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2025 SEA Faculty Meeting Abstract

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Isolation and characterization of bacteriophages infecting Flavobacterium psychrophilum strains isolated from westslope cutthroat trout- Oncorhynchus clarkii lewisi

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The Washoe Park Trout Hatchery (WPTH) located in Anaconda, MT houses the broodstock of westslope cutthroat trout (WCT), *Oncorhynchus clarkii lewisi*, for the Montana Department of Fish Wildlife and Parks (MFWP). This broodstock is maintained for recreational fishing opportunity, and genetic purity of the broodstock is maintained in case of conservational need. Historically WPTH has experienced profound fish mortality from bacterial coldwater disease (BCWD). Losses in excess of 50% in a given production year at WPTH have been observed. *Flavobacterium psychrophilum*, the causative agent of BCWD, is a gram negative, rod-shaped, bacterium1. Bacteriophages (phages) are ubiquitous host specific viruses that infect and kill bacteria. Bacteriophage therapy has been proposed as an alternative to antibiotics in the aquaculture industry and has been shown to reduce mortality during *F. psychrophilum* outbreaks in salmonids. This research aims to isolate and characterize phages infective to WPTH- isolated strains of *F. psychrophilum*.

• Project goals included optimizing soft agar overlay lawn testing conditions of *F. psychrophilium*, discovery of its bacteriophages using direct and enriched isolation techniques, and isolation and characterization of additional strains of F. psychrophilum.

A satellite laboratory has been established at WPTH for continual phage monitoring and bacterial strain isolation. A third strain of *F. psychrophilum* with distinctive colony morphology has been recently isolated from hatchery water. Optimization of top-agar bacterial lawn growth conditions has been established for Tryptone yeast extract salt media (TYES), and several rounds of environmental phage screenings utilizing both direct and enriched isolation methods have been completed utilizing modified Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) protocols.

One phage plaque was observed, and spot testing and transmission electron microscopy have confirmed the isolation of a bacteriophage with myoviridae morphology. Amplification and characterization of this phage along with continued isolation attempts using modified conditions are ongoing. Long-term goals are development of isolated phage(s) for aquacultural phage therapy applications at WPTH.