Received: 21 August 2022 | Revised: 4 January 2023 | Accepted: 23 January 2023

DOI: 10.1002/edn3.395

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COMMENTARY

Best practice guidelines for environmental DNA biomonitoring in Australia and New Zealand

Maarten De Brauwer[1] | Laurence J. Clarke[2] | Anthony Chariton[3] | Madalyn K. Cooper[4] | Mark de Bruyn[5] | Elise Furlan[6] | Anna J. MacDonald[2] | Meaghan L. Rourke[7] | Craig D. H. Sherman[8] | Leonie Suter[2] | Cecilia Villacorta-Rath[9] | Anastasija Zaiko[10,11] | Alejandro Trujillo-González[6]

[1]CSIRO Oceans and Atmosphere, Hobart, Tasmania, Australia

[2]Australian Antarctic Division, Department of Climate Change, Energy, the Environment and Water, Kingston, Tasmania, Australia

[3]School of Life Sciences, Macquarie University, Sydney, New South Wales, Australia

[4]Minderoo Foundation, Perth, Western Australia, Australia

[5]Australian Research Centre for Human Evolution, School of Environment and Science, Griffith University, Nathan, Queensland, Australia

[6]Centre for Conservation Ecology and Genomics, Institute for Applied Ecology, University of Canberra, Bruce, Australian Capital Territory, Australia

[7]Department of Primary Industries, Narrandera Fisheries Centre, Narrandera, New South Wales, Australia

[8]School of Life and Environmental Sciences, Deakin University, Geelong, Victoria, Australia

[9]Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville, Queensland, Australia

[10]Cawthron Institute, Nelson, New Zealand

[11]Institute of Marine Science, University of Auckland, Auckland, New Zealand

Correspondence
Maarten De Brauwer, CSIRO Oceans and Atmosphere, Hobart, TAS 7001, Australia.
Email: maarten.debrauwer@csiro.au

Funding information
Australian Government Department of Agriculture, Fisheries and Forestry; New Zealand Ministry of Business, Innovation and Employment, Grant/Award Number: CAWX1904

Abstract

Environmental DNA (eDNA)-based methods are increasingly used by government agencies to detect pests and threatened species, and for broader biodiversity monitoring. Given rapid technological advances and a growing number of commercial service providers, there is a need to standardize methods for quality assurance and to maintain confidence in eDNA-based results. Here, we introduce two documents to provide best-practice guidelines for Australian and New Zealand eDNA researchers and end-users (available from https://sednasociety.com/publications): the *Environmental DNA protocol development guide for biomonitoring* provides minimum standard considerations for eDNA and environmental RNA projects across the complete workflow, from ethical considerations and experimental design to interpreting and communicating results. The *Environmental DNA test validation guidelines* outline key steps to be used in assay development and validation for species-specific testing and metabarcoding. Both guidelines were developed as an initiative of the Australian Government Department of Agriculture, Fisheries and Forestry and led by the Southern eDNA Society in a collaborative process including multiple consultation rounds with eDNA experts, end-users, and stakeholders to adapt the guidelines to Australian and New

Zealand needs. The aim of these guidelines is not to be prescriptive, but to set minimum standards to support a consistent and best-practice approach to eDNA testing. We anticipate that the guidelines will be reviewed and regularly updated as required. Our aspiration is that these best-practice guidelines will ensure environmental managers are provided with robust scientific evidence to support decision-making.

KEYWORDS
assay development, biosecurity, environmental monitoring, metabarcoding, methods, quality control, standard operating procedures, standardization, test validation

1 | INTRODUCTION

Environmental DNA (eDNA) and RNA (eRNA)-based techniques are methods designed to capture, extract, and analyze traces of genetic material (extracellular or intracellular) from water, soil, air, or other environmental matrices. These methods focus on applications wherein sample collection targets the environment within which species reside, rather than species themselves as an indirect method to detect both macro and microorganisms. As such, eDNA/eRNA-based techniques are now widely used in environmental research and increasingly incorporated into monitoring programs worldwide (Jerde, 2021; McDonald et al., 2020) and offer sensitive, cost-effective approaches to enable detection and monitoring of target species or broad ecological communities without the need to directly observe or handle individual organisms. Advances in genomics technologies and a deeper understanding of how eDNA interacts with the local environment gained over the last decade (Barnes et al., 2014; Collins et al., 2018; Wood et al., 2020) have catalyzed the transition of eDNA methods from basic research tools to key components of monitoring programs in both government and industry (Bani et al., 2020; Darling & Mahon, 2011; McDonald et al., 2020). This has been accompanied by a rapid uptake of eDNA methods in academic research and commercial laboratories, and increasing interest in eDNA technologies from managers and practitioners, who often lack prior experience in using eDNA methods or interpreting eDNA-derived data.

Despite reliance on common methods, different end-users of eDNA data have distinct needs that should be considered during study design (Mathieu et al., 2020; Mize et al., 2019). Short-term research projects can often retain the scope to adapt and change methods as a project progresses to take advantage of new ideas or improved technology. In contrast, long-term monitoring programs and other applied surveys may require use of standardized methods over many years to enable comparison of results across space and time or to ensure compliance with reporting obligations (Lindenmayer & Likens, 2010). With the wide variety of eDNA-based methods available (Deiner et al., 2015), it is crucial that those selected are appropriate to a given question and that their strengths and limitations are clearly defined given the context (Zinger et al., 2019). Although now in demand, eDNA-based methods were previously regarded with a degree of skepticism from some end-users (e.g., Jerde, 2021), highlighting the increasingly important need to maintain confidence by ensuring the use of best practice techniques.

Biomonitoring programs aim to produce data which are both representative of the sampled system and reproducible (Chariton et al., 2016). Given the variety of methods available to users and the diverse, unpredictable nature of eDNA (Barnes & Turner, 2016), there is an increasing need to introduce standardized quality assurance and control measures of laboratory protocols and downstream analyses. This is important to achieve reproducibility and minimize false-negative or false-positive eDNA detections and thus accomplish best practice (Darling et al., 2020; Darling & Mahon, 2011; Trujillo-González et al., 2021; Zaiko et al., 2021). More than a decade of applied and experimental eDNA research has demonstrated that field, laboratory, and analysis protocols must be tailored to account for ecosystem peculiarities (e.g., Pawlowski et al., 2021), target species (e.g., Wacker et al., 2019), and the specific aims of the study and end-users' priorities (e.g., Bani et al., 2020). For example, a false-negative detection in a border biosecurity setting could fail to trigger an immediate response to a threat; on the other hand, a false-positive detection could trigger an unnecessary and financially costly response (Darling et al., 2020, 2021; Trujillo-González et al., 2020). Both instances may arise from a failure to sufficiently establish robust and reliable field, lab, or analysis protocols for the desired purpose. Moreover, technological advances in the field suggest that improved resources allowing for rapid or sensitive eDNA detection will become available over time (e.g., portable qPCR/sequencing technology, Bowers et al., 2021).

Standards set out the specifications and procedures to ensure results are consistent and reliable for a specific method. They need to be endorsed by accredited bodies to be accepted for use in regulatory frameworks. Therefore, designing a "one-size-fits-all" set of standards can lack the flexibility to adapt to changing technologies if not revised regularly. Despite this challenge, national standards for eDNA reporting requirements and terminology have been created in Canada (Gagné et al., 2021). On the other hand, a set of widely accepted best practice guidelines would benefit practitioners, increase the value of eDNA data, and give end-users more confidence in the eDNA monitoring results. If required, best practice guidelines can provide a framework for the development of standards for specific applications.

Best practice guidelines are a set of procedures generally accepted as the most effective way to carry out a method. Setting best practice guidelines for eDNA-based methods is important, especially when incorrect or imprecise results could have financial, ethical, and legal ramifications. While eDNA-based methods can meet legal standards for their reliability, the interface between results and management needs integration for decision-making (Sepulveda et al., 2020). Tools such as decision support trees based on molecular best practice methods that integrate the temporal and spatial trends in eDNA detections can guide managerial actions. Currently, associations of scientists in Japan (aquatic ecosystems), Europe (predominantly freshwater ecosystems), and Canada (aquatic ecosystems) have developed best practice guidelines for practitioners (Abbott et al., 2021; Bruce et al., 2021; Minamoto et al., 2021; Pawlowski et al., 2020). If incorporated consistently, these eDNA methods can be widely applied by government, industry, academic, and citizen scientists for biomonitoring in a robust and reproducible manner.

These published guidelines provide an important template for practitioners globally, but they are not always applicable to Australian and New Zealand contexts. For example, international guidelines seldom acknowledge the ownership and stewardship of First Nation Peoples on the land and waters where eDNA studies occur, which in Australia and New Zealand is a deeply entwined component of environmental management (Handsley-Davis et al., 2021). Additionally, currently published guidelines often focus on aquatic eDNA, with less attention for the applicability of eDNA methods for biomonitoring in other realms (including terrestrial, aerial). Furthermore, Australia and New Zealand span a large latitudinal gradient and are surrounded by a vast and often remote coastline. Their distant geography has historically acted as a barrier to the entry of invasive species, pests, and diseases. However, globalization has led to a dramatic increase in the rates of travel, trade, and movement of potential pest or nuisance species in both countries (e.g., *Trogoderma granarium*; Trujillo-González et al., 2022). Given the high level of species diversity and endemism, as well as specific biosecurity concerns, management practices in Australia and New Zealand must be specific, dedicated, and engaged with environmental stakeholders such as First Nations Peoples during strategic planning, sampling design, and ecological inference (Handsley-Davis et al., 2021).

Here, we describe the Australian/New Zealand best practice guidelines developed by the Southern eDNA Society (SeDNAs) that aim to provide guidance for the development of standard operating procedures (SOPs), including robust eDNA assay development across broad applications (e.g., biosecurity and biodiversity) and across biomes (e.g., terrestrial, marine, and freshwater). Importantly, the guidelines do not make assumptions on the state of the DNA sampled (extracellular or intracellular, tissue fragments, gametes, etc.) and are also not restricted to any specific group of organisms. Our intention is to provide a broad outline of what constitutes current best practice rather than to provide highly prescriptive protocols or standards. This will ensure a consistent approach to the application of eDNA/eRNA testing in Australia/New Zealand, while still allowing practitioners flexibility to tailor their methods so they are appropriate to meet the aims of their work. These guidelines will also increase confidence of the wider community in eDNA technology, given the guidelines are not a static document and will be regularly updated to incorporate new developments in this rapidly advancing field.

2 | GUIDELINE DEVELOPMENT

The Australian/New Zealand best practice guidelines for the use of eDNA/eRNA in biomonitoring were funded by the Australian Government Department of Agriculture, Fisheries and Forestry and developed as a strongly collaborative, community-driven document. Throughout the various design phases, the guidelines received input and reviews from eDNA researchers, government agencies, service providers, and other end-users. The published guidelines represent the efforts of a considerable segment of the Australian and New Zealand eDNA communities.

Informal discussions regarding the design of eDNA standards were started in 2020 by a small group of researchers as part of the development of an Australian–New Zealand eDNA association. The SeDNAs was established in 2021 and included a Standards and Best Practices Committee aiming to design best practice standards for the use of eDNA. Initial meetings with this group and international researchers were used as a base for the development of

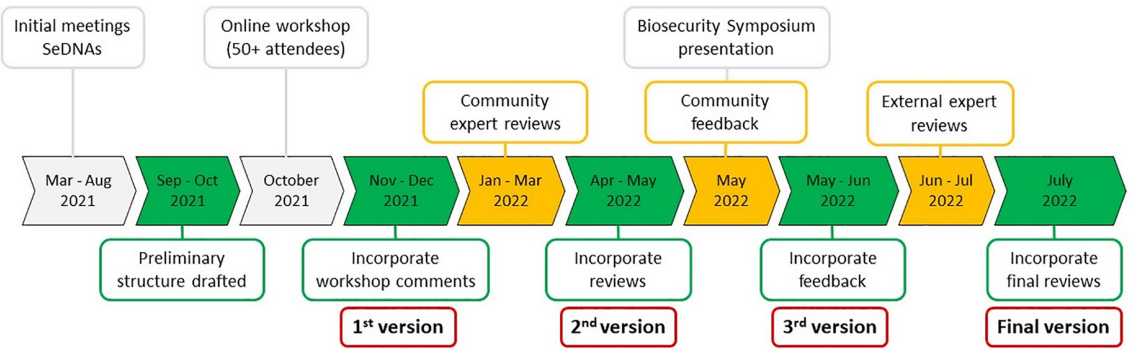

FIGURE 1 Timeline of key stages in the development of the eDNA protocol development guide for biomonitoring and the eDNA test validation guidelines. SeDNAs, Southern eDNA Society.

the guidelines presented here (Figure 1). A 2-day online workshop open to the Aus/NZ eDNA community (attended by 53 participants) was held in October 2021 to shape the content of the guidelines (Figure 1). Community feedback from this workshop was then used by authors Alejandro Trujillo-González and Maarten De Brauwer to design the first draft of the guidelines. This draft was opened for public review for 3 months, after which reviews were incorporated into a second draft. The second draft was presented at both the Australian Biosecurity Symposium and a free, public webinar to elicit a second round of community feedback (Figure 1). Feedback was integrated into a third version of the guidelines. This third version was sent for a final review by four external experts based in Australia/New Zealand. Expert reviews were addressed and integrated into the final version of the guidelines which is now available open access (Figure 1).

We acknowledge that the current version of the guidelines will need to be updated regularly to stay aligned with new developments in the field. As such, we anticipate that the guidelines will be updated and expanded over time as required.

3 | GUIDELINES SUMMARY

The best practice guidelines for environmental DNA/RNA biomonitoring in Australia and New Zealand consist of two separate documents, each with a different focus, but intended to be used in parallel (Table 1). Both the "Environmental DNA protocol development guide for biomonitoring" (*EP guide for biomonitoring*) and the "Environmental DNA test validation guidelines" (*eDNA test validation guidelines*) are published as open access documents and are freely available for download online at the SeDNAs (https://sedna society.com/publications) and at the Australian National Reference Centre (https://www.ecodna.org.au/national-edna-reference-centre-nrc). While focused on eDNA, both documents also provide guidelines for the use of eRNA and the development of eRNA assays.

It is important to note that the goal of both documents is to provide guidance on what are currently considered best practices in a fast-moving field. The guidelines are not intended to be restrictive for the research and development that drives the field forward. Indeed, we acknowledge that research pushing the boundaries of established techniques is essential to advance methods and for a better understanding of what constitutes best practice.

3.1 | Environmental DNA protocol development guide for biomonitoring

The *EP guide for biomonitoring* provides a comprehensive guide to creating SOPs for eDNA/eRNA projects. As such, it is of use to both end-users and researchers. For molecular researchers, the guidelines provide general principles and considerations to guide project development, and information on other important project areas such as communication and ethics. For end-users or clients, the guidelines can provide quality assurance for contracted eDNA/eRNA work by explaining which services and standards can be expected or to help inform staff involved in different phases of an eDNA/eRNA project.

The *EP guide for biomonitoring* covers both single-species (e.g., qPCR) and multi-species (metabarcoding) projects. The document can provide guidance for a range of potential eDNA-based applications such as biosecurity screening, government-mandated monitoring, and environmental assessments, through to research projects, consulting surveys, and citizen science projects.

TABLE 1 Summary of the Australian/New Zealand best practice guidelines documents.

Best practice guidelines for eDNA biomonitoring in Australia and New Zealand		
Document	Environmental DNA protocol development guide for biomonitoring	Environmental DNA test validation guidelines
Aim	Harmonized quality control and minimum standard considerations for creating eDNA/eRNA project SOPs	Harmonized quality control and minimum standard considerations for developing or validating eDNA/eRNA assays for the purpose of single-species or multi-species detection
Audience	Researchers and end-users, particularly at the design phase of a project	Researchers and (for technical questions) end-users, focusing on technical content
Content	Introduction	Introduction
	Principles for conducting an eDNA project	Assay purpose and selection
	Environmental DNA/RNA test protocols	Species-specific assay development and validation
		metabarcoding assay development and validation
	Resources	Resources

The content of the *EP guide for biomonitoring* is divided into two main sections (Table 1). The first section provides general principles for conducting eDNA/eRNA projects, the second section focuses on specific considerations at different project stages. In the first section, "Principles for conducting an eDNA project," we introduce general principles that should be considered when using eDNA-based methods for biomonitoring in Australia or New Zealand. These principles are:

1. *Ensure processes are fit for purpose*: considerations at the design phase of the project
2. *Test and validate processes*: addressed in detail in the "Environmental DNA test validation guidelines" document
3. *Ensure good chain of custody and documentation*: the importance of metadata
4. *Understand the limitations of results*: considerations when interpreting eDNA results
5. *Ensure good communication*: considerations to support best practices with clear communication
6. *Recognize First Nations Peoples' ownership and stewardship*: considerations on including and acknowledging First Nations Peoples in eDNA projects

The second section, "Environmental DNA/RNA test protocols," outlines important considerations for quality assurance and reliable implementation of an eDNA/eRNA project. The protocols encompass the entire workflow and offer users guidelines for each stage, with appropriate controls and measures for independence. These protocols can be used as templates to develop SOPs for specific projects and purposes.

3.2 | Environmental DNA test validation guidelines

The *eDNA test validation guidelines* provide harmonized quality control and minimum standard considerations for developing or validating eDNA/eRNA assays for the purpose of single-species or multi-species detection. eDNA-based methods exist for a broad variety of organisms; however, assay performance has only been superficially tested for most available assays, and few assays are validated to ensure reproducibility (Thalinger et al., 2021). The *eDNA test validation guidelines* offer a guide for the development and use of eDNA and eRNA assays and aim to ensure that surveillance and resource managers are provided with robust scientific evidence to support decision-making. Although these guidelines will help improve the accuracy and reliability of eDNA assays, they were not explicitly designed to provide results for use in compliance and legal situations.

As the *eDNA test validation guidelines* detail key steps to be used in assay development and validation, the content of this document is inherently more technical than the *EP guide for biomonitoring*. The guidelines are therefore likely to be of more direct use to molecular scientists than to end-users. They do, however, provide end-users with quality assurance for operational use of eDNA-based work in Australia and New Zealand.

The *eDNA test validation guidelines* cover information for both single-species (qPCR) and multi-species (metabarcoding) assays. They can provide guidance for potential eDNA-based applications ranging from biosecurity screening and government-mandated monitoring, to environmental assessments, research projects, and consulting surveys.

The *eDNA test validation guidelines* are structured into three sections (Table 1). The first section, "Assay purpose and selection," provides a brief overview of potential purposes for the development of assays. The second and third sections ("Species-specific assay development and validation" and "Metabarcoding assay development and validation," respectively) provide more detail about the development and validation of species-specific and multi-species assays, respectively.

4 | CONCLUSION

As global change accelerates, environmental stressors are reshaping our natural world. Natural resource managers are seeking new ways to tackle important questions about the impacts of change on biodiversity. Molecular toolkits in the form of eDNA and eRNA analyses are increasingly being recognized as providing capacity to address these questions in a cost-efficient manner at speed and scale. However, key considerations surrounding each of these data types are critical, and these should be established at the initial project planning stage.

These key considerations have been scoped by an expert group with the remit to establish guidelines to ensure delivery of the highest quality data for biodiversity management. We present those guidelines here: *EP guide for biomonitoring* and *eDNA test validation guidelines*, from, but not constrained by, an Australian and New Zealand perspective. These guidelines are perceived by our group (members of the Australian and New Zealand eDNA community) as providing a nonrestrictive framework for researchers, resource managers, and other stakeholders to follow to encourage best practice for eDNA and eRNA analyses. We envisage these as dynamic collaborative documents which will be refined and updated when needed to reflect future developments in the field. Our aspiration is that these best-practice guidelines will ensure environmental managers are provided with robust scientific evidence to support decision-making.

AUTHOR CONTRIBUTIONS

MDB and ATG wrote the initial structure of the manuscript, and all other authors contributed in writing and reviewing the final manuscript.

ACKNOWLEDGMENTS

AZ's contribution was supported by New Zealand Ministry of Business, Innovation and Employment funding (CAWX1904—A

toolbox to underpin and enable tomorrow's marine biosecurity system). We would like to acknowledge and thank all co-authors who helped develop and write the guidelines, as well as the 53 experts from across Australia and New Zealand who provided feedback during various stages of the process. The authors thank the four external expert reviewers of the guidelines for their valuable feedback. ATG and MDB would also like to thank Biotext for their services and Paula Doyle for her invaluable role as knowledge broker.

FUNDING INFORMATION

The development of the guidelines was funded by the Australian Government Department of Agriculture, Water and the Environment - now the Department of Agriculture, Fisheries and Forestry (C06421).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

No datasets were generated or analyzed during the current study.

ORCID

Maarten De Brauwer https://orcid.org/0000-0002-9592-979X
Laurence J. Clarke https://orcid.org/0000-0002-0844-4453
Anthony Chariton https://orcid.org/0000-0002-5809-3372
Madalyn K. Cooper https://orcid.org/0000-0003-3078-5534
Mark de Bruyn https://orcid.org/0000-0003-1528-9604
Elise Furlan https://orcid.org/0000-0002-1642-9819
Anna J. MacDonald https://orcid.org/0000-0003-2972-200X
Meaghan L. Rourke https://orcid.org/0000-0003-3709-359X
Leonie Suter https://orcid.org/0000-0002-6434-1766
Cecilia Villacorta-Rath https://orcid.org/0000-0002-1060-5447
Anastasija Zaiko https://orcid.org/0000-0003-4037-1861
Alejandro Trujillo-González https://orcid.org/0000-0002-6376-4978

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How to cite this article: De Brauwer, M., Clarke, L. J., Chariton, A., Cooper, M. K., de Bruyn, M., Furlan, E., MacDonald, A. J., Rourke, M. L., Sherman, C. D. H., Suter, L., Villacorta-Rath, C., Zaiko, A., & Trujillo-González, A. (2023). Best practice guidelines for environmental DNA biomonitoring in Australia and New Zealand. *Environmental DNA*, *00*, 1–7. https://doi.org/10.1002/edn3.395