Objective 1. Determine if reducing the input of myxospores by removal of adult salmon carcasses could be an effective management strategy for decreasing *C. shasta*. Adult salmon contribute large numbers of parasites that result in infection of the worm host. Breaking this cycle may reduce the number of parasites causing infection in the juvenile salmon. The following tasks provide data for this objective:

**Task 1. Test the effects of salmon carcass removal in Bogus Creek.**

*Investigators:* Josh Strange, Yurok Tribe; Scott Foott, USFWS; Jerri Bartholomew, OSU

The Yurok Tribal Fisheries Program completed salmon carcass removal on Bogus Creek in 2009 per study protocols. A total of 1,799 salmon carcasses (predominantly Chinook) were removed from the lower 1 km of Bogus Creek from 10/12/2009 to 11/19/2009. Complete carcass removal occurred daily from Monday to Friday. During this period, automated water sampling sites were maintained and samples collected and filtered twice per week at three locations on Bogus Creek, in the mainstem Klamath River below Bogus Creek, and in the lower Shasta River.

Water samples are undergoing QPCR analysis at OSU to determine myxospore densities. Samples collected mid-November to mid-December have been processed and no *C. shasta* DNA was detected.

Approximately 210 Fall-run Chinook carcass intestine samples were collected from 3 general sites: Bogus Creek, mainstem Klamath River (Iron Gate Hatchery to the confluence of the Shasta River), and within the Shasta River. We have examined 161 samples:

- **Shasta River** - *C. shasta* myxospores detected in 32 / 60 (53%)
- **Bogus Creek** - 9 / 38 (24%)
- **Klamath River** - 13 / 63 (21%)

Spore concentrations in samples from the infected fish have ranged considerably and there are approximately 50 samples remaining to process. We plan to perform QPCR on a subset of the samples to estimate overall *C. shasta* infection. To estimate the viability of the spores, a methylene blue dye exclusion technique is being tested; results to date are inconclusive. Due to an extremely low return of coho salmon in 2009, no coho carcasses were found in the Shasta River, mainstem Klamath River or Bogus Creek, and only 1 mortality was
obtained from Iron Gate Hatchery (IGH). If myxospores are recovered from this carcass, they will be sent to OSU for genotyping.

**Task 2. Determine how long waterborne myxospores survive at different temperatures.**

**Investigator:** Jerri Bartholomew and Sascha Hallett, OSU

Myxospore viability will be determined in polychaete infection experiments, with prevalence of infection in exposed polychaetes providing a measure of “infectivity” or survival of the parasite. Differences in infection levels will be most acute using a threshold infection dose. However, the minimum number of myxospores required to infect a worm is unknown. Therefore, prior to conducting the myxospore viability experiment we performed a dosage experiment; we are currently 6 weeks through the 8 week experiment.

Ten worms were placed in each of 5 wells of a 6-well plate (with sediment). All five wells on a plate were exposed to either 0, 1, 10, 100 or 1000 freshly harvested myxospores per worm. An additional plate of 1 and 10 spores/worm were prepared. Plates were held for 1 month at 17°C, then worms from 3 of the 5 wells from one plate per treatment were examined with a compound microscope for evidence of *C. shasta* development. All worms from each plate were then counted and placed into microfuge tubes and frozen for PCR analysis to determine prevalence of infection. As worm survival was high (~80%), the second 1 and 10 spore/worm plates are being held one additional month.

**Task 3. Describe the timeline of myxospore maturation in adult salmon carcasses.**

**Investigator:** Gary Hendrickson, HSU

As conceived, this portion of the project was to examine production of myxospores of *C. shasta* in post-spawned adult Chinook salmon carcasses. This was to be done by placing carcasses in containment and measuring the quantity of *C. shasta* DNA released into the water over time. For various reasons this simply did not work. The experiments we attempted are as follows:

**Experiment 1:** On October 30, 2009, 40 adult Chinook salmon carcasses were obtained from Iron Gate Hatchery, Yreka, California. Carcasses were collected immediately after spawning by hatchery personnel. They were put on ice and driven back to Humboldt State University. Fork length, weight, and sex were recorded for each carcass, and then wet mounts were prepared from intestinal smears to check for presence of *C. shasta*. None of the wet mounts contained myxospores or pansporoblasts. Suspected trophozoites were observed in six carcasses. These were selected for decomposition experiments.

One carcass was placed in each of six self-contained, re-circulating stream units at the Humboldt State University fish hatchery. Each unit consists of a 15 foot trough with a standpipe at one end which drains water to the sump below. Water is pumped from the sump back up to the head of the unit. Units were filled with water from Fern Lake, a small pond in the forest behind the campus which serves as the water source for the fish hatchery. Units were covered with
screens to keep animals out. Carcasses were then left to decompose. Water samples were to be collected over time, filtered and examined by QPCR for *C. shasta* DNA. QPCR would allow us to determine the quantity of *C. shasta* DNA in the water and, thereby, the number of *C. shasta* myxospores in the water over time. This would allow us to determine the timeline of spore production in the fish host. It would also indicate the number of spores produced by a single carcass.

Numerous technical difficulties arose. Standpipes continuously clogged with algae and fish parts causing the troughs to overflow. There was also great but unexpected variation in flow from one stream unit (pump) to the next. Both of these problems caused troughs to overflow. Having a contained and known water volume was essential for accurately determining the quantity of *C. shasta* DNA in the water and, subsequently, number of myxospores in the water. More importantly, none of the six carcasses ever exhibited any indication of parasite development. Thus, the project was abandoned in an attempt to develop a better system.

**Experiment 2:** The emphasis of our second experiment was to examine myxospore production under differing conditions of temperature, pH, atmospheric gas, and nutrients to identify an environmental factor that might trigger myxospore production.

On November 18, 2009, 22 posterior intestines from adult Chinook salmon were collected from Bogus Creek. Smears of intestinal contents were examined in the field as the posterior intestines were collected. Fork length, sex, and any *C. shasta* stages were recorded for each carcass. While there was some ambiguity in detecting infected fish at times, two posterior intestinal smears clearly showed *C. shasta* myxospores and two clearly showed pansporocysts. Intestinal samples were labeled, placed in bags with a small amount of saline and transported by to Humboldt State University in a cooler.

Three posterior intestines were used in the experiment. The smear from one contained a single myxospore. The others contained only pansporocysts. Each posterior intestine was cut lengthwise, and then sliced into squares about 3 mm x 3 mm. Squares were then placed in one well of 96-well PCR plates. Each well then received water buffered at pH 4, 6, 8, or 10. Plates were then stored in plastic bags containing either air, oxygen, nitrogen, or carbon dioxide. Bags were stored in incubators at 5, 10, 15, or 20°C. This resulted in 64 combinations of gas, temperature, and pH for each of three different fish.

Two additional 96-well plates were set up to determine the effect of nutrient load using Difco Nutrient Broth. Again, water was at pH 4, 6, 8, or 10. Bags were stored in incubators at 5, 10, 15, or 20°C. Nutrient broth was added at three different concentrations. Only one fish was used for nutrient loading.

Smears were made from the wells of the various treatment combinations beginning on day 3. Smears were examined for the presence or absence of myxospores. Every well was examined at least every other day. Over time, several wells showed some suspected trophozoites, however, development
never proceeded beyond that point. At this stage, the run of adult Chinook salmon was over for the year and the experiment could not be repeated.

**Future Plans:** Due to the technical problems encountered, we would like a one-year extension on this portion of the project. We expect to develop a more workable study design. It is our intention to run some preliminary tests on outmigrant juvenile Chinook this spring to develop a technique more likely to work on returning adults in the fall. We expect to develop a scheme for following the time line of myxospore development either in whole carcasses or in posterior intestines. And we expect to develop a better scheme for examining the effects of environmental parameters (pH, temperature, ambient gas, nutrients) on myxospore development. In particular, we would like to identify what triggers myxospore development after the death of the fish host.

**Objective 2.** Integrate data on polychaete population structure with geomorphology data to provide a map of the infectious zone.

**Task 4. Develop a map of the infectious zone taking into account the hydrology and geomorphology of the river.**

*Investigators:* Jerri Bartholomew, OSU; Gordon Grant, USFS

Preliminary field trips were made to look at polychaete distribution and determine sites for the study. We decided to focus hydraulic modeling efforts on one river feature that has been shown to act as habitat for polychaetes, lateral separation eddies. The decision to limit the model to one feature type was influenced by 1) recognition of the time and labor intensive nature of modeling, 2) a desire to ensure a thorough study of the selected site(s) and 3) observation of the unique and biologically relevant character of eddies. Lateral separation eddies are areas of recirculating current and 1-dimensional flow hydraulic models are inadequate for simulating flow within them. Therefore a 2-dimensional model is necessary and a prospective model has been identified. A second field trip was taken to investigate equipment possibilities and collaborations and we are currently discussing equipment use with a group on campus. Collection of bathymetric and velocity data will begin in the late spring 2010 and continue through the summer months.

Concurrent with the hydrologic modeling, samples of sediment will be collected to determine polychaete densities within the eddies and to examine changes in densities as a result of flow variations.

**Objective 3.** Address research gaps in our knowledge of the disease that are critical to understanding the disease and for developing population and epidemiological models.

**Task 5. Evaluate migration rate on ceratomyxosis development in Chinook salmon smolts.**

*Investigator:* Scott Foott, USFWS

Over a three day period in May (27th – 29th), 2009, juvenile Chinook salmon were exposed for 6-8 hrs at the top and bottom of a known infectious reach (I-5 Bridge to Community Center) with cohorts moved through the same 34.25 km reach at
or above the river flow (3.8 - 4.8 km/h). Actinospore concentration was
determined in composite water samples at Community Center and along the
migration route. Post exposure, all groups were held and sampled for
ceratomyxosis infection by PCR, histology and or imprint.

To date, all 372 of 817 samples taken from clinically diseased fish have been
processed and the prevalence of infection in the different exposure groups
ranges between 50% - 95%. Fish not exhibiting clinical signs of ceratomyxosis
by 31 dpe were sampled and tested using PCR; 66% (123/186) were positive.
All water samples were similar (Ct 35) showing that exposure dose was similar
for all the groups. Statistical analysis examining differences between exposure
groups (treatments) have been completed. It appears migration groups tended
to have higher cumulative mortality due to ceratomyxosis than the in-situ groups.

Task 6. Determine parasite transmission to fish under different flows.

Investigator: Jerri Bartholomew, OSU

This task comprises two studies, one conducted in the field and one in the
laboratory.

The field experiment was conducted June 2009 near Beaver Creek in the
infectious zone in mainstem Klamath River. Water velocity during the exposure
was measured, water samples taken and later analyzed by QPCR and gills from
a subset of exposed fish sampled. The remaining fish were observed for infection
at the Salmon Disease Lab (OSU). One set of gills from all fish have been
processed by QPCR but the second set is awaiting in situ hybridization.

The laboratory experiment using swim tubes will be conducted summer 2010.

Task 7. Determine the combined effects of Ceratomyxa shasta exposure and
seawater entry (smoltification) on survivorship of outmigrating Chinook
salmon.

Investigator: Kristen Arkush, UC-Davis

This study requires Chinook that are nearing seawater competency. Fish will be
acquired in late July/early August, 2010, exposed, then trucked to Bodega
Marine Lab for the seawater acclimation period.

Task 8. Determine whether polychaete genetics play a role in supporting the
different parasite strains.

Investigator: Jerri Bartholomew, OSU

Primers and PCR conditions used in earlier Manayunkia studies to amplify the
CO1 gene proved unsuitable for the M. speciosa specimens so we designed and
assessed new combinations. Sixty-four polychaetes have now been sequenced
from sites below and above Iron Gate Dam. All worms collected from the
Klamath River considered morphologically to be M. speciosa differed by 2-3% in
their CO1 gene (41 variable loci over 550 bp) and thus were confirmed to be to
be a single species. The Klamath River worms differed from other Manayunkia
(Russian) species by 15-20%. Genetic relationships were further analyzed using
maximum parsimony and Bayesian phylogenetic methods. Worms from below the dam differed genetically from those above.

We need to acquire more infected worms to include in the genetic comparison with uninfected worms to determine if there is any correlation between infection with *C. shasta* and host worm genotype.

**Objective 4.** Dissemination of information.

**Task 9. A public website will be developed to make current data on infection of fish and parasite densities in water samples available to the fish disease workgroup and interested stakeholders.**

*Investigator:* Jerri Bartholomew and Sascha Hallett, OSU

Following investigator participation at 4 x 2 hour Drupal workshops at OSU, a public website went live in August 2009: [http://microbiology.science.oregonstate.edu/Klamath_River_salmon](http://microbiology.science.oregonstate.edu/Klamath_River_salmon)

Colleagues and known interested parties were notified by email when the site was activated and comments/input were invited.

The site provides an overview of disease reduction in Klamath River salmon, including the problem, goals of potential management actions, impacts, collaborators and funding. There are links to current research (monitoring studies, infectious zone study, Bogus Creek carcass removal study) (which include overviews and reports), the Bartholomew Laboratory, field trips, meetings, news items and website links and journal articles.