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Validate and apply sea lamprey diet analysis using DNA metabarcoding
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ABSTRACT:

DNA metabarcoding approaches can provide an attractive complement to conventional dietary analyses, particularly in hematophagous species like the invasive sea lamprey. This study developed tools to facilitate DNA metabarcoding of sea lamprey digestive contents, evaluated retention of host DNA in the sea lamprey digestive system through controlled experiments, and applied molecular diet analysis to juvenile and adult sea lampreys collected from Lakes Huron, Superior, and Champlain. First, we developed eight blocking primers designed to selectively suppress amplification of the sea lamprey 12S rRNA gene from the mitochondrial genome. The effectiveness of these blocking primers was evaluated through conventional PCR, quantitative PCR, and DNA metabarcoding. While all eight blocking primers successfully suppressed sea lamprey amplification, a subset also led to improved amplification of host DNA in a wild-caught adult sample, suggesting improved performance in field studies. Second, we conducted experiments in aquaria at Hammond Bay Biological Station to evaluate the retention and detectability of host DNA in the sea lamprey digestive tract over fasting periods of up to 30 days and across three different temperatures (5°, 10°, and 15° C). Results showed that host read counts declined quickly after feeding stopped and that longer fasting periods were associated with reduced detection probabilities. Despite declines in read counts, host DNA remained detectable over the duration of the experiment. We were also able to detect multiple hosts in sea lamprey digestive samples when transformers were allowed to feed consecutively on two host species. Finally, we used DNA metabarcoding to characterize diets of juvenile and adult sea lamprey collected from Lakes Huron, Superior, and Champlain. Findings from wild-caught samples indicated that whitefish (coregonines) and lake trout (*Salvelinus namaycush*) accounted for a majority of sequence reads across samples. Across all samples, salmonids, coregonines, and catostomids were the most frequently detected hosts, with higher rates of coregonine detection in juvenile sea lamprey and more frequent catostomid detections in adults. Importantly, taxonomic resolution for some host groups was limited with the 12S marker (e.g., members of the genus *Coregonus* cannot be distinguished from one another). Nonetheless, our study validated the application of DNA metabarcoding for diet analysis in sea lamprey and developed tools (blocking primers, reference databases) that will facilitate future studies. We anticipate that molecular diet analysis projects that use these tools may contribute to a greater understanding of the host fish attacked by sea lampreys, and the resulting damage to Great Lakes fish communities.