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TECHNICAL NOTE

Improving the yield of environmental DNA from filtered aquatic samples

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Abstract
Analysis of environmental DNA (eDNA) through non-invasive sampling continues to gain popularity in the surveillance of organisms. Methodological improvement to ensure maximum DNA recovery from the samples would benefit future studies. We investigated the effects of DNA extraction methods and filter preservation methods on eDNA yield by analyzing field-collected water samples for eDNA of the Eastern Hellbender (*Cryptobranchus a. alleganiensis*). We tested whether the use of bead beating during DNA extraction, which likely facilitates removal of cells trapped on filters, would increase eDNA yield. We also examined whether preservation of filters in ethanol or storage at − 20 °C before extraction would yield more eDNA. Bead beating and preservation at − 20 °C significantly increased the estimated amount of eDNA.

Keywords Bead beating · *Cryptobranchus a. alleganiensis* · Environmental DNA · Eastern Hellbender · Filter preservation method

Analysis of environmental DNA (eDNA) is a promising conservation tool to provide information about biodiversity and the presence/absence of rare organisms. eDNA analysis has been successfully implemented to monitor a variety of species from aquatic vertebrates to insects (Rees et al. 2014). Despite the success of these studies, further efforts are needed to improve methodology used in eDNA analysis (Goldberg et al. 2016; Hinlo et al. 2017).

Filtration of eDNA from aquatic samples using cellulose nitrate filters is a popular and effective capture method (Hinlo et al 2017; Spens et al. 2017; Li et al 2018). However, some cells may still remain trapped in the pores of the filters and may not be fully isolated during extraction. DNA extraction using bead beating, a mechanical disruption technique where small silica beads are vortexed with the sample during DNA extraction, has been shown to be effective in isolating microbial DNA from soil and fecal samples (Yeates et al. 1998; Yu and Morrison 2004). Therefore, bead beating may increase eDNA yield by removing cells trapped on filters and disrupting cell walls more efficiently. In addition to extraction technique, filter preservation methods (− 20 °C vs. ethanol) may also affect eDNA recovery. Ethanol used to preserve biological samples has been shown to contain sample DNA (Shokralla et al. 2010). Thus, filters preserved in ethanol, especially those transported under field conditions, may also lose eDNA into the fluid, negatively affecting eDNA recovery.

The goal of the present study was to investigate the effects of the use of bead beating during DNA extraction and filter preservation methods, and their interactive effect on eDNA yield by analyzing field-collected water samples for eDNA of Eastern Hellbenders (*Cryptobranchus a. alleganiensis*), a fully-aquatic giant salamander species currently in decline.

In August 2017, we collected surface water samples at nine sites along a tributary of the West Branch Susquehanna River (Pennsylvania, USA), where a recent study repeatedly detected hellbender eDNA (Takahashi et al. 2018). From each of nine sites, we simultaneously collected two samples (1 L) from the same location, (18 total samples) that were stored on ice until we reached the lab. We vacuum filtered each 1 L sample through two 0.45 µm nitrocellulose filters, 47 mm diameter (Whatman™) (Fig. 1); we also added two negative controls during filtration as described in Takahashi et al. (2018).

For each sample, we cut the two filters into halves, and a pair of the halves were preserved at − 20 °C, while the other pair were preserved in 95% ethanol (Fig. 1). To

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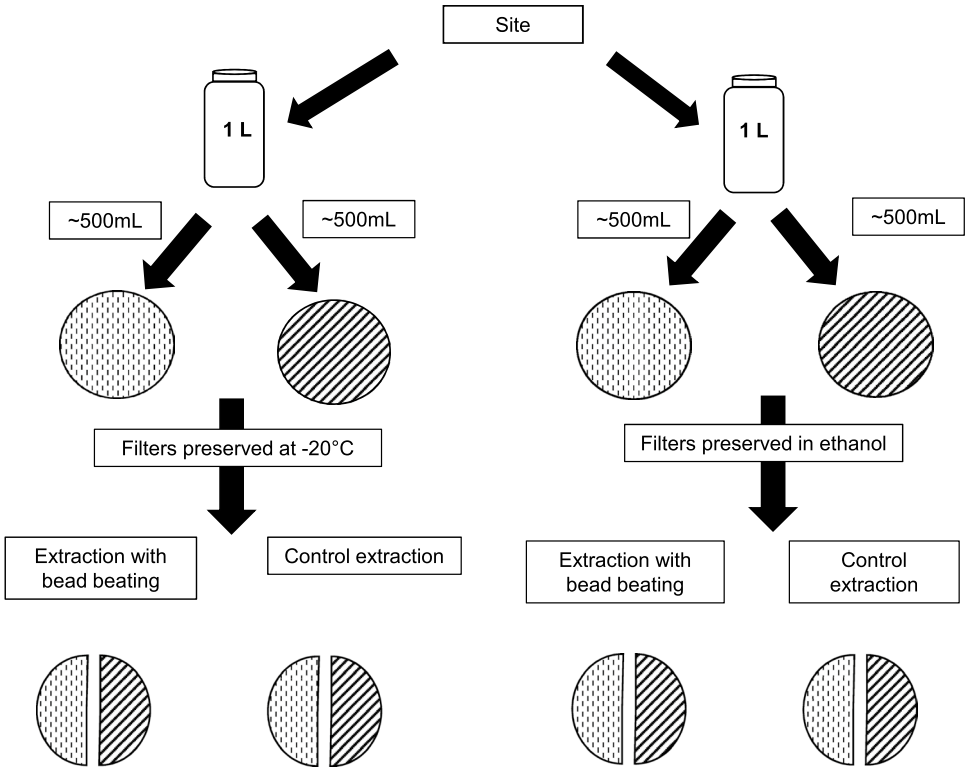

Fig. 1 Sample preparation scheme for water samples collected for eDNA analysis of *Cryptobranchus a. alleganiensis*. To control for discrepancies in eDNA contained between two filters used to filter each sample, one-half of each filter was used for each of the DNA extraction treatments

simulate field collection conditions, filters preserved in ethanol were carried around in a field backpack for 4 days before storing at 4 °C. We performed DNA extraction on the filters following Goldberg et al. (2011).

To test the effect of bead beating on eDNA yield, half of the filters from each preservation method went through the standard DNA extraction protocol described in Takahashi et al. (2018) using DNeasy® Blood and Tissue Kit (Qiagen); the other set of the half filters was used for the bead beating treatment, in which 0.5 g of ZR BashingBead™ (0.1 mm, 0.5 mm) were added to the lysing process of the DNA extraction protocol (Fig. 1). During the lysing process, we vortexed all samples for 10 min.

We performed quantitative PCR (qPCR) on the extracted DNA following Spear et al. (2015). We ran the extracted DNA from the samples in triplicate with PCR negative controls and a series of DNA standards ranging from 0.0001 to 1 ng/µL. To check for PCR inhibition, we included TaqMan™ Exogenous Internal Positive Control in each well during qPCR. We averaged the concentrations of each triplicate to obtain a single concentration per extracted DNA sample. We removed two samples that showed no eDNA concentrations (total sample size 38). To meet the assumptions of parametric tests, we log-transformed the data and conducted a two-way ANOVA to test the effects of the extraction methods and the preservation methods on the estimated concentrations of eDNA.

Bead beating during DNA extraction significantly increased the estimated concentrations of eDNA (0.05779 ± 0.01186 µg/L) compared to the extraction without beading beating (0.02716 ± 0.00662 µg/L) ($F_{1,30}$=15.052, $p = 0.001$, Fig. 2). Preservation at − 20 °C also significantly increased estimated concentrations of eDNA obtained compared to the preservation in ethanol (0.05012 ± 0.01206 vs. 0.03483 ± 0.00774 µg/L; $F_{1,30}$=5.314, $p = 0.028$, Fig. 2). There was no significant interaction between the two ($F_{1,30}$=1.301, $p = 0.263$).

Addition of bead beating to existing DNA extraction protocols provides a simple and cost-effective method to increase eDNA yield. Preservation of filters in ethanol in field conditions significantly reduces eDNA yield compared to storage at − 20 °C. We recommend the use of the additional step of bead beating when expected eDNA concentrations are low or when ethanol is the only option for filter storage during field sampling in order to improve detection probabilities and compensate for DNA lost into the ethanol. As eDNA analyses is an emerging field, numerous capture, preservation and extraction methods are available in addition to those explored in this paper (Hinlo et al 2017; Spens et al. 2017; Li et al 2018). Future studies could examine how much eDNA can be recovered from the ethanol by precipitating and pelleting the remaining DNA.

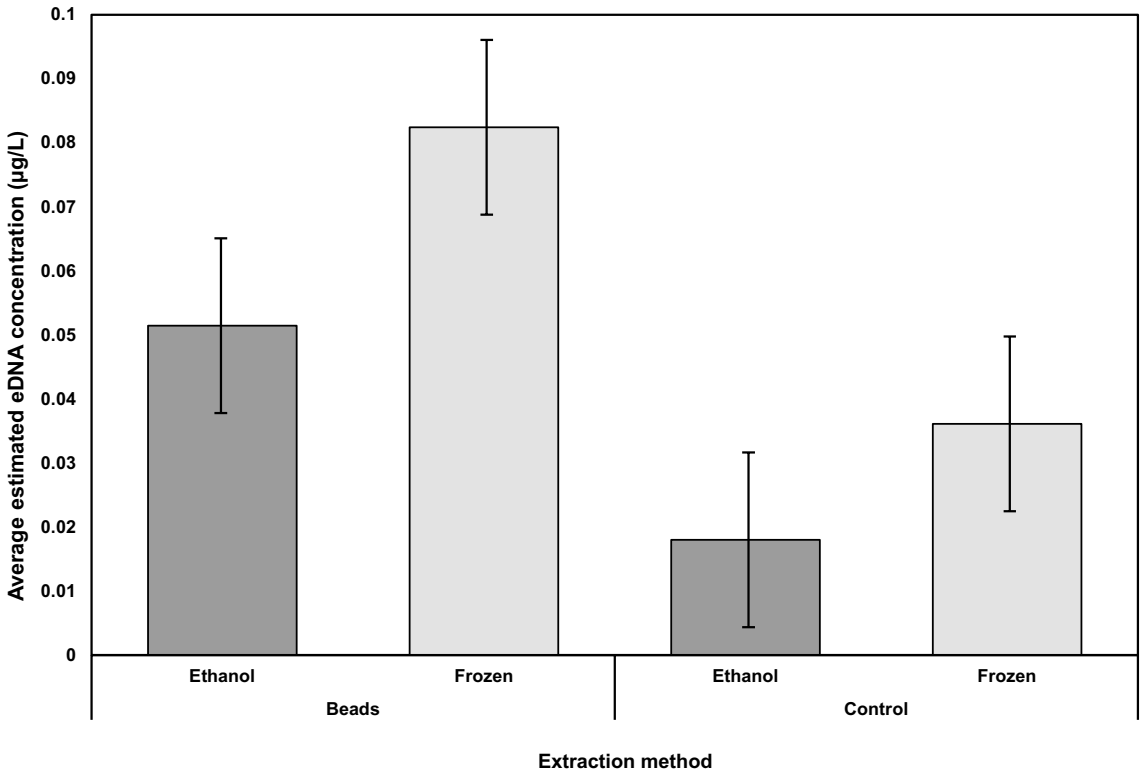

Fig. 2 Effect of extraction and preservation methods on estimated concentrations of eDNA

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