

Comparison of Quantitative PCR and Metabarcoding Methods for the Enumeration of Sockeye Salmon Using Environmental DNA: Year 2 of an Ongoing Study

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Planetary biodiversity is declining at alarming rates. Species conservation depends on knowing what species are present in an environment and in what quantities. A keystone species in Alaska is *Oncorhynchus nerka*, commonly known as sockeye salmon. Traditional methods of quantifying this species are often costly, time-consuming, dependent on expertise, and environmentally invasive. Environmental DNA (eDNA) may provide an alternative quantification method that solves these problems. Our previous project determined that quantitative eDNA metabarcoding could accurately enumerate sockeye salmon in eight creeks in the Wood River watershed of Southwest Alaska. This project continues the previous year's investigation by analyzing the same eDNA samples using a second eDNA analysis technique: quantitative Polymerase Chain Reaction (qPCR), which is potentially more accurate than metabarcoding. Visual survey fish counts served as the gold standard. Samples underwent qPCR processing that produced species abundance estimates from the eDNA. qPCR abundance estimates were compared to those of visual surveys and eDNA metabarcoding. The results strongly supported qPCR's ability to accurately quantify sockeye salmon and established that an eDNA signal no matter the analysis method is a concentration estimate that needs to be corrected for volume surveyed. Visual counts were therefore divided by watershed area before being compared to eDNA counts. Overall, qPCR and metabarcoding methods appear comparable and both capable of serving as an alternative or supplement to traditional methods of species enumeration. However, if metabarcoding is used to quantify abundance across multiple sampling runs, it must be standardized with qPCR to account for variation in sequencing efficiency.