KLAMATH RIVER FISH HEALTH WORKSHOP 2014

River Lodge Conference Center, Fortuna, California

Tuesday, March 4th

AGENDA

and

ABSTRACTS

Funding for support of this annual gathering of Klamath Basin fish health experts was provided by The Fisheries Program for the Arcata Fish and Wildlife Office of the US Fish and Wildlife Service, The Bartholomew Lab of Oregon State University and the Bureau of Reclamation.

AGENDA

8:30am Welcome

8:35am – 9:55am *Ceratomyxa shasta* Monitoring Studies –Salmonids and River Water

8:35am Klamath River Fish Health Monitoring Program 2013: Infection prevalence in juvenile Chinook salmon from the Klamath River basin.
Anne Bolick, Kimberly True, and Scott Foott (USFWS)

8:55am Sentinel fish studies for *Ceratomyxa shasta* infection in 2013
Rich Holt, Ryan Craig, Jerri Bartholomew (OSU)

9:15am Abundance of *Ceratomyxa shasta* in river water samples in 2013
Gerri Buckles on behalf of OSU, Karuk Tribe, Yurok Tribe

9:35am Long-term surveillance of a salmonid parasite by river water sampling and qPCR
Sascha Hallett, Gerri Buckles, Charlene Hurst, R. Adam Ray, Jerri Bartholomew (OSU)

9:55am – 10:15am BREAK

10:15am - *Ceratomyxa shasta* Monitoring Studies cont. - Polychaetes

10:15am The *Ceratomyxa shasta* Hyper-Infectious Zone of the Klamath River: Year-round abundance and infection prevalence of the polychaete host (*Manayunkia speciosa*)
Michael Belchik, Joshua Strange (Yurok Tribe)

10:35am Monitoring invertebrate hosts for *Ceratomyxa shasta*
Julie Alexander, Ryan Craig, Gerri Buckles, Jerri Bartholomew (OSU)

10:55am Mesocosms: Laboratory cultures of *Manayunkia speciosa* in a closed loop system providing a year round source of *Ceratomxya shasta* myxospores
Ryan Craig, Julie Alexander, Stephen Atkinson, Gerri Buckles, Jerri Bartholomew (OSU)

11:15am – 11:55pm Modeling Studies

11:15am Two models, one life cycle: different approaches to understanding complex interactions of the *C. shasta* life cycle
Adam Ray, Jerri Bartholomew (OSU)

11:35am Simulating the spatial distribution of mortality of juvenile Chinook salmon infected with *Ceratomyxa shasta* in the Klamath River
Russell Perry (USGS WFRC), Nicholas Som (USFWS AFWO), Adam Ray (OSU)

12:00 - 1:00pm LUNCH

1:00pm – 1:45pm Modeling Studies cont.

1:00pm A very speciosal collaboration: Using 2D hydrodynamic models and hypothesis-driven sampling designs to predict *Manayunkia speciosa* distribution
Katrina Wright (USFWS AFWO)**, Nicholas Som** (USFWS AFWO)**, Julie Alexander** (OSU), Nicholas Hetrick (USFWS AFWO), Jerri Bartholomew (OSU)

1:45pm – 2:30pm Discussion of critical research questions

Klamath River Fish Health Monitoring Program 2013: Infection prevalence in juvenile Chinook salmon from the Klamath River basin.

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Juvenile Klamath River Chinook (*Oncorhynchus tshawytscha*) experience high prevalence of infection with the myxosporean parasites *Ceratomyxa shasta* and *Parvicapsula minibicornis* during the peak outmigration period of May to July. The USFWS California-Nevada Fish Health Center has monitored myxozoan infections in juvenile Chinook 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. The Fish Health Center utilized quantitative real-time polymerase chain reaction (QPCR) and histology to assess myxozoan POI in juveniles as they migrated from below Iron Gate Dam to the Klamath Estuary, from late March to August 2013.

The seasonal *C. shasta* POI by QPCR in Chinook salmon was 36% overall, however the POI was 46% during the peak outmigration period from May through July. *Parvicapsula minibicornis* POI in Chinook salmon was 76% overall, and 88% for the same outmigration time period. Naturally produced Chinook salmon had a 25% *C. shasta* POI by QPCR and low disease severity based on DNA copy number. The low disease severity result was also seen histologically. Among CWT juvenile Chinook salmon released from Iron Gate Hatchery, *C. shasta* was detected in 46% of fish screened by QPCR. The highest POI for CWT salmon was observed in fish captured after residing in the river 4-5 weeks post hatchery release.

Klamath River Sentinel Fish Studies for *Ceratomyxa shasta* infection in 2013

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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 2013, fish were exposed at selected sites for 3 days in April, May, June, July and September. As in previous years, known *C. shasta*-susceptible rainbow trout stock from Roaring River Hatchery (Oregon Department of Fish and Wildlife) were 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 were held near Beaver Creek and Seiad Valley in May and June and Tully Creek in July. Also, Trinity River Hatchery Chinook were held near Tully Creek in July.

Ceratomyxa 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 suffered high losses and succumbed most rapidly from *C. shasta* despite cessation of the release of this hatchery stock in the watershed in 2011. Compared to sentinel results of 2007-2009, losses of fall Chinook were much lower in 2013 similar to 2010, 2011 and 2012. This is despite the occurrence of slightly higher water temperatures below Iron Gate Dam in the spring of 2013. In April and September, Chinook exposed near Beaver Creek and Seiad Valley suffered no loss from *C. shasta*. At Beaver Creek in May there was 0% loss and 5.2% in June. At Seiad Valley, 9.8% of the Chinook died from *C. shasta* in May and 13% in June. At Orleans, 9.8% of the Chinook died of *C. shasta* in May and 2.5% in June. In July in the Klamath River near Tully Creek, 3% of both the Trinity River Hatchery and Iron Gate Hatchery Chinook died of *C. shasta*. In contrast, coho salmon juveniles exposed near Beaver Creek and Seiad Valley had *C. shasta* loss for May of 25% and 30% and for June, 29% and 45% respectively. In 2013, coho juveniles were more impacted from *C. shasta* than the Chinook. Also, higher infection rates and loss were detected downstream of Beaver Creek near Seiad Valley.

Abundance of *Ceratomyxa shasta* in Klamath River Water Samples 2013

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The myxozoan parasite *Ceratomyxa 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. We have been conducting a monitoring program for 8-years that comprises concurrent sentinel fish exposures and water sampling across 212 river kilometers of the lower Klamath River below Iron Gate Dam.

Molecular analysis of water samples from the lower Klamath River for 2013 showed higher presence of the *C. shasta* parasite than in 2011 or 2012 (but less than high disease years such as 2008). Additionally the hot zone, previously seen at Beaver Creek KBC, appears to be shifting downstream and is more evident now at the Seiad Valley index site.

A longitudinal study was done in 2012 and 2013 in a region flanking Tully Creek to determine the spatial distribution of the parasite in this reach. In weekly monitoring, Tully Creek has consistently had parasite levels between 5-10sp/L for the last 5 years. The longitudinal data showed persistent presence of parasite above 1 sp/L in an area from the confluence of the Trinity River downstream - approx. 35rkms.

Longitudinal studies in the Williamson River occurred in 2009-2013 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 show a drop in parasite density throughout the study area in 2012 and 2013.

Long-term surveillance of a salmonid parasite by river water sampling and qPCR

Sascha Hallett*, Gerri Buckles, Charlene Hurst, R. Adam Ray, Jerri Bartholomew

Oregon State University

Monitoring pathogen levels and predicting related mortality in wild fish populations is difficult. A practical alternative to host sampling is the direct measurement of waterborne parasite stages. *Ceratomyxa shasta* (Myxozoa) causes enteronecrosis in juvenile salmon and trout in the Pacific Northwest and is limiting their recovery in the Klamath River. *C. shasta* has two waterborne stages: actinospores released from freshwater polychaete worms and myxospores from salmonid fishes. In response to high prevalence and severity of *C. shasta* infection in Klamath River salmonids, we developed a parasite monitoring program that included detection of parasite DNA by qPCR of river water samples. We established 5 index sites in the mainstem and 4 sites in tributaries. Weekly, automatic samplers collected and pooled 1L of river water every 2h for 24h. Replicate 1L samples from the pool were filtered and DNA extracted. *C. shasta* was quantified using a TaqMan qPCR which targeted the ssrRNA gene. ITS-1 genotypes were determined using a SYTO9 qPCR and sequencing. We assayed >5000 samples over 7 years. We genotyped a subset of 278 samples, which comprised weekly samples for 1 index site from 2006-2011, and all samples available for the other 4 index sites in 2 years of high total parasite density. We identified spatial and temporal patterns of parasite density and genetic diversity across high-impact and low-impact years. *C. shasta* was detected at all mainstem sites, but levels differed among sites. Levels were low in the tributaries. Typically, parasite density increased in early spring (when salmonids were migrating) and peaked in late spring/early summer. Levels then decreased, but increased again to a lower second peak in late summer/early autumn. Parasite genotypes varied among sites and years. We are now exploring relationships among parasite occurrence, invertebrate and vertebrate host life histories, and water temperature and flow. These data influence management practices and inform epidemiological models and risk assessments.

The *Ceratomyxa shasta* Hyper-Infectious Zone of the Klamath River: Year-round Abundance and infection prevalence of the polychaete host (*Manayunkia speciosa*)

Michael Belchik, Joshua Strange

Yurok Tribe

Within the Klamath River, two myxozoan parasites, *Ceratomyxa shasta* and *Parvicapsula minibicornis*, cause substantial mortality of juvenile salmonids annually (Nichols and Foott 2006). Both of these parasites rely on the freshwater polychaete worm, *Manayunkia speciosa*, as their definitive host (Bartholomew et al. 1997) with salmonid fishes as the alternate host (Bartholomew et al. 1989) and host-specific infectious spores as the vector. The infection prevalence of polychaetes in the highest infectious zone is a fundamental piece of information for determining disease reduction strategies and accurate modeling disease dynamics, but before this study, only limited information regarding the year-round infectivity of these polychaete worms had been collected. This study presents the results of the first year-round survey of the infectivity of these polychaete worms. Polychaete worms were collected with a new modified dredge technique and sorted and analyzed via QPCR by Oregon State University's Pathology Laboratory. Infection prevalence ranged from zero to 25% at the two sites, with the highest infection rate occurring at the Deliverance site in December 2012. Assay inhibition issues affected some of the results.

Monitoring invertebrate hosts for *Ceratomyxa shasta*: 2013

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Phases of the *C. shasta* life cycle that involve the invertebrate host, *Manayunkia speciosa*, are poorly understood. We monitored *M. speciosa* populations at 7 sites on the Klamath River during winter, spring, summer, and fall in 2013. Sites are located throughout the Klamath River basin; up and downstream from dams, and within and outside the 'infectious zone.' We present data on density and size structure of populations and results of molecular assays for the prevalence of *C. shasta* infection. Polychaete densities were highly variable among months and sites, ranging 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 also varied among months (0-2.7%) and sites (detected at 4 sites) and we detected infected polychaetes most frequently from the site near Seiad Valley. This is a contrast to other years when infected polychaetes were more frequently detected from sites located farther upstream. We discuss the results from 2013 monitoring efforts in the context of our long-term data on Klamath River *M. speciosa* collected from 2006 on.

Mesocosms: Laboratory cultures of *Manayunkia speciosa* in a closed loop system providing a year round source of *Ceratomxya shasta* myxospores

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While field based sentinel exposures have provided us with the opportunity to study the presence and effects of *Ceratomyxa shasta* on salmonids, manipulating and controlling variables is difficult during field exposures. We have established a system for maintaining the *C. shasta* year round in *Manayunkia speciosa* (polychaete host) cultures. We use a two tank closed loop system consisting of an upper tank housing polychaetes and a lower tank housing goldfish. The tanks containing polychaetes are fed with a constant flow of water from the fish tank below. The purpose of fish tank below is biological breakdown of ammonia and a supply of micronutrients and filter food for the polychaetes above. The water drains back to the fish tank on outflow to make a closed loop flow-through system. With exception of the control tanks (n=2), *C. shasta* myxospores belonging to genotypes: I (Chinook), IIR (Rainbow Trout), IIC (Coho), or III (Rainbow Trout) are added to polychaete tanks every three weeks (as available). In order to ensure sufficient abundance of polychaetes, the control cultures are restocked 3-4 times a year with field collected polychaetes. The control tanks are used to stock the other tanks with parasite-free worms once parasite DNA levels drop. We have exposed salmonids to our closed loop system water and have successfully infected Chinook, Coho, and Rainbow trout with *C. shasta.* These exposures i) provide us with myxospores that can in turn be used to re-infect the polychaetes in their respective mesocosms, and ii) a source of actinospores and infected *M. speciosa* for other research. As our methods improve, we plan to begin laboratory manipulations of the polychaete cultures to learn more about the life history of *M. speciosa.*

Two models, one life cycle: Different approaches to understanding complex interactions of the *C. shasta* life cycle

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Abiotic factors influence the complex life cycle of *Ceratonoma* (syn *Ceratomyxa*) *shasta*. In the Klamath River the parasite has been linked to decreasing number of retuning adult salmon and can cause high mortality in out-migrating juvenile salmon. We developed two different models to i) predict the effects of *C. shasta* on salmon populations and ii) identify potential management actions to reduce these effects. The first, a mixture cure (statistical) model, is used to predict the effects of abiotic factors (e.g. water temperature, discharge) and *C. shasta* concentration on juvenile Chinook and coho populations. The output values estimates can be incorporated into other salmon population models to refine escapement and harvest quotas and improve salmon management. The second, an epidemiological (mathematical) model is used to understand the interactions between the salmon and polychaete hosts and *C. shasta*. This model "describes" the parasite life cycle as a series of differential equations that are then solved for the basic reproductive number (R_0). The basic reproductive number has an inherent threshold value of 1, below which the disease will unable to persist within a population and can be used to assess control measures. We then conducted a sensitivity analyses to evaluate the effectiveness of potential management actions, by altering values of different parameters and observing how R_0 values respond to those changes. Although different in their approach and development, each model provides real world applications for management of the salmon populations in the Klamath River.

Simulating the spatial distribution of mortality of juvenile Chinook salmon infected with *Ceratomyxa shasta* in the Klamath River

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Juvenile fall Chinook salmon, *Oncorhynchsu tshawytscha*, in the Klamath River become infected with the myxozoan parasite *Ceratomyxa shasta* when the polychaete worm *Manayunkia speciosa* releases actinospores into the water column. *C. shasta* is thought to cause high mortality rates in juvenile salmon in some years, but the population-level effect of this parasite is poorly understood. Over the past two years, Oregon State University, the US Fish and Wildlife Service, and the US Geological Survey have collaborated on a model for the disease dynamics of *C. shasta*. Specifically, disease-related mortality of juvenile Chinook salmon is modeled as a function of three processes: 1) *C. shasta* spore densities in the water column, 2) infection rates of juvenile salmon with *C. shasta*, and 3) mortality rates of infected juvenile salmon. This disease sub-model is being incorporated into a population model of juvenile salmon, the Stream Salmonid Simulator (S^3), which simulates growth, movement, and mortality of salmon from spawning through the juvenile life stages.

Here, we illustrate how casting disease dynamics in the context of a salmon population model can provide insights about the disease ecology of juvenile Chinook salmon. For example, spore density inferred from qPCR analysis of water samples is assumed be composed largely of actinospores during the juvenile salmon outmigration. However, it has been recognized that myxospores produced by juvenile salmon that die from ceratomyxosis might also contribute to these water samples. Understanding where and when water samples might comprise a mixture of actinospores and myxospores requires knowledge of where fish infected with *C. shasta* ultimately die from this parasite. The spatial distribution of *C. shasta*-induced mortality will depend on 1) where fish become infected, 2) the time between infection and mortality, and 3) movement rates of juvenile salmon. We used S^3 for the Klamath River to simulate how these complex processes interact to affect the spatial distribution of mortality caused by *C. shasta*. This application shows how S^3 can be an important tool to help to understand aspects of *C. shasta* dynamics that would be difficult or impossible to measure empirically.

A very speciosal collaboration: Using 2D hydrodynamic models and hypothesis-driven sampling designs to predict *Manayunkia speciosa* distribution

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The demand for effective solutions for managing enteronecrosis (syn ceratomyxosis) in Klamath River salmon has generated interest in using flow manipulation to reduce *M. speciosa* populations. However, evaluating the efficacy of such an action is critical given the importance of water allocation in the Klamath basin. We present a tandem modeling approach to predict the distribution of *M. speciosa* and evaluate the effects of various 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. The 2DHMs were used to spatially describe hydraulic variation and allocate sampling locations across depth and velocity gradients within substrate classes. Benthic samples collected in July 2012 were used to build a statistical model estimating the relationship between physical habitat characteristics and the distribution of *M. speciosa*. The best fitting statistical model demonstrated that in summer, distribution is associated with substrate, as well as depth and velocity conditions during peak discharge (predicted from the 2DHMs) during the immediate water year. This model was then validated using an independent dataset of benthic samples collected in July 2013. We then use the 2DHMs and statistical model to predict the distribution of *M. speciosa* under several alternate peak flow scenarios, and our preliminary results suggest that higher yearly peak flows limit the occurrence of *M. speciosa*.