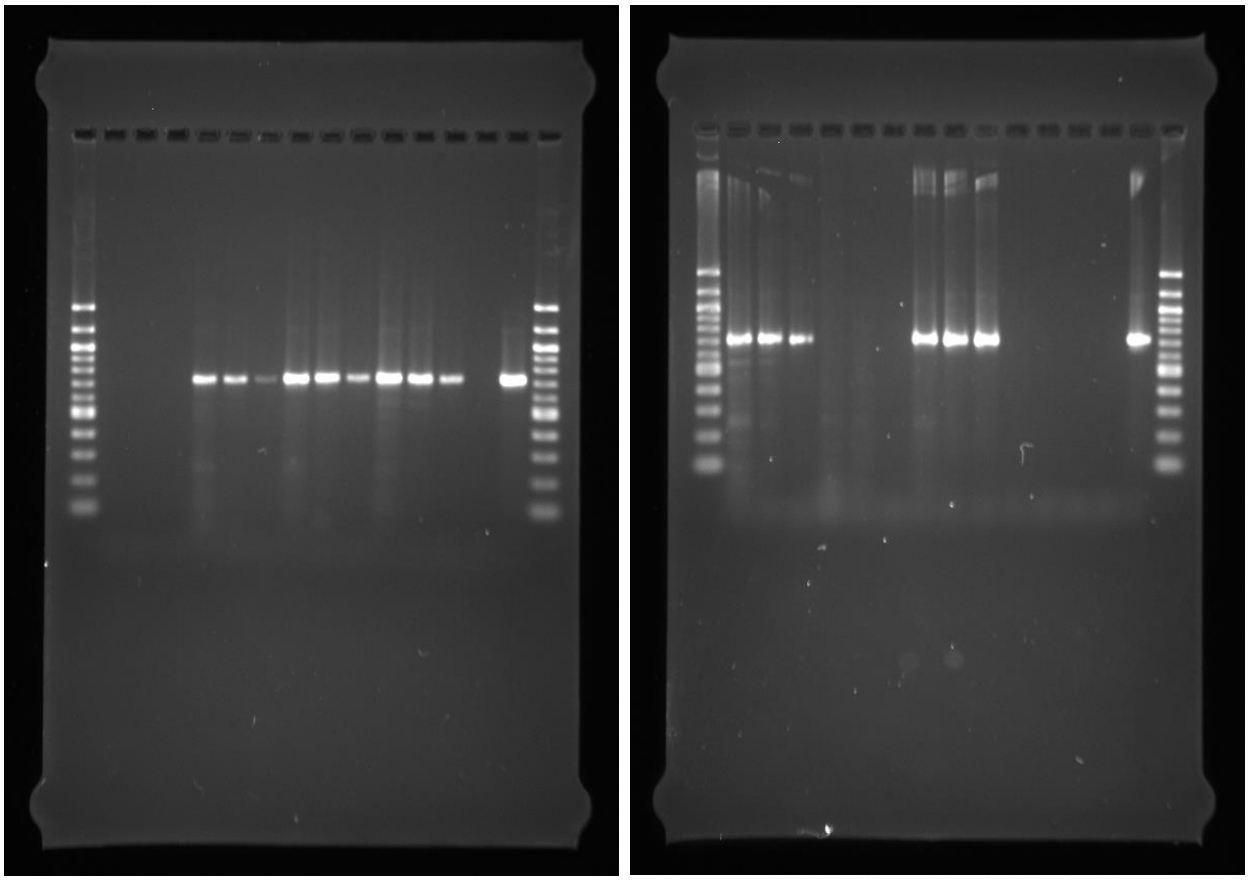
RECLAMATION

Managing Water in the West

Aquatic Species Surveillance Using Environmental DNA (eDNA)

Research and Development Office
Science and Technology Program
Final Report ST-2014-2105-01



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Environmental DNA or eDNA has been defined as *genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material*. The concept originated from the study of micro-organisms (e.g., metagenomics) in environmental samples, but has developed to include eDNA analysis of environmental samples for the detection of rare or elusive macro-organisms. Due to the difficulty of monitoring rare or elusive aquatic species by traditional surveillance methods, eDNA has recently become an increasingly popular surveillance tool. Prominent examples relevant to Reclamation include the detection of endangered species and the tracking of invasive species. However, the shift from traditional visual identification methods to molecular detection assays has great implications for water managers and decision makers. Despite the rapid increase in popularity of eDNA monitoring, there still remain numerous concerns regarding the accuracy and reliability of DNA-based assays in the monitoring of aquatic species.

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Bureau of Reclamation

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Mission Statements

The U.S. Department of the Interior protects America's natural resources and heritage, honors our cultures and tribal communities, and supplies the energy to power our future.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

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Note: This scoping level project was funded in FY14, however, funding was returned when the Environmental Applications and Research Group was tasked to provide priority support for Reclamation construction projects. A summary is provided here at the request of the Science and Technology Program.

Executive Summary

Environmental DNA (eDNA) has been defined as *genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material*.[1] The concept originated from the study of micro-organisms (e.g., metagenomics) in environmental samples, but it has developed to include eDNA analysis of environmental samples for the detection of rare or elusive macro-organisms. Due to the difficulty of monitoring rare or elusive aquatic species by traditional surveillance methods, eDNA has recently become an increasingly popular surveillance tool. Prominent examples relevant to the Bureau of Reclamation include the detection of endangered species and the tracking of invasive species. However, the shift from traditional visual identification methods to molecular detection assays has great implications for water managers and decisionmakers. Despite the rapid increase in popularity of eDNA monitoring, numerous concerns still remain regarding the accuracy and reliability of DNA-based assays in the monitoring of aquatic species.

[1] Source: "Environmental DNA: A Powerful New Tool for Biological Conservation," *Biological Conservation*, March 2015, pp. 83:1-102.

1. Project Synopsis

Environmental DNA (eDNA) has been defined as *genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material*.
[1] The concept originated from the study of micro-organisms (e.g., metagenomics) in environmental samples, but it has developed to include eDNA analysis of environmental samples for the detection of rare or elusive macro-organisms.

Due to the difficulty of monitoring rare or elusive aquatic species by traditional surveillance methods, eDNA has recently become an increasingly popular surveillance tool. Prominent examples relevant to the Bureau of Reclamation (Reclamation) include the detection of endangered species and the tracking of invasive species. For aquatic species, eDNA is usually captured by filtration of large volumes of water where the target organisms may reside. Due to its high level of sensitivity and specificity, molecular assays based on the polymerase chain reaction (PCR) are used to detect eDNA in environmental samples. It involves amplifying a targeted region of the DNA across several orders of magnitude, making it possible to detect and quantify a particular DNA sequence that is species-specific.

The detection of vertebrates using eDNA in water samples was first demonstrated in 2008 (Ficetola et al.). In the years since, interest in using this tool to detect amphibians, fishes, and aquatic invertebrates has grown rapidly, and there are now a large number of published eDNA methods. A short list of recommended reading is included at the end of this summary report.

The PCR laboratory in the Technical Service Center was first proposed and established by the author in 2006 for the early detection of zebra and quagga mussels when a need was identified to provide as much advance warning as possible for the managers of Reclamation water delivery structures. The potential for management or mitigation is highest when invasive species are at the earliest stage of infestation. Since then, the laboratory has expanded the number of molecular biology applications to help address aquatic species that are regulated and/or pose an issue in the operation and maintenance of Reclamation facilities. With few modifications, this laboratory can utilize eDNA protocols as a key component of a surveillance program for aquatic species.

1.1 Decision Framework Issues

Two research questions were posed by this scoping level project:

[1] Source: "Environmental DNA: A Powerful New Tool for Biological Conservation," *Biological Conservation,* March 2015, pp. 83:1-102.

1. Is it possible to perform environmental DNA (eDNA) surveys to test for the presence and distribution of aquatic species (including endangered species) that require our attention as they relate to the operations and maintenance of Reclamation facilities?

2. Is it possible for eDNA to serve as a more sensitive and representative sampling for rare and elusive targets that are impossible or difficult to find using traditional surveillance methods?

The shift from traditional visual identification methods to molecular detection assays has great implications for water managers and decisionmakers. Despite the rapid increase in popularity of eDNA monitoring, numerous concerns still remain regarding the accuracy and reliability of DNA-based assays in the monitoring of aquatic species. These assays were originally developed for research applications, rather than to provide information for an informed regulatory or management decision. Reclamation water managers who are charged with the responsibility of complying with regulations governing endangered species, or who are tasked with protecting facility operation and maintenance, may be faced with the difficulty of trusting results from an assay without the benefit of independent method validation and laboratory accreditation.

In the literature, some of the most common sources of error identified in DNA or eDNA testing include the lack of sensitivity and/or species specificity, insufficient laboratory quality assurance and/or the lack of independent verification of laboratory quality assurance, poorly designed sampling plans and protocols to account for sampling errors, and a lack of scientific understanding of the relationship between DNA persistence/transport vectors versus the actual presence or absence of target species. Although DNA-based methods promise to improve upon traditional monitoring methods, and some water management agencies have made heavy use of these methods, there are still technical impediments and quality assurances that must be resolved before adoption of these tools can be made within a decisionmaking framework.

For a useful assessment of obstacles associated with integrating DNA-based methods into aquatic species management, the reader is directed to this article:

Darling, J.A., and A.R. Mahon. 2011. "From Molecules to Management: Adopting DNA-Based Methods for Monitoring Biological Invasions in Aquatic Environments," *Environmental Research*, 111(7):978-988.

A recent round robin study supported by the U.S. Fish and Wildlife Service demonstrated that DNA-based assays have not reached the "gold standard" of long accepted microscopy methods in terms of both false negative and false positive results. A summary of the results from 19 participating laboratories can be found in:

Frischer, M.E., K.L. Kelly, and S.A. Nierzwicki-Bauer. 2012. "Accuracy and Reliability of *Dreissena* spp. Larvae Detection by Cross-Polarized Light Microscopy, Imaging Flow Cytometry, and Polymerase Chain Reaction Assays," *Lake and Reservoir Management*, 28:265-276.

In this study, all three analytical methods were found to have presence/absence errors, but PCR was the least reliable detection method with 75.8% accuracy. One of the goals of this study was to provide an opportunity for independent performance testing for laboratories using DNA-based methods and to demonstrate that such a program of independent performance testing can be a step toward a comprehensive methodology validation and laboratory accreditation. Validation or certification programs involving DNA-based assays already exist for the testing of human and animal pathogens.

Another source of important information is the National Invasive Species Council (NISC), of which the U.S. Department of the Interior is a member. Expertise inputs are provided to NISC by the Invasive Species Advisory Committee (ISAC). This committee developed and approved a white paper on PCR reliability and laboratory accreditation. It also included a description of the legal and scientific issues surrounding the use of eDNA as an emerging and nonvalidated technology for the detection of Asian carp in the Great Lakes basin.

U.S. Department of the Interior, National Invasive Species Council. May, 2012. "Validation of PCR-Based Assays and Laboratory Accreditation for Environmental Detection of Aquatic Invasive Species." www.invasivespecies.gov.

This white paper recommended a regulated framework for DNA-based assay validation that would increase the reliability of DNA-based assays and the confidence of managers and decisionmakers in the results produced from DNA-based assays.

1.2 Suggested Reading List

The reader is first directed to an upcoming volume of *Biological Conservation* (March, 2015), 83:1-102, Special Issue – "Environmental DNA: A Powerful New Tool for Biological Conservation." These are open access articles that can be found at www.sciencedirect.com. This special issue presents 11 papers describing the application and technical aspects of eDNA methods for the conservation and management of aquatic macro-organisms.

Other recommended articles on eDNA, many of which are open access articles, include:

Barnes, M.A., C.R. Turner, C.L. Jerde, M.A. Renshaw, W.L. Chadderton, and D.M. Lodge. 2014. "Environmental Conditions Influence eDNA Persistence in Aquatic Systems." *Environmental Science & Technology*, *48*(3):1819–27.

Darling, J.A., and M.J. Blum. 2007. "DNA-Based Methods for Monitoring Invasive Species: A Review and Prospectus." *Biological Invasions*, 9(7):751–765.

Dejean, T., A. Valentini, A. Duparc, S. Pellier-Cuit, F. Pompanon, P. Taberlet, and C. Miaud. 2011. "Persistence of Environmental DNA in Freshwater Ecosystems." *PloS one*, 6(8):e23398.

Dejean, T., A. Valentini, C. Miquel, P. Taberlet, E. Bellemain, and C. Miaud. 2012. "Improved Detection of an Alien Invasive Species Through Environmental DNA Barcoding: The Example of the American Bullfrog *Lithobates catesbeianus*." *Journal of Applied Ecology*, 49(4):953–959.

Ficetola, G.F., C. Miaud, F. Pompanon, and P. Taberlet. 2008. "Species Detection Using Environmental DNA from Water Samples." *Biology Letters*, 4(4):423–425.

Foote, A.D., P.F. Thomsen, S. Sveegaard, M. Wahlberg, J. Kielgast, L. Kyhn, and M.T.P. Gilbert. 2012. "Investigating the Potential Use of Environmental DNA (eDNA) for Genetic Monitoring of Marine Mammals." *PloS one*, 7(8):e41781.

Goldbert, C.S., A. Sepulveda, A. Ray, J. Baumgardt, and P. Waits. 2013. "Environmental DNA as a New Method for Early Detection of New Zealand Mudsnails (*Potamopyrgus antipodarum*)." *Freshwater Science*, 32(3).

Goldberg, C.S., D.S. Pilliod, R.S. Arkle, and L.P. Waits. 2011. "Molecular Detection of Vertebrates in Stream Water: A Demonstration Using Rocky Mountain Tailed Frogs and Idaho Giant Salamanders." *PloS one*, 6(7):e22746.

Jerde, C.L., W.L. Chadderton, A.R. Mahon, M.A. Renshaw, J. Corush, M.L. Budny, S. Mysorekar, and D.M. Lodge. 2013. "Detection of Asian Carp DNA as Part of a Great Lakes Basin-Wide Surveillance Program." *Canadian Journal of Fisheries and Aquatic Sciences*, *5*(April):1–5.

Jerde, C.L., A.R. Mahon, W.L. Chadderton, and D.M. Lodge. 2011. "Sight-unseen Detection of Rare Aquatic Species Using Environmental DNA." *Conservation Letters*, 4(2):150–157.

Kelly, R.P., J.A. Port, K.M. Yamahara, and L.B. Crowder. 2014. "Using Environmental DNA to Census Marine Fishes in a Large Mesocosm.: G.E. Hofmann, Ed. *PLoS one*, *9*(1):e86175.

Lodge, D.M., C.R. Turner, C.L. Jerde, M.A. Barnes, L. Chadderton, S.P. Egan, and M.E. Pfrender. 2012. "Conservation in a Cup of Water: Estimating Biodiversity and Population Abundance from Environmental DNA." *Molecular Ecology*, 21(11):2555–2558.

Mahon, A.R., C.L. Jerde, M. Galaska, J.L. Bergner, W.L. Chadderton, D.M. Lodge, and L.G. Nico. 2013. "Validation of eDNA Surveillance Sensitivity for Detection of Asian Carps in Controlled and Field Experiments." M.R. Liles, Ed. *PLoS one*, 8(3):e58316.

Minamoto, T., H. Yamanaka, T. Takahara, M.N. Honjo, and Z. Kawabata. 2011. "Surveillance of Fish Species Composition using Environmental DNA." *Limnology*, 13(2):193–197.

Olson, Z.H., J.T. Briggler, and R.N. Williams. 2012. "An eDNA Approach to Detect Eastern Hellbenders (*Cryptobranchus alleganiensis*) using Samples of Water." *Wildlife Research*, 39:629–636.

Piaggio, A. J., R.M. Engeman, M.W. Hopken, J.S. Humphrey, K.L. Keacher, W.E. Bruce, and M.L. Avery. 2013. "Detecting an Elusive Invasive Species: A Diagnostic PCR to Detect Burmese Python in Florida Waters and an Assessment of Persistence of Environmental DNA." *Molecular Ecology Resources*, 14:374-380.

Pilliod, D.S., C.S. Goldberg, R.S. Arkle, and L.P. Waits. 2013. "Estimating Occupancy and Abundance of Stream Amphibians using Environmental DNA from Filtered Water Samples." *Canadian Journal of Fisheries and Aquatic Sciences*, 1130(January):1123–1130.

Pilliod, D.S., C.S. Goldberg, R.S. Arkle, and L.P. Waits. 2014. "Factors Influencing Detection of eDNA from a Stream-Dwelling Amphibian." *Molecular Ecology Resources*, 14(1):109–116.

Santas, A. J., T. Persaud, B.A. Wolfe, and J.M. Bauman. 2013. "Noninvasive Method for a Statewide Survey of Eastern Hellbenders *Cryptobranchus alleganiensis* Using Environmental DNA." *International Journal of Zoology*, 2013:1–6.

Taberlet, P., E. Coissac, M. Hajibabaei, and L.H. Rieseberg. 2012. "Environmental DNA." *Molecular Ecology*, 21(8):1789–1793.

Takahara, T., T. Minamoto, and H. Doi. 2013. "Using Environmental DNA to Estimate the Distribution of an Invasive Fish Species in Ponds. S. Consuegra, Ed. *PloS one*, 8(2):e56584.

Takahara, T., T. Minamoto, H. Yamanaka, H. Doi, and Z. Kawabata. 2012. "Estimation of Fish Biomass using Environmental DNA." J.A. Gilbert, Ed. *PloS one*, 7(4):e35868.

Thomsen, P.F., J. Kielgast, L.L. Iversen, P.R. Møller, M. Rasmussen, and E. Willerslev. 2012. "Detection of a Diverse Marine Fish Fauna using Environmental DNA from Seawater Samples." *PloS one*, 7(8):e41732.

Thomsen, P.F., J. Kielgast, L.L. Iversen, C. Wiuf, M. Rasmussen, M.T.P. Gilbert, and E. Willerslev. 2012. "Monitoring Endangered Freshwater Biodiversity using Environmental DNA." *Molecular Ecology*, 21(11):2565–73.

Turner, C.R., M.A. Barnes, C.C.Y. Xu, S.E. Jones, C.L. Jerde, and D.M. Lodge. 2014. "Particle Size Distribution and Optimal Capture of Aqueous Macrobial eDNA. *Methods in Ecology and Evolution*, 5(7):676-684.

Wilcox, T.M., M.K. Schwartz, K.S. McKelvey, M.K. Young, and W.H. Lowe. 2013. "A Blocking Primer Increases Specificity in Environmental DNA Detection of Bull Trout (*Salvelinus confluentus*)." *Conservation Genetics Resources*, 6:283-284.

Wilcox, T.M., K.S. McKelvey, M.K. Young, S.F. Jane, W.H. Lowe, A.R. Whiteley, and M.K. Schwartz. 2013. "Robust Detection of Rare Species using Environmental DNA: The Importance of Primer Specificity." *PloS one*, 8(3):e59520.