

**Title:** Unraveling the roles of crayfish ectosymbionts

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**Abstract:** Within freshwater ecosystems, crayfish provide a microhabitat for other organisms. In this study, the relationship of a crayfish host with branchiobdellidan worms (BW) and associated microbial assemblages (MA) was explored. The BW are known to provide a cleaning symbiosis as the BW consume bacteria on crayfish gills. Within a lab-based study, the researchers placed 4 BW on the crayfish carapace and observed the effects of BW on dissolved oxygen (DO) uptake, MA in the form of colony forming units (CFUs/g crayfish), and then sequenced isolated bacteria. The percent change in blotted wet mass showed that the control crayfish grew 26.2% more than the experimental group (with four BW). No significant difference was found in the mean DO consumption between control (0.094 mg/l/g\*2hr) and experimental (0.090 mg/l/g\*2hr) groups. A gill chamber bacterial analysis and characterization showed that the CFUs (log10 CFUs/g) were greater for the experimental group (3.80 log10 CFUs/g) than the control group (2.84 log10 CFUs/g). The isolated bacteria identified revealed two unique MA which were a basis for looking for distinct MA on crayfish with and without BW in a stream.

A second field-based experiment focused on collecting microbial swabs from crayfish and the surrounding environment (water and substrate) in Opossum Creek during the summer of 2018. DNA was extracted and the 16S gene was amplified for DNA sequencing. Sequenced microbes were analyzed for comparisons of MA in relation to stream order, stream environment, and the presence of BW on crayfish. Preliminary Qiime2 analysis of the raw sequencing data revealed significant differences in alpha diversity of the crayfish and the environment, but no significant difference in crayfish with and without BW. Results from these experiments add to our growing understanding of how BW symbionts alter MA on crayfish.