Bureau of Reclamation

Long-Term Fish Disease Monitoring Program In The Lower Klamath River

FY09

Objective 4. **PROVIDE FINER RESOLUTION OF THE INFECTIOUS ZONE IN THE LOWER KLAMATH RIVER**

Collaborators: Yurok Tribe, Karuk Tribe

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BACKGROUND

Ceratomyxosis is a leading disease of Klamath River salmonids, causing mortality in outmigrating juvenile Chinook and coho salmon. Sentinel fish studies conducted at multiple time points over several years have consistently identified the area immediately above Beaver Creek (Rkm 259) as highly infectious, but with mortality in Chinook salmon occurring as far downriver as Seiad Valley (Rkm 208) (Stocking et al. 2006). This pattern is supported by river water sampling, which targets waterborne *Ceratomyxa shasta* spores (Hallett & Bartholomew 2006). However, data from surveys of the parasite's polychaete host, suggest this highly infectious zone may extend upriver to around the confluence of the Shasta River (Rkm 280; itself not a source of the infectious stage of the parasite)(Stocking & Bartholomew 2006). Characterization of the infectious zone is coarse, with consistent data available only from four index sites in the lower river, below Iron Gate Dam. This study aims to more accurately define this zone using developed water sampling and molecular methods (Hallett & Bartholomew 2006). Replicate water samples were collected on three days spanning a six week period at 16 sites along 79 Rkm of the lower Klamath River; a further 6 sites and 76 Rkm were included on the final day. This data produces a longitudinal map of parasite density in this section of the river for each time period which can be used to identify areas that could be targeted for control of polychaete populations.

METHODS

During spring/early summer 2009, water samples were collected in a longitudinal river transect, at 16 approximately equidistant sites, from Seiad Valley to east of the I5 bridge (**Fig. 1**). Sampling occurred on three days, three weeks apart: May 13 (A), June 3 (B) and June 24 (C). Each day, 3 x 1L water samples were collected from each site (exception: only 1 sample was collected at each time point at Rkm 231 and 234 on June 24), three times at three hour intervals. Every other site was sampled simultaneously: either at 9am, 12 noon and 3pm or at 10am, 1pm and 4pm. Samples (total of 144 per day) were chilled during transportation to the Salmon Disease Laboratory, OSU, where they were filtered within 24 hours. On June 24, an additional six downstream sites were included in the study. These were sampled (3 x 1L) once in the morning and once in the afternoon.

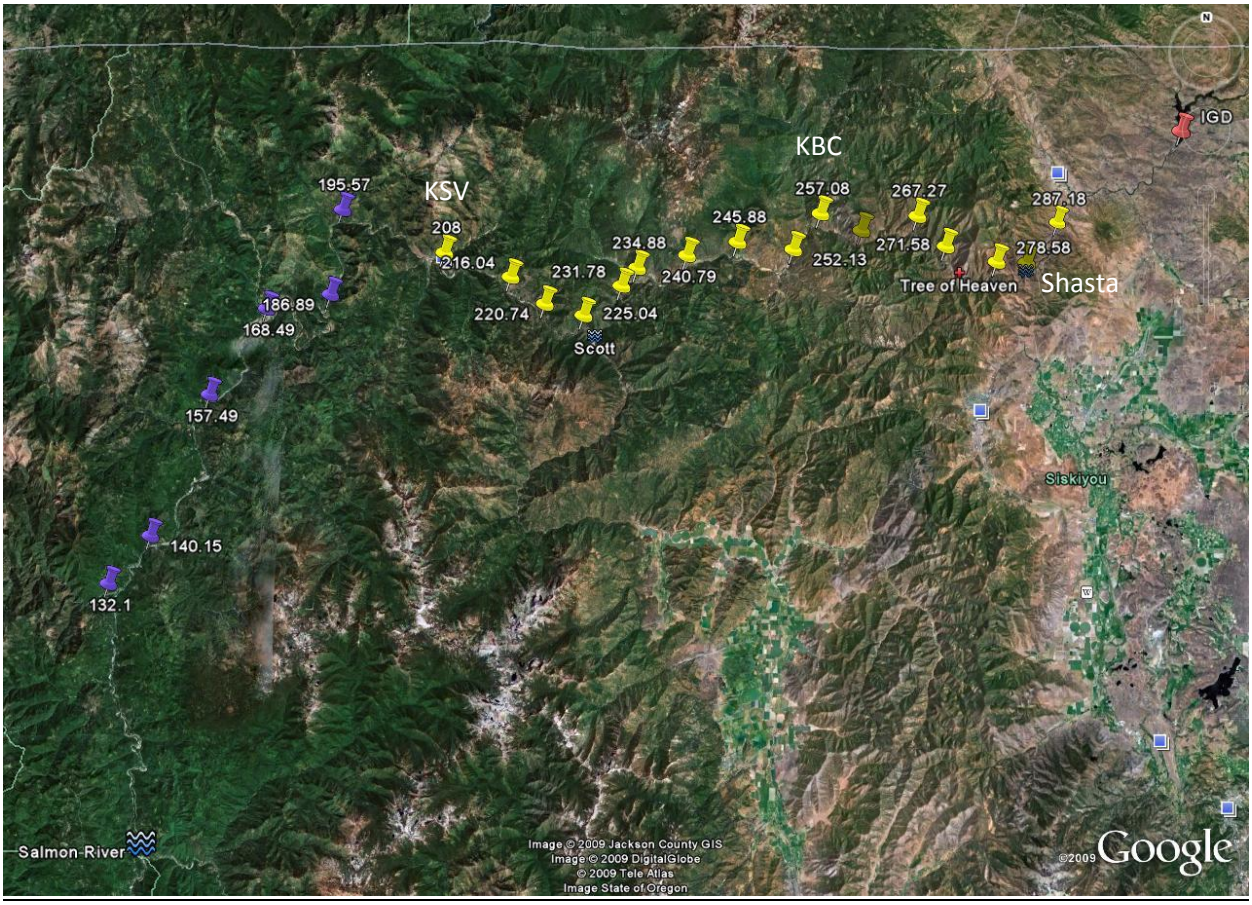

Figure 1. Overview of water sampling sites on the lower Klamath River. The 16 sites from Rkm 208-287 (yellow pins) were sampled on all three dates, May 13, June 3 and June 24, whereas the 6 from Rkm 132-195 (blue pins) were included only on the third sampling day. The Klamath River flows westward into the Pacific Ocean.

In the laboratory, each water sample was filtered through a nitrocellulose membrane using a vacuum pump, the filter paper folded into a microfuge tube and frozen. The filter paper was dissolved in a series of acetone/ethanol washes, removed, and the remaining DNA extracted using a kit (QIAGEN DNeasy Blood and Tissue). Any *Ceratomyxa shasta* DNA present was quantified by a TaqMan PCR assay on a 7300 platform (Applied Biosystems)(Hallett and Bartholomew 2006).

Reference samples with known numbers of *Ceratomyxa shasta* spores were processed in the same manner as the field samples. Positive (tissue) and negative (water) controls were included on each plate. All samples were run in duplicate (i.e. two wells each). Data generated by the SDS software (Applied Biosystems) was exported into Excel where averages and standard deviations were calculated. Any sample whose two wells differed by greater than 1 standard deviation was rerun.

RESULTS and DISCUSSION

Parasite abundance on May 13, June 3 and June 24 is displayed in **Figs 2-4**. All water samples contained *Ceratomyxa shasta* DNA. Levels varied from >1 - >100 spores/L and overall site averages were all >10

spores/L (**Fig. 5, 6**). Daily abundance was consistent at some sites (<1Ct) whereas it fluctuated over 10-fold (>4Ct) at other sites.

Parasite abundance was unusually high in May and decreased at the beginning of June, notably at KBC. Discharge rates from hydrographs don't explain the data, but there was a 9C increase in temperature between sample days. At the end of June the overall pattern had changed and spore abundances were higher at some sites but lower at others.

There was no consistent pattern in abundance between days. Levels increase for about 20 Rkm downstream of the Shasta River confluence to peak east of KBC, thus providing some basis for defining an upstream limit of the infectious zone. On the first two collection days, levels were clearly lower downstream of the confluence of the Scott River, suggesting this as a downstream limit; however, on the third day levels increased markedly both above and below these locations. These levels are more variable between dates than expected, and likely reflect changes in water flow and temperature which affect the polychaete host, release of the parasite from the polycahaete and subsequent survival of the waterborne stage infective to fish. For example, flows from the Scott River (data from Seiad Valley gauge) decreased approximately 1000 cfs over the course of the study and thus contributed less to parasite dilution during the latter sample period.

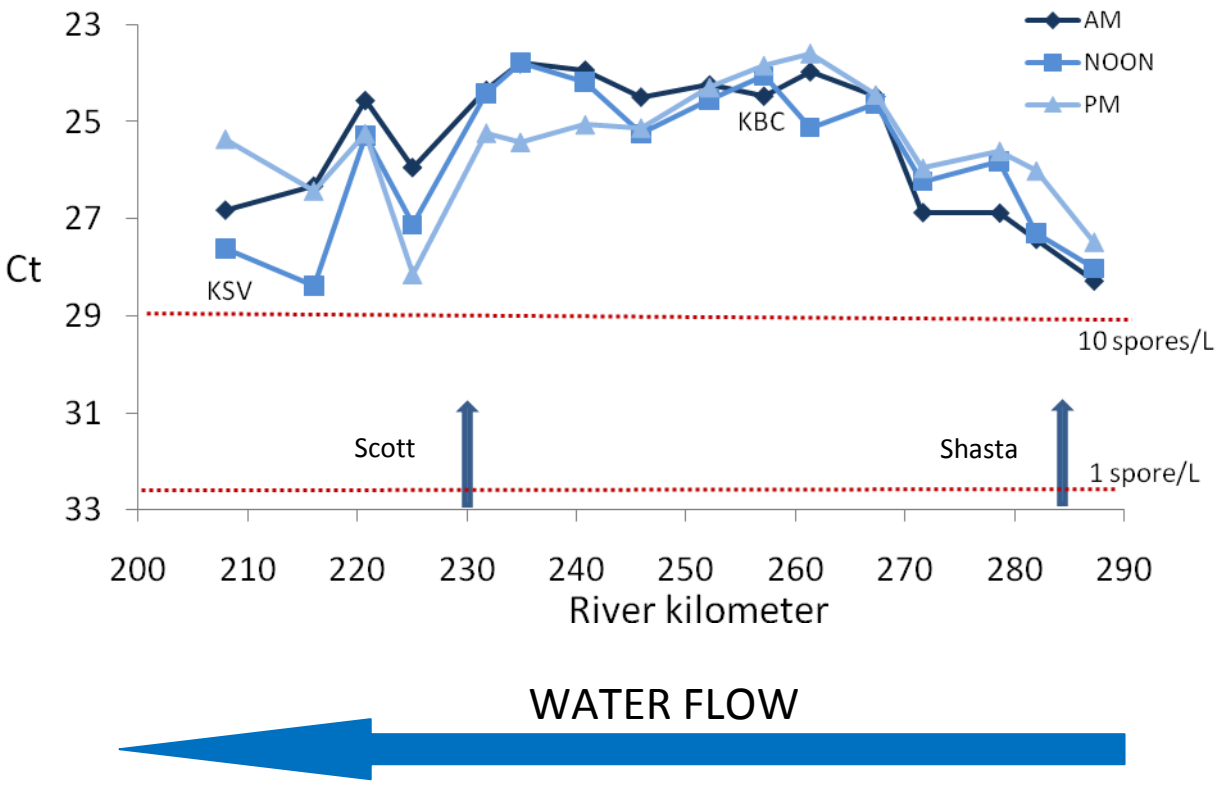

Figure 2. Abundance of *Ceratomyxa shasta* in water samples collected from at 16 lower Klamath River sites. Each data point is the average of 3 x 1L water samples. Samples were taken at 3 time points at

each site, at 9 or 10 am, 12 or 1pm and 3 or 4pm on May 13, 2009 (IZA). The lower the Ct value the more parasite DNA is present. Approx. 3.3 Ct equates to a 10-fold difference in spore numbers.

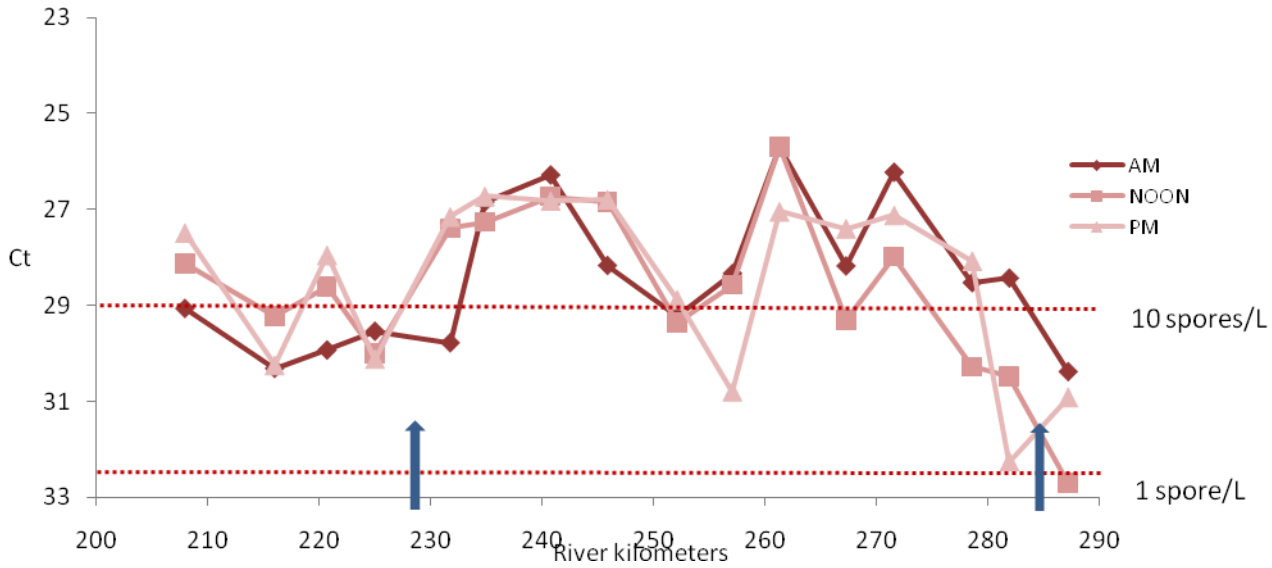

Figure 3. Abundance of *Ceratomyxa shasta* in water samples collected from 16 lower Klamath River sites. Each data point is the average of 3 x 1L water samples. Samples were taken at 3 time points at each site, at 9 or 10 am, 12 or 1pm and 3 or 4pm on June 3, 2009 (IZB). The lower the Ct value the more parasite DNA is present. Approx. 3.3 Ct equates to a 10-fold difference in spore numbers.

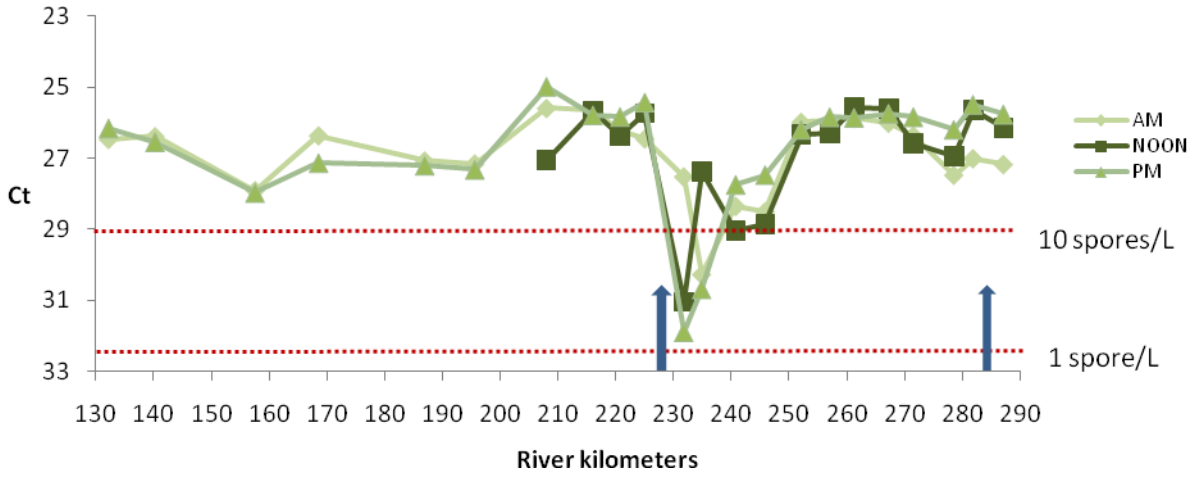

Figure 4. Abundance of *Ceratomyxa shasta* in water samples collected from 16 lower Klamath River sites. Each data point is the average of 3 x 1L water samples. Samples were taken at 3 time points at

each site, at 9 or 10 am, 12 or 1pm and 3 or 4pm on June 24, 2009 (IZC). The lower the Ct value the more parasite DNA is present. Approx. 3.3 Ct equates to a 10-fold difference in spore numbers.

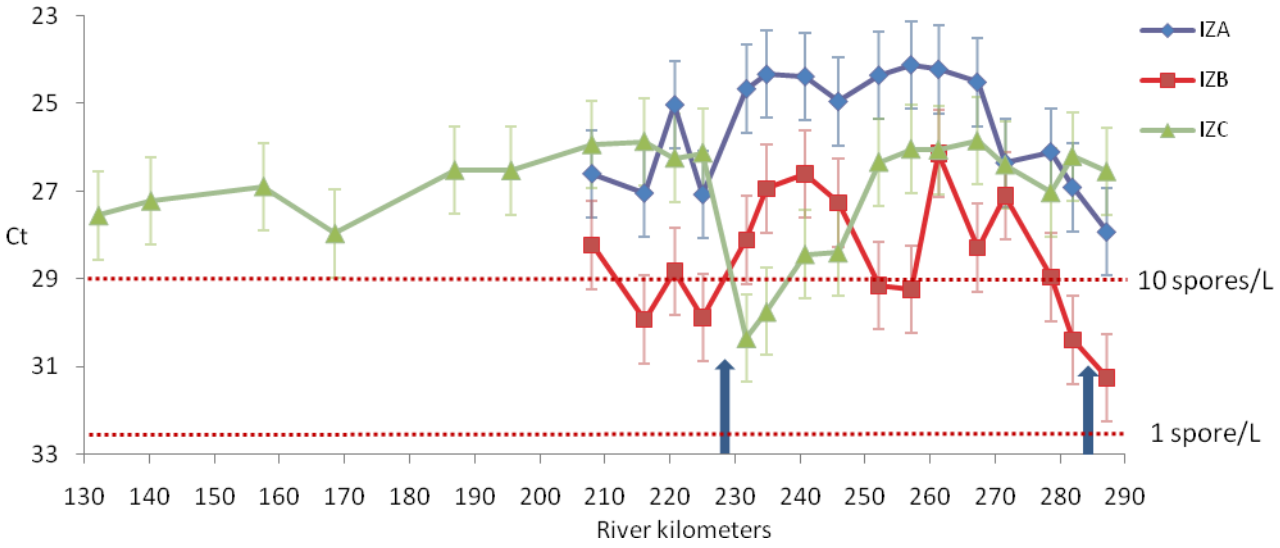

Figure 5. Comparison of abundance of *Ceratomyxa shasta* in water samples collected from 22 lower Klamath River sites on three dates, three weeks apart: May 24 (IZA), June 3 (IZB) and June 24, 2009 (IZC). Each data point is the average of 6-9 x 1L water samples. Samples were taken at 2 time points at the lower 6 sites and at 3 time points at the upper 16 sites. The lower the Ct value the more parasite DNA is present. Approx. 3.3 Ct equates to a 10-fold difference in spore numbers.

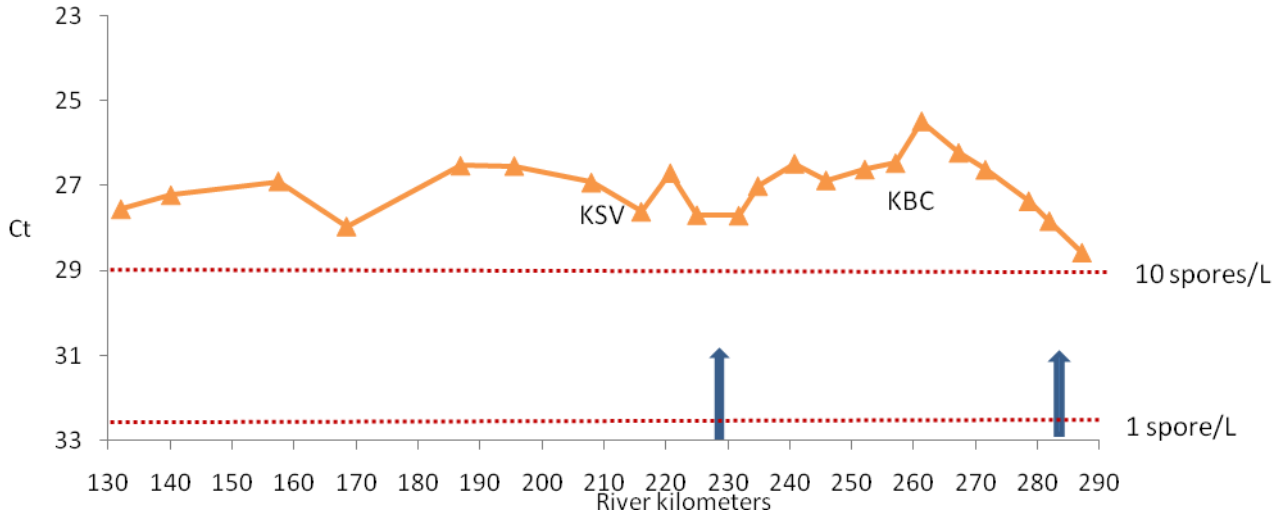

Figure 6. Overall abundance of *Ceratomyxa shasta* in water samples collected from 22 lower Klamath River sites on three dates, three weeks apart: May 24 (IZA), June 3 (IZB) and June 24, 2009 (IZC). Each data point is the average of 6-27 x 1L water samples. The lower the Ct value the more parasite DNA is present. Approx. 3.3 Ct equates to a 10-fold difference in spore numbers. KSV=Seiad Valley index site, KBC = Beaver Creek index site.

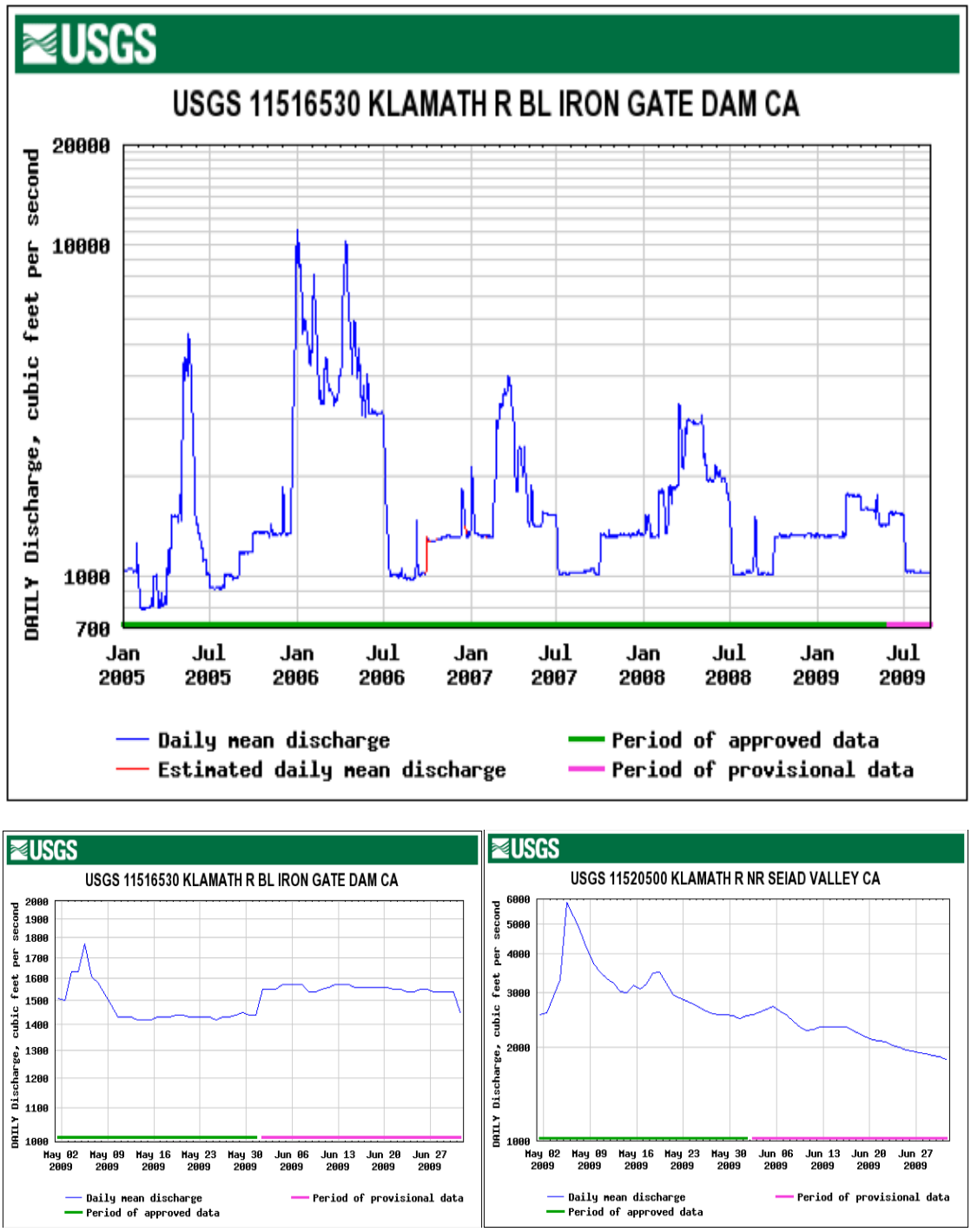

Figure 7. Hydrographs of daily mean discharges from Iron Gate Dam from 2005 – 2009 (upper), Iron Gate Dam May-June 2009 (lower left) and Seiad Valley May-June 2009 (lower right).

This study characterizes spore abundance in the water column, it does not identify the source of the spores ie where the infected polychaetes are located. Decreases in spore abundance e.g. as progress

downstream towards Scott River may be caused by fewer spores being released in this region, or spores being contributed upstream are degrading as they disperse downstream due to high water temperature.

Also, note that water flows have decreased over the past four years (Fig. 7). This may have allowed accumulation of materials suitable for polychaete habitat and conducive to polychaete and parasite propagation.

Acknowledgements

Jill Pridgeon (OSU) helped filter water samples. Steve Christy (OSU) assisted with extraction of water samples. Matt Stinson and Michelle Jordan helped prepare the site map. Hydrograph obtained from http://waterdata.usgs.gov/nwis/.