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Optimizing ethanol-based sample preservation to facilitate use of DNA barcoding in routine freshwater biomonitoring programs using benthic macroinvertebrates

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ABSTRACT

Molecular methods, such as DNA barcoding, have the potential to enhance biomonitoring programs worldwide. Altering routinely used sample preservation methods to protect DNA from degradation may pose a potential impediment to application of DNA barcoding and metagenomics for biomonitoring using benthic macroinvertebrates. Using higher volumes or concentrations of ethanol, requirements for shorter holding times, or the need to include additional filtering may increase cost and logistical constraints to existing biomonitoring programs. To address this issue we evaluated the efficacy of various ethanol-based sample preservation methods at maintaining DNA integrity. We evaluated a series of methods that were minimally modified from typical field protocols in order to identify an approach that can be readily incorporated into existing monitoring programs. Benthic macroinvertebrates were collected from a minimally disturbed stream in southern California, USA and subjected to one of six preservation treatments. Ten individuals from five taxa were selected from each treatment and processed to produce DNA barcodes from the mitochondrial gene cytochrome c oxidase I (COI). On average, we obtained successful COI sequences (i.e., either full or partial barcodes) for between 93 and 99% of all specimens across all 6 treatments. As long as samples were initially preserved in 95% ethanol, successful sequencing of COI barcodes was not affected by a low dilution ratio of 2:1, transfer to 70% ethanol, presence of abundant organic matter, or holding times of up to six months. Barcoding success varied by taxa, with Leptohyphidae (Ephemeroptera) producing the lowest barcode success rate, most likely due to poor PCR primer efficiency. Differential barcoding success rates have the potential to introduce spurious results. However, routine preservation methods can largely be used without adverse effects on DNA integrity.

Full Text

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