Assessing links between water quality, river health and Ceratomyxosis of salmonids in the Klamath River system

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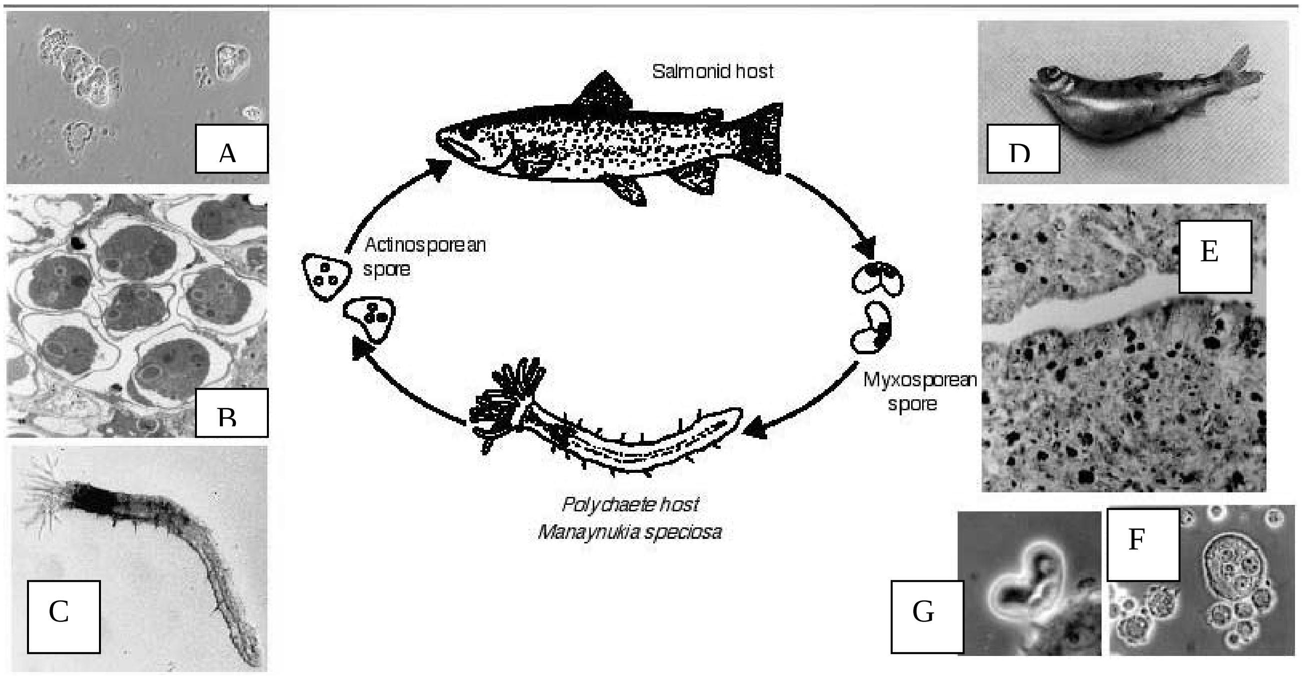
***Ceratomyxa shasta* in the Klamath River.** The myxozoan *Ceratomyxa shasta* has been implicated as a significant source of mortality to out-migrating juvenile Chinook salmon in the Klamath River during recent years (Foott et. al 2002). This again became evident in early May of 2004 when dead and moribund salmon were found in screw-traps along the river between the Beaver Creek confluence and the estuary. Examinations readily identified *C. shasta* as the cause of mortality. A second myxozoan, *Parvicapsula minibicornix* also appeared to be having some effect on these outmigrants. In 2002, the public became aware of a fish kill in the Klamath that implicated low flows, elevated water temperatures, and the combined effect of pathogens (particularly *Ichthyoptherius multifilis* and columnaris) in the deaths of over 33,000 returning adult salmon. Once again, *C. shasta* was present although its role in this outbreak is not clear.

In the Klamath River, as in other river systems in the Pacific Northwest, *C. shasta* exhibits a variable distribution within the main-stem and appears not to be present in the smaller tributaries. In summer 2003, cages containing live trout that are susceptible to the pathogen were placed in various locations within the main-stem Klamath between Beaver Creek and Keno Reservoir. Mortality from *C. shasta* was highest (100%) in fish held at Beaver Creek. The prevalence of infection with *C. shasta* in fish held above Iron Gate dam was variable, but with the exception of fish held in Boyle Reservoir, all infections and mortality occurred in fish held in free-flowing areas of the river. However, it should be noted that conditions were not conducive for keeping trout in cages above the dams due to elevated temperatures and poor water quality. In summer of 2004, the exposures were repeated, with additional sites added below Iron Gate dam. Although, the results are not complete, trends in the data suggest the same: all sentinel fish exposed below the dam's died from *C. shasta* within 90d at a constant holding temperature of 12 ^{o}C. Mortality in fish held above Iron Gate is delayed, suggesting a lower exposure dose. The high infection prevalence among groups held below Iron Gate dam invites the question; why is *C. shasta* such an issue in the Lower Klamath River?

Spore Concentration and Q-PCR. Dr. Sascha Hallet, a post-doctoral researcher at Oregon State University, has recently developed a method of measuring *C. shasta* spore concentrations in water samples. The assay takes advantage of the specificity of primers developed for *C. shasta*, permitting the investigator to amplify the DNA to a minimum detectible level (MDL). The MDL is then back-calculated based on the number of cycles required to achieve the MDL, to estimate the original quantity of target DNA present in the original water sample. The QPCR assay is extremely sensitive, capable of detecting 1/1000th of a *C. shasta* spore in a sample.

Results obtained using this assay on water samples collected from the Klamath River to date correlate with sentinel data, with the highest parasite numbers from samples collected upstream of Beaver Creek, and no detection of parasites from Keno Reservoir. The QPCR was able to detect parasite DNA from all other mainstem samples at varying levels. Because samples are easily collected and can be processed within days, this assay shows great promise as a tool for advancing the study of *C. shasta* on an ecological scale.

Host, Pathogen, and Environment: enter the polychaete. Disease is the result of complex interactions between the host, the pathogen (or parasite) and the environment. Although *C. shasta* is not an exception, the study of it is complicated by the fact that the parasite changes form, and apparently function, and has not one host, but two (note illustration below). Most of what we know about *C. shasta* occurs in the salmonid host, and the polychaete worm is the one aspect of the life cycle that is not well understood. There exists only a few references to this worm and most of those are of incidental findings. Consequently, it is paramount to investigate the ecology of the worm in order to determine its role in the activities of the parasite.



Life cycle of *Ceratomyxa shasta* showing release of the myxospore stage from the infected fish, the polychaete alternate host, and release of the alternate actinospore stage from the polychaete. A. released actinospores, B. electron micrograph of actinospores in the polychaete, C. polychaete, D. infected fish, E. histological section of infected intestine, F. trophozoite stages, G. myxopsore (Bartholomew et al. 1997).

Polychaete habitat summary. In 2003, benthic samples were collected from a variety of habitat types between Beaver Creek and the Keno impoundment, including scrapings of rocks in eddy's, pool, runs, and riffles, as well as collecting fine sediments in reservoirs and pools. The data paved the way for more intensive collections throughout the river basin. The polychaete has been detected from a variety of habitat types such as stable pools where the polychaete inhabits fine sand silt sediments and it can be found amongst freshwater sponge on boulders at the bottom of reservoirs and on bridge pillars. However, the highest densities of polychaetes were always found within a type of attached periphyton. This periphyton is generally made up of species of *Cladophora* (similar in appearance to astro-turf). Polychaete densities within the *Cladophora* easily measure thousands per m^2. Interestingly, the polychaete was always found at the inflow to reservoirs such as Copco, J.C. Boyle, and Iron Gate, remaining consistent with the findings of other investigators (Mackie and Qadri 1971).

Habitat work in 2004 has been conducted with the assistance of numerous collaborators (USFWS, ODFW, Yurok Tribe, Karuk Tribe, USGS) who provided technical help and equipment. Although most samples have not yet been examined, some trends were noted among the samples processed. Standing crops of attached periphytons such as *Cladophora* and other macro-algae were extensive and often thick in many parts of the middle and Lower Klamath basin.

Hypothesis regarding water quality and *C. shasta*: One hypothesis for the high incidence of *C. shasta* in the Klamath is that polychaete populations have increased as a result of an increase in available habitat for the worm. This idea has been proposed for the following reasons. Although not well documented, the polychaete appears to have a broad tolerance to variations in dissolved oxygen, substrate type, current velocity and other physicochemical parameters (Rolan 1974). This would explain the occurrence of the polychaete from the cold oligotrophic lakes of Alaska (Holmquist 1973) to the warm eutrophic waters of the Gulf states (Brehm 1978) and up to the Great Lakes region (Mackie and Qadri 1971). The polychaete inhabits a variety of substrates including fine sediments where polychaete densities in Lake Erie have been reported to reach 45,000 per m^2 and in attached periphytons (Aufwuch) such as *Cladophora*. A number of studies demonstrate the relationship between water quality and macro-algae, and most especially *Cladophora* spp (S.L.Wong and B. Clark 1976, I. Ensminger, 2000, Richard C. Lorenze and Charles E. Herdendorf 1982).

In the Klamath River, polychaete densities in *Cladophora* can reach 1000’s per m^2 and I have noted large “mats” of *Cladophora* in various locations throughout the river system, often covering the entire stream bed. It is the biology and the ecology of *Cladophora* that has led me to propose this hypothesis. *Cladophora* is a well document nuisance species and has become the focus of much research. Investigators have found that this macro-algae has a competitive advantage in nutrient enriched waters and can become the dominant species, frequently covering the entire stream bed and displacing all other macrophytes where it occurs. This prolific, complex, and aggressive species is considered an “ecosystem-reorganizer” capable of altering benthic food webs and centralizing the ecosystem by collecting fine organic matter and creating its own habitat (SchÖnborn 1996). The importance of this macro-algae can not be over-stated and its function in the Klamath River should become a topic of further research. Currently, the Bartholomew lab is documenting polychaete distribution in the Klamath River in relation to physical and biological characteristics and results implicate *Cladophora* as a prime habitat for the polychaete.

Opinion (Stocking): I have spoken with numerous individuals who are strongly affiliated with the Klamath River ranging from life long residents, fishermen, to biologists. All of these individuals, from the headwaters to the mouth, have expressed that the river is changing, and not for the better. Much of this perception is likely due to the amount of publicity the Klamath River has obtained. However, it is not likely that the system has reached a stable equilibrium, but is still reacting to anthropogenic influences such as water diversions, dams and nutrient enrichment. The effects of these disturbances are reflected by the amount of primary production within the Klamath River system, due in part to a upper basin that in not phosphorous limited, explaining the mass production of the nitrogen fixing algae *Aphanizomenon*. Substrata in downstream pools and eddies were often covered with decaying *Aphanizomenon* from upriver reservoirs. The role of organic matter decomposition and nutrient cycling are beyond the scope of this paper, but it is assumed that these nutrients and fine particulates (organic matter) would contribute to in-stream macrophytes and macro-algae production. This cycle is further enhanced by a steady and stable flow regime exhibited within the Klamath River system, which is typical of regulated rivers.

It's important to point out that these problems are not new. Research has been conducted on the Glen Canyon reaches to determine the effects of planned flooding and seasonal flow reductions on aquatic biota (McKinney et al 1999). It is my opinion that base line flows, and moderation of scouring flood events (in addition to nutrient enrichment) would allow the spread of *Cladophora* which in turn creates habitat for the polychaete. Consequently, more vectors within the system increase the opportunity for the parasite to increase in abundance allowing factors such as high parasite concentrations, low water flows during peak runs, and elevated water temperatures to contribute to the disease outbreaks that have been witnessed in the Klamath River system.

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