LONG-TERM FISH DISEASE MONITORING PROGRAM IN THE LOWER KLAMATH RIVER

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Objective 1:
Task 1.1 SELECTION OF INDEX SITES
Two different index sites, Klamathon Bridge (Klamathon River Country Estates) and Young’s Bar, were substituted for R-Ranch and Tully Creek, in 2009.

Task 1.2 SENTINEL FISH EXPOSURES
Sentinel exposures in 2008 were conducted following the same protocol used in previous years to determine:

1. How infection levels this year compare with levels in previous years.
2. If the distribution of the parasite has changed.
3. The relative susceptibility of Klamath River Chinook and coho salmon
4. The effects of temperature on disease progress in Chinook, coho and steelhead
5. The relationship between parasite numbers measured in water samples and biological effects in the different fish species.
Methods:
Exposures were conducted May 13-16, June 17-20 and September 16-19, 2008 for ~72 hrs at locations in the upper and lower river (Figure 1). Exposure sites were those used in previous study years with the following substitutions:
- R-Ranch was replaced with Klamath River Country Estates (Klamathon)
- Tully Creek was replaced with Young’s Bar

Rainbow trout and fall Chinook salmon (IGH stock) were held at all sites; coho salmon (IGH stock) were held only at sites below IGD. After exposure, each group of fish was brought to the Salmon Disease Laboratory (SDL), Corvallis, Oregon, held in well water at water temperatures similar to river temperatures occurring during the 72 hr exposure and observed for loss and disease signs for 90 or more days. The fish were given preventative treatments for external parasites and columnaris disease. In May the sentinel fish were approximately 1-2 g in size, June 2-7 g and September 10-15 g. The post-exposure rearing temperature used in May was 16°C for one month, then increased to 18°C when the June exposure groups were brought to the laboratory. After the September groups were exposed in the river they were held in tanks at 18°C. These temperatures were lower than in 2007 during the same period. All fish that died were evaluated for infection by microscopic examination of a sample from the lower intestine for the presence of myxospores. Additionally, PCR testing was done on those fish that were negative by microscope and also the survivors at termination to get a more complete prevalence of this parasite.

To study the effect of water temperature on C. shasta infections in the Chinook and coho juveniles, during the May and September exposures in the Klamath River near Beaver Creek, 80 fish of each stock were exposed and then each divided into two tanks upon return to the SDL, one receiving water at 13°C and the other at 16 (May) or 18°C (September). A second experiment was included for the June 17-20 exposure near Beaver Creek to look at the effect of 4 water temperatures during the post-exposure holding of 5 stocks of fish at the SDL. Unfortunately, during the first 6 days all groups were initially maintained at 13°C due to a failure of the temperature control equipment at the laboratory. Subsequently, groups were adjusted to each of 4 temperatures including 13, 15, 18 and 21 °C. Replicate tanks of the Chinook and coho were held at each temperature and for both steelhead stocks at 18 and 21°C. Fish were observed as with the previous tests for 90 + days.

Results & Discussion:
Average water temperatures during the 72 hr exposures are shown in Table 1. For June, the water temperature was about 1-2 °C cooler in 2008 than when the exposures were done near Beaver Creek in June 2007. In September, the lower Williamson River had cooled much more than the sites below Iron Gate Dam.

Results of the sentinel exposures in May, June and September are listed in Table 2 and Figures 2-4. The values of percent loss represent fish that had died and were found to have spores of C. shasta or were PCR positive for this parasite.
After the May exposure, greater than 90% of the known susceptible rainbow trout stock from Roaring River Hatchery, Scio, OR died at all locations except for those exposed above HC Boyle Dam at Keno Eddy. The Iron Gate Hatchery (IGH) fall Chinook were affected substantially above Beaver Creek and Seiad Valley. The coho were affected at all tested sites below Iron Gate Dam but most severely above Beaver Creek and Seiad Valley. As in previous years, the Iron Gate Chinook did not suffer loss at the two test sites in Oregon in the lower Williamson River and at Keno Eddy. In contrast, the rainbow died at 97.4% with *C. shasta* infection in the Williamson River.

In June, high loss from *C. shasta* occurred in rainbow trout from all locations except Keno Eddy. Very high loss, much more than seen in previous years, occurred in the IGH fall Chinook and coho held above Beaver Creek and Seiad Valley.

In September, once again the rainbow trout died at a high rate due to *C. shasta* at all sites except Keno Eddy. Much lower loss of the IGH fall Chinook occurred above Beaver Creek and Seiad Valley compared to the May and June exposures. But the IGH coho loss remained high near Beaver Creek and Seiad Valley and about 25% at above and below what is considered the “hot zone” for *C. shasta*.

**TABLE 1.** Average Klamath River water temperatures (°C) at sentinel sites during the 72-hour fish exposures in 2008.

<table>
<thead>
<tr>
<th>Site</th>
<th>May 13-16</th>
<th>June 17-20</th>
<th>September 16-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Williamson River</td>
<td>14.5</td>
<td>17.1</td>
<td>13.3</td>
</tr>
<tr>
<td>Keno Eddy</td>
<td>15.7</td>
<td>19.5</td>
<td>18.6</td>
</tr>
<tr>
<td>Klamathon</td>
<td>15.3</td>
<td>18.2</td>
<td>18.7</td>
</tr>
<tr>
<td>Near Beaver Creek</td>
<td>16.0</td>
<td>19.1</td>
<td>18.9</td>
</tr>
<tr>
<td>Seiad Valley</td>
<td>14.1</td>
<td>18.4</td>
<td>19.7</td>
</tr>
<tr>
<td>Orleans</td>
<td>12.2</td>
<td>17.6</td>
<td>19.7</td>
</tr>
<tr>
<td>Young’s Bar</td>
<td>Not done</td>
<td>17.2</td>
<td>Not done</td>
</tr>
</tbody>
</table>

**TABLE 2.** Per cent loss of fish found to be infected with *Ceratomyxa shasta* after exposure at various Klamath River sentinel sites for 72 hours in May, June and September 2008 and subsequently held for 90 or more days at the Salmon Disease Laboratory in a 16-18°C water supply. ND = no fish were exposed. RbT = rainbow trout, Chk = Chinook salmon.
FIGURE 2. Percent mortality with *C. shasta* infection of susceptible rainbow trout and Iron Gate Hatchery Chinook and coho salmon held at exposures sites in the Klamath River in May 2008. Coho salmon were not held at the Williamson River and Keno sites in the upper basin.

![May 2008 mortality chart](image)

FIGURE 3. Percent mortality of susceptible rainbow trout and Iron Gate Hatchery Chinook and coho salmon held at exposures sites in the Klamath River in June 2008. Coho salmon were not held at the Williamson River and Keno sites in the upper basin.

![June mortality chart](image)
FIGURE 4. Percent mortality of susceptible rainbow trout and Iron Gate Hatchery Chinook and coho salmon held at exposures sites in the Klamath River in September 2008. Coho salmon were not held at the Williamson River and Keno sites in the upper basin.

Summary of the May, June and September sentinel results:

- The hot zone for severe infections of ceratomyxosis occurred in the Klamath River below Iron Gate Dam near Beaver Creek. This is in agreement with previous year’s results, showing that the infectious area is stable.
- In both May and June, fall Chinook juveniles held at Beaver Creek suffered ~85-92% loss with *C. shasta* infection after 72 h exposure in the river and then held at 16-18°C; this is significantly higher than in previous years. In September, the Chinook loss was much lower (12.8%).
- Mortality with *C. shasta* infection in coho salmon held near Beaver Creek during May and June was 68 and 84%; also in September the coho loss with *C. shasta* was 71.4%, this is significantly higher than in previous years.
- Losses in fall Chinook and coho salmon were lower downstream of Seiad Valley, as in previous years but losses with *C. shasta* infection were still above 25% during in September above the “hot zone” at Klamathon and below at Orleans.
- As in previous years, non-native rainbow trout had high loss at all sites except for Keno Eddy (above JC Boyle Dam).
- Also, Iron Gate Hatchery fall Chinook did not suffer loss from *C. shasta* in the lower Williamson River despite the high mortality in rainbow trout.
Effect of post exposure rearing water temperature on infections of *C.shasta* in Klamath River fish stocks.

Results of the comparison of percent loss of Chinook and coho with *C.shasta* infection when exposed near Beaver Creek in May and September and held at the laboratory at two different water temperatures are shown in Table 3. The known susceptible rainbow trout stock consistently suffered high loss even at the lower temperature of 13°C. For the Chinook and coho salmon, the loss was high at both temperatures but was higher in those fish held at the greatest water temperature. In September, the coho exposed near Beaver Creek and held at 18°C suffered a severe *Flavobacterium columnare* infection after being brought to the SDL. About 40% were lost to this disease. For those surviving the columnaris disease and kept at 18°C, 71.4% died and were found to be infected with *C. shasta*. The portion of coho held at 13°C had only an 8.6% loss. The general trend appears to be that the warmer the water temperature after exposure the higher the loss in Chinook and coho with *C. shasta* infections.

**TABLE 3.** Effect of water temperature during post-exposure holding at the Salmon Disease Laboratory on percent loss of fish with *Ceratomyxa shasta* infections incurred during the May and September 72 hour exposure of fish in the Klamath River near Beaver Creek. p.e. = post exposure, IGH = Iron Gate Hatchery.

<table>
<thead>
<tr>
<th>Exposure Month</th>
<th>Water temp. during p.e. rearing (°C)</th>
<th>Rainbow Trout</th>
<th>IGH fall Chinook salmon</th>
<th>IGH coho salmon</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>13</td>
<td>100</td>
<td>75.0</td>
<td>49.0</td>
</tr>
<tr>
<td></td>
<td>16-18</td>
<td>100</td>
<td>84.6</td>
<td>68.3</td>
</tr>
<tr>
<td>September</td>
<td>13</td>
<td>97.0</td>
<td>2.4</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>93.9</td>
<td>12.8</td>
<td>71.4</td>
</tr>
</tbody>
</table>

Results of the study to look at effect of four post-exposure rearing water temperatures after fish were exposed near Beaver Creek on June 17-20, 2008 are shown in Figure 5. Rainbow trout at all temperatures suffered nearly 100% loss to *C. shasta*. The highest loss of IGH fall Chinook and coho occurred at 18 and 21°C but was quite high even at 13 and 15°C. Irrespective of fish species, the higher the water temperature, the greater the percent loss of fish with *C. shasta* infection. Data on the mean day to death are shown in Table 4. Chinook had a shorter time to death than any of the other stocks. This was the highest loss of IGH fall Chinook juveniles that we have observed in a three day exposure at the site above Beaver Creek during the last few years. The IGH coho died at a high rate at all temperatures but both steelhead stocks had the greatest resistance. At both 18 and 21°C, the Trinity River Hatchery steelhead appeared to have a slightly higher percent loss.
FIGURE 5. Effect of post-exposure water temperature rearing on *Ceratomyxa shasta* infections in stocks of fish exposed near Beaver Creek on June 17-20, 2008.

Table 4. Effect of water temperature on the mean day to death during post-exposure holding at the Salmon Disease Laboratory for stocks of fish held for 72 hr in the Klamath River near Beaver Creek during June 17-20, 2008. IGH = Iron Gate Hatchery, TRH = Trinity River Hatchery.

<table>
<thead>
<tr>
<th>Water Temp. (°C)</th>
<th>Rainbow Trout</th>
<th>IGH fall chinook salmon</th>
<th>IGH coho salmon</th>
<th>IGH steelhead trout</th>
<th>TRH steelhead</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>32.7</td>
<td>30.6</td>
<td>34.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>30.5</td>
<td>24.1</td>
<td>26.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>18</td>
<td>24.5</td>
<td>19.3</td>
<td>25.2</td>
<td>40.0</td>
<td>35.0</td>
</tr>
<tr>
<td>21</td>
<td>18.2</td>
<td>16.4</td>
<td>17.5</td>
<td>35.0</td>
<td>34.2</td>
</tr>
</tbody>
</table>

Summary of water temperature results:
- IGH fall Chinook and coho suffered very high loss at all four temperatures tested. The 72 h exposure in the Klamath River during June 17-20 demonstrated a severe challenge level at that time. The IGH fall Chinook had the shortest mean day to death at each temperature.
- The total loss for each species in the post-exposure rearing of the fish was not greatly reduced by the lower water temperature of 13°C. Also, all groups were held at 13°C for 6
days after exposure due to equipment failure but even with this cool water exposure prior to being elevated for the remaining holding at 21, 18 or 15°C, losses from *C. shasta* were severe.

- Both the IGH and TRH steelhead juveniles were more resistant to *C. shasta* infection, which may indicate a somewhat lower resistance for this stock. At 21°C, loss in the TRH steelhead was 13.6% compared to 4.0% for IGH steelhead.
- 98-100% of the susceptible rainbow trout died with *C. shasta* infections at all water temperatures.

**Task 1.3 WATER SAMPLE COLLECTION AND PARASITE DENSITY DETERMINATION**

Water samples were taken to determine:

1. The spatial and temporal distribution of *Ceratomyxa shasta* in the Klamath River.
2. How abundance this year compares with levels in previous years.
3. If the distribution of the parasite has changed.
4. The relationship between parasite numbers measured in water samples and biological effects in the different fish species.

**Methods:**

Sampling efforts were increased this year and over 1000 water samples were collected over a 6 month period from March 30–Sept 19. At one site, Orleans, sampling continued through the fall and winter to generate one full year’s data. Sampling occurred at 5 mainstem sites: Klamathon Bridge (KKB)(note that this was selected as alternate site to R-Ranch which was inaccessible this year), Orleans (KOR), Beaver Creek (KBC), Seiad Valley (KSV) and Young’s Bar (YB) (note that this was an alternate site 5 miles downstream to Tully Ck as this was not accessible this year); and at 5 tributaries: Shasta (SHR), Salmon (SMN), Scott (SCT), and Trinity (TRN)(see Figure 1 for site locations).

This year, 4 automatic water samplers, ISCOs, were set up at 4 of the 5 mainstem sites (all except Tully Ck). These were programmed to collect 1L from the river every 2 hours for 24 hours twice per week (starting 8am Sunday and Wednesday). The total sample (12L) was then mixed manually and 4 x 1L samples taken, filtered and frozen. Manual sampling occurred at the remaining sites once a week, every other week. For this, 4 x 1L grab samples were taken from the river at one time point, filtered and frozen.

Water samples were also taken at sentinel fish sites during the exposures in May, June and September. 4 x 1L samples were taken at the start and end of exposure and at Beaver Creek they taken throughout exposure period (24 h intervals) using the ISCO.

DNA was extracted from 3 of the 4 frozen 1L samples collected at each site. An inhibition test (combined with *Parvicapsula minibicornis*) was performed on one sample from each site then all three samples were assessed for presence of *C. shasta*. Each sample was run in duplicate and sample pairs with values having a standard deviation greater than 1 were rerun. Positive and negative controls were included in each QPCR run. Samples that were undetected were assigned
a Ct value of 42 and included in the average. Each data point on a graph represents the average of the 3 water samplings at that time point; error bars display the standard deviation.

**Results & Discussion:**

*Ceratomyxa shasta* was absent or present in very low quantities (less than 1 spore/L) in all water samples collected in March (*Figure 6*), but became present (greater than 1 spore/L) at all mainstem sites, except Klamathon, in April. All mainstem sites were positive for the parasite in May. Levels peaked in June (Figure 1) at over 100 spore/L, then decreased in July and August, but increased again in September.

In wild Chinook salmon, the USFWS found a higher prevalence of infection, intensity of infection and shorter mean day to death in fish sampled in June compared to May (Fogerty, pers comm.). This is consistent with levels of parasite detected in water samples. However, in mixed origin Chinook, the peak in infection levels was detected in May but the decrease the following sampling was likely the result of the influx of newly released uninfected hatchery fish (Nichols, USFWS, pers comm.).

*FIGURE 6. Abundance of Ceratomyxa shasta in water samples in March (lowest abundance) and June (highest abundance) 2008 at various mainstem and tributary sites. Sites are displayed from west to east. Each data point on a graph represents the average of the 3 water samples at that time point. The dotted line represents approximately one spore per liter.*
Beaver Creek remained the ‘hottest’ spot from which samples were collected, with levels of over 100 spores per liter recorded in June (Figure 7). Above Beaver Creek, levels were low (around 1 spore per liter), and below Beaver Creek abundance decreased westward. This is likely a combination of dilution from the tributaries and disintegration of the fragile actinospore stage. Only small quantities of parasite (less than 1 spore per liter) were ever detected in the tributaries. The most was in the Trinity River and this was the only tributary in which *C. shasta* infected fish were found (2/158; Nichols, USFWS, pers comm.).

FIGURE 7. Abundance of *Ceratomyxa shasta* in water samples from Beaver Creek collected March through September 2008. Each data point on a graph represents the average of the 3 water samples at that time point; error bars display the standard deviation. The dotted line represents approximately one spore per liter.
This was the first year samples were collected all year round at one mainstem location, Orleans. Previously, sampling stopped while the parasite was still present (Sept) and it was unknown if the parasite persisted throughout the year. At Orleans, the pattern of abundance was similar to that recorded at Beaver Creek, although levels were lower overall (Figure 8). After a peak in September (around 10 spores/L), parasite abundance then dropped to less than 1 spore/L and no spores were detected by the end of November.

FIGURE 8. Abundance of *Ceratomyxa shasta* in water samples from Orleans collected March through November 2008. Each data point on a graph represents the average of the 3 water samples at that time point; error bars display the standard deviation. The dotted line represents approximately one spore per liter.

Mortalities attributable to *C. shasta* in sentinel Chinook salmon occurred only in groups exposed at Beaver Creek and Seaid Valley; these were 2 of the 3 sites at which the parasite was most abundant (Figure 9). No mortalities occurred in Chinook exposed in the Williamson River, despite equally high levels of parasite. This supports previous indications for the presence of 2 different strains of the parasite in the lower and upper Klamath River that differ in host preference. Parasite levels at Orleans were insufficient to cause mortality in Chinook but were just below those that did induce mortality in fish exposed elsewhere. This indicates that the threshold for mortality in Chinook salmon is approximately 10 spores/L; this threshold was the same as last year. Although less than 10 spores/L is insufficient to cause mortality in Chinook, levels less than 1 spore/L proved lethal for susceptible non-native rainbow trout (at all sites except Keno Eddy) and spore abundance in water was proportional to mean day to death in this fish. At Keno Eddy, *C. shasta* was present in water samples at 1 spore/L, however no mortalities occurred in fish exposed here, including the usually susceptible rainbow trout. This suggests a different strain of the parasite is present at this location.

FIGURE 9. Abundance of *Ceratomyxa shasta* in water samples collected during sentinel fish exposures in May, June and September 2008. Each data point on a graph represents the average of the 3 water samples at that time point. The dotted line represents approximately one spore per liter.
The distribution of the parasite has remained similar between years, only the relative abundance between years has fluctuated. With the exception of Klamathon Bridge, parasite abundance in water was higher in 2008 than 2007, consistent with mortalities observed in sentinel Chinook (Figure 10).


Task 1.4 POLYCHAETE ABUNDANCE AND INFECTION PREVALENCE
Polychaete samples were collected at three sites: Up river from the I-5 Bridge ~.5 Rkm, at the Tree of Heaven campground, and down river from the Seiad Creek confluence (~5 Rkm). Three
separate samples were collected at each site in May, June and September, using a Hess sampler. The samples have been preserved in alcohol and will be examined for population density, prevalence of infection and qualitative demographics.

**Task 1.5 PROJECT COORDINATION**

In December, 2007, a meeting was held in Corvallis, OR with members of the OSU and USFWS laboratories and Yurok tribe; Karuk tribe representatives joined us via conference call. Attendees presented their current research and discussion of protocols and further research questions ensued as well as the selection of sites for sentinel exposures and water sampling.

**Objective 2.**

**Task 2.1 IDENTIFICATION OF CRITICAL UNCERTAINTIES IN OUR UNDERSTANDING OF PARASITE TRANSMISSION**

While there is a solid baseline of data for *C. shasta* infections in the Klamath, there are still some vital gaps that need to be filled. One such gap is the transmission efficiency of the actinospore stage to the salmonid host. Adam Ray plans to conduct a study this summer that will provide information for this parameter. If the transmission efficiency appears to be significant to the perpetuation of this disease; then future studies will be conducted to examine the importance of environmental factors on the transmission of actinospores. Another area where more information is needed is a more intensive survey of returning adult salmon and their potential contributions of myxospores to the river. Finally, information about the demographic makeup of the polychaete populations could be used in a leslie matrix to identify the most fragile stage in the life history. Biologists can then focus management strategies to target those delicate stages.

**Task 2.2 MODELING DISEASE TRANSMISSION IN THE KLAMATH RIVER**

Adam Ray, under the supervision of Dr. Phillippe Rossignol, has developed a preliminary mathematical model of disease transmission to examine the basic host-parasite interactions necessary for *C. shasta* to complete its life cycle. The model focuses on the transmission of the actinospore stage to the fish and subsequent propagation of the parasite (*Figure 11*). From this model, we will be able to determine a basic reproductive number ($R_0$) for ceratomyxosis and also determine which parameters may be the most important in disease transmission and therefore would be targets for interrupting the parasite life cycle. This model will help organize data that has been collected, identify data gaps, and provide insight as to which parameters are susceptible to management actions that may reduce the impact of this disease on the out-migrating juvenile salmonids of the Klamath River.

*FIGURE 11: Epidemiological model of ceratomyxosis developed for the Klamath River. The grayed-in areas are parameters that estimates were obtained for in the summer of 2008.*
Methods:
In summer 2008, field exposures were conducted in the Klamath River using Chinook salmon to determine parasite infectious dose and resulting spore production. These experiments were designed to provide data which can be inserted into the epidemiological model designed for ceratomyxosis. In June and September, IGH Fall Chinook were exposed to the main stem Klamath River, just up river from the confluence of Beaver Creek, for varying lengths of time. In both months, Chinook were exposed for 72, 48 and 24 hours, and an additional 16 hour exposure in September. The water velocity was recorded for each of the eight live cages, every two hours to estimate the total volume of water experienced by the fish. An ISCO water sampler collected daily water samples (1 liter/2 hours) for QPCR analysis to quantify the actinospore dose encountered by the Chinook. After the exposure periods, fish were transferred to the SDL and monitored for signs of infection, for up to 90 days post exposure. Sick and moribund fish were visually examined for the myxospore stage. If visually positive the intestine and kidney were excised, homogenized, and a sub-sample was placed on a hemocytometer to enumerate the myxospores produced. If visually negative, a piece of intestinal tissue was collected and assayed by PCR. Water samples, at the SDL, were collected from four aquaria that contained Chinook exposed for 72 hours. These samples will be analyzed by QPCR to determine if and when infected juvenile Chinook begin to release myxospore back into the water column.

Results:
Chinook mortality increased relative to the period of exposure in the Klamath River (Figure 12). Exposures in June resulted in 100% mortality with exposure doses exceeding 140 million spores;
in September mortality was 37% (Figure 12). Myxospore production by infected Chinook was inversely proportional to the duration of their exposure (Figure 13).

FIGURE 12- Percent mortality and approximate dose of *C. shasta* experienced by IGH Chinook exposed at Beaver Creek for 72, 48, 24, and 16 (Sept. only) hours.

FIGURE 13. Production of myxospores from infected IGH Chinook exposed for 72, 48, 24, and 16 (Sept only) hours.

Acknowledgements: The Karuk and Yurok tribes assisted with water sample collection.