Investigating the apparent absence of polychaetes (*Manayunkia speciosa*) in the Shasta River: distribution of vectors for myxozoan fish diseases

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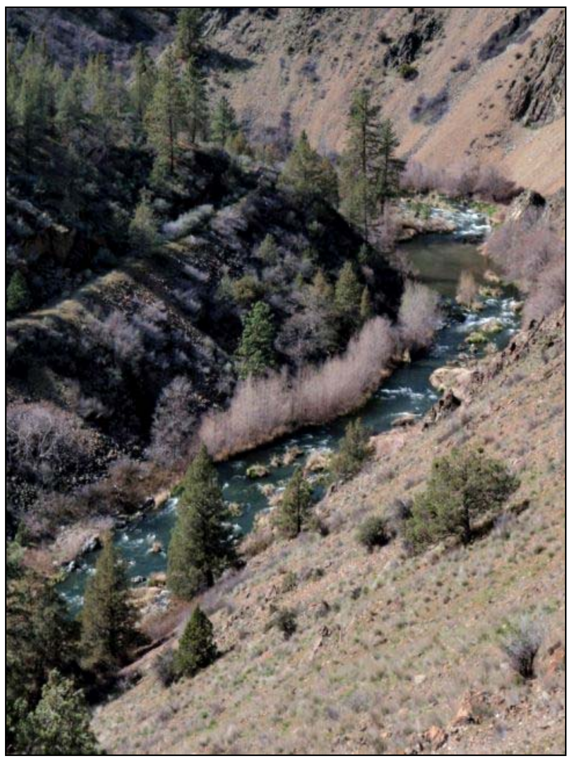

Canyon reach on the lower Shasta River 2010.

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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 the Shasta and Scott rivers. However, polychaete worms are either absent or of unknown determined 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 with 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. This report presents findings of our second and final year of sampling for polychaetes in the Shasta River.

Methods

Our approach to determining the presence or absence of polychaetes in the Shasta River during the summer of 2009 and spring of 2010 consisted of visual inspection of cobbles and woody debris for the characteristic tubes constructed by polychaetes. Any suspicious material was then examined with a 4x magnification hand lens to determine if polychaetes tubes were indeed present. In the event of positive sighting, material would be collected and preserved in ethanol for later verification in the laboratory using microscopes and QPCR to determine myxozoan infection prevalence.

Sampling that we conducted during previous years in the mainstem Klamath River in conjunction with researchers from the US Fish and Wildlife Service, Oregon State University, and Humboldt State University gave us the experience needed to visually inspect natural substrate and quickly 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. This approach required us to quickly inspect as much substrate as possible in our target reaches; therefore we opted to float the river using small inflatable kayaks. This investigation method also had the advantage of avoiding trespassing on private property by accessing polychaete inspection areas via boat on a navigable waterway with the right to pass guaranteed by the California State Constitution (Article 10, §4), the federal Public Trust Doctrine, and court precedent.

Our target reaches for polychaete surveys in 2009/2010 were: 1) the canyon reach from the Highway 162 crossing near Yreka to the confluence of the Klamath River; and, 2) the Big Springs Reach from the confluence of Big Springs Creek to the crossing at County Road A-12 (Figure 2). The canyon reach was chosen because it is closest in proximity to the Klamath River a most likely to have polychaetes based on their limited dispersal abilities, and the Big Springs reach was chosen because it remains relatively cool (i.e. below the lethal temperature threshold for polychaetes) throughout the summer due to the influence of Big Springs Creek as compared to downstream and upstream reaches of the Shasta River. Surveys were conducted in the Shasta River during the summer (2009) when polychaetes tubes were at their full size in the Klamath River and therefore most easily visible if present in the Shasta River, and again the following spring (2010) prior to the onset of irrigation season (April 1st). Spring surveys were chosen in order to have enough water to conduct boat based surveys in the Shasta River but late enough in the season to have recognizable polychaete tubes in the Klamath River and therefore in the Shasta River as well if polychaetes were indeed present. Cobbles and woody debris were visually inspected opportunistically or every 100 m at minimum.

Results

A one mile long area of the Shasta River canyon reach was surveyed by foot in the summer of 2009. In the spring of 2010, approximately 20 stream miles were surveyed in the canyon and Big Springs reaches. No polychaete worms, their tubes, or other evidence of their presence were observed during these surveys (Table 1).

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 most locations and often completely covered any hard substrates such as cobbles and small boulders (Figure 3). 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 macro-invertebrates typical to Klamath River tributaries. However, the density of snails in the Shasta River was noticeably higher than in the Klamath River, and woody debris in the Shasta River were typically grazed clean by an abundance of snails (Figure 4).

Discussion

Inspections conducted in lower and middle reaches of the Shasta River in the summer of 2009 and the spring of 2010 did not detect any evidence of the presence of the polychaete worm, *M. speciosa*, consistent with our study results from 2008. While the investigation was not exhaustive, the amount of inspection conducted should have detected polychaetes had they been present. By contrast, polychaete worms were readily collected and identified in the mainstem of the Klamath River using the same inspection methods. The consistency of results from our investigations in 2008, 2009, and 2010 leads us to conclude that the Shasta River is not infective for myxozoan fish diseases because it lacks populations of polychaetes.

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 and snails, 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 firmly supported by the available evidence.

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 (i.e. Big Springs reach). Yet our sampling in those upstream reaches did not detect any polychaetes either. Most reaches of the Shasta River possess a super abundance of macrophytes and elongated epiphytic algae, which smothers the hard substrates in the channel, thereby greatly reducing the preferred stable substrates of polychaetes. In the Klamath River, polychaetes rapidly colonize woody debris, often dominating the invertebrate assemblage on colonized pieces of wood, which are typically covered with a dense amount accumulated organic material providing ideal material to construct their tube casings. In contract, wood debris in the Shasta River were observed to be typically covered with a high abundance of snails, which had grazed their surface clean. 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 almost certainly due to their historic absence from the Shasta River 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, Pit, and Columbia Rivers.

The conclusion that the Shasta River does not possess populations of polychaetes is also supported by the lack of *C. shasta* DNA detectable in water samples from the lower Shasta River during summer months (S. Hallett, Oregon State University, personal comm.). Caution should be applied when solely 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 in 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 from polychaetes in the Klamath River (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 extremely 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 River 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. Fortunately, this goal is not further complicated as the Shasta River has been confirmed to lack populations of the polychaete worm *M. speciosa*, which are vectors of the myxozoan diseases *C. Shasta* and *P. minibicornis*.

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Rick Stocking of Dr. Jerri Bartholmew’s lab at Oregon State University deserves credit for his original research on polychaetes in the Klamath River basin that has provided the foundation of knowledge for further research on polychaetes in the basin. Yurok Tribal Fisheries technicians Jamie Holt and Rocky Erickson assisted with fieldwork for this project. Funding for this project was provided by the US Bureau of Reclamation’s Klamath Basin Area Office and the Pacific Coastal Salmon Recovery Fund.

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Tables and Figures

Table 1. Dates and locations of polychaete inspections in the Shasta River during 2009 and 2010.

Location	Sampling Type	Stream Miles	Date Surveyed	Polychaetes
Canyon Reach	visual inspection	1	8/1/2009	none
Canyon Reach	visual inspection	8	3/17/2010	none
Big Springs Reach	visual inspection	12	3/18/2010	none

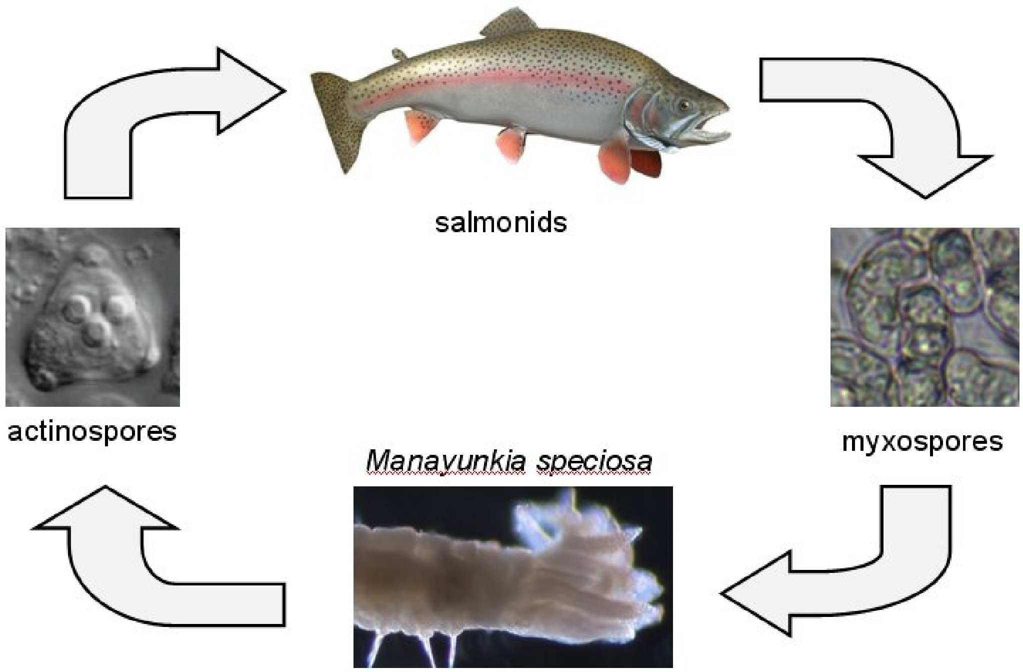

Figure 1. Life cycle of *Ceratomyxa shasta* and *Parvicapsula minibicornis* showing the alternate polychaete host *Manayunkia speciosa* (adapted from Stocking 2006).

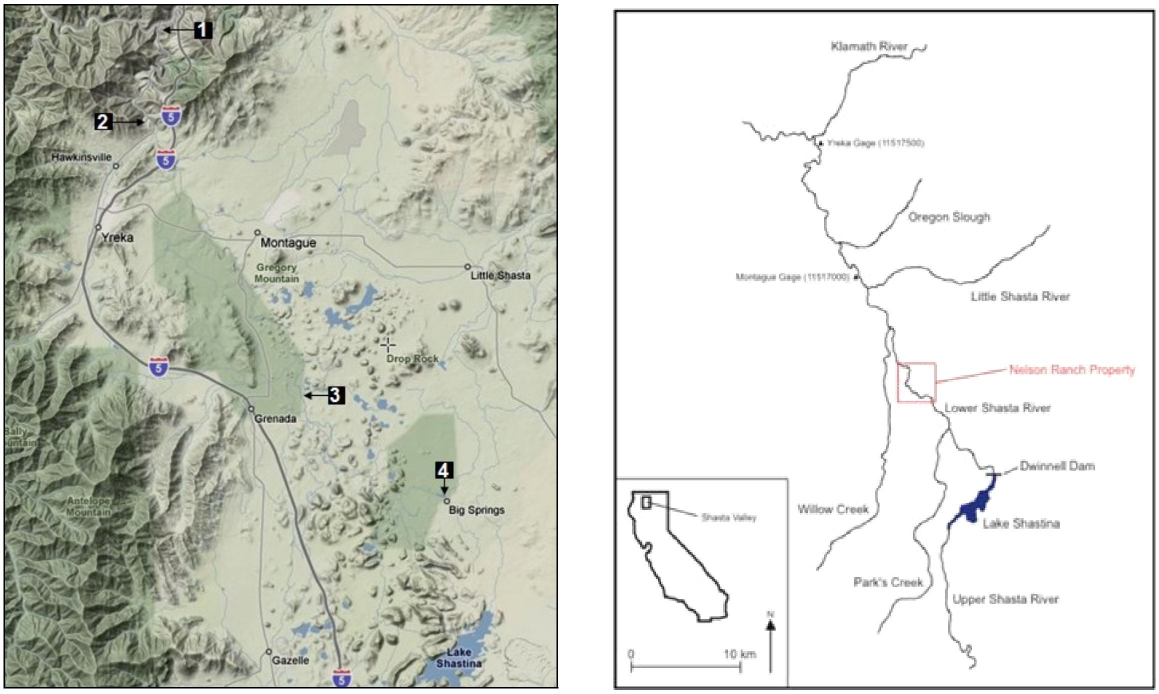

Figure 2. Maps of the Shasta River study area. Areas surveyed in 2009/2010 were the canyon reach from point 2 to 1, the Big Springs reach from point 4 to 3.

Figure 3. Photographs showing the heavy amount of elongated epiphytic algae (top, caynon reach 2010) and riparian emergent vegetation (bottom, Big Springs reach 2010) that typically smother hard substrates in the Shasta River potentially reducing the quality and quantity of habitat for polychaetes in contrast to the Klamath River.

Figure 4. Photograph showing the heavy amount of organic matter covering a piece of woody debris in the Klamath River favored by polychaetes (top, Klamath River just above the Shasta River confluence 2010) in contrast to a piece of woody debris in the Shasta River that has been grazed clean by an abundance of snails (bottom, Big Springs reach 2010).