

Considerations for future environmental DNA accreditation and proficiency testing schemes

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Funding information

National Environmental Science Programme (NESP), Northern Australia Environmental Resources Hub; Australian Government Department of Agriculture, Water and the Environment, Grant/Award Number: Biosecurity Innovation program ID 182020

Abstract

Environmental DNA (eDNA) monitoring has revolutionized the way biodiversity is surveyed and has been proposed as a reliable method to inform management decisions. Recognition of eDNA techniques as reliable tools to inform management and biosecurity require stringent standards to assess sample quality and reliability of results. Laboratories can have their workflows assessed and certified through accreditation and be involved in proficiency testing schemes provided to test the accuracy and precision of molecular methods. Currently, there is only one eDNA-based proficiency testing scheme designed to test competency of laboratories in amplifying eDNA from the Great Crested Newt, *Triturus cristatus* (Laurenti, 1768) in water samples. This test, however, is a closed scheme currently run by invitation only to laboratories in the United Kingdom. Given the paucity of eDNA-based proficiency schemes to ensure high-quality services, this commentary discusses how future proficiency testing schemes could be designed to assess eDNA sample quality and reliability on detection results for environmental management and biosecurity applications. We discuss the use of tissue-derived and synthetic oligonucleotides as reference materials, the need for proficiency testing schemes to assess the capacity of analytical facilities to determine sample quality as well as accurately detecting trace eDNA in blind samples and discuss the context in which fit for purpose eDNA testing schemes could be designed. To complement the future development of eDNA proficiency testing schemes, we provide firsthand accounts and lessons learned while developing the current Great Crested Newt eDNA proficiency scheme. Lastly, we highlight current limitations in standardizing rapidly improving eDNA-based techniques and discuss disadvantages to accreditation and standardization from an Australian-centered perspective as a means to promote an active debate on the topic of future eDNA accreditation and proficiency testing.

KEYWORDS

accreditation, International standard, International Organization for Accreditation, monitoring, surveillance

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1 | INTRODUCTION

Environmental DNA (eDNA) monitoring has revolutionized the way biodiversity is surveyed (Ficetola et al., 2008; Goldberg et al., 2014, 2016; Harper et al., 2019; Rees et al., 2014, 2015). A myriad of eDNA protocols and techniques have been developed to improve eDNA isolation from environmental samples, greatly diversifying eDNA applications in species surveillance (Tsuiji et al., 2019). Detection of DNA from targeted species in environmental samples (e.g., water, soil, and air) has provided great insight on the occurrence of rare or endangered species (Bylemans et al., 2017; Jerde et al., 2011; Laramie et al., 2014; Lintermans, 2016), allowed for early detection of invasive species (Bylemans et al., 2016; Piaggio et al., 2014) and improved biodiversity estimates and surveillance (Barnes & Turner, 2016; Jerde et al., 2011; Minamoto et al., 2012; Thomsen et al., 2012). Environmental DNA techniques are being increasingly advertised as promising tools to inform environmental management (Bruce, 2018; Ji et al., 2013; Kelly et al., 2014; Zaiko et al., 2018). Multiple studies have demonstrated the utility of eDNA-based monitoring of rare and invasive species, wherein eDNA detection often preceded visual detections of targeted species (Darling & Mahon, 2011; Jerde, 2019; White et al., 2020). Indeed, the question is no longer if the techniques work, but rather if reproducibility can be achieved to inform management decisions (Cristescu & Hebert, 2018; Harper et al., 2019; Zaiko et al., 2018).

Studies have considered the benefits and limitations of eDNA workflows (i.e., a series of steps, which include eDNA capture, preservation, extraction, amplification, sequencing, and analysis) to improve the reliability of eDNA methods (Cristescu & Hebert, 2018; Ficetola et al., 2015; Goldberg et al., 2016; Zaiko et al., 2018). Workflows must be designed to reduce the proliferation of false positive (Trujillo-González et al., 2020) and false-negative results (Furlan & Gleeson, 2016; Zaiko et al., 2018), ensure efficiency at each step of the eDNA workflow to improve recovery of DNA (Hinlo et al., 2017; Murray et al., 2015; Pilliod et al., 2014), and improve the sensitivity of eDNA analyses (Cristescu & Hebert, 2018; Goldberg et al., 2016). Most importantly, eDNA methods were recently reviewed within a legal framework to inform invasive species management, suggesting that the implementation of eDNA methods in decision making requires molecular best practices that integrate temporal and spatial trends in eDNA-based results relative to human risk tolerance (Sepulveda et al., 2020).

Stringent and highly scrutinized workflows are needed to deliver consistent and repeatable molecular results that can reliably inform management. For this purpose, laboratories can have their workflows assessed and certified through accreditation following guidelines of the International Organization for Accreditation (ISO; www.iso.org) and the International Electrotechnical Commission (IEC; www.iec.ch), which form the specialized system for worldwide standardization and are recognized by regulating entities globally. Recently, facilities of Bureau Veritas based in Guelph, Canada, obtained ISO/IEC 17025 accreditation for environmental DNA testing, becoming the first laboratory to formally receive international

accreditation to undertake eDNA-based testing and calibration services with 28 quantitative polymerase chain reaction (qPCR)-based eDNA accredited assays (Bureau Veritas, 2020). However, there are currently no publicly registered producers of certified reference materials (ISO 17034 accreditation) or proficiency testing scheme providers (ISO/IEC 17043 accreditation) that can assess the competence and reliability of eDNA-based workflows.

Assessing the competency of laboratories and their eDNA-based molecular methods will be a key component for widespread incorporation of eDNA-based applications in management. Given the importance of the topic and the need for active debate and deliberation (Loeza-Quintana et al., 2020; Tang et al., 2018), we provide an Australian-based perspective on the topic of species-specific eDNA proficiency testing. In doing so, we base our perspective and recommendations using guidelines from the International Organisation for Standardisation (ISO) and Principles of Good Laboratory Practice (GLP) from the Organisation for Economic Co-operation and Development (OECD, 1997) as recognized by the Australian National Association of Testing Authorities (NATA, www.nata.com.au). In addition, we use the "Technical advice note for field and laboratory sampling of great crested newt (*Triturus cristatus*) environmental DNA" (Biggs et al., 2014) from the food, water, and environmental Proficiency Testing Scheme Fapas® from Fera Science Ltd in the United Kingdom (test WC1067, DEFRA, 2019) as a framework for future eDNA-based proficiency testing schemes. This commentary offers the perspective of leading eDNA experts and laboratories across Australia and abroad, providing a unified vision of value to the international debate and application of eDNA proficiency testing schemes.

2 | ISO/IEC 17025 ACCREDITED FACILITIES UNDERTAKING EDNA-BASED SERVICES

The ISO/IEC 17025 Accreditation is a common requirement requested by regulating entities worldwide for laboratories providing standard tests and calibration services (ISO, 2019c). It is important to consider that the activities for which accreditation is granted do not necessarily comprise all activities the facility performs. As such, accredited facilities may have methods and techniques complementary to eDNA-based methods, but this does not necessarily indicate competency in eDNA techniques. Beyond following good-practice protocols and training, accredited laboratories undertaking eDNA-based testing services should ensure sample quality is suitable for eDNA testing and assays have been optimized for eDNA-based testing (i.e., target short gene fragments and can detect low-copy DNA; see also Thalinger et al., 2021), and minimize the risk of contamination from post-PCR products which can greatly affect the quality and certainty of eDNA results (Lahoz-Monfort et al., 2015; Zaiko et al., 2018).

Sample quality control is a crucial component of molecular testing to ensure reliable results regardless of the technique. In the case of eDNA, quality control involves a wide range of best-practice

protocols to minimize false-negative results (Furlan & Gleeson, 2016) while using a sampling method complementary to the environmental conditions and the abundance of targeted organisms (Goldberg et al., 2016; Taberlet et al., 2018). Although accredited facilities can indeed provide consultation prior to sample collection or offer eDNA services that include sample collection; ensuring that users collect eDNA using a suitable method may well be outside the scope of fee-for-service testing by accredited facilities. Nonetheless, accredited facilities would as part of their ISO/IEC 17025 accreditation have quality control standard operating procedures (SOPs) applicable to other molecular services that are complementary and suitable to eDNA-based testing. Specifically, quality control for the purpose of eDNA would test for degradation and inhibition, two common factors present in eDNA that could compromise amplification (Schrader et al., 2012) (Figure 1). Ideally, laboratories should have physically separate working areas designated specifically for pre-PCR and PCR procedures (Figure 1). The PCR working area should strictly be used to amplify pre-PCR preparations and should employ a strict unidirectional workflow, wherein movement of users is restricted to minimize cross-contamination (e.g., movement from pre-PCR to post-PCR only, dedicated consumables and personal protective equipment for each working space). Tubes or plates that have undergone amplification in the post-PCR room should under no circumstances be opened or introduced in the pre-PCR working area (Figure 1).

3 | ENVIRONMENTAL DNA STANDARDS

The steps that lead to eDNA analysis are crucial for a comprehensive framework of quality assurance and the confidence users have in eDNA methods in an operational context. Species detection is contingent upon detection probability during eDNA sampling (Ficetola et al., 2015; Schmidt et al., 2013; Schultz & Lance, 2015), which is a complex interplay between eDNA shedding, decay, transport, and resuspension in the system (Sansom & Sassoubre, 2017). Sampling methodologies must consider environmental factors that dilute, transport (e.g., water flow), and degrade (e.g., temperature, pH, bacterial activity) eDNA, as well as factors affecting sampling accessibility (Goldberg et al., 2016; Harper et al., 2019; Robson et al., 2016). As such, standardizing eDNA workflows may not be appropriate across habitat types as ecosystems vary considerably in their biological, physical, and chemical properties (Harper et al., 2019). However, it is paramount to establish clean and consistent field collection protocols that minimize contamination (Goldberg et al., 2016) and improve sampling efficiency of standard collection methods (Loeza-Quintana et al., 2020; Thomas et al., 2018). For example, the Smith-Root ANDe™ system is an integrated eDNA collection equipment for eDNA that allows trained users to collect water samples following a standardized collection program (i.e., pump pressure, flow rate, filter-pore size, sample volume; Thomas et al., 2018). Integrated build for purpose collection technologies would improve

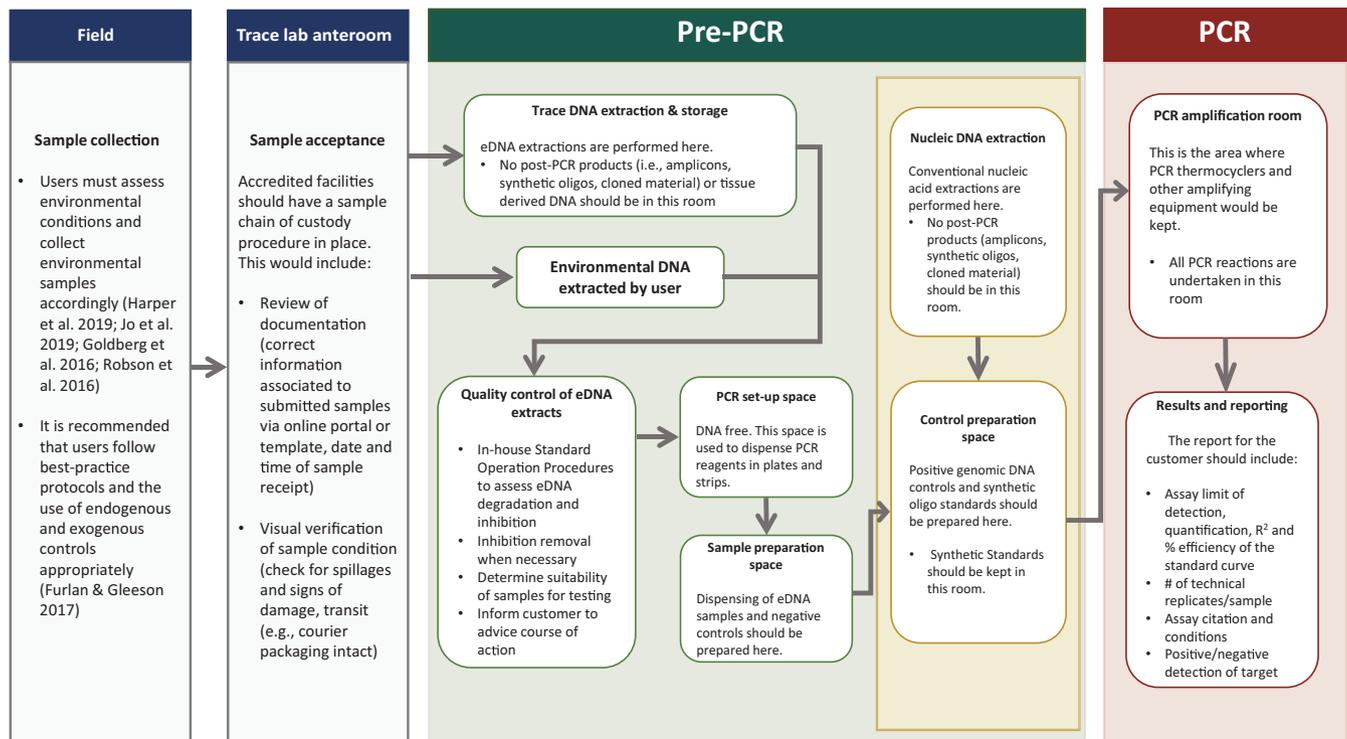


FIGURE 1 Environmental DNA one-directional workflow designed to prevent cross-contamination of genomic DNA and PCR products with trace eDNA samples. Three laboratory spaces are recommended for eDNA testing: one room or separate laboratory for eDNA extraction and qPCR setup (green); one space or separate laboratory for nucleic acid extraction and synthetic oligo handling (yellow); and one separate area for PCR where thermocyclers are located and qPCR product is handled (red)

the standardization of eDNA collection protocols fine-tuned for specific environments (Loeza-Quintana et al., 2020).

Multiple efforts to achieve standardization of eDNA methods are well underway (Loeza-Quintana et al., 2020; European Cooperation in Science and Technology, 2016). For example, the European Cooperation in Science and Technology (EU COST) developed DNAqua-net (www.dnaqua.net), a project created to standardize DNA-based approaches for integration into the Water Framework Directive of the European Union (Directive 2000/60/EC, European Parliament, 2000; see also Leese et al., 2018), the Canadian Standards Association is currently assessing the need for standardized eDNA surveillance protocols (Helbing & Hobbs, 2019), and the European Standards Organisation has a dedicated technical committee for DNA and eDNA methods (CEN/TC 230/WG 28 - DNA and eDNA methods). Similarly, the Government of the United Kingdom commissioned the development of protocols and regulations to provide eDNA proficiency tests for accredited facilities at the national level (Tang et al., 2018), and the British Columbia Ministry of environmental provides a standardized eDNA protocol for freshwater aquatic ecosystems (British Columbia Ministry of Environment (BCME), 2017). Lastly, various international workshops have provided essential platforms for researchers, stakeholders, companies, and government officials to discuss the advancement of eDNA standardization (Loeza-Quintana et al., 2020). Outcomes from these workshops suggest that communication and networking between trailblazing experts and companies in the field of eDNA is essential to develop platforms that facilitate the development of eDNA standardized techniques (Loeza-Quintana et al., 2020), as well as reporting of results, while addressing current limitations affecting eDNA standardization (Tang et al., 2018).

4 | ENVIRONMENTAL DNA PROFICIENCY TESTING

Current ISO proficiency scheme guidelines indicate that accredited laboratories must enroll and demonstrate satisfactory performance in proficiency testing programs for all tests conducted in the laboratory and for which a suitable program is available. Where no such program is available, the laboratory must demonstrate suitable validation and control procedures for the test method. The food, water, and environmental Proficiency Testing Scheme Fapas® from Fera Science Ltd in the United Kingdom (www.fapas.com) offers proficiency testing for the great crested newt (GCN) in the UK, in which laboratories can validate their eDNA techniques to detect *Triturus cristatus* (Laurenti, 1768) eDNA in water samples (test WC1067, DEFRA, 2019). Briefly, the test requires that involved facilities determine the presence of *T. cristatus* eDNA in a total of ten blind samples. The samples are randomly selected from four categories: negative samples, inhibited samples, low-level GCN samples, or high-level GCN samples. Although multiple laboratories offering GCN eDNA services in the UK advertise the results of this proficiency testing scheme, technical details of the test itself are not publicly advertised

in the Fapas catalogue of proficiency tests (www.fapas.com) or in the European Proficiency Testing Information System (EPTIS, 2020). Instead, it is a closed scheme currently run by invitation only to laboratories engaged with Natural England. This proficiency tests highlight four important aspects that must be debated for the benefit of future proficiency testing scheme providers.

Firstly, should reference material be collected or created for the purpose of eDNA proficiency testing? Future reference material for the purpose of proficiency testing can be designed by ISO 17034 accredited companies (ISO, 2019d) to be fit-for-purpose. That been said, most proficiency testing scheme providers do not use ISO 17034 for their production of materials, and rather use in-house standardized materials. In the case of the GCN test, reference material is collected by swabbing live *T. cristatus* specimens (Biggs et al., 2014); however, the authors indicate that for the purpose of standardization, future efforts to create eDNA reference material should explore the utility of synthetic oligo standards (Biggs et al., 2014). Indeed, multiple laboratories currently use synthetic oligonucleotide standards to accurately quantify DNA copy number and constitute important sample collection and laboratory-based cross-contamination controls in eDNA-based detection methods (Bylemans et al., 2017; Furlan et al., 2015; Wilson et al., 2016). Although collecting reference material from live or recently deceased specimens is a common practice (e.g., standardized Global Biological Index PTS IBGN:82 in EPTIS 2020, Great Crested Newt- WC1067 in DEFRA, 2019, and Wildlife Genetics Proficiency Testing Program -Test # 021815 in SWFS 2015), using synthetic oligo standards within the context of eDNA proficiency testing would allow providers to assess: (1) the presence of cross-contamination between samples; (2) determine the limits of detection achieved by each involved participant following consistent methods (see Klymus et al., 2020;) in detecting trace amounts of eDNA; and (3) enforce minimal standards in detecting synthetic eDNA matrices by testing extraction and inhibition proficiency.

Secondly, should proficiency testing assess the capacity of analytical facilities to determine sample quality as well as accurately detecting trace eDNA in blind samples? As stated before, degradation and inhibition are two common factors that could compromise eDNA amplification that should be identified prior to testing through quality control. Accredited and non-accredited facilities inherently have quality control protocols regardless of the technique; however, given the impact that degradation and inhibition have on eDNA-based analyses, future schemes should test quality control measures as part of proficiency assessments (Figure 2). Environmental DNA samples can be a complex mixture of DNA from multiple sources (prokaryotes and eukaryotes) containing DNA templates that are differentially degraded. Therefore, DNA degradation of the template of interest (i.e., species-specific assay) should be investigated. Examining the A260/A280 wavelength ratio of samples via spectrophotometry, followed by standard agarose gel visualization can provide an overall assessment of the template degradation in an eDNA extract. Similarly, a qPCR assessment that targets different fragment lengths of the gene region of interest developed for the species-specific assay can provide a thorough investigation of target

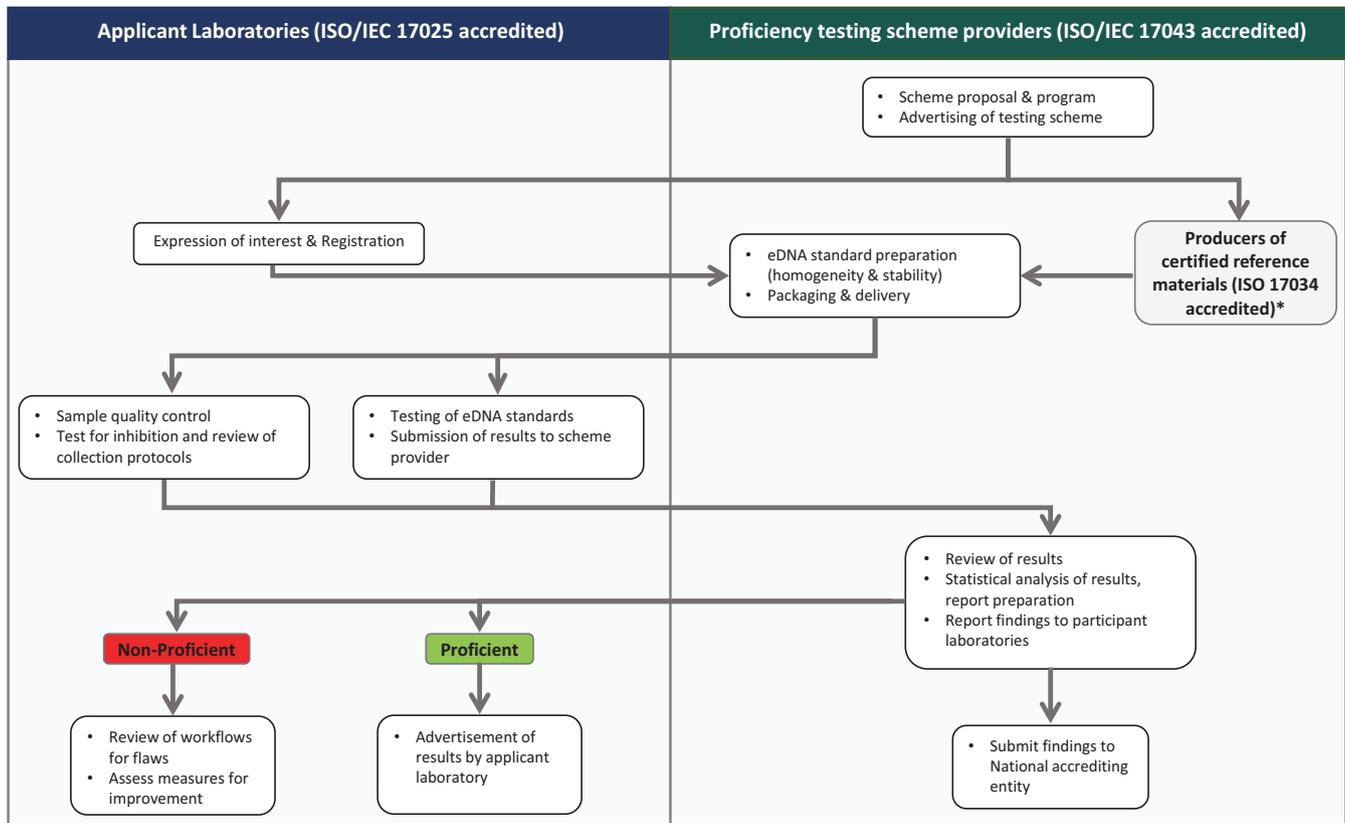


FIGURE 2 Proficiency testing scheme flowchart. *Proficiency testing scheme providers may alternatively develop in-house standardized genomic material as part of their proficiency testing schemes

DNA template degradation (Deagle et al., 2006). Inhibition, in conjunction with assessment of degradation, can be accessed via qPCR with SYBR-based PCR chemistry on a real-time PCR instrument with a dilution series of the eDNA extract with a minimum of two dilution points (i.e., neat and 1:10) of the submitted samples. Evaluation of the cycle threshold (C_T) values, where eDNA extracts free of inhibition should behave quantitatively (i.e., 3.3 C_T shift between dilution points), would show inhibited samples having delayed amplification (Bylemans et al., 2017; Murray et al., 2015; White et al., 2020). In the same way, the use of internal positive controls can also assess delayed amplification (Conte et al., 2018; Furlan et al., 2015; Trujillo-González et al., 2020). Some commercial DNA polymerase master mixes contain substances that can effectively counteract most qPCR inhibition from eDNA extracts (tested by Kreuzer et al., 2000 and more recently by Jane et al., 2015) and their use by accredited laboratories should be enforced. Moreover, if inhibition was detected in eDNA extracts, an inhibitor removal step or a dilution of the sample should be carried out in order to avoid false-negative detections (Schrader et al., 2012). Including inhibition testing and removal as quality control in eDNA proficiency testing would also assess the capacity of analytical facilities to determine sample suitability before testing, informing customers on the possibility of false-negative detections (Furlan & Gleeson 2017) and how that risk was minimized.

Thirdly, in what contexts should eDNA testing schemes be designed fit for purpose? For example, the GCN proficiency test is based

on a species-specific validated assay and method designed for the purpose of improved surveillance of the great crested newt (DEFRA, 2019; Biggs et al., 2014). Great crested newts are a European protected species, and their eggs, breeding sites, and resting places are protected by law in the United Kingdom, where construction activities require a mitigation license from Natural England, which requires an environmental assessment using eDNA among other detection methods (Government of the United Kingdom, 2020). The context of this proficiency test is highly specific, with the premise of approving laboratories in the United Kingdom to offer eDNA-based services to assess the presence or absence of GCN. Future proficiency tests may well follow this same context and technical framework (test WC1067, DEFRA, 2019), targeting species of importance with a potential regulatory framework. In this context, stakeholders that would benefit from eDNA-based proficiency schemes would include governmental authorities as well as state and federal agencies requiring accreditation for the purpose of environmental testing and surveillance. Alternatively, proficiency testing schemes can be designed without the context of regulatory frameworks, offering a wider range of laboratories to achieve proficiency in eDNA testing as a method, rather than proficiency in detecting specific species. In this context, stakeholders would include private companies, primary industries, and non-regulatory requiring environmental surveys to assess biodiversity with high-quality assurance (see Thalinger et al., 2021), rather than ensuring law compliance. It is unclear if given the variable nature

of ecosystems and matrices from which eDNA can be collected, schemes could be designed to test proficiency in extracting methods; however, standardized extraction methods could well open opportunities for eDNA extraction proficiency schemes in the future.

Lastly, what considerations and quality assurance are needed for the inherent development of standard operating procedures in proficiency testing schemes? As eDNA-based monitoring continues to develop, more and more methods will be designed and tested to fill specific niches to sample, extract, and test eDNA. This so far has been a thriving aspect of eDNA-based research and has provided the basis to expand the application of the field (Tsuji et al., 2019). Within the context of proficiency testing schemes, however, methods will require standardization to ensure quality compliance and control. Such implication entails that proficiency testing scheme providers will mandate a selected Standard Operating Procedure (SOP) fit for the purpose of the testing scheme. There are three potential approaches under which SOPs could be developed or implemented within the context of proficiency testing: 1. countries may already have harmonized eDNA-based methods suitable for comparison across different testing facilities that can be implemented by scheme providers (such is the case of the GCN proficiency test; DEFRA, 2019), 2. accredited facilities could incorporate previously developed testing schemes as well as associated SOPs and use them for the purpose of testing eDNA-based proficiency rather than fit-for-purpose eDNA testing, or 3. providers could design SOPs with reproducible methodologies that encompass complete eDNA-based workflows (i.e., sample collection, eDNA extraction, amplification, and confirmation of detection) and, most importantly, that test the capacity of involved facilities in accurately detecting eDNA and maintain suitable quality assurance and compliance.

5 | LESSONS LEARNED FROM THE FAPAS EDNA PROFICIENCY TESTS

The Fapas eDNA GCN proficiency test was initiated in 2016–2017 at the request of a stakeholder group comprising Natural England and facilities offering a commercial eDNA GCN testing service. The specificity of this application meant that a closed scheme model (not openly publicized) was most appropriate to ensure full engagement of the stakeholders. This was the first experience in proficiency testing for the majority of participants and fewer than 10 laboratories in the UK were in a position to participate. The timing of the proficiency test was also critical, as surveillance activities in the UK for GCN are undertaken during their breeding season (March–July) and laboratories were keen to complete the proficiency test prior to this period, necessitating production of samples in simulated pond water, with or without coloration and a PCR inhibitor. Real pond water was avoided to ensure that heavily incurred eDNA and other matrix complications would not adversely affect the ability of laboratories to detect GCN eDNA. The proficiency test has been run annually since 2017, a more frequent proficiency test exercise was decided by the stakeholders to be unnecessary.

The proficiency test was designed to be qualitative (detect or not detect) but with sample preparation at nominally low, medium, or high eDNA levels. This was intended to provide a semi-quantitative aspect to the proficiency test and to address the requirement for 12 replicates to confirm a positive detection. It is important to note that the commercial service laboratories were not accredited to ISO 17025 for the eDNA method. The driver was the need for confidence in the eDNA method for surveillance under license compared to the traditional trapping method for confirming the presence of GCN in development sites.

The sampling was a question for debate at stakeholder meetings. Although all the commercial testing providers follow the Natural England sampling protocol, all differ in the sampling containers used. The first proficiency test used a standardized sampling tube for all participants which were filled from the bulk water samples at Fapas for distribution. Subsequent proficiency tests required that the participants send to Fapas their own sampling bottles, which complicates the preparation exercise and the packaging for returning the samples to each participant. Sampling tubes are pre-filled with ethanol to preserve the eDNA in transit. For courier transportation within the UK, this is not problematic but the ethanol content (60% per sample) would have implications for international shipping if such a proficiency test was to attract international participants.

The question is raised above about the availability of reference materials. A proficiency test is run at a single point in time and non-preserved bulk proficiency test samples might not be sufficiently stable for *ad hoc* use as a reference material. The more recent proficiency tests required participants to send to Fapas two sets of sample kits per sample type; one set to be used for the proficiency test, the other set to be retained by the participant for post-proficiency test troubleshooting or quality control purposes during the GCN surveillance season. In the absence of formal reference materials, this is the next-best solution to this question.

6 | ENVIRONMENTAL DNA REFERENCE CENTRE: AUSTRALIA

Current efforts to harmonize and better implement eDNA-based techniques to inform management rely heavily on science-based assessments supported by governmental entities (e.g., DNAqua-Net in Europe; <https://dnaqua.net>). The Australian Government, as the authorized Competent Authority, is establishing national eDNA testing capability for environmental and biosecurity risk management purposes. To achieve their goal, an appropriate and responsive governance system must be in place. One of the initiatives of the Australian national capability program is the establishment of a National eDNA Reference Centre and eDNA Collaborating Centre network.

The National eDNA Reference Centre will play a critical role as a key strategic partner to sustain national capability and critical competencies at the highest levels. For instance, it will function as a national center of expertise and to facilitate standardization of sampling and testing techniques relevant to the specified target

species, as well as designing and validating eDNA-based molecular assays of current and emerging pests/pathogens and target species of environmental and biosecurity concern. The eDNA Reference Centre, in collaboration with members of the eDNA Collaborating Centre network, will also develop, update, and evaluate new Standard Operating Procedures and National Environmental DNA Test Protocols for the sampling, identification, control, and exclusion testing of the specified target species. In doing so, the center will also provide and update guidelines on the collection, design, storage, and use of biological reference material. Lastly, the eDNA Reference Centre will provide scientific and technical training for public and private stakeholders and laboratory personnel in Australian and international partner laboratories, as well as designing, updating, and administering Proficiency Testing Schemes for species of national importance. Establishing the eDNA Reference Centre to better provide nationally responsible confirmatory testing services as required will also encourage research-based testing and dissemination of fit-for-purpose eDNA-based guidelines and best practice standards, while encouraging the participation of associated laboratories and organizations in scientific and technical studies.

7 | DISADVANTAGES TO STANDARDIZATION

There are several disadvantages to standardizing rapidly evolving techniques such as eDNA-based workflows. Approving a recognized international standard takes a considerable amount of time and expertise. International standards are normally carried out through ISO technical committees, which are appointed by the ISO Technical Management Board and involve specialized leading experts in the field and theory of specific subject areas (ISO, 2019a). The ISO/TC 147: water quality and ISO/TC 207: environmental management technical committees could be involved in developing eDNA international standards. Standard eDNA molecular methods could be developed by the ISO/TC 147/SC 5: biological methods sub-committee, while standard eDNA sampling methods could be developed by the ISO/TC 147/SC 6: sampling sub-committee within the ISO/TC 147 (ISO, 2019b). Ideally, ISO could create a technical committee for eDNA in collaboration with leading experts in eDNA; however, developing a standard usually takes at least 3 years, a timeframe in which the speed and development of eDNA-based methods and techniques may well surpass the quality of the standard. Similarly, the timeframe required to accredit an eDNA assay may render this approach prohibitive in certain circumstances (e.g., a new incursion requiring rapid emergency surveillance).

High costs can act as an additional deterrent preventing laboratories from seeking accreditation. In Australia, for example, NATA accreditation for ISO/IEC 17025 to a previously non-accredited facility has an estimated cost of AUD \$18,000.00, which includes the review of documentation by a NATA officer, an on-site advisory visit from NATA lead assessors, and an on-site initial assessment by NATA lead and technical assessors. NATA accreditation has an

assessment cycle of three years, which includes a surveillance visit at 18 months followed by a reassessment at 36 months (NATA, 2020a). In the same way, ISO 17043 accredited facilities require further accreditation for each newly accredited proficiency scheme, as well as maintaining routine equipment calibration and intermediary checks (NATA, 2020b). Costs needed to ensure compliance and proficiency may be beyond the scope of laboratories undertaking research.

Lastly, it is crucial for governmental authorities to understand the current limitations of eDNA-based testing before using the method to assess compliance or make decisions about environmental and biosecurity risk management in the national interest. Laboratories may well be ISO/IEC 17025 accredited and proficiency testing schemes can be designed as outlined in this commentary; however, false-negative detection in the field remains a limiting factor when targeting high priority species with low abundances (Furlan et al., 2019). Policy involving the use of eDNA requires an accurate assessment of how applicable and fit-for-purpose eDNA-based testing is in the required context. Not understanding the limitations of eDNA (see Furlan & Gleeson 2016; Furlan et al., 2019; Trujillo-González et al. 2020) before risk management policy and legislation is written may well hinder eDNA research rather than advancing the need for accreditation and standards.

8 | CONCLUSION

Environmental DNA proficiency testing schemes need context, standards, and an understanding of how eDNA-based assessments will frame reproducible eDNA methods in the future. We highlight the importance of quality control to assess inhibition and degradation in eDNA samples and how proficiency testing schemes could be designed to assess these measures as well as laboratory proficiency in reliably detecting eDNA. Proficient eDNA service providers could give private and governmental entities confidence in eDNA methods, allowing regulating entities to routinely ensure providers use technically feasible, precise, and repeatable eDNA standard methods.

ACKNOWLEDGMENTS

Dr Alejandro Trujillo-González was funded by the Biosecurity Innovation Program from the Australian Government Department of Agriculture, Water and the Environment. Dr Cecilia-Villacorta Rath was funded by the Australian Government's National Environmental Science Programme (NESP), Northern Australia Environmental Resources Hub. We thank Professor Stephen Sarre, Dr Susan Wagner, and Mr Matthew Jeromson for their constructive advice and valuable comments during planning stages of this commentary paper.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception of the study (ATG), writing of the manuscript (all authors).

DATA AVAILABILITY STATEMENT

There are no data associated with this commentary paper.

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REFERENCES

- Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, 17, 1–17. <https://doi.org/10.1007/s10592-015-0775-4>
- Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Griffiths, R. A., Foster, J., Wilkinson, J., Arnett, A., Williams, P., & Dunn, F. (2014). *Analytical and methodological development for improved surveillance of the Great Crested Newt. Defra Project WC1067*. Freshwater Habitats Trust.
- British Columbia Ministry of Environment. (2017). Environmental DNA Protocol for Freshwater Aquatic Ecosystems. Prep. Hemmera Envirochem Inc. Comp. Hobbs, J., Vincer, E., Goldberg, C. Version 2.2, Accessed. Retrieved from <https://www.hemmera.com/wp-content/uploads/2018/08/171115-eDNA-protocol-V2.2.pdf>
- Bruce, K. (2018). DNA metabarcoding of invertebrates to evaluate outcomes of ecological restoration. *Practice*, 99, 13–17.
- Bureau Veritas. (2020). Metals, Minerals & Environmental: Schedule of Services & Fees 2020. Environmental DNA, pg 55. Accessed 17 August 2020. Retrieved from https://commodities.bureauveritas.com/sites/g/files/zypfnx241/files/media/document/Metals%20Minerals%20and%20Environmental_2020_Fee_%20Schedule_MINING_CAD.pdf
- Bylemans, J., Furlan, E. M., Hardy, C. M., McGuffie, P., Lintermans, M., Gleeson, D. M., & Gilbert, M. (2017). An environmental DNA-based method for monitoring spawning activity: A case study, using the endangered Macquarie perch (*Macquaria australasica*). *Methods in Ecology and Evolution*, 8, 646–655. <https://doi.org/10.1111/2041-210X.12709>
- Bylemans, J., Furlan, E. M., Pearce, L., Daly, T., & Gleeson, D. M. (2016). Improving the containment of a freshwater invader using environmental DNA (eDNA) based monitoring. *Biological Invasions*, 18, 3081–3089. <https://doi.org/10.1007/s10530-016-1203-5>
- Conte, J., Potoczniak, M., & Tobe, S. (2018). Using synthetic oligonucleotides as standards in probe-based qPCR. *BioTechniques*, 64, 177–179. <https://doi.org/10.2144/btn-2018-2000>
- Cristescu, M. E., & Hebert, P. D. N. (2018). Uses and misuses of environmental DNA in biodiversity science and conservation. *Annual Review of Ecology, Evolution, and Systematics*, 49, 209–230. <https://doi.org/10.1146/annurev-ecolsys-110617-062306>
- Darling, J., & Mahon, A. (2011). From molecules to management: Adopting DNA-based methods for monitoring biological invasions in aquatic environments. *Environmental Research*, 111, 978–988. <https://doi.org/10.1016/j.envres.2011.02.001>
- Deagle, B. E., Eveson, P. J., & Jarmon, S. N. (2006). Quantification of damage in DNA recovered from highly degraded samples – a case study of DNA in faeces. *Frontiers in Zoology*, 3, 11. <https://doi.org/10.1186/1742-9994-3-11>
- Analytical and methodological development for improved surveillance of the Great Crested Newt, and other pond vertebrates - WC1067. United Kingdom of Great Britain and Northern Ireland. Accessed 16 April 2019. Retrieved from <http://sciencesearch.defra.gov.uk/Default.aspx?Module=More&Location=None&ProjectID=18650>
- European Cooperation in Science and Technology (2016). CA15219 - *Developing new genetic tools for bioassessment of aquatic ecosystems in Europe*. Accessed 23 January 2020. Retrieved from <https://www.cost.eu/actions/CA15219/#tabs|Name:overview>
- European Proficiency Testing Information System (2020). *PT scheme database*. Accessed 2 October 2020. Retrieved from <http://www.eptis.org/index.htm>
- Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, 4, 423–425. <https://doi.org/10.1098/rsbl.2008.0118>
- Ficetola, G. F., Pansu, J., Bonin, A., Coissac, E., Giguët-Covex, C., De Barba, M., Gielly, L., Lopes, C. M., Boyer, F., Pompanon, F., Rayé, G., & Taberlet, P. (2015). Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Molecular Ecology Resources*, 15, 543–556. <https://doi.org/10.1111/1755-0998.12338>
- Furlan, E. M., & Gleeson, D. (2016). Improving reliability in environmental DNA detection surveys through enhanced quality control. *Marine and Freshwater Research*, 68, 388–395. <https://doi.org/10.1071/MF15349>
- Furlan, E. M., Gleeson, D., Hardy, C. M., & Duncan, R. P. (2015). A framework for estimating the sensitivity of eDNA surveys. *Molecular Ecology Resources*, 16, 641–654. <https://doi.org/10.1111/1755-0998.12483>
- Furlan, E. M., Gleeson, D., Wisniewski, C., Yick, J., & Duncan, R. P. (2019). eDNA surveys to detect species at very low densities: A case study of European carp eradication in Tasmania, Australia. *Journal of Applied Ecology*, 56, 2505–2517. <https://doi.org/10.1111/1365-2664.13485>
- Goldberg, C. S., Strickler, K. M., & Pilliod, D. S. (2014). Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. *Biological Conservation*, 183, 1–3. <https://doi.org/10.1016/j.biocon.2014.11.040>
- Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., & Gilbert, M. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, 7, 1299–1307. <https://doi.org/10.1111/2041-210X.12595>
- Government of the United Kingdom (2020). *Great crested newts: protection and licences*. Accessed 12 October 2020. Retrieved from <https://www.gov.uk/guidance/great-crested-newts-protection-surveys-and-licences>
- Harper, L. R., Buxton, A. S., Rees, H. C., Bruce, K., Brys, R., Halfmaerten, D., Read, D. S., Watson, H. V., Sayer, C. D., Jones, E. P., Priestley, V., Mächler, E., Múrria, C., Garcés-Pastor, S., Medupin, C., Burgess, K., Benson, G., Boonham, N., Griffiths, R. A., ... Hänfling, B. (2019). Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiologia*, 826, 25–41. <https://doi.org/10.1007/s10750-018-3750-5>
- Helbing, C. C., & Hobbs, J. (2019). *Environmental DNA standardization needs for fish and wildlife population assessments and monitoring*. CSA group; standard research report. Accessed 14 July 2021. Retrieved from
- Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. (2017). Methods to maximise recovery of environmental DNA from water samples. *PLoS One*, 12, e0179251. <https://doi.org/10.1371/journal.pone.0179251>
- International Organization for Standardization (2019). *ISO/IEC 17025:2017: General requirements for the competence of testing and calibration laboratories*. Accessed 10 April 2019. Retrieved from <https://www.iso.org/standard/66912.html>
- International Organization for Standardization (2019). *Technical committees*. Accessed 10 April 2019. Retrieved from <https://www.iso.org/technical-committees.html>

- International Organization for Standardization (2019). *How we develop standards*. Accessed 10 April 2019. Retrieved from <https://www.iso.org/developing-standards.html>
- International Organization for Standardization (2019). *ISO 17034:2016: General requirements for the competence of reference material producers*. Accessed 10 April 2019. Retrieved from <https://www.iso.org/standard/29357.html>
- Jane, S., Wilcox, T., McKelvey, K., Young, M., Schwartz, M., Lowe, W., Letcher, B., & Whiteley, A. (2015). Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology Resources*, 15, 216–227. <https://doi.org/10.1111/1755-0998.12285>.
- Jerde, C. L. (2019). Can we manage fisheries with the inherent uncertainty from eDNA? *Journal of Fish Biology*, 2019, 1–13. <https://doi.org/10.1111/jfb.14218>.
- Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, 4, 150–157. <https://doi.org/10.1111/j.1755-263X.2010.00158.x>.
- Ji, Y., Ashton, L., Pedley, S. M., Edwards, D. P., Tang, Y., Nakamura, A., Kitching, R., Dolman, P. M., Woodcock, P., Edwards, F. A., Larsen, T. H., Hsu, W. W., Benedick, S., Hamer, K. C., Wilcove, D. S., Bruce, C., Wang, X., Levi, T., Lott, M., ... Yu, D. W. (2013). Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecology Letters*, 16, 1245–1257. <https://doi.org/10.1111/ele.12162>.
- Kelly, R. P., Port, J. A., Yamahara, K. M., Martone, R. G., Lowell, N., Thomsen, P. F., & Crowder, L. B. (2014). Harnessing DNA to improve environmental management. *Science*, 344, 1455–1456. <https://doi.org/10.1126/science.1251156>.
- Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., Jackson, C. A., Lance, R. F., Mangan, A. M., Monroe, E. M., Piaggio, A. J., Stokdyk, J. P., Wilson, C. C., & Richter, C. A. (2020). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*, 2(3), 271–282. <https://doi.org/10.1002/edn3.29>.
- Kreuzer, K., Bohn, A., Lass, U., Peters, U., & Schmidt, C. (2000). Influence of DNA polymerases on quantitative PCR results using TaqMan™ probe format in the LightCycler™ instrument. *Molecular and Cellular Probes*, 14, 57–60. <https://doi.org/10.1006/mcpr.1999.0284>.
- Lahoz-Monfort, J. J., Guillera-Arroita, G., & Tingley, R. (2015). Statistical approaches to account for false-positive errors in environmental DNA samples. *Molecular Ecology Resources*, 16, 673–685. <https://doi.org/10.1111/1755-0998.12486>.
- Laramie, M. B., Pilliod, D. S., & Goldberg, C. S. (2014). Characterizing the distribution of an endangered salmonid using environmental DNA analysis. *Biological Conservation*, 183, 29–37. <https://doi.org/10.1016/j.biocon.2014.11.025>.
- Leese, F., Bouchez, A., & Altermatt, F. (2018). Why we need sustainable networks bridging countries, disciplines, cultures and generations for aquatic biomonitoring 2.0: A perspective derived from the DNAqua-Net COST action. *Advances in Ecological Research*, 58, 63–99.
- Lintermans, M. (2016). Finding the needle in the haystack: comparing sampling methods for detecting an endangered freshwater fish. *Marine and Freshwater Research*, 67, 1740–1749. <https://doi.org/10.1071/MF14346>.
- Loeza-Quintana, T., Abbott, C., Heath, D., Bernatchez, L., & Hanner, R. (2020). Pathway to increase standards and competency of eDNA surveys (PISCeS)—Advancing collaboration and standardization efforts in the field of eDNA. *Environmental DNA*, 2(3), 255–260. <https://doi.org/10.1002/edn3.112>.
- Minamoto, T., Yamanaka, H., Takahara, T., Honjo, M. N., & Kawabata, Z. (2012). Surveillance of fish species composition using environmental DNA. *Limnology*, 13, 193–197. <https://doi.org/10.1007/s10201-011-0362-4>.
- Murray, D. C., Coghlan, M. L., & Bunce, M. (2015). From Benchtop to Desktop: Important Considerations when Designing Amplicon Sequencing Workflows. *PLoS One*, 10(4), e0124671. <https://doi.org/10.1371/journal.pone.0124671>.
- National Association of Testing Authorities (2020). *NATA procedures for accreditation*. Accessed 8 September 2020. Retrieved from <https://www.nata.com.au/phocadownload/gen-nata-docs/NATA-procedures-for-accreditation.pdf>
- National Association of Testing Authorities (2020). *General Accreditation Criteria Proficiency Testing Scheme Providers*. Accessed 8 September 2020. <https://www.nata.com.au/phocadownload/gen-accreditation-criteria/ISO-IEC-17043-Standard-Application-Document.pdf>
- Organisation for Economic Co-operation and Development (1997). *OECD Principles of Good Laboratory Practice*. Accessed 18 September 2020. Retrieved from [http://www.oecd.org/officialdocuments/publicdisp/laydocumentpdf/?cote=env/mc/chem\(98\)17&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisp/laydocumentpdf/?cote=env/mc/chem(98)17&doclanguage=en)
- Parliament, E. (2000). Directive 2000/60/EC of the European parliament and of the council: Establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities*, L327, 1–72.
- Piaggio, A. J., Engeman, R. M., Hopken, M. W., Humphrey, J. S., Keacher, K. L., Bruce, W. E., & Avery, M. L. (2014). Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA. *Molecular Ecology Resources*, 14, 374–380. <https://doi.org/10.1111/1755-0998.12180>.
- Pilliod, D. S., Goldberg, C. S., Arkle, R. S., & Waits, L. P. (2014). Factors influencing detection of eDNA from a stream-dwelling amphibian. *Molecular Ecology Resources*, 14, 109–116. <https://doi.org/10.1111/1755-0998.12159>.
- Rees, H. C., Gough, K. C., Middleditch, D. J., Patmore, J. R. M., & Maddison, B. C. (2015). Applications and limitations of measuring environmental DNA as indicators of the presence of aquatic animals. *Journal of Applied Ecology*, 52, 827–831. <https://doi.org/10.1111/1365-2664.12467>.
- Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, 51, 1450–1459. <https://doi.org/10.1111/1365-2664.12306>.
- Robson, H., Noble, T., Saunders, R., Robson, S., Burrows, D., & Jerry, D. (2016). Fine-tuning for the tropics: application of eDNA technology for invasive fish detection in tropical freshwater ecosystems. *Molecular Ecology Resources*, 16, 922–932. <https://doi.org/10.1111/1755-0998.12505>.
- Sansom, B. J., & Sassoubre, L. M. (2017). Environmental DNA (eDNA) shedding and decay rates to model freshwater mussel eDNA transport in a river. *Environmental Science & Technology*, 51, 14244–14253. <https://doi.org/10.1021/acs.est.7b05199>.
- Schmidt, B. R., Kéry, M., Urusenbacher, S., Hyman, O. J., & Collins, J. P. (2013). Site occupancy models in the analysis of environmental DNA presence/absence surveys: a case study of an emerging amphibian pathogen. *Methods in Ecology and Evolution*, 4, 646–653. <https://doi.org/10.1111/2041-210X.12052>.
- Schrader, C., Schielke, A., Ellerbroek, L., & John, R. (2012). PCR inhibitors—occurrence, properties and removal. *Journal of Applied Microbiology*, 113(5), 1014–1026. <https://doi.org/10.1111/j.1365-2672.2012.05384.x>.
- Schultz, M. T., & Lance, R. F. (2015). Modelling the sensitivity of field surveys for detection of environmental DNA (eDNA). *PLoS One*, 10, e0141503. <https://doi.org/10.1371/journal.pone.0141503>.
- Sepulveda, A., Nelson, N., Jerde, C., & Luikart, G. (2020). Are environmental DNA methods ready for aquatic invasive species management? *Trends in Ecology & Evolution (Amsterdam)*, 35, 668–678. <https://doi.org/10.1016/j.tree.2020.03.011>.
- Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For biodiversity research and monitoring* (p. 253). Oxford University Press.

- Tang, C. Q., Crampton-Platt, A., Townend, S., Bruce, K., Bista, I., & Creer, S. (2018). *Development of DNA applications in Natural England 2016/2017*. Natural England Commissioned Reports. Number 252.
- Thalinger, B., Deiner, K., Harper, L. R., Rees, H. C., Blackman, R. C., Sint, D., Traugott, M., Goldberg, C. S., & Bruce, K. (2021). A validation scale to determine the readiness of environmental DNA assays for routine species monitoring. *Environmental DNA*, 00, 1–14. <https://doi.org/10.1002/edn3.189>.
- Thomas, A., Howard, J., Nguyen, P., Seimon, T., & Goldberg, C. (2018). ANDe™: A fully integrated environmental DNA sampling system. *Methods in Ecology and Evolution*, 9, 1379–1385. <https://doi.org/10.1111/2041-210X.12994>.
- Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., Orlando, L., & Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, 21, 2565–2573. <https://doi.org/10.1111/j.1365-294X.2011.05418.x>.
- Trujillo-González, A., Becker, J. A., Huerlimann, R., Saunders, R. J., & Hutson, K. S. (2020). Can environmental DNA be used for aquatic biosecurity in the aquarium fish trade? *Biological Invasions*, 22(3), 1011–1025. <https://doi.org/10.1007/s10530-019-02152-0>.
- Tsuji, S., Takahara, T., Doi, H., Shibata, N., & Yamanaka, H. (2019). The detection of aquatic macroorganisms using environmental DNA analysis—A review of methods for collection, extraction, and detection. *Environmental DNA*, 1, 99–108. <https://doi.org/10.1002/edn3.21>.
- White, N., Guzik, M., Austin, A., Moore, G., Humphreys, W., Alexander, J., & Bunce, M. (2020). Detection of the rare Australian endemic blind cave eel (*Ophisternon candidum*) with environmental DNA: implications for threatened species management in subterranean environments. *Hydrobiologia*, 847(15), 3201–3211. <https://doi.org/10.1007/s10750-020-04304-z>.
- Wilson, C. C., Wozney, K. M., & Smith, C. M. (2016). Recognizing false positives: synthetic oligonucleotide controls for environmental DNA surveillance. *Methods in Ecology and Evolution*, 2016(7), 23–29. <https://doi.org/10.1111/2041-210X.12452>.
- Zaiko, A., Pochon, X., Garcia-Vazquez, E., Olenin, S., & Wood, S. (2018). Advantages and Limitations of Environmental DNA/RNA Tools for Marine Biosecurity: Management and Surveillance of Non-indigenous Species. *Frontiers in Marine Science*, 5(322), 1–17. <https://doi.org/10.3389/fmars.2018.00322>.

How to cite this article: Trujillo-González, A., Villacorta-Rath, C., White, N. E., Furlan, E. M., Sykes, M., Gossel, G., Divi, U., & Gleeson, D. (2021). Considerations for future environmental DNA accreditation and proficiency testing schemes. *Environmental DNA*, 3, 1049–1058. <https://doi.org/10.1002/edn3.243>