Fisheries and Oceans Canada | Pêches et Océans Canada
Ecosystems and Oceans Science | Sciences des écosystèmes et des océans

National Capital Region

Canadian Science Advisory Secretariat
Science Advisory Report 2020/058

ADVICE ON THE USE OF TARGETED ENVIRONMENTAL DNA (eDNA) ANALYSIS FOR THE MANAGEMENT OF AQUATIC INVASIVE SPECIES AND SPECIES AT RISK

Figure 1. Sampling for eDNA offshore in the Bay of Fundy.

Context:

There is growing interest in using environmental DNA (eDNA) methods for biological monitoring and to support the implementation of federal, provincial, and territorial legislation (e.g., Aquatic Invasive Species Regulations, Species at Risk Act*). However, the lack of robust guidelines and reporting standards for eDNA has affected the confidence of natural resources managers to apply eDNA results in support of management actions.*

Fisheries and Oceans Canada, through the Aquatic Invasive Species and Species at Risk Programs, and the federally-, provincially- and territorially-led National Aquatic Invasive Species Committee (NAISC), has identified the need for guidance on the use of eDNA to support management decisions and provide insights into the distribution of aquatic organisms. The Aquatic Invasive Species and Species at Risk Programs, together with NAISC, submitted a formal request for Science Advice on eDNA in 2019.

This Science Advisory Report is from the July 6-8, 2020 National Peer Review Meeting on the Guidance on the Use of Targeted Environmental DNA (eDNA) Analysis for the Management of Aquatic Invasive Species and Species at Risk national peer review meeting. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

SUMMARY

- Environmental DNA (eDNA) herein refers to DNA extracted from environmental samples (e.g., water, biofilms, air, sediment, gut contents, feces) and analyzed for biological

monitoring and surveillance. This advice focuses on targeted eDNA approaches that selectively detect DNA of aquatic organism(s) of management interest. As quantitative PCR (qPCR) is currently the most common and widely-used targeted eDNA approach, it is the focus of this advice.

- When targeted eDNA methods are validated adequately, they can be used to indirectly infer the presence or absence of target organism(s). DFO and DFO clients are increasingly using eDNA approaches for monitoring programs and decision-making.
- This advice is a first effort towards enabling consistent and transparent communication and reporting between eDNA end-users and service providers (e.g., DFO Science or third-party). This advice builds towards national and international reporting standards and consensus.
- The numerous strengths of eDNA approaches (e.g., non-destructive, non-intrusive, sensitive) make them an ideal management tool for detection and monitoring of organisms that often are challenging to detect using conventional surveillance methods, such as aquatic invasive species and species at risk.
- An eDNA Guidance Document and Reporting Template are included with this advice, without being overly prescriptive, as complementary tools to support sound, science-based decisions and increase confidence in eDNA studies by providing the information necessary for managers to understand eDNA study design, implementation, and interpretation.
- The validity of eDNA data and results relies on a robust study design, which is specific to project objectives and best developed early and in consultation with managers/end-users, ecologists or researchers, and eDNA service providers. Study design should include sufficient and ecologically-appropriate field sampling for the target organism(s), contamination prevention, and appropriate use of field and laboratory controls to test for potential error(s).
- To ensure consistency in reporting and appropriate interpretation of data, the Guidance Document includes an eDNA validation scale for assessing the rigor of eDNA assays and an example of an eDNA detection decision tree to facilitate interpretation of qPCR results.
- eDNA results must be interpreted in the context of the management objectives and risk tolerances. Interpretation criteria should be selected in consultation with resource managers/end-users, eDNA experts, ecologists, and other relevant experts, and in consideration of relevant environmental factors.

INTRODUCTION

Conventional biological monitoring methods (e.g., visual surveys, trawling) can be resource intensive, logistically challenging, and destructive to species and/or ecosystems under study. Environmental DNA (eDNA) detection is a rapidly growing field that holds the potential to address and overcome many of the challenges or inherent limitations of conventional methods. Environmental DNA herein refers to genetic material that is extracted from environmental samples (e.g., water, biofilms, air, sediment, gut contents, feces) and analyzed for biological monitoring and surveillance.

Globally, eDNA approaches increasingly are being incorporated into biological monitoring and surveillance programs. The DFO Aquatic Invasive Species and Species at Risk Programs, and the federally-, provincially-, and territorially-led National Aquatic Invasive Species Committee (NAISC) have been early adopters and end-users of eDNA approaches for biological monitoring, early detection, and to support legislative requirements (e.g., *Aquatic Invasive Species Regulations*, federal *Species at Risk Act*); however, there have been challenges integrating eDNA into management decision-making processes. The lack of standardized and validated eDNA methods has resulted in end-users being concerned or uncertain about the validity of eDNA evidence, especially when there is potential high economic, social, and political impact associated with acting on eDNA evidence (Sepulveda et al. 2020). The need to establish standardized practices is recognized by many groups regionally, nationally, and internationally as a limitation to management and regulatory uptake, but standardization is challenging due to rapid technological advancements in eDNA detection and the complexity of environmental samples (Goldberg et al. 2016).

While national and global efforts towards eDNA standardization are underway (e.g., CSA 2019), guidance is needed for end-users who are using, or considering the use of, eDNA results in support of day-to-day decision-making. Aquatic Invasive Species and Species at Risk Program managers approached DFO Science for Science Advice to encourage consistent reporting of methods and improve communication of eDNA results between end-users and scientists. As outlined in the Terms of Reference, the purpose of this Science Advisory process is to produce peer-reviewed guidance on eDNA methodologies, standardized language for eDNA terms and concepts, and minimum reporting requirements for eDNA studies; a first step towards standardized practices.

ANALYSIS

Contextual considerations

The ability to confidently and reliably implement eDNA for decision-making and regulatory purposes will improve with time, experimentation, and increased experience with integration of eDNA results into management decisions. Environmental DNA methods are relatively new and developing (Goldberg et al. 2016; Baillie et al. 2019). This advice focuses on targeted eDNA approaches (i.e., eDNA approaches that selectively detect the DNA of single species or taxon) that are more established and less complex than semi-targeted approaches (i.e., methods assessing whole communities, such as eDNA metabarcoding) which are newer, and more experimentally complex. Although there are several technologies available that can be used in targeted eDNA studies, the focus of this advice is on quantitative polymerase chain reaction (qPCR) because it is the most common and widely-used targeted eDNA approach, due to its cost-effectiveness and current accessibility.

It is important to recognize that eDNA is an indirect approach to monitoring the occurrence of species, and eDNA detection is not the same as direct species observation (Lacoursière-Roussel and Deiner 2019). In this Science Advisory process, an eDNA "detection" is a measure of the presence of target DNA in a sample, not the presence of the target species. However, with sufficient spatial and temporal replication and reproducibility, eDNA results can be used to infer that a species likely was present in a given area. Inferring a species' absence also is possible using eDNA. However, similar to conventional monitoring methods, inferring absence requires extensive and repeated sampling often in combination with other sources of biological information and expert opinion.

There are both benefits and limitations to eDNA being an indirect measure of presence/absence. Targeted eDNA approaches have many benefits for effective and reliable monitoring of present and past biodiversity in aquatic systems (Thomsen and Willerslev 2015). Sampling for eDNA generally avoids the handling, disturbing, or killing of target macro-species (e.g., fish, mussels), and often has no or limited impact on a species' habitat. Environmental DNA approaches are sensitive and accurate, thus are well suited and often cost-effective for species that are challenging to monitor (e.g., rare, cryptic) using conventional methods (Sigsgaard et al. 2015). For some species, eDNA detection will be the only viable option to survey the species (e.g., remote areas, endangered species, early detection, large-scale survey). Successful eDNA detection also can help to guide future work in other ecosystems. The genetic data generated through eDNA approaches complements, but does not necessarily replace, conventional methods that have their own strengths and limitations. In terms of limitations, eDNA approaches are subject to many of the same inferential pitfalls as conventional monitoring methods, and issues associated with environmental or experimental variation; therefore controls for error must be used. False-positive (detection of eDNA of a species when that species actually is absent) and false-negative (failing to detect the eDNA of a species when that species actually is present) errors can occur and affect the reliability of results (see Table 1 for more information).

eDNA Guidance Document

To respond to the request for Science Advice, a Guidance Document with standardized language and minimum reporting requirements for eDNA-qPCR workflows was developed as a communication tool and resource for Aquatic Invasive Species and Species at Risk Program end-users. It also can be used by eDNA scientists to better communicate results, uncertainty, and confidence to end-users. The Guidance Document is used best when both end-users and science experts work together to weigh how uncertainty might be reduced throughout an eDNA study.

Concepts, terminology, and minimum reporting requirements provided in the Guidance Document are important for reducing uncertainty in the interpretation of results in so far as standardized reporting guidelines will minimize the variation of reported measures/parameters (among eDNA service providers) that ultimately will facilitate the interpretation of results among different end-users and/or eDNA service providers. The Guidance Document encompasses DFO Science and external science expertise, the state of knowledge on eDNA from the scientific literature, and national and international best practices. It expands upon a previous DFO state of knowledge paper on eDNA (Baillie et al. 2019) and builds towards national and international standardization by providing a model for enhancing the use of eDNA results in decision-making. There are four main steps in the eDNA-qPCR workflow (Figure 2). The state of knowledge on steps 1-3, which includes designing, conducting, and analyzing eDNA results, is more established than for step 4, which includes the interpretation of results for management;

therefore the eDNA Guidance Document accompanying this Science Advisory Report focuses on steps 1-3.

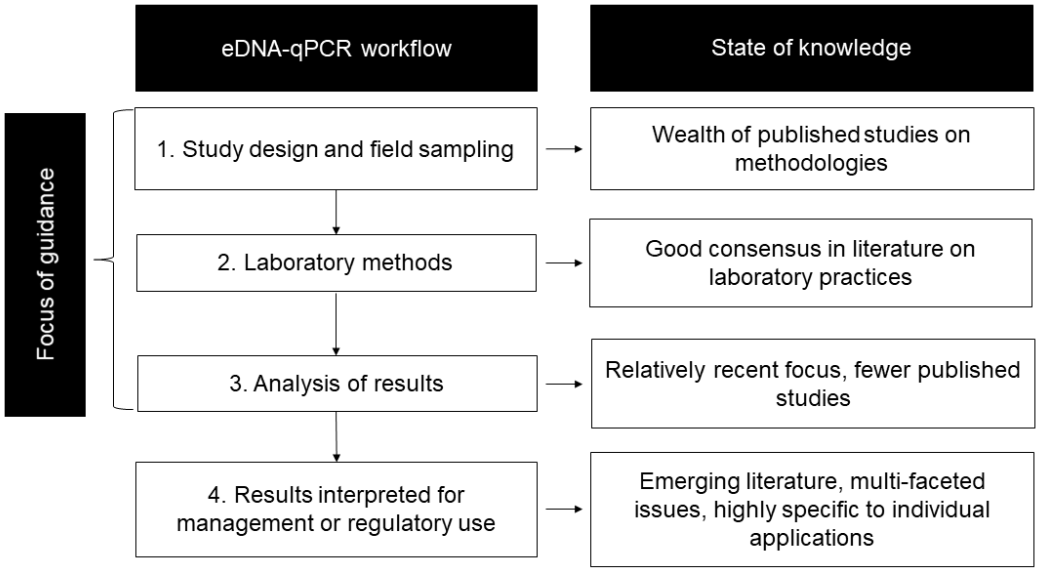

Figure 2. Focus of the eDNA Guidance Document content based on the state of knowledge of four main steps of the qPCR-eDNA workflow.

Step 4, in which eDNA results are interpreted for management or regulatory use, is challenging to standardize because inferring the presence/absence of a target organism is highly specific to individual applications. While qPCR results reliably can report whether or not the target organism's DNA was detected in a tested sample, there are no set criteria for the minimum proportion of samples or replicates necessary to infer the presence/absence of an organism (Goldberg et al. 2016). Since data interpretation is species- and ecosystem-specific, end-users are advised to consult eDNA experts, ecologists, and other relevant experts on a case-by-case basis to optimize species detection and discuss uncertainties in data interpretation of eDNA results.

The minimum reporting requirements in the eDNA Guidance Document and Reporting Template are designed to address a wide range of eDNA sampling and analysis protocols using qPCR and focus on what elements need to be reported, while not being prescriptive about specific methodologies. End-users should ensure that all of the minimum reporting requirements are met as these are the essential criteria that demonstrate the scientific integrity of an eDNA study. Improved and consistent reporting will allow for comparative studies to be undertaken in the future, which can further advance eDNA methodologies for decision-making and regulatory purposes.

Terms and concepts provided in the glossary of the Guidance Document are important for end-users to understand eDNA methods and results. Glossary terms should be used as consistently as possible by eDNA scientists when communicating results. Ambiguous terms such as "weak positive" or "suspect positive" that are not included in the Guidance Document should be defined as an "inconclusive detection" for end-users and avoided.

Study design considerations

The first step in the eDNA-qPCR workflow is study design. Although collecting environmental samples might be straightforward, complex considerations must be taken into account to optimize the study design. Aquatic Invasive Species and Species at Risk Program end-users are advised to discuss their study goals or objectives with a suitable eDNA service provider(s) and other relevant eDNA experts early in project development as part of a communication plan to ensure proper study design and methods.

Determining an appropriate eDNA service provider(s)

The complete workflow of an eDNA study may include various components completed by separate entities, organizations, or teams. For the purpose of communication and reporting, the term ‘eDNA service provider’ refers to the project manager (e.g., DFO Science, third-party consultant) responsible for communicating results to the end-user that requested the study.

Laboratory service providers should be able to demonstrate suitable qualifications, experience level, and clean laboratory conditions. Adherence to Good Laboratory Practices (GLP; see OECD 1998), or eDNA studies conducted in accordance with alternative GLP standards, with strong quality assurance/quality control (QA/QC) measures can increase the probability of accurate detection. End-users requesting eDNA services should evaluate, with a high level of stringency, the suitability of potential service providers and reach out to eDNA experts and other experts as needed during the assessment stage of the contracting process. Note that an eDNA service provider may be involved only to the point of delivery of the qPCR results or aid in the interpretation of eDNA results.

Minimum reporting requirements included in the Guidance Document can be used by end-users to encourage eDNA service providers to report consistently on essential components of the eDNA-qPCR workflow. The goal here is to relay to service providers that certain information would be considered critical knowledge; especially when management decisions based on eDNA results, and the impacts of those decisions, are significant. Thus, before entering into an agreement with a service provider, it should be established whether or not there are barriers to the release of critical knowledge (e.g., protocols, methods) for proprietary reasons. If key reporting items are missing or unclear, the end-user should confirm and clarify them with the eDNA service provider.

Communication plans

It is recommended that a communication plan be developed before a study commences. A communication plan summarizes the expected flow of information between all parties involved in an eDNA study from study design to the notification of eDNA results. A key component of a communication plan is to exchange information and establish clear roles, responsibilities, and expectations among end-users, eDNA service provider(s), and other supporting scientific experts (i.e., ecologists, taxonomists). Communication plans should incorporate the risk tolerances of end-users based on the consequences of results, as this will affect the criteria, thresholds, and notification period for determining whether target eDNA has been detected.

Field sampling considerations

Field sampling involves collecting, processing (e.g., filtration), and preserving environmental samples. Environmental samples often are preserved until DNA can be extracted and analyzed in a laboratory or in the field using portable and/or automated devices. Sound field sampling is critical to optimizing the likelihood of collecting target eDNA from aquatic environments. The

number of samples (and replicates), sampling locations, and methods chosen to collect and preserve eDNA will directly impact the species detection probability, and thus the validity of results. Management objectives and risk tolerance are important factors in field sampling and end-users should convey these factors to an eDNA service provider(s) at the beginning of a study. Experience and past studies should ultimately guide and inform method selection. A pilot study is recommended for each new application of eDNA (e.g., a new geographic location or season) to validate the study design, field sampling protocols, qPCR assay validation, and use of controls are appropriate for detecting the target eDNA.

Figure 3 shows environmental and biological influences on the rates at which DNA persists, disperses, degrades, and accumulates in an environment. The biology of the species of interest and environmental factors are key considerations when choosing sampling locations, frequency, timing, and methods. Temporal, spatial, and seasonal variability in species distribution, behaviour, and abundance will impact eDNA detection probability (Harrison et al. 2019; Jerde et al. 2019). Environmental factors influence the detection probability by affecting both species biology and the eDNA itself. Sampling protocols and processing can differ for different species and sampling locations. Sampling locations should be carefully selected to optimize detection probability based on target species ecology and environmental variability. For aquatic invasive species, sampling locations may reflect natural or anthropogenic vectors, or domains of biological pathways by which invasive species are introduced into new environments.

Logistical constraints

Similar to any study, field sampling and sampling effort often are limited by logistical constraints. Site access, crew availability and experience, remoteness of sites, and hazardous or challenging conditions are important considerations for eDNA studies. These factors often influence timing of sampling, sampling locations, choice and availability of equipment, sample volume, sample depth, and sample storage, which in turn affects eDNA yield and detection probability (Jerde et al. 2019). For example, increasing the volume/weight of the environmental sample improves eDNA yields and probability of detecting target organism(s) eDNA, lowering the risk of false negatives (Hunter et al. 2019). However, there are many specific applications where small sample volumes are appropriate or logistically feasible (e.g., logistical constraints, when using autonomous vehicles). The degradation rate of eDNA also impacts sample quality and increases the likelihood of false negatives; therefore, the length of time taken to process and preserve eDNA samples must be reported. A non-optimal field sampling due to logistical constraints will have consequences for results interpretation.

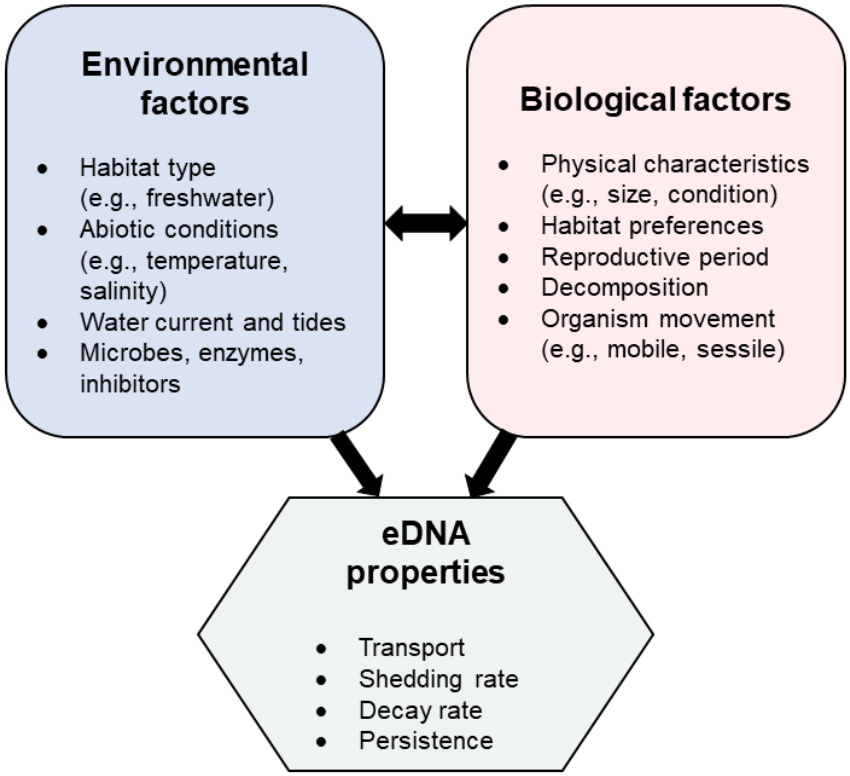

Figure 3. Environmental and biological considerations that interact and affect properties of eDNA. These factors should be considered during field sampling.

Replication and reproducibility

Replication is used to increase confidence in results and assess reproducibility necessary for many statistical modelling applications. The choice of how many replicate samples are needed depends on the specific application. More replication generally increases species detection and confidence in results, but also increases sampling effort and cost. While replication is recommended at most workflow stages (e.g., sample, station, site, qPCR assay), it is not necessarily needed at all stages depending on management objectives, logistical constraints, and risk tolerances.

Controls

Controls are an essential component of eDNA study design; without controls, eDNA results cannot be interpreted meaningfully. It is important to include controls in every eDNA study or survey, and to include them across all stages of the workflow when logistically feasible; however, the specific number of controls needed will depend on the particular application and study objectives (Bohmann et al. 2014). Controls need to be included to detect and report on the potential for false positives and false negatives. Positive controls ensure that protocols are working effectively to detect possible false negatives, while negative controls can be used to identify sources of contamination and detect false positives. A positive control generally includes adding a known concentration of DNA with a different sequence from the target DNA into a blank sample at different points in the eDNA workflow. Negative controls or “blanks” contain no DNA of interest and also are included throughout the workflow.

Results of controls should be described in sufficient detail so end-users can understand the level of uncertainty before making a management decision. Special attention should be paid to whether controls have passed or failed. There is no clear consensus on criteria to assess controls for eDNA approaches and criteria may vary among eDNA service providers. Generally, in many laboratories, if a positive control fails (i.e., no detection), that step of the protocol is repeated until it is optimized and succeeds. If there is unexpected DNA amplification in a negative control, sometimes all associated samples will be omitted from the results. In other laboratories, however, the highest level of amplification in the negative control may be treated as a minimum threshold, and only amplification above this level in samples will be interpreted as a detection. At present, a best practice is to request that control results be described transparently and consistently.

eDNA laboratory methods

Laboratory analysis of eDNA samples begins with DNA extraction - a method to purify or isolate DNA using physical and/or chemical methods. Different DNA extraction protocols will vary in their ability to collect target eDNA. The DNA isolation technique should lead to efficient extraction with good quantity and quality of DNA for qPCR analysis. Quantitative PCR is a technique used to selectively amplify a target segment of DNA by matching it with genetic markers designed for the species or taxon of interest. An advantage of qPCR over regular PCR is that it can be used to both detect DNA at low concentrations and quantify the amount of DNA in a sample.

Rigorous scientific validation that precedes implementation of any qPCR-based eDNA tool in management decision-making is instrumental to minimize uncertainty and enable meaningful application and interpretation of eDNA analyses (Bustin et al. 2009; Sepulveda et al. 2020). A qPCR-based eDNA assay, and its validation, involves the complete set of protocols from field sampling to laboratory analysis and to the interpretation of results. To ensure appropriate interpretation of data, the Guidance Document includes a 5-level validation scale developed by Thalinger et al. (2020 PREPRINT), that ranks the accuracy, sensitivity, and reliability of eDNA methods for targeted eDNA detection (Figure 4). Confidence in qPCR assay performance improves along the scale but other factors also are relevant, such as the repeatability of methods and reproducibility of results. It is important to recognize that the level of qPCR-based eDNA assay validation is associated tightly with field components and varies between species; therefore validation testing must be performed in the geographic location and substrate in which the eDNA assay will be used for management decisions.

Level-1 and Level-2 validation represent the initial *in silico* (computer-based primer design) and *in vitro* (laboratory-based primer optimization) testing phases, respectively, but not the *in situ* (field sampling of eDNA) testing phases for an eDNA-qPCR assay. These first two levels focus on development and testing of genetic markers to make certain they are specific to the organism of interest (i.e., they do not amplify unwanted species or subspecies). At these stages, the assay has not reached a validation level high enough to determine whether or not target DNA is present/absent.

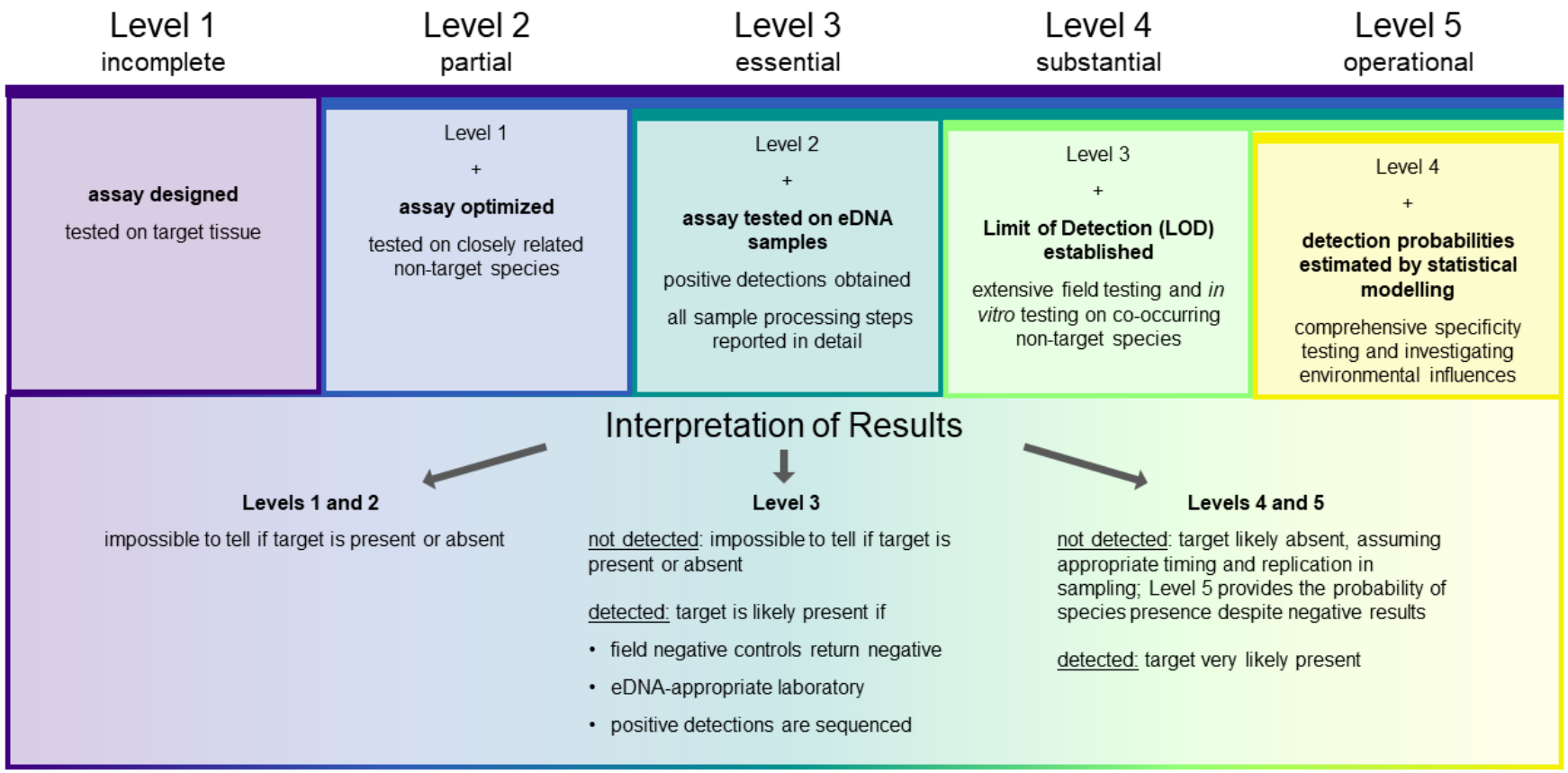

Figure 4. A 5-level validation scale developed by Thalinger et al. 2020 (PREPRINT) to aid evaluation of eDNA assays and appropriate interpretation of results. For each of the levels, the main accomplishments in the validation process and appropriate interpretation of results are provided. See Thalinger et al. 2020 for the minimum criteria at each level.

Level-3 validation requires that the assay be performed on eDNA samples collected from the field (*in situ*) in areas where the target organism is known to be present/absent in order to evaluate its performance under natural conditions. If the eDNA assay is shown to successfully detect the target organism *in situ*, then the assay becomes operational (i.e., it becomes possible to tell whether the eDNA of an organism(s) of interest can be detected in the study area) as long as workflow and standard operating procedures are followed strictly. Therefore, Level-3 validation is an essential step to determine whether or not target DNA is present/absent. For studies that are more exploratory or inform management decisions that are low risk, Level-3 validation may be sufficient to tell if target DNA is likely present in a given area.

For Level-4 validation, the limit of detection (LOD), which is the lowest concentration of target eDNA that can be detected with a level of confidence (95% detection rate is the standard confidence level), is recommended as a minimum detection criterion (Klymus et al. 2019). The limit of quantification (LOQ) is another qPCR measure that may be used with or instead of LOD when a higher detection criterion is needed (i.e., lower risk tolerance; see Klymus et al. 2019). The use of an LOD or LOQ eDNA detection threshold means that a Level-4 assay can be considered highly validated and represents a higher level of confidence in eDNA results than that of a Level-3 assay. Level-4 validation is sufficient to determine whether or not target DNA is very likely present in a given area.

Level-5 validation is considered fully operational for presence/absence of a species/taxon, and is most ideal when supporting decision-making with high resource and/or socio-political consequences; however there are only a few examples where targeted species-specific assays have reached Level-5 validation (e.g., the US Asian Carp Program; USFWS 2019). Level-5 validation is not a static destination and can downgrade should environmental variables change over time (Thalinger et al. 2020).The time and resources needed to achieve Level-3 to Level-5 validation are highly variable depending on the scale and scope of the study. If there are no genetic markers available for a target species, they will need to be designed, developed, and tested as indicated at each validation level. It may take months to achieve a Level-2 validated assay and multiple years to achieve a Level-3 validated assay. Comparably, Level-4 validation often involves many studies and intensive experimentation to understand how to improve eDNA detection in the study area. Moving from Level-4 to Level-5 involves several years of testing and substantial resources. If genetic markers or an eDNA-qPCR workflow is available, it is still important to test and validate a qPCR assay in the planned study area.

Analysis of qPCR assay results for eDNA detection

While there is no consensus about how qPCR results should be analyzed, there are two main approaches used to analyze results: i) detection decision trees, ii) statistical approaches. For both approaches, the eDNA service provider needs to carefully define the criteria for a sample to be considered as “detected”, “not detected”, or any other classification that may be required in a specific study at each level of replication (e.g., site, station). It is recommended that the approach used for eDNA detection be developed as part of a communication plan, especially for studies with lower risk tolerance.

In this Science Advisory process, the recommended minimum reporting information, or criteria, were selected to enhance interpretation and transparency of qPCR assay results, while acknowledging that eDNA methods are evolving. Details on all positive and negative controls should be reported so that the end-user can understand the robustness of the study, regardless of their discipline and experience with eDNA. An overview of qPCR assay results should be provided to the end-user(s) to allow for assessment independent from that of the eDNA service

provider. It is considered acceptable that an eDNA service provider limits their reporting to the QA/QC analysis, however, the service provider also can provide an analysis or interpretation of the qPCR assay results.

To help support managers, an example detection decision tree was developed (Figure 5). This example shows key components of a detection decision tree and is complementary to other decision trees developed for specific eDNA applications or programs. Note that the example decision tree is not intended as a standard for any given eDNA detection study. The number of qPCR replicates (upper box Figure 5) and the number of samples, stations, and sites (lower box Figure 5) could be set to any agreed upon minimum number, and could change over time as information is learned about the study system.

In the example decision tree, Level-4 to Level-5 validation (see Figure 4) is the minimum level that must be attained to permit a “detected” or “not detected” status designation because the criteria for detection was chosen as the level of detection that meets or exceeds the LOD parameters at the sample, site, and station level. The term “inconclusive detection” is used when the sample amplified but its signal was lower than the LOD (i.e., the weight of evidence does not meet the level of detection), and further work is required (e.g., resampling, repeated testing).

Results at any stage of an eDNA study, or qPCR assay workflow, can be affected by false positive and false negative errors (Table 1). The Guidance Document and minimum reporting requirements are intended to help address sources of error by ensuring that end-users have the information needed to understand how they may have originated, and how they may be addressed. Status designations of eDNA studies (“detected”, “inconclusive detection”, or “not detected”) have the potential to vary depending on study objectives, results, and the risk tolerance of end-users; therefore, it is not possible to provide specific guidance on interpretation of eDNA assay results for management or regulatory use. Depending on the status designation of a study, further sampling and repeated eDNA testing, either with the same or amended study design, may be needed to corroborate the available evidence in order to increase confidence in results. Confirmation of eDNA results may be required by alternative survey methods focused at the site(s) of eDNA detection(s), resampling, use of an additional qPCR assay, and/or DNA sequencing of a subset of qPCR products.

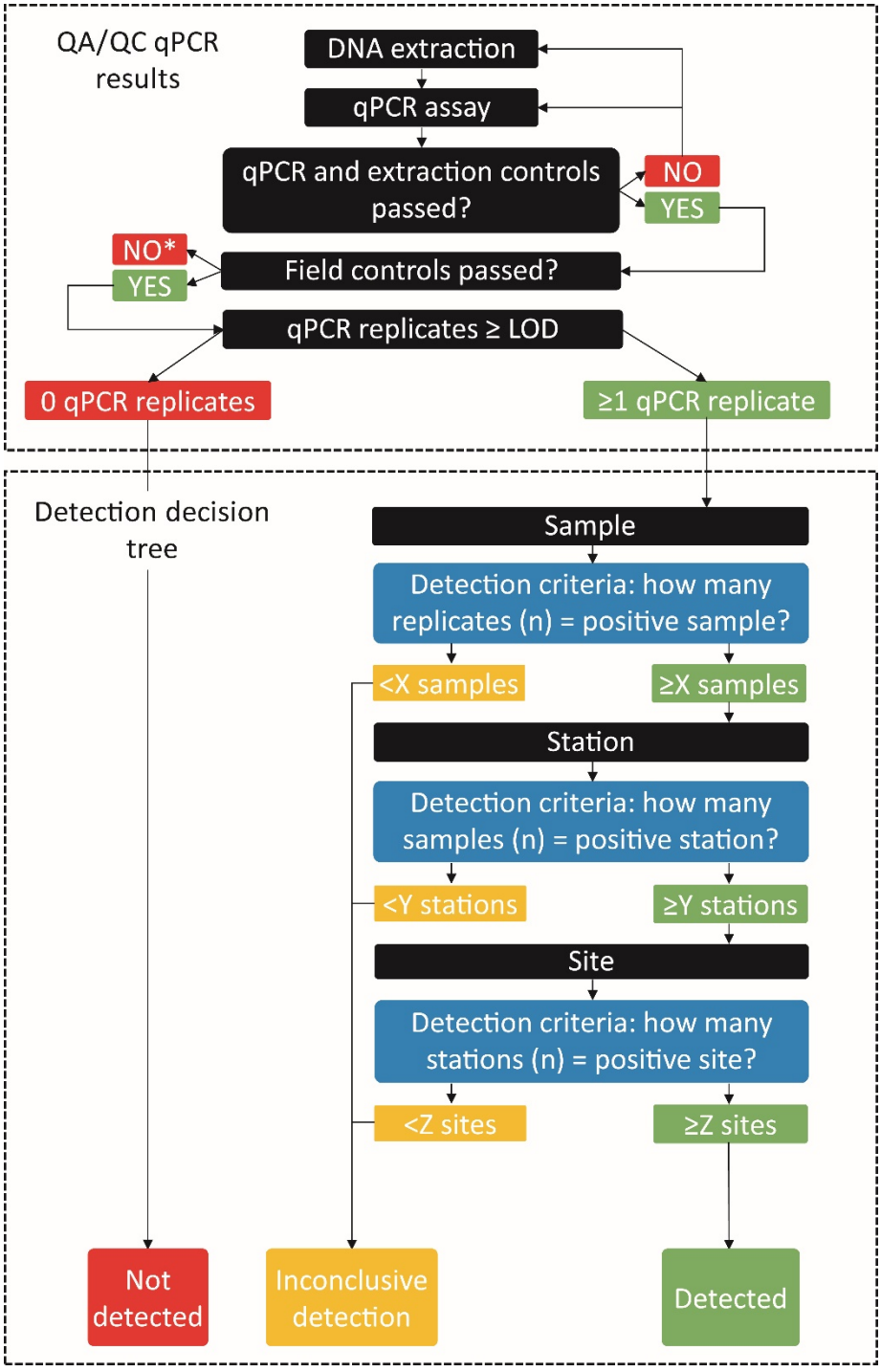

Figure 5. Example of a decision tree for interpreting eDNA qPCR results. In the upper dotted box, final qPCR results are generated by verifying controls. The lower dotted box is a detection decision tree, within which the criteria applied are variable (as denoted by X, Y, and Z) and may or may not differ for each level, as they will be set according to study objectives and the risk tolerance of the end-user. The asterisk associated with field controls that did not pass indicates the need for a discussion between the end-user and eDNA service provider.

Table 1. Possible status designations for eDNA results based on whether the target species is present or absent. These designations should be discussed in the context of management objectives and risk tolerance.

	eDNA not detected	**eDNA detection inconclusive**	**eDNA detected**
Species is **present**	• Environmental samples were not collected at the right time and/or location for the target species. • DNA degraded in the field, in storage, or between extraction and amplification. • DNA was not efficiently extracted from environmental samples. • Amplification was not successful (e.g., inhibition).	• Sporadic amplifications that failed set 'detected' thresholds may have been due to low target abundance resulting from physical and biological processes (i.e., distance from source, low DNA shedding rate, low target organism abundance, inhibitory compounds, DNA degradation).	✓
Species is **absent**	✓	• Samples might have been contaminated in the field and/or laboratory. • Amplification of a closely related species' DNA.	• eDNA released by a dead organism(s) (e.g., in ballast water) was amplified. • eDNA persisted from an early introduction event where the species did not establish (i.e., relict signal). • eDNA was transported from a distant source. • Contamination in the field or/and laboratory. • Amplification of a closely related species' DNA.

Sources of Uncertainty

Environmental DNA is an indirect measure of presence/absence of target DNA and cannot distinguish between live or dead organisms (e.g., fecal matter, digested remains from predators). Certainty in inferring presence/absence can be improved through repeated temporal and spatial sampling of the area and careful attention to document any observed dead organisms or possible outside sources of eDNA. In parallel, not detecting the eDNA does not necessarily indicate the absence of a target species; target eDNA might not have been collected even when present, the species may have a low DNA shedding rate, or technical issues might have occurred.

There are several analytical sources of uncertainty from DNA extraction, qPCR assay validation and operationalization, and interpretation of results. Uncertainty can be introduced during field sampling if there is an insufficient number or volume of samples, not enough spatial and/or temporal sampling to successfully detect the target organism(s), and/or contamination. The sensitivity of eDNA methods requires stringent GLP, QA/QC procedures, and rigorous testing to reduce sources of DNA cross-contamination and increase confidence in eDNA detection.

Other sources of uncertainty include knowledge gaps about the persistence and distribution of eDNA from different sources in different environments. Attaining a high level of certainty requires ongoing surveys and testing in a given ecological system at a particular location.

CONCLUSIONS AND ADVICE

Environmental DNA is particularly well-suited for rapid detection of aquatic invasive species, species at risk, and other species that often are underestimated or underrepresented using conventional monitoring methods. Environmental DNA analysis has many cost-effective applications and can provide information to managers and policy-makers that complements other conventional monitoring methods and supports the conservation of aquatic species and ecosystems.

Targeted eDNA-qPCR approaches can provide effective and reliable detection methods for the DNA of aquatic organisms when properly designed, thoroughly validated, and consistently reported among end-users and eDNA service providers. Minimum reporting requirements and standard language included in the Guidance Document to provide a communication tool and comprehensive resource to guide Aquatic Invasive Species and Species at Risk Program end-users through the entire eDNA-qPCR workflow. Minimum reporting requirements are intended to be used by end-users to understand the criteria important for eDNA study status designation, and to encourage eDNA service provider(s) to deliver results in a consistent manner. Consistent reporting of eDNA studies will allow for more comparative studies to be undertaken that can help address knowledge gaps and advance eDNA methodologies for decision-making and regulatory purposes.

The field of eDNA is relatively new and under rapid development spurred by its numerous beneficial applications. This Science Advisory process brings DFO towards national and international reporting standardization and consensus, representing a first effort towards consistent and transparent communication of eDNA results.

It was concluded that if most or all of the above practices for designing and reporting on an eDNA study are in place, eDNA results should be considered in policy and management actions and eDNA should be another tool for detection, monitoring, and managing species of interest.

OTHER CONSIDERATIONS

Other eDNA approaches not included in this request for advice, such as Droplet Digital Polymerase Chain Reaction (ddPCR), may be as valid as qPCR approaches and have their own detection criteria, validation steps, and outputs that should be reported in as much detail as possible. Some of the minimum reporting requirements outlined here regarding field sampling and analysis may be applicable to other eDNA approaches.

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SOURCES OF INFORMATION

This Science Advisory Report is from the July 6-8, 2020 National Peer Review Meeting on the review of the Guidance on the Use of Targeted Environmental DNA (eDNA) Analysis for the Management of Aquatic Invasive Species and Species at Risk. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

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Canadian Science Advisory Secretariat (CSAS)
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ISSN 1919-5087

Correct Citation for this Publication:

DFO. 2020. Advice on the Use of Targeted Environmental DNA (eDNA) Analysis for the Management of Aquatic Invasive Species and Species at Risk. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2020/058.

Aussi disponible en français :

MPO. 2020. Avis sur l'utilisation des analyses ciblées d'ADN environnemental (ADNe) pour la gestion des espèces aquatiques envahissantes et des espèces en péril. Secr. can. de consult. sci. du MPO, Avis sci. 2020/058.