic Animal Health Laboratory.............................................................................................................18
FIGURE 1.1.6. H&E stained section of a yearling coho eye showing a normal area of retina (left) and an area infected with *C. shasta*. ........................................................................................................................................18
FIGURE 1.1.7. Cumulative loss of IGH Chinook and coho exposed in sentinel cages for 72 hr in May 2015 near Beaver Creek and Seiad Valley in the Klamath River. ........................................................................................................19
FIGURE 1.1.8. Cumulative per cent loss associated with *C. shasta* in rainbow trout at six sentinel sites in May 2015. ...........19
FIGURE 1.1.9. Comparison of percent loss from *C. shasta* of juvenile IGH Chinook salmon (upper figure) and coho salmon (lower figure) at six index sites in May of 2007-2015. 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. ..................................................................................................................20
FIGURE 1.1.10. Comparison of percent loss from *C. shasta* in juvenile rainbow trout exposed for 72 hr during May 2007-
2015 at seven different Klamath River sentinel index sites and held for more than 60 days. 

FIGURE 1.1.11. Percent *C. shasta*-associated mortality of sentinel Iron Gate Hatchery juvenile Chinook 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 (K15), near Beaver Creek (KBC), Seiad Valley (KSV) and Orleans (KOR) in the Klamath River for 72 hours June 15-18, 2015. Also, coho from Iron Gate 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. 

FIGURE 1.1.12. Cumulative loss of *C. shasta* in IGH Chinook and coho salmon exposed for 72 hr near Beaver Creek (KBC) and Seiad Valley (KSV) in June 2015. 

FIGURE 1.1.13. Cumulative percent loss associated with *C. shasta* in rainbow trout following exposure at seven sentinel sites in June 2015. 

FIGURE 1.1.14. 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 2007-2015. 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 1.1.15. Comparison of percent *C. shasta* mortality for rainbow trout exposed in June 2007-2015 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. 

FIGURE 1.1.16. Percent mortality of rainbow trout (Rbt) and IGH fall Chinook salmon exposed September 18-21, 2015 at two Klamath River sentinel index sites, near Beaver Creek (KBC) and Seiad Valley (KSV) and held for 63 days post-exposure at 18°C. 


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 hr in April, May, June and September (when conducted) in years 2007-2015. 

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 hr in April, May, June and September (when conducted) in years 2007-2015. 

FIGURE 1.1.20. Percent loss with *C. shasta* infections of sentinel Iron Gate Hatchery juvenile Chinook and coho salmon exposed near Beaver Creek and Seiad Valley in the Klamath River for 72 hours prior to an anticipated river pulse-flow event planned in May. Fishes were held at the Aquatic Animal Health Laboratory and monitored for 71 days post-exposure. Fish in which no myxospores were observed had an intestinal sample PCR-tested. 

FIGURE 1.2.1. Density of *Ceratonova shasta* in water samples collected at the Beaver Creek index site from 2009-2015 showing the unprecedentedly high levels in 2015. Each data point is the average of 3 x 1L water samples. The upper graph is scaled to show relatively lower spore levels. 

FIGURE 1.2.2. D
FIGURE 1.2.3. Spatial and temporal density of Ceratovina shasta in water samples collected at Klamath River mainstem index sites in 2015. Each data point is the average of 3 x 1L water samples. The lower graph is scaled to show lower spore values. .................................................................................................................................................................................. 33
FIGURE 1.2.4. Temporal abundance ............................................................................................................................................................................................................................................................................... 34
FIGURE 1.2.5. Spores per liter of genotypes I (Chinook; orange) and type II (coho; blue) at Beaver Creek in 2015. Each dot is the average of three 1 L water samples. The gray dots and shading represent the detection limit of 5% of the total signal, which possibly masked detection of low levels of type II (blue) at some timepoints. .......................................................................................................................................................................................................................................................... 35
TABLE 1.2.1. Density of Ceratovina shasta in 1 L water samples (average of 3 1L; range) collected at the start and end of sentinel fish exposures. ........................................................................................................................................................................................................................................................................................................... 36
FIGURE 1.2.6. Density of Ceratovina shasta at Beaver Creek and Seiad Valley in May 2015 collected during a pre-pulse-flow event. Each dot is the average of three 1 L water samples. ........................................................................................................................................................................................................................................................................................................... 36
FIGURE 1.3.1. Locations of monitoring sites from upstream (KED) to downstream (KDB) 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, KTOH near the Tree of Heaven Campground, KBC near Fisher’s RV park, KSV near Seiad Valley, and KOR at Dolan’s Bar fishing access near Orleans CA. ........................................................................................................................................................................................................................................................................................................... 38
FIGURE 1.3.2. Densities of Manayunkia speciosa (left), the polychaete host of C. shasta at 7 monitoring sites in 2015, from top to bottom, monitoring sites include KED and KJB in the upper basin, KI5, KTH and KBC in the mid basin, and KSV and KOR in the lower basin. Discharge (right plots, denoted in blue) and water temperature (denoted by grey solid line) were obtained from USGS gaging stations and OSU Hobo temperature loggers deployed near the sampling sites. ........................................................................................................................................................................................................................................................................................................... 39
TABLE 1.3.1. Prevalence of C. shasta in M. speciosa at 7 monitoring sites in 2015. ........................................................................................................................................................................................................................................................................................................... 40
TABLE 1.3.2. Estimated densities of C. shasta infected M. speciosa at 7 monitoring sites in 2015. ........................................................................................................................................................................................................................................................................................................... 41
Figure 5.1.2.1. Map of the Klamath River showing the study area from Iron Gate Dam to Seiad Valley. Red dots show the USGS discharge stations at IGD, Shasta, Scott, and Seiad Valley stations. Green dots show the stations at Tree of Heaven, Beaver Creek, and Community Center where bathymetry data have been measured. Blue arrows also show the major tributaries joining the Klamath River. ........................................................................................................................................................................................................................................................................................................... 44
FIGURE 5.1.2.2: Estimated bathymetry (blue solid line) vs measured data (red dashed line) for a) Tree of Heaven, b) Beaver Creek, c) Community Center, and d) 10 miles upstream of Shasta Rive. ........................................................................................................................................................................................................................................................................................................... 46
FIGURE 5.1.2.3: Accumulative flow from Seiad Valley station (black solid line) comparing with flow from IGD (red dotted line), adjusted flow from Scott River (green dash-dot line), flow from Shasta River (orange thick dashed line), and sum of all inflows to the system (blue dashed line) for 2015. ........................................................................................................................................................................................................................................................................................................... 46
FIGURE 5.1.2.4: Simulated and measured water surface elevation versus flow discharge for a) Tree of Heaven, b) Beaver Creek, and c) Community Center. The blue dashed line shows the measured data and red solid line shows the simulated water surface elevation. ........................................................................................................................................................................................................................................................................................................... 47
FIGURE 5.1.2.5: Comparison between measured water column temperature and EFDC simulated depth-averaged water column temperature. The blue dashed-line shows the measured data and red solid line shows the modeled water column temperature. ........................................................................................................................................................................................................................................................................................................... 48
TABLE 5.1.2.1: Dam release scenarios tested for calculating the travel time at Seiad Valley. cms=m³s⁻¹. ........................................................................................................................................................................................................................................................................................................... 48
Figure 5.1.2.6: Water age at Seiad Valley for Scenarios with 40 cms base flow for 2 days, then dam release of 60 cms for a) 6hr (blue solid line), b) 9hr (green dashed line), c) 12hr (red dotted line), and d) 24hr (orange dot-dash line).

Figure 5.1.2.7. Travel time to Seiad Valley versus dam release flow with base flow of 28 cms (green dashed line), 56 cms (blue dotted line), 84 cms (orange dot-dash line), and 112 cms (black solid line) for dam release period of a) 6hr, b) 9hr, c) 12hr, and d) 24hr.

Table 5.1.2.2: Concentration reduction of spores for various dam release scenarios.

Table 5.1.2.3: The effect of dam release on maximum water temperature of Seiad Valley. RSVR: Reservoir, Water temp*: Water temperature after dam release.

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).
Research outcomes

Objective 1. 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

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

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) IS 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 and near Seiad Valley

Task 1.1. Methods
Sentinel fish exposures were conducted according to the sites and schedule. There were four exposures for 72 hr each at up to seven index sites (Figure 1.1.1) in the lower and upper Klamath River mainstem in 2015 during the following dates: April 6-9, May 11-14, June 15-18 and September 18-21. 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 all sites. Klamath River fall Chinook juveniles from Iron Gate Hatchery (IGH) (California Department of Fish and Wildlife) were held at all sites except for one location, the Lonesome Duck Resort on the Williamson River. Juvenile coho salmon were not available in 2015 from IGH because they did not have sufficient fish to meet their production goals. However, a limited number of coho salmon yearlings from IGH were available for sentinel exposures near Beaver Creek and Seiad Valley in May and 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 0.5-1.5g, in May 2-3g, June 3-7g and in September 15-20g. The yearling coho salmon ranged from 23-30g during the May and June exposures.
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-hr exposure. However, 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 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 for prevention of 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 succumbed later than five days after they were brought to the laboratory.
Methods specific to each of the exposures are listed as follows:

April 6-9 exposures: 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. Susceptible rainbow trout and IGH fall Chinook were exposed in the Klamath River at two lower mainstem sites, upstream of the Beaver Creek confluence (KBC; 40 of each species) and near Seiad Valley (KSV; 40 of each species). The river water temperatures during exposure ranged from 9-11°C so all fishes were held at 13°C upon return to the laboratory during the post-exposure rearing.

May 11-14 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 (KIS), near Beaver Creek (KBC), Seiad Valley (KSV) and Orleans (KOR). Susceptible rainbow trout (40 fish/cage) and IGH Chinook (40 fish/cage) were held at all six sites. Yearling Coho salmon from IGH (30 fish/cage) were also held near Beaver Creek and Seiad Valley. Upon return to the AAHL, groups were reared at 16°C water temperature. The river water temperature averaged 12-14°C in the upper river and 15-16°C at the lower river sites.

June 15-18 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. Chinook and susceptible rainbow trout (40 fish of each species/cage) were exposed at six sites but only rainbow trout (40 fish/cage) were held at Lonesome Duck Resort on the lower Williamson River. Coho yearlings (30 fish/ cage) were held in the river only near Beaver Creek and Seiad Valley. After the 72 hr exposure, all groups were transported to the AAHL, reared in 18°C well water and monitored for loss from *C. shasta*. In the Williamson River, the average water temperature during the June sentinel exposures was 17-18°C but at Keno eddy and sites below Iron Gate Dam the water temperature was very high, averaging about 22°C.

September 18-21 exposure: Similar to our April exposures, IGH fall Chinook and the Roaring River Hatchery *C. shasta*-susceptible triploid rainbow trout (40 fish of each species) were exposed in the Klamath River near Beaver Creek and near Seiad Valley. After the 3-day exposure, fishes were reared in well water at 18°C at the AAHL and monitored for ceratomyxosis. The river water temperatures during exposure averaged 18°C.

**Task 1.1. Results and Discussion**

Average water temperatures during the 72-hr exposures at all sites and the laboratory post-exposure rearing temperature are shown in Table 1.1.1. Average water temperatures were 10°C during the exposure in April, ranged from 12-15°C in May, 17-23 °C in June, and 18°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 2008-2015. Water temperatures in the spring of 2015 appear to be generally higher in March, late April, the first half of May and early June than previous years.
TABLE 1.1.1. Average Klamath River water temperature (°C) at sentinel sites during the 72 hr fish exposures in 2015.

<table>
<thead>
<tr>
<th>Site</th>
<th>April 6-9</th>
<th>May 11-14</th>
<th>June 15-18</th>
<th>Sept 18-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Williamson R-WMR</td>
<td></td>
<td>11.6</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td>Williamson R-WLD</td>
<td></td>
<td></td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>Keno Eddy-KED</td>
<td>13.7</td>
<td>22.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klamath R I5-KI5</td>
<td>15.2</td>
<td>20.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beaver Creek-KBC</td>
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<td>15.1</td>
<td>21.5</td>
<td>18.4</td>
</tr>
<tr>
<td>Seiad Valley-KSV</td>
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<td>15.2</td>
<td>22.6</td>
<td>18.5</td>
</tr>
<tr>
<td>Orleans-KOR</td>
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<td>22.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lab rearing</td>
<td>13</td>
<td>16</td>
<td>18</td>
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</tr>
</tbody>
</table>

FIGURE 1.1.2. Average daily water temperatures during the months of March-September during the years 2008-2015 at the sentinel site near Beaver Creek 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 2015 and are shown for each month in Figures 1.1.2-1.1.4. 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 in 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 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 attributable to infection by *C. shasta* by site and fish species in 2015 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 63-68 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.

<table>
<thead>
<tr>
<th>Exposure dates</th>
<th>Exposure site</th>
<th>IGH Chinook</th>
<th>IGH coho</th>
<th>Rainbow trout</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 6-9</td>
<td>KBC-10°C</td>
<td>10.3</td>
<td></td>
<td>15.0</td>
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<tr>
<td></td>
<td>KSV-10°C</td>
<td>12.2</td>
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<td>29.7</td>
</tr>
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<td>May 11-14</td>
<td>WMR-12°C</td>
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<td>100</td>
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<tr>
<td></td>
<td>KED-14°C</td>
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<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>KI5-15°C</td>
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<td>78.9</td>
</tr>
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<td></td>
<td>KBC-15°C</td>
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<td>13.8</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>KSV-15°C</td>
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<td>100</td>
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<td></td>
<td>KOR-15°C</td>
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<td>100</td>
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<td>June 15-18</td>
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<td></td>
<td>KED-22°C</td>
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<td>0</td>
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<td></td>
<td>KI5-20°C</td>
<td>40.5</td>
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<td>92.1</td>
</tr>
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<td>KSV-23°C</td>
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<td></td>
<td>KOR-23°C</td>
<td>39.5</td>
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<td>76.5</td>
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<tr>
<td>September 18-21</td>
<td>KBC-18°C</td>
<td>2.4</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>KSV-18°C</td>
<td>5.1</td>
<td></td>
<td>97.6</td>
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April 6-9 exposure (Figure 1.1.2): By termination, 10.3% of the Chinook that were exposed near Beaver Creek and 17% at Seiad Valley had become moribund. For the susceptible rainbow trout, 17.5% of those exposed near Beaver Creek and 29.7% from those exposed at Seiad Valley had become moribund. Microscopic examination to determine if myxospores of *C. shasta* are present in lower gut material of all fish that died and the PCR testing of gut tissue from all fish that were negative microscopically was done. All moribund Chinook exposed near Beaver Creek were positive (10.3% of all the Chinook held post-exposure), and 12.2% of the Chinook from Seiad Valley. For the rainbow trout, six fish of seven that died (15%) of those exposed near Beaver Creek and 29.7% from Seiad Valley were positive for *C. shasta*. By early April, with only a 72-hr exposure, more than 10% of the IGH Chinook and more than 15% of the rainbow trout had died with *C. shasta* infections.

When comparing the *C. shasta* loss of IGH Chinook exposed in early April during 2015 with previous years that were exposed during the third or fourth week of the month, since 2009 (Figure 1.1.3) at KBC, in 2009 about 16% of the Chinook died. No Chinook have died in April from 2010-2013, but in 2014 7% died and 10% in 2015. Also, in April 2009 and 2014, the rainbow trout loss was just under 100% and about 80% in 2010 but low in 2011, 2013 and 2015.
May 11-14 exposure (Figures 1.1.4-10): After 68 days of rearing, all groups were euthanized with MS222 and enumerated. Juvenile Chinook exposed at the Nature Conservancy site on the Williamson River and at Keno Eddy in the upper river experienced no loss during the post-exposure rearing. At the I5-bridge site in the lower river, Chinook had a low loss of 2.6% (1 fish) that was positive for *C. shasta* spores (Figure 1.1.4). Chinook exposed near Beaver Creek incurred a 66.7% loss with 59.0% positive for *C. shasta* compared to 95.2% loss (90.5% were *C. shasta*-positive) at Seiad Valley. Chinook exposed in the river near Orleans had a 25.0% loss and 22.5% were positive for *C. shasta*. Sentinel exposures of the Chinook in May 2015 resulted in higher losses compared to Chinook held at those sites in May in the last couple of years. More Chinook juveniles than coho salmon yearlings became moribund. *Ceratonova shasta* infections occurred in 13.8% of coho (all that had died) exposed near Beaver Creek and 26.7% of the coho salmon held at Seiad Valley (30% had died). For the coho yearlings exposed in May 2015 at the two sites, losses from *C. shasta* were much lower than what had occurred in 2014 (in May 2014, 48.5% at Beaver Creek and 93.3% at Seiad Valley). However, *C. shasta* had a greater effect than what is reflected in the fish that died during the post-exposure rearing. Many of the coho yearlings exposed at either site that survived the 68-day post-exposure rearing had either one or both eyes damaged by the parasite. The affected eyes were opaque, probably resulting in near or complete blindness. An example of a yearling coho with an affected eye is shown in Figure 1.1.5-6. A histological section showing normal retina tissue and *C. shasta* infected areas of the retina are shown in Figure 1.1.6. For the yearling coho exposed near Beaver Creek, 14 of 25 (56%) surviving fish had opaque damaged eyes while at Seiad Valley it was 13 of 21 (62%) fish. We have not completed our microscopic and PCR testing of these fish but expect most fish with opaque eyes will be positive for *C. shasta*. If we include these fish with damaged eyes along with those that died during the post-exposure rearing, coho exposed near Beaver Creek and Seiad Valley in 2015 may have a final *C. shasta* prevalence of about 62% and 70%, respectively. *Ceratonova shasta* infections were found in 97-
100% of the susceptible rainbow trout exposed at all sites except Keno Eddy (0% C. shasta infections) and I5-Bridge (78.9% loss with C. shasta infections) in May 2015.

FIGURE 1.1.4. Percent C. shasta-associated mortality of sentinel Iron Gate Hatchery juvenile Chinook 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 (KIS), near Beaver Creek (KBC), Seiad Valley (KSV) and Orleans (KOR) in the Klamath River for 72 hours May 11-14, 2015. Also, coho from Iron Gate Hatchery were held during the same time near Beaver Creek and Seiad Valley. Fishes were held at the OSU Aquatic Animal Health Laboratory and monitored for 68 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, Chinook exposed at Seiad Valley died most rapidly (Figure 1.1.7). At both Seiad Valley and Beaver Creek, the Chinook began dying in less than 20 days and reached very high level of nearly 90% at Seiad Valley and 59% near Beaver Creek before 40 days of post-exposure rearing. The yearling coho at both locations suffered much lower loss (26.7% at Seiad Valley and 13.8% near Beaver Creek) and most loss occurred after 35-40 days of post-exposure rearing. The coho loss associated with _C. shasta_ probably would have been greater with extended rearing since many of the survivors after the 68 days holding had affected eyes. Rainbow trout exposed at all locations in May 2015 became infected with _C. shasta_, except at Keno Eddy. The fish exposed at WMR died with _C. shasta_ infections most rapidly of all sites tested (Figure 1.1.8). Rainbow trout exposed at KSV, KBC and KOR died at the second fastest rate and losses were slightly lower near KIS.
FIGURE 1.1.7. Cumulative loss of IGH Chinook and coho exposed in sentinel cages for 72 hr in May 2015 near Beaver Creek and Seiad Valley in the Klamath River.

FIGURE 1.1.8. Cumulative per cent loss associated with C. shasta in rainbow trout at six sentinel sites in May 2015.
When comparing the May exposure loss with *C. shasta* in IGH Chinook and coho salmon at the upper Klamath River sites from 2007 to 2015, no infections were detected in the Williamson River (WMR) and only a low prevalence of infection was detected in Chinook in 2010 at KED (Figure 1.1.9). Sentinel exposures of coho salmon at WMR were only done in May in two years and never were done at Keno Eddy. Below Iron Gate Dam before 2015, the greatest loss of Chinook occurred in 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. In May 2010-2013, Chinook salmon exposed for 72 hr 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, extremely high loss of Chinook with *C. shasta*-infection of 90.5% the greatest level observed in our sentinel fish monitoring occurred at Seiad Valley and also a high level of 59% near Beaver Creek. In 2014, the highest impact and loss from *C. shasta* was observed in the coho in May with 93% succumbing with this parasite at the Seiad Valley site and 48.5% near Beaver Creek. This high loss of coho is similar to that observed in 2007 and 2008. In May 2015, the coho yearling loss with *C. shasta* infections was much lower than 2014; 13.8% near Beaver Creek and 26.7% at Seiad Valley, however, the impact of *C. shasta* on the coho in 2015 was greater than just mortality during the post-exposure rearing because many of the surviving coho had severe eye lesions likely caused by *C. shasta*.

![Graph of percent loss from C. shasta of juvenile IGH Chinook salmon (upper figure) and coho salmon (lower figure) at six index sites in May of 2007-2015. 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.](image-url)
The juvenile rainbow trout sentinel fish percent loss with *C. shasta* infection for seven sites in May 2007-2015 in the Klamath River watershed (Figure 1.1.10) was very high for most sites. Two exceptions to this were the Klamathon (which was shifted to near the Interstate 5 Bridge (KIS) 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 KIS and 0% at KED.

**FIGURE 1.1.10.** Comparison of percent loss from *C. shasta* in juvenile rainbow trout exposed for 72 hr during May 2007-2015 at seven different Klamath River sentinel index sites and held for more than 60 days.

**June 15-18 exposure (Figures 1.1.11-15):** During the June 2015 sentinel exposures, river water at many sites reached very high daily temperatures. A few fish from several sites died of columnaris disease just after they were brought to the laboratory, however, because river water temperatures exceeded 22°C at Keno Eddy and Seiad Valley after the fish were placed in cages at those sites, high losses of the sentinel fish with *Flavobacterium columnare* infections occurred. At Keno Eddy, 51% of the rainbow trout and 69% of the Chinook died during the sentinel exposure or within five days after arrival at the laboratory. Coho salmon yearlings exposed near Seiad Valley suffered 85% loss from *F. columnaris*. On August 24, after 67 days of rearing of the June exposure groups at the AAHL, all groups were euthanized with MS222 and enumerated.

None of the juvenile Chinook exposed in the upper Klamath River watershed at the Nature Conservancy site on the Williamson River and at Keno Eddy died with *C. shasta* infections. Of those fishes that were exposed at several lower river sites, the Chinook experienced about 40% losses with *C. shasta* infections which was slightly lower than in May but similar to June 2014 Chinook loss near Beaver Creek and Seiad Valley (Figure1.1.11). Chinook exposed near I5-Bridge had a 47.6% loss and 40.5% were *C. shasta* positive, Beaver Creek, 48.8% died.
and 46.3% positive compared to only 7.7% loss and all that died were positive at Seiad Valley. Chinook exposed in the river near Orleans had a 39.5% \( C. \) shasta loss. Why the loss for Chinook at Seiad Valley was so low compared to other lower river sites is unknown. The average water temperature at Seiad Valley and Orleans were similar at 22.6°C but the maximum water temperature was greatest and reached 24.3 °C at Seiad Valley compared to 23.1°C at Orleans.

In June, as in May, fewer coho yearlings than Chinook juveniles died with \( C. \) shasta infections during the post-exposure rearing and this is much lower than coho losses in June 2014. Coho exposed near Beaver Creek had 11.1% loss and all were infected with \( C. \) shasta and at Seiad Valley, one fish died or 25% that was PCR positive for \( C. \) shasta (only 4 fish from Seiad Valley were held in the post-exposure rearing due to severe losses from columnaris disease which began during the sentinel exposure). Similar to the coho yearlings held near Beaver Creek in May, many of those that survived the 67-day post-exposure at the AAHL had damaged opaque eyes that likely were due to \( C. \) shasta infections. Of 21 surviving coho yearlings, 12 (57%) had one or both eyes damaged. The examination of these fish for \( C. \) shasta is in progress.

The susceptible rainbow trout exposed at all sites experienced losses of 92-100% except at Keno Eddy and Orleans during their holding at the AAHL. No rainbow trout exposed at Keno Eddy were positive and 76.5% of those exposed near Orleans were \( C. \) shasta positive.

![Bar chart](chart.png)

**FIGURE 1.1.11.** Percent \( C. \) shasta-associated mortality of sentinel Iron Gate Hatchery juvenile Chinook 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 IS Bridge (KIS), near Beaver Creek (KBC), Seiad Valley (KSV) and Orleans (KOR) in the Klamath River for 72 hours June 15-18, 2015. Also, coho from Iron Gate 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 67 days post-exposure. Fish in which no myxospores were observed had an intestinal sample PCR-tested.
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. A reduction in *C. shasta* infection in susceptible rainbow trout was not detected in the sentinel fish exposures at the WMR and WLD sentinel sites tested in 2012-2015.

The cumulative loss of IGH Chinook and coho salmon exposed for 72 hr in June is shown in Figure 1.1.12. An early loss on day seven of one yearling coho exposed at Seiad Valley did not have myxospores but was positive by PCR. Because there was only four coho after the sentinel exposure and the first five days of post-exposure rearing, this fish represented a 25% loss. Prior to day 20, the Chinook exposed near Beaver Creek began to experience loss with *C. shasta* infection that reached 46.3%. The coho yearlings exposed near Beaver Creek and the juvenile Chinook at Seiad Valley experienced a very low loss with *C. shasta* infection, 11.1% and 7.7% respectively and the last losses occurred between 40-50 days after holding.

The cumulative loss of rainbow trout exposed at seven Klamath River sentinel sites in June 2015 show the most rapid loss occurring at the two Williamson river sites, WMR and WLD followed by KBC, KSV and KI5 (Figure 1.1.13). At most sites, fish had begun to die about 20 days after exposure.
During the June sentinel exposures, the greatest loss of Chinook 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 1.1.14). In June 2010-2013, Chinook salmon exposed for 72 hr incurred low losses from C. shasta, i.e. less than 20%. In 2014, Chinook loss was greater then 40% at Beaver Creek and Seiad Valley. In 2015, Chinook loss was near 40% from KIS, KBC and KOR. 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 was at the 40% level at KIS, and thus resulting in an expansion of the infectious zone from KIS to KOR with the exception of Seiad Valley.

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 KBC and 44.8% at KSV 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 KBC and KSV 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.

The rainbow trout sentinel loss for seven sites in June 2007-2015 in the Klamath River watershed (Figure 1.1.15) show greater than 90% loss at most locations. Rainbow trout exposed at Keno Eddy and KIS show in some years there is moderate C. shasta loss and other years very low.
FIGURE 1.1.14. 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 2007-2015. 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.
September 18-21 exposure (Figures 1.1.16-17). The September exposure of Iron Gate Hatchery Chinook and susceptible rainbow trout near Beaver Creek and Seiad Valley provided C. shasta results very similar to those of the 2014 September exposure (Figure 1.1.16). Only 5.1% of the Chinook exposed at Seiad Valley had C. shasta associated mortality and 2.4% of those exposed near Beaver Creek. Greater than 97% of the exposed rainbow trout at each site had C. shasta infections.
Comparison of percent *C. shasta* infections in Chinook salmon exposed in September of 2007-2015 at selected sites in the Klamath River basin shows that generally *C. shasta* infections in this month are very low (Figure 1.1.17). Only in 2007, 2008, 2014 and 2015 were sentinel Chinook found to become infected, but at a low level. Exposures in other years were negative.
Comparison of sentinel results for the IGH Chinook and coho salmon exposed at KBC in 2007 - 2015 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 at KBC. In 2014, loss of both Chinook and coho at KBC was much greater than 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.

![Comparison of C. shasta mortality of Juvenile IGH fall Chinook and coho salmon exposed in the Klamath River near Beaver Creek for 72 hr in April, May, June and September (when conducted) in years 2007-2015.](image)

The comparison of *C. shasta* mortality of juvenile IGH fall Chinook and juvenile or yearling coho salmon exposed at KSV for 72 hr in May and June of years 2007-2015 show similar results as the comparison for the same years at KBC (Figure 1.1.19). No comparison can be made for Chinook and coho in 2007 since no coho salmon were exposed at KSV in that year. The coho salmon loss from *C. shasta* in 2011 and 2013 was much higher than the Chinook. Also, in 2013 and 2014 there appears to be a slight downstream shift of greater *C. shasta* rate at KSV compared to KBC. 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 May since we began sentinel studies.

28
Pulse-Flow exposures: To examine the effect of a pulse flow of river water on infections of *C. shasta*, sentinel studies with IGH Chinook juveniles and coho salmon yearlings were conducted on May 8-11 (72 hr) prior to an anticipated pulse-flow event at both Beaver Creek and Seiad Valley index sites. During May and June of 2015, exposures planned during a pulse-flow event did not occur because those involved in Klamath River water management decided that they did not have sufficient water in the upper watershed to provide a pulse-flow through the lower river.

In the pre-pulse exposure on May 8, thirty fish of each species (juvenile Chinook and yearling coho salmon) were held at each site for 72 hr. After the exposures, the fishes were transported to the AAHL, reared at 16°C water temperature and monitored for *C. shasta* infections. Fishes received medicated food to prevent bacterial infections and formalin baths for parasites. All fish were euthanized on July 21 (71 days of post-exposure rearing) with MS222 and enumerated.

Losses were highest for the Chinook compared to the coho at both Beaver Creek and Seiad Valley (Figure 1.1.20). At Beaver Creek, mortality in Chinook salmon as a result of *C. shasta* infection was higher (93.3%) than near Seiad Valley (80%). Coho salmon yearlings had *C. shasta* loss of 40% near Beaver Creek and 43.3% at Seiad Valley, whereas a similar 72 hr exposure during May 11-14 resulted in a coho loss of 13.8% exposed near Beaver Creek and 26.7% at Seiad Valley. It is interesting that water temperatures averaged 16.6°C near Beaver Creek and 17.2°C at Seiad Valley during the May 8-11 exposure but 15.1 °C near Beaver Creek and 15.2°C at Seiad Valley during May 11-14. From past sentinel studies with juvenile coho it appears elevated water temperatures can cause greater infections of *C. shasta* in coho but other factors such as the number of parasite actinospores per liter of river water may vary significantly over a few days.
FIGURE 1.1.20. Percent loss with C. shasta infections of sentinel Iron Gate Hatchery juvenile Chinook and coho salmon exposed near Beaver Creek and Seiad Valley in the Klamath River for 72 hours prior to an anticipated river pulse-flow event planned in May. Fishes were held at the Aquatic Animal Health Laboratory and monitored for 71 days post-exposure. Fish in which no myxospores were observed had an intestinal sample PCR-tested.

Task 1.2. Determine parasite density in water samples to include data collection during spring outmigration 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 IS 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 IS fish trap (KIS), Orleans (KOR) and Tully Creek (KTC), from March through October 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 to May. The use of automated water samplers and the field assistance provided by the Karuk and Yurok tribes allowed weekly collections of 24-hr composite water samples. The ISCOs were programmed to begin sampling at 8 am and 1 L was collected from the river every 2 hr for 24 hr, then the total sample was mixed manually and 4 x 1 L samples taken. All samples were chilled until filtered, within 24 hr 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 May this was at six sites, June at seven sites and in April and September samples were collected at Beaver Creek and Seiad Valley only.

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). Project funds this year enabled the acquisition of a new Real-Time PCR instrument (ABI StepOne Plus thermocycler) to replace the broken previous machine. We modified the original TaqMan assay (of Hallett and Bartholomew 2006) to develop a duplex C. shasta/IPC assay. This allowed 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 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. Each data point on the graphs represents the average of three 1 L water samples collected at that time point.

**Task 1.2. Results and discussion**

Molecular analysis of water samples from the lower Klamath River in 2015 showed higher presence of *C. shasta* than in any other year since monitoring began in 2006 (Figure 1.2.1). In 2015, levels were up to an order of magnitude higher than the previous highest level of ~100 spores/L in 2007-9 at Beaver Creek.

![Graph showing density of *Ceratonova shasta* in water samples collected at the Beaver Creek index site from 2009-2015](image)

**FIGURE 1.2.1.** Density of *Ceratonova shasta* in water samples collected at the Beaver Creek index site from 2009-2015 showing the unprecedentedly high levels in 2015. Each data point is the average of 3 x 1L water samples. The upper graph is scaled to show relatively lower spore levels.

Additionally, spore levels peaked sooner in 2015 (April) rather than May/June (Figure 1.2.2). The ‘infectious zone’, originally associated only with Beaver Creek (KBC), continued to extend downstream to the Seiad Valley KSV index site (Figure 1.2.3, 1.2.4).
FIGURE 1.2.2. Density of Ceratonova shasta in water samples at Klamath River mainstem index sites in 2014 (orange) and 2015 (blue).
FIGURE 1.2.3. Spatial and temporal density of *Ceratonova shasta* in water samples collected at Klamath River mainstem index sites in 2015. Each data point is the average of 3 x 1L water samples. The lower graph is scaled to show lower spore values.
FIGURE 1.2.4. Temporal abundance of *Ceratonova shasta* in water samples collected at six Klamath River mainstem index sites April - June 2015. Sites are ordered downstream to upstream, left to right (the Klamath River flows west into the Pacific Ocean). Each data point is the average of 3 x 1L water samples. The highest spore level zone appears to shift downstream from April to May to June.
Previous research by the Bartholomew Lab (Atkinson and Bartholomew 2010a,b) revealed that there are multiple genotypes of C. shasta simultaneously present in the Klamath River. These genotypes differentially cause disease 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 2015, the majority of spores at all timepoints was genotype I (Chinook), with only a small proportion of type II (coho). At KBC, the coho mortality threshold of 5 spores/L type II was first exceeded in early May. Levels were 5-20 type II spores/L until mid-May, then about 5 type II spores/L until mid-June. Levels decreased to no detection of type II after late June. At KSV, the threshold was first exceeded in late April (20-50 type II spores/L) to mid-May; levels then tapered to hover around 5 type II spores/L for all of June.

Historically, the highest densities of total C. shasta in river water samples have been approximately 100 spores/L. Based on tests with reference samples of varying proportions of type I and type II, we determined that the limit of detection of type II in a predominantly type I sample, was 5% (i.e. 5 spores/L, which is coincidentally the mortality threshold for coho). In 2015, the extraordinarily high levels of type I spores in April and May (Fig. 1.2.5) meant that our detection limit for type II in the sample was above the coho mortality threshold. In response to this, we are now developing a qPCR assay that targets only type II spores, independent of other geotypes in the sample.

FIGURE 1.2.5. Spores per liter of genotypes I (Chinook; orange) and type II (coho; blue) at Beaver Creek in 2015. Each dot is the average of three 1 L water samples. The gray dots and shading represent the detection limit of 5% of the total signal, which possibly masked detection of low levels of type II (blue) at some timepoints.
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-hr period as for the ISCO-collected samples.

**TABLE 1.2.1. Density of Ceratonova shasta in 1 L water samples (average of 3 1L; (range)) collected at the start and end of sentinel fish exposures.**

<table>
<thead>
<tr>
<th>SITE</th>
<th>FISH IN April 6</th>
<th>FISH OUT April 9</th>
<th>FISH IN May 11</th>
<th>FISH OUT May 14</th>
<th>FISH IN June 15</th>
<th>FISH OUT June 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>WMR</td>
<td>41 (21-71)</td>
<td>43 (26-53)</td>
<td>69 (59-86)</td>
<td>43 (12-81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WLD</td>
<td>7 (1-10)</td>
<td></td>
<td>26 (12-42)</td>
<td>43 (27-65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KED</td>
<td>160 (143-196)</td>
<td>67 (48-82)</td>
<td>382 (274-455)</td>
<td>326 (92-560)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIS</td>
<td>75 (68-80)</td>
<td>206 (178-260)</td>
<td>170 (160-192)</td>
<td>401 (365-456)</td>
<td>550 (501-567)</td>
<td></td>
</tr>
<tr>
<td>KSC</td>
<td>139 (113-168)</td>
<td>547 (494-592)</td>
<td>1730 (870-3105)</td>
<td>167 (96-227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KOR</td>
<td>491 (318-660)</td>
<td>830 (680-925)</td>
<td>331 (77-552)</td>
<td>170 (168-186)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In anticipation of a pulse-flow event in May, water samples were collected by ISCO (1L every 2 hours and three sets combined for a 6-hr block) concurrent with a sentinel fish exposure at KBC and KSV (Figure 1.2.6).
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

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 (February), spring (June), summer (July), and fall (October). 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 (KIS), Tree of Heaven Campground (KTH), Fisher’s RV park near Beaver Creek (KBC), Sead 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 229 cm², 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 (20cmx28cm, Wildco, FL) and randomly selecting three 25cmx25cm 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 (KDB) 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, KTOH near the Tree of Heaven Campground, KBC near 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 m² 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 (Figure 1.3.2): Polychaete population dynamics differed among river sections and seasons in 2015. Overall, densities were highest at sites in the upper river section (KJB and KED) compared to sites in the other river sections and densities increased with temperature and persisted through the fall. This result was consistent with observations from 2013-2014, but densities were higher overall in 2014 and 2015 compared to 2013.

In the middle river section, polychaete densities were highly variable: At KI5 (RKM 287), densities were lowest in the winter but increased rapidly by the spring sampling period, peaked in summer and remained high in the fall. At TOH (RKM 281), densities were low in winter, peaked in the spring, and decreased from summer to fall. The decreases in the summer and fall at this site may be explained by competition with algae- polychaetes were heavily encrusted with algae by mid-summer that was still present in fall. At KBC, densities were lowest in winter and peaked in summer. These results differ from results from 2013-2014, when we observed polychaete
densities at sites in the middle river section to be correlated with temperature; lowest in winter and highest in summer. The results for the 15 sites (highest densities in the fall) may have important implications for *C. shasta* persistence because myxospores may be deposited by returning adult salmon proximal to this location. In the lower river section, densities were low relative to densities at sites in the other river sections until fall. This result is partially explained by the inverse relationship between density and discharge; by summer and fall, differences in discharge between the middle and lower river sections are negligible thus densities may be highest in summer and fall. The low densities in summer may be explained by the sedimentation from the flow event that occurred in the Scott River in summer 2015. This event resulted in ~1 m of fine sediment being deposited on polychaetes at KSV prior to the summer sampling (late July).

**FIGURE 1.3.2.** Densities of *Manayunkia speciosa* (left), the polychaete host of *C. shasta* at 7 monitoring sites in 2015, from top to bottom, monitoring sites include KED and KJB in the upper basin, K15, KTH and KBC in the mid basin, and KSV and KOR in the lower basin. Discharge (right plots, denoted in blue) and water temperature (denoted by grey solid line) were obtained from USGS gaging stations and OSU Hobo temperature loggers deployed near the sampling sites.
Prevalence of infection (POI) and the density of infected polychaetes: there were season and site specific differences in populations (Table 1.3.1). Prevalence of infection was highest at KI5 in winter and spring, and at KTH in fall. Results from summer are pending analysis. These results contrast with those from 2014 when we detected higher prevalence in late winter (March) samples than spring, summer, or fall samples at all sites.

TABLE 1.3.1. Prevalence of C. shasta in M. speciosa at 7 monitoring sites in 2015.

<table>
<thead>
<tr>
<th>Site</th>
<th>Prevalence (%) of C. shasta infection in polychaetes by site and month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Late Feb/March</td>
</tr>
<tr>
<td>KOR</td>
<td>1.3</td>
</tr>
<tr>
<td>KSV</td>
<td>1.3</td>
</tr>
<tr>
<td>KBC</td>
<td>0.7</td>
</tr>
<tr>
<td>KTH</td>
<td>0.3</td>
</tr>
<tr>
<td>KI5</td>
<td>5.0</td>
</tr>
<tr>
<td>KJB</td>
<td>0.3</td>
</tr>
<tr>
<td>KED</td>
<td>0</td>
</tr>
</tbody>
</table>

Density, prevalence of infection and the environments of monitoring sites: Infected polychaetes were detected at all but three site_sampling period combinations in 2015, KOR (June), KSV (late July), and KED (Feb). Moreover, prevalence of infection was frequently detected at levels >1.0%. These results contrast with results from previous years when disease risk to fish was lower. For example, in 2010-2013 the distribution of infected polychaetes was more limited and infected polychaetes were detected fewer sites and at lower prevalence. However, in 2014, when disease risk to fish was more similar to 2015, infection was detected in polychaetes at all but 7 site_date combinations (KOR in June, and late July, KBC in June, KTH and October, and KJB and KED in June and October).

In 2015, densities of polychaetes and prevalence of C. shasta infection were higher at middle river sites, KI5, KTH, and KBC by mid June than in previous years (with the exception of 2014).
TABLE 1.3.2. Estimated densities of *C. shasta* infected *M. speciosa* at 7 monitoring sites in 2015.

<table>
<thead>
<tr>
<th>Site</th>
<th>Estimated number of polychaetes/m² by site and month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Late Feb/March</td>
</tr>
<tr>
<td>KOR</td>
<td>1,194</td>
</tr>
<tr>
<td>KSV</td>
<td>1,708</td>
</tr>
<tr>
<td>KBC</td>
<td>1,413</td>
</tr>
<tr>
<td>KTH</td>
<td>646</td>
</tr>
<tr>
<td>KIS</td>
<td>12,940</td>
</tr>
<tr>
<td>KJB</td>
<td>6,608</td>
</tr>
<tr>
<td>KN</td>
<td>0</td>
</tr>
</tbody>
</table>

Analyses examining relationships among water year, fish disease risk, and polychaete data (density, prevalence of infection, and density of infected polychaetes) from 2010-2015 are in progress. We are building predictive models using these data, and plan to test the models using data we collected from 2006-2009 when possible, and 2016-2018.

**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., 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 2015. However, one was anticipated and thus one pre-pulse flow monitoring study involving both sentinel fish exposures and water sampling occurred. These efforts are shared under Task 1.1.

**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 genotypes. For this, we need well-annotated transcriptome sequences and a high quality reference genome. In 2015 we worked on quality assessing and assembling both our *C. shasta* draft genome (genotype II – rainbow trout) and transcriptomes (genotype I – Chinook, and the two biotypes of II - rainbow and coho). We discovered significant contamination issues in those transcriptome data, which made gene reconstruction and identification difficult. The contamination stemmed from the underlying parasite nature of *C. shasta*: as transcriptomes must be from living organisms (with viable RNA) we had to take samples from living, infected fish. Unfortunately, all resulting *C. shasta* transcriptome sequences had high levels of fish compared with parasite. To solve this problem, we re-sampled genotype II from a series of infected rainbow trout, and did extensive pre-sequencing quality and quantity assessment of the RNA. We used *C. shasta* and fish qPCRs to
assess which samples had the highest *C. shasta* to fish ratio, then used gel and bioanalyzer assays to determine which of those had the best RNA. At the end of 2015 we submitted our best *C. shasta* sample for transcriptome library preparation and sequencing; (we received the data in early 2016 – initial analysis shows it to be of excellent quality, with low % fish). Once we have this new reference genotype II transcriptome, we will re-sequence genotypes O, I and II (coho) with a higher probability of generating useful data.

In 2015 we discovered an important aspect of genotypes II and III. Previously, we have observed that these two genotypes frequently co-occur in infected rainbow trout and occasionally Coho salmon. In early 2015, they also showed up in our lab polychaete worm cultures, which we are using to generate 'pure' genotype material for genomics and transcriptomics. To better understand this potential contamination problem, we genotyped individual parasite spores from fish whose bulk genotype was a mixture of II and III. Surprisingly, individual parasite spores were also mixed genotype II and III, meaning that "II" and "III" are actually just genetic variants of the same strain of *C. shasta*, and should no longer be regarded as distinct genotypes. This discovery simplifies our search for an assay that distinguishes between the different genotypes, because it shows we do not need to distinguish II from III as they are both the same. This finding is being written up for publication.

**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**

We continued work on the epidemiological model that was piloted in 2013. In parallel, we developed a model to predict effects of discharge on parasite spores (5.2 particle transport and water temperature models) based on data gaps highlighted by the epidemiological model to identify data gaps. We plan to further modify the model in 2016 to be able to examine the effects of polychaete host demography and parasite genotype.

**Task 5.1.2. Spore transport and water temperature models**

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.

**Task 5.1.2 Methods**

**Study area and data collection**

The study area included river kilometers (RKM) 304 to 128. The Shasta and Scott Rivers are two main tributaries that join the main-stem along the study site.

![Map of the Klamath River](image)

Figure 5.1.2.1. Map of the Klamath River showing the study area from Iron Gate Dam to Seiad Valley. Red dots show the USGS discharge stations at IGD, Shasta, Scott, and Seiad Valley stations. Green dots show the stations at Tree of Heaven, Beaver Creek, and Community Center where bathymetry data have been measured. Blue arrows also show the major tributaries joining the Klamath River.

We developed our model using data obtained from different sources. The flow measurements were obtained from the United States Geological Survey (USGS) gauge Stations #11516530 at IGD, #11517500 at Shasta River, #11519500 at Scott River, and #11520500 at Seiad Valley. The atmospheric data including the precipitation, air temperature, relative humidity, wind speed, and wind direction were collected at Collins Baldy (CLB) and Slater Butte (SRB) stations. The water temperature measurements were collected from Karuk tribe water resources measurements at IGD, Seiad Valley and Shasta River. The bed profile was collected from a bathymetry and LIDAR survey by U.S. Department of the Interior, Bureau of Reclamation (USBR). The bathymetry data was conducted by two boats using a Multi-beam ADCP interfaced with GPS. However, data gaps exist along the river due to data collections issues (e.g. shallow water) and GPS coverage. Woolpert also conducted a series of cross sections for 3-mile gap between I-5 to Shasta River. Bathymetry data at Tree of Heaven, Beaver Creek, and Community Center sites were collected using GPS and echo sounder with error tolerance of approximately ±0.03 m [7]. These data were used to validate the generated bathymetry for missing areas along the river. The water surface elevation from LIDAR data and water depth were obtained from USBR. However, they were not collected at the same date, so they can not directly be used to calculate the bathymetry.
Simulation model: The Environmental Fluid Dynamic Code (EFDC) is a commonly used model for simulating up to three-dimensional flow as well as fate and transport processes in surface water bodies such as rivers, lakes, reservoirs, wetlands, estuaries, and coastal ocean regions [8-10]. EFDC contains three functional modules including hydrodynamics, water quality, and sediments-contaminants components that have been fully integrated into a single source code. It supports Cartesian and curvilinear orthogonal horizontal coordinates and stretched or sigma vertical coordinates. EFDC solves the continuity and momentum equations for the flow and transport equations for temperature.

Water age: Water age is defined as the time required for a particle to be transported from its boundary to the specific location. If the initial water age is zero, the water age at a desired location represents the travel time from the point that water was released to the arrival point. Water age was calculated based on tracer and age concentration [11]:

\[
\frac{\partial c(t,\vec{x})}{\partial t} + \nabla (uc(t,\vec{x})) - Kc(t,\vec{x}) = 0
\]

(1)

\[
\frac{\partial \alpha(t,\vec{x})}{\partial t} + \nabla (ua(t,\vec{x})) - K\alpha(t,\vec{x}) = c(t,\vec{x})
\]

(2)

where \(c\) is the tracer concentration, \(K\) is the diffusivity tensor, and \(\alpha\) is the age concentration.

Lagrangian Particle Transport: Dynamic Solutions - International LLC (DSI), has developed a version (EFDC_DSI) of the code that includes the Lagrangian Particle Transport (LPT) sub-model. The differential equations for the Lagrangian movement of particles are as follows [12]:

\[
dx = dx_{\text{drift}} + dx_{\text{random}} = \left( u + \frac{\partial u}{\partial x} \right) dt + \sqrt{2D_H dt} (2P - 1)
\]

(3)

\[
dy = dy_{\text{drift}} + dy_{\text{random}} = \left( v + \frac{\partial v}{\partial y} \right) dt + \sqrt{2D_H dt} (2P - 1)
\]

(4)

\[
dz = dz_{\text{drift}} + dz_{\text{random}} = \left( w + \frac{\partial w}{\partial z} \right) dt + \sqrt{2D_v dt} (2P - 1)
\]

(5)

where \((x,y,z)\) are Lagrangian coordinates of a particle, \(P\) is a random number from a uniformly distributed random variable generator having mean of 0.5, and \(D_H\) and \(D_v\) are the horizontal and vertical diffusion coefficients, respectively. Particles were released at two areas on the main stream, and different dam release scenarios were tested to estimate the concentration of particles in the study area. The results from these tests would help decision makers to take the appropriate action when the concentration of actinospores (the stage that infects salmon) increases. Moreover, identification of hotspots where myxospores (the stage that infects the sessile invertebrate host) settle out will be useful for designing effective monitoring and management approaches when coupled with predictive models for worm hosts (already developed, Alexander et al., in press).

Water temperature: Water temperature is another factor that significantly increases \(C.\ shasta\) related mortality in salmonids. The high temperature especially during the summer can affect fish health in general in shallow rivers such as Klamath River. The most important factors that affect the temperature of shallow water bodies include the solar radiation, the air temperature, and the wind speed. Reservoirs temperature is typically cooler than the water temperature of river due to deeper water, so releasing cool water from dam to the river can be considered as an effective method to decrease the water temperature of the river and decrease the risk of fish death. Different scenarios were considered to investigate how water temperature changes at study sites with different flows released from the IGD. After calibrating the temperature model, four sample days during the year with different temperature were selected to explore the maximum water temperature reduction. The results from these scenarios will allow managers to evaluate the benefits of releasing water from the dam when aiming to reduce water temperature as a method to reduce the effects of disease. Such a scenario may occur following the release of juveniles from the hatchery because hatchery releases account for the majority of Chinook in this system.
Task 5.1.2 Results

Bathymetry data generation: There are bathymetry data gaps for 6 areas along the river (11.5 km of river length). LIDAR data has provided a dense set of elevation data for the study area. Since LIDAR typically uses a near-infrared laser to map the land, it cannot penetrate the water to measure the bathymetry. Therefore, available LIDAR data provides the water surface elevation. Even though water surface elevation and water depth are available for the entire river length, they have been collected at the different dates and times. Depth of water from LIDAR data was subtracted from the water surface elevation to roughly estimate the bathymetry. The results were then compared to actual bathymetry at Tree of Heaven, Beaver Creek, Community Center, and 10 miles upstream of Shasta River. Results show that if water surface elevation provided by LIDAR data is adjusted by 30 cm, the overall root mean square errors between generated bathymetry and actual data is less than 42 cm (Figure 5.1.2.2). Furthermore, the data set across the river is not dense enough, so the nearest neighbor interpolation was used to fill the missing areas.

1.1. Mass balance

Shasta River and Scott River are considered as the main tributaries of the Klamath River. The cumulative water flowing into the river (from IGD, Shasta River, and Scott River) was calculated and then compared with the flow at Seiad Valley station (Figure 5.1.2.3). Results show that there is a noticeable difference between the inflow to the river and the outflow which means there is amount of water that has not been considered. The possible reasons are:

1) The Scott River station is located 30 km upstream of the location that Scott River joins the Klamath River. Hence, this station only accumulates the water draining from 72% of the Scott Basin area. Assuming the
uniform soil type and land use for Scott Basin, the flow at this station was adjusted as:

\[ Q_{Scott_{adj}} = Q_{Scott} \times (1 + \frac{A'}{A_T}) \]  

(6)

where \( Q_{Scott_{adj}} \) is the total flow at the Scott Basin outlet, \( A' \) is the area of basin that does not drain to the USGS station at Scott River, but it drains to the outlet of basin, and \( A_T \) is the total area of Scott Basin.

2) The water draining to the Lower Klamath Basin has not been measured by the USGS stations. Therefore, using a back calculation, the missing water was calculated using the flow measurements at Seiad Valley and sum of the inflows from IGD, Shasta River, and adjusted flow of Scott River. This flow was then distributed among the other major tributaries in Lower Klamath Basin including Beaver Creek, Horse Creek, and Grider Creek (Figure 5.1.2.1). The calculated yearly cumulative runoff from Lower Klamath Basin is 65% of the adjusted runoff from Scott Basin. Rational method was used to compare the runoff from Lower Klamath and Scott basins and to validate the results from back calculation. Assuming the same rainfall for both basins, the runoff for Lower Klamath Basin can be estimated as

\[ Q_{R,LK} = \left( \frac{C_{R,LK}}{C_{R,S}} \times \frac{A_{LK}}{A_S} \right) Q_{R,S} = \left( \frac{0.18}{0.23} \times \frac{1920}{2105} \right) Q_{R,S} = 0.71 Q_{R,S} \]  

(7)

where \( Q_R \) is the runoff, \( C_R \) is the runoff coefficient for rational method, \( A \) is the area of basin, and the subscripts \( LK \) and \( S \) are referencing to Lower Klamath Basin and Scott Basin respectively. The small difference between results (9%) is expected to be from the uncertainty in estimating the runoff coefficient, assumption of the same rainfall for both basins, and water coming from other sources such as ground water.

Model calibration

Water surface elevation: The water surface elevations have been measured for different flow discharges at three sites including Tree of Heaven, Beaver Creek, and Community Center. The simulated water surface elevation (blue solid line) was compared with observations (red dashed line) in Figure 5.1.2.4. Comparison of the modeled results and the field measurements produced a root mean square error of 0.09 meter.

![Figure 5.1.2.4](image)

**FIGURE 5.1.2.4:** Simulated and measured water surface elevation versus flow discharge for a) Tree of Heaven, b) Beaver Creek, and c) Community Center. The blue dashed line shows the measured data and red solid line shows the simulated water surface elevation.

Water column temperature: The period March 22\textsuperscript{nd}, 2015 to September 15\textsuperscript{th}, 2015, when the time series of temperature at Seiad Valley were available, was used to calibrate the temperature model. Model parameters were adjusted using EFDC in order to achieve the best agreement with water temperature measurements. **FIGURE 5.1.2.** shows the model predictions and observations at Seiad Valley. It indicates very good correlation between the measured (blue dashed line) and the modeled (red solid line) water column temperature. Statistical analysis shows an overall RMSE of 0.89°C, which is within the satisfaction range for water temperature simulation using EFDC.
**Water age:** In this section, different hypothetical dam release scenarios were tested to estimate the water age at Seiad Valley. If the initial value of water age is set to zero, the water age would represent the travel time from IGD to the study sites. It helps decision makers operating the dam in order to decrease the risk of fish mortality. More than 100 scenarios were tested using different flows and release periods according to Table 5.1.2.1.

**TABLE 5.1.2.1: Dam release scenarios tested for calculating the travel time at Seiad Valley. cms=m³ s⁻¹**

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Flow (cms)</td>
<td>28 - 71</td>
</tr>
<tr>
<td>Dam release flow (cms)</td>
<td>28 - 156</td>
</tr>
<tr>
<td>Dam release period (hr)</td>
<td>6 - 24</td>
</tr>
</tbody>
</table>

Figure 5.1.2.6 shows the water age at Seiad Valley for four scenarios listed in TABLE 5.1.2.1. In these scenarios, base flow is set at 40 m³ s⁻¹ (cms) for 2 days, followed by a dam release of 60 cms for 6, 9, 12, and 24 hr for each scenario. As is seen in this figure, water age starts from zero and increases with time. Small values of water age represent the travel time for water particles closer to the site. Water age increases for particles moving from farther location to the site which means they have been in system for longer time. Then, the water age reaches to the constant value of 1.26 days, which means that all the particles have arrived to this location and any new particle released to the system takes 1.26 days (30.3 hr) to arrive to the Seiad Valley. After 11.3 hours from dam release (day 2.47), the water at Seiad Valley is mixing with the particles with higher speed from the 60 cms dam release (negative wave) which decreases the water age at Seiad Valley. However, the new particles have not arrived to the site until the graph reaches to the minimum value. The water age then increases to the water age corresponding to the base flow when the dam closes and all the new water to the system arrive to the site.
Figure 5.1.2.6: Water age at Seiad Valley for Scenarios with 40 cms base flow for 2 days, then dam release of 60 cms for a) 6hr (blue solid line), b) 9hr (green dashed line), c) 12hr (red dotted line), and d) 24hr (orange dot-dash line).

The same analysis was repeated for all the scenarios.

Figure 5.1.2.6. shows the travel time to Seiad Valley for 6hr, 9hr, 12hr, and 24hr dam release period. Results show that for 6hr dam release period, if total flow is higher than 170 cms, the release period doesn’t affect the travel time to the Seiad Valley. These limits for 9hr, 12hr, and 24hr release periods are 112 cms, 56 cms, and 28 cms respectively.

FIGURE 5.1.2.7. Travel time to Seiad Valley versus dam release flow with base flow of 28 cms (green dashed line), 56 cms (blue dotted line), 84 cms (orange dot-dash line), and 112 cms (black solid line) for dam release period of a) 6hr, b) 9hr, c) 12hr, and d) 24hr.

Particle Tracking: The peak of actinospores observed during the spring while the peak of myxospores observed during the fall. The actinospores are neutral buoy but myxospores settling by velocity of 0.4 m/day. 15,000
particles, representing the parasites spores, were released at two areas on the main stream, downstream of IGD and close to Shasta River, under different dam scenarios. Results show that the concentration of spores reduces as they move downstream because spores are stuck in the dry cells or settle down which is consistent with the observations. The concentration reduction of parasites was then estimated in TABLE 5.1.2.2. The concentration of actinospores at study sites mentioned in TABLE 5.1.2.2 decreases as flow increases. However, the concentration of myxospores increases for low base flows because most of the spores are settled down or get stuck in the dry cells before arriving to the study sites, but after releasing dam from water, higher velocity scour the spores and move them to the downstream. Hence, the concentration of spores is low for low base flows and before dam release for these sites and it increases as the water is released from dam.

### TABLE 5.1.2.2: Concentration reduction of spores for various dam release scenarios

<table>
<thead>
<tr>
<th>Tested sites</th>
<th>Base Flow (cms)</th>
<th>Dam release (cms)</th>
<th>Concentration reduction of Actinospores (%)</th>
<th>Concentration reduction of Myxospores (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close to Shasta River</td>
<td>30</td>
<td>55</td>
<td>40</td>
<td>-220</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110</td>
<td>56</td>
<td>-170</td>
</tr>
<tr>
<td></td>
<td></td>
<td>170</td>
<td>61</td>
<td>-130</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>55</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>170</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Tree of Heaven</td>
<td>30</td>
<td>55</td>
<td>23</td>
<td>-400</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110</td>
<td>43</td>
<td>-290</td>
</tr>
<tr>
<td></td>
<td></td>
<td>170</td>
<td>53</td>
<td>-210</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>55</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110</td>
<td>31</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>170</td>
<td>42</td>
<td>38</td>
</tr>
</tbody>
</table>

**Water temperature:** Various dam release scenarios were considered to investigate the effect of releasing cool water from the dam on the water temperature at Seiad Valley station. TABLE 5.1.2.3 shows the flows and water temperature of selected days and compares the results after dam release scenarios.

### TABLE 5.1.2.3: The effect of dam release on maximum water temperature of Seiad Valley. RSVR: Reservoir, Water temp*: Water temperature after dam release

<table>
<thead>
<tr>
<th>Date</th>
<th>13-Mar</th>
<th>28-Apr</th>
<th>27-Jun</th>
<th>10-Sep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Flow (cms)</td>
<td>30</td>
<td>37</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Max. water Temp (°C)</td>
<td>11.8</td>
<td>17.1</td>
<td>26.6</td>
<td>22.9</td>
</tr>
<tr>
<td>Avg. Released RSVR¹ Temp (°C)</td>
<td>8</td>
<td>13</td>
<td>20.4</td>
<td>18.4</td>
</tr>
<tr>
<td>Dam Release (cms)</td>
<td>55</td>
<td>85</td>
<td>55</td>
<td>85</td>
</tr>
<tr>
<td>Period (hr)</td>
<td>6</td>
<td>12</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Water temp* (°C)</td>
<td>11</td>
<td>10.2</td>
<td>10.9</td>
<td>10</td>
</tr>
</tbody>
</table>

**Conclusion**

A three-dimensional hydrodynamic model was built and integrated with CE_QUAL_W2 model and Lagrangian Particle Tracking model to investigate the possible management actions to protect salmonid. The results of the model highlighted the following findings:

a) For flows greater than a threshold, travel time is only reliant on the total flow and can be estimated using equation 8.

b) The concentration of spores is higher at upstream and decreases as they move downstream. Also, releasing 170 cms can decrease the concentration of spores up to 60%. However, in case of low base flows, the concentration of myxosporides increases after releasing water because the higher velocity move the spores to the downstream.

c) Releasing cool water from the dam could be an effective method to decrease the water temperature. Results show that by releasing 55-85 cms from dam, water temperature at Seiad Valley can be decreased from 1° C to 4 C dependent on the reservoir temperature. Also, it was concluded that dam releases period is not an effective factor in reducing the water temperature.

**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.

- **Task 6.2.** Quantify parasite levels in water samples.

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

High water temperatures in 2015 prevented these studies. High levels of microcystin prevented sampling in late summer 2015. We plan to work with the Yurok tribal biologists to determine an appropriate time to sample this reach for polychaetes.

**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 used a tandem modeling approach to predict the distribution of *M. speciosa* and evaluate the effects of three 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, and July 2014; these will be used for validation. Samples collected in 2012 were used to build a statistical model estimating the relationship between physical habitat characteristics and the distribution of *M. speciosa*. We have 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 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. Consequently, this year we will run simulations and capitalize on the natural and managed peak flood event that occurred in March 2016 (>10,000 cfs from IGD) and plan to validate the polychaete predictive model under this context.

We are in the process of building predictive models for density and prevalence of infection to examine how flow regimes may affect these factors in the polychaete host.

**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.

Management actions that target the polychaete host are desirable because of the high conservation value of salmonid hosts and the logistical constraints associated with targeting waterborne parasite stages. Salmonid whirling disease (also caused by a myxozoan parasite that alternately infects an invertebrate host) has been successfully managed in hatcheries through actions that reduce obligate invertebrate host densities. Reducing densities of *M. speciosa* may be one method for managing ceratomyxosis. One action that has been proposed to reduce *M. speciosa* population densities involves manipulating the discharge from Iron Gate Dam to increase flow heterogeneity. A total of 4 habitats in the infectious zone are monitored for the presence of polychaete tubes every year.

**7.3 Results and Discussion:**
Polychaete tubes were observed at the reach 1 pool and eddy sites and the reach 2 pool and eddy sites in 2015. This is in contrast to 2014, when polychaetes were not observed inhabiting fines in the reach 2 sites. These habitats will continue to be monitored in 2016 to determine the effects of the March 2016 flood event on recolonization.

**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. Dataset synthesis has begun. Exploratory analyses examining correlations between water temperature and discharge have been completed and we plan to develop models to predict polychaete responses to these factors. We plan to link these to the epidemiological model in 2016.

**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 2013-2014. We plan to add data from 2015 and 2010-2013 and then validate these relationships using data collected 2006-2009.

**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 are using a series of models to examine the risk of salmonid enteronecrosis (ceratomyxosis), the disease caused by *C. shasta*. We plan to link a series of models designed specifically for the Klamath River system including a fine-scale climate change model to predict future stream temperatures and discharge (Perry et al. 2011), a 2-D hydraulic model coupled with a statistical model to predict changes in polychaete populations.
under different river discharge scenarios (Task D.2.4, above), a degree-day model to predict the potential number of generations per year under different thermal regimes (Chiaramonte 2013), and an epidemiological model to quantify the risk of disease in the salmon host under the different climate scenarios (Ray 2013).

The models and their outputs will be linked together to predict changes in disease severity in salmon as a result of C. shasta infection under different future climate scenarios. In addition to these Klamath-specific models there is a long-term data set on the intensity and distribution of C. shasta infections in juvenile salmon. The focus of this task is to examine the effect 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 also be made 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: to be 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; to be 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 2016.

**Acknowledgements**
The Karuk and Yurok tribes assisted with water sample collection and filtration. Jamie Graen, Damien Barrett and Kate Threlfall (OSU) assisted with qPCR. California Department of Fish and Game (Keith Pomeroy and crew at the Iron Gate Hatchery) provided Klamath River fall Chinook and coho salmon juveniles for our sentinel studies. Roaring River Hatchery, Oregon Department of Fish and Wildlife, Scio, provided susceptible rainbow trout. We are grateful to land owners who allowed us access to conduct the sentinel studies and water sampling: The Nature Conservancy (Klamath Falls) and Lonesome Duck Resort (Chiloquin), OR; The Sportsman’s Park Club near Keno, OR; Fisher’s RV Park at Klamath River, CA; Wally Johnson, Seiad Valley; Sandy Bar Resort, Orleans, CA.
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Atkinson and Bartholomew (2010b) Spatial, temporal and host factors structure the Ceratomyxa shasta (Myxozoa) population in the Klamath River Basin. Infection, Genetics and Evolution 10:1019-1026


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