IWEG

International Workshop on Environmental Genomics

Workshop Report 6
eDNA at the Frontlines of Global Environmental Challenges
June 17th-18th, 2021

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Executive Summary

The 6th annual International Workshop on Environmental Genomics (IWEG) was held virtually and hosted in St. John's, NL, Canada on June 17th and 18th, 2021. This was the second virtual IWEG due to ongoing travel restrictions and health concerns related to the global COVID-19 pandemic. This year's workshop focused on the theme "*eDNA at the Frontline of Global Environmental Challenges*" with the goal of addressing how environmental genomics can provide solutions to understanding our rapidly changing environment. The workshop brought together participants from across the globe representing stakeholders across many sectors, including the oil and gas industry, environmental consulting, regulatory agencies, and academia, to discuss advances in the field of environmental genomics and the use of eDNA to address major global challenges. Most participants (88%) had experience working with environmental genomics tools or were leading their own environmental genomics research programs (see Appendix I for participant survey results).

This year's workshop included a keynote presentation from Dr. Katherine Dafforn (Macquarie University), four presentation sessions, and a discussion panel each day. On day 1, panelists were Sherry Walker (Fisheries and Oceans Canada), Jordan Angle (ExxonMobil, USA), Sunniva Aagaard (Norwegian Environment Agency, Norway), and Francisco Chavez (Monterey Bay Aquarium Research Institute, USA). On day 2, IWEG panelists were Donald Baird (Environment and Climate Change Canada), Lucie N'Guessan (ExxonMobil, USA), Susanna Theroux (Southern California Coastal Water Research Project, USA), Thomas Merzi (TotalEnergies, France), and Mehrdad Hajibabaei (University of Guelph & CEGA, Canada). Six central themes were identified from the two-day workshop:

Internationally coordinated eDNA programs address global challenges

With the push towards ecosystem-based monitoring, no other method can provide a measurement of biodiversity across all domains of life and across large spatial and temporal scales like environmental genomics. Many pressing environmental issues, from assessing the impacts of climate change to planning for sustainable industrial development, could be better addressed at a global scale. This necessitates using a scalable approach, such as environmental genomics, as well as coordination between groups in various countries. Currently, environmental genomics practices and procedures vary between groups around the world. Cooperation and standardization are crucial to apply eDNA to large-scale coordinated programs and to advance ecosystem monitoring forward to meet the environmental challenges of today.

Democratizing environmental genomics tools enhances uptake

eDNA practitioners can make environmental genomics tools more accessible to stakeholders and the general public. Co-developing projects with partners and regulators and getting them involved at the early planning stages will improve partner and regulator understanding of all the steps and considerations for a successful environmental genomics project. Making data accessible and providing the appropriate tools to interpret the data allows end-users to take full advantage of the new information. Community-based projects empower local and Indigenous communities to collect samples and receive biodiversity data from locations that are important to them, while simultaneously generating data that can be used for research and monitoring.

Environmental genomics to the rescue in a post-pandemic world

Field sampling could not take place for many projects over the past year due to limited access to remote locations and vulnerable communities or because it was not safe for large teams to come together with the threat of COVID-19. In a post-pandemic world, where concerns about large field work teams travelling large distances may remain, there is an opportunity for eDNA sampling to become the preferred approach for biodiversity monitoring. The urgency of the pandemic also led to vaccine development and regulatory approval in under a year, showing that regulatory agencies can adapt their policies and make rapid decisions when needed. The environmental genomics community can lean on these examples of regulatory flexibility to bring environmental genomics to the mainstream of environmental monitoring.

Overcoming the limitations of incomplete reference databases

In most regulatory frameworks, taxonomic information is critical to support decision-making processes thus, improving reference databases is a priority for environmental genomics practitioners. Iterative approaches that identify gaps in reference databases and then intentionally fill these gaps provide an efficient approach to populating reference databases. Alternatively, taxonomy independent analyses use DNA sequences directly to measure diversity and can overcome the challenges of incomplete reference databases, but new ecological indices are needed to take advantage of the additional information contained in DNA sequences. With this approach, high-resolution, highly diverse DNA datasets have the potential to reveal more than taxonomic data.

eDNA adds value to biomonitoring beyond species detections

While the primary application of environmental genomics is currently for species detection and identification, novel layers of community data are also available using environmental genomics approaches, such as genetic biodiversity (e.g., unique sequences) and ecosystem function (e.g., metagenomics, metatranscriptomics). Additionally, current standard monitoring practices are expensive and time consuming, often resulting in inconsistent observations over time of subsets of the biodiversity (e.g., fish, plankton, etc.). Environmental genomics offers an opportunity to gain a deeper understanding of biodiversity by increasing the frequency and scale of monitoring efforts while simultaneously increasing taxonomic breadth. Developing and communicating the added value of eDNA in biomonitoring will open new possibilities for the application of environmental genomics.

Increasing demand for near real-time monitoring

There continues to be an increase in the uptake of environmental genomics in regulatory agencies across the globe, with an increased focus on real-time monitoring. This is especially relevant for invasive species, where early detection and a quick turnaround of results can greatly facilitate enforcement and, potentially, prevent species from establishing in a new region. Real-time monitoring could also be used to detect endangered species and enforce regulations in an area while the species is present. Technology advances are creating more rapid eDNA tests, but those results must be able to hold up in a court of law before they can successfully be used in enforcement. The environmental genomics community can look to forensic science for guidance on admissibility of DNA-based evidence and development in this direction.

eDNA at the frontline of global environmental challenges

Keynote Address by Dr. Katherine Dafforn

Coastal Cities: Monitoring and Managing Urban Impacts Below the Waterline with Environmental Genomics

The keynote address of this year's workshop was given by Dr. Katherine Dafforn of Macquarie University. Coastal zones around the globe are under intense pressure from human activities which alter natural conditions, physically modify the landscape, and introduce contaminants. Katherine's research aims to improve the monitoring and management of urban coastal zones by using environmental genomics for various applications, including monitoring the effects of contaminants in estuaries, mitigating the impacts of stormwater runoff in harbours, improving wastewater management, and documenting the effects of wildfires on coastal regions.

Across all these projects, environmental DNA-based measures of microbial biodiversity were highly effective in identifying biological responses to environmental stressors. In estuaries, environmental genomics was more sensitive than morphology-based surveys for identifying trends in biodiversity along environmental gradients. The genomics data revealed a new group of indicator taxa for estuary condition and health that is not typically detected in conventional surveys. A study of industrial contaminants in stormwater runoff demonstrated that the diversity and function of sediment microbes changed in response to storm events and proximity to stormwater runoff drains. For improved wastewater management, environmental genomics revealed the association between the microbial community and the amount of wastewater pollution of rivers and identified potential indicator species linked to effluent concentrations. Finally, Katherine used the massive wildfire in 2019-2020 in New South Wales as an example of a land-based stressor impacting aquatic systems. Preliminary results from this eDNA time series study show changes in microbial biodiversity are correlated with environmental variables such as how much of the site was burned.

Katherine's research is a fantastic example of placing eDNA directly at the frontline of global environmental challenges. Stressors like industrial contaminants and the increased frequency and scale of wildfires are worldwide concerns. Environmental genomics offered a highly sensitive and efficient method to examine the impacts of such stressors. Katherine is working with regulators to use these techniques and data to inform management decisions in response to these broad-scale challenges.

Theme Discussion

What steps are you taking to place your environmental genomics projects in the broader context of the global environmental challenges?

Participants discussed pervasive challenges in accurately and consistently measuring the condition of a site and visualizing the health or degradation of an environment. Moving beyond taxonomic lists to take advantage of the additional information in environmental genomics data, such as community function and dynamics, can improve our assessment of ecosystem quality. The impacts from human activities extend across biomes and trophic levels but monitoring impacts across the tree of life is challenging. Participants noted that identifying and monitoring many small, diverse organisms, like zooplankton, remains a challenge using the traditional morphological approach. eDNA broadens the scope of biodiversity monitoring in rapidly changing environments by enabling widespread, monitoring of all organisms in a system, including those taxonomic groups that are traditionally difficult to identify.

Moving beyond scientific practice, social barriers to widespread, global adoption of environmental genomics were addressed. Participants discussed their efforts to shift perspectives and change behaviours of end-users and stakeholders. Increasing the adoption of a new technology requires raising awareness, creating connections, and generating engagement across industries and stakeholders, including the public. It is a universal challenge to build trust in a new technology, but trust facilitates uptake and more widespread use by helping to provide a “social license” to use eDNA.

How do you see your work contributing to the solutions needed to understand our rapidly changing environment?

Several participants emphasized the potential and value of global coordinated efforts to increase the range and scope of environmental genomics datasets. The International Association of Oil and Gas Producers Joint Industry Programme is a big opportunity for oil and gas industry members to leverage the diversity of work sites and resources to contribute to advancing the technology and understanding changing environments. Similarly, coordination can take the form of data integration across environmental genomics projects within other large multinational organizations. Participants also called for increased joint efforts across sectors between industry, regulators, and academia.

Technical solutions to these global challenges include advances in genomic tools in the areas of population genetics, transcriptomics, biomass quantification, and automation both in the lab and field. Environmental genomics overcomes taxonomic identification challenges in environmental monitoring because eDNA signatures are the same at all life stages and can be recorded and tracked across sites even for undescribed organisms using unique sequences. Participants also described new data analysis solutions to support data interpretation, including methods to integrate eDNA with other data streams such as acoustic surveys, new visualizations for complex systems, tools for network and trophic analysis, and methods to identify novel bioindicators from eDNA. The expanded data analysis toolkit also includes new, open-source software and plans for long term support to help more users take advantage of developments.

Lastly, participants highlighted their solutions to the social barriers hindering widespread deployment of this new technology for monitoring changes in the environment. This included better marketing strategies, initiatives to raise awareness and increase adoption of the technology, planning for knowledge transfer and outreach in projects, and fostering stronger relationships between indigenous groups, government, and academia to build a new environmental management framework. Tools like public data portals that make eDNA records globally accessible in a user-friendly manner also help to generate engagement and democratize environmental genomics. Outreach helps people connect with the science and visualize what eDNA can do to help improve environmental stewardship, thus helping build support and trust in the technology, ultimately leading to acceptance and uptake in the long term.

Technical Advances in Environmental Genomics

FIELD ADVANCES

Sample collection in the field is often the most time-consuming step in environmental genomics workflows. Improving the efficiency of sample collection has the potential to greatly increase throughput in environmental genomics workflows. IWEG 2021 saw presenters share several advancements towards this goal. Rob Beiko from Dartmouth Ocean Technologies described an exciting new tool: an eDNA sampler which can be mounted to a buoy, a vertical profiler or other platforms for autonomous water sample collection and filtration. He also demonstrated its use in an eDNA workflow for the detection of harmful algae blooms. Carolina Berdugo (Imperial Oil) shared that Imperial is supporting Canadian Oil Sands Innovation Alliance on the development of a portable eDNA device for species detection in the field, which is in the early stages of planning.

While new technologies reduce effort in the field by creating sample collection tools, other approaches are adapting sampling designs to increase the efficiency of data generation and useability of data. For example, Meredith Everett shared research from the National Oceanic and Atmospheric Administration (NOAA) where they are developing workflows to detect new and unknown species using an iterative process of sampling eDNA, identifying unknown sequences, and then returning to re-sample the unknowns.

LABORATORY ADVANCES

A variety of new quantitative PCR (qPCR) assays for eDNA were presented at this year's workshop. qPCR relies on the careful design and validation of specific primers and probes to detect target species. Assay development requires cost and time investment prior to conducting a study, therefore the more assays that are publicly available for various species, the easier it is to implement qPCR-based eDNA studies on those species. Most new qPCR assays presented this year targeted species of commercial interest, invasive species, or species of health concern. More specifically, qPCR was used to detect species that form harmful algal blooms as demonstrated by Rob Beiko. Katherine Dafforn showed qPCR being used to detect *Vibrio* bacteria which produce a toxin affecting swimmers. Sara Cowell (Fisheries and Oceans Canada) discussed the use of qPCR to detect invasive zebra and quagga mussels. Andrew Shelton (Northwest Fisheries Science Centre NOAA) presented on the large-scale monitoring of commercially harvested Pacific Hake using qPCR. For this project, qPCR was used alongside conventional methods to quantify the relationship between eDNA signal and biomass to support fisheries monitoring.

In the metabarcoding approach, eDNA molecules from a broad range of species are amplified at once in a single assay, often detecting species from many phyla in parallel, and then next-generation sequencing (NGS) is used to obtain sequences used for taxonomic identification. The generation of broad taxonomic data provides a wealth of information, however, there are opportunities in the workflow for optimization to generate more accurate and comprehensive biodiversity data. For example, Mike Bunce (New Zealand Environmental Protection Agency) presented research using multiple metabarcoding markers, up to 10-12 markers run in parallel from nuclear and mitochondrial regions. This approach generates more consistent detection across the broadest possible taxonomic spectrum and generates more accurate taxonomic identifications. Research presented by Anders Lanzen (AZTI) showed that additional replication at the sampling and extraction stages for sediment samples increased the biodiversity captured from a single site. Several efforts are ongoing to fill gaps in reference databases and improve taxonomic assignment of metabarcoding data. These projects are focused on generating reference sequences for species their respective study areas, the eastern Pacific and the North Sea. Nicole Fahner (Centre for Environmental Genomics Applications) demonstrated how genome skimming can be used to generate reference sequence data. Genome skimming is an efficient method to build reference databases because it generates full mitogenomes and full-length barcoding genes simultaneously.

In addition to more commonly used eDNA approaches, eRNA-based metatranscriptomics are also being used to monitor ecosystem remediation as demonstrated by Katherine Dafforn. eRNA has been primarily used for microbial studies thus far, but interest in this technique for monitoring a broader range of species in increasing. This tool provides functional information alongside taxonomic information, which is very valuable in monitoring ecosystem health.

DATA ANALYSIS ADVANCES

Several presenters highlighted their approaches to analyzing and interpreting large biodiversity genomics datasets. Network analyses, which build on species presence data to enable inferences on interactions between species and ecosystem structure, were prominently featured in several presentations. Using agricultural and freshwater systems, Teresita Porter (Natural Resources Canada) demonstrated how networks can be used to find potential mutualisms, prey-predator interactions, and competition, and how they can be used to assess the robustness and stability of ecosystems. Incorporating trait data can further enhance networks and elucidate ecosystem function. Anders Lanzen and Leire Garate (AZTI) demonstrated how networks can be used to identify bioindicator taxa that respond to environmental change or anthropogenic impacts (e.g., offshore oil extraction). These indicator taxa can be used to monitor and gauge impacts on entire ecosystems. Katherine Dafforn demonstrated how changes in the abundance of keystone microbial taxa were linked to microbial community shifts in response to wastewater effluent concentration in Sydney Harbour. Wendy Monk (Environment and Climate Change Canada) demonstrated another approach to analyzing biodiversity genomics datasets using hierarchical occupancy models. Occupancy modelling is a powerful approach to estimate occupancy while accounting for imperfect detection. It can be applied to single species or multi-species metabarcoding datasets. With the appropriate study design, this approach can be used to estimate the relative abundance of taxa across sites.

Pairing environmental genomics data with other detection methods (e.g., physical capture of specimens, acoustic detection, and ROV camera detection) generates more robust results and takes advantage of the strengths of each method. For example, Haila Schultz (University of Washington) presented results from eDNA and net tow zooplankton surveys and suggested that pairing eDNA surveys for rapid results with net tows to ground truth taxonomic identifications can provide an efficient approach to survey zooplankton communities.

Box 1. Data Analysis Tools & Resources

Several of the data analysis approaches shared at IWEG 2021 require additional complementary data to be integrated with eDNA data to augment the analysis and interpretation. Network analyses can be supplemented with information from available databases such as species interactions from **GLOBI**, (globalbioticinteractions.org); lotic species functional traits from **USGS**, (pubs.usgs.gov/ds/ds187/); and ecological functions of prokaryotes from **Faprotax**, (loucalab.com/archive/FAPROTAX). Programs used for, or in conjunction with, network analyses included **Netshift** (web.rniapps.net/netshift/), **igraph** (igraph.org), **cytoscape** (cytoscape.org), and **wTO R package** (cran.r-project.org/web/packages/wTO/index.html).

DISCUSSION & FUTURE DIRECTIONS

KNOWLEDGE GAPS

Taxonomy-dependent analyses are limited by incomplete reference databases as well as inconsistent quality of the existing references (e.g., not all sequences are linked to vouchers). The efforts to build existing databases are fragmented and databases will not be complete for all taxa globally anytime soon. Restricting eDNA analyses to sequences with database matches is akin to working with one hand tied behind your back. Developing taxonomy independent analyses can circumvent this problem allowing us to work from the sequences directly. Taxonomy-free methods present different challenges, such as how to best communicate the data without the innate linkage to a name and ecological information of a species. Additionally, regulators would like to see backward compatibility with existing methods to have some continuity with long term datasets. Developing taxonomy-free indices or metrics that are comparable with conventional indices can help with this transition. Even with the exciting option for taxonomy-free solutions, efforts should be made to build strong reference databases and generate sequences for indicator species.

There are also many gaps in our understanding of eDNA dynamics in the environment (e.g., how it enters the environment, what determines persistence versus degradation, etc.) and further research in this area will allow more informed interpretation of eDNA data. Studies with multiple methods of detection (e.g., acoustics, cameras, etc.) and international data systems to combine information across projects will help fill these knowledge gaps and provide more confidence.

DESIGNING FOR LARGE SCALE

Cooperation among users is needed to achieve large-scale programs to measure environmental change on a global level. The environmental genomics community should learn from each other's efforts to guide new efforts and develop bigger programs. National and/or international standardization will facilitate cooperation and data sharing among groups.

Research and development projects are underway to create "eDNA sensors" that can do autonomous collection and analysis to return data rather than samples. Automation such as this can greatly increase throughput enabling large scale projects.

To increase the sensitivity of detecting broad-scale ecosystem change, eDNA data should be integrated with conventional methods and survey efforts, as opposed to just comparing methods. Multiple measures, such as acoustic, video, and eDNA surveys, deployed simultaneously will generate added value and provide new insights.

QUANTITATIVE ANALYSIS

While there are still knowledge gaps preventing accurate abundance or biomass estimation from eDNA, environmental genomics approaches can be used to assess relative abundance with the appropriate study design and modeling approach (e.g., occupancy modeling). The accuracy and reliability of quantitative eDNA models for target organisms will improve as our understanding of the fate and transport of eDNA in the environment develops. Conducting ground truthing studies with quantitative models will ensure that these models generate high-quality monitoring data. This can be challenging as conventional methods may not yield appropriate data for ground truthing. For example, relative abundance measures from conventional benthic surveys have huge confidence intervals which makes it challenging to use for benchmarking.

There is a false sense of comfort that counts of individuals in a conventional survey are accurate and meaningful. Environmental genomics methods have created an opportunity to step away from this conceptual approach and look at an ecosystem more holistically. For example, environmental genomics approaches can generate functional metrics for whole ecosystems. Depending on the questions or goals of the study, different types of quantitative data will be more valuable.

Progress Towards Standardization

Multiple standardization efforts are underway for environmental genomics applications. Government agencies, service providers, and international research collaboratives are all developing guidelines and standards for sampling and laboratory processing that will be transferable across various applications of this technology. Efforts are focused on standardizing key steps in the workflow (e.g., sample collection, negative controls, assay validation, reporting) and providing overarching guidelines as opposed to enforcing standards at each and every step. A key point of discussion was whether standardization necessitates practitioners all following identical procedures or whether comparable, consistent, reproducible outcomes can achieve the same goals. Given the breadth of methods currently being used, end-to-end standardized procedures would likely prove more limiting to the advancement of environmental genomics than advantageous. There was also debate in whether method standardization should depend on cross-validating eDNA results with conventional methods or if another point of reference could be used. Conventional methods are highly varied, and each method has its own inherent bias. Should these methods be held as the gold standard or are there other ways of validating eDNA measures?

Standardization efforts range in scale from local to global. For example, as an R&D centre and a service provider with a broad client base, CEGA developed a standard metabarcoding workflow that has been used across multiple collaborative projects to generate biodiversity data. On a national scale, federal regulators such as Fisheries and Oceans Canada have developed sampling and processing guidelines for industrial and research applications. On a global scale, international research collaboratives, such as DNAqua-Net, are working towards implementing their own standardized approach. The International Association of Oil and Gas Producers is also developing a set of standards, guidelines, and recommendations in consultation with experts to decide when and how to implement environmental genomics across their range of projects and sites.

DISCUSSION & FUTURE DIRECTIONS

FACILITATING ACCEPTANCE

Building trust between practitioners and end users will facilitate regulatory acceptance so it is essential to have open communication. Standards, especially in reporting, can foster open communication between managers and practitioners by presenting eDNA results consistently and accurately conveying confidence levels in the data. However, standardization should not be a roadblock to acceptance of the technology. Environmental genomics can be employed now to address various research and monitoring questions.

Environmental genomics methodologies are rapidly evolving as new techniques and data become available. The implementations of standards should not impede innovation and development in the field. Operational labs devoted to processing samples are needed to focus on optimization for high throughput and implementing standards. Research labs can focus on improving methodologies and advancing the science. If environmental genomics will be used for enforcement purposes as a line of evidence for issuing charges, operational labs should have the capacity to support the legal system in evaluating the genomics evidence.

By Sector

INDUSTRY

Researchers continue to demonstrate the applicability of genomics as an assessment tool in the oil and gas industry to meet regulatory guidelines. Examples include the use of sediment eDNA metabarcoding in the Metamon project (a collaboration project with the Norwegian Research Centre, ikerbasque, Equinor, Total and Forskningsrådet) to evaluate the biological status of benthic environments and assess impacts from oil extraction. This project highlighted the role of microbes in marine ecological processes and how they could be leveraged for environmental monitoring. The Metamon project is also refining the eDNA approach by testing different DNA markers to select the optimal marker for efficient monitoring of benthic communities. Work conducted by Imperial in collaboration with CEGA, showed that genomic technologies can assess the effectiveness of land reclamation strategies by comparing species composition between old and new reclamation practices and undisturbed areas. Other environmental genomics tools (i.e., transcriptomics) can aid in investigating the biological processes involved in degradation of organic waste products and reducing their toxicity.

A major effort to advance the use of environmental genomics in the oil and gas industry comes from the International Association of Oil and Gas Producers Environmental Genomics Joint Industry Program (IOGP JIP-34). Jordan Angle (ExxonMobil) provided updates on the recent activity of the IOGP JIP-34 working to synthesize guidance documentation for the reliable application of these environmental genomics tools across the landscape of environmental monitoring and management activities relevant to the industry. They have recently released a white paper summarizing the current capabilities of genomics for monitoring purposes, including baseline assessments, detection of key species, remediation and restoration, and real time on-site measurement analysis. Future priority themes will include sampling standards and guidelines, laboratory processing guidelines, and considerations for compliance and impact monitoring.

GOVERNMENT & INSTITUTIONS

Government agencies are essential to advance eDNA research and to integrate eDNA into monitoring programs. Representatives from several government agencies shared recent research advances and described how their agencies are approaching eDNA monitoring programs.

The advancement of eDNA research within government was first discussed by Teresita Porter, who presented work with Agriculture and Agri-Food Canada in which they used network analysis to better understand the associations between bacterial and fungal soil microbiomes and high organic matter as an indicator of soil health. Andrew Shelton outlined NOAA's large eDNA sampling effort along the Pacific coast of the United States. Using Pacific Hake as a proof-of-concept species, eDNA showed similar indices of abundance as compared to acoustic trawl data at large spatial scales. This shows promise for developing indices of abundance for other species, especially those which lack significant historical data. Nicole Fahner presented on the continuing collaborative effort between the Centre for Environmental Genomics Applications and Fisheries and Oceans Canada. The goal of this research was to characterize fish biodiversity in the remote, deep Labrador Sea via metabarcoding of sediment and water samples. Collecting both sample types is necessary to obtain a more complete picture of biodiversity, as unique fish species were found in each sediment and water samples. Wendy Monk presented three case studies from Environmental and Climate Change Canada showing how they are moving beyond eDNA generated taxa lists and integrating eDNA with existing knowledge to provide a more well-rounded interpretation of the ecosystem. These case studies highlighted methods to estimate relative abundance with frequency of detection, using historical aerial photos and GIS to link taxonomy and traits to function, and discussed how researchers are working in collaboration with Indigenous groups to develop a genomic-ethnobotanical study design.

As the confidence in environmental genomics methods increases, eDNA is progressively being integrated into more monitoring programs in countries around the globe. Willie Duncan (Scottish Environmental Protection Agency) discussed how Scotland is incorporating eDNA data into their biodiversity protection initiatives in the form of a new biomonitoring project. The Scottish DNA hub will span several different environments over temporal and spatial scales with the goal of providing a more complete picture of the biodiversity condition, ecosystem heath, and resilience. A collaboration between University of Guelph and Natural Resources Canada was presented by Teresita Porter. The Ecobiomics project is building capacity throughout the federal government to characterizing invertebrate and microbial communities using metagenomics for environmental assessments, monitoring and remediation. The project “Expanding Pacific Research and Exploration of Submerged Systems” (EXPRESS); a collaborative effort of between NOAA, the United States Geological Survey, the Bureau of Ocean Energy Management, and others, was discussed by Meredith Everett (NOAA). The project aims to use eDNA to increase survey effort in the exploration of US west coast deep-sea environments to facilitate more informed, scientifically-sound, decision making. An aspect of this effort is the survey of coral and fish habitat via both traditional methods and eDNA to establish environmental baseline data for these vulnerable communities and to improve the available reference sequences for this region. Michael Bunce described an accessible, bottom-up approach to eDNA in which easy-to-use syringe-based sampling kits are provided to community groups and schools by the New Zealand Environmental Protection Authority. Efforts such as these are important in fostering a sense of value, connection, and stewardship of the environment while generating biodiversity data across a broad scale. A case study of the Canadian federal government's response to an aquatic invasive species (zebra mussels) entering Canada via aquarium moss balls was presented by Sara Cowell. A national response was launched to minimize the environmental impact of this infected product import, including exploring the use of eDNA monitoring to identify infected products since visual ID is difficult.

DISCUSSION & FUTURE DIRECTIONS

BUILDING TRUST & GENERATING ENGAGEMENT

eDNA practitioners should set up for success with clear, honest communication with end users right from the start of a project by explicitly discussing details including what research questions will be addressed, study design considerations, protocols, and any limitations. There's a balance between demonstrating the power of the new technology and overselling it. End users must understand the distinction between methods that are validated and what is strictly R&D. This is a fast-moving field so practitioners should be prepared with up-to-date information. If practitioners codesign studies with regulators and managers to meet their needs, it will demystify the approach and empower them to interpret it. Building trust with end users facilitates regulatory acceptance so it is essential to have this open communication with the whole team. And in return, if practitioners strive to better understand the regulatory contexts in which decision makers operate, they can frame the results or technology accordingly. Working together in a collaborative manner builds positive relationships with stakeholders. It's more productive to view working relationships as "partnerships" where each side is contributing towards the technology development instead of creating a dichotomy between us and them. This co-development model and open communication creates a more sustainable path forward in the technology development.

Trust also means ensuring results are reliable and robust. Type I (false positive) and Type II (false negative) errors are a concern for decision makers using environmental genomics results. To build trust, environmental genomics practitioners must communicate these errors and the source of the errors, as well as use standard protocols, guidelines, and accredited laboratories to minimize sources of error and manage risks in an open, consistent manner. There is a need for better tools to interpret this relatively complex data, including new visualizations and translating data into something actionable for decision making.

To generate engagement with new users, clear communication and positioning are important. Kahlil Lawless (Illumina, Inc.) shared marketing techniques that can help increase exposure, understanding, and adoption of environmental genomics approaches. Implementing such techniques can accelerate the adoption of eDNA across applications. When communicating with a new audience, eDNA practitioners should start with the basics (e.g., what is the technology?) and fundamentals (e.g., cells have DNA and DNA contains information) of the science. The COVID-19 pandemic has given everyone a reference point for some methods (e.g., PCR). This new familiarity provides a starting point to help make linkages to eDNA-based approaches. Showing examples of similar projects is a good way to convince stakeholders of the value and utility of eDNA-based methods and build trust.

OPPORTUNITIES FOR ENVIRONMENTAL GENOMICS

There are several applications of environmental genomics with great potential to increase uptake: establishing baselines, monitoring ecosystem-level change, assessing the success of protection and restoration efforts (e.g., Marine Protected Area monitoring), and detecting species at risk and invasive species. For invasive species detection in particular, this approach can be a highly sensitive, early warning detection system and while targeted species approaches are more readily standardized than metabarcoding, enforcement applications of eDNA (e.g., wildlife forensics) depend on accredited labs to perform the analyses which we do not have yet, nor do we have a system or framework to provide accreditation for this type of work. Environmental genomics can also be used for applications that were not possible with conventional methods (e.g., microbes and pathogen detection) or where a less invasive (non-destructive) sampling method is preferred (e.g., endangered species and sensitive ecosystems). Environmental genomics is also cheaper and cost effectiveness is a big driver of uptake. Furthermore, eDNA can be added to “ships of opportunity” for little additional cost or effort. It is also safer (less exposure of staff to field risks) and it produces a more holistic data set. Samples gathered for environmental genomics can yield DNA that be archived for future applications and surveys. These features make it appealing across many applications.

Environmental genomic case studies and pilot projects have been ongoing for a decade now. It is difficult to fund two approaches simultaneously (traditional method and new method) so practitioners have been strategic with existing funding. To move past this transitional phase into longer programs, it is time to focus on more systematic or programmatic integration of eDNA. For example, eDNA and pelagic acoustic surveys can be synergistically combined into an operational survey to estimate relative abundance of species in pelagic acoustic signals. As environmental genomic technologies continue to develop, they will create more opportunities to facilitate environmental monitoring. For example, continuous remote sensing instruments or methods to sequence samples on site will circumvent the need to store and ship samples, enabling eDNA technologies to be applied on increasingly broad scales.

MAXIMIZING THE IMPACT OF THE DATA

Agencies need to hire in-house experts that understand environmental genomics data for both technical interpretation and for regulatory implementation. Just as agencies have ecologists and environmental scientists on staff, if an agency is interested in adopting environmental genomics methods, then they should have molecular ecologists on staff to provide the required knowledge base. Practitioners can also take steps to help regulators interpret environmental genomics data by making the data relatable or comparable to past work or surveys. For example, reports can include familiar analyses that regulators are already comfortable with, like indicator species.

Regulatory agencies need to develop the capacity to store, analyze, and reanalyze the huge volume of data from environmental genomics along with a versioning system to track analyses and reference databases used for taxonomy assignment. Building this capacity will allow agencies to take full advantage of the data now and in the future as bioinformatics approaches evolve and reference databases grow. Biobanks for physical samples and DNA extracts can also be set up to provide backward compatibility for eDNA methods as the technology continues to evolve and the methods improve.

Regulators need to understand the uncertainties in environmental genomics data and how to communicate those uncertainties when talking about the results. This underscores the importance of communication between practitioners and end-users. Environmental genomics is also revealing previously unknown uncertainties in the current methods and bringing those uncertainties to the foreground. Despite this, it is always much more difficult to displace an established method because people are comfortable with its limitations.

COVID-19 Impacts and Outlook

COVID-19 Impacts and Outlook

Participants shared lessons learned, successes from the past year, and their perspectives on how the global pandemic has impacted the use of environmental genomics.

- During the pandemic, field work was very limited due to additional health and safety constraints and limited access to remote locations and areas with vulnerable communities.
- In the post-pandemic world, field work might still be limited as people are hesitant to travel or unwilling to allow access to their properties. Where environmental genomics needs a very small team for field collection compared to conventional surveys, this could be an opportunity for switching to eDNA sampling to continue with surveys despite limitations.
- Community-based monitoring can facilitate work across a huge geographic scale, and this type of survey approach was resilient to pandemic-based travel restrictions because local partners and community groups collected samples. This collaborative network approach could be considered for future projects in place of a typical top-down approach.
- The pandemic may have also influenced how society thinks and talks about science. For example, diagnostic tests were developed and approved in months and even vaccine

manufacturing and approval happened within a year, showing that regulatory approval can be rapid! This was unthinkable a few years ago. Additionally, environmental genomics uses similar methods to COVID-19 testing. More people than ever know what qPCR is and the environmental genomics community can use this when explaining the science of eDNA to help make it relatable.

- The COVID-19 pandemic has shown how distrustful people can be of basic science. This emphasizes how important it is to put effort into building trust in new technologies and not to assume that new technologies will be embraced based on scientific merit alone.

Retrospective Analysis

Looking back on six years of IWEG

For the past six years, IWEG has provided a yearly forum for practitioners, users, and stakeholders to discuss innovations and developments within the field of environmental genomics and to support the adoption of this approach for biomonitoring efforts. Over the years, the conversation around environmental genomics has shifted to reflect the current state of the field and high-priority issues for real-world applications. The themes of each year's IWEG reflect these changes, where the focus in early years was on understanding the science and translating it into applications (2016: *Understanding The State of the Science and Research Opportunities*; 2017: *Transitioning to Real World Applications for Environmental Characterization and Monitoring*). The discussion then shifted towards how the genomics community can facilitate regulatory acceptance of this approach and increase accessibility to broader range of users (2018: *Building a Path to Regulatory Acceptance of Evidence from Ecogenomics*; 2019: *Scalable Solutions*; 2020: *Generating Engagement with Genomic Data*). The formation of the International Association of Oil & Gas Producers Joint Industry Program on Environmental Genomics highlights the advancements made in industry application and acceptance. This year's workshop looked at environmental genomics on a broad scale to expand the scope and applications of this approach to tackle pressing global issues (2021: *eDNA at the Frontline of Global Environmental Challenges*).

In addition to the main theme of IWEG, several other important topics arose during the workshop each year. Among these, a number of recurring discussion items stand out. Standardization has been cited each year as an important goal to build confidence with regulatory agencies and accelerate advancements in environmental genomics. At early IWEG events, the focus was on standardizing bioinformatics software and increasing reproducibility during analysis. Over the years, this discussion broadened to include standardizing efforts throughout the environmental genomics workflow starting with sample collection. The push for standardization does not demand that everyone follow identical protocols, but will generate best practices, minimum reporting standards, and validation for alternative workflows to ensure that data generated are reliable, reproducible, and comparable. While it remains a central discussion topic at IWEG, this does not mean that there have been no advancements towards standardization. Indeed, participants have reported on several efforts underway to standardize environmental genomics approaches. Thus far, these efforts have primarily focused on qPCR-based eDNA workflows but will undoubtedly tackle metabarcoding workflows next. **The ongoing development of standards will increase confidence among stakeholders and increase uptake of this approach for biomonitoring efforts.**

Another discussion topic that has consistently come up during the yearly workshops is the importance of building and maintaining reference databases. Accurate and complete reference databases form the backbone of assay development and taxonomic assignment with metabarcoding data. Many projects have drastically improved reference database coverage for their study area through focused barcoding. **Despite targeted efforts, reference databases are still lacking for many taxonomic groups and environments on a global scale**. Given the enormous challenge of populating reference databases for all organisms and environments, there has been much discussion around developing taxonomy-free approaches for environmental genomic data analysis, particularly at recent IWEG events. Biodiversity can be measured directly from DNA sequences (e.g., OTUs, ESVs). **These high-resolution, highly diverse DNA datasets have the potential to reveal more than taxonomic data provided that new ecological indicators are developed to tap into these data**. This research area has been highlighted as requiring more effort and development to enable regulators to take advantage of this taxonomy-free data.

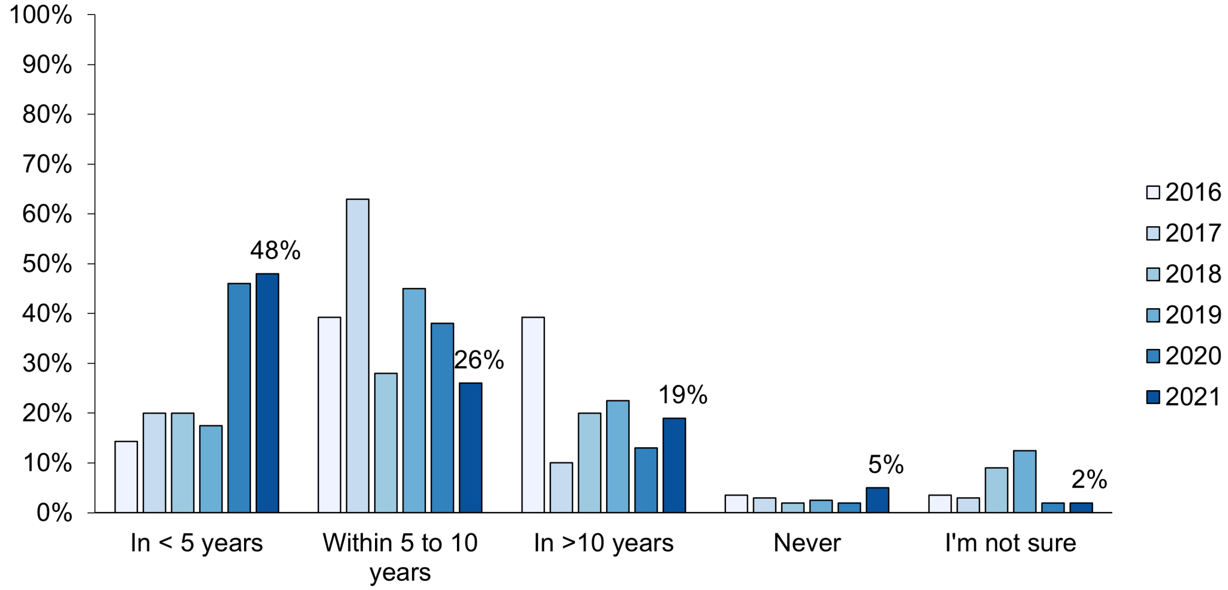


Figure 1 Reponses from IWEG participants to survey question: “When do you see environmental genomics becoming the primary approach to characterize and monitor biodiversity in the marine/aquatic environment?” between 2016 and 2021.

At each IWEG, participants were surveyed on the outlook for the adoption of environmental genomics as a biomonitoring tool. These six years of responses provide additional insight into how the field has progressed over this time period. Participants in the workshop varied between years but consistently included a mix of government, academia, industry, and others (e.g., consulting, commercial sector). There was a small decline in the proportion of participants from the oil and gas industry and an increase in the other category over the 6 years of IWEG. For a breakdown of IWEG participants over time and responses to all survey questions, see Appendix I. The responses to some key questions are discussed below.

The timeline for when IWEG participants see environmental genomics becoming the primary approach to biodiversity assessment has shortened, with <5 years now the most common response for both aquatic and terrestrial environments. This reflects the progress and technological advances made over the last six years as well as the continued uptake of genomics approaches by regulatory agencies and industry during this period. Throughout all years, participants thought environmental genomics would become the primary approach to biodiversity characterization in aquatic systems sooner than in terrestrial systems (Figure 1 & 2). There was also a higher proportion of participants that were unsure about the timeline for adoption of environmental genomics in terrestrial environments. Given that eDNA research has been more active in aquatic systems compared to terrestrial system, it is unsurprising that there is less certainty surrounding environmental genomics as a biomonitoring tool in terrestrial systems. This highlights the need for more research and development into using eDNA in terrestrial systems.

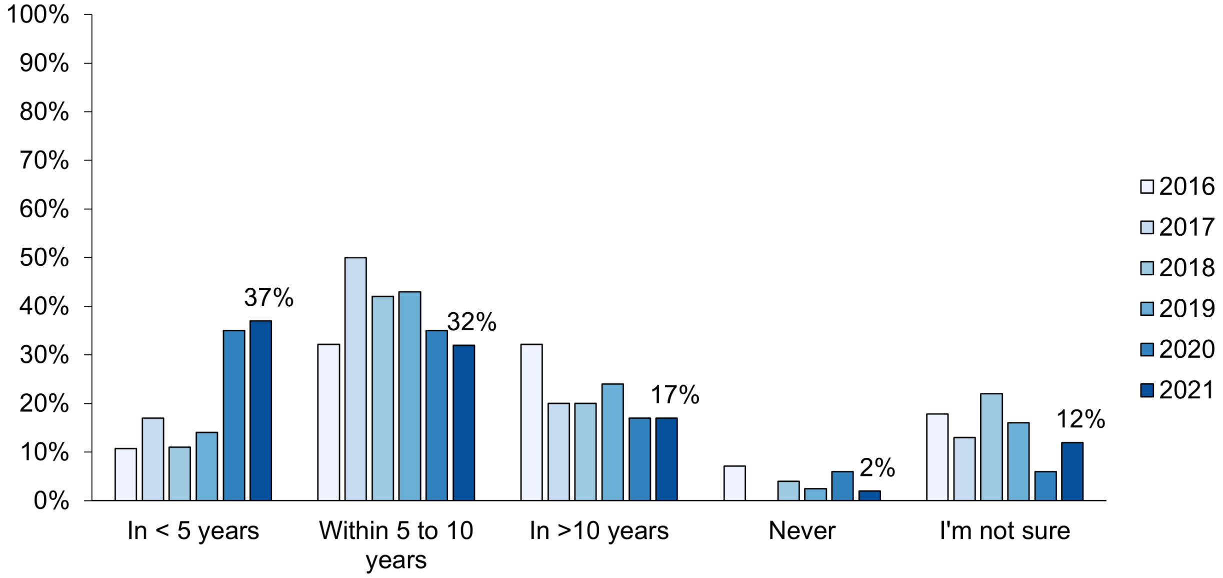


Figure 2 Reponses from IWEG participants to survey question: "When do you see environmental genomics becoming the primary approach to characterize and monitor biodiversity in the terrestrial environment?" between 2016 and 2021.

Even so, there was a shift over time, with an increasing proportion of participants believing environmental genomics will become the primary approach to biomonitoring in terrestrial environments in the short-term. This shift in perspective is encouraging because it shows that progress has been made towards integrating environmental genomics into biodiversity characterization efforts and suggests that there are fewer barriers to future adoption of this

Uncertainty in utility of deliverables and regulatory acceptance have both been consistently considered the biggest barriers to introducing environmental genomics into monitoring and assessment efforts. Throughout all survey years, these were the two most common responses (Figure 3). However, in the last two years, there has been a flip from more participants choosing uncertainty in utility of deliverables as the biggest barrier to more participants choosing regulatory acceptance as being the biggest barrier. This suggests that awareness of genomics approaches and their potential value is increasing but it is taking time for regulators to adapt to this new data stream. While representing a smaller proportion of responses, the cost to run a pilot project has steadily increased in frequency as a response. These obstacles all need to be addressed to facilitate uptake of environmental genomics in monitoring programs and biodiversity assessment efforts.

While there are still barriers to overcome, looking back on the advances that have been made since the first IWEG is very encouraging. We have seen great uptake of eDNA approaches by regulatory agencies and industry. Standardization efforts are in development to facilitate

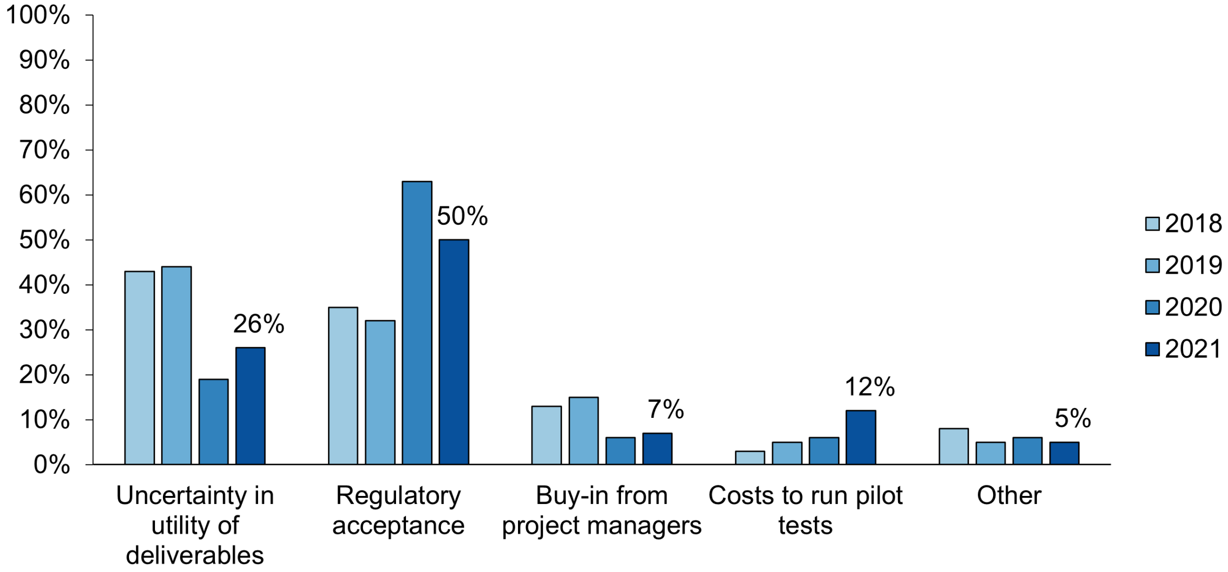


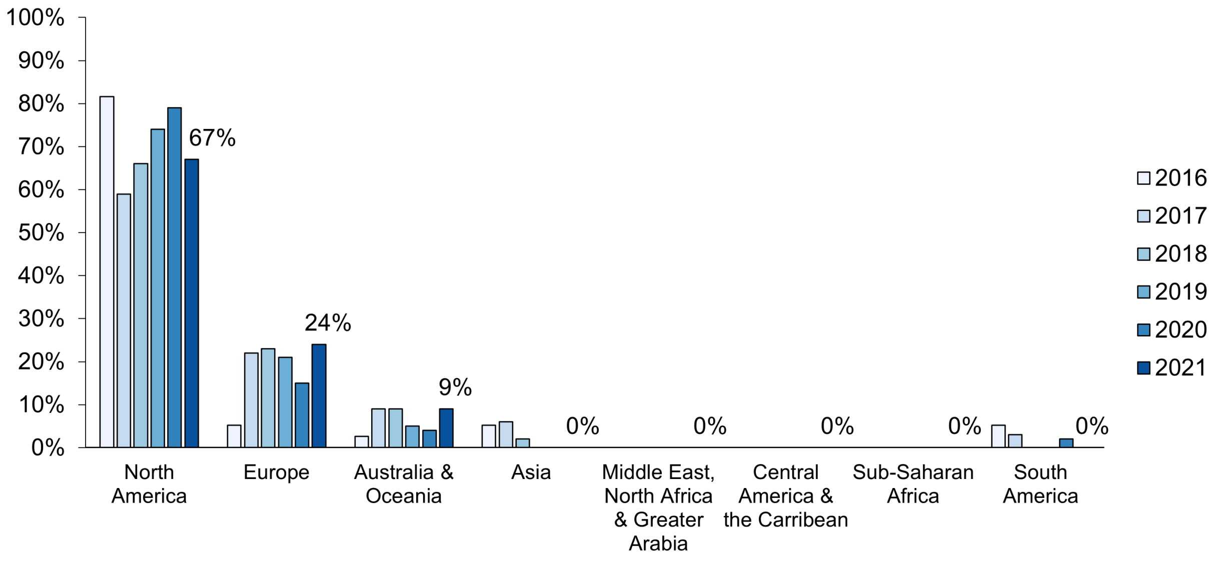
Figure 3 Reponses from IWEG participants to survey question: "What are the biggest barriers to introducing environmental genomics in new and existing environmental assessment and monitoring efforts?" between 2018 and 2021.

continued adoption of this approach and advancements in technology. Ongoing research is focusing on developing tools for regulators to take full advantage of environmental genomics data. All of this has contributed to an optimistic shift in participants' outlook for the future uptake of environmental genomics. The environmental genomics community will have an exciting next six years as the field continues to grow.

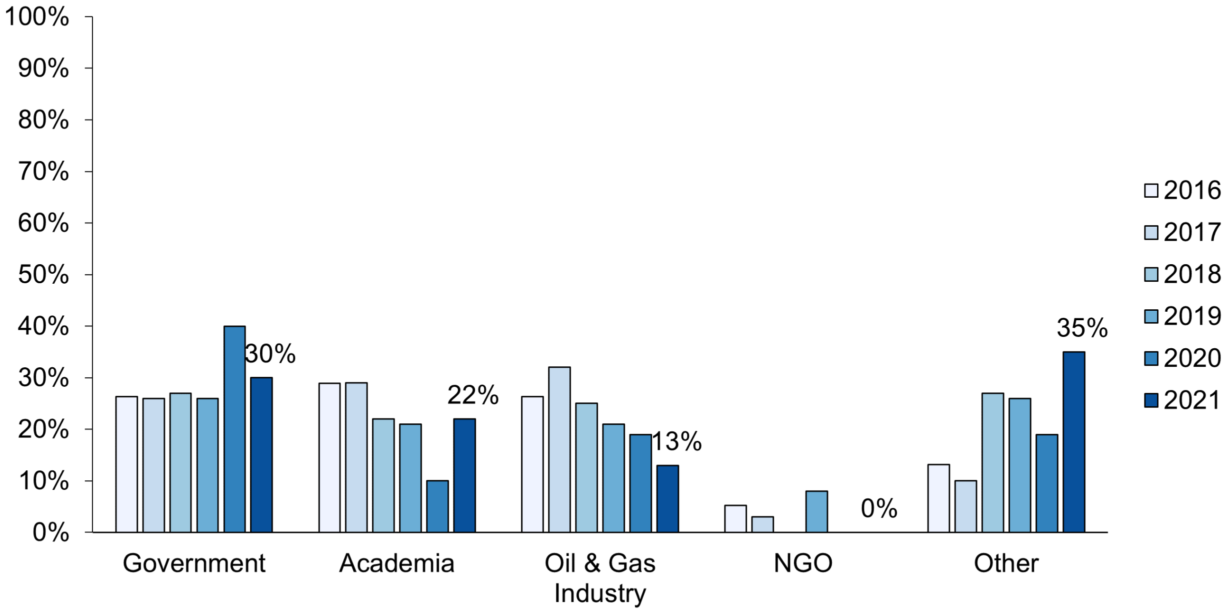
Appendices

Appendix A—Survey Questions

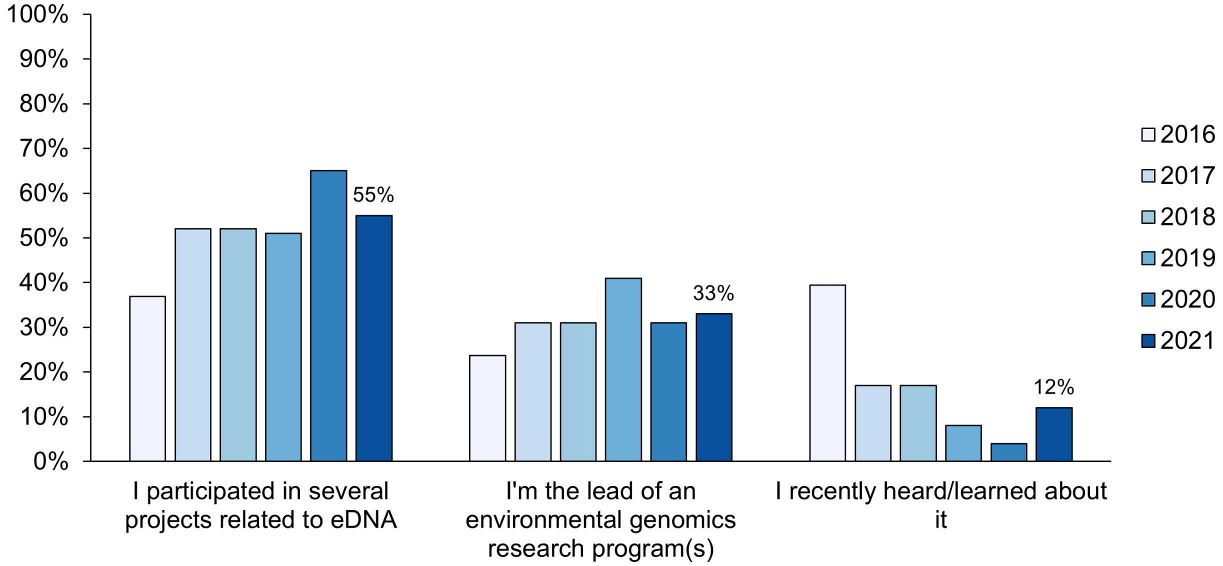
Which region are you from?



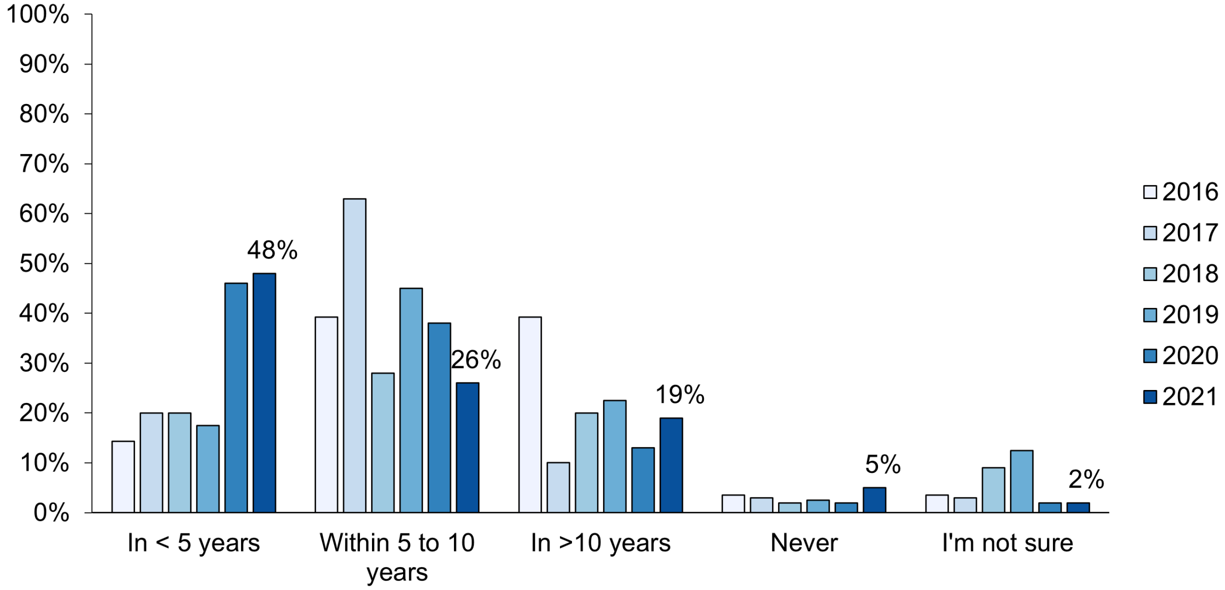
What group do you represent?



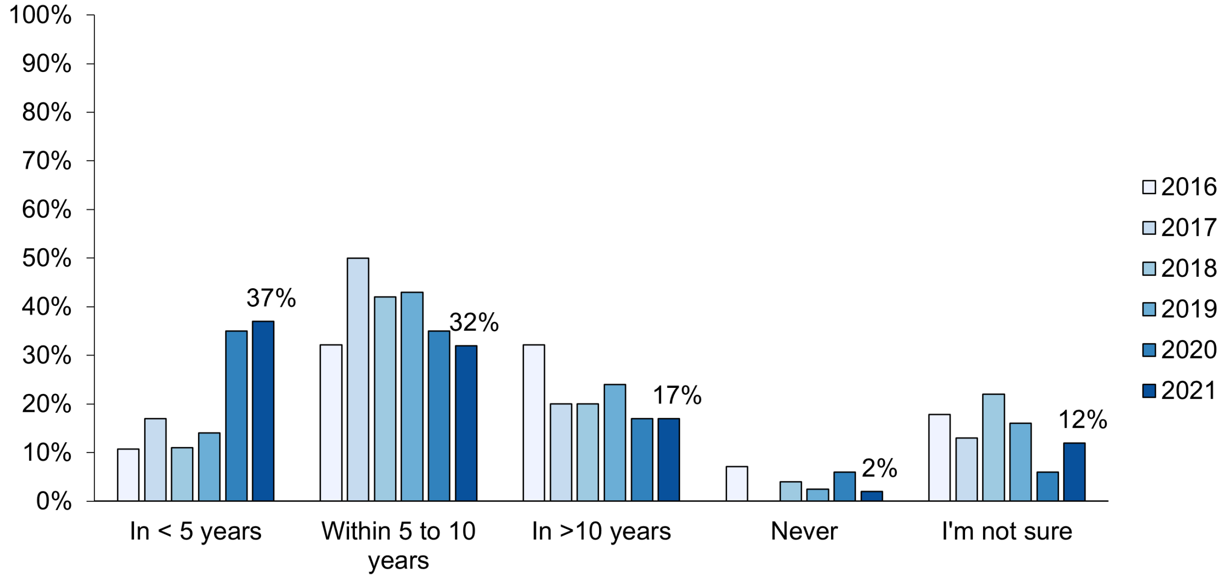
How familiar are you with environmental genomics?



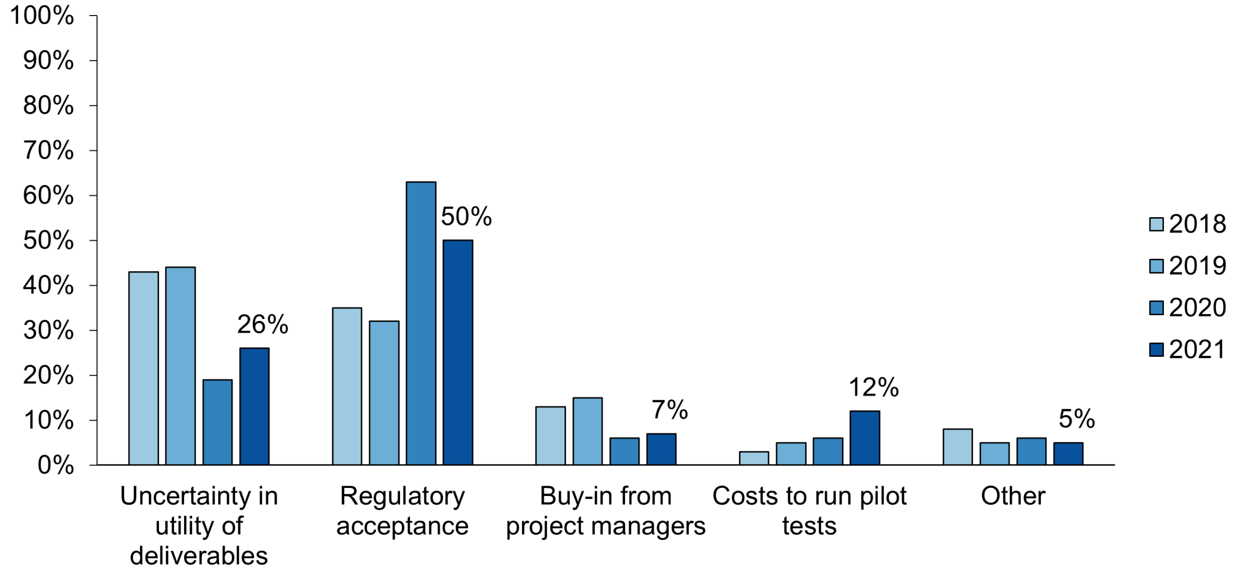
When do you see environmental genomics becoming the primary approach to characterize and monitor biodiversity in the marine/aquatic environment?



When do you see environmental genomics becoming the primary approach to characterize and monitor biodiversity in the terrestrial environment?



What are the biggest barriers to introducing environmental genomics in new and existing environmental assessment and monitoring efforts?



From a regulatory perspective, what is the highest priority?