



**Conference Abstract** 

# An assay validation framework to compare and evaluate targeted environmental DNA assays for routine species monitoring

Bettina Thalinger<sup>‡,§</sup>, Kristy Deiner<sup>I,¶</sup>, Lynsey Rebecca Harper<sup>#</sup>, Helen C Rees<sup>n</sup>, Rosetta Charlotte Blackman<sup>«,I</sup>, Daniela Sint<sup>§</sup>, Michael Traugott<sup>»</sup>, Caren S. Goldberg<sup>^</sup>, Kat Bruce<sup>×</sup>

‡ Centre for Biodiversity Genomics, University of Guelph, Guelph, Canada

§ Department of Zoology, University of Innsbruck, Innsbruck, Austria

- ¶ Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, United States of America
- # Liverpool John Moores University, Liverpool, United Kingdom
- ¤ RSK ADAS Ltd, School of Veterinary Medicine and Science, The University of Nottingham, Loughborough, United Kingdom
- « Eawag, Dübendorf, Switzerland
- » University of Innsbruck, Innsbruck, Austria
- ^ School of the Environment, Washington State University, Pullman, United States of America
- \* Nature Metrics Ltd, Egham, United Kingdom

Corresponding author: Bettina Thalinger (bettina.thalinger@gmail.com)

Received: 01 Feb 2021 | Published: 04 Mar 2021

Citation: Thalinger B, Deiner K, Harper LR, Rees HC, Blackman RC, Sint D, Traugott M, Goldberg CS, Bruce K (2021) An assay validation framework to compare and evaluate targeted environmental DNA assays for routine species monitoring. ARPHA Conference Abstracts 4: e63836. https://doi.org/10.3897/aca.4.e63836

#### Abstract

Environmental DNA (eDNA) analysis utilises trace DNA released by organisms into their environment for species detection and is revolutionising non - invasive species and biodiversity monitoring. However, this technology requires rigorous validation along the whole workflow – from field sampling to statistical analysis – to ensure appropriate and meaningful interpretation of results. Targeted eDNA assays are often validated within a specific system and with particular aims, but without fulfilling predefined criteria. Consequently, their applicability beyond initial development often remains undetermined. Additionally, there tends to be poor understanding of the uncertainties and limitations associated with already published assays and thus potentially inappropriate interpretation of the results they produce. The lack of a "gold standard" limits the incorporation of

© Thalinger B et al. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

<sup>|</sup> University of Zurich, Zurich, Switzerland

targeted eDNA assays into species monitoring and policy making by end-users and is therefore key for the future implementation of eDNA-based surveys.

Here, we present a framework (<u>https://edna-validation.com/</u>) and user-friendly criteria for the classification of assays, which is based on previous validation efforts. A 5 - level assay validation scale ("incomplete" to "operational") was defined by reviewing the current eDNA literature and conducting a meta-analysis on sampling, laboratory practices, detection limits, and detection probabilities. The so far published single species eDNA assays were reviewed for their performance in this new framework and we identified steps within the validation process that often remain untouched. Finally, we provide guidance for end - users as to which criteria are most important for validation and suggest how results obtained from assays at different levels of the validation scale should be interpreted.

### **Keywords**

assessment, species-specific, quantitative PCR, digital PCR, endpoint PCR, species detection

## Presenting author

**Bettina Thalinger** 

## Presented at

1st DNAQUA International Conference (March 9-11, 2021)