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Special Issue Article: Environmental DNA

Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms

Caren S. Goldberg [a,*], Katherine M. Strickler [a], David S. Pilliod [b]

[a] School of the Environment, Washington State University, 100 Dairy Rd., Pullman, WA 99164, United States
[b] U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, 970 Lusk St., Boise, ID 83706, United States

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ABSTRACT

The discovery that macroorganisms can be detected from their environmental DNA (eDNA) in aquatic systems has immense potential for the conservation of biological diversity. This special issue contains 11 papers that review and advance the field of eDNA detection of vertebrates and other macroorganisms, including studies of eDNA production, transport, and degradation; sample collection and processing to maximize detection rates; and applications of eDNA for conservation using citizen scientists. This body of work is an important contribution to the ongoing efforts to take eDNA detection of macroorganisms from technical breakthrough to established, reliable method that can be used in survey, monitoring, and research applications worldwide. While the rapid advances in this field are remarkable, important challenges remain, including consensus on best practices for collection and analysis, understanding of eDNA diffusion and transport, and avoidance of inhibition in sample collection and processing. Nonetheless, as demonstrated in this special issue, eDNA techniques for research and monitoring are beginning to realize their potential for contributing to the conservation of biodiversity globally.
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1. Introduction

Since Ficetola et al. (2008) first demonstrated that detection of vertebrates using environmental DNA (eDNA) in water samples was possible, interest in using this tool for the biological conservation of fishes, amphibians, and aquatic invertebrates has grown rapidly. The potential for eDNA methods to improve detection sensitivity and cost efficiency over aquatic field surveys was recognized early on, and eDNA detection of macroorganisms from water samples was quickly demonstrated to work across aquatic systems (Goldberg et al., 2011; Jerde et al., 2011; Thomsen et al., 2012a,b). Research has since addressed three major topics: the detection of endangered species, the tracking of invasive species, and the refinement of field and laboratory methods (e.g., Barnes et al., 2014; Mahon et al., 2013; Wilcox et al., 2013). This special issue presents a body of research from scientists working to further advance the application of eDNA methods for conservation and management of aquatic macroorganisms. The 11 papers included in this issue focus on the technical aspects of analyzing eDNA samples, eDNA production and degradation, and applications of eDNA in detection and management programs for endangered fishes and amphibians.

The field of eDNA analysis of samples for detection of macroorganisms developed from the study of micro-organisms in environmental samples as well as from the fields of ancient DNA (e.g., Willerslev and Cooper, 2005) and microbial source tracking (Harwood et al., 2013). While the goal of micro-organismal analysis of water and soil samples is often to characterize all or a subset of biodiversity in samples (Taberlet et al., 2012), the detection of macroorganisms from eDNA samples has, to date, primarily focused on a small set of target endangered and/or invasive species. A promising area of future conservation research will bridge this gap by providing novel ways to study complex ecological interactions such as relationships between ecosystem structure and disease dynamics across trophic levels. Thomsen and Willerslev (2015) review the history of eDNA analysis in the context of these related fields.

2. Processes affecting eDNA detection

Three major processes affect the detection of eDNA: production, transport, and degradation. The rate of production of eDNA from macro-organisms has been quantified in few cases so far (Goldberg et al., 2013; Pilliod et al., 2014; Takahara et al., 2012; Thomsen et al., 2012b) and may be affected by the size, health, sex, and density of organisms. Klymus et al. (2015) found that diet

* Corresponding author. Tel.: +1 509 335 3673.
E-mail address: caren.goldberg@wsu.edu (C.S. Goldberg).

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had a large impact on the eDNA production of two species of carp (*Hypophthalmichthys* spp.) while temperature did not, indicating that the majority of eDNA was shed from the gut lining. Additionally, Spear et al. (2015) used quantification of eDNA to investigate the reproductive status of rare hellbenders (*Cryptobranchus alleganiensis*). These authors found evidence of increased production of eDNA during the breeding season, even in a lone captive animal, indicating the potential of using eDNA quantification for determining reproductive status of individuals in the wild. Complicating inference about individuals from eDNA quantification, however, is the large amount of variation among individuals and through time in eDNA production. Strickler et al. (2015) found a 13-fold difference in the amount of eDNA shed by American bullfrog (*Lithobates catesbeianus*) tadpoles held under the same static conditions, and Pilliod et al. (2014) found a similar pattern, with shed rates varying 6-fold among Idaho giant salamanders (*Dicamptodon aterrimus*) in containers. Over a longer time period, Klymus et al. (2015) found high variability through time for similar-sized carp kept in flowing tanks, which averaged out among individuals.

The process of transport or diffusion of eDNA in lentic and lotic systems is critical for sampling design and spatial inference. Diffusion can be limiting in lentic systems (Takahara et al., 2012), indicating that widespread spatial sampling may be important for high detection rates. In lotic systems, detection rates can be high (Goldberg et al., 2011; Pilliod et al., 2013) but spatial inference (i.e., distance upstream that a sample represents) is unknown, although environmental DNA of macroorganisms has been detected up to 12 km from established populations (Deiner and Altermatt, 2014). Laramie et al. (2015) quantified the eDNA of chinook salmon (*Oncorhynchus tshawytscha*) in relation to stream location and found no consistent relationship between stream distance and eDNA concentration. This indicates that eDNA is not accumulating in downstream reaches, but is instead being removed through processes such as settling or destruction from physical forces. This hypothesis is further supported by the work of Jane et al. (2014), who found that the distance eDNA traveled from the source was reduced at low flows.

As eDNA settles, it may be adsorbed to soil particles that delay degradative processes. Turner et al. (2015) elucidate how sediment-adsorbed DNA molecules can persist in freshwater systems much longer than dissolved or suspended DNA. This finding suggests that high flow or other events that disturb stream or water body sediments could re-suspend eDNA up to 6 months later, which could give a false positive for species presence; most suspended eDNA does not last more than 14–60 days (Dejean et al., 2011; Pilliod et al., 2014; Strickler et al., 2015; Thomsen et al., 2012a,b). Turner et al. (2015) suggest avoiding sediments when collecting water for eDNA analysis to meet temporal inference assumptions.

In addition to the removal processes of settling or destruction, eDNA degradation may have a strong influence on how much eDNA is present in a system. Strickler et al. (2015) conducted an experiment to determine the effects of UV-B, temperature, and pH on eDNA degradation rate and found that eDNA lasted longest in colder, darker, and more alkaline conditions. They also found that the three factors were interactive in their effects, indicating they were likely operating through the bacterial community. These findings have implications for the influence of shading, season, and water chemistry when designing eDNA studies to detect species in the wild.

Together, these papers inform our understanding of the processes affecting eDNA detection and indicate the need for further studies in these areas to inform study design that maximizes detection rates and allows for accurate inferences from eDNA detections. In particular, investigations into the variation within and among species in eDNA shedding rates, the processes influencing how far eDNA travels away from a source, and the biotic factors influencing eDNA degradation rate are all important topics for future research.

3. Sample collection and analysis

There are a number of methods for collecting and analyzing eDNA samples, including precipitation of small volumes (Ficetola et al., 2008; Thomsen et al., 2012b) and filtration of larger volumes (Goldberg et al., 2011; Jerde et al., 2011). Filtration provides the advantage of collecting DNA from larger volumes of water, but loses dissolved DNA that may provide increased detection, as reviewed in Deiner et al. (2015). There are also variants on extraction methods, including chloroform-based extraction (Renshaw et al., 2014), physical disruption of cells for lysis (Jerde et al., 2011), and silica-based extractions (Goldberg et al., 2011). Selection of collection and extraction methods can greatly affect eDNA detection of target organisms. Deiner et al. (2015) provide a comparison of filtering and extraction techniques and recommend filtering and extraction with the Qiagen DNeasy® Blood & Tissue kit for detection of macroorganisms. However, their results also indicate that this may not be the best method for characterizing micro-organism biodiversity.

One of the major challenges in eDNA sample analysis is that substances from the rest of the environment are also contained in the sample. While many of these substances are removed during extraction (and potentially filtering) procedures, some are co-extracted and may inhibit the PCR reaction. If this problem goes undetected, samples may be classified as negative when they actually contain the DNA of the target organism. McKee et al. (2015) demonstrate that a silica-based inhibitor removal kit may provide higher rates of detection when compared with 5- and 10-fold dilutions, although some eDNA (approximately 25%) is lost in the process.

Another challenge in eDNA collection is the ability to process and preserve samples at remote field sites. Water samples have been retained on ice for up to 24 h without reducing eDNA detection (Pilliod et al., 2013), and results from Strickler et al. (2015) indicate that keeping samples cool greatly reduces eDNA degradation rate. Takahara et al. (2015) took this a step further and found that freezing and then thawing samples significantly reduced detection. Interestingly, this pattern did not hold when an enzyme formulated for environmental samples was used, indicating that the freeze/thaw process may have increased inhibition rather than causing eDNA fragmentation. This observation is supported by further testing indicating that increasing the concentration of a sample in the PCR reaction also reduced detection. Together, these studies indicate that whether inhibition interferes with eDNA analyses may depend on collection, preservation, and analysis methods. Identifying work flows that maximize eDNA collection while avoiding or compensating for co-extracted inhibitors is essential for forwarding the field of eDNA detection.

4. Conservation applications

Environmental DNA methods are rapidly, though not always smoothly (Darling and Mahon, 2011), moving from technical breakthrough to widespread application for conservation and management. A number of studies have shown that eDNA detection is more sensitive than field surveys for rare and elusive species (Dejean et al., 2012; Jerde et al., 2011; Pilliod et al., 2013). Sigsgaard et al. (2015) add the weather loach (*Misgurnus fossilis*), an endangered freshwater fish, to this list. Laramie et al. (2015) documented the distribution of the endangered chinook salmon

across vast watersheds and demonstrated how repeat sampling for eDNA can be used to document the arrival of migratory species or assess the success of habitat restoration or repatriation for salmon in river networks. Another potentially powerful application of eDNA methods is to estimate population abundance from the concentration of eDNA in water samples; the relationship between biomass of individuals and eDNA concentration has been shown in both lentic (Takahara et al., 2012) and lotic (Pilliod et al., 2013) systems. However, this relationship may not hold for every species, especially at low densities, as is demonstrated by Spear et al. (2015), and needs further investigation before these measures may be ready to inform conservation and management.

An extremely promising aspect of eDNA survey methods is the ability to use the efforts of people that are not taxonomically trained to collect widespread samples. Biggs et al. (2015) present the results of a highly successful test of a citizen science collection protocol for eDNA sampling of an endangered amphibian. Their careful instructions and sample collection kits led to high rates of detection, demonstrating that there is great opportunity in using citizen scientists to contribute to the conservation of species though eDNA collection efforts.

5. Conclusions

Full implementation or supplementation of eDNA methods into survey and monitoring programs will take time, but dedicated professionals around the world are rapidly advancing these methods closer to this goal. This set of papers is at the forefront of technical and applied advances in eDNA sample collection, processing, analysis, and inference. The collection also identifies areas in further need of investigation, including technical aspects of sample collection, DNA extraction, and sample analysis; investigations of production, transport, and degradation; and the overcoming of sample inhibition. Environmental DNA methods have immense promise for highly sensitive, non-invasive detection of target species, including those of conservation concern. These papers take us several steps closer to incorporating eDNA methods into standard survey and monitoring protocols for species of concern around the world.

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