KLAMATH RIVER FISH HEALTH WORKSHOP 2015
Karuk Community Center, Yreka, California
Tuesday, March 24th

AGENDA

8:30am Welcome & Overview

Jerri Bartholomew & Scott Foott

8:40am Ceratonova shasta: Evolution Of (How We Perceive) A Parasite

Jerri Bartholomew, Stephen Atkinson (OSU)

9:00am Klamath River Fish Health Monitoring Program 2014: Infection prevalence in juvenile Chinook salmon from the Klamath River basin

Kimberly True, Anne Bolick, Scott Foott (USFWS)

9:20am Klamath River Fish Health Monitoring Program 2014: Disease severity, mortality and annual trends in juvenile Chinook salmon from the Klamath River basin

Kimberly True, Anne Bolick, Scott Foott (USFWS)

9:40am Sentinel fish studies for Ceratonova shasta infection in 2014

Rich Holt, Ryan Craig, Gerri Buckles, Sascha Hallett, Jerri Bartholomew (OSU)

10:00am Abundance of Ceratonova shasta in river water samples in 2014

Gerri Buckles on behalf of OSU, Karuk Tribe, Yurok Tribe

10:20-10:40am BREAK

10:40am Monitoring and research on the invertebrate host, Manayunkia speciosa: 2014 update

Julie Alexander, Ryan Craig, Gerri Buckles, Jerri Bartholomew (OSU)

11:00am Monitoring a myxozoan parasite for management: 2014 Pulse Flow Event

Sascha Hallett, Rich Holt, Ryan Craig, Julie Alexander, Gerri Buckles, Stephen Atkinson, Jerri Bartholomew

11:20am Extending the sentinels: exploring the effects of prolonged exposure on the survival of salmonids in the Klamath River

Nicholas Som (USFWS AFWO), Russell Perry (USGS WFRC)

11:40am Updates to the Stream Salmonid Simulator (S3) as it relates to disease caused by C. shasta

Russell Perry, John Plumb (USGS WFRC); Nicholas Som, Nicholas Hetrick (USFWS AFWO); Thomas Hardy (Texas State University)
12:00 – 1:00pm  LUNCH

1:00pm  Towards a hydrodynamic and water quality model of the lower Klamath River  
       Amir Javaheri, Meghna Babbar-Sebens (OSU)

1:20pm  An Outbreak of Ichthyophthirius in Adult Salmon in the Klamath and Trinity Rivers in the Fall of 2014  
       Michael Belchik (YTFP)

1:45-3:45pm  Discussion of critical research questions
Ceratonova shasta: Evolution Of (How We Perceive) A Parasite

Jerri L. Bartholomew, Stephen D. Atkinson

Department of Microbiology, Nash Hall 226, Oregon State University, Corvallis, OR 97330

Ceratomyxa shasta is a long-standing taxonomic outlier to all other Ceratomyxa spp. It is histozoic (rather than coelozoic), has a freshwater life cycle (as opposed to marine) and is phylogenetically distant. The recent description of a new myxozoan species from freshwater sticklebacks led us to propose erecting a new genus, Ceratonova, to contain the two species: C. shasta n. comb. and C. gasterostea n. sp. This redescription provided the opportunity to look back at the changes in our understanding of this parasite and forward to what we are learning from new approaches. While our knowledge of some aspects of C. shasta, such as the life cycle, have changed dramatically, the geographic and host ranges have not. However, new information about the invertebrate host provides an explanation for the unusually static and restricted geographic range of C. shasta. Similarly, a better understanding of parasite genetics is informing us about host-parasite co-evolution. Our knowledge is expanding rapidly, with molecular diagnostic methods providing tools for predicting disease impacts on wild populations and epidemiological approaches providing critical insights about alternatives for disrupting disease dynamics. Sequencing of the genome and transcriptome is providing insights into differences between the host-specific parasite genotypes as well as into the basic biology of the parasite.
Klamath River Fish Health Monitoring Program 2014: Infection prevalence in juvenile Chinook salmon from the Klamath River basin.

Kimberly True, Anne Bolick, Scott Foot

USFWS, California-Nevada Fish Health Center
24411 Coleman Fish Hatchery Rd.
Anderson, CA 96007

The USFWS California-Nevada Fish Health Center has monitored myxozoan infections in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Klamath basin since 2005, focusing on prevalence of infection (POI) of *Ceratonova shasta* and *Parvicapsula minibicornis* in natural and hatchery-origin Chinook salmon. Juvenile Klamath River Chinook salmon were collected over the peak outmigration period (April to August 2014) from 4 major reaches of the Klamath River and the Estuary. Fish tissues were assayed by quantitative polymerase chain reaction (QPCR) and histology for myxosporean parasite infection levels and severity.

The seasonal *C. shasta* and POI by QPCR in natural Chinook salmon was 78% overall, however the POI was 81% during the peak outmigration period from May through July. *Parvicapsula minibicornis* POI in Chinook salmon was 91% overall, and 92% for the same outmigration time period. Naturally produced Chinook salmon had much higher *C. shasta* prevalence of infection by QPCR in 2014 (76%) compared to 2013 (25%). Among CWT juvenile Chinook salmon released from Iron Gate Hatchery, *C. shasta* was detected in 79% of fish screened by QPCR.

Prevalence of infection will be presented with emphasis on *C. shasta* and *P. minibicornis* by Klamath River reach and fish origin (natural or hatchery origin Chinook).
The USFWS California-Nevada Fish Health Center has monitored myxozoan infections in juvenile Chinook salmon (Oncorhynchus tshawytscha) in the Klamath basin since 2005, focusing on prevalence of infection (POI) in natural and hatchery-origin Chinook salmon, including an emphasis on coded-wire tagged (CWT) fish with known residency period in the Klamath River.

C. shasta infection levels and disease severity will be presented with emphasis on environmental conditions in 2014, parasite infectious load (quantity of parasite DNA), and diagnostic casework in out-migrant CWT juvenile Chinook salmon. Among coded-wire tagged (CWT) juvenile Chinook salmon released from Iron Gate Hatchery (IGH), C. shasta was detected in 79% of fish screened by QPCR. The highest C. shasta prevalence of infection observed was 92-96% in IGH CWT Chinook salmon residing 1-2 Weeks At Large (WAL) at time of recapture. Iron Gate Hatchery Chinook salmon had higher parasite infectious load, measured as quantity of C. shasta DNA present in intestinal tissue, in 2014 compared to 2013.

Diagnostic casework in June involving a mortality event in the Scott to Shasta (K3) reach found approximately 80% of IGH CWT juvenile Chinook salmon examined at the mouth of Ti Creek in end stage clinical disease, including myxospore production and mortality. Weeks at Large (WAL) analysis demonstrated many of these clinically diseased juvenile Chinook salmon had relatively short exposure periods post hatchery release. Annual trends in severity of C. shasta and climate year type and will be discussed in regard to continued drought conditions in the basin.
The myxozoan parasite *C. shasta* has been implicated as a significant source of mortality for salmonid fishes below Iron Gate Dam. Fish sentinel studies were conducted to develop a multiyear dataset on *C. shasta* infection prevalence in both Klamath River Chinook and coho salmon exposed at selected locations to monitor how changes in flow, water temperature and other variables alter parasite infection rate. In 2014, fish were exposed at up to six sites including two in the upper basin, the lower Williamson River and Keno Eddy, and four sites below Iron Gate Dam including near the I5-bridge, near Beaver Creek, Seiad Valley and Orleans, for 3 days in April, May, June, and September. As in previous years, known *C. shasta*-susceptible rainbow trout stock from Roaring River Hatchery (Oregon Department of Fish and Wildlife) was held at all sites. Klamath River fall Chinook from Iron Gate Hatchery (California Department of Fish and Wildlife) were held at all sites except for one location in the Williamson River. A limited number of coho salmon juveniles from Iron Gate Hatchery was held near Beaver Creek and Seiad Valley in May and in June at those same sites plus Orleans.

Chinook exposed in late April near Beaver Creek and Seiad Valley had 7% *C. shasta*-associated loss and 2.4 and 5% respectively in September. Compared to recent years’ sentinel exposures near Beaver Creek and Seiad Valley, the infection rates of *C. shasta* in juvenile Chinook and coho during May and June 2014 were very high. In May, ceratomyxosis developed in 40.7% of Chinook exposed near Beaver Creek, 32.5% at Seiad Valley and 5.0% near Orleans. The coho were even more severely infected with *C. shasta* than the Chinook with a loss 48.5% in fish exposed near Beaver Creek and 93.3% at Seiad Valley. In June, Chinook exposed near Beaver Creek incurred a 40% *C. shasta* loss compared to 42, 46 and 52% in the groups held separately in three cages at Seiad Valley and 5% near Orleans. In June, as in May, more coho juveniles than Chinook developed ceratomyxosis: 66.7% in coho exposed near Beaver Creek, 72.4% at Seiad Valley and 32.1% at Orleans. In May and June, none of the juvenile Chinook exposed in the upper Klamath River watershed at the Nature Conservancy site on the Williamson River or at Keno Eddy developed ceratomyxosis. Near I5 Bridge, the exposed Chinook in May had only a 2.6% *C. shasta* loss and none in June.
When comparing *C. shasta*-associated mortality in juvenile Chinook and coho during sentinel studies from 2007-2014, the impact of *C. shasta* in 2014 is similar to 2007-2009 when high losses also occurred. In 2014, the loss of coho (93%) in May at Seiad Valley was greater than any previous year (see figures below). *Ceratonova shasta* infections were detected in susceptible rainbow trout in all months tested and at all sentinel sites including the upper and lower Klamath River. Rainbow trout exposed in the lower Williamson River continued to suffer high losses and succumbed most rapidly from *C. shasta* despite cessation of the release of this hatchery stock in the watershed in 2011.
Abundance of *Ceratonova shasta* in Klamath River Water Samples 2014

Gerri Buckles*, Sascha Hallett, Jerri Bartholomew

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The myxozoan parasite *Ceratonova shasta* is a significant pathogen of juvenile salmonids in the Pacific Northwest of North America and is limiting recovery of Chinook, *Oncorhynchus tshawytscha*, and coho, *O. kisutch*, salmon populations in the Klamath River. As part of an extensive *C. shasta*-monitoring program that includes sentinel fish exposures, polychaete sampling and water sampling, in 2006 we established five index sites on the main stem of the lower Klamath River below Iron Gate Dam. Abundance of waterborne stages of *C. shasta* was determined by molecular analysis of river water samples. Automatic samplers collected river water for 24-hours approximately weekly from April through October at three sites and year round at 2 sites. The water samples were retrieved each week and filtered by Karuk and Yurok tribal biologists and sent to OSU for molecular analysis (quantification of total *C. shasta* and genotyping). Data are shared online at: http://microbiology.science.oregonstate.edu/content/monitoring-studies.

Molecular analysis of water samples from the lower Klamath River for 2014 showed higher presence of the *C. shasta* parasite than in 2011 through 2013 (but less than high disease years such as 2008 and 2009). Additionally the hot zone, previously seen at Beaver Creek KBC, appears to be expanding downstream to the Seiad Valley index site. By genotyping water samples, the amount of Type-II spores present can be determined. Previous combined sentinel fish exposures and water sampling indicated a 40% mortality threshold of 5sp/L for coho. With the exception of the first two weeks of June, Type-II spores were present at greater than 5sp/L from late April through early July at both Beaver Creek and Seiad Valley.

In addition to the lower Klamath River sampling, longitudinal studies in the Williamson River occurred in 2009-2014 to monitor changes associated with stocking practices and to establish pre-dam removal data. Levels above 10sp/L were present below Sprague Creek during the stocking of susceptible Rainbow trout in Spring Creek. This practice was stopped in 2011 and longitudinal studies for 2012 and 2013 showed a drop in parasite density throughout the study area. In the 2014 longitudinal study in mid-July, the area below Sprague Creek showed a return to spore densities above 10sp/L.
Klamath River Basin 2014
Monitoring and research on the invertebrate host, *Manayunkia speciosa*: 2014 update

**Julie Alexander**¹, Ryan Craig², Gerri Buckles¹, Jerri Bartholomew¹,²

¹ Microbiology Department, Oregon State University, Corvallis, OR 97330  
² J.L. Fryer Salmon Disease Laboratory, Oregon State University, Corvallis, OR 97330

Phases of the *C. shasta* life cycle that involve the invertebrate host, *Manayunkia speciosa*, are poorly understood and should be better characterized before we can expect to develop effective management actions. This presentation provides an overview of monitoring and modeling datasets as well as laboratory experiments that were conducted in 2014. We monitored *M. speciosa* populations at 7 sites on the Klamath River during winter, spring, summer, and fall in 2014. Sites are located throughout the Klamath River basin; up and downstream from dams, and within and outside the 'infectious zone.' Polychaete densities ranged from <10 m⁻² to >100,000 m⁻² however, we observed the highest densities of *M. speciosa* in the J.C. Boyle bypass reach. Median polychaete size was largest in spring and progeny were most abundant in summer at all but one site where we did not detect differences among months. Prevalence of infection varied among months and sites (detected at 4 sites) and we detected infected polychaetes most frequently from the site proximal to I5 Bridge. This is a contrast to last year when infected polychaetes were more frequently detected from sites located farther downstream. We discuss the results from our monitoring efforts in the context of our long-term data on Klamath River *M. speciosa* collected from 2006 on and report on the modeling and laboratory experiments that are underway.
Ceratonova shasta causes enteronecrosis in juvenile salmonids in the Pacific Northwest of North America and is associated with population-level impacts in the Klamath River. Transmission of this freshwater myxozoan parasite occurs through waterborne stages: actinospores, released from polychaete worms, develop into myxospores in salmonid fishes. In response to the high prevalence and severity of C. shasta infection in Klamath River salmonids, we developed a parasite monitoring program that included sentinel fish exposures, invertebrate host sampling and molecular quantification of waterborne stages in river water samples (total parasite density and ITS-1 genotypes). A management goal for the system is to reduce salmonid mortality to below 40%. Parallel water sampling and sentinel fish exposures indicated that 10 spores of type I/L reach this threshold in Chinook whereas 5 spores of Type II per liter cause mortality in coho. Furthermore, lab temperature studies indicated that a threshold of 16°C was important for disease severity in coho. Thus, in 2014 we were requested to expedite quantification and genotyping of C. shasta in water samples from April through June. In April 2014, type II surpassed 5 spores/L and in May river temperatures surpassed 16°C. In response in an effort to reduce disease severity in outmigrating juvenile salmonids, water was released from Iron Gate Dam May 27 – a ‘pulsed flow event’. We monitored the parasite before, during and after the pulse flow event at two index sites, Beaver Creek and Seiad Valley, through water samples, sentinel fish exposures, free-ranging fishes and polychaete sampling.

Sentinel exposures of Iron Gate Hatchery Chinook and coho salmon juveniles (30 fish of each species) were conducted just prior to the flow event on May 23-26 (72 hr) and again during the event on May 27-30. After the exposures, the fish were transported to the Salmon Disease Laboratory, reared at 18°C water temperature and monitored for
C. shasta infections for 60 days. Losses were highest for the coho compared to the Chinook at both sites. At Beaver Creek, C. shasta-mortality in Chinook salmon was higher pre-flow (61.3%) than post-flow (44.8%). Mortality in coho at this site did not appear to be affected by the flow event (76.7% pre-flow; 80.0% post-flow). At Seiad Valley, C. shasta-related mortality did not appear to be affected by the change in flow for either species (Chinook: 40.0% pre-flow, 43.3% post-flow; coho 75.9% pre-flow, 82.1% post-flow).

Out-migrant Chinook: Tagged Iron Gate Hatchery Chinook (2 on May 27-29, 35 first week in June) were collected by US Fish and Wildlife Service biologists near Walker Bridge and the Kinsman Trap, transported to the Salmon Disease Laboratory and monitored for C. shasta. Both the first 2 Chinook became moribund with C. shasta and 77.1% of the second group during rearing; Microscopic examination of lower gut material and PCR testing revealed a C. shasta infection prevalence of 57.1%.

Water samples were collected daily, beginning three days prior to the event and ending on May 30. Samples were collected every 2h using automated samplers, and pooled to make a 6h composite sample that was assayed using a C. shasta-specific qPCR. Data were more complete for Seiad Valley. At both sites there was a decrease in spores/L during the first day of the flow, although densities remained greater than 10 spores/L. By the end of the week, densities exceeded pre-flow levels at both sites.

Polychaete densities were measured on boulder (n=8; 4 per site) and fine (n=8; 4 per site) substrates at two sites immediately prior to the pulse flow (PRE; 3 days before ramp up began) and immediately after the pulse flow (POST; one week after the flow ramped down). The sites included KTH (rkm 281) and a site near the Klamath Community Center (rkm 263). Polychaetes were detected in all PRE samples collected from boulder and fines. However, polychaetes were detected only in samples collected from boulder substrates in POST flow samples. We observed interesting differences between the PRE and POST samples: First, we observed a decrease of 100-1000 polychaetes per m² on fine substrates between the PRE and POST sampling periods, which may indicate the elevated flows had some effect on polychaetes inhabiting fine substrate at these two sites. However, by June, we detected polychaetes on fine sediments at these sites at densities similar to PRE densities. Second, we observed an increase in polychaete densities on boulder substrates between the PRE and POST flow sampling periods, corresponding with an increase of approximately 50,000 polychaetes per m² on boulder substrates. The increase is most likely related to the elevated water temperatures that occur naturally at this time of year, rather than flow changes.
Extending the sentinels: exploring the effects of prolonged exposure on the survival of salmonids in the Klamath River.

Nicholas A. Som, US Fish and Wildlife Service, Arcata Fisheries Program

Russell W. Perry, USGS Western Fisheries Research Center, Columbia River Research Laboratory

Sentinel exposure experiments have dramatically refined our knowledge regarding the mortality response of salmonids to C. shasta. In the traditional sentinel experiment, caged juvenile fish are first placed in the Klamath River to experience in situ water temperatures and actinospore concentrations. After three days, the fish are transported to a laboratory where they are held in tanks with water containing no actinospores, and temperatures that are experimentally controlled. These experiments have demonstrated that warmer temperatures and higher concentrations of actinospores are associated with lower rates of survival and shorter durations until fish succumb to their disease. Recently, it has been hypothesized that exposure duration might also be associated with rates of survival. To test this hypothesis, we planned an experiment in which juvenile Chinook and Coho Salmon were placed in the Klamath River for 1, 3, 5, and 7 days. After exposure, fish were again reared under controlled conditions at a laboratory. In this presentation, we will summarize the findings of an analysis aimed to explore the effects of exposure duration, actinospore concentrations, and water temperatures on survival. Additionally, we will compare the results in the context of the two species considered, and discuss implications for future monitoring.
Updates to the Stream Salmonid Simulator (S3) as it relates to disease caused by *C. shasta*.

**Russell W. Perry**, USGS Western Fisheries Research Center, Columbia River Research Laboratory

John M. Plumb, USGS Western Fisheries Research Center, Columbia River Research Laboratory

Nicholas A. Som, US Fish and Wildlife Service, Arcata Fisheries Program

Nicholas Hetrick, US Fish and Wildlife Service, Arcata Fisheries Program

Thomas Hardy, Texas State University

Mortality caused by *C. shasta* is thought to significantly reduce populations of juvenile Chinook salmon in the Klamath River, yet empirically quantifying mortality caused by disease is difficult. The Stream Salmonid Simulator (S3) is a dynamic simulation model that tracks movement and mortality due to different causes. A primary challenge has been incorporate infection and mortality processes due to *C. shasta* into the model. Recent updates to the model include tracking populations from different sources separately. By tracking source populations, we can now gain an understanding of how differences in migration timing among different populations interact with temporally varying spore concentrations to influence infection and mortality for each population. In this presentation, we highlight the current status and additions to S3, with particular focus on how S3 can be used to gain an understanding of disease caused by *C. shasta*. 
Towards a hydrodynamic and water quality model of the lower Klamath River

Amir Javaheri, Meghna Babbar-Sebens

Civil and Construction Engineering Department, Oregon State University, Corvallis, OR 97330

The myxozoan parasite *Ceratonova shasta* is responsible for high mortality in juvenile salmon in the lower Klamath River below the Iron Gate Dam. Water temperature is an important factor that affects the mortality rates of salmons especially Coho salmon juveniles. Prediction of the flow discharge, water temperature and parasite density can determine the areas with higher risk of mortality and identify management actions that could decrease disease effects. Numerical methods are effective tools to predict the behavior of complex aquatic systems such as rivers. A three-dimensional hydrodynamic model of Klamath River is being developed to estimate the flow discharge, velocity and temperature of water as well as parasite concentration. The accuracy of model is reliant on different model parameters and variables that will need to be calibrated and regularly updated to reproduce changing aquatic conditions accurately. Different observations such as the spatial and temporal abundance of the parasite monitored by the Bartholomew Lab at Oregon State University, atmospheric data, water temperature and flow discharge from USGS stations will be used to improve the model accuracy and update the model frequently. This study will also use an ensemble Kalman filter data assimilation methodology to automate model updating using data from heterogeneous sources.
In the fall of 2002, a massive fish kill occurred in the lower Klamath River. Several reports and clinical examination of the fish indicated that the proximal cause of the epidemic was a disease condition brought on by the protozoan ectoparasite *Ichthyophthirius multifiliis*. Commonly known as “ich” or whitespot disease, this parasite caused the death of somewhere between 34,000 and 78,000 adult Chinook salmon that year. Since that time, the Yurok Tribal Fisheries Program has monitored adult salmon returning to the river, and in drought conditions, additional flows have been provided from Trinity Reservoir. Adult Chinook are gillnetted from the river, and the gills are visually inspected for ich with a microscope while fresh. From 2004 through 2013, no ich was detected out of hundreds of Chinook salmon captured per year. In early September 2014, ich was detected for the first time since 2003 in the lower Klamath River. Ich levels quickly reached emergency flow release criteria (>5% of fish with >30 ich/gill arch) and emergency flows were initiated. Despite these water releases, ich levels continued to climb, ultimately reaching levels of over 900/gill arch in some fish with infection prevalence approaching 100%. Almost 400 fish were inspected for ich and presence was verified by USFWS fish pathology experts. Results of the ich sampling over time and by geographic location will be presented as well as a new method for quantifying ich levels.
Predicting the effects of climate change on *Ceratonova* (syn *ceratomyxa*) *shasta* in Klamath River salmon

Adam Ray¹, Julie Alexander², Jerri Bartholomew²

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Climate related shifts in water temperatures and precipitation patterns will likely have important effects on the dynamics of *Ceratonova shasta* in Klamath River salmonids. However, predicting the magnitude and direction of specific responses is challenging. We present future disease dynamics given three climate change scenarios for ceratomyxosis in Klamath River salmonids. Using a model ensemble, we predicted responses of *Ceratonova* (syn *Ceratomyxa*) *shasta* in three different types of water years (dry, median, wet) for three different future climate scenarios (hot/dry, moderate/median, and cold/wet) selected from 2020-2060, in the Klamath River CA, USA. The ensemble uses outputs from Global Climate Models (GCMs) as inputs for hydraulic and water temperature models, which are used as inputs for predictive statistical models. Outputs from the predictive models provide values for parameterizing the epidemiological model, which outputs an estimated basic reproductive number (R₀) for each climate scenario. The resultant R₀ values were scaled relative to empirical data collected in 2008 (high mortality in salmonids) and 2011, (low mortality) in the Klamath River. The majority of predicted future R₀ values were similar to the 2008 value, which provides compelling evidence that *C. shasta*-induced mortality will increase and remain high in the Klamath River.
Meeting Location and Parking:

Karuk Community Center
(also known as Karuk Tribal Housing Authority)
1836 Apsuun Drive
Yreka, CA 96097

Parking is available in the Karuk Community Center parking lot.

Google Maps Link*

*NOTE: MapQuest does not place the address accurately*

Directions:

**From the South:**
Take I-5 North
Take CA-3 - Exit 773 - Yreka
Left onto CA-3
Right onto South Main / CA-3
Right onto Oberlin Rd
Right onto Comstock Dr
Left onto Campbell Ave
Right onto Dove Ln
Right onto Apsuun Dr
1836 Apsuun Dr

**From the North:**
Take I-5 South
Take CA-3 - Exit 773 - Yreka
Right onto CA-3
Right onto South Main / CA-3
Right onto Oberlin Rd
Right onto Comstock Dr
Left onto Campbell Ave
Right onto Dove Ln
Right onto Apsuun Dr
1836 Apsuun Dr

Karuk Community Center Internet Availability
Internet will be available to attendees in the community computer room.
An open wireless connection will **not** be available due to limited bandwidth.