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Environmental DNA as a Tool for Invasive Species Detection and Management

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Environmental deoxyribonucleic acid (eDNA) is broadly defined as any DNA that is present within a given environment and that might be collected in samples of environmental media. Recent application of eDNA has focused primarily on DNA shed by animals and plants in water, sediment, air, or soil. Environmental DNA samples can be analyzed to detect the presence of DNA from one or more target species or to characterize biodiversity in an ecological community. Use of eDNA sampling makes it possible to infer the presence of non-native or invasive species even when only one or a few individuals are present in the surrounding environment.[1]

Environmental DNA sampling allows for precise, DNA marker-based taxonomic identification, including taxa that are small, cryptic, and/or difficult to distinguish morphologically. In comparison with traditional collection and identification methods, such as electrofishing, seine or trawl nets, trapping, or visual surveys, eDNA techniques and sampling strategies may be highly targeted and sensitive for rare species or those that are at low densities in the sampling area. The collection of eDNA samples can be cost-effective, less intrusive to the ecosystem, and safer for wildlife and field staff.[2] Environmental DNA sampling may facilitate monitoring efforts in hard to access areas and can justify the need for additional traditional sampling methods. Finally, the unused volume of DNA extract and any unconsumed portion of the filter (e.g., in instances when DNA is extracted from only half of a filter) are archivable for future eDNA analyses targeted at both the initial species of interest, along with other species that might become of concern.

Application of eDNA sampling may be particularly beneficial for initial detection, identification, and pathway delimitation of introduced species, tracking their spread, or monitoring for survivors of eradication efforts.[3] For example, management agencies may employ eDNA sampling to monitor for new introductions of watch-list species in high-risk areas. Eradication or control is more feasible when new species introductions, or expansion of existing invasive species, are detected early, while their numbers are small. Following treatments to eradicate an invasive species in a particular area, eDNA can help determine whether the targeted invasive species' eDNA may still be present, and whether re-treatment is necessary. As eDNA methods are further refined, additional applications could include routine analysis of priority pathways of introduction, such as ships' ballast water and imported shipments of aquatic organisms. Once fully developed for routine use, *in situ* automated robotic eDNA

[1] The terminology in this white paper focuses on the application of eDNA to invasive species, however these techniques can be applied to a broad range of species of concern including non-natives whose potential impacts have yet to be determined.

[2] JA Darling, "How to learn to stop worrying and love environmental DNA monitoring," *Aquatic Ecosystem Health & Management* 22 (2019): 440-451, https://doi.org/10.1080/14634988.2019.1682912.

[3] For example, see CS Goldberg, et al. "Critical considerations for the application of environmental DNA methods to detect aquatic species," *Methods in Ecology and Evolution* 7 (2016): 1299-1307; and RE Valentin, et al. "Early detection of invasive exotic insect infestations using eDNA from crop surfaces," *Frontiers in Ecology and the Environment* 16 (2018): 265-270, https://doi.org/10.1002/fee.1811.

samplers on water gages or autonomous vehicles could allow for greater temporal sampling and real-time monitoring for non-native or invasive species.[4]

As with any monitoring technique, eDNA methods require thoughtful planning and evaluation of many factors for effective use. The sampling strategy and assay used must be carefully selected based on the species of interest, density and distribution of the target(s), local conditions, and empirical estimation of detection limits to ensure detection of rare targets. Sampling strategies, interpretation of results, and corresponding management goals should be considered in the early stages to guide experimental design.[5] The field of eDNA is not unique in requiring interpretation of results; traditional methods often include interpretation, sometimes with significant uncertainty. As the eDNA field and associated guidance are rapidly evolving, specialized laboratories with appropriate technical capacity and controls, as well as knowledgeable subject matter experts, play key roles in ensuring that sampling, analyses, and interpretation of results meet best practices.

This white paper focuses on eDNA tools for invasive species monitoring and detection by U.S. Federal agencies. It builds on the work of an interagency task team under the auspices of the National Invasive Species Council (NISC), which developed a technical report for invasive species managers entitled, "Strategic considerations for invasive species managers to utilize environmental DNA (eDNA): if, when, and how."[6] This white paper summarizes the findings of the technical report and provides specific considerations for NISC member agencies and other interested parties. Federal agencies can best incorporate eDNA sampling as an invasive species detection tool by building on successful applications and established programs, and by recognizing and taking advantage of the unique aspects of this relatively new, but potentially powerful method. To advance such efforts, federal agencies should consider work in two key areas:

- Capitalizing on existing investments and programs related to eDNA monitoring
- Facilitating implementation of eDNA sampling for invasive species surveillance

Capitalizing on existing investments and programs related to eDNA monitoring

Federal programs in the United States have been instrumental in advancing the field of eDNA and its application to invasive species detection and management. Here we highlight some existing eDNA programs and initiatives being implemented by federal agencies or involving significant engagement of federal personnel. It should be noted that this is a representative list and that there are many federal laboratories not listed that also provide important eDNA sampling research and management support. Awareness of these initiatives and coordination among federal and non-federal invasive species management efforts would ensure that such efforts continue to grow and advance the collective expertise of NISC agencies and their partners on eDNA and its applications. Additional programs and initiatives are outlined in the supporting technical paper.

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[4] BK Hansen, et al. "Remote, autonomous real-time monitoring of environmental DNA from commercial fish," *Scientific Reports* 10 (2020): 13272, https://doi.org/10.1038/s41598-020-70206-8; and KM Yamahara, et al. "*In situ* Autonomous Acquisition and Preservation of Marine Environmental DNA Using an Autonomous Underwater Vehicle," *Frontiers in Marine Science*, 6:373 (2019), doi:10.3389/fmars.2019.00373.

[5] CL Jerde, et al. Guidance for Environmental DNA Sampling Design and Effort. (Ann Arbor: Great Lakes Fisheries Commission, 2019); and TM Wilcox, et al. "Comment: the importance of sound methodology in environmental DNA sampling," *North American Journal of Fisheries Management* 38 (2018): 592-596, https://doi.org/10.1002/nafm.10055.

[6] JT Morisette, et al. "Strategic considerations for invasive species managers in to utilize environmental DNA (eDNA): if, when and how," *Management of Biological Invasions* 12:3 (2021): 747-775. https://doi.org/10.3391/mbi.2021.12.3.15.

Federal invasive species programs and initiatives would benefit from capitalizing on existing investments and programs related to eDNA, including:

NOAA 'Omics Strategy[7]: The National Oceanic and Atmospheric Administration (NOAA) recently developed an agency-wide 'Omics strategy, which provides a framework to advance the application of a suite of advanced research methods (e.g., eDNA, genomics, transcriptomics, proteomics, metabolomics) to address mission priorities. It explicitly references invasive species and more generally prioritizes work on enhancing infrastructure, transitioning research to applications, and expanding partnerships. **Coordination with other federal agencies could further leverage the invasive species and partnership goals in NOAA's 'Omics Strategy and Strategic Plan contributing to collective federal eDNA efforts to track and better manage invasive species.**

USGS eDNA NAS Database Standards: The Non-indigenous Aquatic Species (NAS) database is the primary source of aquatic invasive species distribution data in the United States, drawing on data from dozens of federal, state, and non-governmental partners. The U.S. Geological Survey (USGS) used broad stakeholder engagement to develop field, laboratory, and reporting data and metadata standards for display of eDNA survey results in the NAS Database. Establishment of eDNA standards allows reliable eDNA data to be integrated with existing physical observation data in the NAS Database. **By applying these standards, community-derived eDNA data can be displayed in the NAS Database and participating federal agencies can contribute to improving the comprehensive knowledge of non-native aquatic species distributions in the United States.**

USFS Aquatic eDNAtlas: The U.S. Forest Service (USFS) National Genomics Center for Wildlife and Fish Conservation worked with partners to develop the Aquatic eDNAtlas. The Aquatic eDNAtlas, is an open-access database using crowd-sourced field surveys that provide eDNA sampling results with precise spatial location information on native and invasive aquatic species in the United States. The database currently houses over 22,000 data points generated in partnership with federal and non-federal entities. **Future surveillance efforts should seek to integrate eDNA sampling results into stable databases such as the Aquatic eDNAtlas and/or NAS Database.**

The Government eDNA Working Group (GEDWG): GEDWG is an informal interagency working group comprised of eDNA scientists from numerous federal, state, provincial, and local agencies and universities. The group is focused on the exchange of technical information, including sharing best practices, lessons learned, and new advances. GEDWG can serve as a resource for addressing technical issues and fostering communications across the community of subject matter experts working with eDNA. This could include further engagement with other national and international efforts developing and accessing eDNA applications. Current information on joining GEDWG can be found at the USGS eDNA CoP page. **Participation in GEDWG can assist with inter-agency coordination that promotes skill development and information sharing on the latest developments in the field.**

ANS Task Force: The Aquatic Nuisance Species Task Force (ANS Task Force) works through its membership and regional panels to coordinate efforts related to aquatic invasive species management, including the application and limitations of eDNA. This includes linkages to individual federal agency efforts (see USGS NAS Database and USFS eDNAtlas), along with work by its regional panels. For example, the Western and Mississippi River Basin Regional Panels both have eDNA working groups that foster coordination and information sharing between technical experts and managers. **The ANS Task Force regional panels can serve as a valuable resource for exchange of information and coordination**

[7] K Goodwin, et al. *NOAA 'Omics Strategy: Strategic Application of Transformational Tools*. (Silver Spring: National Oceanic and Atmospheric Administration, 2020) doi: https://doi.org/10.25923/1swn-rj62.

of efforts by federal and state agencies as well as other partners in the application of eDNA sampling for priority invasive species.

USFWS Invasive Carp eDNA Program: The U.S. Fish and Wildlife Service (USFWS) Whitney Genetics Laboratory (WGL) has been building eDNA capacity to monitor bighead and silver carp eDNA since 2013 in response to concerns about the movement of invasive carp towards the Great Lakes. Working with many partners, the WGL has become a high throughput eDNA facility with a formal Quality Assurance Program Plan and Communications Plan essential to understanding how results will be interpreted and results will be reported in a consistent and transparent manner agreed upon within the partnership. The USFWS established an **Invasive Carp eDNA Community of Practice that engages laboratory and field staff from federal and state agencies and other partners to provide a forum for managers and biologists to connect and exchange information on invasive carp eDNA monitoring activities, accomplish training, and standardize methods (field, lab, and reporting) that will be a valuable resource for a broad range of partners.**

USFWS eDNA Training: The U.S. Fish and Wildlife Service (USFWS) developed a training course entitled *FWS-CSP2000a Emerging Topics in Conservation Science Workshop – eDNA* to provide resource professionals with the background and tools to evaluate issues where eDNA data can play a role in the management of plants and animals. **Delivered through the National Conservation Training Center, the course can serve as a tool to develop capacity in evaluating eDNA information, assessing appropriate environmental data collection and analysis methods, understanding the benefits and limitations of applying eDNA methods to management issues; and developing study designs using best management practices.**

USFWS Gene Sequencing and Marker Development: The USFWS has funded the Northeast Fishery Center and Whitney Genetics Laboratory to complete mitogenome sequencing of aquatic invasive species (from both native and introduced portions of the range) and develop qPCR markers for aquatic invasive species eDNA projects. Work focuses on species new to the United States or at risk of secondary spread into new watersheds, especially those listed as injurious under the Lacey Act (e.g., Wels Catfish) or that are vectors for OIE-reportable diseases (e.g., Signal Crayfish and Spiny-cheek Crayfish). **Future research into eDNA and other marker development and validation as well as continued research in genetic technologies for use as tools for invasive species management may lead to more sensitive detection methods to inform decision-making for intensive invasive species management actions**.

International programs: Significant interest in eDNA exists outside the United States, including its application to invasive species challenges. For example, Fisheries and Oceans Canada has developed guidance on minimum reporting standards, terminology, and interpretation of data.[8] At the regional level, cooperation between U.S. federal and Canadian partners has been a critical component of successful eDNA surveillance for invasive carp in the Great Lakes. **Federal support for engagement of U.S. eDNA researchers and practitioners with other international experts and national programs could allow for more rapid dissemination of standards and best practices and comparisons of methods on a global scale.**

[8] Canadian Science Advisory Secretariat, *Advice on the Use of Targeted Environmental DNA (eDNA) Analysis for the Management of Aquatic Invasive Species and Species at Risk, Science Advisory Report 2020/058* (Ottawa: Division of Fisheries and Oceans, 2020) https://www.dfo-mpo.gc.ca/csas-sccs/Publications/ResDocs-DocRech/2021/2021_019-eng.html.

Facilitating implementation of eDNA sampling for invasive species surveillance

As with any survey tool, managers need to understand both the strengths and limitations of eDNA approaches to employ it effectively. Sampling strategies are based on clear guidance from the manager on the biology, distribution, and density of the species of concern, as well as the habitat and spatial extent of the study area. Because the detection of an organism's DNA in the environment may mean they are present, were recently present, or are present in connected habitats, full communication and interpretation of results require a detailed understanding of management agency goals. Reviewing possible outcomes with researchers and considering management response options and thresholds based on risk tolerance in advance will facilitate survey design, inform decision-making, and provide clarification to the public. Management goals will inform decisions on the specific eDNA approach(es) to be employed, the minimum/required level of qualifications for the eDNA laboratory and techniques to be used, detection tolerance modeling requirements, and how results will be interpreted and conveyed to inform management actions.

The ability to make these management decisions is tied to critical technical and laboratory capacity to process results, provide quality assurance and quality control, and maintain appropriate data and informational records. Guidance from experienced eDNA practitioners (e.g., direct consultation, well-established practices) and close adherence to quality-assurance and quality-control guidelines for sample collection, processing, and preservation are critical given the technical nature and continuous advances in eDNA methods. Pilot studies may also be used to optimize eDNA methods for local conditions and species of interest, and to identify potential challenges with sampling, processing, analysis, and/or data interpretation prior to larger scale studies.

Several federal agencies already have incorporated eDNA sampling as an established method for specific projects, employing standard protocols for field sampling, laboratory analysis, and interpretation of results (e.g., USFWS Quality Assurance Project Plan).[9] The use of such protocols, or minimum guidelines, developed through interagency coordination and in consultation with non-federal partners, could help in comparing and selecting appropriate methods and interpretation of data, including minimizing and alerting to incidents of false positive or false negative results. However, eDNA methods are rapidly evolving with technical and methodological advances in all aspects of eDNA surveys that can inform both onsite applications, as well as broader analyses at landscape, regional, and national scales. Therefore, agencies need to have a method for rapidly updating protocols to keep pace with the evolving science.

The use of eDNA in regulatory enforcement will require additional consideration. Regulatory agencies at the state and federal levels may want to consider requirements for the use of eDNA in the regulatory context, including method validation, required sampling for species of interest, and technical expertise and capacity.

Environmental DNA methodology can be adapted to the particular life histories of target species and idiosyncrasies of different systems. However, such variation can be challenging for managers and stakeholder groups seeking to gauge the quality and efficacy based on standards for sampling, processing, analyzing, and interpreting eDNA. This underscores the opportunity to leverage the expertise and experience found within individual federal agencies to benefit other federal agencies and non-federal partners. There is a critical need for properly resourced mechanisms to facilitate ongoing communication and information exchange among the community developing and applying eDNA techniques for invasive species monitoring (e.g., GeDWG).

[9] U.S. Fish and Wildlife Service, *Quality Assurance Project Plan: eDNA Monitoring of Bighead and Silver Carps,* prepared for USFWS Great Lakes Region 3 (Bloomington, MN: USFWS, 2022) https://www.fws.gov/sites/default/files/documents/eDNA-QAPP-2022-Whitney-Genetics-Lab.pdf.

Operational implementation of eDNA sampling for invasive species surveillance would benefit from:

1. Coordination among federal agencies and non-federal partners regarding how to interpret eDNA sampling results for invasive species, how to report these results to stakeholders, when to take management action, and which parties are responsible for these roles. This should include processes and procedures for reporting results, as well as a communication plan that clearly conveys project goals, protocols, and intended outcomes to the public and impacted stakeholders.
2. Development of best practices and minimum competency requirements for eDNA analysis laboratories, potentially including laboratory accreditation or certification, cross-laboratory validation of analysis results, and establishment of quality assurance protocols that facilitate interoperability across laboratories.[10]
3. Studies that optimize new and existing sampling and analytical approaches, including development of best practices for single-species assays and community-based analyses, revalidation of field and laboratory workflows, development of eRNA tools, and tests of reproducibility across laboratories.
4. Maintenance and expansion of federally sponsored and publicly accessible databases for invasive species eDNA data and metadata (e.g., NAS Database, Aquatic eDNAtlas, Genomic Observatories Meta-Database (GEOME)). This should include:
    i. Integration of eDNA markers, sampling results and physical samples in public, accessible, generalized, and eDNA-specific databases and genomic sample archives.
    ii. Post-study archiving of environmental samples at appropriate depositories (e.g., through the Global Genome Initiative), including expansion of infrastructure for long-term archival and curation of samples.
    iii. Identification of appropriate reference data repositories including vouchered reference specimens and sequence data for target species.
5. Integration of eDNA capacity with data streams produced by environmental data acquisition tools (e.g., remote sensing, ground sensors, drones), as well as application of statistical methods for comparing results across tools for robust detection (e.g., occupancy modeling).
6. Periodic technical reports or guidance documents stored together in an easily accessible location to help practitioners employ the most current best practices for sampling, assay development, results interpretations, etc.

Conclusion

The intent of this white paper is to provide NISC member agencies with a fundamental understanding of the nature of eDNA as a tool for invasive species management, the types of information to consider when gauging eDNA efforts, and areas where agencies can promote and utilize increasingly powerful eDNA capabilities for meeting invasive species challenges. Actively pursuing the recommendations set forth in this paper will help build on existing programs for invasive species surveillance and eDNA sampling, improve operational implementation across agencies, and ensure that sufficient technical capacity is maintained to meet agency goals.

[10] A Trujillo-González, et al. "Considerations for future environmental DNA accreditation and proficiency testing schemes," *Environmental DNA*, 3:6 (2021) https://doi.org/10.1002/edn3.243.

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