Shasta River Polychaete Survey 2008

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Introduction

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* (Figure 1), as one of their obligate hosts (Bartholomew et al. 1997) with salmonid fishes as the other host (Bartholomew et al. 1989) (Figure 2). Patchy but dense populations of these polychaetes have been indentified within the mainstem Klamath River, particularly between Iron Gate Dam and the Scott River confluence. However, polychaete worms are either absent or of unknown status in the Klamath River's tributaries (Hendrickson et al. 1989; Stocking et al. 2006; Stocking and Bartholomew 2007). Of the Klamath River's tributaries, the Shasta River is perhaps the most likely to possess populations of polychaetes because it has most of the same attributes that favor polychaetes in the mainstem Klamath River. These attributes include high nutrients loads, suitable physical habitat for polychaetes, a relatively stable flow regime with minimal winter flooding and year round flow, and adult salmonids that are likely infected and could therefore deliver myxospores to infect any polychaetes present. Yet prior to this study, the only polychaetes documented in the Shasta River was one individual located in the first pool above its confluence with the Klamath River during a relatively limited survey (K. Cummins, Humboldt State University, personal comm.).

The objective of this study was to conduct a thorough survey to confirm the presence or absence of polychaetes (*M. speciosa*) in the Shasta River, and if present, to determine levels of infection with *C. shasta*. If the Shasta River is indeed not infective for myxozoan pathogens, it is critical to determine if this is because polychaete populations are absent, in extremely low abundance, or are present but uninfected. Determining which scenario is most likely occurring will provide important clues to assist with identify factors that control polychaete distribution and ultimately the geography of myxozoan infectivity among Klamath River salmonids.

Methods

We used three approaches to sample for the presence or absence of polychaetes at multiple locations in the Shasta River during the summer and fall of 2008: 1) gently scrubbing natural substrate and using a fine mesh modified kick net to collect loosened material and invertebrates; 2) deploying artificial substrates designed to favor polychaete colonization; and, 3) visual inspection and scraping of cobble and woody debris. A "sample" consisted of the contents of one artificial substrate or multiple pieces of washed or scraped substrate from an immediate area. This material was combined into a single, pooled sampled. All collected samples were then inspected using dissecting microscopes at 20x magnification to detect and enumerate any polychaetes present.

For the first approach, we gently scrubbed cobbles in a bucket of water or in the current with our hands to dislodge attached material and invertebrates, which were then collected with a modified kick net with 63 micron nylon mesh nylon. Collected material and

invertebrates were then placed into locking plastic bags and preserved with isopropyl alcohol (70%) for later inspection the following week. The second approach consisted of deploying artificial substrates in the Shasta River within habitats that appeared favorable for polychaete colonization. The substrates were deployed for a period of weeks to allow colonization by invertebrates including any potential polychaetes in the vicinity. The artificial substrates consisted of bricks wrapped in nylon window screen, which provides suitable anchoring spaces for filter feeding invertebrates such as polychaetes (Figure 3). These substrates were successfully used to sample polychaetes in the Klamath River previously and concurrently during the summer of 2008. The final approach consisted of floating the Shasta River canyon from near Yreka to its confluence with the Klamath River and sampling cobble and woody debris. Polychaete sampling that we conducted in the mainstem Klamath River in conjunction with the US Fish and Wildlife Service gave us the experience needed to visually inspect natural substrate and determine if any polychaete colonies were present, or likely to be present, based of the appearance of clusters of their tubes and the matted algae, *Cladophora* spp., which provides favorable microhabitat. During our float in the Shasta River canyon, we used 4x magnification hand lens to inspect numerous cobbles and pieces of wood and scraped off any *Cladophora* or material that looked like potential polychaetes tubes. Sampled material was placed into locking plastic bags along with river water to keep any invertebrates and polychaetes alive for subsequent inspection several days later using dissection microscopes.

We inspected all sampled material for the presence or absence of polychaetes using dissecting microscopes at 20x magnification. The inspection process consisted of placing an appropriate amount of the sample onto a square gridded Petri dish. Each sub-sample was then completely inspected using a search pattern that followed the grid lines of the Petri dishes. Fine tweezers were used to move debris or isolated invertebrates for closer inspection. Any look-alike invertebrates were placed into 1.5 mil tubes for a second opinion by another inspector. This process was repeated until all material from each sample was inspected. In the event that any polychaetes were observed in the samples, they would be preserved in ethanol to provide an evidentiary record.

Results

A total of 43 aquatic invertebrate samples were collected at six different study site locations in the Shasta River during the summer and fall of 2008 (Table 1). The six different collection areas were distributed over the course of the Shasta River from Dwinell Dam (i.e. Lake Shastina) to its confluence with the Klamath River (Figures 4 - 7), although a greater proportion of samples came from the Shasta River canyon because of its proximity to known populations of polychaetes in the mainstem Klamath River. Emergent aquatic vegetation (e.g. bull rushes) was common at all sampling locations, while submerged aquatic vegetation (macrophytes such as water crowfoot and milfoil) was super abundant in locations upstream of the Shasta River canyon and often completely covered any hard substrates such as cobbles and small boulders (Figure 5). Of the 43 total samples, nine were collected from artificial substrates deployed for an

average of 74 days, 16 were collected using the kick net method of washing hard substrate, and 18 were collected by visual inspection and scraping of cobble and woody debris. All sampled material was inspected for the presence or absence of polychaetes using dissecting microscopes at 20x magnification but no polychaetes were observed, either whole or in part. No other evidence of polychaetes, such as empty tube casings, was observed either. However, invertebrates commonly observed in conjunction with polychaetes in the Klamath River, such as nematodes, midges, snails, and hydras were observed along with a variety of other aquatic macroinvertebrates typical to Klamath River tributaries.

Discussion

Sampling conducted in lower, middle, and upper reaches of the Shasta River in the summer and fall of 2008 did not detect any evidence of the presence of the polychaete worm, *M. speciosa*. While the sampling was not exhaustive or consistent at all collection sites, the amount of sampling conducted should have detected polychaetes had they been present. By contrast, polychaetes worms were readily collected and identified in the mainstem of the Klamath River concurrently using the same methods. In the course of sampling in the Klamath River we discovered that polychaetes colonies could be readily identified on substrate using the naked eye or a handheld magnifying lens because of the characteristic appearance of their tubes. In the Klamath River, polychaetes were also found to colonize woody debris, often dominating the invertebrate assemblage on colonized pieces of wood. During our float survey of the Shasta River canyon we relied on the method of visually inspecting cobbles and woody debris with a magnifying glass and scraping off any *Cladophora* or material that looked like polychaetes tubes. While subjective, we believe this is the quickest and most reliable method to survey long stream segments for the presence or absence of polychaetes assuming experienced personnel are present.

The apparent lack of polychaetes in the Shasta River could be due to the following reasons: 1) rare abundance and patchy distribution leading to non-detection; 2) present day habitat characteristics, such as high summer water temperatures and low water flow or super-abundance of macrophytes, preclude their survival; or, 3) historic absence maintained due to their poor dispersal ability. Of these possible explanations, the third is the most plausible and best supported by available evidence.

If polychaete populations in the Shasta River were extremely rare and patchy, it would be difficult to rule out their existence with 100% certainty with one year of sampling. However, their ability to produce significant numbers of infectious actinospores would probably also be negligible. In addition, as previously discussed, we believe that our sampling efforts were sufficient to detect at least some individual polychaetes if populations existed in the Shasta River. Previously, one individual polychaete worm was found by other researchers at the first pool in the Shasta River above its confluence with the Klamath River (K. Cummins, Humboldt State University, personal comm.). However, during flood events water from the Klamath River mixes with the Shasta River

and can flow into this pool, potentially washing polychaetes from the Klamath River into this pool where a few could occasionally settle out and survive to form intermittent and low abundance colonies. The fact that we were not able to detect any polychaetes in this pool or in the riffle immediately downstream is consistent with this hypothesis.

In general habitat conditions for polychaetes in the Shasta River appear favorable, including a stable flow regime (relatively little winter flooding and year round flow) and high nutrients. However, it could be argued that low flow and high temperatures in the summer could result in inhospitable summer conditions that could preclude polychaete survival. We do not believe this is true because the Shasta River flows year round resulting in a stable inter-annual minimum surface water elevations in pools, and while water temperatures do get very warm (e.g. 30ºC), upstream reaches remain cooler during the summer. Yet our sampling in those upstream reaches did not detect any polychaetes either (i.e. Nelson Ranch). Upstream reaches of the Shasta River do possess a super abundance of macrophytes, which smother the hard substrate of the river bed, thereby reducing but not eliminating the preferred hard substrate of polychaetes. Viewed in total, habitat characteristics for polychaetes in the Shasta River are not as favorable as they generally appear but should not preclude their establishment and survival either.

The apparent absence of polychaetes populations in the Shasta River is most likely due to their historic absence from the Shasta basin being maintained by their poor dispersal abilities. *M. speciosa* can crawl along substrates or float with currents, but has no ability to swim upstream against currents and are thus poor at dispersing. This likely explains why their distribution on the Pacific coast of North America is restricted to large, old, antecedent rivers such as the Klamath and Columbia Rivers.

The absence of polychaetes in the Shasta River is also supported by the lack of *C. shasta* DNA detectable in water samples from the lower Shasta River during summer months, including in 2008 (S. Hallett, Oregon State University, personal comm.). Caution should be applied when using this data to infer the absence of polychaete populations in the Shasta River since uninfected polychaetes or populations sufficiently far upstream would not be detected with this method. In addition, no sentinel fish exposures have been conducted in the Shasta River at this date to test for the presence of infectious actinospores, which is another method to validate the myxozoan infectivity of a body of water that also provides evidence for or against the presence of polychaete populations.

From the perspective of fish health, the apparent absence of polychaetes from the Shasta River is important because it confirms that juvenile salmonids exiting the Shasta River each spring are uninfected with *C. shasta* when they enter the Klamath River and are subsequently subjected to highly infectious doses of actinospores (Nichols and Foott 2006). A substantial portion of coho and Chinook salmon juveniles leave the Shasta River as fry in the spring (CA Department of Fish and Game, unpublished data) as conditions in the Shasta River begin their annual deterioration with the onset of the irrigation season and summer weather. Depending on the year, conditions in the Klamath River can be deleterious in terms of relatively low flow releases from upstream reservoirs, warm water temperatures, and high abundance of infectious actinospores; all

of which can combine to greatly reduce the survival probability of juvenile salmonids entering the Klamath River from the Shasta basin. Improving conditions in the mainstem Klamath River for juvenile salmonids in order to reduce the infection prevalence and mortality from *C. shasta* is a necessary step to maintaining and restoring salmonid populations in the Shasta River.

Tables and Figures

Table 1. Dates and locations of polychaete survey sampling in the Shasta River during 2008.

Site ID #	Location	Sampling Type	Date Deployed	Date Removed	Days	# of Samples	Polys
4	Nelson Ranch	artificial substrate	5/29/2008	8/14/2008	77	3	no
1	Webb Ranch	artificial substrate	5/29/2008	8/12/2008	75	3	no
1	Webb Ranch	kick net	8/13/2008	na	na	9	no
4	Nelson Ranch	artificial substrate	8/14/2008	10/23/2008	70	3	no
5	Dwinell Dam	kick net	8/14/2008	na	na	3	no
5	Dwinell Canal	kick net	8/14/2008	na	na	1	no
3	Meamber Ranch	kick net	8/14/2008	na	na	3	no
3	Meamber Ranch	scrape	10/23/2008	na	na	3	no
4	Nelson Ranch	scrape	10/23/2008	na	na	3	no
2	Canyon Reach	scrape	10/24/2008	na	na	12	no

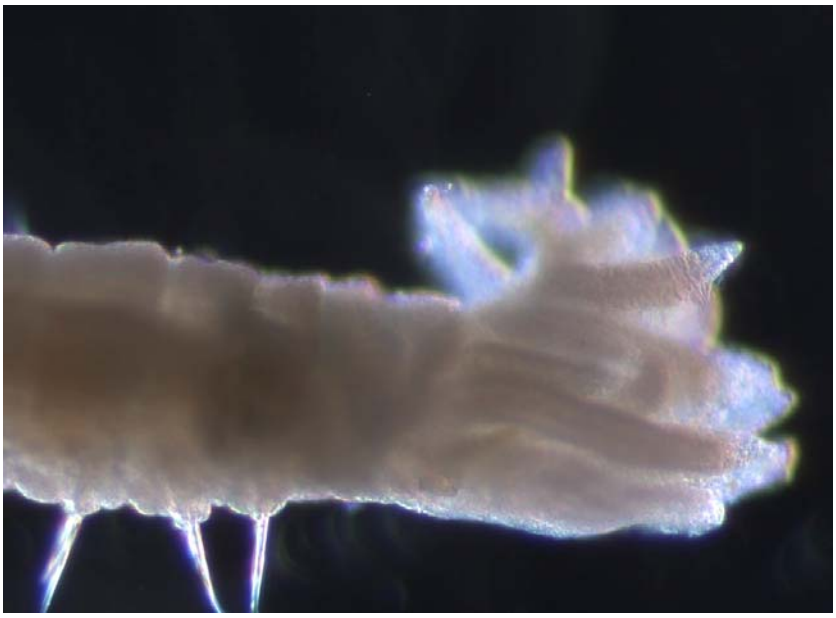

Figure 1. Photograph of polychaete collected in the mainstem Klamath River during the summer of 2008 at 40x magnification. This polychaete has ejected from its tube and its characteristic feeding crown (right) and dorsal spines are visible (bottom left).

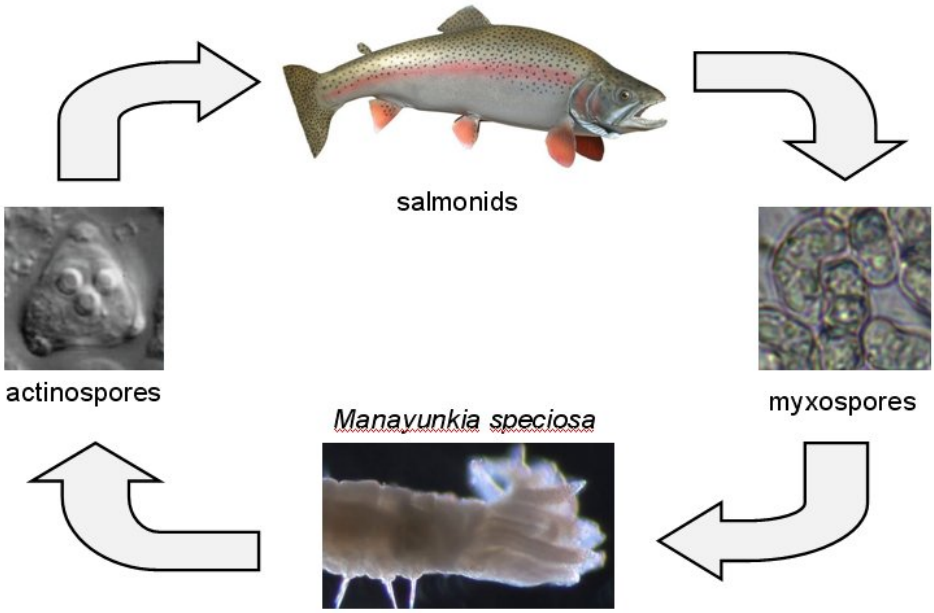

Figure 2. Life cycle of *Ceratomyxa shasta* and *Parvicapsula minibicornis* showing the alternate polychaete host *Manayunkia speciosa* (adapted from Stocking 2006).

Figure 3. Picture of an artificial substrate (i.e. brick wrapped in nylon window screen) deployed in low velocity edge water habitat in the Shasta River at the Webb Ranch (site #1), 2008.

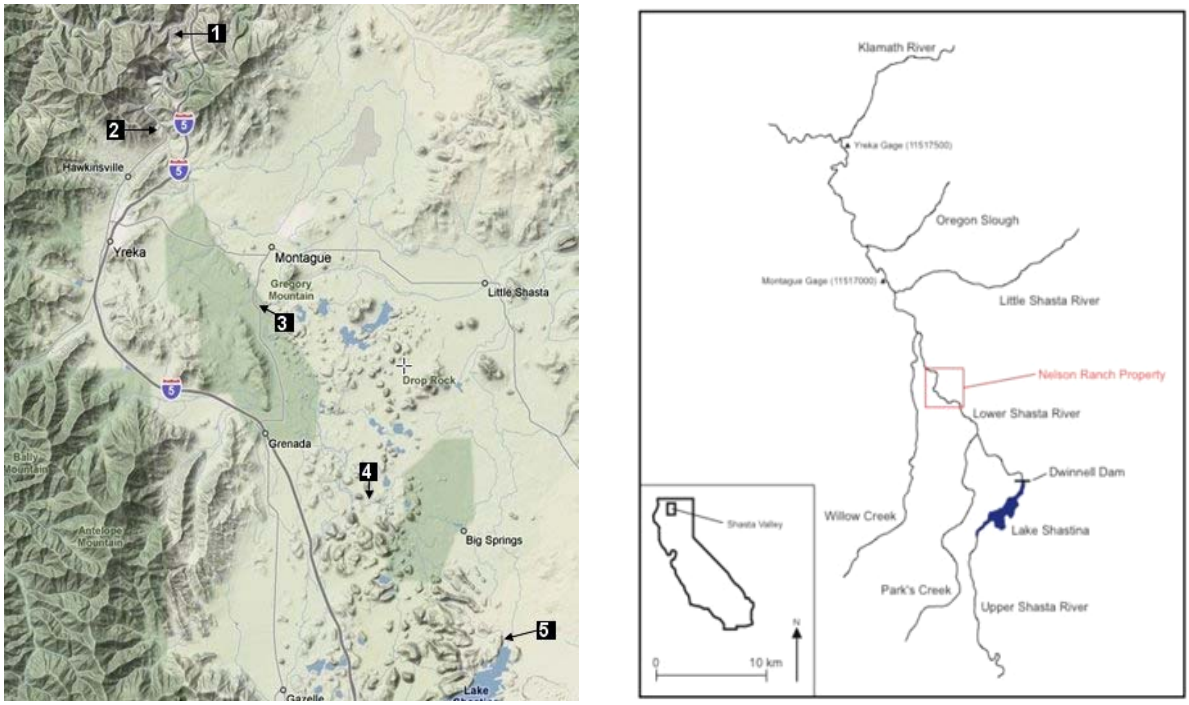

Figure 4. Maps of the Shasta River study area. Sampling locations are as follows with numbers corresponding to the site ID # in Table 1: 1) confluence of the Shasta River with the mainstem of the Klamath and approximate location of Webb Ranch; 2) top of the Shasta River Canyon which ends at its confluence with the Klamath; 3) Meamber Ranch; 4) Nelson Ranch; and, 5) Shasta River below Dwinell Dam and Dwinell canal.

Figure 5. Photograph of the Shasta River at the sampling site on the Nelson Ranch on Aug 14th, 2008 showing the extensive submerged and emergent aquatic vegetation and lack of cobble and boulder substrate that is typical of the Shasta River above its lower canyon.

Figure 6. Photograph of the Shasta River (and old water diversion weir) at the Meamber Ranch on Aug 14th 2008.

Figure 7. Photograph of the Shasta River a short distance below Dwinell Dam on Aug 14th 2008.

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