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Proceedings of the National Peer Review for Guidance on the Use of Targeted Environmental DNA (eDNA) Analysis for the Management of Aquatic Invasive Species (AIS) and Species at Risk (SAR)

Meeting dates: July 6-8, 2020
Location: Virtual meeting

Chairpersons: Shauna Baillie & Sophie Foster
Editors: Karine Robert and Shannan May-McNally

Fisheries and Oceans Canada
200 Kent Street
Ottawa, Ontario K1A 0E6

March 2024

Canada

Foreword

The purpose of these Proceedings is to document the activities and key discussions of the meeting. The Proceedings may include research recommendations, uncertainties, and the rationale for decisions made during the meeting. Proceedings may also document when data, analyses or interpretations were reviewed and rejected on scientific grounds, including the reason(s) for rejection. As such, interpretations and opinions presented in this report individually may be factually incorrect or misleading, but are included to record as faithfully as possible what was considered at the meeting. No statements are to be taken as reflecting the conclusions of the meeting unless they are clearly identified as such. Moreover, further review may result in a change of conclusions where additional information was identified as relevant to the topics being considered, but not available in the timeframe of the meeting. In the rare case when there are formal dissenting views, these are also archived as Annexes to the Proceedings.

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SUMMARY

A Fisheries and Oceans Canada (DFO) Canadian Science Advisory Secretariat (CSAS) National Advisory Process was held virtually from July 6-8, 2020. The focus of the meeting was to provide peer-reviewed science advice and guidance on the use of targeted Environmental DNA (eDNA) analysis for the management of Aquatic Invasive Species (AIS) and Species at Risk (SAR).

The meeting included discussion around how to improve eDNA reporting standards through study design, sample analysis, and results reporting. The discussion was guided by several presentations given by the lead authors of the associated working paper or led by the meeting co-chairs. This Proceedings document is the record of meeting presentations, discussions, recommendations, and conclusions. A Research Document and a Science Advisory Report were produced following the conclusion of the meeting.

INTRODUCTION

Environmental DNA (eDNA) analysis is a non-intrusive, sensitive, and often cost-effective biological monitoring approach that is becoming increasingly used for the detection of species. However, the complexity and rapid evolution of eDNA has created challenges for managers tasked with decision-making involving the use of eDNA technologies. Given the continuous development of the eDNA methods and applications, the Department identified a need for guidance for DFO managers who are using, or considering the use of eDNA results in support of everyday decision-making on Aquatic Invasive Species (AIS) and Species at Risk (SAR).

The peer-review of the “Guidance on the Use of Targeted Environmental DNA (eDNA) Analysis for the Management of Aquatic Invasive Species (AIS) and Species at Risk (SAR)” was held virtually, from July 6–8, 2020. The meeting commenced with a co-chair welcoming the participants and providing an overview of the Canadian Science Advisory Secretariat (CSAS) peer-review process, guiding principles, science advisory products, and timelines. The co-chair also reviewed the Terms of Reference (Appendix 1), which outlined the purpose of the meeting: to provide Science Advice on the use of targeted eDNA approaches (with respect to eDNA sampling, detection, and analysis) for AIS and SAR to encourage more consistent reporting and interpretation of eDNA results, by evaluating the draft Research Document (i.e., the working paper) which was tabled at the meeting and drafting a Science Advisory Report. The discussion was guided by four presentations by the lead authors of the working paper and discussion led by the co-chairs. For each section of the working paper that was presented, a reviewer was identified prior to the meeting who would initiate the discussion by addressing three pre-identified questions:1) What clarification/major changes are required?; 2) What gaps, if any, exist in the draft Research Document?; and 3) What else could be flagged to the decision makers?

A total number of 26 meeting participants consisted of experts from Fisheries and Oceans Canada (DFO), including Science, SAR and AIS programs; Scottish Environmental Protection Agency (SEPA); United States Geological Survey (USGS); United States Department of the Interior; and the University of Guelph (Appendix 2). The meeting agenda is provided in Appendix 3.

PRESENTATION OF THE RESEARCH DOCUMENT: INTRODUCTION AND SECTION I: eDNA GUIDANCE AND REPORTING TEMPLATE

Presented by: Cathryn Abbott

SYNOPSIS OF PRESENTATION

The presenter introduced the working paper and gave an overview of the first section which covered the eDNA guidance and reporting template. The presentation focused on the purpose of the eDNA guidance development, detailing the goals of the science advice as well as the scope of the guidance document. The presentation concluded with an overview of the eDNA reporting template and contextual considerations.

DISCUSSION

The reviewer assigned to this section's discussion began by addressing the three opening questions.

1. The reviewer pointed out that the working document is too technical for the intended general audience, therefore it was suggested to include resources for people that may be less comfortable with technical information.
2. It was suggested to include good laboratory practices (GLP) in the working paper.
3. It was suggested to include recommendations regarding eDNA data archiving in the working paper.

There were discussions pertaining to the appropriate level of detail and technical terminology in the working document. It was suggested to use more lay language in the guidance, particularly for those sections where the work may be completed by non-specialists (e.g., field sampling). As different sections of the guidance will be used by different people, the level of language in each section should be chosen accordingly.

Participants then discussed the need to include GLP in the working document. The point was made that clarifying supporting documentation would be preferable for ensuring that service providers have Standard Operating Procedures (SOPs), and that they follow GLP. A participant pointed out that "service provider" had not been defined in the guidance, and other participants agreed that "service provider" should be explicitly defined in the working document.

Although this guidance was not designed to be prescriptive, a participant suggested adding information on minimum standards to provide a baseline target for managers. Although minimum standards were not prescribed in the guidance for every study scenario, the reporting template will provide suggested minimum data reporting criteria. A participant also suggested having discussions on archiving of samples and data, suggesting that authors include this information in the working document.

PRESENTATION OF THE RESEARCH DOCUMENT, SECTION II: STUDY DESIGN AND eDNA SAMPLE COLLECTION

Presented by: Nellie Gagné & Anaïs Lacoursière

SYNOPSIS OF PRESENTATION

Section II: The presentation provided an overview of study information and design, including eDNA sample collection. The importance of appropriate study design was discussed, including considerations such as accounting for the relevant ecosystem as well as the end goal of the project (these aspects can help improve the likelihood of eDNA detection). The presentation concluded with an overview of some crucial aspects of results reporting and interpretation.

DISCUSSION

The reviewer that was identified to start this section's discussion began by addressing the three opening questions.

1. It was questioned whether the term "study design/experiment" should be changed to "surveillance design", for clarifying that it can be used in an operational context and not solely in a research context.
2. It was suggested to include specific information about AIS and domains (i.e., pathways of introduction) in the guidance.
3. The reviewer suggested adding more detail on the intended actions or decisions related to eDNA surveillance in the document.

The reviewer concluded the discussion of the three opening questions by praising the overall quality of the proposed guidance document.

Following the reviewer's comments, a participant suggested to specify in the guidance that the first step in any good eDNA study is to understand the goals/consequences/actions once an AIS is found.

The group had lengthy discussions on the level of detail that should be included in the guidance regarding the type and quantity of controls needed in order to better estimate the eDNA detection probability. Given that controls can vary among species and studies, the guidance was originally designed to be less specific on the type and quantity of controls needed in order to be as inclusive as possible and consider many different possibilities. A participant noted that, although the number of controls was not specified in the guidance for flexibility purposes, it would be useful to include more information regarding the importance of controls. It was also suggested that the guidance include information regarding the inclusion of sample(s) from a proxy or site where the target species is known to live as a positive control. This was suggested because it is logistically difficult to assess the false negative detection rate and that an increase in the number of controls could help strengthen results. The group also agreed to include a statement in the guidance on the beneficial impact that an increase in the number of controls at the site level has on the ability to detect contamination. The more negative controls there are at the site level, the more possible it is to determine the sources of contamination. It was also agreed to better communicate in the guidance how controls impact both quality assurance and quality control, and ultimately the results.

A participant noted that the ambiguity around the document's definition of 'false positive' should be addressed. In the glossary, 'false positive' is defined in terms of the organism and not DNA. It was suggested to make the definition as explicit as possible by adding definitions that have not been considered (e.g., DNA).

PRESENTATION OF THE RESEARCH DOCUMENT, SECTION III: LABORATORY METHODS

Presented by: Geneviève Parent

SYNOPSIS OF PRESENTATION

Section III: eDNA sample analysis – laboratory methods of the working document was presented. The presentation focused on the reporting requirements of DNA extraction and qPCR assay. The qPCR assay was presented in more detail including an overview of the explanations for managers that are found in the working document (i.e., what is a qPCR assay? multiplex qPCR assays, level of assay validation, and results interpretation and PCR inhibition).

DISCUSSION

The reviewer that was identified to start this section's discussion began by addressing the three opening questions.

1. The reviewer inquired on whether it would be preferable to include optional primers and probes in the working document for the qPCR assay.
2. The reviewer asked for clarifications on specificity testing and inquired on whether this reporting refers to *in vitro* or Sanger sequencing to confirm some of the positive results. The reviewer thought that most of the essential information was captured well.
3. The point was brought forward that it would be helpful to require the service provider to include more specific information on assay validation in the reporting template. It was acknowledged that most assays may not have been validated across all environments or regions where it is being used, and therefore it would be necessary to increase the level of validation.

Participants had lengthy discussions on both the process and level of validation that would be required. The group generally agreed with the 5-level validation scale that was presented, pending clarifications on certain points. It was agreed that it would be helpful for managers to include additional explanations in the guidance as to when and why a specific level of validation would be required. The group also agreed that it needs to be clarified in the document that validation is not linked to a given assay, but rather to the area studied (i.e., it is not possible to transfer validation level amongst geographic locations). It was pointed out that the proposed guidance seems to generally correspond to the European Union DNAqua-Net validation stages. It was added that it would be important to confirm that the level of validation scale in the document aligns with the DNAqua-Net one, and to highlight any differences between the two.

A participant pointed out that there is currently a lot of ambiguity in the literature with respect to specificity testing and suggested that the guidance address this by tasking the service provider with providing more details on assay validation and methods (e.g., DNA extraction protocols). It was mentioned that study outcome may be confused with assay validation (levels 4 and 5) and participants suggested the inclusion of additional context in the document to avoid confusion. It was pointed out that asking for a lot of details may lead to vague answers and it was thus suggested to set up a communication plan (involving the service group, stakeholder, researcher, and client), in which finer details could be provided. Participants agreed that the guidance requires clarification as to what an eDNA service provider is, and what they are responsible for.

The group also agreed on adding details to some steps of the laboratory methods, while recognizing the need to balance having sufficient information for interpretation with the desire to avoid producing a document that is too complex and cumbersome for practical use.

PRESENTATION OF THE RESEARCH DOCUMENT, SECTION IV: REPORTING eDNA RESULTS

Presented by: Mark Coulson

SYNOPSIS OF PRESENTATION

Section IV: Reporting eDNA results was presented which included an overview of controls and eDNA results reporting and the working document closing statements. The results reporting section was presented in more detail and included an overview of an example of a decision tree for qPCR results interpretation for eDNA detection. The presentation ended with an overview of the working document and concluding remarks.

DISCUSSION

The reviewer that was identified to start this section's discussion began by addressing the three opening questions.

1. The reviewer suggested including information on what decisions need to be made *a priori* as opposed to *a posteriori*.
2. The need to clarify what is repeatable vs reproducible results was highlighted.
3. It was suggested to determine what decisions need to be made in discussion with management up front before the study commences. It was added that this may need to be done for each study or project, but those criteria would need to be determined on an individual basis.

Participants had lengthy discussions on the contents of this section including requesting clarification on why reproducible results are important (it was noted that they increase confidence in results among other things). It was then suggested to integrate the caveats on eDNA methods into the main text, rather than in the conclusion, as it would avoid ending this section on a negative note. It was pointed out that the information on a pilot study could be added to the conclusion section.

A participant mentioned that it would be beneficial to include temporal aspects in the box entitled *Interpretation of qPCR results for eDNA detection* (decision tree), so both spatial and temporal aspects can be taken into consideration. Discussions occurred regarding the limit of quantification (LOQ) and the limit of detection (LOD). Participants wondered whether LOQ should be included in the decision tree given that it is not really addressed in the guidance document. It was suggested to include some information on LOQ that would allow the service provider to have a general sense of the confidence that should be put in the lab derived LOQ values when using eDNA quantities for ecological inference and management decisions. A suggestion was made for a new text box on the use of replication that would include LOD linked with results confidence.

The discussion continued on technical aspects of the guidance. A participant mentioned that guidance would be needed on the necessary number of qPCR cycles and on how this information might impact the interpretation of results. It was suggested to incorporate ancient DNA as an example. It was also suggested to undertake beta testing, so that any issues could be identified by researchers.

The group then discussed any additional information that should be included in the guidance document from a management perspective. More guidance on how managers should identify and quantify risk tolerance was requested. A participant outlined the need for more communication between the service provider and manager, and to clearly define their roles. It

was proposed to add a new box in which a communication plan (between end-users, eDNA service providers, eDNA specialists, and ecologists) would be explained.

CONCLUDING DISCUSSION

The authors presented an overview of the major proposed additions to the guidance document, based on the comments received during the meeting for a concluding discussion. The group engaged in open, in-depth discussions regarding the document, and attempted to identify any additional gaps. Overall, the discussions led to changes to the working document, which increased the clarity of wording and terminology regarding the use of targeted eDNA analysis. The changes suggested by the group will result in a more user-friendly document, which clearly outlines how to use eDNA to inform decision making in the context of AIS and SAR management. The co-chairs asked if there were any volunteers that could review the updated research document and template to assess whether reviewer comments were sufficiently addressed. It was mentioned that external reviewers are critical in the peer-review process.

The co-chairs asked whether the proposed changes were approved. Consensus was reached on the suggested changes to address comments that were raised during this meeting. The working paper was upgraded to a Research Document to be published on the CSAS website.

CONCLUSION OF THE SCIENCE ADVISORY PROCESS

Towards the end of the third day, all three objectives from the Terms of Reference were discussed in plenary. The Science Advisory Report conclusions in bullet point form were then drafted and reviewed. Participants agreed that the summary bullets covered the main points; consensus had been reached. The chair summarized the remaining sections and structure of the Science Advisory Report and laid out next steps. Participants agreed with the approach suggested by the Co-Chairs.

APPENDIX 1: TERMS OF REFERENCE

Guidance on the use of targeted environmental DNA (eDNA) analysis for the management of Aquatic Invasive Species and Species at Risk

National Peer Review – National Capital Region

July 6-8, 2020
Virtual meeting

Chairpersons: Shauna Baillie and Sophie Foster

Context

Environmental DNA (eDNA) is defined herein as DNA that can be extracted from bulk environmental samples, such as water, biofilms, or sediment, and analyzed to infer presence or absence of species in an ecosystem. eDNA detection is developing rapidly as a sensitive and non-invasive genetic method that can be used to monitor the occurrence and distribution of aquatic species. However, the rapid evolution of eDNA technologies, coupled with the complexity of environmental samples, has led to challenges with the estimation of uncertainty associated with the results of eDNA studies.

Fisheries and Oceans Canada (DFO), through the Aquatic Invasive Species (AIS) and Species at Risk (SAR) programs, has identified the need for guidance on the use of eDNA in support of decision making for the management of aquatic species and ecosystems. This need also was recognized by the National Aquatic Invasive Species Committee (NAISC), which hosts members from each province and territory to promote national coordination and collaboration on AIS-related issues. While some guidelines, best practices, and standards for eDNA currently exist (see Goldberg et al., 2016), the scientific community continues to advance towards standardization of eDNA research and practices (e.g., Canadian Standards Association, 2019). As applications for eDNA methods continue to be researched and developed, guidance is needed for DFO managers who are using, or considering the use of, eDNA results in support of day-to-day decision making on AIS and SAR.

Through this CSAS process, key terms and concepts relating to targeted eDNA approaches (i.e., using species-specific assays, in contrast to semi-targeted approaches, such as metabarcoding) will be defined, and reporting guidelines and a reporting template for communicating eDNA results to management will be developed. A DFO state of knowledge paper on eDNA (Baillie et al., 2019) will be used as a background document for developing this guidance.

Objectives

The goal of this CSAS process is to provide guidance on the use of targeted eDNA approaches (with respect to eDNA sampling, detection, and analysis) for AIS and SAR to encourage more consistent reporting and interpretation of eDNA results. Specific objectives are to:

Define the scientific terms and concepts associated with eDNA technologies and techniques;

Provide minimum reporting guidelines and a reporting template for management and eDNA practitioners with considerations related to: (i) study design; (ii) field methods; (iii) laboratory methods for targeted eDNA approaches; and (iv) results; and

Provide an accompanying guidance document for items included in the reporting template, with a brief explanation of associated limitations, caveats, and best practices.

Expected Publications

- Science Advisory Report
- Research Document(s)

Expected Participation

- Fisheries and Oceans Canada (DFO)
- Provincial/territorial governments facilitated through NAISC

References

Baillie, S.M., McGowan, C., May-McNally, S., Leggatt, R., Sutherland, B., and Robinson, S. 2019. Environmental DNA and its applications to Fisheries and Oceans Canada: National needs and priorities. Can. Tech. Rep. Fish. Aquat. Sci. 3329: xiv + 84p.

Canadian Standards Association 2019. Environmental DNA standardization needs for fish and wildlife population assessments and monitoring.

Goldberg, C., Turner, C., Deiner, K., Klymus, K., Thomsen, P., Murphy, M., … Taberlet, P. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods in Ecology and Evolution, 7(11): 1299-1307.

APPENDIX 2: LIST OF MEETING PARTICIPANTS

Name	Affiliation
Abbott, Cathryn	Fisheries and Oceans Canada, Pacific Region
Baillie, Shauna (co-chair)	Fisheries and Oceans Canada, National Capital Region
Bajno, Robert	Fisheries and Oceans Canada, Ontario and Prairie Region
Carpentier, Julie	Fisheries and Oceans Canada, National Capital Region
Coulson, Mark	Fisheries and Oceans Canada, National Capital Region
Cowell, Sara	Fisheries and Oceans Canada, National Capital Region
Dietrich, Charise	Fisheries and Oceans Canada, National Capital Region
Duncan, Willie	Scottish Environmental Protection Agency (SEPA)
Ferrante, Jason	United States Geological Survey (USGS)
Foster, Sophie (co-chair)	Fisheries and Oceans Canada, National Capital Region
Gagne, Nellie	Fisheries and Oceans Canada, Gulf Region
Gertzen, Erin	Fisheries and Oceans Canada, Pacific Region
Hamilton, Lorraine	Fisheries and Oceans Canada, Maritimes Region
Hanner, Bob	University of Guelph
Howland, Kimberley	Fisheries and Oceans Canada, Ontario and Prairie Region
Kristmanson, James	Fisheries and Oceans Canada, National Capital Region
Lacoursière, Anaïs	Fisheries and Oceans Canada, Maritimes Region
Leblanc, Francis	Fisheries and Oceans Canada, Gulf Region
May-McNally, Shannan	Fisheries and Oceans Canada, National Capital Region
Morisette, Jeffrey	US Department of the Interior
Parent, Geneviève	Fisheries and Oceans Canada, Quebec Region
Robert, Karine	Fisheries and Oceans Canada, National Capital Region

Name	Affiliation
Sepulveda, Adam	United States Geological Survey (USGS)
Silverio, Cassandra	Government of British Columbia
Valentin, Alexandra	Fisheries and Oceans Canada, Quebec Region
Walker, Sherry	Fisheries and Oceans Canada, National Capital Region

APPENDIX 3: MEETING AGENDA

DAY 1, JULY 6TH	
12:30–12:40(PM)NST 12:00-12:10(PM) AST 11:00–11:10(AM) EST 10:00–10:10(AM) CST 9:00 – 9:10 (AM) MST 8:00 – 8:10 (AM) PST 4:00–4:10 (PM) BST	Welcome and introductions (*Sophie Foster)*
11:10 – 11:20	Introduction to CSAS national peer-review science process (*Sophie Foster)*
11:20 – 11:30	Context: Request for advice and development of guidance and standards for eDNA to support management applications *(Shauna Baillie)*
11:30 –12:00	Introduction and Section I: eDNA Guidance and Reporting Template (*Cathryn Abbott*)
12:00 – 13:15	Discussion (1)
13:15 – 14:00	Break or lunch
14:00 –14:30	Section II: Study design and eDNA sample collection (*Nellie Gagne/ Anaïs Lacoursière*)
14:30 – 15:45	Discussion (2)
15:45 – 16:00	Break
16:00 –16:20	Additional discussion
16:20 – 16:30	Closing remarks and plan for following day (*Shauna Baillie / Sophie Foster*)

DAY 2, July 7th	
12:30–12:40(PM)NST 12:00-12:10(PM) AST 11:00–11:10(AM) EST 10:00–10:10(AM) CST 9:00 – 9:10 (AM) MST 8:00 – 8:10 (AM) PST 4:00–4:10(PM) BST	Opening remarks and review of previous days discussion (*Shauna Baillie / Sophie Foster)*
11:10 – 11:40	Section III: eDNA sample analysis – laboratory methods (*Geneviève Parent*)
11:40 – 13:00	Discussion (3)
13:00 – 13:45	Break or lunch
13:45 – 14:15	Section IV: Reporting eDNA results (*Mark Coulson*)
14:15 – 15:30	Discussion (4)
15:30 – 15:45	Break

DAY 2, July 7th	
15:45 – 16:15	Additional discussion
16:15 – 16:30	Closing remarks and plan for next day *(Shauna Baillie / Sophie Foster)*

DAY 3, July 8th	
12:30–12:40(PM)NST 12:00-12:10(PM) AST 11:00–11:10(AM) EST 10:00–10:10(AM) CST 9:00 – 9:10 (AM) MST 8:00 – 8:10 (AM) PST 4:00–4:10(PM) BST	Summary of discussions and plan for the day (*Shauna Baillie/Sophie Foster)*
11:10 – 11:20	Overview of structure of Science Advisory Report (*Shauna Baillie / Sophie Foster*)
11:20 – 13:00	Drafting of Science Advisory Report (ALL)
13:00 – 13:45	Break or lunch
13:45 – 15:20	Consensus on summary bullets of Science Advisory Report (ALL)
15:20 – 15:30	Closing remarks (*Shauna Baillie / Sophie Foster*)