Fisheries Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Review article

Freshwater fisheries assessment using environmental DNA: A primer on the method, its potential, and shortcomings as a conservation tool

Nathan T. Evans*, Gary A. Lamberti

Department of Biological Sciences, University of Notre Dame, 100 Galvin Life Sciences Center, Notre Dame, IN, 46556, United States

ARTICLE INFO

Handled by George A. Rose

Keywords:
Environmental DNA
eDNA
Biodiversity
eDNA metabarcoding
Bioassessment

ABSTRACT

Conservation of fishes is dependent on accurate and precise data about the distributions and population status of at-risk species. Moreover, effective management of fisheries requires that data be collected in ecologically and politically actionable timeframes. Environmental DNA (eDNA) analysis, the extraction and identification of DNA from environmental samples, is a relatively new bioassessment method with the potential to improve species detection probabilities and efficiency compared to traditional capture or observation-based sampling approaches. Here, we provide a primer on eDNA analysis as it pertains to fisheries ecology and conservation. We provide a summary of the history and current status of eDNA analysis with particular attention paid to the study of fishes. We highlight the major advances that have transformed eDNA analysis into an application-ready tool that can assist fisheries professionals in achieving research and conservation goals. Furthermore, we provide an overview of the limitations of eDNA as they pertain to fisheries science.

1. Introduction

Global declines in fisheries are among the critical conservation challenges of the 21st century (Worm et al., 2006; Dudgeon, 2010; Coulthard et al., 2011). Extinction rates for freshwater biota, including fishes, exceed those of even the most highly impacted terrestrial fauna and flora (Ricciardi and Rasmussen, 1999; Sala, 2000). Declines in aquatic biodiversity threaten to negatively impact the health and livelihoods of millions of people around the world who depend on ecosystem services provided by aquatic biota (Díaz et al., 2006; Brummett et al., 2013). Threats to global fish diversity and sustainability emanate from diverse mechanisms including overexploitation, flow modification, water pollution, habitat degradation, genetic pollution, climate change, and invasive species introductions (Revenga et al., 2005; Dudgeon et al., 2006; Suski and Cooke, 2007). The breadth and diversity of these threats to fish diversity makes the conservation of freshwater fisheries logistically challenging.

Among the challenges faced by researchers and managers working to conserve fishes is the need to efficiently and cost effectively collect accurate and precise data on the distributions and population status of fish species in an ecologically and politically actionable timeframe (Thomsen and Willerslev, 2015). Traditional fisheries assessment methods rely extensively on capture or observations of fishes via the use of nets, traps, electrofishing, angling, hydroacoustics, and visual observation (Murphy and Willis, 1996; Bonar et al., 2009). These effort-intensive sampling strategies can, in some cases, fail to provide fisheries scientists with the necessary data to effectively and efficiently implement conservation actions. Traditional fisheries assessment methods can be inadequate or provide less than ideal information for several reasons including (1) issues with morphological identification of closely related and 'cryptic' species, (2) low detection probabilities stemming from the inherent inefficiencies of underwater sampling and the mobility of organisms, and (3) challenges of gear deployment in certain circumstances (Bayley and Peterson, 2001; Mackenzie and Royle, 2005). Environmental DNA (eDNA) surveillance of macrofauna is a recently developed tool that holds potential to improve fisheries assessments and, therefore, conservations success by reducing systematic errors in inference about species richness resulting from low detection probabilities and species misidentifications.

Environmental DNA is the genetic material that can be extracted from an organism's environment rather than from the organism directly. In recent years, the scientific literature demonstrates a growing interest in the use of eDNA to monitor aquatic and semi-aquatic populations of organisms including fishes (Appendix 1 in the Supplementary data). The potential for eDNA surveillance to improve detection-per-unit-effort over traditional assessment methods is an exciting prospect to many researchers, managers, and conservationists. Those focused on monitoring rare and elusive species, such as

* Corresponding author. Present address: Southeast Environmental Research Center, Florida International University, 250L Marine Science Building, North Miami, FL, 33181, United States.
E-mail address: nevans@fiu.edu (N.T. Evans).

http://dx.doi.org/10.1016/j.fishres.2017.09.013
Received 21 February 2017; Received in revised form 6 September 2017; Accepted 14 September 2017

threatened and endangered species, as well as invasive species, welcome alternatives that improve species detection and ultimately conservation success. While much remains to be learned regarding how eDNA persists in and reacts with the surrounding environment, as well as how to best optimize eDNA surveys, the available published literature illustrates that eDNA has reached a level of scientific maturity that it is now a viable tool to aid fisheries professionals in achieving conservation objectives (e.g., Laramie et al., 2015; McKelvey et al., 2016; Evans et al., 2017a)

This paper provides a summary of the status and future of eDNA as it specifically pertains to fisheries science. The goal of this review is to provide classically-trained fisheries biologists and ecologists with a fundamental understanding of how eDNA can be used improve fisheries assessment and increase conservation success. This review builds on the fisheries-centric content provided by Jones (2013) and provides additional details about methods and advancements in the field. More extensive reviews of the history, associated technologies, analyses, and applications of eDNA are provided by Rees et al. (2014), Bohmann et al. (2014), and Thomsen and Willerslev (2015). We emphasize the elements of eDNA that can enhance traditional fisheries methods to achieve better understanding of fish distributions and community composition.

2. The history and basics of eDNA as an assessment method

The use of DNA, isolated from water samples, to detect macroorganisms grew out of the study of microorganism diversity in environmental samples as well as microbial source-tracking and ancient DNA analysis (Goldberg et al., 2015; Thomsen and Willerslev 2015). Researchers began to use eDNA to study microbial diversity in the natural environment in the 1980s (Thomsen and Willerslev, 2015). However, it was not until the early 2000s that researchers began to utilize eDNA for the assessment of macroorganisms. The first applications of eDNA to macroorganisms were to assess the diversity of mammals, birds, and plants in ancient sediments (Willerslev et al., 2003). Within a few years, the detection of an invasive amphibian (American bullfrog *Rana catesbeiana*) in freshwater samples (Ficetola et al., 2008) illustrated the potential of eDNA to detect aquatic vertebrates. Additional studies quickly demonstrated that eDNA can successfully detect the presence of other vertebrate species including fishes (e.g., Dejean et al., 2011; Evans et al., 2016; Jerde et al., 2011; Kelly et al., 2014; Laramie et al., 2015; Takahara et al., 2013; Thomsen et al., 2012a; 2012b; Wilcox et al., 2013), amphibians (e.g., Biggs et al., 2015; Dejean et al., 2012; Goldberg et al., 2011; Pilliod et al., 2013; Thomsen et al., 2012b), mammals (Foote et al., 2012; Thomsen et al., 2012b), and reptiles (Piaggio et al., 2014; Davy et al., 2015; Hunter et al., 2015). Additional applications focused on detection of diverse aquatic invertebrates (Thomsen et al., 2012b; Goldberg et al., 2013; Deiner and Altermatt, 2014; Tréguier et al., 2014).

Studies detecting the presence of macroorganisms differ from those aimed at microorganisms in that the source of DNA for microbes is often obtained from whole organisms present in environmental sample while the DNA obtained for macroorganisms is only present in the form of cellular remains and free DNA (Thomsen and Willerslev, 2015). Environmental DNA assessment of aquatic macroorganisms (referred to as "eDNA analysis" from this point forward) from water samples involves six basic steps: (1) marker selection and primer design, (2) sample collection, (3) sample preparation, (4) DNA extraction, (5) DNA amplification, and (6) eDNA screening/detection calling (Fig. 1). A summary of each step is provided below.

2.1. Marker selection and primer design

Environmental DNA analysis is based on the detection of species-specific genetic marker sequences that consist of relatively short fragments of mitochondrial DNA (mtDNA) that typically span 80–250 bp

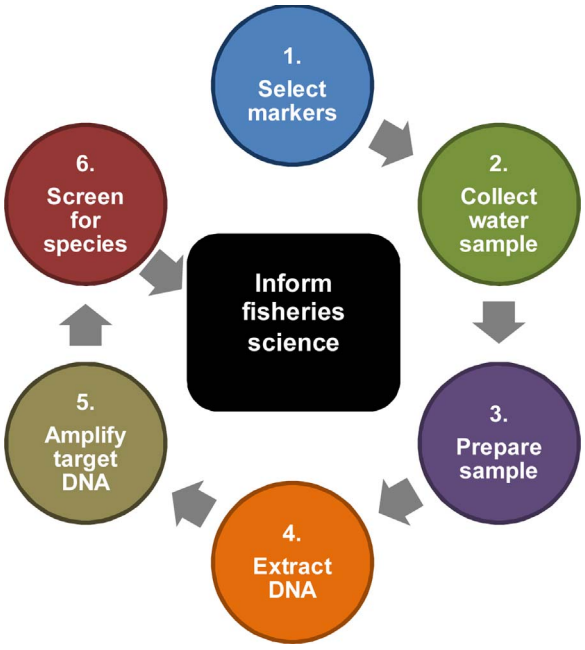

Fig. 1. Major steps associated with processing aquatic environmental DNA (eDNA) samples.

(Bohmann et al., 2014). Mitochondrial DNA is preferred over nuclear DNA in eDNA analysis due to its much greater copy number per cell, which increases the chance of detection in environmental samples where DNA is often degraded and in low concentrations (Rees et al., 2014). Moreover, the majority of eDNA in water samples is likely contained within mitochondria or small cells rather than as free DNA (Turner et al., 2014a). Selection of a marker is based on the species being targeted for detection and the eDNA screening platform being utilized. The goal of marker selection is to utilize a portion of the target species mitochondrial genome that is species-specific, is of the proper fragment size, and is amenable to accurate primer binding (Bohmann et al., 2014; Rees et al., 2014). Once the marker region is selected, forward and reverse PCR primers (short nucleotide sequences that bind to template DNA and function as a starting point for DNA replication) and associated probes, if performing quantitative PCR (qPCR) or digital PCR (dPCR), are designed. These primers are designed to ensure high target specificity with no base pair mismatches for the target species and as many mismatches as possible for any closely-related or co-occurring species (Wilcox et al., 2013).

2.2. Sample collection

Methods for collecting water samples for eDNA analysis vary among studies. Water can be dipped from the surface, or slightly below the surface, by hand (e.g., Jerde et al., 2011), collected at depth using limnological water samplers (e.g., Van Dorn sampler; Eichmiller et al., 2014), or pumped via peristaltic pump into a sample container (e.g., Goldberg et al., 2011). All collection methods require a high degree of care to avoid contaminating samples with target DNA from sources other than the body of water being sampled. Sampling equipment and bottles should be sterile or decontaminated with chlorine bleach (sodium hypochlorite, NaClO) prior to sampling. Researchers often bleach any equipment that comes into contact with the water sample prior to sampling via a minimum 10-min exposure to 10% chlorine bleach solution to sanitize any DNA present (Prince and Andrus, 1992). Additionally, sealed bottles of distilled or reverse-osmosis water, termed "cooler blanks" or "field controls" are often carried into the field by researchers to function as full process indicators of contamination (Jerde et al., 2011; Turner et al., 2015). Cooler blanks are treated the