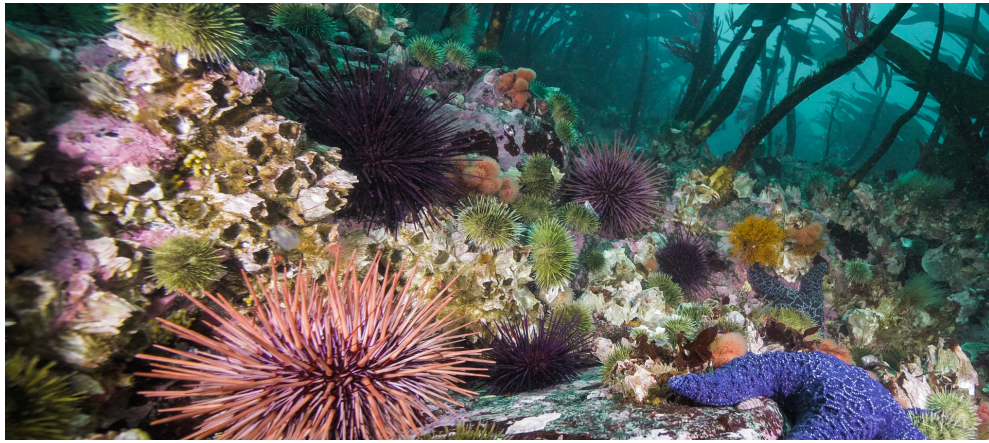
Introduction to
Using eDNA for Environmental Management

So, You're Interested in Using eDNA...

It is hard to open environmental news these days without seeing mention of 'eDNA'. **One might say - it's everywhere!** But what exactly is it? And how might it help you in your work?

Environmental DNA - or **eDNA** - is very simply put, DNA that can be found in the environment, including soil, sediment, water, and air. Organisms are constantly shedding bits of themselves - such as dead skin, mucous, or waste products - into their surroundings. The DNA in this shed organic matter, along with organismal DNA from microscopic organisms such as protists and bacteria, makes up eDNA. When a sample of water is collected, the eDNA within can be analyzed to determine what different species were present or recently passed through those waters, even if those species were not observed with the naked eye.



The use of eDNA is becoming increasingly popular across the world, including in the **Northeast Pacific Ocean Region.** Approaches to collect and detect eDNA are now providing rapid, accurate, and cost-effective means of identifying marine organisms - from marine mammals to fish, invertebrates, and harmful algal blooms - and are being used to build valuable baseline marine biodiversity datasets. But, despite the growing use of eDNA and increasing evidence of its effectiveness to address a wide range of research questions, the incorporation of eDNA-based approaches into **environmental management applications** is still in evolution. There are so many possibilities for application of eDNA to marine monitoring and management, including Marine Protected Area (MPA) and Indigenous Protected and Conserved Area (IPCA) monitoring and management; threatened species detection; invasive species detection; and environmental restoration. The more the technology is understood by marine managers and stewards, and the more the needs of communities are understood by scientists, the greater the successful mobilization of this evolving science for management will be.

Whether you're a marine steward or manager brand new to eDNA, or are familiar with the technology and science but would like to learn more, this 'eDNA primer' will hopefully point you towards greater understanding of this powerful tool.

Some Common Questions

About eDNA

"What are the benefits of using eDNA?"

There are many benefits to using analysis of eDNA data to support conservation and management efforts, including that it is a **simple, non-invasive survey method** to implement in the field; it is **cost-effective** relative to many other survey methods; and, it can be relatively **easily incorporated** into existing monitoring regimes, either to supplement, verify, or complement other sampling methods. eDNA can be sampled relatively quickly of broad geographic scales, from terrestrial to freshwater to marine environments, and analyzed to accurately determine presence/absence of a suite of biota and can also be used to specifically target individual species of interest (e.g. invasive species, threatened species, etc.).

"What exactly can be detected in each eDNA sample?"

There are two different lab-based approaches that can be used to interrogate the species present in samples collected during an eDNA-based field survey:

(1) **Individual target species** can be detected using **quantitative PCR (qPCR)**, in just the same way a COVID qPCR test detects the virus in a sample. For this approach, species-specific PCR primers are designed so that they yield specific, quantitative estimates of the DNA present for the target species. This approach is used when there is a single focal species of interest and you would like information on the relative abundance of that species through space or time. Note that a similar approach using different PCR instruments is called digital or droplet digital PCR (dPCR or ddPCR).

(2) **Multiple species or taxonomic groups** can be detected from one eDNA sample using **DNA metabarcoding**. This type of analysis is based on the use of broad-spectrum PCR primers; the choice of primers defines which group of species' DNA is targeted and amplified. One can target fish DNA, for example, by using PCR primers specific to fishes. Like any sampling method, metabarcoding will imperfectly reflect the cross-section of biodiversity present in a sample – some species will be over-represented, for example, and some species will get missed – but in general metabarcoding yields an in-depth view of the target taxonomic groups present in a sample. This approach is used for broad biodiversity surveys, such as when you would like a species list of all fish living in a particular location.

"Can you tell the abundance of populations from an eDNA sample?"

Not yet. The number of sequences detected in DNA metabarcoding depends on many factors, including the sampling method and location, the PCR protocol and its efficiency, the size and DNA shedding rate of the organisms, DNA degradation rates, and the fate and transport of DNA molecules in the environment. Nevertheless, it is clear that where more of a species is present, more of its DNA is likely to be found in the environment. Linking these more qualitative eDNA-derived observations to quantitative visual or acoustic estimates is one of the present frontiers of eDNA research.

"How far can eDNA travel in the marine environment? What size of an area does a sample reflect? How long does eDNA remain in the environment?"

Advection, mixing, decay, and settling all impact the transport of eDNA in marine environments. Despite the potential for long transport with ocean currents, eDNA signatures have been found to differ on spatial scales as small as meters in coastal environments, depth scales as small as 5m, and temporal scales as short as a few hours depending on the environment. In general, aquatic eDNA from a given individual can be detected for hours to days. Ultimately, sampling locations with different water transport dynamics will experience different spatial and temporal variability of eDNA signatures, but as a rule, eDNA signals generally reflect the biota in the immediate vicinity at the time of sample collection.

“What species can we detect with eDNA?”

All organisms leave genetic traces in their environments, meaning that eDNA can be used to study **any species or taxonomic group** of interest. In practice eDNA techniques have been used to identify a wide range of organisms including fish, mammals, marine invertebrates, and insects. In the case of targeted species-specific assays, a new primer set may need to be developed and optimized to detect a target species, if no such existing primer set is already available. For community-based metabarcoding studies, some species that are small, rare, or transient may be more challenging to detect as their DNA signal can be swamped by that of more abundant species.

An additional complication when it comes to species detection is how well the DNA of species of interest matches the metabarcoding primer set. If the primers used are poor matches for your species of interest you may not detect it even if its DNA is present in the sample – just as using the wrong tool for any job will result in suboptimal results.

“How do the results of eDNA surveys depend on the reference database?”

This question is specific to DNA metabarcoding studies. To resolve communities from eDNA, **we use a reference database to match each recovered DNA sequence** with the corresponding species or taxonomic group name. A reference database is simply a large table for matching unknown environmental sequences to a set of sequences from known species; like a Rosetta Stone it helps us turn raw data into usable information. Because not every species is in this database, and because some species are highly similar in their genetic markers, the level of taxonomic resolution and our certainty in this association (called annotation) can be affected, leading to an array of possible annotations for some sequences. In a typical metabarcoding survey in the NE Pacific region, one can expect several sequences of fish to annotate only to genus and sometimes only to family with confidence. When this happens, different types of reporting can handle this taxonomic annotation uncertainty. For other groups where the reference databases are less complete, such as marine invertebrates, the proportion of sequences with species-level annotations may be much lower.

“How confident can I be in what we find or don’t find?”

As with any sampling method, false positive and false negative species detections do occur with the methods used to analyze the eDNA in a sample. **The degree to which these influence the results can be addressed with attention to study design and laboratory methods.** For example, if high-confidence information about presence and absence for a focal species is essential for a study, then a targeted species-specific assay (qPCR) would be best to provide the highest confidence for that species. Also, when using multi-species approaches, it is important to make sure the species you want to track are adequately detected by the metabarcoding assay chosen. In general, a user can choose between high-accuracy data for a single focal species versus generating data for an entire taxonomic group with some degree of false positives (picking up erroneous signals when species are not really there) or false negatives (missing species that truly are there).

Getting Started: An eDNA Study Workflow

What goes into an eDNA-based study?

The figure below depicts the **typical workflow** when undertaking an eDNA project, from sampling collection in the field once you've designed your study to bioinformatics once your samples have been processed in the laboratory. **Following this is a list of questions for you to ask yourself and eDNA service providers when starting an eDNA-based project.** Together, all of these resources will help you move towards a better understanding of how to use eDNA as a tool for environmental monitoring, stewardship and management.

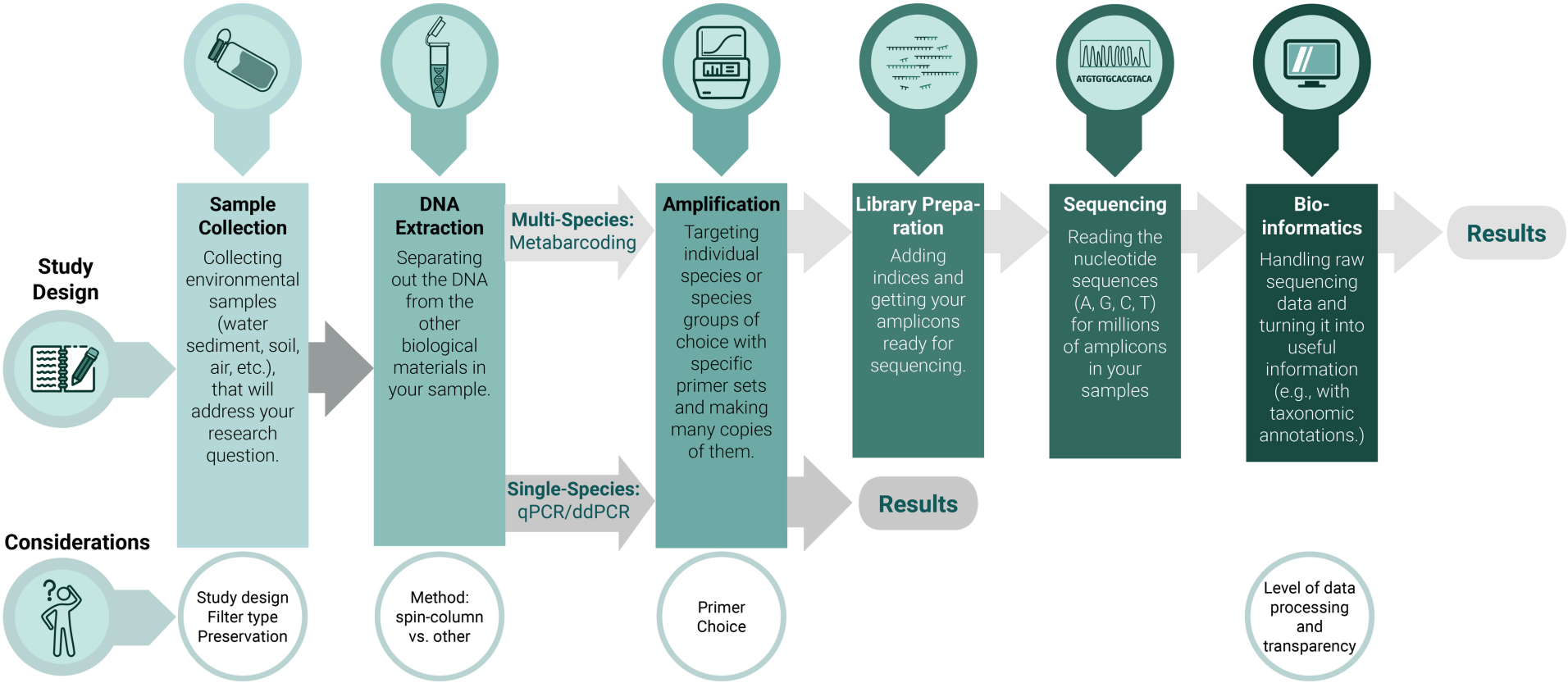


Figure 1. A typical workflow when undertaking an eDNA project includes these main steps. After sample collection and DNA extraction, the resulting extract can be used for multiple subsequent analyses. Single-species analysis with quantitative PCR (qPCR) or digital-droplet PCR (ddPCR) yields results after the amplification step. Multi-species analysis, using metabarcoding, requires additional steps in preparing samples for sequencing and in processing the large amount of resulting data. Circles below each step show several options or decisions that may bear discussion with a service provider.

Questions for You and for Service Providers

*For each question, the associated step in the overall workflow (Figure 1) is depicted via icon(s).

Questions for YOU before meeting with eDNA service provider:

What is the question you are trying to answer with your study?

For example, if you are interested in biodiversity at a site, ask yourself: Are there species or taxonomic groupings that you are more interested in than others? Are you interested in biodiversity shifts over time? Over space? The clearer you understand your needs, and can communicate them, the more valuable your dialogue with a provider(s) will be.

Do you need quantitative estimates about one (or a few) species, or presence/ absence information about many species?

If you are interested in a single species, even when it is low in local biomass, quantitative PCR (qPCR) is likely a good route for your study. If you're interested in the presence/ absence of multiple species, DNA metabarcoding is more likely the better avenue. In all cases, you should find out if there is a user-ready primer set developed already, or if it will require development.

How confident would you like to be in your results? What is the risk of missing something?

A degree of replication may be desirable to assess your results in a quantitative way. For example, if positive controls indicate that your assay detects a target species 99% of the time when the species is present, field replicates may be redundant. On the other hand, if a species is rare or stochastically detected, it might make sense to collect and process several replicates, both in the field and in the lab, particularly where it is important to compare within-site vs. between-site sampling variability. In a multi-species survey, more samples means you will less-often miss species that are low in abundance or that don't sequence efficiently, and will give you greater power to detect change over time or over space. If the risk of missing a species of interest is very high, qPCR rather than metabarcoding is most usually recommended, often deploying at least 3 and up to 8 technical replicates. If the risk of missing low-abundance species in a multi-species survey is high, or the risk of missing small changes in community composition is high, taking more field and technical replicates for metabarcoding (see below) is recommended.

Questions to ask your eDNA service provider:

Do you customize assays or test assays for my species of interest as part of your services? If not, is there a fee to test and optimize assays to make sure my species of interest will be detected?

This is an important question to ask, if you're interested in single species detection (e.g., you're focused on a threatened or invasive species).

How do you report limits of detections and results from controls (positive and negative)?

It's important to know how certain the service provider is in their ability to detect your species of interest. This certainty can be assessed by the limits of detection and quantification. It is important to understand and report these values when using single- species quantitative approaches (q/ddPCR) as knowing these values influences how you interpret results. For example, you may have a sample that falls below the limit of quantification, but is still detectable as present.

What will I receive back from the facility? For example, will I have access to the raw data files as well as processed data files?

Ultimately, it is important for you, as the user, to understand the data products provided by the service provider and how they generated them. Ideally, you will obtain both the processed (summarized) data from your provider, as well as the raw, unprocessed data. In the event that raw data is not shared, for metabarcoding (i.e.multi-species) assays, it is crucial to make sure the service provider provides a table of sequence abundance (i.e. read number) per sample, the exact sequence recovered in every sample, as well as the taxonomic identification of each sequence. Ideally, they also provide the frequency of each unannotated sequence per sample as well. The reference databases used to annotate a taxonomic identification to every sequence are constantly growing. Therefore, there may be a time in the future, after you receive your data, that you may want to re-annotate (identify) the sequences with newer genetic reference databases. This is only possible if you obtain the DNA sequences detected in your samples.

Do you check samples for inhibition before processing? If so, how do you deal with inhibition?

PCR inhibitors are any factors - commonly tannins and other secondary compounds that get extracted alongside DNA - that prevent the amplification of your target gene of interest. They can be something that was present in your sample itself or something that was added during sample extraction and inadequately removed during DNA purification. A variety of methods can be employed to check for inhibition and attempt to mitigate it (sample dilution, sample clean-up kits, etc.). It is good to discuss with providers what, if any, strategies will be used to deal with PCR inhibition should a sample prove to be challenging to amplify.

How many technical replicates will be run for each sample? Will you provide the data for technical replicates separately?

There are two main types of replicates often employed in studies where eDNA is collected. Biological or field replicates are a set of samples collected at the same location at nearly the same time, meant to capture the natural variability in the system at the time of sample collection. These are on the sample collector to obtain. Another type of replicate is a technical replicate. Technical replicates are employed in the lab to capture the variability in the analytical procedure itself. Consider your sample of extracted DNA: unless the DNA within that sample is perfectly distributed in solution, there will be some variation in what strands of DNA are pulled out of the master sample and put into the tube for the PCR.

Therefore, to accommodate this variability within a sample, multiple PCRs of the sample can be run and these replicate PCRs would be considered technical replicates. In single-species assays (qPCR), these replicate PCRs may increase the chances of detecting your species of interest. This is especially useful when you are going after rare species in your system, where the target DNA may be a very minor component of the total environmental DNA pool. If you run a PCR on that sample 8 times instead of 2 times, you'll have a higher likelihood of detecting that rare strand of DNA. It is important for a service provider to tell you how many technical replicates they run for your samples, and to provide the results for each technical replicate separately or at least the percentage of technical replicates in which your target species was detected to allow for proper estimates of that species in your sample. In multi-species assays (metabarcoding), replicate PCRs can increase the chances of detecting species of low relative biomass with low relative primer-binding efficiency, and it is important for the service provider to tell you how many technical replicates they run for your samples, and if they pool those replicates (so you can't see the results of each) or are able to report them separately.

MULTIPLE SPECIES, metabarcoding only:

Is your reference database appropriate for my research question, target species, or taxonomic groups?

Our ability to identify the source of DNA sequences obtained from an eDNA sample is only as good as the sequence reference database used. If metabarcoding, make sure you check with your service provider that your species of interest can be detected by their assay and are present in the reference database they are using to assign IDs to sequences detected. Or perhaps your application doesn't demand species-level assignments, and genus- or family-level annotation would suffice. In any event, asking this question ensures you will get usable data back from the provider.

Do you measure cross-contamination and if so how do you correct for it?

Cross-contamination pertains to the case where DNA from other samples being processed at the same time or that have been processed in the laboratory in the past contaminate a different sample, changing the original composition of that sample. It is important to ask service providers about their methods for running (and reporting) control samples that would reveal any cross-contamination, if present – and what method they might use to subtract out any signal of cross-contamination from your data.

Will my samples be run with other clients' samples on the same sequencer? If so, are the samples expected to have similar taxa or completely different?

There is always a small chance of cross-contamination during sequencing with other samples when they are run together in the same 'pool'. To avoid this, you could request that your service provider run your samples on their own, likely at a greater cost to you. Another option is to ensure that the potential sources of cross-contamination would be so obvious they could be removed after the fact, e.g. if your samples are from a marine environment and other samples were from terrestrial or freshwater habitats.

Looking for
More Information?

The information about eDNA presented above is just a start! If you're interested in learning more, **Appendix I** provides some additional resources that you might like to explore and **Appendix II** provides a non-exhaustive list of eDNA service providers.

Appendices

Appendix I: Additional Resources

*This appendix is not meant to be exhaustive and will likely quickly become outdated. Still, we hope that it may be helpful for some to have these resources collated.

Organizations / Initiatives:

Ocean Biomolecular Observing Network (OBON): https://obon-ocean.org/

The eDNA Collaborative: https://www.ednacollab.org/

Hakai Institute Integrated Coastal Observatory (ICO): https://ico.hakai.org/

Pacific eDNA Coastal Observatory (PECO): https://peco-project.weebly.com/

West Coast Ocean Biomolecular Observing Network: https://evsatt.github.io/WC-OBON_Website/

Informational Resources:

*Listed alphabetically

eDNA Explorer:
eDNA Explorer brings together eDNA projects from around the world, allowing beginners and experts alike to easily analyze, share, and explore eDNA data, and connect with a comprehensive dataset of biodiversity. See their FAQ page.

eDNA Resources:
This eDNA Resources site is currently managed by Washington State University with a mix of content that was created by WSU for the site as well as eDNA resources contributed by the eDNA research community.

United Nations Educational, Scientific and Cultural Organization (UNESCO):
UNESCO has an "Environmental DNA Expeditions" initiative that is a global, citizen science initiative to help measure marine biodiversity, and the impacts climate change might have on the distribution patterns of marine life, across UNESCO World Heritage marine sites. They have compiled a helpful FAQ about eDNA.

National Oceanographic and Atmospheric Administration (NOAA):
NOAA 'Omics is dedicated to leveraging cutting edge biotechnology to benefit a myriad of national priorities including fisheries management, food and water safety, species and habitat conservation, seafood consumer protection, and natural products discovery. Novelists and researchers alike can find resources on how NOAA utilizes eDNA here.

Great Lakes Fishery Commission:
The Great Lakes Fishery Commission coordinates fisheries research, controls the invasive sea lamprey, and facilitates cooperative fishery management among state, provincial, tribal, and federal agencies. This factsheet focuses on the uses and limitations of eDNA in fisheries management.

Parks Canada:
Parks Canada scientists work to conserve Canada's environment. Together with indigenous knowledge holders, they create and manage protected areas, protect species and habitats, and restore natural areas, all in the face of climate change. Increasingly Parks Canada is employing eDNA as a tool for monitoring species distributions in parks across Canada.

United States Geological Survey (USGS):
USGS's Water Science School offers information on many aspects of water, along with pictures, data, maps, and an interactive center where you can give opinions and test your water knowledge. Check out their eDNA page to learn more about eDNA and how it is used by the USGS.

Southern California Coastal Water Research Project:
The Southern California Coastal Water Research Project (SCCWRP) is leading the incorporation of eDNA tools into aquatic ecosystem management. With that, they produced an informative eDNA Primer background document and webinar about eDNA and its applications.

Woods Hole Oceanographic Institute (WHOI) Ocean Twilight Zone (OTZ):
The Ocean Twilight Zone project combines exploration and research with policy and broad engagement on the ocean's twilight zone, so that we can make informed, thoughtful choices about our ocean, framed by equitable and sustainable use of this shared resource. Check out their eDNA explained page for more eDNA information.

Select Peer-Reviewed Papers:

*This publication list is by no means comprehensive but rather is meant to be a springboard for knowledge building in the field of eDNA research and applications.

Bass, D., Christison, K.W., Stentiford, G.D., Cook, L.S.J., & Hartikainen, H. (2023). Environmental DNA/ RNA for pathogen and parasite detection, surveillance, and ecology. Trends in Parasitology, 39(4), 285-304. https://doi.org/10.1016/j.pt.2022.12.010

Darling, J. A., Jerde, J. L., & Sepulveda, A. J. (2021). What do you mean by false positive? Environmental DNA, 3, 879–883. https://doi.org/10.1002/edn3.194

Gold, Z., Wall, A.R., Schweizer, T.M., Pentcheff, N.D., Curd, E.E., Barber, P.H., Meyer, R.S., Wayne, R., Stolzenbach, K., Prickett, K., Luedy, J., & Wetzer, R. (2022). A manager's guide to using eDNA metabarcoding in marine ecosystems. PeerJ, 10:e14071 https://doi.org/10.7717/peerj.14071

Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., Spear, S.F., McKee, A., Oyler-McCance, S.J., Cornman, R.S., Laramie, M.B., Mahon, A.R., Lance, R.F., Pilliod, D.S., Strickler, K.M., Waits, L.P., Fremier, A.K., Takahara, T., Herder, J.E. and Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods in Ecology and Evolultion, 7, 1299-1307. https://doi.org/10.1111/2041-210X.12595

Lee, K. N., Kelly, R. P., Demir-Hilton, E., Laschever, E., & Allan, E. A. (2024). Adoption of environmental DNA in public agency practice. Environmental DNA, 6, e470. https://doi.org/10.1002/edn3.470

Morisette J., Burgiel S., Brantley K., Daniel W.M., Darling J., Davis J., Franklin T., Gaddis K., Hunter M., Lance R., Leskey T., Passamaneck Y., Piaggio A., Rector B., Sepulveda A., Smith M., Stepien C.A., Wilcox T. (2021). Strategic considerations for invasive species managers in the utilization of environmental DNA (eDNA): steps for incorporating this powerful surveillance tool. Management of Biological Invasions, 12(3), 747–775, https://doi.org/10.3391/mbi.2021.12.3.15

Nagarajan, R.P., Bedwell, M., Holmes, A.E., Sanches, T., Acuña, S., Baerwald M., Barnes M.A., Blankenship S., Connon R.E., Deiner K., Gille D., Goldberg C.S., Hunter M.E., Jerde C.L., Luikart G., Meyer R.S., Watts A., Schreier A. (2022). Environmental DNA Methods for Ecological Monitoring and Biodiversity Assessment in Estuaries. Estuaries and Coasts, 45, 2254–2273 https://doi.org/10.1007/s12237-022- 01080-y

Shea MM, Kuppermann J, Rogers MP, Smith DS, Edwards P, Boehm AB. 2023. Systematic review of marine environmental DNA metabarcoding studies: toward best practices for data usability and accessibility. PeerJ, 11:e14993 https://doi.org/10.7717/peerj.14993

Select Technical Reports:

U.S. Fish and Wildlife Service developed the Environmental DNA (eDNA): Best Management Practices for Project Planning, Development, and Application document. This guide addresses the need for comprehensive eDNA studies standardization, specifically through the lens of a conservation management agency.

Fisheries and Oceans Canada developed the Guidance on the Use of Targeted Environmental DNA (eDNA) Analysis for the Management of Aquatic Invasive Species and Species at Risk document providing science advice on eDNA qPCR and dPCR to support decision making on aquatic species and ecosystems.

Training Opportunities (online, on demand, free):

eDNA Collaborative: YouTube Channel: https://www.youtube.com/channel/UC4vek6-y_lQlXRQwEBbA-UA

eDX: ETHx: Environmental DNA: Sensing the Diversity of Life and Assessing Ecosystem Health course: https://www.edx.org/course/edna-01x
*Note that there is also a paid version of this course and lifetime access to materials

Salish Sea Research Center - Genomics in Tribal Communities:
https://www.salishsearesearchcenter.com/outreach

Paid Courses:

U.S. Fish and Wildlife Service: Environmental DNA: Study Design, Implementation, and Application:
https://www.fws.gov/course/environmental-dna-study-design-implementation-and-application

Appendix II: eDNA Service Providers

*Please note this list is not exhaustive and we do not endorse any single company. We hope to provide a place for practitioners to start looking for service providers.

Name	Location	Website	Core Business
AllGenetics	Spain	https://www.allgenetics.eu/company	End-to-end eDNA analysis and consulting
Applied DNA Sciences	USA	https://adnas.com/	Clinical diagnostics
Applied Genomics LTD	UK	https://appliedgenomics.co.uk/	End-to-end eDNA analysis and consulting
Biome-id	Germany	https://www.biome-id.com/	End-to-end eDNA analysis and consulting
Bureau Veritas	Canada	https://www.bvna.com/environmental-ih-laboratories/services/environmental-dna	End-to-end eDNA analysis and consulting
eDNATech	Canada	https://ednatec.com/	Programmatic eDNA sampling and analysis
EnviroDNA	Australia	https://www.envirodna.com/	End-to-end eDNA analysis and consulting
Genidaqs	USA	https://genidaqs.com/	End-to-end eDNA analysis and consulting
Identifica	Portugal	https://identificabio.com/	Environmental diagnostics
Jonah Ventures	USA	https://jonahventures.com/	End-to-end eDNA analysis and consulting
NatureMetrics	UK	https://www.naturemetrics.co.uk/	End-to-end eDNA analysis and consulting
SimplexDNA	Switzerland	https://www.simplexdna.com/	End-to-end eDNA analysis and consulting
Sinsoma	Austria	https://www.sinsoma.com/en/	End-to-end eDNA analysis and consulting
Spygen	France	https://www.spygen.com/	End-to-end eDNA analysis and consulting
WilderLab	New Zealand	https://www.wilderlab.co.nz/	End-to-end eDNA analysis and consulting

Acknowledgements

This document was collaboratively produced by members of the following partnering institutions who came together to host a public Webinar and invitational Workshop on 'Mobilizing eDNA for Environmental Management in the Northeast Pacific' in 2024.

• The eDNA Collaborative • The Ocean Decade Collaborative Centre for the Northeast Pacific • The Hakai Institute • Fisheries and Oceans Canada (DFO) • National Oceanic and Atmospheric Administration (NOAA 'Omics) • McGill University's Sunday Lab •

It was produced to support marine resource managers and stewards throughout the Northeast Pacific Ocean Region - a region that is incredibly diverse in its ecosystems and its people, and has been home to Indigenous peoples since time immemorial. The authors and contributors respectfully acknowledge the First Nations and Tribal stewards with deep connections to this place who are sustainably managing their lands and waters, as they have for generations.

Photo credit: Keith Holmes, Hakai Institute
All remaining photos taken by Grant Callegari, Hakai Institute