Toward a National eDNA Strategy for the United States

Abstract

Environmental DNA (eDNA) data makes it possible to measure and monitor biodiversity at unprecedented resolution and scale. As use-cases multiply and scientific consensus grows regarding the value of eDNA analysis, public agencies have an opportunity to decide how and where eDNA data fits into their mandates. Within the United States, many federal and state agencies are individually using eDNA data in various applications and developing relevant scientific expertise. A national strategy for eDNA implementation would capitalize on recent scientific developments, providing a common set of next-generation tools for federal natural-resource management and public-health protection. Such a strategy would avoid patchwork and possibly inconsistent guidelines in different agencies, smoothing the way for efficient uptake of eDNA data in federal management. Because eDNA analysis is already in widespread use in both ocean and freshwater settings, we focus here on applications in these environments. However, we foresee broad adoption of eDNA analysis to meet many resource management issues across the nation because the same tools have immediate terrestrial and aerial applications.

Introduction

The United States federal government manages living resources for the benefit of its citizens at a vast scale. Sustainable use of these resources requires federal agencies to detect and monitor many species of commercial interest (e.g., fisheries, timber) and potential threats (e.g., invasive species, pathogens), and to assess shifts in biodiversity in a changing climate all while balancing the environmental impacts of their decisions. Responsible management accordingly requires understanding species distributions, how their abundances change over space and time, and how they adapt to pollution, harvesting, and large-scale stressors such as climate change.

The mismatch between the scale of such tasks and the resources available to address them is increasingly apparent. For example, at present, maintaining a single research vessel to monitor our coasts and Great Lakes costs between US\$2.2-\$40 million per year; the United States has more such vessels than any other nation (Luis Valdes & Intergovernmental Oceanographic Commission, 2017) and other critical environmental-monitoring infrastructure is similarly expensive. Such high costs limit our ability to provide the data needed to improve earth-system modeling and prediction in the face of rapidly changing environmental conditions. Accordingly, there is substantial opportunity for more economically efficient approaches to large-scale biological observation.

Biologists have made tremendous strides over the past decade, learning to collect and analyze the genetic material constantly generated and shed by living organisms. This ambient genetic information – encoded in environmental DNA, or eDNA – reflects the species present in a given place and time and greatly enhances our ability to assess biology in much the same way that remote sensing has revolutionized our perspective on agriculture, oceanography, hydrology, chemistry, and landscape ecology, with applications from weather forecasting to GIS. Importantly, genetic information allows direct measurement of biology and biological responses, as opposed to using chemical and physical oceanographic proxies.

eDNA data has become increasingly applicable to management as technology has matured, throughput has grown, and costs have declined – sequencing one megabase of DNA cost nearly \$5300 in 2001 and was less than \$0.006 in 2021 (Wetterstrand, 2021) – so that large numbers of samples can now be analyzed quickly and cheaply. Widespread methods of analyzing eDNA currently include single-species assays using quantitative PCR (qPCR) or digital PCR (dPCR), and multi-species amplicon sequencing (metabarcoding); qPCR studies have become common over the past decade to track the movement, abundance, and interactions of species over increasingly broad geographic scales (Beng & Corlett, 2020; Miya, 2022), and eDNA metabarcoding work has begun to generate multispecies and community-level views of the same phenomena.

The scientific and technological gains of the past decade make eDNA analysis ready for

use as a practical management tool at a federal scale (Lodge, 2022), and federal agencies have individually developed eDNA applications to meet their own mandates (see examples below). In some applications, the use of eDNA is now a powerful complement to traditional biological monitoring techniques, and in other applications is replacing more expensive and slower traditional techniques (Evans et al., 2017; Qu & Stewart, 2019). As a result, the European Union and nations elsewhere are moving quickly toward standardized eDNA implementation for ecosystem management – as reflected in multiyear efforts funded by Eu-ropean Cooperation in Science and Technology (DNAqua-NET; Leese et al. 2018), and by the European Biodiversity Partnership (e.g., eWhale; https://ewhale.eu/). Canada, for ex-ample, has implemented a cross-sector national standard through the Canadian Standards Association (CSA Group) accredited by the Standards Council of Canada for eDNA re-porting requirements and terminology (Gagné et al, 2021). Other high-profile national- and international-scale documents and applications include examples from Finland (Norros et al. 2022), Australia and New Zealand (De Brauwer et al. 2022; Trujillo-González 2021), UNESCO (2023), and elsewhere.

An eDNA strategy for the United States would capitalize on the last 15 years of eDNA research and development. Such a strategy would harmonize the application of eDNA techniques across agencies, encouraging consistent standards and guidelines as the relevant techniques mature, and thus would avoid a patchwork of inconsistent policies in different agencies. This unified strategy would smooth the path for efficient tools that would lead natural-resources management into the future. Moreover, it could ensure agency practice keeps pace with the dynamism of scientific, technological and industry advances, creating mechanisms to improve the accuracy, reliability and sensitivity of eDNA, broadening species and habitat coverage, while reducing costs. Indeed, many federal statutes require agencies to use the best available technology to meet mission needs, keeping pace with the best available science and most effective methods as they continue to evolve.

81 Management-Ready Applications

eDNA analysis offers a means of improved decision support for environmental management. A national strategy could foster the institutional conditions to ensure that comprehensive and sustained use of eDNA analysis remains salient (answering questions important to decision makers), scientifically credible, and legitimate (standing up to legal scrutiny) (Clark et al., 2016). Increased deployment of consensus eDNA applications could, for example, accelerate U.S. national priority programs including NOAA's large-scale effort to characterize the nation's offshore exclusive economic zone (National Strategy for Ocean Mapping, Exploration, and Characterization (NOMEC)), the goal to conserve 30% of national lands and waters by 2030 (Exec. Order No. 14008, 2021), the National Nature Assessment (Exec. Order No. 14072, 2022), the USGS Biothreats Program, the Department of Interior National Early Detection and Rapid Response (EDRR) Framework, and many others.

Applications of eDNA around which scientific consensus exists fall generally into three categories.

First, using qPCR or digital PCR to detect individual target species at low population density has repeatedly been shown to be more sensitive, faster, and cheaper than traditional biological surveillance and monitoring tools. Applications include the early detection of invasive species, imperiled species, and indicator species.

Within the U.S. government, the Environmental Protection Agency (EPA) led the way with molecular methods to provide rapid water-quality assessments, partnering with academic labs and other agencies to develop qPCR assays for assessing fecal indicator bacteria and markers for sources of fecal contamination (e.g., USEPA 2015, USEPA 2019); identical methods have subsequently been used for COVID detection in wastewater streams (Boehm et al., 2022; Soller et al., 2022). The U.S. Geological Survey (USGS) and EPA – among more than a dozen federal agencies represented on the interagency National Invasive Species Council – leveraged qPCR assays into powerful methods for detecting invasive animal species (Darling, 2019), NISC 2022). USGS, U.S. Fish & Wildlife Service (FWS), and several state agencies have led the effort to monitor invasive carp species; this is perhaps the most well-developed eDNA monitoring program, which has vastly improved our ability to detect harmful species at scale (FWS 2022). eDNA data have similarly proved invaluable in assessing the success of restoration projects – as USGS researchers showed in tracking native salmon reoccupying upstream habitat following the removals of the Elwha dams in

Washington state (Duda et al., 2021).

Second, eDNA metabarcoding makes it possible to assess many species and trophic levels at once, an approach that provides more comprehensive species richness assessments vastly more quickly and cheaply than traditional biological monitoring tools (Andres et al., 2023). These kinds of data are often necessary for environmental-impact assessment under the National Environmental Policy Act (NEPA), state equivalents, and environmental assessments required by other statutes. For example, multispecies eDNA data are being used for environmental assessments in offshore-energy projects permitted by the Bureau of Ocean Energy Management (BOEM 2022).

Third, in many circumstances, eDNA data can provide a useful index of population size – where more of a species is present, more of its DNA is inevitably present – although estimating organismal abundance in an absolute sense remains an area of active research (Shelton et al., 2022; Yates et al., 2021). NOAA, in collaboration with academic partners have demonstrated the quantitative value of eDNA for commercial fisheries at continental scales along both the Atlantic (Metabarcoding; (Stoeckle et al., 2021)) and Pacific (qPCR; (Shelton et al., 2022)) coasts – in both cases, eDNA data closely reflected species' abundance trends estimated by traditional net or acoustic methods. Similarly, a NOAA study quantified a threatened salmon population using qPCR data, where the molecular data captured the same trends with less uncertainty than the traditional (and more labor-intensive) seine nets (Shelton et al. 2019). Moreover, this observation that "more animal = more DNA" is repeatable and robust across DNA isolation methods and markers (Jo and Yamanaka 2022).

In sum, it is clear that across many federal agencies, eDNA is facilitating more efficient and comprehensive data collection, and adding new information to critical resource monitoring, management and conservation. A unified national strategy would consolidate the field's technological gains, harmonize the nascent efforts already in process in different agencies, and facilitate the standardization of methods for widespread management applications.

139 From Research to Management

A national eDNA strategy could accelerate agency uptake of consensus applications (see examples in Figure 1) and, by supporting ongoing research, guide further development of eDNA-based methods for natural-resources management. In particular, government agencies (as opposed to academic researchers, NGOs, or private-sector actors) are uniquely situated to minimize externalities arising from the incentives of individual actors and to generate public benefits efficiently. Accordingly, we highlight these roles below, setting out categories of actions in which a national eDNA strategy would likely require support.

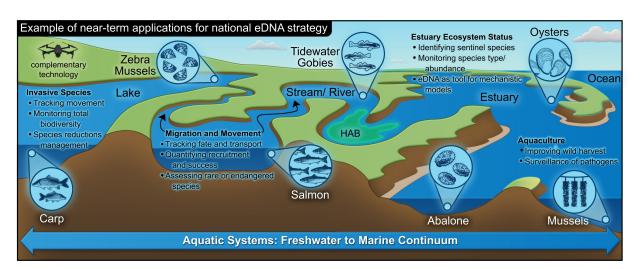


Figure 1: Examples of near-term applications that could be widely implemented under a national eDNA strategy.

Coordination

Although some federal agencies have developed plans to accelerate the routine use of eDNA and other molecular techniques in aquatic systems (Goodwin, 2020a, 2020b; Morisette et

al., 2021; United States. National Oceanic and Atmospheric Administration, 2021) – and although eDNA data has survived judicial scrutiny and has supported federal rulemakings under the Endangered Species Act (ESA; see below) – on the whole, there appears to be a spectrum of acceptability of eDNA data across different agencies. Several key steps would facilitate the high-level harmonization in eDNA policy across federal agencies, with subsidiary benefits to a range of non-federal entities, avoiding confusion and making uptake more efficient.

- Clear Statement of Acceptability. eDNA data have featured significantly in several federal court cases and regulatory decisions (Endangered and Threatened Wildlife and Plants; Endangered Species Status for Black Warrior Waterdog and Designation of Critical Habitat, 2018; Michigan v. U.S. Army Corps of Engineers, 2011), signaling legal acceptability in the contexts of invasive- and endangered-species management. Insofar as eDNA data reflect the species present in or near the sampled environments, for legal purposes, eDNA data are merely another form of biological information and give results analogous to existing survey methods. A formal interagency position statement would clarify the legal status of eDNA data in the federal context and could usefully characterize the minimum attributes of acceptable data (Bustin et al., 2009), and would open the door to broader-scale eDNA applications in the private sector by reducing regulatory uncertainty.
- Mechanisms for Coordination. High-level coordination is a key function of federal governance and would channel existing momentum and provide helpful guidance for state and local governments as well as the judiciary, with core goals being rapid dissemination of the best practices and avoiding inconsistent, ad-hoc policies. Specific activities might include interagency working groups perhaps facilitated by the White House Office of Science & Technology Policy (OSTP), the Council on Environmental Quality (CEQ) or a similar administrative entity to develop guidance and, if necessary, rules for the use of eDNA data under statutes such as the ESA, Marine Mammal Protection Act (MMPA), and National Environmental Policy Act (NEPA), among others. Similar guidance would apply to federal duties under treaties such as CITES and overarching goals such as improving invasive-species biosecurity.
- Common Sets of Best Practices. Developing and distributing best practices for every phase of eDNA analysis, from sampling design, equipment, and collection through interpretation and metadata standards, would consolidate recent advances and identify horizons of opportunity for eDNA-based monitoring. Such an effort would capture evolving science from existing agency practice and from emerging efforts in Canada, the EU (Bruce et al. 2021), and other international entities. As a recent nonpartisan Congressional Research Service Report (Kuiken et al. 2022) notes, such shared resources "could aid in research collaboration, interoperability of reference databases, and quality control, as well as affect how data is analyzed, shared, and used." Quantifying repeatability and reliability of assays for example, via intercalibration experiments using standard reference materials and proficiency testing (as is common, for example, in public health and forensic sciences) would be an important element of the process of developing best practices.
- Mechanisms for Continuous Improvement. Even the best technologies of a given era are eventually superseded, particularly in areas of rapid development such as eDNA analysis. Consequently, there is a danger of developing overly prescriptive sets of best practices and protocols; dynamic science can quickly render such static requirements outmoded, saddling agency scientists with substantial opportunity-costs, unable to benefit from everadvancing techniques (Blancher et al., 2022). This mismatch between the pace of science and the pace of implementation rules is a general problem in administrative law, which federal statutes often solve by including ratcheting mechanisms requiring the use of the best available technologies what-

ever those technologies are at the time of implementation. A national eDNA strategy could include similar best-available-technology mechanisms or other ways of ensuring agency practices keep pace with evolving science, in part by supporting lab-intercalibration studies and certification processes, and by developing clear ways of assessing methodological improvement over current best practices (Bland & Altman, 1986). Certification is a function of government in fields ranging from consumer-product safety to pharmaceuticals, encompassing both products and processes. Wildlife forensics certification, for example, is an existing analog for eDNA process certification, and at least one private eDNA-services company has received international certification for its quality-management system (Ocean News 2022).

Capacity-Building

Adopting eDNA as an information source for routine management will require developing capacity within agencies and across sectors. Federal agencies do a significant amount of capacity-building in the form of primary research (e.g., NOAA and USGS science centers, USDA research-driven sub-agencies), collaboration with academic and other researchers, and outright funding (National Science Foundation, National Institutes of Health). We therefore focus the itemized needs below on mechanisms for deepening and leveraging existing federal commitments as agencies work to move eDNA techniques into routine practice.

- Research & Development. Increasing and coordinating research-and-development across federal eDNA efforts would help expand genetic monitoring from the scale of individual projects into routine, systemic use. Substantial support is necessary to promote and fund collaborative projects, particularly those at first that follow standardization guidelines, and produce freely accessible protocols. Specific activities might include (1) integrating eDNA monitoring into existing surveys perhaps in combination with existing survey techniques, as is beginning to happen at NOAA and other agencies (e.g., Shelton et al. 2022) and generalizable validation exercises via comparison with "gold-standard" metrics (e.g., field samples from populations of known size), (2) investing in the development of scalable, automated sample-collection and analysis platforms, including data-processing, visualization, and data management, and (3) building capacity in the federal workforce to carry out these efforts and to build up long-term, routine use within agencies.
- Public-Private Partnerships. As noted above, federal efforts to advance technology- development often reduce risk to private-sector firms while generating public benefit. Explicitly endorsing the use of eDNA in decisionmaking would create demand for eDNA equipment, supplies, and services, incentivizing private-sector investment and helping to expand a market sector with considerable potential for job creation and economic growth. Promoting eDNA training and workforce-development could be a key area of public-private partnership, and entering into fixed-price contracts for reagents and equipment from preferred vendors for example, via the General Services Administration's GSA Advantage service would create price certainty and scaling incentives for manufacturers. Further, clear guidance on outsourcing requirements would help private-sector eDNA-service firms several of which already exist and are growing rapidly in the domestic and international spheres develop efficient analysis chains.
- Infrastructure. Much of the infrastructure for a genomic revolution in biological monitoring is already in place at federal laboratories and universities nationwide, as well as in a budding private sector. Here again, however, a national strategy could create far more value than currently exists by helping overcome the individualized incentives of the relevant actors. For example: (1) developing standard reference materials for common eDNA assays, as is common in the public-health sphere and many other fields (2) providing state, local, and tribal training perhaps including durable Centers of

Excellence in regions of strategic interest – and (3) building out interoperable databases (below, "Technology Needs") are important for spurring widespread adoption of eDNA-based monitoring techniques, but these measures are often beyond the scope of any one institution's normal activities.

- Communications. Clear communication between researchers, stakeholders, environmental managers, and the broader community is critical to the success of eDNA method adoption. Early experiences with eDNA surveys for invasive carp species revealed significant concern around – and misinterpretation of – the monitoring results. Developing communication plans will help to inspire and involve the broader community in eDNA work, increasing comprehension of the methods and maximizing acceptance. These communication guidelines can include details on sampling and analytical methods, probabilistic survey design, interpretation of false positives/negatives (Darling et al. 2021), and decision-making in the face of uncertainty (Sepulveda et al., 2022). Additionally, a key motivation in generating communication guidance is ensuring perfect is not the enemy of the good – we should not limit uptake of powerful molecular tools because all unknowns are not yet answered. Understanding limitations and benefits, performance of eDNA compared to traditional approaches, and how the resulting eDNA data can be used, allows for risk-based assessments in how and where eDNA approaches can be applied now. Improved communication will lead to better comfort with and therefore accessibility of these tools.
- Ethics. New sources of information bring with them concerns about the scope of its appropriate and ethical uses. A national strategy could work toward transparent, public assessments of concerns surrounding privacy, the ownership of information, potential misuse (and safeguards against it), and filtering of data for sensitive species.

Technical Needs

Although eDNA analysis is already used for many management applications (see examples above), existing uses only hint at the potential for eDNA as a source of environmental information. Developing this potential more fully will require advances – and investments – in both conceptual models and tools fit for purpose. Below we include descriptions of some of the most pressing technical needs as eDNA moves from research into implementation at a national scale. These needs are not specific to government, and indeed many nongovernmental research groups around the world are already engaged in developing relevant information. A review of these efforts is beyond the present scope, however to the extent that a national eDNA strategy would include priority areas for research and development, the below areas stand out as among the most important.

Conceptual Models

- Fate and Transport. Better information on the ways in which an eDNA signal changes over space and time will enable us to better link observations to management needs. Because DNA molecules degrade in the environment over time, and because DNA can be transported away from its source organisms, management applications that require precision in space and time will require that we understand the likely combined effects of degradation and transport (Harrison et al. 2019). For example, fate-and-transport models can show how far upstream a species is likely to be given the detection of its eDNA at a particular point. Such information is also required for identifying sources and pathways of invasive species and biological contaminants (Andruszkiewicz et al., 2019; Ellis et al., 2022).
- **Abundance**. Organismal abundance is often necessary information for management for example, to determine the allowable level of take of a commercially important species, or to populate data-layers that agencies use for risk assessment under NEPA, ESA, MMPA, and other federal environmental statutes. Translating eDNA concentrations into known abundances of

organisms is an important area of eDNA research because each eDNA template molecule does not typically exist in a one-to-one relationship with an organism. Developing models of the ways in which eDNA is generated, collected, and detected will substantially improve our ability to link molecules to organisms in a quantitative way (Rourke et al., 2022).

Tools

- Mechanical Sampling Tools. Many species of management concern are rare (e.g., endangered species) or occupy difficult-to-sample habitats (e.g., marine mammals in the open ocean or in deep water). Adequately sampling for these species is a mechanical challenge in terms of acquiring the eDNA samples. The existence of robust mechanical and statistical (below) sampling tools will be critical for reliably surveying many species and ecosystems (Simmons et al., 2022).
- Statistical and Bioinformatics Tools. Some eDNA methods, in particular metabarcoding, produce large amounts of data that must be filtered, sorted, and analyzed to provide usable biological information. Such processing broadly referred to as bioinformatics is a necessary and specialized skill set that, at present, is often in short supply (but see Hakimzadeh et al. in review). Moreover, appropriately interpreting the data obtained from eDNA analysis requires a robust quantitative foundation, and available statistical methods are often misapplied to eDNA data. Statistics for understanding rare events (e.g., qPCR detections of scarce molecules) and compositional data analysis, for example, presently demand specialized expertise; responsibly scaling eDNA analysis into routine monitoring will require user-friendly statistical tools.
- Targeted Assays. Developing a standard library of eDNA assays specifically targeting species of management interest would put mutually compatible, off-the-shelf tools into the hands of a wide array of users (see Takahashi et al. 2023). This would enable national-scale surveys of economically important species, imperiled species, invasives, and so on, the results of which would become the substance of interoperable data repositories (see below). Reliable assays have known specificity and sensitivity, consistent with existing information standards (Bustin et al., 2009)
- In Silico PCR Models. With potentially limited target DNA in an environmental sample, Much eDNA research relies on the amplification of targeted gene-regions of interest using PCR. Predictive computer-based models of the PCR process would exponentially accelerate the development of targeted assays and broad-spectrum metabarcoding primer sets, enabling researchers to rapidly screen for useful assays out of trillions of theoretical possibilities. At present, in silico PCR only partially predicts the real-world behavior of oligonucleotide primers (So et al., 2020).
 - Reference Databases. Accurately identifying the species detected by eDNA metabarcoding requires a complete, curated database of relevant taxonomic and sequence information. Presently available nucleotide databases (such as the National Center for Biotechnology Information (NCBI), hosted by the National Institutes of Health; the Barcode of Life Database (BOLD), Silva, Midori, and others) would benefit from systematic curation and development in their taxonomic and geographic coverage. Priorities for improved databases might include groups of special management concern (e.g., dozens of ESA-listed coral species around the Indo-Pacific), for which monitoring is currently difficult. Expanding collections of reference specimens would facilitate the development of new assays and the taxonomic interpretation of eDNA metabarcoding results. Where populations within species differ genetically, population-level databases may also be desirable (Juhel et al., 2020; Weigand et al., 2019).

- Analysis and Visualization Tools. Policy-relevant science requires raw data to be converted into interpretable answers to management questions. At present, most tools for the analysis and interpretation of eDNA data are project-specific, custom fragments of code not intended for broader use. Thankfully, a wide range of visualization tools already exist for clinical and water-quality assessments, and these can be adapted for conveying management progress with eDNA-based assessments.
- Interoperable Data and Sample Repositories. Current eDNA data-management requires individualized bioinformatics effort to synthesize data siloed across private and public repositories (e.g., NCBI). A means of acquiring eDNA data from many, widely distributed sources would enable continental-scale analysis of biological data, akin to the tools with which meteorological data are processed today. Archives for extracted eDNA samples would enable future analyses, as analytical approaches and reference databases improve over time or new questions arise, particularly in the context of climate change and the loss of biodiversity (Zizka et al., 2022).

39 Conclusion

Dozens of federal agencies require vast amounts of information about the location and abundance of wildlife and other living natural resources, and the same kinds of information is critically important to a wide spectrum of stakeholders. Historically, generating such data has been cumbersome, costly, and slow – and as a result many species and areas remain essentially unsurveyed. The advent of eDNA analysis positions societies to transform the ways ecosystems and changes to those ecosystems are monitored at a wholesale level; examples include the ways offshore oil and gas operators monitor their operations, port operators dredge, fisheries open and close, researchers explore and track ecological changes, coastal developers assure the public that their works operate responsibly, and governments nominate areas for protection and restoration and evaluate their success.

In short, the analysis of eDNA may significantly improve how many federal agencies do business by permitting them to track, report, and archive biological information at (sometimes unprecedented) spatial and temporal scales relevant to natural-resource management. The relevant technologies have matured to the point at which many of these applications have already begun to come online; a national eDNA strategy would consolidate and harness these innovations for the public benefit, bringing natural-resources management into the twenty-first century.

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