IWEG

International Workshop on Environmental Genomics

NEW HORIZONS FOR ENVIRONMENTAL GENOMICS APPLICATIONS

JUNE 21 – 22, 2023

eDNATec

CEGA Centre for Environmental Genomics Applications

E&P ENVIRONMENTAL GENOMICS RESEARCH PROGRAMME

An IOGP programme

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Introduction

The 8th annual International Workshop on Environmental Genomics (IWEG) was hosted in St. John's Newfoundland and Labrador, Canada from June 21st – 22nd, 2023.

This year's workshop focused on technical innovations and new applications of environmental genomics technologies, with the theme: New Horizons for Environmental Genomics Applications. Additionally, many talks and discussions followed up on the previous 2022 IWEG theme of Standardization and Evaluation of eDNA Approaches. The workshop opened with a keynote presentation on the importance of international standards by Dr. Kristian Meissner of the Finnish Environment Institute. The workshop included three presentation sessions with presenters from Fisheries and Oceans Canada, United States Geological Survey, the International Association of Oil and Gas Producers Environmental Genomics Joint Industry Program, US Army Corps of Engineers, TotalEnergies, Dalhousie University, AZTI, Illumina, Dartmouth Ocean Technologies Inc., McLane Research Labs, Ocean Diagnostics, and the Centre for Environmental Genomics Applications. The workshop also featured an interactive group discussion about Technology Readiness Assessments and a panel discussion on how eDNA and environmental genomics can be used to meet UN Biodiversity Conference COP15 goals. The workshop agenda is included in Appendix A.

Keynote Address

Dr. Kristian Meissner, development manager of the Marine and Freshwater Solutions unit at the Finnish Environment Institute, Syke, and chair of the European CEN TC230 working group delivered the keynote address on the importance of international standards.

As the use of environmental genomics technologies for ecosystem assessment increases around the globe, we risk running into issues of data reliability and comparability. Ecosystem health is an international goal and thus requires international solutions. Without international standards, global-scale comparisons may be impossible to conduct in the future. The European Union Water Framework Directive (EU WFD) makes a great case study for reflecting on the importance of standardization. The EU WFD did not include a universal set of standard assessment procedures, instead opting to give member states the authority to determine appropriate assessments for various environment types. Thus, over 300 aquatic ecological assessment methods were developed for use in Europe to meet the EU WFD goals. As a result, the assessments from different members of the EU WFD were incomparable and required complex intercalibrations which took several years to develop. This serves as an example of why we need to consider international standardization sooner rather than later.

The process of creating standards is time intensive, requiring several rounds of negotiations and validation steps. The standardization process should also involve proficiency testing within labs and between labs to ensure the validity of results. The International Organization for Standardization (ISO) is an independent, non-governmental organization that can facilitate the development and publication of consensus-based international standards. Such standardization could improve public trust in environmental genomics technologies and increase their adoption. International standards should not only focus on quality, but also inclusivity. It is in everyone's interest to establish a harmonious system of methods and data structures that are accessible to all stakeholders globally.

Applications of Environmental Genomics

Presenters highlighted government, academic, and industry projects that are using genomics technologies to study the environment for a variety of end goals.

Government researchers in Canada and the United States are using eDNA for early detection of aquatic invasive species in both freshwater and marine systems. In the US, there are early-stage projects using eDNA metabarcoding to determine eukaryotic assemblages from soil and predict geographic origin. Another project showcased metabarcoding of aquatic eDNA to map fish species in data-poor freshwater systems throughout Newfoundland and Labrador. The presentation sessions demonstrated the broad applicability of eDNA metabarcoding.

One topic that was frequently discussed is how to address the hesitation of decision makers to adopt environmental genomics solutions. Skepticism about the reliability and reproducibility of environmental genomics metrics is one of the main roadblocks to widespread use. As such, it is important to establish minimum standards for how samples are collected and handled in the field, processed in the laboratory, and analyzed with bioinformatic procedures to ensure reliability and reproducibility. The International Association of Oil and Gas Producers and their partners are currently working to publish industry guidelines for applying environmental genomics technologies to ecological assessments. Similarly, the iTrackDNA project aims to promote the standardization of eDNA methods. Results from inter-lab calibration studies from the iTrackDNA phase 1 tests were similar across labs, but one lab with high variance in results highlighted the need to ensure all technicians are proficient. Another way to improve the reliability of environmental genomics studies is to improve the reference databases that are used in bioinformatic processing pipelines. Custom reference databases are emerging as a way to improve the accuracy of taxon assignments from eDNA metabarcoding data. Compared to pipelines that query massive DNA sequence repositories, those that use curated reference databases can have built-in quality control through additional verification steps taken while building the database. For example, the new NAMERS (Novel Applied eDNA Metabarcoding Reference Sequences) database includes a web portal of whole mitogenomes and nuclear ribosomal DNA cistrons for freshwater fish in British Columbia. Every sequence in the NAMERS database has a museum-cataloged voucher specimen with detailed metadata and the morphological species identity was verified using standard COI barcoding. These initiatives to develop standard methodologies and improve reference database curation foster increased trust in environmental genomics metrics.

What are the new horizons for environmental genomics technologies?

We have seen widespread adoption of eDNA as a tool since the first annual IWEG in 2016. While the use of eDNA metabarcoding for biodiversity assessment and qPCR for single-species detections are becoming quite common, there are several instrumental and methodological innovations that could take a foothold in the field of environmental genomics.

Advancements in sampling and analytical technologies are making environmental DNA and RNA samples easier to collect and sequence. The workshop included presentations on three new automated eDNA samplers: the Dartmouth Ocean Technologies automated eDNA sampler, the RoCSI high sample count eDNA sampler by McLane Research Laboratories, and the Ascension eDNA sampler by Ocean Diagnostics. The adoption of automated samplers could greatly increase the quantity of environmental data that is collected, especially from remote locations which are difficult to access. Widespread use of automated sampling could also provide eDNA datasets with more spatial and temporal coverage than ever before. However, it is important to carefully consider cost/benefit of deploying these new tools for widespread applications. Various benchmarking analyses will ensure optimal use of emerging tools. In addition to advancements in the way samples are collected, improvements to laboratory instruments could improve the quality and quantity of data available. Illumina's sequencing advancements continue to make data more accessible through faster processing times and simplified instrument operation and maintenance.

Much of the discussion of new horizons focused on methodological developments which may give environmental genomics practitioners better insights into organisms and their ecosystems. Some presentations explored alternatives to standard mitochondrial amplicon approaches to eDNA, with one presentation exploring the use of nuclear elements to better-detect rare species and another presentation showcasing metagenomics for environmental assessment. Other presentations highlighted bioinformatics developments that are improving the accuracy and scope of eDNA metabarcoding data. As discussed in the above section, customized sequence databases are improving the taxonomic assignment capabilities of metabarcoding analysis pipelines. The workshop also included presentations showing novel applications of network analysis using environmental genomics to assess holistic ecosystem health. Trophic networks built with eDNA biodiversity data can provide similar indicators to conventionally derived networks for the number of nodes, number of links, maximum/minimum chain length, connectance, and connectivity. The networks can be adapted to provide similar estimates for redundancy and keystone species. Networks built with eDNA typically include a broader range of organisms than what is usually feasible for conventionally derived networks. In addition to improving analysis of new samples, these types of advancements in methodologies may provide opportunities for eDNA practitioners to reanalyze archived environmental genomics data with new reference databases or new functional network approaches.

Interactive session

During the interactive session, workshop attendees applied Technology Readiness Assessment (TRA) to new and emerging environmental genomics technologies.

TRA is a method to describe the maturity of technologies. TRA was developed to assess space system technologies and is published in the ISO 16290:2013 standard, but TRA can also be generalized to apply to other types of research innovation. The goal of the session was to identify which environmental genomics technologies are the most mature and to discuss what additional research is needed to make less mature technologies ready for implementation. This exercise was chosen because of the workshop's focus on New Horizons for Environmental Genomics Applications. The assessment process involves identifying Critical Technology Elements (CTEs), assigning Technology Readiness Levels (TRLs) to each CTE, and developing a technology maturation plan to address gaps in readiness.

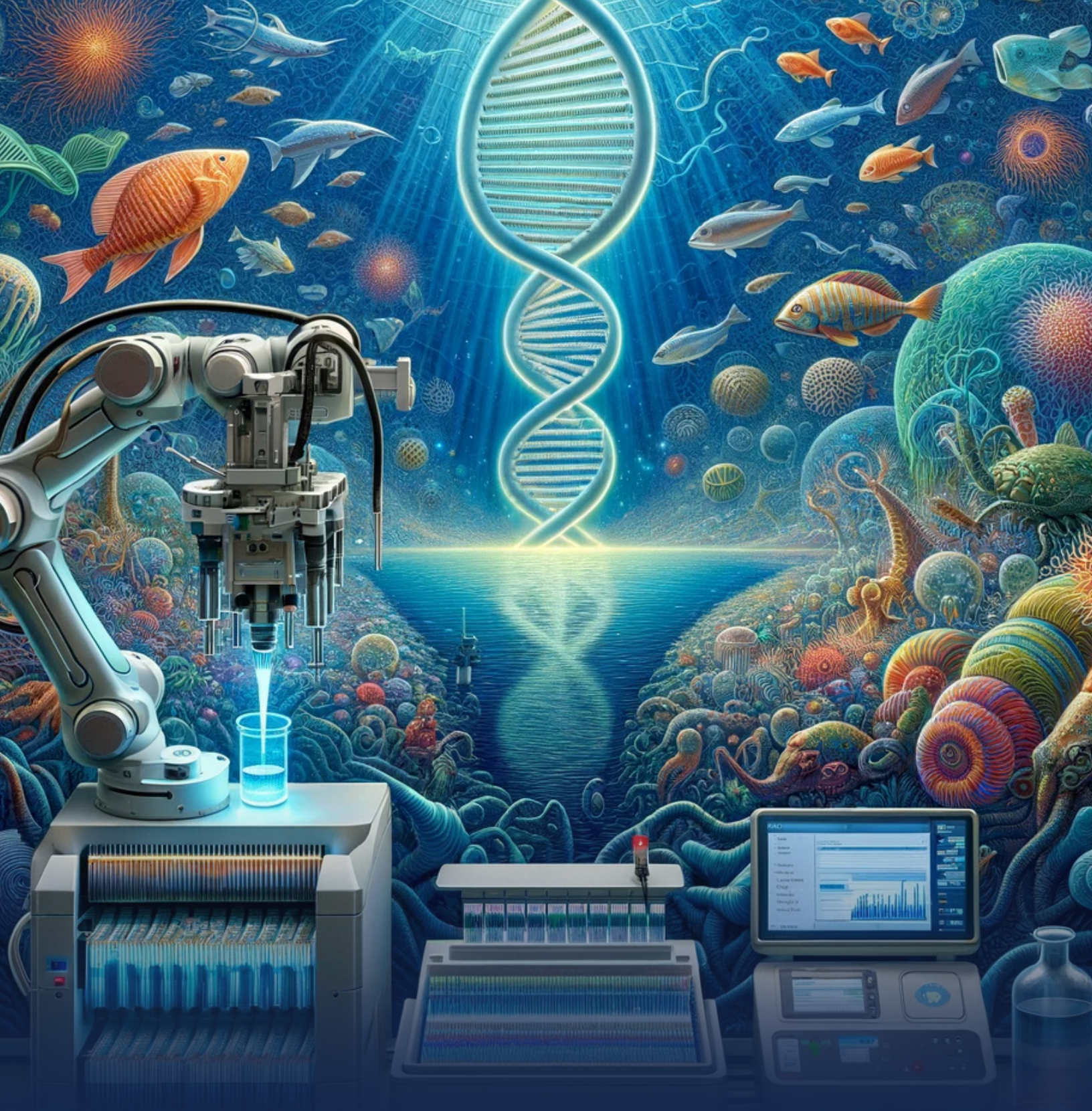
There are nine levels of technology readiness:

TRL	Description
1	Basic principles observed
2	Technology concept formulated
3	Experimental proof of concept
4	Technology validated in lab
5	Technology validated in relevant environment
6	Technology demonstrated in relevant environment
7	System prototype demonstration in operational environment
8	System complete and qualified
9	Actual system proven in operational environment

Interactive session discussions are summarized in **Appendix B**. Five technologies were evaluated during the interactive session:

1. Automated Sampling
2. In Situ Analysis
3. Population Assessment via eDNA
4. Quantification & Biomass Estimation
5. Metagenomics & Metatranscriptomics

Workshop attendees self-selected TRL discussion groups based on their expertise and interests. Each group discussed a specific use case for their technology so conversations could center around specific problems and solutions. After determining a scenario, the groups identified CTEs for their technology. A technology element is considered to be a CTE if it is both critical to the operation and also a new or novel application of that element. The technologies differed in the number of CTEs that were identified by the groups. Quantification & Biomass Estimation had only two CTEs assigned, whereas the group discussing Automated Sampling identified 5 CTEs. The groups then determined the TRL for each CTE of their technology. This was a challenging step and, in some cases, groups identified a TRL range rather than a single TRL for a CTE. Based on the average TRL score of CTEs, Automated Sampling is the technology that is the most mature out of the five technologies that were discussed. Population Assessment via eDNA had the lowest average TRL score and the group recommended more studies comparing eDNA-based and tissue-based population genetic structure assessments. Overall, the TRL exercise was a great opportunity for experts to discuss these emerging technologies and come up with plans to guide research and development.



Discussion Panel

The discussion was moderated by Mehrdad Hajibabaei (CEGA & University of Guelph), and the panel included Thomas Merzi (TotalEnergies), Daniel Doolittle (Integral Consulting), Richard Lance (US Army Engineer Research and Development Center), and Cynthia McKenzie (Fisheries and Oceans Canada). The panel discussed eDNA as a means of fulfilling some of the targets set forth at the UN Biodiversity Conference, COP15, which was held in Montreal, Quebec from December 7 – 19, 2022.

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COP15 Target 2: Ensure that **by 2030 at least 30 percent of areas of degraded terrestrial, inland water, and coastal and marine ecosystems are under effective restoration,** in order to enhance biodiversity and ecosystem functions and services, ecological integrity and connectivity**.**

How can currently available environmental genomics tools be leveraged to support this target?

There is a dearth of post-implementation monitoring to evaluate the effectiveness of restoration programs. Long-term post-restoration monitoring is especially rare, usually due to a lack of funding. Automated sampling and metabarcoding can be easier to scale than conventional ecosystem assessment methods, and this scalability may make it more feasible to monitor restoration efforts on larger temporal and spatial scales.Considering environmental reconnections as a mosaic, the expanded spatial scale may improve our ability to ascertain the effectiveness of mitigation actions on restoring ecological connectivity. Environmental genomics will be an effective tool to aid in monitoring restoration activities. For example, eelgrass habitat restoration projects have shown value in combined approaches that augment conventional beach seine monitoring with eDNA monitoring and traditional knowledge to evaluate efficacy over long time frames.

Are there any roadblocks or barriers that exist for eDNA practitioners and/or managers in applying these tools to support this target?

Panelists acknowledged that the target itself is not clearly defined and that this ambiguity may act as a roadblock to applying environmental genomics. For example, what qualifies as degraded? At what threshold would we consider restoration effective? Should efficacy be assessed based on ecosystem functions, genus or species compositions, genetic variation within populations, etc.? These uncertainties could complicate the process of planning environmental genomics projects.

Another roadblock is that most eDNA work has been focused on aquatic environments, whereas much of the restoration activity associated with this target will likely be terrestrial. This mismatch may limit the applicability of current environmental genomics applications for this target. Additionally, the diversity present in areas that are in need of restoration monitoring may not be well represented in reference databases.

What advances in environmental genomics approaches are on the horizon that can support this target?

Non-aquatic sample types such as soil eDNA can be explored to address the roadblock of terrestrial applications, and the continuing efforts to improve reference databases will be helpful as current databases are not representative of the eukaryotic diversity found in terrestrial soils. Network analysis and metagenomics developments may also support this target. Specifically, these technologies can be applied to evaluate if ecological integrity and ecosystem functions and services are responsive to restoration efforts.

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COP15 Target 8: Minimize the impact of climate change and ocean acidification on biodiversity and increase its resilience through mitigation, adaptation, and disaster risk reduction actions, including through **nature-based solution and/or ecosystem-based approaches,** while minimizing negative and fostering positive impacts of climate action on biodiversity.

How can currently available environmental genomics tools be leveraged to support this target?

In order to understand the impacts of climate change, we need more baseline information. Environmental genomics can be applied to establish baseline measurements before mitigation actions are initiated[1]. In some systems, it may be possible to estimate historic biodiversity baselines from ancient eDNA retrieved from sediments and traditional knowledge. Having more comprehensive estimates of historic biodiversity compositions could provide better guidance on how to implement nature-based solutions.
Additionally, mitigation efficacy could be improved by considering the genotypes of organisms planted or introduced during restoration activities. Organisms could be selected based on their genetic diversity and tolerance to stressors related to climate change and ocean acidification, and thus have better survival and reproductive success. This will require more research into population genomics, epigenetics, transcriptomics, and ecotoxicology.
Biodiversity metrics based on environmental genomics surveys could be added to the framework used by the oil and gas sector for carbon sequestration. This could help to ensure that carbon sequestration activities are supporting biodiversity goals. There are lots of opportunities to team up with carbon sequestration projects to try and develop eDNA-based monitoring metrics.

Are there any roadblocks or barriers that exist for eDNA practitioners and/or managers in applying these tools to support this target?

Resource allocation towards nature-based solutions needs to be increased. To encourage this shift, we need better metrics (financial, risk-management, etc.) that can be used to demonstrate the benefits of ecosystem-based approaches over conventional methods. Environmental genomics could be included as a tool to build these metrics.

What advances in environmental genomics approaches are on the horizon that can support this target?

Metatranscriptomics or epigenetics may be leveraged to give indicators of ecosystem health sooner than metabarcoding-based biodiversity indicators can. Metagenomics could be used to investigate intraspecific variability and associated resilience. Furthermore, network analysis could be applied to these data to see what makes some taxa more resilient to climate change impacts.

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[1] The PhytoArk project is a great example of this (https://phytoark.com/).

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COP15 Target 9: Ensure that the **management and use of wild species are sustainable**, thereby providing social, economic and environmental benefits for people, especially those in vulnerable situations and those most dependent on biodiversity, including through sustainable biodiversity-based activities, products and services that enhance biodiversity, and protecting and encouraging customary sustainable use by indigenous peoples and local communities.

How can currently available environmental genomics tools be leveraged to support this target?

Network analyses of environmental genomics data could be used to assess the impacts of management actions on ecosystem functioning and ecosystem services. As we move to ecosystem-based management approaches, environmental genomics will be useful for not only providing estimates of taxonomic richness, but also population structure, and food web indicators. Environmental DNA sampling can also be more accessible than conventional biological surveys and thus facilitate more collaboration between communities, regulators, universities, etc. Additionally, genomics could democratize the ability for members of the public to check the source of fish in supermarkets and verify they came from sustainably harvested regions.

Are there any roadblocks or barriers that exist for eDNA practitioners and/or managers in applying these tools to support this target?

Environmental DNA is great for targeted detections and biodiversity assessments, but it is not reliable for abundance estimations or quantifying individuals in a natural setting. That is a major limiting factor in the context of wild species management. More research is necessary to determine the possibilities and limitations of environmental genomics for wild species management.

What advances in environmental genomics approaches are on the horizon that can support this target?

Information on population structure and age composition of wild populations can be important for the sustainable management of wild species. There are some emerging approaches that may be used to assess population structure from environmental samples. Additionally, DNA methylation markers have been used to estimate age from fish tissues, and it would be interesting to explore the suitability of methylation analysis for age estimates from eDNA.

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COP15 Target 15: Take **legal, administrative or policy measures to encourage and enable business**, and in particular to ensure that large and transnational companies and financial institutions **regularly monitor, assess, and transparently disclose their risks, dependencies and impacts on biodiversity**, … in order to progressively reduce negative impacts on biodiversity, increase positive impacts, reduce biodiversity-related risks to business and financial institutions, and promote actions to ensure sustainable patterns of production.

How can currently available environmental genomics tools be leveraged to support this target?

It is evident that environmental genomics technologies will be increasingly used to support regulatory decisions as government agencies continue to develop plans for incorporating eDNA data into existing systems. Transnational corporations can have thousands of sites in a variety of environments that could require annual impact assessments. Environmental genomics tools can enable companies to efficiently run assessments and regularly report whether they are reaching biodiversity objectives.

Are there any roadblocks or barriers that exist for eDNA practitioners and/or managers in applying these tools to support this target?

We may run into challenges with integrating eDNA data with existing datasets, especially for large multinational companies. Environmental genomics can allow us to monitor changes at scales not possible with conventional methods, so there may be pushback to adding this new level of surveillance. Additionally, because eDNA practitioners do not operate under a universal set of published standards, this hinders public trust and legal actionability. The pandemic highlighted the importance of public trust in the bioinformatic and molecular lab processes. We saw government bodies rapidly adopt eDNA/eRNA wastewater Covid-19 surveillance strategies which shows rapid acceptance is possible when a new technology is deemed vital. The environmental genomics community has not been as effective at communicating our confidence in the protocols and pipelines.

Implementing biodiversity risk-reduction requirements on the private sector could be a very long process. We've seen ballast water restrictions which took decades to come into full effect, and biofouling mitigation regulations are proving to be an equally complex process. This should be something we consider when evaluating the feasibility of applying eDNA to this target.

This image was generated with the assistance of AI.

COP15 Target 15: Continued

What advances in environmental genomics approaches are on the horizon that can support this target?

International standardization guidelines would greatly improve our ability to take legal, administrative, or policy measures to encourage or require companies to monitor and disclose their impacts on biodiversity. Standardization can inhibit customization and innovation, so minimum requirements could offer some standardization without limiting scientific progress. Cross-lab validation experiments should also be an expectation in the standardization of new and emerging methods.

What developments in environmental genomics are most exciting to you at the moment? How do you see these progressing over the next three years?

Panelists noted that it will be exciting to see methods improve for determining population genetic structure from eDNA samples. Emerging bioinformatics pipelines for assessing population structure from metabarcoding data could be used to reanalyze much of the existing archived data. Other exciting developments are the ongoing efforts to improve reference sequence databases and functional databases as this will enable better taxonomic and functional assignment capabilities and more trust in the data. Panelists also discussed interest in innovative sources of eDNA, such as collecting DNA from meteorological filters already in use. Environmental genomics could remove some of the logistical excuses for not surveying environments and thus lead to better conservation of global biodiversity. It is exciting to see our understanding of remote environments continue to grow.

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Appendix A

Workshop agenda.

AGENDA

DAY 1 - WEDNESDAY, JUNE 21st, 2023

08:30 – 09:00	Registration
09:00 – 09:30	Welcome & Introductions
09:30 – 10:15	**Kristian Meissner (Finnish Environment Institute),** Keynote Speaker *Why do we need international standards for molecular methods?*
10:15-10:45	*Coffee Break*
10:45 – 12:15	SESSION 1 – Standards and environmental management
	Katy Klymus (Columbia Environmental Research Center) *Developing lab standard procedures and guidelines to improve uptake of eDNA methods by resource managers: An overview of two national-scale projects*
	Cynthia McKenzie (Fisheries and Oceans Canada) *Sampling Strategies to Enhance the Use of Molecular Tools for Early Detection of Aquatic Invasive Species in High Risk Harbours*
	Michael Marnane (IOGP Environmental Genomics Joint Industry Program | Chevron Technical Centre) *Improving uptake and confidence in the application of environmental genomics within the oil and gas industry*
12:15 – 13:00	*Lunch Break*
13:00 – 15:00	BREAKOUT SESSION
	Technology Readiness Assessment for new eDNA Tools
15:00 – 15:30	*Coffee Break*
15:30 – 17:00	SESSION 2 – Metabarcoding and community analysis
	Richard Lance **(Environmental Laboratory, US Army Engineer Research and Development Center, US Army Corps of Engineers)** *Treading New Ground: Exploring Soil eDNA Signal and Its Potential Utilization*
	Thomas Merzi (TotalEnergies) *Trophic Networks Assessment from Metabarcoding Data*
	Samantha Crowley (Dalhousie University) *Assessing uncertainty in eDNA metabarcoding data using simulations*
17:00-17:30	Day 1 Wrap-up

DAY 2 - THURSDAY, JUNE 22nd, 2023

09:00 – 10:30	SESSION 3 – New horizons: Genomics
	Samantha Beal (Dalhousie University) *SINEs for the times: short interspersed nuclear elements increase ability to detect rare species via eDNA*
	Ion Abad-Recio (AZTI) *Beyond Amplicon Sequencing*
	Muneesh Kaushal (CEGA) *NAMERS: a purpose-built reference DNA sequence database to support actionable eDNA metabarcoding results*
10:30 – 11:00	*Coffee Break*
11:00 – 13:00	SESSION 4 – New horizons: Technology
	Hussein Daoud (Illumina) *Illumina Sequencing Technology update for Environmental Genomics*
	Robert Beiko (Dalhousie University / Dartmouth Ocean Technologies, Inc.) *Deploying the Dartmouth Ocean Technologies Inc. automated eDNA sampler in the marine environment*
	Timothy Shanahan (McLane Research Labs) *Presenting the RoCSI, a new high sample count eDNA sampler*
	Ethan Edson (Ocean Diagnostics) *New enabling technologies for the in situ sampling and collection of eDNA in the coastal zone*
13:00 – 14:00	*Lunch Break*
14:00 – 15:30	Panel Discussion
15:30 – 16:00	Closing Remarks
18:30 – 23:00	**Special Event at THE ROOMS** *Cocktail Reception*

Thank you for Attending IWEG 2023

Please join us for a **Cocktail Reception** at **The Rooms**

Reception: 6:30 PM – 11:00 PM

Event Location:

ST. JOHN'S CONVENTION CENTRE
50 News Gower Street
St. John's, NL Canada

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Reception Location:

THE ROOMS
9 Bonaventure Ave
St. John's, NL Canada

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Appendix B

Summaries from breakout session TRL exercises.

Automated Sampling

Example scenario: Automated sampling in water

Step	TRL	Rationale for TRL Assignment	Addressing Technology Gaps
Preservation of Samples	TRL 9	Current automated samplers have proven sample preservation methods.	Current automated samplers have proven sample preservation methods.
Cross-Contamination prevention	TRL 9	Current automated samplers have proven strategies for avoiding cross-contamination.	N/A
Mobility	Not CTE	Not a novel application	
Long term deployment	TRL 5	Mixed testing on long-term deployment.	Tests on biofouling effects.
Sample capacity	TRL 9	Current automated samplers can take multiple samples, but more sample capacity could be beneficial.	Increasing size and/or number of samples.
Environmental operating parameters	TRL 1 – 9	Automated sampling technologies have been demonstrated in a range environmental conditions.	Some automated sampling technologies still need validation in high temperature and high particulate conditions. Additionally, we need tests on how biofouling may affect performance.
Power Supply	Not CTE	Not a novel application	

In situ analysis

Example scenario: Estimate returning migration of salmon in a river

Step	TRL	Rationale for TRL Assignment	Addressing Technology Gaps
Sampling	Not CTE	Not a novel application	
In situ DNA extraction	TRL 7	In-field DNA extraction on environmental DNA samples has been completed in various environments and would just need final validation testing for this application.	Round-robin multi-lab investigations could be used to validate the technology and establish minimum performance targets.
On-site qPCR Amplification	TRL 6 – 7	In situ qPCR has been documented in relevant environments, but the technology would need to undergo testing for this specific application and targets.	Multi-lab comparisons could be used to validate the technology and establish minimum performance targets.
Analysis	Not CTE	Not a novel application	
In Situ Interpretation	TRL 3 – 4	The interpretation of results from qPCR analysis in situ has not (to our knowledge) been validated for this application in a relevant environment.	Cross-lab method comparisons could be used to establish minimum standards for this element of the technology.

Population assessment via eDNA

Example scenario: Tracking populations of fish spatially and temporally using eDNA

Step	TRL	Rationale for TRL Assignment	Addressing Technology Gaps
Sampling Design	TRL 3	New sampling designs are required for population tracking using eDNA since there is no established method. We need to establish the appropriate locations for sample collection and understand the impact of hydrodynamic factors due to the persistence and dispersal of eDNA in the environment.	We would like to see a side-by-side comparison of population tracking using eDNA and traditional methods. This would increase the overall TRL and address concerns by scientists about using an eDNA method. A comparative study is needed to: • Validate and optimize the sampling approach. • Determine the feasibility and benefits of eDNA-based population assessment. • Assess population tracking accuracy and validate the ability to discern populations via eDNA. • Understand the complexities of population tracking with eDNA and define the scope of applications.
DNA extraction	Not CTE	Not a novel application	
PCR amplification	Not CTE	Not a novel application	
DNA sequencing	TRL 4 – 5	Sequencing technology is relatively advanced for population analysis using eDNA. Validation is needed to ensure accurate differentiation of populations and to address sequencing errors.	
Population Discernment	TRL 2	More proof-of-concept studies are needed to effectively discern populations using eDNA.	
Reference Databases	TRL 6 – 9	The TRL for this element varies by project. Relevant databases exist, but they might not be populated with the required data depending on the species of interest. The reference database technology itself has a high TRL, but there is a scalability challenge for populating these comprehensive population databases because this requires collecting individuals and sequencing their DNA.	

Quantification & Biomass Estimation

Example scenario: Assessing emerging fisheries in the Arctic for food security: quantifying abundance and biomass of Icelandic Scallop using eDNA

Step	TRL	Rationale for TRL Assignment	Addressing Technology Gaps
Sampling – water filtration with desiccated filters	TRL 7	Desiccated filters have been used for eDNA sampling, but (to our knowledge) they have not been tested and verified for use for Icelandic scallop eDNA collection in the remote Arctic environment.	We could conduct species-specific assessments of filter compatibility as well as multi-lab proficiency testing to verify the technology meets minimum standards.
DNA extraction	Not CTE	Not a novel application	
qPCR	TRL 4	Abundance and biomass estimation via qPCR has been shown to work for some organisms in a lab setting. However, the technology has not been validated for use for quantifying Icelandic scallops from field environments.	We could test qPCR quantification of Icelandic scallops in laboratory tanks. The next step would be to run field tests in environments with known biomass or population size estimates, such as in commercially managed scallop beds in Europe.
Data analysis	Not CTE	Not a novel application	

Metagenomics & Transcriptomics

Example scenario: Assessing microbial composition

Step	TRL	Rationale for TRL Assignment	Addressing Technology Gaps
Sampling	TRL 6	eDNA/eRNA sampling methods for metagenomics and metatranscriptomics have not been validated in all proposed study environments.	Sampling should be demonstrated and validated in the planned operational environments.
Nucleic Acid Extraction	TRL 6	Nucleic Acid extraction methods still need to be demonstrated to work for some environmental sample types, particularly for RNA.	More studies can be done to find the best conditions for eRNA extraction from environmental samples.
Library Preparation	TRL 7	Library preparation methods for metagenomics and metatranscriptomics assessments from environmental samples have been developed and demonstrated, but have not been tested for all possible applications.	This element could be integrated into SOPs or other regulatory environments.
Bioinformatics	TRL 5	Pipelines for metagenomics and metatranscriptomics have been developed and tested with specific applications, but may undergo changes for broader compatibility.	Existing pipelines should be critically evaluated by non-biased evaluators. The code used in bioinformatics pipelines should also include some standardized elements.