

COMMENTARY

The Second National Workshop on Marine eDNA: A workshop to accelerate the incorporation of eDNA science into environmental management applications

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Abstract

The Second National Workshop on Environmental DNA was held on September 12–15, 2022, at the Southern California Coastal Water Research Project (SCCWRP) in Southern California and was focused on transitioning eDNA from research to management applications. The Workshop was attended by 150 people in-person and an additional 200 more online. Workshop attendees represented a broad cross-section of disciplines and backgrounds, including research scientists, state, and federal agencies, and those in the environmental management sector. This diverse collection of attendees assembled with the goal of achieving cross-sector collaboration and working together to identify the necessary next steps to move eDNA methods into the management application mainstream. The Workshop structure included a Training Day oriented towards environmental managers and those new to eDNA science, to facilitate a common ground for discussions on subsequent days. The Plenary Day focused on case studies about eDNA applications and culminated with a roundtable panel discussion with local, state, and federal agency representatives on eDNA method readiness and the road to method adoption. Among the key takeaways from the Workshop was bridging the communication gap between researchers and managers because scientists often focus on technical details and the unknowns, giving the impression that eDNA science is not yet mature, whereas managers want to hear consensus statements about readiness and a roadmap for method adoption, including standard operating procedures, lab accreditation, and unified sequence libraries. This outcome was a clear directive for many scientists in attendance that it is time to stop letting perfect be the enemy of good and to focus future efforts on method harmonization and a national strategy towards method adoption. The Workshop concluded with a working session of invited participants to identify key priorities and needs to achieve the goals highlighted in the Workshop discussions.

KEY WORDS

barcoding, bioinformatics, conservation management, ddPCR, digital droplet PCR, environmental DNA, metabarcoding, practical implementation, qPCR, quantitative PCR, sequence libraries

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1 | INTRODUCTION

1.1 | Background and aims

Environmental DNA (eDNA) is becoming an increasingly popular tool to detect aquatic organisms in biomonitoring and bioassessment programs, with tremendous growth in the number of peer-reviewed publications demonstrating its potential. That growth was aided in part by the First National Conference on Marine Environmental DNA (<https://phe.rockefeller.edu/eDNAMarine2018/>), which was held on November 29–30, 2018, and helped establish a vision for eDNA as a powerful, dependable, and efficient tool for ocean observation. A large part of developing that vision involved discussion among leading scientists about ongoing scientific advances and technical hurdles that needed to be addressed.

The Second National Workshop was held on September 12–15, 2022, nearly 4 years later, after being postponed due to COVID-19 pandemic restrictions. Reflecting on the rapid evolution of the field in that extended time between workshops, the second one shifted focus from technical hurdles, many of which had been addressed in the intervening years, to steps that needed to be undertaken to transition eDNA from research laboratories into the application by the management community. The workshop was sponsored by four federal agencies and two philanthropic foundations looking to create a unified playbook for method adoption across an array of sectors. Figure 1 is the Workshop logo.

The Second Workshop was held at the Southern California Coastal Water Research Project Authority (SCCWRP), a research institute formed more than 50 years ago to facilitate the transition of scientific advances into water quality management applications. There were 150 in-person attendees and 200 more online, with workshop attendees representing a broad cross-section of the eDNA user community. Approximately half of the participants were research scientists, and the other half comprised management practitioners or potential practitioners. The practitioner audience was

broad, including federal, state, and local government agencies, environmental advocacy groups, and industry (Figure 2). The Workshop goal was to enhance dialog across this diverse audience about what next steps are needed to move eDNA measurements into the management application mainstream.

1.2 | Agenda overview

The Workshop included traditional oral and poster scientific sessions for knowledge transfer (i.e., through reviews of the latest technical advances and eDNA tools) but primarily focused on developing agency roadmaps for the incorporation of eDNA methods at the federal, state, and local levels.

The first day of the Workshop was coined “Training Day” and was oriented towards environmental managers and novice users to create familiarity with eDNA technology and facilitate a common foundational understanding so they could actively participate in sessions on the following days. The second day was a “Plenary Day” consisting of invited speakers and an agency roundtable discussion. Both days also featured poster sessions to maximize opportunities for engagement and collaboration among scientists, early career researchers, agency representatives, and stakeholders. Following the public workshop, 36 invited participants held 2 days of discussion aimed at addressing the barriers to widespread adoption identified during the Panel Discussion.

2 | HIGHLIGHTS: SECOND NATIONAL WORKSHOP ON MARINE eDNA

2.1 | Training day

The Training Day introduced participants to the basics of eDNA collection, processing, and interpretation. The morning involved plenary talks introducing eDNA approaches and applications covering: (a) The basics of eDNA for environmental managers; (b) Descriptions of example eDNA applications, including invasive and endangered species monitoring, biodiversity, bioassessments, and more; and (c) Time, costs, and infrastructure needed to implement an eDNA program. This session also covered commonly-asked questions regarding how quickly eDNA degrades in aquatic systems, eDNA fate, and transport dynamics and estimating species abundances with eDNA. Like any tool, eDNA-based approaches are not perfect and are evolving, but their application for biomonitoring and ocean conservation can be immediate and impactful. Recordings of the morning session are available on the Workshop website.

The afternoon featured three rotating demonstrations on (1) eDNA field sampling, (2) lab sample preparation and measurement techniques, and (3) computational bioinformatic processing. For the field sampling session, Austen Thomas demonstrated the Smith-Root, Inc. backpack samplers and their citizen science samplers. Dr. Peter Thielen of Johns Hopkins University's Applied Physics Lab

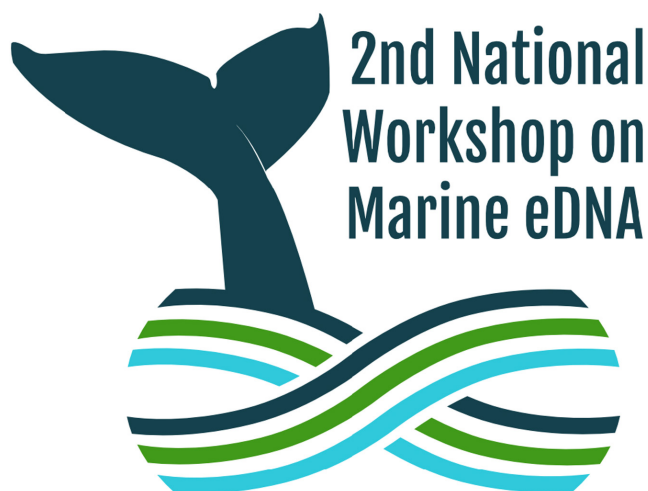
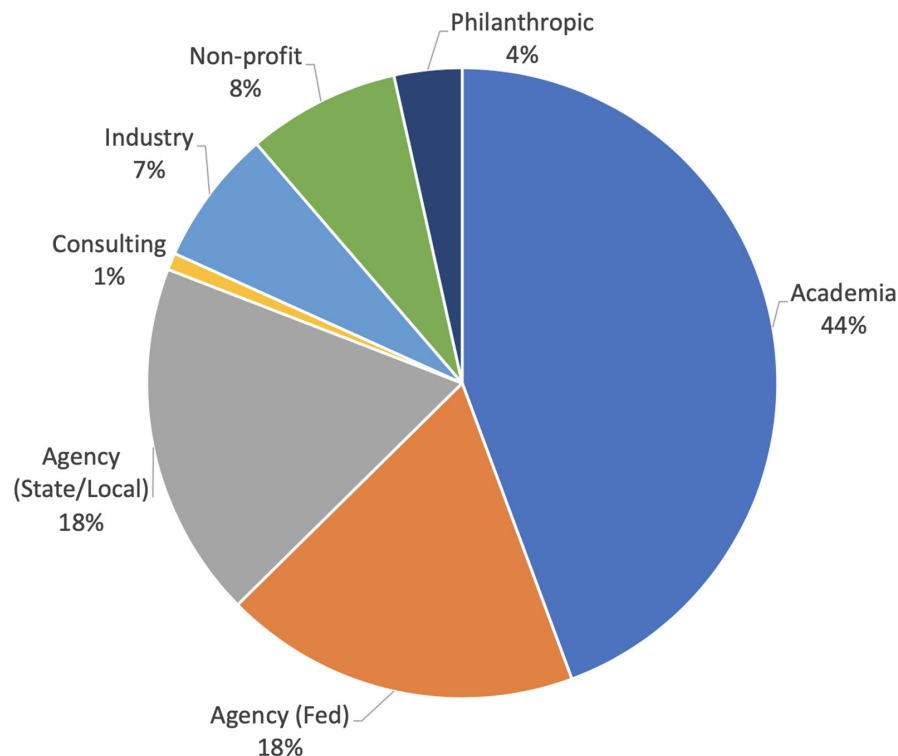


FIGURE 1 Logo for Second National Workshop on Marine Environmental DNA, designed by S. Theroux

FIGURE 2 Sector participation in the Second National Workshop on Environmental DNA



(APL), demonstrated his light-weight, low-cost, quiet, and efficient portable system of single to three small pumps housed in a water-proof Pelican case, which is designed to be self-put together by end-user laboratory groups. Dr. Joseph Craine from Jonah Ventures (<https://jonahventures.com/>) demonstrated a commercial end-to-end sampler system, which has a single-use water syringe sampler with 25 mm disks (about $\frac{1}{2}$ of the surface area as Sterivex filters) that can be returned to Jonah Ventures for DNA metabarcode sequencing and targeted applications.

The session on laboratory techniques demonstrated four steps: (1) Collection, (2) Concentration, (3) Extraction, and (4) Analysis. Emphasis was placed on how scientists rely on field and lab blanks (negative controls), protection of the sample from contamination and from degradation, as well as controls and best practices for sample processing, DNA extraction, and analyses.

The bioinformatics session presented best practices for molecular assays, including qPCR and digital PCR (ddPCR) assays to identify and quantify single taxa, and targeted metabarcoding assays to discern multiple taxa/species or population variants (haplotypes). Participants learned about the balance between specificity and breadth, and the importance of regional reference species databases for the most accurate reference sequence library comparisons. This session also featured demonstrations on the DNA explorer tool (<https://www.ednaexplorer.org>), which provides an accessible eDNA experience that can empower users. There also was a demonstration of Phinch 2.0 (<http://phinch.org>), an interactive, exploratory, visualization framework for eDNA data sets, which employs visual displays to accompany bioinformatics analyses to promote data exploration and interpretation.

2.2 | Plenary day

The Plenary Day began with opening remarks from the White House Office of Science and Technology Policy Deputy Director for Climate and the Environment, Dr. Jane Lubchenco, who highlighted the opportunities that marine eDNA science has to support the nation's goals of biodiversity assessment, conservation, and marine management. Dr. Lubchenco reiterated that biodiversity data are crucial to smart decision-making, both within and beyond the U.S. exclusive economic zone. Although current existing techniques for surveying biodiversity can provide critical information not afforded by eDNA, Dr. Lubchenco emphasized that those generally are limited in capacity to provide rapid assessments of a full spectrum of biodiversity within and across systems. eDNA will be a powerful complement to other modes of assessing biodiversity. She concluded by challenging the workshop participants to develop and apply eDNA techniques to the nation's big goals "of protecting the ocean effectively, producing from it sustainably, and benefiting from it equitably." Dr. Lubchenco's full remarks are available at [whitehouse.gov](https://www.whitehouse.gov).

Inspired by this national call for advancing eDNA, the plenary session continued with invited talks focused on applications of eDNA methods for routine monitoring, from fisheries stock assessment to marine mammals to harmful algae blooms, and transitioning eDNA into use by managers at the local, state, national, and international levels. The afternoon concluded with a panel discussion with state and federal agency representatives, as detailed below. All of the talks and the Panel discussion are available on the Workshop website.

2.2.1 | Key applications and science advances

The first plenary session featured talks that highlight applications of eDNA science for routine monitoring. These applications included fisheries stock assessment, marine mammal monitoring, invasive species monitoring, harmful algal blooms, and bioassessment. The talks highlighted the utility of eDNA methods for enhancing bio-monitoring via improved precision for species identifications, sensitive early warning detections of low abundance taxa, and resolving closely related or cryptic species complexes. Likewise, the sessions highlighted the utility of rapid detection methods and the combinations provided by multiple lines of evidence, either from morpho-taxonomic approaches combined with eDNA methods or multiple eDNA-based approaches such as qPCR combined with DNA metabarcoding. Finally, the review of the successful DNAquaNet efforts (<https://dnaqua.net/>) to optimize and standardize DNA metabarcoding methods for bioassessment provided a clear example for U.S. efforts to parallel.

2.2.2 | Standardization

The next plenary session highlighted the standardization and best practice efforts that are evolving globally, and the limitations of these standardization efforts as they become increasingly siloed within monitoring programs or regions. Changes that were called for include increased collaboration among agencies to harmonize standardization activities, the focus of standardization DNA method metadata reporting to synchronize methods when technical approaches may differ, and efforts to build staff capacity, build lab capacity (including lab certification/accreditation, intercalibration, and proficiency tests), provide training opportunities, adopt data interpretive tools, and work in partnership with industry. Collaboration across sectors can entail joint pilot demonstration studies with regulatory communities. Lastly, there was a focused discussion on the legality of eDNA approaches, as first highlighted in the invasive bigheaded carps (bighead carp *Hypophthalmichthys nobilis* and silver carp *H. molitrix*) qPCR monitoring (Jerde et al., 2011), which was deemed admissible in court and has been supported in subsequent legislations. There also is an Endangered Species Act (ESA) example that used eDNA for the 2018 federal listing of the black warrior waterdog *Necturus alabamensis* (a salamander native to the Tennessee River watershed) by the U.S. Fish and Wildlife Service (2018), whose critical-habitat designation relied in part on qPCR data, along with the 2021 delisting of the snail darter fish *Percina tanasi* (U.S. Fish and Wildlife Service, 2022).

2.2.3 | Tools to facilitate management application

The final plenary session focused on the increasing availability of tools that could help automate eDNA method collection and otherwise expand the ability of monitoring programs to adopt eDNA

methods at scale. These sessions emphasized the importance of eDNA lab accreditation procedures, and the challenges that arise when inferences from eDNA approaches diverge from results obtained by established methods. Additionally, this session highlighted advancements in commercial capabilities (e.g., Jonah Ventures) that provide user-friendly sampling methodologies and DNA sequencing technologies to help build capacity in large monitoring programs, such as the California Surface Water Ambient Monitoring Program (SWAMP), which has adopted these shelf-ready tools for water eDNA sampling and analyses. Additional technological innovations include those that automate eDNA sampling for enhanced spatial and temporal scales, such as those provided by the Monterey Bay Aquarium Research Institute (MBARI), which is endeavoring to create accurate, timely, and comprehensive assessments of aquatic environments at large scales through the use of autonomous gliders equipped with DNA collection and processing capability. Lastly, the session highlighted the advancements in our understanding of eDNA fate and transport dynamics in the environment and the role that these measurements will play in the enhancement of predictive models and eDNA interpretation.

2.2.4 | Agency roundtable

The Plenary Day capstone was an agency roundtable panel discussion, with panelists selected from a range of federal, state, and local agencies that are exploring how eDNA fits into their respective agency's monitoring and management programs. Panelists were Dr. Michael Weise, Office of Naval Research (ONR); Dr. Kelly Goodwin, National Oceanic and Atmospheric Administration (NOAA); Dr. Chris Meyer, Smithsonian National Museum of Natural History (NMNH); Dr. John Darling, Environmental Protection Agency (EPA); Johnathan Bishop, California State Water Resources Control Board; and Dr. Peter Vroom, the City of San Diego Department of Public Utilities. The agency representatives were asked, "Where is your agency in eDNA adoption?"

Dr. Kelly Goodwin said that NOAA is actively exploring eDNA as a tool for a variety of mission applications because it provides a tractable approach to monitor biodiversity at a large scale. She noted impediments to implementation that go beyond basic technical development; for example, there is a shortage of expertise in molecular biology and bioinformatics within the agency. Because this is a relatively new area of science, there is a need to recruit a diverse cadre of young scientists to accelerate the operational use of eDNA in NOAA. She also felt that engaging professional scientific communicators would improve how eDNA methodology and utility are explained to leadership, stakeholders, and the public.

Dr. Chris Meyer said that adoption within the Smithsonian will be easier because it is more of a "Discovery and Exploration" institution and not a monitoring agency. Because the Smithsonian supports the increase and diffusion of knowledge across its units, adapting and serving new technologies is a natural extension of its mission. In particular, the Smithsonian's National Museum of

Natural History (NMNH) serves as the nation's "Library of Life", which will be needed for translating the sequence-based knowledge generated from eDNA. He discussed the importance of DNA reference sequence libraries to accompany the voucher specimens in the NMNH, which constitute 2/3 of every U.S. marine species, both vertebrates and invertebrates. He noted the growing popularity of BioBlitzes to document and voucher local diversity. Meyer underscored the need for (a) trusted, curated, voucher-based, DNA reference data sets, (b) longer DNA amplicons, (c) evaluation of specific targeted markers, and (d) preservation of DNA extractions from both specimens and ecosystems in museums and other repositories.

Dr. John Darling indicated that because his agency does regulation, there will be legal questions associated with the use of eDNA but expects that those will be overcome. He felt that one of the first applications will be in bioassessment using diatoms, as their identification from traditional morphological techniques has been challenging. Darling emphasized that although EPA develops tools, most of their implementation occurs at the state level. He stated that it is necessary to build partnerships with states for the widespread adoption of eDNA usage.

Jonathan Bishop commented that he was getting mixed messages from the workshop. From some of the speakers, particularly ones on the Panel, he was hearing that we are close to eDNA management use readiness. On the other hand, most of the speakers emphasized the things not yet known and the need to overcome these potential obstacles before adoption. He said that "as the least technically adept person on the Panel, but as one of the key decision-makers as to whether California will begin using eDNA for management application," he needed greater clarity from the research community. He recognized the need to talk to managers, translating complex stuff into simple talking points. He also stated the need for scientists to produce the typical products associated with most new methods: (a) quantification of lab reproducibility, typically through inter-laboratory calibrations, (b) standard operating procedures (SOPs), and (c) a lab accreditation program, which together will add confidence to eDNA adoption. He further noted that there has to be clear justification for the costs of re-training the state's technical employees and for outfitting laboratories with new equipment.

Dr. Peter Vroom mirrored a number of Bishop's points, emphasizing that he would need to answer the question about how much cost adoption would add to citizen water bills, and what are the benefits of that added cost. He added that he needed greater clarity about whether these new types of analyses could be absorbed by existing staff, what training will be necessary, and who would be available to conduct that training. He indicated that San Diego was large enough to absorb those costs, particularly if supported by regulatory agencies such as the one run by Bishop, but such would be more challenging for smaller cities. He stated that the scientific development community needs to evolve its messaging to help address these questions. He finished by offering hope that his team undertook wastewater-based epidemiology for COVID using droplet digital PCR (ddPCR), working in collaboration with the U.S. Centers

for Disease Control and Prevention (CDC), which could serve as a model for the adoption of these new eDNA techniques.

Dr. Michael Weise noted that the first use case for eDNA within the Navy is to enhance understanding about marine mammals, providing more information than they presently get from other monitoring technologies, such as passive acoustics. Weise indicated that his agency was moving towards adoption, but still had technical questions regarding eDNA fate and transport questions for marine mammals. He also emphasized the need to translate science into language that managers can more readily understand.

2.3 | Poster presentations

The Workshop featured 24 poster presentations, including both in-person and virtual posters. The topics ranged from the eDNA Collaborative, to invasive species, marine mammals, eDNA decay rates, and more. All poster abstracts and select virtual posters can be viewed on the Workshop website.

2.4 | Breakout sessions

In the 2 days following the Workshop, a subset of attendees gathered to identify the key hurdles to eDNA method adoption in a series of breakout sessions of smaller working groups. The ~36 participants were from state and federal agencies, industry and academic sectors, as well as philanthropic and nonprofit organizations, who together discussed the key impediments to eDNA methods adoption and solutions for overcoming them. The participants identified four key areas for future activities:

1. *DNA method standardization*: Current efforts to develop eDNA standardization methods have become siloed within disciplines and agencies. Additionally, a majority of these standardized methods (e.g., the Department of Fisheries and Oceans (DFO), Canada, and U.S. Fish and Wildlife Service (FWS)) have focused on qPCR approaches, leaving a vacuum for the standardization and best practice guidelines for DNA metabarcode sequencing. Additionally, many monitoring programs are hampered by the absence of lab accreditation and proficiency testing programs to evaluate the performance of standardized methods and how they are applied by various groups/labs. The establishment of formal Quality Assurance/Quality Control (QA/QC) procedures for eDNA sampling and analyses would help to provide the foundation for the broadscale adoption of these methods.
2. *DNA reference libraries*: Gaps in DNA reference libraries are limiting the full potential of eDNA approaches (Weigand et al., 2019). In particular, incomplete reference libraries for marine vertebrates, marine invertebrates, and algae, including invasive, protected, and commercial species, have limited the transition of bioassessment tools from traditional microscopy to DNA-based approaches. There is great potential to leverage existing monitoring programs

and existing sample archives such as those maintained by the Smithsonian National Museum of Natural History and the NOAA CalCOFI program, to collect voucher specimens and fill in reference sequence library gaps.

3. **Communication:** Building off the Panel discussion, enhancing communication between researchers and managers was identified as a critical need for the successful adoption of eDNA methods in routine biomonitoring and environmental decision-making. In the past, scientists have concentrated on an outsized focus on the “known unknowns” of eDNA, particularly key limitations, with less emphasis on the elements in which there is significant confidence. Co-designing eDNA studies alongside managers and stakeholders was identified as a recommended approach, particularly for the development of Demonstration Projects.
4. **A National Implementation Strategy:** As this was a national workshop initiated at the request, and with funding from federal agencies, there was a recognition of the need for a national eDNA strategy to inform federal actions. Proposed strategy goals could include coordinated interagency efforts and resources to advance biodiversity monitoring that supports the Blue Economy. Other contributions include creating jobs, helping regional development, informing natural resource management about climate change, developing mature, new, and emerging science and technologies to advance biodiversity monitoring, and adopting minimum standards for broad applicability and uptake.

3 | TAKE-HOME MESSAGES AND UPCOMING

Across multiple presentations and discussions, the key messages that evolved from the Second National Marine eDNA Workshop were: (1) methodology, including that eDNA sampling, preservation, and bioinformatic pipelines are rapidly becoming more standardized and less costly, facilitating agency adoption, and (2) we are at the point of method readiness and need to communicate that effectively. We cannot let perfection be the enemy of good, as there are management needs that can be met effectively at this moment, and we will miss this critical opportunity if we cannot effectively communicate that message, and (3) the time is now to capture the power of eDNA methods and analyses for use in management decisions. The Workshop participants emphasized the importance of laboratory standards and certification, where possible, at the state level, along with federal agency guidelines.

An upcoming special issue for the journal *Environmental DNA* is planned, based on the presentations and outcomes of the workshop, with guest editors Dr. Ryan Kelly, Dr. Rachel Meyer, and Dr. Susanna Theroux, along with journal Editor Dr. Louis Bernatchez and Associate Editor Dr. Carol Stepien. The special issue will assemble a collection of articles focused on topics that push beyond the application of eDNA for species detection purposes and towards real-life, practical management applications. This special issue will highlight recent advances in eDNA methods for routine biomonitoring and

bioassessment, as well as a series of articles, focused on overcoming these and other hurdles towards the widespread adoption of eDNA methods. The aim is to specifically highlight research that is focused on leveraging data from eDNA (and eRNA) for informing environmental management decision-making and a roadmap for broadscale standardization and agency implementation. Five key themes are planned for this Special Issue:

1. Applications of eDNA methods for routine monitoring and assessment.
2. Fate and transport of eDNA in the environment.
3. Determining species abundance from molecular data.
4. Global DNA reference databases.
5. Regulatory hurdles to eDNA method implementation.
6. Standard methods for eDNA sample collection, analysis, and interpretation.

The Special Issue should appear in 2023 in *Environmental DNA*. The special issue will include about 15 contributions on the proposed subjects, ranging from review/synthesis papers to original research contributions. These papers will become available online as soon as they are ready for publication.

4 | RESOURCES

- Workshop website: <https://tinyurl.com/marineedna>
- Workshop eDNA 101 primer
- Slack channel: <https://tinyurl.com/eDNAslack>
- Workshop Posters: <https://tinyurl.com/marineednaposters>

AUTHOR CONTRIBUTIONS

ST and SW led the workshop. CS wrote the first draft and covered the workshop meeting for the journal *Environmental DNA*. All authors contributed to editing.

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CONFLICT OF INTEREST

The authors of this workshop report were not all part of the organizing committee, as CAS represented the journal *Environmental DNA*. The organizing committee has read and approved this workshop meeting report.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed.

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