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Pathway to Increase Standards and Competency of eDNA Surveys (PISCeS)—Advancing collaboration and standardization efforts in the field of eDNA

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Abstract

This special issue features eleven articles presented at the Pathway to Increase Standards and Competency of eDNA Surveys (PISCeS) conference in October 2018. The prominent theme in this special issue is methodological developments to enhance species and community characterization using eDNA, covering tree main scientific trends: validation of eDNA detections, methodological innovations in eDNA research, and standardization of eDNA practices. Articles in this special issue cover advances on single-species detection and metabarcoding, and examine eDNA methods standardization. Strong collaboration among academics, regulators, and industry present in the eDNA community is evidenced by the research presented here.

KEYWORDS
eDNA detections validation, eDNA practices standardization, metabarcoding, methodological advances, single-species detection

1 | INTRODUCTION

Twelve years have passed since the first published study using environmental DNA (eDNA) for conservation purposes—detection of the invasive American bullfrog (*Lithobates catesbeianus*) in controlled and field conditions as proof of concept (Ficetola, Miaud, Pompanon, & Tab erlet, 2008). Since then, the number of published studies describing species presence/absence detections using eDNA analysis has been increasing at a rapid rate (Tsuji, Takahara, Doi, Shibata, & Yamanaka, 2019). eDNA analyses have been applied in diverse systems (including terrestrial, freshwater and marine environments) and to answer a variety of questions, from the detection of target organisms using quantitative PCR (qPCR) (e.g. Buxton, Groombridge, Zakaria, & Griffiths, 2017; Currier, Morris, Wilson, & Freeland, 2018; Davy, Kidd, & Wilson, 2015; Hunter et al., 2015;

Lacoursière-Roussel, Rosabal, & Bernatchez, 2016), droplet digital PCR (ddPCR) (e.g., Baker, Steel, Nieukirk, & Klinck, 2018; Doi et al., 2015; Evans, Shirey, Wieringa, Mahon, & Lamberti, 2017; Mauvisseau et al., 2019), and even gene editing methods (e.g., CRISPR-Cas, Williams et al., 2019) to community structure studies using metabarcoding with high-throughput sequencing (HTS) (e.g., Balasingham, Walter, Mandrak, & Heath, 2018; Günther, Knebelsberger, Neumann, Laakmann, & Martínez Arbizu, 2018; Kelly, Port, Yamahara, & Crowder, 2014; Leduc et al., 2019; Valentini et al., 2016).

Recently, the field of eDNA has gained more attention from conservationists, recognizing not only its benefits, but also remaining challenges. For instance, although many methodological approaches have been developed for collecting and processing eDNA samples (Thomas, Howard, Nguyen, Seimon, &

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Goldberg, 2018; Tsuji et al., 2019), improved understanding of the ecology of eDNA—its origin, state, transport, and fate (Barnes & Turner, 2016)—is needed to inform best practices of eDNA collection, analyses, and study design. Ultimately, a better understanding of eDNA ecology and developing best practices will aid to predict taxa presence/absence and distributions. Despite this and other challenges (e.g., PCR inhibition), the application of eDNA-based technology is already beginning to influence conservation and management decisions (e.g., Kelly, Port, Yamahara, Martone, et al., 2014). However, there is considerable work to be done before eDNA surveys can be fully accepted by policy makers. Only when eDNA surveys become broadly accepted by regulatory agencies will the industry sector justify directing budgets away from conventional field surveys. Hence, a continued collaboration between researchers, regulators, and other stakeholders is necessary to promote methods standardization and a framework to assess the quality of methods employed in order to support the acceptance of eDNA surveys (Helbing & Hobbs, 2019).

The analysis of eDNA has been the topic of numerous literature reviews (e.g., Barnes & Turner, 2016; Bohmann et al., 2014; Deiner et al., 2017; Díaz-Ferguson & Moyer, 2014; Rees, Maddison, Middleditch, Patmore, & Gough, 2014; Thomsen & Willerslev, 2015; Tsuji et al., 2019), special issues (Goldberg, Strickler, & Pilliod, 2015; Tab erlet, Coissac, Hajibabaei, & Rieseberg, 2012), and conferences (e.g., The national conference on marine environmental DNA 2018; Environmental DNA workshop 2019; Monitoring biodiversity using environmental DNA 2019). To support this recent momentum of eDNA science, Pathway to Increase Standards and Competency of eDNA Surveys (PISCeS) conference was organized in 2018 and held at University of Guelph, Canada, to advance the application of eDNA for conservation and management within a regulatory framework.

This special issue features eleven articles presented by academia, regulators, and industry at the PISCeS conference in October 2018. Methodological developments to enhance species and community characterization using eDNA is a prominent theme in this special issue. Three main scientific trends related to this theme described in detail below include validation of eDNA detections, methodological innovations in eDNA research, and standardization of eDNA practices. Most articles cover advances on single-species detection (Gasparini, Crookes, Prosser, & Hanner, 2020; Hernandez et al., 2020; Hobbs et al., 2020; Klymus et al., 2019; Laporte et al., 2020; Roux et al., 2020; Skinner, Murdoch, Loeza-Quintana, Crookes, & Hanner, 2020; Thomas et al., 2019) while two articles focus on eDNA metabarcoding for describing communities (Morey, Bartley, & Hanner, 2020; Mychek-Londer, Balasingham, & Heath, 2019), focusing on the detection of invasive species (Hernandez et al., 2020; Mychek-Londer et al., 2019; Roux et al., 2020; Thomas et al., 2019) and species-at-risk (Gasparini et al., 2020; Hernandez et al., 2020; Hobbs et al., 2020; Mychek-Londer et al., 2019; Skinner et al., 2020). This special issue examines eDNA methods standardization (Klymus et al., 2019; Nicholson et al., 2020; Roux et al., 2020) as they apply to different study environments including close-controlled laboratory studies (Morey et al., 2020; Skinner

et al., 2020; Thomas et al., 2019), freshwater (Gasparini et al., 2020; Hobbs et al., 2020; Laporte et al., 2020; Mychek-Londer et al., 2019; Nicholson et al., 2020; Thomas et al., 2019), and marine (Morey et al., 2020; Roux et al., 2020; Skinner et al., 2020) systems. Finally, the strong collaboration among academics, regulators, and industry that is present in the eDNA community is evidenced by the research in this special issue (Roux et al., 2020; Skinner et al., 2020; Thomas et al., 2019).

2 | VALIDATION OF EDNA DETECTIONS

A still-growing area of eDNA research is the proof of concept for targeted and metabarcoding analytical approaches for detecting specific species and whole communities, respectively. Gasparini et al. (2020) validated the utility of an eDNA qPCR assay to monitor the imperilled wavy-rayed lampmussel (*Lampsilis fasciola*). Using caged *L. fasciola* individuals placed in an area where the species was known to be absent, they evaluated eDNA detection rates at several downstream sampling distances. Their study demonstrated that unionid mussels could be successfully detected in lotic systems (Gasparini et al., 2020). However, the authors warn of reduced eDNA detection when species abundance is low and/or when sampling too far downstream. The authors also encourage the use of internal positive controls (IPC) to test for PCR inhibition and to avoid false-negative results due to the presence of PCR inhibitors in environmental samples. Similarly, Hobbs et al. (2020) demonstrated the advantage of eDNA surveys to characterize the distribution of the Rocky Mountain tailed frog (*Ascaphus montanus*) in British Columbia, Canada. The greater species detection probabilities achieved using eDNA compared with conventional visual surveying techniques resulted in expanding the known range of the species to include by newly inhabited streams. The streams have been recommended for designation as Wildlife Habitat Areas—species-specific area designations for habitat conservation—for mapping of critical habitat for conservation of the Rocky Mountain tailed frog in Canada. Their study also evaluated mitigating false negatives through testing for qPCR inhibition and sample degradation. Overall, the authors support the efficiency and effectiveness of eDNA surveys for monitoring aquatic amphibians (Hobbs et al., 2020). Hernandez et al. (2020) developed and validated 60 targeted qPCR assays to monitor invasive, threatened, or exploited aquatic species including fish, amphibians, reptiles, mollusks, and crustaceans. The success of eDNA-targeted species detection relies on the specificity of the assays and their sensitivity to amplify DNA from the species of interest while avoiding false positive amplification of nontarget taxa (Hernandez et al., 2020). The results of specificity testing suggest the assays can be broadly used in North America. This is of great value to conservation efforts as the target species are the focus of ongoing monitoring programs (Hernandez et al., 2020).

Additionally, Laporte et al. (2020) documented dispersion patterns of eDNA in the St. Lawrence River using caged Brown trout (*Salmo trutta*). The authors sampled 500m upstream and at several

downstream distances from the caged fish. Using a species-specific qPCR assay, their results revealed positive detection of the species only downstream and as far as 5,000m. To further investigate eDNA dispersion patterns, the authors used prediction models for quantification of 2D dispersion. They found that a model incorporating low lateral mixing and a downstream flow in direct line provided the best fit.

Two studies in this special issue validate the use of eDNA metabarcoding in mapping aquatic communities. Mychek-Londer et al. (2019) used eDNA metabarcoding to determine the spatial distributions of native species-at-risk and Aquatic Invasive Species (AIS) of invertebrates in two major Great Lakes tributaries. Using a universal set of primers for invertebrates targeting the COI gene, the authors identified species-at-risk in both sampled tributaries. The study also revealed several AIS previously unreported in the sampling locations. At many sampling sites, the presence of AIS eDNA overlaps with, or was near to, sites with detections of at-risk native species. The presence of AIS could have negative impacts on the native species and ecosystem. The results from this study highlight the analytical power of eDNA metabarcoding and its potential to direct conservation and management strategies (Mychek-Londer et al., 2019). eDNA metabarcoding of marine biodiversity was assessed by Morey et al. (2020) through a pilot study at Ripley's Aquarium in Toronto, Canada. Using previously published primer sets for COI, 12S, and 16S markers, their study only recovered around 50% of target species and 80% of target genera within the closed marine system when combining all three markers. The authors highlight important limitations and knowledge gaps that must be addressed before eDNA metabarcoding can be employed for monitoring diverse marine systems (Morey et al., 2020).

3 | METHODOLOGICAL ADVANCES IN EDNA RESEARCH

Methodological developments are a dominant trend in this special issue. Two contributions focus on the validation of methods for the rapid and effective detection of eDNA on-site using portable handheld qPCR devices (Skinner et al., 2020; Thomas et al., 2019). Thomas et al. (2019) presented a protocol for the rapid eDNA detection of AIS. Their protocol included the collection and processing of eDNA samples on-site using a backpack portable eDNA filtration system (eDNA-Sampler) combined with a handheld qPCR device (Biomeme) to produce eDNA species detections in as quick as one hour (Thomas et al., 2019) in a field setting. Their technique was validated in laboratory and field conditions using the invasive freshwater New Zealand mudsnail (*Potamopyrgus antipodarum*) as target species. According to their study, the on-site eDNA-Sampler/Biomeme system generates consistent results comparable to a laboratory-based protocol (i.e., both protocols achieved target detectability at the same eDNA dilution levels) (Thomas et al., 2019). Similarly, Skinner et al. (2020) conducted mesocosm experiments to determine the accuracy and

precision of eDNA detections of a marine species-at-risk (Striped bass *Morone saxatilis*) using a handheld qPCR device compared with a conventional laboratory-based qPCR protocol. In their study, the authors demonstrated the success of both platforms in detecting eDNA from the target species (Skinner et al., 2020). However, unlike Thomas et al. (2019), Skinner et al. (2020) showed lower estimates of eDNA concentration from the portable handheld compared with the laboratory-based qPCR platforms. In their study, the authors also used a time series experiment to determine the persistence/decay rate of eDNA signal demonstrating that striped bass eDNA can be reliably detected in a water sample within 24 hr of collection. Lastly, despite the variation in eDNA concentrations between the handheld and laboratory-based qPCR platforms, both showed a positive relationship between eDNA concentrations and striped bass stocking densities. Their results validate the utility of on-site eDNA methods for identifying spatial and temporal distributions of the marine fish (Skinner et al., 2020).

4 | STANDARDIZATION OF EDNA PRACTICES

Current published eDNA studies are highly variable regarding field sampling and laboratory methods, assay development, data interpretation, and metadata reported. Research in this special issue highlight specific areas that lack standard methods and reporting across the field of eDNA (Klymus et al., 2019; Nicholson et al., 2020; Roux et al., 2020). Klymus et al. (2019) emphasized the need for improved accuracy in interpreting eDNA results and propose achieving this through standardized methods and reporting for the limit of detection (LOD) and limit of quantification (LOQ) in single-species qPCR eDNA assays. Well-defined qPCR assay performance metrics will also facilitate the assessment of eDNA data across studies and provide resource managers with a rigorous foundation for decision-making (Klymus et al., 2019). Another area where standardization is deficient is in metadata reporting. Nicholson et al. (2020) reviewed 160 eDNA freshwater studies and assessed whether or not they could be replicated using the reported data. The authors evaluated, and identified important data gaps, in 10 metadata categories: time, space, environmental conditions, sampling method, filtration method, sanitation, controls, extraction method, PCR conditions, and statistical analyses. According to their review, the best reported parameter (in 99.2% of the analyzed papers) was habitat type within the metadata category space. Other most commonly reported parameters are PCR data (i.e., PCR cycles, primer sequences), extraction equipment, negative controls, and sampling information such as sample volume, filter size, filter preservation method, year of sampling, and sampling location. Environmental conditions at the time of sampling (i.e., UV, precipitation, and wind) are the least reported parameters among studies (Nicholson et al., 2020). This review demonstrates the high variability in metadata reported among eDNA studies and the need for standardized data collection and reporting protocols.

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In an effort to advance eDNA protocol standardization, Roux et al. (2020)analytically validated a qPCR assay using an approach consistent with that recommended by the World Organization for Animal Health (OIE) for assays used to detect globally listed animal diseases (International Office of Epizootics Aquatic Animal Health Standards Commission, 2016). The assay was thoroughly laboratory-validated for the detection of highly invasive European green crab from water samples and bulk DNA extracted from plankton samples and was field-tested in both Canada and Australia (Roux et al., 2020). This study provides an example of how established guidelines from another field of study can be leveraged to promote the uptake of consistent and defensible approaches among eDNA practitioners. Uniformity in the development and validation of eDNA qPCR assays will enable defensible comparisons among studies, improve replicability of methods among labs, and facilitate clear communication of assay characteristics to end-users.

5 | CHALLENGES IN EDNA RESEARCH

Three main challenges in eDNA research are identified in this special issue: PCR inhibition, appropriate selection of primers for eDNA metabarcoding, and absence of standardization of eDNA methods. PCR inhibition occurs when environmental compounds (e.g., sediments, suspended particles, inorganic substances) or a high density of target DNA, interfere with PCR at the molecular level and prevent or significantly diminish amplification of the target region (Lance & Guan, 2019; Skinner et al., 2020). PCR inhibition was also the main drawback of the on-site qPCR detection approaches tested by Thomas et al. (2019) and Skinner et al. (2020).

Lack of amplification alone does not provide sufficient information to distinguish whether there is inhibition, absence of target DNA, or another process at work. In this special issue, PCR inhibition is demonstrated in both freshwater (Gasparini et al., 2020; Hobbs et al., 2020; Thomas et al., 2019) and marine studies (Roux et al., 2020; Skinner et al., 2020), with encouragement for the use of an internal positive control (IPC) to identify inhibition (see Gasparini et al., 2020; Klymus et al., 2019; Skinner et al., 2020; Thomas et al., 2019). Specially, inhibition can be identified when a delay or complete absence of IPC amplification occurs in a specific reaction well (Gasparini et al., 2020; Skinner et al., 2020; Thomas et al., 2019). Other suggested approaches to test for inhibition presented here are by spiking the eDNA samples with an artificial positive control (gBlock) (Roux et al., 2020) and by using an IntegritE-DNA^TM test (Hobbs et al., 2020). The most common and overall successful approach to remove inhibitors is by diluting the problematic samples (Gasparini et al., 2020; Skinner et al., 2020; Thomas et al., 2019). Although there are ways of dealing with PCR inhibition, an important issue remains which is the variability in inhibition among sampling sites and seasons. This suggest heterogeneous patterns of inhibitors in the environment (Gasparini et al., 2020; Hobbs et al., 2020; Roux et al., 2020; Thomas

et al., 2019) which should be further investigated to accurately interpret the results from eDNA surveys.

Another important challenge identified in this special issue is the appropriate selection of primers in eDNA metabarcoding studies to maximize taxa recovery (Morey et al., 2020; Mychek-Londer et al., 2019). Choosing a specific set of primers can affect taxa recovery in community studies and even universal primers can be subject to primer bias (i.e., preferential binding to specific taxa) as pointed out by Mychek-Londer et al. (2019). The use of different primer sets for a single genetic marker could improve species recovery; however, the use of multiple genetic markers would provide superior biodiversity coverage from environmental samples as demonstrated by Morey et al. (2020).

Lastly, the absence of methodological and reporting standards in eDNA research has been remarked in several articles presented in this special issue (Klymus et al., 2019; Nicholson et al., 2020; Roux et al., 2020). The acceptance of eDNA surveys by government and industry highly depends on the establishment of standardized protocols. The challenges identified in this special issue reflect issues frequently discussed among eDNA researchers and are important research areas. Methodological development and standardization in the field of eDNA should include field survey design and bioinformatic methods for processing high-throughput sequence data from eDNA metabarcoding studies. This discussion will continue as novel research emerges and further discussion forums and collaborations among academics, regulators, and industry are established.

6 | MOVING EDNA COLLABORATIONS FORWARD

Biomonitoring surveys using eDNA are an innovative option that can improve taxon detectability, minimize cost, and increase the timeframes available for environmental surveys. eDNA can complement conventional survey methods thereby improving conservation outcomes. Adoption of eDNA surveys by regulated communities is currently held between research and regulatory acceptance. Notably, eDNA technologies are still viewed by regulators as "emerging and developing" (EPRI, 2018). The field of eDNA needs to advance to a consensus on methods standardization from researchers, and then, government agencies may be in a better position to confidently accept the results from eDNA surveys. Once industry has authorization from regulatory agencies to use this survey method, the widespread adoption, and consistent application of eDNA will result in increased benefits for biodiversity conservation.

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REFERENCES

Baker, C. S., Steel, D., Nieukirk, S., & Klinck, H. (2018). Environmental DNA (eDNA) from the wake of the whales: Droplet digital PCR for detection and species identification. *Frontiers in Marine Science*, *5*(133), https://doi.org/10.3389/fmars.2018.00133

Balasingham, K. D., Walter, R. P., Mandrak, N. E., & Heath, D. D. (2018). Environmental DNA detection of rare and invasive fish species in two Great Lakes tributaries. *Molecular Ecology*, *27*(1), 112–127. https://doi.org/10.1111/mec.14395

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, *17*(1), 1–17. https://doi.org/10.1007/s10592-015-0775-4

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., ... de Bruyn, M.. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution*, *29*(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003

Buxton, A. S., Groombridge, J. J., Zakaria, N. B., & Griffiths, R. A. (2017). Seasonal variation in environmental DNA in relation to population size and environmental factors. *Scientific Reports*, *7*(46294), https://doi.org/10.1038/srep46294

Currier, C. A., Morris, T. J., Wilson, C. C., & Freeland, J. R. (2018). Validation of environmental DNA (eDNA) as a detection tool for at-risk freshwater pearly mussel species (Bivalvia: Unionidae). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *28*(3), 545–558. https://doi.org/10.1002/aqc.2869

Davy, C. M., Kidd, A. G., & Wilson, C. C. (2015). Development and validation of environmental DNA (eDNA) markers for detection of freshwater turtles. *PLoS One*, *10*(7), 1–14. https://doi.org/10.1371/journal.pone.0130965

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., ... Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, *26*(21), 5872–5895. https://doi.org/10.1111/mec.14350

Díaz-Ferguson, E. E., & Moyer, G. R. (2014). History, applications, methodological issues and perspectives for the use environmental DNA (eDNA) in marine and freshwater environments. *Revista De Biología Tropical*, *62*(4), 1273–1284. https://doi.org/10.15517/rbt.v62i4.13231

Doi, H., Takahara, T., Minamoto, T., Matsuhashi, S., Uchii, K., & Yamanaka, H. (2015). Droplet digital polymerase chain reaction (PCR) outperforms real-time PCR in the detection of environmental DNA from an invasive fish species. *Environmental Science & Technology*, *49*(9), 5601–5608. https://doi.org/10.1021/acs.est.5b00253

EPRI (Electric Power Research Institute, Inc.). (2018). Important considerations for the use of environmental DNA in the electric power industry. Report Number: 3002013775. https://www.epri.com/#/pages/product/000000003002013775/?lang=en-US.

Evans, N. T., Shirey, P. D., Wieringa, J. G., Mahon, A. R., & Lamberti, G. A. (2017). Comparative cost and effort of fish distribution detection via environmental DNA analysis and electrofishing. *Fisheries*, *42*(2), 90–99. https://doi.org/10.1080/03632415.2017.1276329

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, *4*(4), 423–425. https://doi.org/10.1098/rsbl.2008.0118

Gasparini, L., Crookes, S., Prosser, R. S., & Hanner, R. (2020). Detection of freshwater mussels (Unionidae) using environmental DNA in riverine systems. *Environmental DNA*. https://doi.org/10.1002/edn3.71

Goldberg, C., Strickler, K., & Pilliod, D.. (Eds.). (2015). Environmental DNA: A powerful new tool for biological conservation.

Günther, B., Knebelsberger, T., Neumann, H., Laakmann, S., & Martínez Arbizu, P. (2018). Metabarcoding of marine environmental DNA based on mitochondrial and nuclear genes. *Scientific Reports*, *8*, (14822), . https://doi.org/10.1038/s41598-018-32917-x

Helbing, C. C., & Hobbs, J. (2019). Environmental DNA standardization needs for fish and wildlife population assessments and monitoring. Canadian Standards Association, 1–41. Retrieved from https://www.csagroup.org/wp-content/uploads/CSA-Group-Research-Environmental-DNA.pdf.

Hernandez, C., Bougas, B., Perreault, A., Simard, A., Côté, G., & Bernatchez, L. (2020). 60 specific eDNA qPCR assays to detect invasive, threatened, and exploited freshwater vertebrates and invertebrates in Eastern Canada. *Environmental DNA*. https://doi.org/10.1002/edn3.89.

Hobbs, J., Adams, I. T., Round, J. M., Goldberg, C. S., Allison, M. J., Bergman, L. C., ... Helbing, C. C. (2020). Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus*, in British Columbia, Canada, using environmental DNA methods. *Environmental DNA*. https://doi.org/10.1002/edn3.82

Hunter, M. E., Oyler-McCance, S. J., Dorazio, R. M., Fike, J. A., Smith, B. J., Hunter, C. T., ... Hart, K. M. (2015). Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive burmese pythons. *PLoS One*, *10*(4), e0121655. https://doi.org/10.1371/journal.pone.0121655

International Office of Epizootics Aquatic Animal Health Standards Commission. (2016). Manual of diagnostic tests for aquatic animals (7th Ed.).

Kelly, R. P., Port, J. A., Yamahara, K. M., & Crowder, L. B. (2014). Using environmental DNA to census marine fishes in a large mesocosm. *PLoS One*, *9*(1), e86175.

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*(6191), 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., ... Richter, C. A. (2019). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*. https://doi.org/10.1002/edn3.29

Lacoursière-Roussel, A., Rosabal, M., & Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: Variability among capture methods and environmental conditions. *Molecular Ecology Resources*, *16*(6), 1401–1414. https://doi.org/10.1111/1755-0998.12522

Lance, R. F., & Guan, X. (2019). Variation in inhibitor effects on qPCR assays and implications for eDNA surveys. *Canadian Journal of Fisheries and Aquatic Sciences*, *77*(1), 23–33. https://doi.org/10.1139/cjfas-2018-0263

Laporte, M., Bougas, B., Côté, G., Champoux, O., Paradis, Y., Morin, J., & Bernatchez, L. (2020). Caged fish experiment and hydrodynamic bidimensional modeling highlight the importance to consider 2D dispersion in fluvial environmental DNA studies. *Environmental DNA*. https://doi.org/10.1002/edn3.88.

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Leduc, N., Lacoursière-Roussel, A., Howland, K. L., Archambault, P., Sevellec, M., Normandeau, E., ... Bernatchez, L. (2019). Comparing eDNA metabarcoding and species collection for documenting Arctic metazoan biodiversity. *Environmental DNA*, *1*(4), 342–358. https://doi.org/10.1002/edn3.35

Mauvisseau, Q., Davy-Bowker, J., Bulling, M., Brys, R., Neyrinck, S., Troth, C., & Sweet, M. (2019). Combining ddPCR and environmental DNA to improve detection capabilities of a critically endangered freshwater invertebrate. *Scientific Reports*, *9*(14064), https://doi.org/10.1038/s41598-019-50571-9

Morey, K. C., Bartley, T. J., & Hanner, R. H. (2020). Validating environmental DNA metabarcoding for marine fishes in diverse ecosystems using a public aquarium. *Environmental DNA*. https://doi.org/10.1002/edn3.76

Mychek-Londer, J. G., Balasingham, K. D., & Heath, D. D. (2019). Using environmental DNA metabarcoding to map invasive and native invertebrates in two Great Lakes tributaries. *Environmental DNA*. https://doi.org/10.1002/edn3.56

Nicholson, A., McIsaac, D., MacDonald, C., Gec, P., Mason, B. E., Rein, W., ... Hanner, R. H. (2020). An analysis of metadata reporting in freshwater environmental DNA research calls for the development of best practice guidelines. *Environmental DNA*. https://doi.org/10.1002/edn3.81

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*(5), 1450–1459. https://doi.org/10.1111/1365-2664.12306

Roux, L. D., Giblot-Ducray, D., Bott, N. J., Wiltshire, K. H., Deveney, M. R., Westfall, K. M., & Abbott, C. L. (2020). Analytical validation and field testing of a specific qPCR assay for environmental DNA detection of invasive European green crab (*Carcinus maenas*). *Environmental DNA*. https://doi.org/10.1002/edn3.65

Skinner, M., Murdoch, M., Loeza-Quintana, T., Crookes, S., & Hanner, R. (2020). A mesocosm comparison of laboratory-based and on-site eDNA solutions for detection and quantification of striped bass (*Morone saxatilis*) in marine ecosystems. *Environmental DNA*. https://doi.org/10.1002/edn3.61

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. (2012). Environmental DNA. *Molecular Ecology*, *21*(8), 1789–2050. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Thomas, A. C., Howard, J., Nguyen, P. L., Seimon, T. A., & Goldberg, C. S. (2018). ANDe™: A fully integrated environmental DNA sampling system. *Methods in Ecology and Evolution*, *9*(6), 1379–1385. https://doi.org/10.1111/2041-210X.12994

Thomas, A. C., Tank, S., Nguyen, P. L., Ponce, J., Sinnesael, M., & Goldberg, C. S. (2019). A system for rapid eDNA detection of aquatic invasive species. *Environmental DNA*. https://doi.org/10.1002/edn3.25

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA - An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019

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*(2), 99–108. https://doi.org/10.1002/edn3.21

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., ... Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*(4), 929–942. https://doi.org/10.1111/mec.13428

Williams, M.-A., O'Grady, J., Ball, B., Carlsson, J., Eyto, E., McGinnity, P., ... Parle-McDermott, A. (2019). The application of CRISPR-Cas for single species identification from environmental DNA. *Molecular Ecology Resources*, *19*(5), 1106–1114. https://doi.org/10.1111/1755-0998.13045