Review

Summary, Overview, and Insights from the 3rd U.S. National Marine Environmental DNA Workshop (2024)

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Summary, Overview, and Insights from the 3rd U.S. National Marine Environmental DNA Workshop (2024)

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Abstract: The Third U.S. National Marine Environmental DNA Workshop on June 2–5, 2024 brought together researchers, practitioners, and policymakers to overview and discuss: (1) environmental (e)DNA – defined as "DNA in the environment" – as a strategic U.S. national priority, (2) eDNA tool readiness and approaches to support decision-making, (3) emerging eDNA technologies, and (4) plans for implementation and adoption for environmental monitoring and ecosystem assessment "from microbes through whales". Uses and applications of eDNA have been revolutionizing exploration, measurement, and monitoring of biodiversity across ecosystems, including the marine and freshwater aquatic environments emphasized in the Workshop, as well as terrestrial and aerial systems. The Workshop featured the launch of the new U.S. National Aquatic Environmental DNA Strategy from the White House Office of Science and Technology Policy (2024), with aquatic defined as freshwater to ocean waters, across all salinities; the Strategy was developed using outputs and a taskforce from the 2022 Second Workshop. The 2.5-day Third Workshop included speaker presentations, panel discussions, and networking on eDNA tools, applications, use, upscaling, and their future. The Workshop led into Capitol Hill Ocean Week (CHOW) events (June 5–6, 2024), during World Ocean Week. Note that this summary paper represents the author's take-away view and interpretation, and not necessarily the overall views or consensus of the U.S. Government, Smithsonian Institution, workshop organizers, or speakers.

Keywords: biodiversity; environmental DNA; eDNA; marine eDNA; aquatic eDNA; metabarcoding; qPCR; quantitative PCR; GenBank; NCBI; Ocean Science; species diversity; species richness; microbiome; World Ocean; eRNA; artificial intelligence

1. Introduction

The three U.S. biennial National Workshops on Marine Environmental DNA (eDNA) (held in 2018, 2022, and 2024) brought together researchers, practitioners, and policymakers to present and discuss emerging and existing eDNA technologies and their adoption, with eDNA broadly defined as "DNA in the environment". The Third Workshop (June 3–5, 2024) [1] (Figure 1) in this series featured strategies and implementation priorities, with the release of the new U.S. National Aquatic Environmental DNA Strategy [2] issued from the White House Office of Science and Technology Policy. Aquatic was defined in the Strategy as including fresh through ocean water environments (across all salinities: rivers, lakes, streams, estuaries, bays, oceans, and seas). The Strategy was developed using outputs and a task force from the 2022 Second Workshop [3,4]. The Strategy and the Third Workshop objectives aim to move forward the implementation of eDNA as an accepted method for determining the presence of organisms in the environment, identifying them to species (or closest possible taxonomic level), and for evaluating species richness and species diversity across aquatic ecosystems. Jointly hosted by the Johns Hopkins Applied Physics Laboratory (APL) and the National Museum of Natural History, Smithsonian, the Third Workshop's four major subthemes [1] were to overview and discuss:

(1) Strategic U.S. national priorities;

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(2) eDNA technology readiness to support decision-making;
(3) Emerging technologies within the strategic framework; and,
(4) Implementation and adoption: from research to resource management.

The First National Marine eDNA workshop, held at Rockefeller University in New York City in November 2018, focused on where the eDNA science was, along with its emerging tools and uses; it largely featured overview talks summarized by selected government agency and university scientists, and was attended by about 100 invitees (including this author, who has attended and participated in all three of the National eDNA workshops). The Second Workshop held at the Southern California Coastal Water Research Project (SCCWRP) in Costa Mesa, California in September 2022 focused on laboratory and analysis procedures used for eDNA science, how these techniques can be applied by federal, state, and local managers and industry, and what are the means to transition the science to implementation (summarized in [3]). The Second Workshop was attended by industry users (about 50%), in addition to agency managers at the federal, state, and local levels (25%), and university researchers (25%). The Second Workshop featured presentations and demonstrations on the various techniques and approaches (including sampling in the field, laboratory quantitative (q) PCR and metabarcoding, quality control, and data analyses), how to best train personnel, and effective ways to communicate with end-users and the public. An invitee-only session following the Second Workshop then formulated an invited writing group of selectee government agency and university scientists, who published their recommendations [4] and informed the new U.S. National Aquatic Environmental DNA Strategy [2} that was rolled out during the Third Workshop [1].

For the Third Workshop (Figure 1), Days 1 and 2 were hosted by and held at Johns Hopkins University's Applied Physics Laboratory (APL) in Laurel, Maryland, led and coordinated by APL's Dr. Peter Thielen, and attended by about 372 in person and with a larger online presence [1]. The capstone Day 3 of the Workshop was hosted by and held at the National Museum of Natural History (NMNH), Smithsonian Institution in Washington, D.C., as a half-day prelude to the Capitol Hill Ocean Week (CHOW) events in Washington D.C. on June 5–6 [5]. The overview summary given here aims to provide a general perspective for scientists, students, and the public who could not attend the Third Workshop and/or desire a capstone that is available to a broader audience across the World. This summary paper also gives background information and how to access the reference materials discussed in the Third Workshop (see References).

Figure 1. Logo of the 3rd National Workshop on Marine eDNA.

2. Plenary Talk: Workshop introduction and the roll-out of the U.S. National Aquatic eDNA Strategy (Day 1)

The Workshop's opening welcome on June 3, 2024 was given by Dr. Andrew C. Merkle (APL' s mission area Executive for Research and Exploratory Development) who highlighted advances from APL's climate research [6] and genomics sequencing data for disease surveillance stemming from the Covid19 pandemic. Dr. Merkle commented that focus at APL on eDNA has been on "from bench to boat" and "field-forward analysis" moving towards on-site DNA data analysis and developing methodology for results in hours from hand-held devices.

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The Workshop's plenary address featured the release of the National Strategy on Aquatic eDNA by Dr. Jane Lubchenco (Distinguished Professor at Oregon State University and Deputy Director for Climate and Environment in the White House Office of Science and Technology Policy (OSTP)). Dr. Lubchenco had addressed the Second Workshop virtually and spoke at this Third Workshop in person, first highlighting that there is one interconnected "World Ocean" rather than separate oceans and seas, noting connectivity and shared resources across the World. Dr. Lubchenco outlined how eDNA and other Omics tools and techniques have progressed to allow "Actionable Environmental Information" to be collected more efficiently and rapidly, including rapid identifications of the "culprits" causing disease outbreaks in the ocean, such as sea star wasting disease and harmful algal blooms (HABs), and the assessment of the growing impacts of CO_2, and its consequences. Dr. Lubchenco highlighted how the U.S. is using eDNA to advance science applications and that this release of the National Strategy on Aquatic eDNA by OSTP provides a roadmap as to how eDNA should no longer be considered an experimental tool and now is ready for "prime time" to explore, map, and monitor aquatic life. eDNA thus offers "a window into the lives of organisms that are poorly documented, from bacteria to vertebrates, providing unprecedented knowledge of aquatic life". The increasing capability of automated sensing and sampling using buoys, drones, autonomous vehicles, and satellites offers growing means to sample remotely, cost-effectively, safely, with less environmental disturbance of habitats and species, and moving towards near-real time data processing. She emphasized the need for shared technological knowledge, targeted training and expertise, and increased diversity, equity, inclusion and accessibility for all.

The National Aquatic eDNA Strategy aims to "advance fast, low-cost, and effective eDNA technologies to understand life in the ocean and how it's changing", as stated by Dr. Lubchenco. The Strategy document [2] notes that "analyzing the DNA in a body of water to identify the species present is much more efficient than conducting traditional censuses of different species". The eDNA Strategy outlines opportunities to improve and deploy eDNA processes to inform the development of more effective ocean policies. The three stated goals in the eDNA Strategy are to:

(1) **"Coordinate Across Sectors to Facilitate Integration of Aquatic eDNA into Decision Making.** This involves engaging cooperative mechanisms to align and promote standards and best practices for eDNA workflows across sectors, contributing technical readiness recommendations for priority applications and locales, and unifying communication strategies to enhance scientific literacy and data interpretation across all sectors;

(2) **Build Capacity, Infrastructure, and the Research Enterprise Needed to Employ Aquatic eDNA Technology at Scale** by improving human capacity with training and education, meeting technical demands through infrastructure development — ranging from national sample repositories to interoperable data management structures, and supporting research and development to bolster and transition eDNA science into sustained operations; and

(3) **Advance Coordinated Aquatic eDNA Observations to Aid Assessments in U.S. Waters** by harmonizing the extensive collection and delivery of eDNA data needed for robust and trustworthy metrics about aquatic health across the nation, resulting in an eDNA network to support national priorities and actions and to inform decisions that promote aquatic life and resilient ecosystems."

The Strategy's guidelines advocate that through coordinated sampling, implementation, infrastructure, and research, eDNA measurements and analyses can be employed at larger scales to generate high-quality and reliable environmental assessments to protect and use natural resources that build public trust [2]. Dr. Lubchenco further stated that this Strategy, under Goal 3, will be used to "Aid comprehensive assessments in U.S. waters, by:

(a) Identifying priority sites and applications for aquatic eDNA sampling,

(b) Implementing technological advances to build operational capacity, and

(c) Operationalizing biological resource data for societal benefit".

As also introduced by Dr. Lubchenco, during the CHOW (Capitol Hill Ocean Week) on June 5, 2024 [6], OSTP additionally released two other complementary new 2024 U.S. ocean health strategies:

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The National Ocean Biodiversity Strategy [7] and The U.S. National Strategy for a Sustainable Ocean Economy [8].

3. Strategic National Priorities for Aquatic eDNA, Adoption, and Alignment for Implementation

The following are synopses and notes about the ensuing presentations and talks from Days 1 and 2, with emphasis on those points that have significantly moved beyond what already was covered in the prior two National Workshops on Marine eDNA in 2018 and 2022 (see [3]) and what already has been extensively reported in the scientific literature about eDNA. Please note that these talk summaries reflect the insights and interpretation of the author.

3.1. Strategic National Priorities for Marine and Aquatic eDNA

Dr. Steve Weisberg (Executive Director of the Southern California Coastal Water Research Project Authority (SCCWRP), who hosted the Second National Marine eDNA Workshop in 2022 [3] spoke on, "A call to action: Products from the Second Workshop, expectations from the Third", advocating for voicing what can be agreed upon and for taking up the points of the new National Aquatic eDNA Strategy, with an impending agreed-upon implementation plan. SCCWRP is a research consortium formed by 14 California water quality agencies to ensure a solid scientific foundation for their management activities, including developing molecular tools to support environmental monitoring, which has been engaged in all three Workshops and serves as a state and local example and catalyst for implementing eDNA biotechnology.

Dr. Mike Weise (U.S. Navy Office of Naval Research (ONR), Marine Mammals and Biology Program Officer, and co-chair of the federal eDNA Task Team), presented an enhanced overview of the National Aquatic eDNA Strategy goals, further explaining the assessment objectives. Dr. Weise stated, "There is an urgent need for improved biological survey tools to meet the scale, scope and management/monitoring needs in the U.S., and eDNA is an exciting tool that has come of age and can meet the need…The National Aquatic eDNA Strategy sets the stage for the co-design of implementation milestones, including harmonized technical approaches, coordinated observations, and aligned communication strategies…This network of shared information will result in a nationwide eDNA network to inform decisions that promote resilient ecosystems."

Dr. Adam Sepulveda (Research Ecologist, U.S. Geological Survey (USGS) Northern Rocky Mountain Science Center), highlighted "Environmental DNA communication strategies", noting the variability among different eDNA samples and differential results obtained from various samplers and techniques, along with the need to separate noise from signal and to clearly state so. He highlighted the use of the MBARI (Monterey Bay Aquarium Research Institute) ESP (Environmental Sample Processor) 3 auto sampler [9] which currently can process up to 60 samples, can autonomously extract DNA, and has been deployed at many (primarily freshwater) stream monitoring sites across the U.S (see [10] for an example from this research).

Dr. Margaret Leinen (Director of Scripps institution of Oceanography, University of California, San Diego) commented on the need for international coordination in her talk on, "Communications and coordination from national to international". Dr. Leinen presented about OBON (Ocean Biomolecular Observing Network) and the United Nation's Decade of Ocean Science for Sustainable Development, 2021–2030 projects [11], highlighting the international spirit towards eDNA usage and procedures. Specifically, OBON through UNESCO (the United Nations' Intergovernmental Oceanographic Commission) during the 2021–2030 Decade of Ocean Science [11] is working during to develop "a global system that will allow science and society to understand ocean life like never before, enabling communities to detect biological hazards like harmful algal blooms (HABs) and pathogens, and will be a key component of next-generation ocean observing systems".

Dr. Christopher Meyer (Research Zoologist and Curator of Mollusks, Smithsonian Institution, Museum of Natural History), spoke on "Building human capacity, infrastructure, and the research enterprise for eDNA, commenting that, "How do we start getting these data into ecosystem models?" is a big question. He further emphasized that samples and their preservation and archival "constitute irreplaceable snapshots" and that it is important to have them "be Artificial Intelligence (A.I.) ready".

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Some of the other points raised by Dr. Meyer included the need to "enhance infrastructure to meet technical demands, including:

(a) Improving field, laboratory and informatics infrastructure;
(b) Better, faster, scalable sampling devices for all habitats;
(c) Clean, high-throughput capacity (in labs and sequencing facilities);
(d) Curated, voucher-based reference libraries;
(e) Long-term biorepositories for verification and reuse; and,
(f) Coordination of data workflows, computational resources, and information management".

Dr. Susanna Theroux (Senior Scientist, SCWRP), who also spoke extensively at the Second Workshop [3], as well as gave the plenary presentation for the Sixth Annual eDNA Technical Exchange Workshop 2023 conference [12], talked on "Research to implementation: from national to local scales", discussing the need to close reference data gaps. Dr. Theroux used the example of the Intertidal Biodiversity DNA Barcode Library funded by the state of California's Ocean Protection Council, for which the state has allocated $9 million U.S. in response to climate change pressures and species range shifts. Key partners for that effort are the Los Angeles County Museum of Natural History, the Smithsonian National Museum of Natural History, and the Scripps Institution of Oceanography biological repositories.

Angela McMellen Brannigan (National Invasive Species Council (NISC) Technical Advisor, which is multi-agency), reinforced the need to prioritize invasive species to develop needed reference markers for surveillance and monitoring (see [1e]). Her talk on the "National Environmental Detection and Rapid Response Framework (EDRR): Advancing early detection of invasive species using environmental DNA", highlighted that the global annual costs of biological invasions are estimated to exceed $423 billion. The mission of the EDRR Framework [14] is to "find and eradicate invasive species new to the U.S. or those demonstrating secondary spread by coordinating across federal and non-federal partners and investing in innovative approaches for surveillance, data integration, and response capabilities for natural resource management".

Broadening the Workshop to an international audience, Dr. Michael Bunce (Chief Science Advisor, New Zealand Department of Conservation, and Professor at Curtin University) lamented that 75% of New Zealand waters are not swimmable, pointing out the need for accessibility of eDNA sampling, eDNA data reuse, improvement of the ability to determine quantitative signals in the data, and the use eDNA to inform models, such as models for wastewater. He recommended a recent paper in the journal Science on, "The positive impact of conservation action", which states that in two-thirds of cases, conservation either improved the state of biodiversity or at least slowed declines, with interventions targeted at species and ecosystems, such as invasive species control, habitat loss reduction and restoration, protected areas, and sustainable management, being highly effective [15]. Dr. Bunce extended invitations for attending the Southern eDNA Society's upcoming February 18–21, 2025 Environmental DNA conference in New Zealand, whose mission is, "Promoting science and industry collaboration across Australia and New Zealand to advance best practice eDNA methods and adoption in government, private and community sectors" [16].

Dr. Kelly Goodwin (Chair of the NOAA 'Omics Working Group and Vice-Chair of the NOAA Science and Technology Synergy Committee) spoke about, "Summary of request for information (RFI) and agency feedback as a foundation for implementing the National Aquatic eDNA Strategy". She pointed to the need for training for collection and analysis of eDNA, quality control, accreditation, validation, dedicated lab facilities for eDNA analysis, and biorepositories. She stated to the need to remove silos, partner with industry, and include terrestrial eDNA, along with freshwater and marine eDNA. Dr. Goodwin addressed both of the previous Workshops (see [3,4]). Specific points raised included the needs for:

(1) "Data infrastructure and interoperability, including data storage, analysis, visualization, and sharing of data and metadata – including bioinformatic tools, portals, and database for ASVs (amplicon sequence variants);
(2) Training – provide eDNA workforce training and proficiency enhancement;

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(3) Sampling – seek automated solutions, low-cost sampling solutions, and better filtration for dilute targets and turbid conditions;
(4) Accreditation – develop process and guidance for Accreditation/Certification/Validation/Quality Analysis-Quality Control (QA-QC);
(5) Continued Research and Development – continued assay development and study topics to include: fate and transport, eRNA, comparisons with standard monitoring, parameterize uncertainty, and population information from eDNA,
(6) Hubs – establish technology hubs and centers of excellence to accelerate technology development and provide inclusive training,
(7) Reference materials – develop standard reference materials,
(8) Reference sequences – expand the database of genetic markers for vouchered species of interest. Generate and maintain reference sequence libraries,
(9) Lab facilities – develop dedicated lab facilities for eDNA analysis,
(10) Biorepositories – deposit and maintain voucher samples and their tissues and DNA in well-referenced museum facilities, and
(11) Partnerships, including with industry for analytical scale-up, cost reduction (economy of scale), standardization, and market development."

Dr. Goodwin further highlighted the development of quality-controlled databases, with expansion to metabarcoding, specifically, "to develop QA/QC procedures to avoid false positive and false negative reporting into databases, and to execute procedures for data interpretation and communication of data, particularly for low-copy metabarcoding for invasive species in databases." (Note: a recent example of QA/QC for metabarcoding of invasive species in plankton samples is provided in [17]).

3.2. Broader Adoption–Models and Lessons Learned for eDNA

Dr. Alison Watts (University of New Hampshire, Department of Civil and Environmental Engineering) spoke about "Is eDNA Ready for use – and are we ready to use it?", asking what is its "Deployment, development, and research value?". She gave examples from NERR (National Estuarine Research Reserves), which have added eDNA for fishes and other eukaryotes to an existing, standardized water quality monitoring program. Dr. Watts also discussed adoption readiness, and issues with species misidentifications, missing species, consistency with other methods, regulatory acceptance and difficulties setting up programs and interpreting the results (Figure 2).

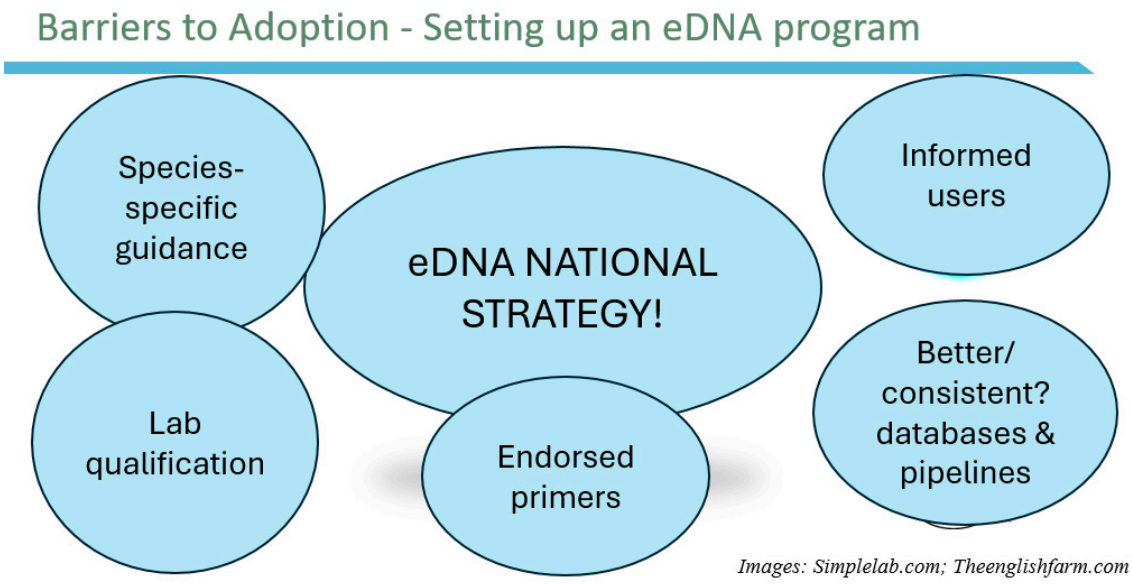

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Figure 2. Potential barriers to setting up an eDNA program, as anticipated to be aided by the National eDNA Strategy. Courtesy of Dr. Alison Watts.

Jennifer Skidmore (NOAA National Marine Fisheries Service (NMFS) Office of Protective Resources (OPR)) talked on, "eDNA Permitting for NOAA species under the Marine Mammal Protection Act (MMPA) and the Endangered Species Act (ESA), relaying that a federal permit is not needed to sample eDNA in U.S. waters, as long as marine mammals are not harassed or disturbed.

Sarah Brown (Senior Scientist, California Department of Water Resources (DWR)) spoke about, "Unlocking eDNA potential for water management in California" and the implementation plan for California, which presents a working model for other entities [18]. DWR eDNA applications include monitoring and evaluation for,

(a) Rare species, to
    (1) Inform Endangered Species Act compliance, and
    (2) Allow research on the species without capture or take;
(b) Invasive and nuisance species,
    (1) Examples: nutria, dreissenid (zebra and quagga) mussels, to verify local eradication,
    (2) Example: to develop tools to evaluate impacts of nuisance species, such as toxic algae;
(c) Biodiversity assessment, to
    (1) Efficiently track species assemblages in response to habitat restoration or species reintroduction; and,
(d) Pathogen monitoring, to
    (1) Establish pathogen presence and prevalence.
    (2) Example: *Ceratonova shasta* near salmon hatcheries".

Brown updated methodology and findings presented in the Second Workshop about projects [3] on using eDNA monitoring for the federally protected Delta smelt (*Hypomesus transpacificus*), an endemic fish that only occurs in the San Francisco Bay estuary, and for the state-threatened longfin smelt (*Spirinchus thaleichthys*) population in San Francisco Bay estuary, to accompany traditional monitoring. She again updated on monitoring nutria (*Myocastor coypus*) in California using eDNA, which is an invasive burrowing aquatic rodent that is difficult to capture and to monitor with traditional sampling.

Dr. Brooke Penaluna (Research Fisheries Biologist, Pacific Northwest Research Station, U.S Department of Agriculture (USDA) Forest Service (USFS), presented, "Learning from Freshwaters: Stream biodiversity, cryptic and rare species, and management implications", relaying how streams that have reported fish populations, generate more protection actions, and how eDNA has been more sensitive than electrofishing sampling in determining the upper limit of fish distributions in these studies.

John A. Hagan (Habitat Ecologist, Northwest Indian Fisheries Commission (NWIFC) representing four tribes and six major watersheds in western Washington state, explained the, "Coastal Watershed Assessment Project: eDNA as a freshwater habitat assessment tool and its potential for tribal fisheries management". He said that the fish species are managed by watershed, giving examples from ongoing eDNA projects for the Olympic mudminnow, Chinook salmon, and Pacific salmon in the Pacific Northwest, which are in addition to traditional sampling and monitoring programs.

3.3. Alignment for Impactful eDNA Research and Implementation

Furthering international perspective, Dr. Kristian Meissner (Development Manager, Finnish Environment Institute SYKE) spoke on the "International eDNA Standardization Task Force" [19] of GEO BON (Group on Earth Observations, Biological Observation Network [20] as an inclusive platform that was founded on World Standards Day in 2023. He said that "almost half of the European experts agree that the key limiting factor to molecular method implementation is the lack of standards and funding". Dr. Meisner advocated the need for building international consensus for

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methodology and adoption. He cited three possible options on how to move forward in developing standards:

(1) "Reinforce cooperation on international standards,
(2) Develop national standards, or
(3) Let others do this first."

At present, standards for eDNA methodology and analyses are being developed in Europe, Canada, the USA, Japan, Australia and New Zealand, and others.

Dr. Caren Helbing (Professor of Biochemistry and Microbiology, University of Victoria, Canada), talked on, "Enhancing confidence in environmental DNA qPCR-based assays through national standards and creating proficiency testing frameworks", featuring the "iTrackDNA" program [21] (which she co-directs) and the Canadian National Standards [22] for accreditation of laboratories through the **Standards Council of Canada** to ISO 17025 standards for eDNA testing. The stated goal of iTrackDNA is "to decrease uncertainty in environmental monitoring and assessments through the following objectives:

(1) Promote standardization of eDNA methods,
(2) Reduce ecological survey time and costs,
(3) Create widely available software for modeling regional biodiversity,
(4) Raise end-user proficiency regarding the use of eDNA methods, and
(5) Promote mainstream eDNA best practices in management, policy, and regulations".

Dr. Helberg commented that iTrackDNA allows for innovation and change to occur, with baseline reporting requirements, performance criteria for quantitative (q)PCR analyses, and a 3rd standard in the works for field sampling methodology. There is a 3-year plan for the iTrackDNA interlab validation program, using synthetic DNA and targeted assays. She described the qPCR validation testing and processes they are using for participatory laboratories.

Dr. Zachary Gold (NOAA Oceanic and Atmospheric Research (OAR)) spoke about, "Operationalizing metabarcoding best practices and standardization: A NOAA 'Omics perspective" stating that there are now 40 data sets in the Ocean Biodiversity Information System (OBIS), which is a global open-access data and information clearing-house on marine biodiversity for science, conservation and sustainable development [23]. He cited the example of the 75-year Pacific krill (*Euphausia pacifica*) time series that was just this year made publicly available from CalCOFI (California Cooperative Oceanic Fisheries Investigations, which has been "observing the California Current since 1959 to understand and predict the effects of climate change [24], providing information on how biodiversity is responding to changing ocean conditions. Dr. Gold further commented that "1/3 of monitored species have no barcodes for identifying them to species, including mysid shrimps and myctophid lanternfishes". Dr. Gold said, "NCBI GenBank [25] is a 'parking lot' for sequences, how can it be curated?", referring to the mis-identifications and missing taxonomic information in GenBank that can thwart correct species assignments (see [17]). He further commented that since sequencing mitochondrial genomes now can cost as little as $150 each, much sequence information from mitogenomes will be forthcoming to improve identifications.

Dr. Tobias Frøslev (Associate Professor, GLOBE Institute, University of Copenhagen, Denmark), of the Global Biodiversity Information Facility (GBIF) Secretariat, spoke on "DNA-based biodiversity Data in GBIF" regarding sharing data and making it standardized and accessible. "The Global Biodiversity Information Facility is an international network and data infrastructure funded by the world's governments and aimed at providing all with open access to data about all types of life on Earth" [26]. It is run through nodes for participating countries, such as the U.S. node at GBIF-US through the National Museum of Natural History, Smithsonian Institution and the U.S. Geological Survey (USGS). GBIF provides data-holding institutions around the world with common standards, best practices, and open-source tools —enabling the sharing of information about where and when species have been recorded. The tools incorporate diverse sources for species information, including museum collections, DNA barcodes, and smartphone photos, based on data standards including Darwin Core [27], which forms the basis for the bulk of GBIF.org's index of hundreds of millions of species occurrence records. Dr. Frøslev brought forward the difficulty of getting data into

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databases, in standard formats, and publishing the data, demonstrating with a prototype metabarcoding tool [26} (https://edna-tool.gbif-uat.org/).

3.4. Emerging Technologies within the Strategic Framework

Dr. Susanne E. Craig (Senior Scientist, NASA Goddard Space Flight Center (GSFC)) presented on, "Bringing environmental monitoring concepts to reality", outlining the "NASA PACE (Plankton, Aerosol, ocean, Cloud Ecosystem) Mission: A hyperspectral view of the Ocean ecosystem", which will see a microscopic view of the ocean from space [28]. For aquatic applications, PACE is employing advanced multi-spectral radiometry and spectroscopy to detect: phytoplankton community compositions and productivity, harmful versus beneficial algal blooms, aerosol contribution to biogeochemical cycles, material exchanges among air, land, and sea, and effects on ocean and human health. PACE aims to aid management and understanding of natural resources, fisheries, aquaculture, ecosystem and watershed health, along with coastal tourism. Dr. Craig also discussed high throughput imaging technologies, for cell identification and enumeration, e.g., imaging flow CytoBot plus machine learning algorithms, for determining phytoplankton community composition, coupled with autonomous eDNA collection and analytical platforms for coupling and verification. This presentation offered an impressive overview of new spectral imaging biotechnology with satellites, coupled to other autonomous platforms and artificial intelligence, to enhance information from eDNA for understanding oceanographic processes and linkages among aquatic, areal, and terrestrial ecosystems.

Dr. Kevan Yamahara (Research Specialist, Monterey Bay Aquarium Research Institute (MBARI)) spoke about, "Unlocking the potential: Exploring the diversity of eDNA sampling: Instrumentation", stating that there are no single sensors or sampler technology that can answer all questions in all ecosystems (see [9]). Dr. Yamahara presented on the instrument renaissance and what is currently commercially available, as well as research and development gaps, and needs for validation and benchmarking. Providing an example of "moving from cutting edge to monitoring transects", he co-authored the Preston et al. (2024) [29] research that sampled eDNA autonomously on the uncrewed surface vessel Saildrone from California to Hawaii, analyzing 52 eDNA samples, which were later analyzed using metabarcoding of the mtDNA COI (cytochrome oxidase I) barcode gene region. Dr. Yamahara concluded that there is "a lot of instrumentation in development, but few are currently commercialized" and that "developers need to balance scientific curiosity and novel developments, with routine monitoring needs".

3.5. Emerging eDNA and eRNA Topics

Dr. Ryan Kelly (Professor of Marine and Environmental Affairs, University of Washington) gave a talk titled, "Learning to count (Organismal abundance and eDNA)", co-authored with Dr. Andrew Shelton (NOAA Fisheries), which discussed the challenges with moving beyond qPCR to metabarcoding and measuring relative abundance of species from eDNA samples. Some of the issues include: (a) primer amplification bias for some taxa, which can be addressed, or evaluated by using different primers and/or using calibration with mock communities and standards, (b) physical loss and transport of eDNA in the environment, with knowledge to be gained from particle tracking analyses in ocean ecosystems, and (c) degradation of eDNA from biological agents – termed biological loss. Dr. Kelly's conclusions were that physical loss of eDNA in ocean ecosystems is much larger than biological loss, with estimates from dolphins and salmon indicating that biological loss is only about 5%.

Dr. Meghan Parsley (Postdoctoral Scholar, Washington State University) presented, "Environmental RNA (eRNA): Progress and potential for population dynamics and demographics", outlining that only a small percentage (less than 1%) of the total nucleic acids in environmental samples are from macro-organisms (with most being micro-organisms). Moreover, eRNA in samples tends to degrade much faster than eDNA, and its possible applications are the "opportunity to determine population status or condition, including age structure, sex ratios, stress and health" (Stevens and Parsley, 2023) [30]. Among types of eRNA, ribosomal eRNA is the most abundant, and

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messenger eRNA is less abundant but provides more diverse information about gene expression. Mitochondrial messenger eRNA is more abundant, while nuclear messenger eRNA is detectable but rarer. A recent paper by Parsley and Goldberg (2023) [31] indicates that life stages of amphibians can be distinguished from eRNA samples using gene-specific assays, paving the way for future applications in oceanic species.

3.6. eDNA Fate and Transport, and eDNA Data Models

Luke McCartin (Ph.D. Student, Lehigh University) spoke about how, "Temperature controls the degradation rate of marine eDNA", finding that higher temperatures are correlated with prevalent and rapid changes in bacterial community diversity, abundance and composition, implying possible impacts with ocean warming. Dr. Eily Andruszkiewicz Allan (Chief Scientist, University of Washington and the eDNA Collaborative) talked about "Localized eDNA transport models". The eDNA Collaborative is a privately-funded group (by Oceankind and the Packard Foundation) directed by Dr. Kelly, which "seeks to accelerate eDNA research through ongoing original research and by building a network of practitioners."

Dr. Jeremy Bruch (Physical Oceanographer, Johns Hopkins APL), presented, "Large scale eDNA modeling", asking, "If we had constant real-time eDNA capabilities, then what?", saying that this will allow development of accurate models for understanding:

(a) "How long has the eDNA been there and where did it originate from?
(b) Can sampling reconstruct the properties of source emission?
(c) What about continuous source emissions vs. intermittent releases (e.g., skin cells vs. excrement)?, and
(d) What are the model requirements for eDNA prediction?"

Dr. Rachel Meyer (Adjunct Assistant Professor, University of California, Santa Cruz) outlined the, "eDNA Explorer Platform", which is a commercial platform [32] whose mission is to "make eDNA fast, easy and accurate", by creating organism lists and insights in days, integrating eDNA, geospatial and GBIF (Global Biological Information Facility) [26] data, and identifying "more organisms accurately and with high confidence". eDNA Explorer provides organism lists for metabarcodes, with modeling using maximum likelihood to analyze biodiversity of samples, as well as other features.

Matthew Biddle (Physical Scientist, NOAA/IOOS (Integrated Ocean Observing System) OBIS-USA) presented, "OBIS (Ocean Biodiversity Information System): Incorporation of eDNA data". He explained that both OBIS [23] and GBIF [26] endorse a joint strategy and action plan for marine biodiversity data, with GBIF incorporating all biodiversity data, and OBIS being specific for ocean biodiversity data, with both being built on the same meta(data) model. The U.S. node is OBIS-USA, which is managed by the US Geological Survey (USGS) and advises the U.S. scientific community on biodiversity informatics, data, and standards.

4. Capstone Talk by Dr. Enric Sala (Pristine Seas Program), and a Panel of Agency and Industry Representatives About the Use of eDNA

On day 3 of the Workshop, in relation to the Smithsonian's Life on a Sustainable Planet Initiative and the Ocean DNA Initiative at the Smithsonian Institution's National Museum of Natural History [33], the Smithsonian Institution's Undersecretary of Science, Dr. Ellen Stofan, welcomed and introduced the capstone keynote speaker of the Third Workshop, Dr. Enric Sala, leader of the National Geographic Society's National Geographic's Pristine Seas Program [34]. Pristine Seas has been employing eDNA sampling at its sites to set baselines and demonstrate what healthy ocean ecosystems should look like. Working with local stewards and communities, this program aims to provides an example of how to engage people and nature to deliver science to inform management. For example, Figure 3 illustrates the use of eDNA for siting locations for analysis, and Figure 4 illustrates the take-over of reefs that are environmentally degraded by pathogens (see [35]), which can be monitored and analyzed using eDNA.

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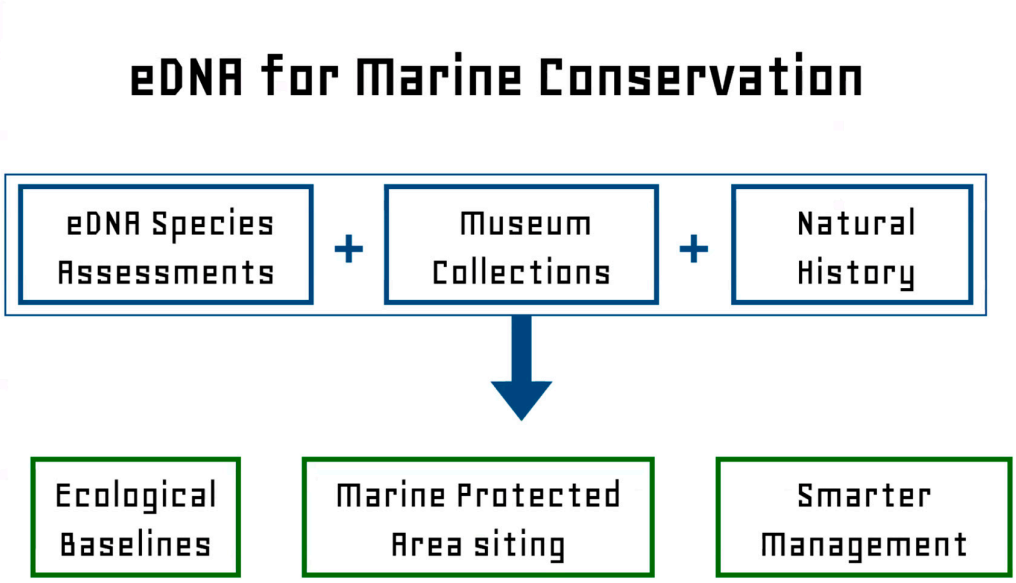

Figure 3. The use of eDNA for monitoring and siting locations of Marine Protection Areas, as discussed by Dr. Sala.

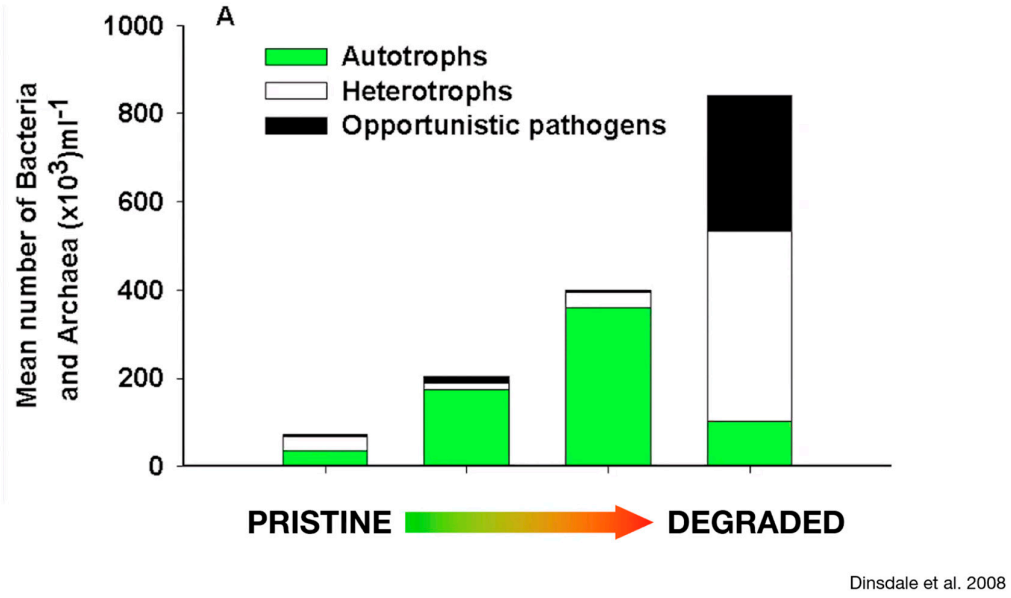

Figure 4. Example of potential comparative reef and restoration processes that can be improved using eDNA analysis, shown by Dr. Sala in his address, illustrating that pristine reefs have fewer opportunistic pathogens with data from Dinsdale et al. (2008)[35] (copyright-free).

Dr. Sala's presentation was followed by a set of short presentations by agency and industry representatives about how they foresee the power and impact of environmental DNA in their day-to-day operations. All in all, the Third National Workshop on Marine eDNA revealed extensive progress in methodology, acceptance, use, and applications for world-wide ecosystem species presence, diversity, variability, and adaptation beyond what was available at the time of the Second

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Workshop in 2022 [3]. Growing needs for the future include additional international coordination and communication, which this paper aspires to contribute to.

Note that this summary paper is the take-away view and opinion of the author and her perspectives on the conference and its presentations and discussions and does not necessarily represent the overall view of the Smithsonian Institution, the conference organizing committee, or other entities.

5. Conclusions and Summary

There has been marked and exciting progress during the past two years in eDNA and eRNA sampling, analytical methodology, utilization in ecological studies, and acceptance in the scientific and management communities, as evidenced in this Third Workshop. Especially intriguing is the potential for growing international engagement and cooperation to save the resources of the World Ocean and freshwater ecosystems in the face of anthropogenic pressures, using eDNA sampling and remote analyses coupled with real-time in-situ processing and A.I. This Workshop's applications also are essential to our understanding of terrestrial and aerial ecosystems, and hold inspirational promise for extraterrestrial explorations. Note that this summary overview paper is the take-away view and opinion of the author and her perspectives on the conference and its presentations and discussions and does not necessarily represent the overall view of the Smithsonian Institution, the Workshop organizing committee, the presenters, attendees, or other entities. Further notes and outcomes of the break-out sessions are anticipated to be made available by the Third Workshop organizers.

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