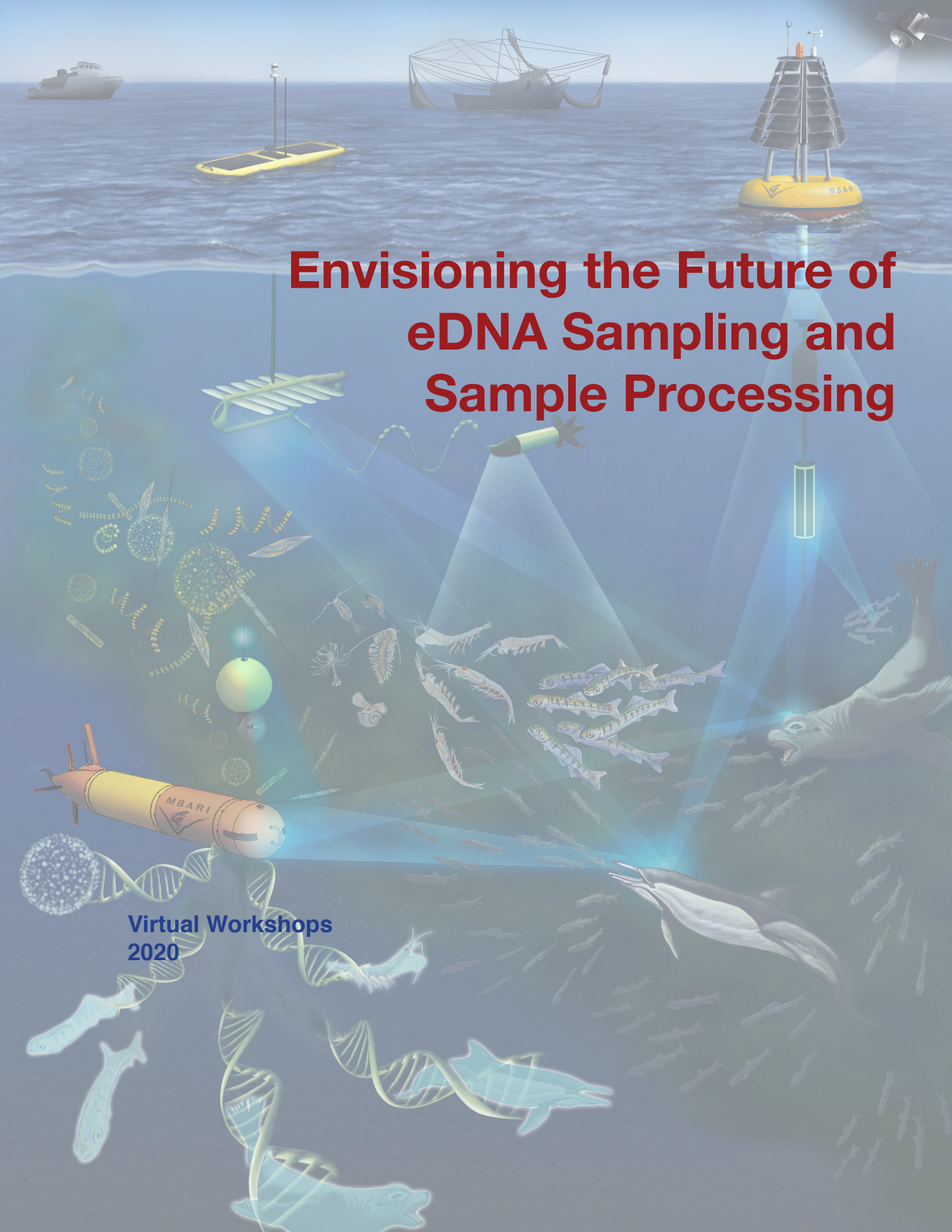

Envisioning the Future of eDNA Sampling and Sample Processing

Virtual Workshops
2020

Workshop Proceedings

Alliance for Coastal Technologies

ENVISIONING THE FUTURE OF eDNA SAMPLING AND SAMPLE PROCESSING WORKSHOP

Remote Meeting

23 June 2020

20 September 2020

This workshop was organized and hosted by the Alliance for Coastal Technologies (ACT) and sponsored by the National Oceanic and Atmospheric Administration (NOAA)/US Integrated Ocean Observing System (IOOS) and Marine Biodiversity Observation Network (MBON)

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ALLIANCE FOR COASTAL TECHNOLOGIES

One of the greatest challenges that NOAA faces in incorporating advanced technologies is bridging the Technology Readiness Level gap between developmental and operational instrumentation. Efforts dedicated to maturing observing technologies to operational readiness through rigorous and relevant testing, while simultaneously building user confidence and capacity, continue to be critical. Building on almost two decades of experience in facilitating the development and adoption of environmental observing instrumentation, the Alliance for Coastal Technologies (ACT, www.act-us.info), proposes to work in collaboration with U.S. IOOS Program Office and Regional Associations (RAs), IOOS federal and non-federal partners, local and regional resource managers, academic researchers and the private sector to improve operational observation capabilities through the quantification of existing instrument performance, and the introduction of new technologies, and enhanced communications. ACT's mission is to foster the creation of new ideas, new skills, new technologies, new capabilities, and new economic opportunities in support of the sustained national IOOS.

ACT was established by NOAA in 2001 to bring about fundamental changes to environmental technology innovation and research to operations practices. ACT achieves its goal through specific technology transition efforts involving both emerging and commercial technologies with the explicit involvement of resource managers, small and medium-sized firms, world-class marine science institutions, and NOAA and other Federal agencies. ACT's core efforts are:

1) Technology Evaluations for independent verification and validation of technologies,
2) Technology Workshops for capacity- and consensus-building and networking, and
3) Technology Information Clearinghouse including an online Technologies Database.

ACT is a leader in the evaluation of commercial and emerging ocean, coastal and freshwater sensing technologies. ACT's Technology Evaluations employ an ISO/IEC 17025:2005 compliant process to generate sensor performance data of known and documented quality through an open, inclusive, and transparent process that is responsive to the *users' operational needs*. Evaluations focus on classes of instruments to demonstrate capabilities/potential of emerging technologies, provide unequivocal verification of performance specifications for commercial technologies, and/or provide validation of instrument operational qualifications that meet users or observing system requirements. Laboratory and field testing are carried out under reproducible, well-understood conditions, which allows manufacturers to assess and improve components, configurations, and designs as necessary. Since 2004, ACT has evaluated nearly 90 sensors from 32 international companies. Results of ACT Technology Evaluations also have provided important insights to users on how to interpret data provided by in-situ instrumentation and thus how to appropriately quantify various environmental parameters. The ACT Evaluations provide independent assurance that basic science understanding, forecasting, and management decisions are based on accurate, precise, and comparable observing data, while minimizing the risk of artifacts and problems associated with young technology.

ACT Technology Workshops have addressed the capabilities of existing operational technologies (e.g., dissolved oxygen and salinity) and needs for new technological solutions to address specific global environmental issues (e.g., nutrients pollution and ocean acidification). Encouragement of the private sector as participants not only provides users with opportunities to

better understand technology options, but also helps technology providers to better understand customers' needs.

The ACT Information Clearinghouse includes all Technology Evaluation and Workshop reports (as downloadable PDFs) and a stakeholder driven database that compiles and inventories information on observing technologies. The Technology Database now connects users with over 400 companies and nearly 4,000 commercial instruments, which increases awareness of technology customers, users, regulators and policymakers of available technology options.

EXECUTIVE SUMMARY

The Alliance for Coastal Technologies (ACT), in coordination with the U.S. Integrated Ocean Observing System (NOAA-IOOS) and the U.S. Marine Biodiversity Observation Network (MBON), sponsored a series of two workshops entitled "Envisioning the future of eDNA sampling and sample processing." In response to the COVID-19 pandemic, the workshop series convened virtually on 23 June and 20 September 2020. The workshop series was narrowly focused on addressing the challenges and needs during eDNA collection and processing. Specific questions included:

1. Does the "state of the science" in eDNA collection and processing apply across aquatic systems and applications? If not, which methods are scalable? Which methods need to be system/application-specific?
2. Are we using the right tools to collect and process eDNA samples?
3. What technological innovations (i.e., materials, consumables, gear) are needed to advance the use of eDNA across systems and applications?
4. How can we better document sample collection from field metadata to lab processing and sample storage?

Both workshops were attended by invited participants from academic research, federal and state agencies, management, and the technology development/transfer sector who are engaged in environmental DNA (eDNA) research and development. Uses of eDNA for research and monitoring ranged from building a bigger picture of biodiversity and communities to detecting a single species or group of species, while technology developers were mainly interested in developing better methods and tools to address users' needs.

During Workshop #1, participants primarily identified barriers to sample collection and processing. The main barriers identified in Workshop #1 included:

1. Contamination, primarily during sample collection and processing
2. Collecting representative samples at appropriate temporal and spatial resolutions
3. Clogging of filters, which causes slow filtering speeds and increases sampling time
4. Tradeoffs in size-fractionation of eDNA and open vs. closed filtration systems
5. Continued need for standard methods, standard operating procedures, especially ones that incorporate reporting metadata, blanks and standards, and quality control

Participants in Workshop #2 learned about and discussed several solutions to some of the barriers identified in the preceding workshop. The different technologies addressed a few of these challenges by incorporating:

1. Autonomous sampling
2. Room temperature preservation
3. Automatic recording of metadata
4. Reduction in waste
5. Pre-sampling estimations of effort

Additional time was spent brainstorming what ideal solutions might look like for barriers in the pipeline still in need of innovation. Priority next steps for improving these technologies include cost and waste reduction, identifying and developing "fit for purpose" solutions, and maintaining lines of communication between engineers and practitioners to scope and, ultimately, test appropriate tools and/or methods.

INTRODUCTION

Worldwide biodiversity has been rapidly declining due to anthropogenic impacts. Increasing incidents of biological invasions and the loss or modification of natural habitats continue to negatively impact native flora and fauna. The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services reported that up to 1 million species worldwide are threatened with extinction, many within decades (IPBES, 2019). Thus, monitoring of biodiversity, invasive species, and endangered/imperiled species has become a focal point in global environmental conservation. Consistent observation of target species or communities over large temporal and spatial scales is needed for effective management and conservation efforts. However, this is difficult when considering the abundance and diversity of species across disparate localities and environments and can be especially challenging in remote regions like polar oceans and the deep sea.

Over the past few decades, the application of environmental DNA (eDNA) metabarcoding has become a powerful tool in detecting organisms in their environments and has allowed the study of whole communities from a single sample. eDNA is DNA originating from cells, tissues, and extracellular DNA that can be extracted from the environment via water, air, or soil (e.g., Ficetola et al. 2008, Díaz-Ferguson 2014, Ruppert et al. 2019). When compared to traditional barcoding, metabarcoding allows for faster, more accurate detection of multiple species at once, but can be limited by reference databases (Ruppert et al. 2019) and may be less sensitive in detecting some species (Deiner et al. 2017). For more specific targets, use of eDNA samples in quantitative PCR (qPCR) allows for quantification of species that are of interest due to their rarity (e.g., McKelvey et al. 2016), status as endangered (e.g., Thomsen et al. 2012) or invasive (e.g., Goldberg et al. 2013, Takahara et al. 2013), or negative effects on human or animal health (e.g., Liu et al. 2020).

The emergence of eDNA methodology is creating opportunities for scientists to develop increasingly better ways to capture, process, and preserve eDNA samples for subsequent extraction, sequencing, and interpretation steps. With most new technologies or methods,

however, uncertainties, inefficiencies, and/or inconsistencies exist. For example, several reviews have recently advised eDNA practitioners to use caution when collecting and processing samples and interpreting results (see Goldberg et al. 2016, Deiner, et al. 2017, Ruppert et al. 2019, Zinger et al. 2019). These include suggestions to 1) conduct pilot studies for target species and environments to optimize sample volume, collection method, and sampling design; 2) create consistent field collection protocols to limit contamination due to the sensitive nature of eDNA methods; 3) better understand and report primer biases during PCR amplification; 4) develop extensive primer/reference sequences databases; and 5) critically interpret and report results to minimize misrepresentation of species presence.

Since the use of eDNA to detect organisms of interest in aquatic environments is an area of rapid and promising development in both basic and applied research and management, users of these tools have created working groups or initiatives to evaluate solutions to overcome these barriers. One example is the interagency Government eDNA Working Group (GEDWG), a group of U.S. scientists working with a wide variety of federal, state, provincial, tribal, and municipal agencies. For the past four years, GEDWG has organized the annual eDNA Technical Exchange Workshop that highlights methods for eDNA sample collection, DNA isolation, marker and assay design and testing, and quality control metrics. The Technical Exchange Workshop also convenes a section dedicated to translating eDNA results into management decisions and communicating eDNA results to managers. Other groups, such as those involved with the Marine Biodiversity Observing Network (MBON), have published method evaluations and recommendations for best practices. For example, Djurhuus et al. (2017) evaluated filtration and extraction methods for eDNA biodiversity assessments across trophic levels and found that extraction method can result in significantly different views of community structure, with some molecular targets more sensitive than others. Such contributions to the scientific literature continue to build the foundation for standard operating procedures (SOPs) for current and future researchers that can help inform the decision-making and experimental-design process.

The ACT eDNA Virtual Workshop Series built upon the progress made by these, and other groups and efforts to focus on first steps in the eDNA "pipeline." The "pipeline" describes four different phases of eDNA analyses including 1) sample collection, 2) lab processing (including sequencing), 3) bioinformatics, and 4) data interpretation. Several ongoing efforts[1] tackle downstream steps in the pipeline (i.e. steps 2-4). Fewer groups are specifically focusing on earlier stages of the pipeline. DNAqua-Net is one such effort in which a multi-disciplined consortium of researchers that serve on five different working groups to address all areas within the pipeline. Smaller regional groups, such as the Molecular Methods Working Group of the California Water Quality Monitoring Council, have formed to identify best practices and provide resources to various stakeholders for interpreting and distributing molecular-based data.

The workshop scoping group (see Appendix A) therefore decided to focus the ACT eDNA workshop series on initial steps in the pipeline, specifically sample collection, concentration, and preservation of samples for later eDNA analyses. This focus was also chosen to best leverage ACT expertise in building consensus around technological needs that may be brought to bear on

[1] Marine Biodiversity Observation Network (MBON); GLOMICON; Genomics Standards Consortium, Oceans Best Practices, NOPP groups, National Microbiome Data Collective, Interagency Working Group on Biological Data Sharing; and others

limitations in current methods and tools. The ACT partnership has conducted numerous technology evaluations and demonstrations, which inform users on the performance of existing and novel technologies. The various workshops organized by ACT have also helped build consensus to further the development of useful tools while also facilitating communication between technology developers, manufacturers, and users. With this track record in facilitating consensus building, testing new technologies and tools, and providing that information to the public, the potential for transformative innovation to tackle these first steps in the eDNA pipeline is an opportunity that ACT is well suited to lead.

WORKSHOP SERIES OVERVIEW

The purpose of the workshop series was to foster discussion about the challenges and obstacles to collecting, concentrating, and preserving samples for eDNA analyses. This objective was accomplished by inviting participants from a broad range of regional and national agencies and institutions (see scoping group and participant lists, Appendix A and B, respectively) to contribute a breadth of knowledge and experience on the state of the science in eDNA and current challenges and/or opportunities for innovation. Prior to each workshop, short surveys were sent to participants to gather information to help guide the agenda and direction of each event. Charge questions were also presented to workshop participants in advance of each workshop, with breakout sessions used to facilitate open discussions and recommendations. Consensus was built following presentation of breakout recommendation by the workshop participants. Each workshop balanced open discussion with facilitated breakout sessions guided by charge questions led by designated facilitators.

The second workshop also incorporated short presentations by technology and/or method developers on novel solutions to overcome challenges, including efficient collection and concentration of samples. At the start of each workshop, a short introduction of the overall effort was given by the workshop organizer and leader, Dr. Beth Stauffer (University of Louisiana at Lafayette and ACT partner) and was followed by a presentation of the pre-workshop survey results. Results from the pre-workshop surveys, discussion of charge questions, and recommendations for next steps are described below.

ENVISIONING THE FUTURE OF EDNA SAMPLING AND SAMPLE PROCESSING, VIRTUAL WORKSHOP #1

Pre-workshop 1 Survey:

The pre-workshop survey asked participants to identify the sector in which they work, main interests in using eDNA, and their targets and environment(s) of interest. They were also asked to describe what they believe are the most limiting steps in the collection and processing of samples for eDNA and how sufficient their current approaches and tools are in meeting their needs.

The majority of respondents (n=24) were from research (63%) and technology development/transfer (25%), while 3 participants identified as "other" or "all of the above." Participants were mainly interested in studying biodiversity (41%) and targeting single species (37%). Most respondents were sampling in the ocean (32%) and estuary/coastal (29%) environments versus freshwater. The speed of collection of clean, representative samples (27%) and filtration steps/filters (31%) were identified as the most limiting steps in eDNA collection and processing, respectively.

Charge Question 1: **What are the main barriers to collecting a clean, representative sample in your environment of interest? Are there certain environments that are harder to collect samples from than others**?

1. *Contamination during field collection is a main concern.* This is especially true when samples are collected by field personnel who are unfamiliar with molecular techniques and may not be taking the necessary precautions to limit contamination, especially that from human DNA.

- In order to minimize contamination, researchers typically spend much of their time disinfecting equipment and materials between samples, and even when these disinfecting protocols are in place, they may not be followed the same way among different personnel.
- Conducting extreme flushes between samples tends to be inadequate in limiting sample cross-over/contamination (e.g., when sampling communities of ubiquitous microbes using autonomous platforms).
- One solution would be manufacturing enough 3D printed parts to have new materials for every sample collected; a downside to this solution is the production of plastic waste.
- Another approach to address contamination is to run many blanks to account for any contamination and cross-over in samples. The results from these blank runs should be incorporated into data reporting in subsequent steps in the pipeline (i.e., sequencing, bioinformatics, interpretation).

2. *Another concern was the ability to capture representative samples across relevant temporal and spatial scales.* This was of greatest concern when balancing efficient use of field time and effectively sampling target groups. Finding balance between more sampling sites with transport effects (i.e., by not filtering on-site) or fewer sites with reduced transport effects (i.e., by filtering on-site) was also highlighted.

- One of the potential solutions discussed was using pre-loaded filter devices that could be taken into the field or on a ship. These provide one unit per sample and therefore eliminates the need for decontamination steps between samples. Ideally these devices would be balanced with a 'green' approach to minimize non-renewable materials in production and disposal.

Charge Question 2: **What are the main barriers to concentrating a representative sample for your purposes? Are there certain samples that are harder to concentrate than others?**

The group discussing this charge question outlined several major barriers to concentrating samples for eDNA analyses. These were positioned as a set of conflicting issues that, at present, represent trade-offs in effective, accurate eDNA sample processing.

1. *The form, or size fraction, of eDNA when collected is a primary challenge.* While prescreening can help with apportioning size fractions, there are concerns about wasting or missing eDNA that may be present in unused fractions.

2. *The use of open (able to be dismantled and reused) vs. closed (single unit, often single use) filter systems both have advantages and disadvantages,* for example:

- Ease of use in the field (advantage: closed)
- Ease of extracting in the lab (advantage: open)
- Cost (advantage: open) and increased plastic waste (advantage: reusable)

3. *Clogging of filters can lead to slow speeds and insufficient volumes, especially in environments with high particle loads.*

- With one exception (see below), all participants rely on filtration to concentrate samples, though it is notable that across the group filter pore sizes range from 0.2 µm - 5 µm.
    - The other participant utilizes a Subsurface Environmental Sampler (Montana Emergent Technologies), which uses an internal chamber to collect, grow, and ultimately evaluate microbes *in situ*. Within the internal chamber a media can be added that attracts microorganisms, enables biofilm formation and could be used to attract and concentrate other forms of eDNA. The Subsurface Environmental Sampler can be closed and sampled through swagelok fittings in the bottom.

Charge Question 3: **How well do you trust that samples from other groups are collected and concentrated in a consistent, documented manner?**

1. *The general consensus was that there is little sample sharing occurring at present, and that such sharing is impractical given current practices.* Participants felt limits on sample sharing stemmed from more than metadata reporting requirements, which seems to be relatively well defined at this stage.

- Variation in quality control practices among groups was a main barrier to sharing samples or data, with the following concerns and recommendations:
    - Application of blanks and standards should be routinely used to improve consistency and validate accuracy.
    - Ideally, community-specific standards (prokaryote/eukaryote microbes, metazoan) need to be developed to assess efficacy of eDNA processing pipelines.
    - Spike-in standards should be used at extraction. There is interest in developing whole organism standards that could be spiked-in at the sample concentration stage to assess extraction efficiencies and downstream community characterizations.
    - Contamination control steps should be documented with sample collection to identify potential sources of human contamination (critical for microbial, pathogen tracking)

and control for sample carryover (e.g., repeat sampling, transect sampling, diverse habitat sampling in single field session).
    - Filters can be subdivided to provide cross-calibration of a particular probe set or sequencing strategies.
- For *large-scale monitoring programs* employing eDNA for habitat-community description, samplers should be trained and field-tested on SOPs to ensure consistency in sampling (filter type, sample volumes, metadata).

ENVISIONING THE FUTURE OF eDNA SAMPLING AND SAMPLE PROCESSING, VIRTUAL WORKSHOP #2

Pre-workshop 2 Survey:
The pre-workshop survey asked participants to identify themselves as users of eDNA data, developer/providers of eDNA sampling/processing technologies, or both. Users were asked to classify their job fields, define how they use eDNA, and describe how efficient they believe their current methodology to be. Developers were asked to detail what they understand to be the biggest barriers to eDNA sample collection and concentration and any solutions that they are developing or find promising.

The majority of respondents (n=13) were both users and developers (69%) of eDNA sampling/processing technologies. Most users identified filtration (46%) as the rate limiting step, with many users (27%) wanting more efficient concentration/filtering but were also unsure what an improvement would look like (37%). Developers understood filtration, cost, manual labor, contamination, and plastic waste to be the biggest barriers to innovations in efficient and appropriate eDNA sample collection and concentration.

Summary of Workshop Presentation Topics
- **"Jonah Ventures," Noah Fierer, Jonah Ventures**, presented a brief introduction to Jonah Ventures focusing on what they have learned about eDNA sampling and sample processing from running many thousands of aquatic eDNA samples over the past few years.
- **"Large volume eDNA extraction and inhibitor removal," Maggie Hunter, U.S. Geological Survey**, presented on improving eDNA yield and quality through the development of novel large volume eDNA extraction and inhibitor removal protocols.
- **"Some thoughts on the collection and extraction of samples for eDNA analysis," Jeff Bowman, Scripps Institution of Oceanography**, presented on the challenges in addressing both microbial and metazoan eDNA sampling and analysis needs, and on new methodological developments that can lead to higher throughput and lower cost analysis.
- **"An Overview of Pall Filtration Products," Lori Euler, Pall Laboratory**, presented an overview of the flexible filtration options Pall can offer labs doing eDNA work and how Pall can work with end users on custom solutions for their needs.
- **"eDNA Sampler: A fully integrated environmental DNA sampling system," Austen Thomas, Smith-Root**, presented efforts to develop user-friendly eDNA sampling tools.

- **"The Environmental Sample Processer," Kevan Yamahara, Monterey Bay Aquarium Research Institute**, presented a brief overview of the Environmental Sample Processor (ESP) and applications of its use for eDNA sampling in both fresh and marine waters.
- **"Lab-on-chip microfluidics," Vincent Sieben, Dartmouth Ocean Technologies, Inc**., presented their lab-on-chip sensors for in situ chemical analysis and their eDNA sampler for the OceanAware project, which will see 15 phosphate analyzers and 10 eDNA samplers deployed in an aquaculture context for harmful algal bloom monitoring.

All presentations can be accessed as PDFs here.

Charge Question 1: ***What challenges do the presented innovations address? What aspects could be improved on or are still lacking?***

Challenges addressed:

1. *Autonomous sampling* can address issues with user contamination and problems with sampling over large spatial and/or temporal scales. Autonomous samplers also enable high frequency sampling, thereby addressing concerns about sample variability.
2. *Room temperature preservation without the use of chemicals* would solve many issues such as lack of or limited freezer space. Such preservation methods would make sampling available to a larger number of people, including community-based science efforts.
3. *Lab-on-chip* approaches were also of interest since they included DNA plus water chemistry, addressing limitations associated with tying eDNA and metadata records together throughout the pipeline.
4. *Automatic recording of metadata* during sampling addresses issues with standardizing metadata records for eDNA samples.
5. Many participants appreciated seeing a *reduction in waste* in some of the presented solutions.

Suggested improvements:

1. *Lowering the cost of the technology.* This was a primary concern around the use of autonomous or other expensive platforms and approaches. Also, some improvements that come with standardizing protocols require expensive equipment (e.g., centrifuge for larger tubes, bead beater) that may not be available in small academic lab, or other, budgets. Government agencies can do more long-range planning to factor in these costs, but it can become limiting for academia or other users.
2. *More "Fit for Purpose" solutions are needed.* Several comments underscored that the "one size fits all" approach does not work for many applications of eDNA methods. A recent and timely example - coronavirus detections in wastewater versus in air (e.g., Al Huraimel, 2020 and references therein) - highlighted this need for different approaches.
3. *More mobile, portable, and/or autonomous platforms* at lower price points with filter/extraction/sequence capabilities. Being able to transmit data back to shore in near real-time would be a "pie in the sky" solution for eDNA collection. Portable technology for river systems or more complex technology for ocean applications could allow these tools to be applied at different spatial scales, as needed.

4. *Next steps should bring together engineers and biologists to create a list of specifications* that tools can be built around. A workshop participant mentioned that although there are many eDNA workshop activities occurring right now, none seem to have an engineering angle to them.

Charge Question 2: ***What would be most helpful to you in terms of improving the current way you are collecting, concentrating, and preserving eDNA samples?***

1. *The development of a handheld/autonomous device to augment or replace Niskin bottles.*
   - Sampling the deep or open ocean comes with challenges, such as having limited reproducibility due to the limited number of bottles on a CTD rosette.
   - Another significant challenge is sampling at depth without contamination during the process of getting the equipment and sample back onto the vessel.

2. *Optimizing sampling design before sampling occurs.*
   - Pilot studies for new areas or targets are beneficial but can be costly. Such pilots are only valuable if the seasonality/potential environmental variability (especially in dynamic systems) between pilot and actual sampling are consistent, predictable, and/or can be considered.
   - Empirical tools such as occupancy modeling for optimal sample volume, replicates, etc. may help overcome the challenges of pilot studies.

3. *A clearinghouse/repository of information and best practices.* Such a resource would allow for new researchers/agencies coming onto the eDNA scene to not have to reinvent (or retest) methods/protocols (e.g., that filters perform equally for prokaryotes).

4. *A Methods "Bake-off"* (i.e., similar to the ACT demonstration or verification model, https://www.act-us.info/evaluations.php)
   - *Test the main methods and tools for sample collection, concentration and preservation in a standardized framework*. Such an activity could be transformational for standardizing methods for longer-term (i.e., time series) projects where consistency and efficiency are key.
   - Such an effort should utilize standard, spiked samples and unified downstream processing (extraction and cleanup, metabarcoding/sequencing, etc.) to only test these early pipeline methods.

Consensus Building Discussions

- Around the issue of contamination and the need to run blanks or standards as controls, the point was made that *data on quality control is often decoupled from the downstream eDNA data.* These data streams must be consistently coupled from sample collection through interpretation.

  - One way these data issues have been dealt with in the past are by requiring some minimum quality control data to be included in repository uploads[2].
- Around *issues of filtration* and the tradeoffs inherent in finding an effective and economical solution, the suggestion of using peristaltic pumps or serial filtration were also discussed.
- Finally, there was again a fair bit of discussion in the larger group around the fact that many of these challenges are context-dependent and *there is no "one size fits all" approach to eDNA sampling and processing.*
  - Rather, a continuum of methods currently exists, and there are use- and/or target-specific considerations that influence where along that continuum users place themselves. More effort should be put into understanding what these shared uses are, optimizing them within the community, and also building more use-specific communities of practice who can benefit from shared experiences.

COMMUNITY RECOMMENDATIONS AND CHARGES FOR ACT

Based on the input, discussions, and feedback from this workshop series, the following recommendations were made to continue to advance early stages in the eDNA pipeline:

1. Build consensus on the requirements needed for building the next generation of efficient, cost-effective, and appropriate eDNA sample collection and processing instruments. To accomplish this, communication, consensus-building, and ultimately collaboration between researchers, technology and methods developers, and end-users must be maintained in focused, goal-oriented meetings and workshops moving forward.
2. ACT should foster the design and support for necessary cross-lab and cross-method comparisons focused on existing AND emerging sample collection technologies. Such an effort should capture the impact of these tools and methods on standardization of eDNA collection, sequence data quality, and downstream interpretation by diverse end users. Publicly accessible reports verifying or demonstrating performance of these tools and methods must be provided to help researchers and users select suitable and robust approaches for eDNA collection and concentration.
3. Continue to develop standard practices, protocols, and tools that are well suited for shared targets (e.g., multiple/single species/taxa, abundant/rare) and environments (e.g., deep or open ocean, estuary, soil, river). Such SOPs will continue to enhance consistency across groups of eDNA practitioners and lower the bar for incoming eDNA researchers while also allowing for a tailored approach depending on the use of eDNA data in research, monitoring, and management.

[2] e.g., by NCBI. Standards for doing this with eDNA data could be based on the MIQE guidelines promulgated for qPCR data (Goldberg et al. 2016)

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