Received: 1 July 2020 | Revised: 16 February 2021 | Accepted: 19 February 2021

DOI: 10.1111/ddi.13262

BIODIVERSITY RESEARCH

Diversity and Distributions WILEY

Screening marker sensitivity: Optimizing eDNA-based rare species detection

Zhiqiang Xia[1] | Aibin Zhan[2,3] | Mattias L. Johansson[1,4] | Emma DeRoy[1] | Gordon Douglas Haffner[1] | Hugh J. MacIsaac[1,5]

[1]Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON, Canada

[2]Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China

[3]University of Chinese Academy of Sciences, Beijing, China

[4]Biology Department, University of North Georgia, Oakwood, GA, USA

[5]School of Ecology and Environmental Science, Yunnan University, Kunming, China

Correspondence
Hugh J. MacIsaac, Great Lakes Institute for Environmental Research, University of Windsor, N9B 3P4 Windsor, ON, Canada. School of Ecology and Environmental Science, Yunnan University, Kunming, China.
Email: hughm@uwindsor.ca

Zhiqiang Xia, Great Lakes Institute for Environmental Research, University of Windsor, N9B 3P4 Windsor, ON, Canada.
Email: xiab@uwindsor.ca

Funding information
National Natural Science Foundation of China, Grant/Award Number: 31622011; Canada Research Chairs; Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grants

Editor: Ramiro Logares

Abstract

Aim: Environmental DNA (eDNA)-based techniques are useful tools in disciplines such as conservation biogeography at local to global scales since they provide promising methods to locate organisms at low abundance. Here, we raise a largely overlooked issue that the marker (primer pairs and/or probes) sensitivity of eDNA-based detection should be optimized and reported to improve detection performance and result interpretation.

Location: Global.

Methods: We analysed 250 articles published between 2008 and 2019 that sought to detect animals from environmental water samples using species-specific markers to identify effort required.

Results: Most (66.0%) studies used newly designed markers, and real-time quantitative PCR dominated the studies (72.4% of articles). The use of quantitative PCR increased significantly over time ($p = .016$), while conventional PCR decreased significantly ($p = .005$). In 82.4% of studies using newly designed markers, researchers did not screen their chosen markers for sensitivity, and 46.7% of these studies did not report the limit of detection (LoD). Limited knowledge of sensitivity screening and LoD was also found among aquatic species on the list of the world's worst alien invasive species, and many studies used published markers without such knowledge, potentially propagating errors.

Main conclusions: The rapidly growing use of eDNA-based detection of low-abundance species requires well-designed protocols to improve sensitivity. Knowledge of the limits of eDNA technology is imperative, particularly when applied to conservation biogeography studies for detecting non-indigenous or endangered species. Our results highlight the currently inadequate sensitivity screening of genetic markers used in most studies, contrasting the transition to highly sensitive PCR methods. Along with ongoing calls for standardization in the eDNA methods, we add that newly designed markers be screened to determine and optimize sensitivity before use to reduce the uncertainty of detection and benefit future applications within or beyond areas of their development.

KEYWORDS
aquatic ecosystems, environmental DNA, false negatives, limit of detection, marker screening, optimization, PCR method, sensitivity

1 | INTRODUCTION

Rare (low-abundance) species are critical for maintaining ecosystem functioning and services (Dee et al., 2019; Lyons et al., 2005). Fast and accurate detection of rare species—such as newly introduced non-indigenous species or endemic endangered species—has attracted the attention of ecologists and policymakers alike, as it underpins management (Bohmann et al., 2014; Dee et al., 2019; Jerde et al., 2011; Mehta et al., 2007; Sepulveda et al., 2020; Trebitz et al., 2017). For example, one fundamental question for conservation biogeographers is whether an introduced, non-indigenous species can establish and form self-sustaining populations in new areas, the answer to which is critical to triggering rapid responses. However, the abundance of the introduced propagules may be extremely low and be restricted to limited geographical ranges, challenging many traditional detection methods and impeding conservation attempts (Darling & Mahon, 2011; Dee et al., 2019; Harvey et al., 2009; Hoffman et al., 2011; Zhan & MacIsaac, 2015). Environmental DNA (eDNA)-based techniques have emerged as promising tools for targeting these species in a non-destructive manner while promising enhanced sensitivity. These techniques have the dual advantages of high environmental prevalence and ease of extraction from bulk environmental samples (Bohmann et al., 2014; Taberlet et al., 2012). By using properly selected protocols, it is possible to discriminate a single species or profile an entire community from environmental samples (Bohmann et al., 2014). The former, which is the focus of the present study, is achieved by using species-specific primer pairs (and/or probes) of chosen genetic markers (hereafter markers) to amplify single species through PCR.

Like many emerging techniques, eDNA-based species detection is imperfect and is affected by an array of technical issues that need to be disentangled (Ficetola et al., 2016; Roussel et al., 2015). For example, a typical application of eDNA for detecting species from aquatic environments comprises multiple steps, and problems at any of these stages may impede detection and lead to false interpretations (Bohmann et al., 2014; Goldberg et al., 2016; Song et al., 2020). False negatives (type II errors), for instance, lead to incorrect assessments for "undetected" endangered species or non-native species, leading to delayed conservation or prevention strategies, respectively (Darling & Mahon, 2011; Furlan et al., 2016; Xiong et al., 2016). Such uncertainties may impede full embracement of eDNA methods by policymakers and managers to trigger conservation applications (e.g. invasive species management) (Jerde, 2019; Sepulveda et al., 2020). Many case studies, particularly in aquatic systems, have focused on technical issues including primer design (Farrington et al., 2015; Wilcox et al., 2013), sample handling (Mächler et al., 2016; Moyer et al., 2014; Takahara et al., 2015), DNA extraction (Deiner et al., 2015), inhibition abatement (McKee et al., 2015) and selection of PCR method (Wood et al., 2019; Xia, Johansson, et al., 2018), thereby offering useful solutions to advance eDNA-based species detection. A recent review of critical considerations in detecting aquatic species *via* eDNA methods established important guidelines for such steps (Goldberg et al., 2016). For marker selection, existing studies focus mostly on specificity for single species-based applications or generality for community-based (or multispecies) applications (Deagle et al., 2014; Goldberg et al., 2016; Wilcox et al., 2013; Zhan et al., 2014). However, surprisingly little attention has been paid to screening sensitivity of markers, for which high sensitivity is usually expressed as a low limit of detection (LoD). This may unwittingly undermine the detection sensitivity of a given eDNA-based species detection protocol, which can be assessed as the lowest amount of target DNA that can be detected with 95% probability (Schultz & Lance, 2015). A developed protocol is likely—possibly inevitably—to be applied in subsequent studies. This has broad implications in conservation biogeography studies because developed and published markers can then be used by researchers in any locations where the species may occur (e.g. a transoceanic introduced non-indigenous species). A well-screened marker with robust sensitivity thus creates legacy value beyond the time and location of its origin.

To call attention to marker selection from a sensitivity perspective, we conducted a meta-analysis and addressed a deficit that insufficient attention has been paid to screening and reporting of the sensitivity of species-specific markers, which may harm the efficacy of eDNA as a tool in detecting low-abundance species. We first focused on marker sensitivity screening and the PCR method selection, which have been demonstrated to influence detection performance. We then identified limitations that result from a dearth of information regarding LoD—which we define as the lowest amount of target DNA that can be detected—of markers used for eDNA-based species detection. These issues may result in use of suboptimal assays, thereby inflating false negatives and delaying management strategies. We demonstrated this by examining eDNA detection studies for species on the "100 of the world's worst invasive alien species" (Lowe et al., 2000).

2 | METHODS

2.1 | Literature surveys

To generate a quality data set to address the issues above, we focused on studies using species-specific markers to detect animal species from water samples because these studies represent the most extensive application of eDNA-based methods for detecting low-abundance species. Specifically, we searched the ISI

Web of Science (WoS) using "eDNA" or "environmental DNA" and "species detect*" as keywords from 2008 through 2019, which encompasses the latest studies since macro-organism detection *via* eDNA was first used (Ficetola et al., 2008). We narrowed our survey to 11 WoS categories that comprise the majority of articles in aquatic ecosystems (Table S1, supporting information). Through paper-by-paper identification, we removed articles that were beyond the scope of this study, such as those exclusively focusing on species barcoding (i.e. not real environmental samples) or detecting algae and bacteria, etc., or that purely focusing on concept validations with aquarium-based samples (i.e. validations in field samples were retained), resulting in 250 retained articles (Tables S1–2).

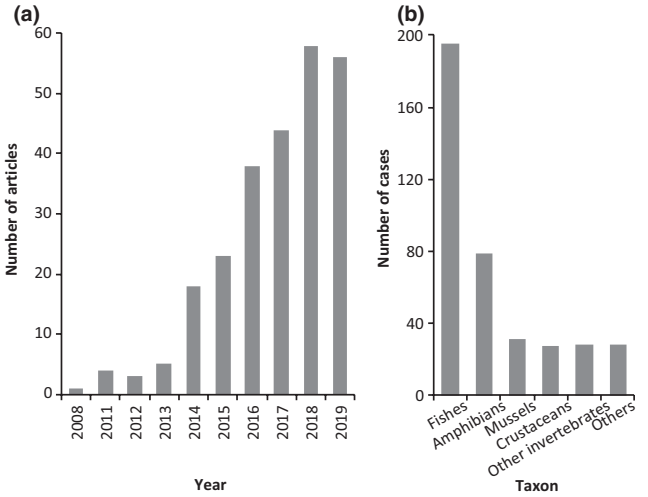

FIGURE 1 Annual number of articles on species detection from aquatic environmental DNA samples using species-specific markers (a) and distribution of study cases for each taxonomic group therein (b), showing results of a total number of 388 cases from 250 articles recorded in Web of Science

2.2 | Criterion extraction

For each article, criteria pertaining to source of marker used (i.e. designed in study, cited from existing study or combined when a mixed source is identified), status of sensitivity screening of markers ([i.e. screened if testing multiple candidate genes, or multiple primer sets, or any other preliminary measures to ensure that relatively highly sensitive markers were selected], or unscreened if no such measures were considered [including testing of multiple candidates for specificity without examining LoD]), whether LoD of the marker used was reported (i.e. reported or unreported), PCR method (i.e. conventional PCR [cPCR, including nested PCR], real-time quantitative PCR [qPCR, including dye-based and probe-based qPCR], droplet digital PCR [ddPCR] or combined when different methods were used in a single article) were extracted. We examined the methods and results of each article to extract the above information, and we also examined their supplementary materials if relevant information was not available in the main article but was directed to them. Articles that studied multiple species were broken down to single species, resulting in a case-based data set of 388 species, which we used to calculate the prevalence of taxonomic groups (Tables S1–2). For cases that used cited assays, corresponding information was incorporated from the original studies. To investigate the evolution of these efforts, we used time series linear models to relate the portion (%) of each criterion to the year of publication, with the analysis performed in R (version 3.6.2) (R Core Team, 2019) and the time series analysis using "dplyr" package (version 0.8.3). Since no records were identified in 2009–10, and only one was available in 2008 (see results), we used articles in 2011–19 for time series analysis to avoid missing values.

2.3 | Examples of the worst invasive species

To highlight potential problems of unscreened (i.e. as to sensitivity) markers being used in later studies, we reviewed articles detecting aquatic animal species listed on the "100 of the world's worst invasive alien species" (Lowe et al., 2000). We considered these species as examples because they represent concerns globally, which was critical to avoid biases in species selection and to simplify literature survey. We believe that studies on the selected species represent a snapshot of how sensitivity screening of markers is treated at a broad scale, though we might have missed some species not on the list but which have received extensive studies such as the Asian carp (Jerde, 2019). We identified articles in which the aquatic animal species on the list were detected *via* eDNA approaches using originally designed markers by using the same keywords for constructing the above article data set, together with species name (either Latin or common name). We retained several markers not originally designed for eDNA samples *sensu stricto* (e.g. plankton samples or gut content) because we found subsequent studies used them for eDNA samples (see results). Based on these records, we calculated the number of times each original marker was used in other studies. To achieve this, we reviewed all articles citing each of these original articles and only retained studies using the marker.

3 | RESULTS

3.1 | Increasing popularity of eDNA methods for species detection

The number of eDNA-based publications on detection of species from water samples using species-specific markers has increased rapidly, from one in 2008 to 56 in 2019 (Figure 1a). eDNA detection was most commonly employed with fish, accounting for 195 of 388 cases (50.3%), followed by amphibians (79, 20.4%), mussels (31, 8.0%), crustaceans (27, 7.0%) and other invertebrates (28, 7.2%) (Figure 1b).

3.2 | Marker source, PCR method, sensitivity screening and LoD reporting of markers

Overall, 66% studies (165 of 250) used newly designed and 30.4% (76) used cited markers for target species, while 3.6% (9) used both (Figure 2a). Neither the use (%) of cited nor newly designed makers demonstrated significant changes over time (p-values >.55) (Table S3, supporting information). A total of 48 (19.2%) studies used cPCR, though its proportional use declined significantly during 2011–19 ($F_{1,7}$ = 15.7, p = .005, R^2 = .692) (Figure 2b; Table S3). In contrast, 181 (72.4%) studies used qPCR, which increased significantly ($F_{1,7}$ = 10.1, p = .016, R^2 = .591) during the same time period (Figure 2b; Table S3). ddPCR was only used in a small number of studies (7, 2.8%) and demonstrated no significant changes over time (p = .122) (Figure 2b; Table S3). Among the 165 studies that used newly designed markers, only 26 (15.8%) studies reported sensitivity screening to optimize LoD of markers used, while 139 (84.2%) studies failed to do so (Figure 2c), and neither pattern changed over time (p = .186 and 0.298) (Table S3). In addition, 88 (53.3%) studies reported marker LoD, while 77 (46.7%) failed to do so (Figure 2d), with neither pattern changing over time (p = .289 and 0.320) (Table S3). The format of reported marker LoD was highly inconsistent across studies (Table 1).

3.3 | Detection of the "worst invasive alien species"

Twenty aquatic animal species are listed in the "100 of the world's worst invasive alien species" (Lowe et al., 2000). As of 31 December 2019, 14 of these species have been recorded in Web of Science as having been detected at least once each from eDNA samples (including plankton samples). Brown trout, bullfrog and common carp were each represented by more than one marker, resulting in a total of 19 species-specific markers (Figure 3; Table S4). Three (17.6%) of these markers were screened to achieve low LoD and thereby optimize detection sensitivity, and LoD was reported for nine (47.4%) of them. Seven (36.8%) of the markers were subsequently used in other studies, even though only one (14.3%) had been screened previously for sensitivity and two (28.6%) had LoD reported (Figure 3). In addition, a few markers (e.g. fishhook water flea, green crab) not originally designed for eDNA samples *sensu stricto* were also used in later studies for eDNA sample-based detection (Figure 3; Table S4).

4 | DISCUSSION

Rapid and accurate locating of rare species is vital for conservation applications (e.g. rapid response to introduced non-indigenous species). eDNA-based techniques serve as promising tools for detecting

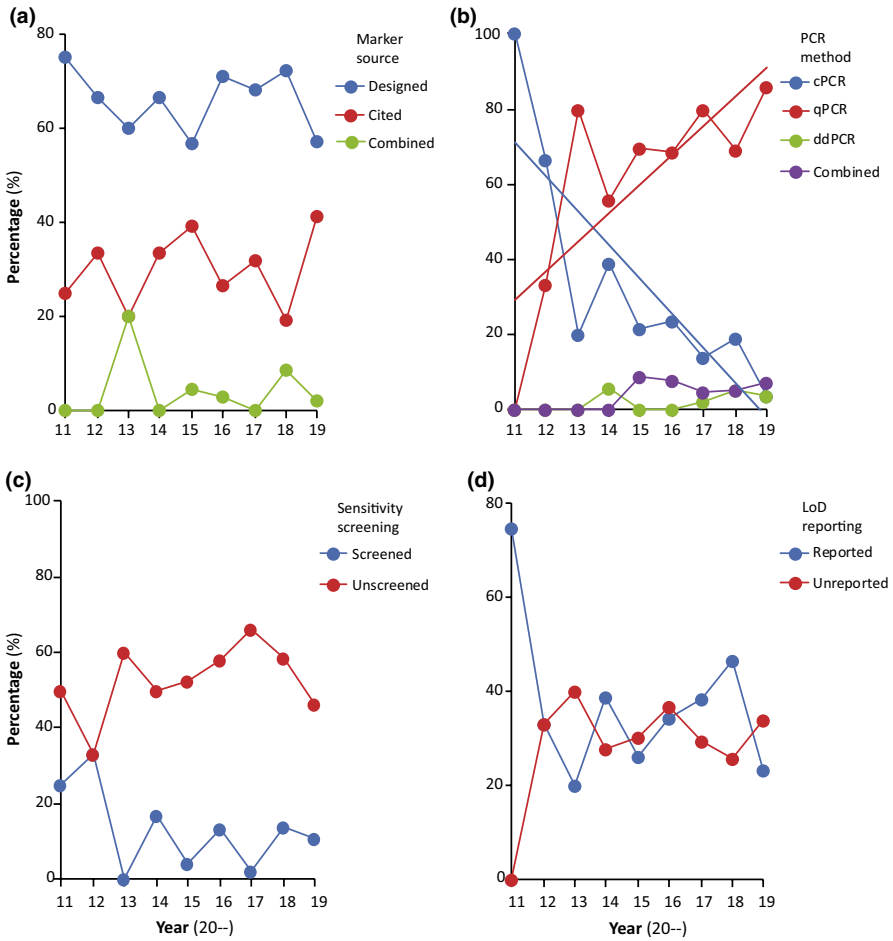

FIGURE 2 Changes of marker sources (a), PCR methods (b), status of sensitivity screening (c) and limit of detection reporting (d) in literature on species detection using eDNA methods. (a) and (b) are based on 250 articles, among which 165 articles using designed markers exclusively were used for calculating (c) and (d). Time series linear models were used to relate each criterion to time (year), and solid regression lines indicate significant changes at p = .05 level. One study in 2008 was not shown to avoid missing values in 2009–10 to simplify time series analysis

TABLE 1 Current report formats for detection limit of markers in literature and suggested report formats for single species detection from eDNA samples

	PCR methods	
	cPCR	**qPCR**
Current formats	0.5 ~ 0.01 ng, 7.25×10^{-11} ng/μl, 1 zooid, 5 D-hinge-stage larvae, 50 copies	1 individual per 100 m^2, 2 copies, 3 copies per reaction, 1 larva, 0.013–3.2 pg/μl, <10 copies 4 μl^{-1}
Suggested formats	# of copies	# of copies

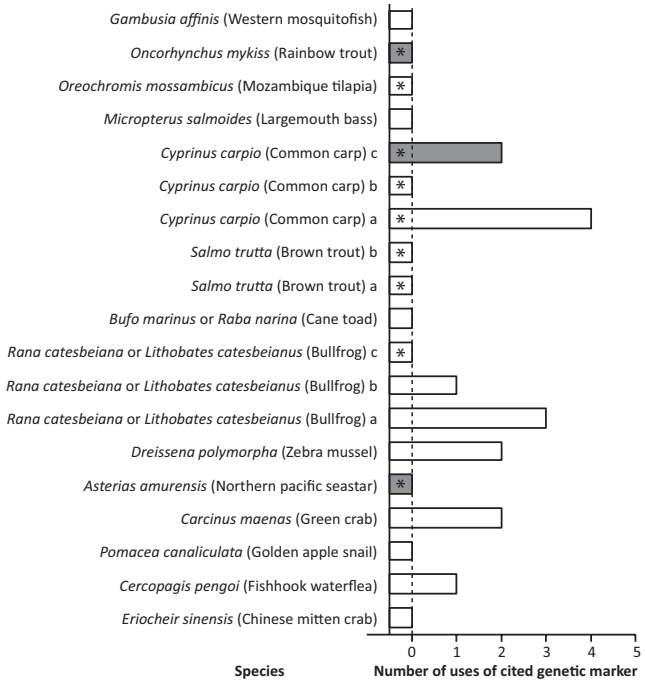

FIGURE 3 Number of uses in other studies for 19 designed markers of 14 aquatic animal species on "the 100 worst invasive species in the world" (Lowe et al., 2000). Grey bars represent markers that were screened for sensitivity to optimize detection, while white bars were unscreened markers. An asterisk indicates that the limit of detection (LoD) of the markers was reported, otherwise it was unreported. The dashed line indicates that the genetic marker has accumulated no uses as of 31 December 2019 (Web of Science)

low-abundance species and are rapidly growing in popularity in research (Figure 1a) though conservation managers seem hesitant to fully embrace such tools, notably due to uncertainties associated with eDNA results (Jerde, 2019; Sepulveda et al., 2020). Use of optimal markers following sensitivity screening of multiple candidates can reduce false negatives (e.g. Farrington et al., 2015; Xia, Zhan, et al., 2018). Despite this, an overwhelming 84.2% of articles that used newly designed markers seemingly overlooked this crucial step, contrasting the increasing use of more sensitive detection platforms such as qPCR (Figure 2b, Table S3). Furthermore, the proportion using sensitivity-screened markers did not change over time (Figure 2c, p =.186, Table S3), which seems more problematic than the low overall rate per se given the increasing number of eDNA applications. Similarly, 46.7% of studies using newly designed markers did not report their LoDs, a pattern that did not decline over time (Figure 2d, p =.288; Table S3). The absence of knowledge on LoD prevents researchers from identifying the boundary at which false-negative rate increases, which in turn compromises eDNA-based species detection programmes. For example, two recent studies detecting fishes using both eDNA methods and the American Fisheries Society's standard sampling assessment (gillnetting, boat electrofishing and snorkelling) reported overall lower detection rates with eDNA than with standard methods (Perez et al., 2017; Ulibarri et al., 2017). Use of eDNA-based technique as a detection tool was challenged in these programmes, partially owing to the lack of optimization of both sampling methods and assay design (Perez et al., 2017; Ulibarri et al., 2017). In contrast, a recent study reported an improvement in detection performance for Asian carp following a switch to more sensitive markers (Song et al., 2020). To some extent, the above examples imply false negatives associated with eDNA methods, which may delay the initiation of conservation strategies (e.g. rapid response to invasive species). Therefore, eDNA-based species detection requires careful protocol design and execution to improve confidence in the results obtained (Furlan et al., 2016; Jerde, 2019; Jerde et al., 2011; Loeza-Quintana et al., 2020; Sepulveda et al., 2020; Song et al., 2020).

High marker sensitivity is typically expressed by a low LoD, which has a theoretical minimum at one copy per PCR (Bustin et al., 2009). Though it may be challenging to achieve this theoretical limit, eDNA markers should be screened to achieve an LoD as low as possible to maximize detection probability. In a more applied perspective, a lower LoD for eDNA-based detection can result in a greater probability of a rare species being detected through eDNA earlier than traditional detection (Jerde, 2019). However, prior knowledge about LoD is almost impossible until tested (Farrington et al., 2015; Xia, Zhan, et al., 2018). Therefore, a sensitivity screening step is required to obtain a robust marker with a low LoD. To achieve this, designing multiple candidate markers for sensitivity screening may be required. Different genes with multiple markers based on each can increase the probability of finding highly sensitive ones. Different marker design platforms can also be considered to generate different high-score candidates, which may exhibit varied amplification efficiency as these design tools often stem from different algorithms (Doi et al., 2015; Goldberg et al., 2016). Even though further studies are required,

the above options can allow multiple markers to be generated and screened to identify ones with low LoD. Furthermore, any selected markers should undergo strict validation (i.e. in silico, in vitro and in situ) to confirm specificity and sensitivity before formal use (Darling & Mahon, 2011; Goldberg et al., 2016; Klymus et al., 2019; Langlois et al., 2020; Roussel et al., 2015; Wilcox et al., 2013). It is important to keep in mind that highly sensitive markers are sensitive to trace levels of target eDNA regardless of its source, thus requiring strict quality control to avoid contamination (Darling & Mahon, 2011; Goldberg et al., 2016).

We observed that designed markers were used more frequently than cited ones in almost all years (Figure 2a), although over 36% of the original markers in the "worst aquatic invaders" case study were utilized in at least one subsequent study (Figure 3). Given the developing stage of eDNA methods and a large number of important species yet to be detected *via* eDNA, it is likely that even more studies will use literature-based markers in the future. The proliferation of species being barcoded and shared through public platforms and databases such as the Barcode of Life Database System (BOLD) and the National Center for Biotechnology Information (NCBI) highlights the importance of online databases of quality marker information. In addition, a marker deemed suitable for one study may be considered unsuitable in subsequent studies. For example, new candidate markers are required if an existing marker does not pass specificity tests when used in novel geographical locales in which different sympatric species are present than those used at the original design site (Wilcox et al., 2013). We expect that a similar situation applies to marker sensitivity (see below for more discussion), and both require associated information reported. Almost 53.3% of studies reported the LoD of their assays used, though formatting varied across studies (Table 1). Diverse characterization of the LoD and reporting formats can also impede their use in future studies (Bustin et al., 2009; Darling & Mahon, 2011) because they render difficult comparisons of marker efficiency for a single species or among laboratories (Klymus et al., 2019). Therefore, it is vital to ensure that the sensitivity of newly designed markers be screened, tested and reported in publications (MacDonald & Sarre, 2017; Veldhoen et al., 2016). In addition, their testing and reporting should be formatted in a standard manner such as the number of copies of the target fragment, which can be derived by using gBlocks gene fragments (i.e. synthesized DNA fragments, Langlois et al., 2020). For example, Klymus and colleagues (2019) reported comprehensive guidelines in calculating and reporting LoD for qPCR, which dominates current eDNA-based applications (Figure 2b).

In addition to enhanced efforts in sampling, inhibitor abatement and quality control (Wilcox et al., 2013; Wood et al., 2019), the use of robust markers and more sensitive detection platforms provide additional room to improve detection of low-abundance species from eDNA samples (Song et al., 2020; Xia, Zhan, et al., 2018). However, it is important to interpret the changes of criteria investigated in the present study from an evolving perspective. For example, the increasing use of qPCR may reflect the collective pursuits of highly sensitive and quantitative tools (Doi et al., 2015; Hunter et al., 2016; Xia, Johansson, et al., 2018). Our study may have underestimated the proportion of studies using sensitivity-screened and LoD-known markers if the authors did, in fact, test but not report these results. Given the rapid growth and development and broad research application of eDNA, such cases are possible. However, as shown in the most invasive species examples, originally designed and reported markers are likely to be used in future studies—possibly for different aims and in different geographical locales—and thus are of great future value in conservation biogeography studies. Even though the sensitivity required for a marker may vary across research purposes and geographical locales, reporting of sensitivity screening and LoD is essential to allow future users to decide whether to adopt existing markers or design new ones.

eDNA-based methods are becoming technically mature and have acceptable reliabilities in many cases. The lack of standardized efforts in methods development and validation represents a critical obstacle of eDNA methods between the laboratory and field. Standard technical protocols in laboratory and field, and efficient communication between eDNA scientists and conservation managers, can facilitate the transition of eDNA methods from research to application (Klymus et al., 2019; Langlois et al., 2020; Loeza-Quintana et al., 2020; Sepulveda et al., 2020). Goldberg and colleagues (2016) established comprehensive guidelines and recommended minimum information that should be considered in eDNA-based aquatic species detection. Sepulveda et al., (2020) proposed a decision-support framework to assist decision-making in aquatic species management based on eDNA results. Additionally, we encourage authors to screen sensitivity and report LoD for any newly developed markers. As rare species detection becomes increasingly based upon eDNA-based techniques, we believe that such routine efforts can add additional value to their work by benefitting future users and advance the use of eDNA in conservation studies and applications.

ACKNOWLEDGEMENTS

We are grateful for the constructive comments of two anonymous reviewers on previous versions of this work. Funding was provided by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants and Canada Research Chairs to GDH and HJM, and the National Natural Science Foundation of China (31622011) to AZ.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ddi.13262.

DATA AVAILABILITY STATEMENT

All supporting information can be found online for this article.

ORCID

Zhiqiang Xia https://orcid.org/0000-0002-9201-1043
Aibin Zhan https://orcid.org/0000-0003-1416-1238

REFERENCES

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, *29*, 358–367.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J. O., & Wittwer, C. T. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, *55*, 611. https://doi.org/10.1373/clinchem.2008.112797

Darling, J. A., & Mahon, A. R. (2011). From molecules to management: Adopting DNA-based methods for monitoring biological invasions in aquatic environments. 2011. *Environmental Research*, *111*, 978–988.

Deagle, B. E., Jarman, S. N., Coissac, E., Pompanon, F., & Taberlet, P. (2014). DNA metabarcoding and the cytochrome c oxidase subunit I marker: Not a perfect match. *Biology Letters*, *10*, 20140526.

Dee, L. W., Cowles, J., Isbell, F., Pau, S., Gaines, S. D., & Reich, P. B. (2019). When do ecosystem services depend on rare species? *Trends in Ecology & Evolution*, *34*, 748–758. https://doi.org/10.1016/j.tree.2019.03.010

Deiner, K., Walser, J. C., Mächler, E., & Altermatt, F. (2015). Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation*, *183*, 53–63.

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*, 5601–5608. https://doi.org/10.1021/acs.est.5b00253

Farrington, H. L., Edwards, C. E., Guan, X., Carr, M. R., Baerwaldt, K., & Lance, R. F. (2015). Mitochondrial genome sequencing and development of genetic markers for the detection of DNA of invasive bighead and silver carp (*Hypophthalmichthys nobilis* and *H. molitrix*) in environmental water samples from the united states. *PLoS One*, *10*, e0117803. https://doi.org/10.1371/journal.pone.0130965

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., Taberlet, P., & Coissac, E. (2016). How to limit false positives in environmental DNA and metabarcoding? *Molecular Ecology Resources*, *16*, 604–607.

Furlan, E. M., Gleeson, D., Hardy, C. M., & Duncan, R. P. (2016). A framework for estimating the sensitivity of eDNA surveys. *Molecular Ecology Resources*, *16*, 641–654.

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., & Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, *7*, 1299–1307.

Harvey, C. T., Qureshi, S. A., & MacIsaac, H. J. (2009). Detection of a colonizing, aquatic, non-indigenous species. *Diversity and Distributions*, *15*, 429–437.

Hoffman, J. C., Kelly, J. R., Trebitz, A. S., Peterson, G. S., & West, C. W. (2011). Effort and potential efficiencies for aquatic non-native species early detection. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*, 2064–2079.

Hunter, M. E., Dorazio, R. M., Butterfield, J. S. S., Meigs-Friend, G., Nico, L. G., & Ferrante, J. A. (2016). Detection limits of quantitative and digital PCR assays and their influence in presence in-absence surveys of environmental DNA. *Molecular Ecology Resources*, *17*, 221–229.

Jerde, C. L. (2019). Can we manage fisheries with the inherent uncertainty from eDNA? *Journal of Fish Biology*, *98*(2), 341–353. 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

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. (2019). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*, *2*(3), 271–282. https://doi.org/10.1002/edn3.29

Langlois, V. S., Allison, M. J., Bergman, L. C., To, T. A., & Helbing, C. C. (2020). The need for robust qPCR-based eDNA detection assays in environmental monitoring and species inventories. *Environmental DNA*. https://doi.org/10.1002/edn3.164

Loeza-Quintana, T., Abbott, C. L., Heath, D. D., Bernatchez, L., & Hanner, R. H. (2020). Pathways to increase standards and competency of eDNA Surveys (PISCeS)- advancing collaboration and standardization efforts in the field of eDNA. *Environmental DNA*, *2*, 255–260. https://doi.org/10.1002/edn3.112

Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000). 100 of the world's worst invasive alien species: A selection from the global invasive species database, *12*, Auckland: Invasive Species Specialist Group.

Lyons, K. G., Brigham, C. A., Traut, B. H., & Schwartz, M. W. (2005). Rare species and ecosystem functioning. *Conservation Biology*, *19*, 1019–1024. https://doi.org/10.1111/j.1523-1739.2005.00106.x

MacDonald, A. J., & Sarre, S. D. (2017). A framework for developing and validating taxon-specific primers for specimen identification from environmental DNA. *Molecular Ecology Resources*, *17*, 708–720.

Mächler, E., Deiner, K., Spahn, F., & Altermatt, F. (2016). Fishing in the water: Effect of sampled water volume on environmental DNA-based detection of macroinvertebrates. *Environmental Science & Technology*, *50*, 305–312. https://doi.org/10.1021/acs.est.5b04188

McKee, A. M., Spear, S. F., & Pieerson, T. W. (2015). The effect of dilution and the use of a post-extraction nucleic acid purification column on the accuracy, precision, and inhibition of environmental DNA samples. *Biological Conservation*, *183*, 70–76.

Mehta, S. V., Haight, R. G., Homans, F. R., Polasky, S., & Venette, R. C. (2007). Optimal detection and control strategies for invasive species management. *Ecological Economics*, *61*, 237–245.

Moyer, G. R., Díar-Ferguson, E., Hill, J. E., & Shea, C. (2014). Assessing environmental DNA detection in controlled lentic systems. *PLoS One*, *9*, e103767.

Perez, C. R., Bonar, S. A., Amberg, J. J., Ladell, B., Rees, C., Stewart, W. T., Gill, C. J., Cantrell, C., & Robinson, A. T. (2017). Comparison of American fisheries society (AFS) standard fish sampling techniques and environmental DNA for characterizing fish communities in a large reservoir. *North American Journal of Fisheries Management*, *37*, 1010–1027.

R Core Team (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Roussel, J. M., Paillisson, J. M., Tréguier, A., & Petit, E. (2015). The downside of eDNA as a survey tool in water bodies. *Journal of Applied Ecology*, *52*, 823–826.

Schultz, M. T., & Lance, R. F. (2015). Modelling the sensitivity of field surveys for detection of environmental DNA (eDNA). *PLoS One*, *10*, e0140503.

Sepulveda, A. J., Nelson, N. M., Jerde, C. L., & Luikart, G. (2020). Are environmental DNA methods ready for aquatic invasive species management? *Trends in Ecology & Evolution*, *35*, 668–678.

Song, J. W., Schultz, M. T., Casman, E. A., Bockrath, K. D., Mize, E., Monroe, E. M., Tuttle-Lau, M., & Small, M. J. (2020). A probabilistic model for designing and assessing the performance of eDNA sampling protocols. *Molecular Ecology Resources*, *20*, 404–414.

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental DNA. *Molecular Ecology*, *21*, 1789–1793.

Takahara, T., Minamoto, T., & Doi, H. (2015). Effects of sample processing on the detection rate of environmental DNA from the Common carp (*Cyprinus carpio*). *Biological Conservation*, *183*, 64–69.

Trebitz, A. S., Hoffman, J. C., Darling, J. A., Pilgrim, E. M., Kelly, J. R., Brown, E. A., & Schardt, J. C. (2017). Early detection monitoring for aquatic non-indigenous species: Optimizing surveillance, incorporating advanced technologies, and identifying research needs. *Journal of Environmental Management*, *202*, 299–310.

Ulibarri, R. M., Bonar, S. A., Rees, C., Amberg, J. J., Ladell, B., & Jackson, C. (2017). Comparing efficiency of American Fisheries Society standard snorkeling techniques to environmental DNA sampling techniques. *North American Journal of Fisheries Management*, *37*, 644–651.

Veldhoen, N., Hobbs, J., Ikonomou, G., Hii, M., Lesperance, M., & Helbing, C. C. (2016). Implementation of novel design features for qPCR-based eDNA assessment. *PLoS One*, *11*, e0164907.

Wilcox, T. M., McKelvey, K. S., Young, M. K., Jane, S. F., Lowe, W. H., Whiteley, A. R., & Schwartz, M. K. (2013). Robust detection of rare species using environmental DNA: The importance of primer specificity. *PLoS One*, *8*, e59520.

Wood, S. A., Pochon, X., Laroche, O., von Ammon, U., Adamson, J., & Zaiko, A. (2019). A comparison of droplet digital polymerase chain reaction (PCR), quantitative PCR and metabarcoding for species-specific detection in environmental DNA. *Molecular Ecology Resources*, *19*, 1407–1419.

Xia, Z., Johansson, M. L., Gao, Y., Zhang, L., Haffner, G. D., MacIsaac, H. J., & Zhan, A. (2018). Conventional versus real-time quantitative PCR for rare species detection. *Ecology and Evolutions*, *8*, 11799–11807.

Xia, Z., Zhan, A., Gao, Y., Zhang, L., Haffner, G. D., & MacIsaac, H. J. (2018). Early detection of a highly invasive bivalve based on environmental DNA (eDNA). *Biological Invasions*, *20*, 437–447.

Xiong, W., Li, H., & Zhan, A. (2016). Early detection of invasive species in marine ecosystems using high-throughput sequencing: Technical challenges and possible solutions. *Marine Biology*, *163*, 139.

Zhan, A., Bailey, S. A., Heath, D. D., & MacIsaac, H. J. (2014). Performance comparison of genetic markers for high-throughput sequencing-based biodiversity assessment in complex communities. *Molecular Ecology Resources*, *14*, 1049–1059.

Zhan, A., & MacIsaac, H. J. (2015). Rare biosphere exploration using high-throughput sequencing: Research progress and perspectives. *Conservation Genetics*, *16*, 513–522.

BIOSKETCH

Our research focuses on a wide variety of questions in invasion ecology, with special emphasis on aquatic ecosystems. Major research interests of the research team include but are not limited to early detection, risk assessment, local adaptation and species distribution modelling of invasive species.

Author contributions: ZX and HJM conceived the study. ZX collected the data and drafted the manuscript. AZ, MLJ and HJM edited the manuscript. All authors reviewed and approved the final version of the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Xia Z, Zhan A, Johansson ML, DeRoy E, Haffner GD, MacIsaac HJ. Screening marker sensitivity: Optimizing eDNA-based rare species detection. *Divers Distrib*. 2021;27:1981–1988. https://doi.org/10.1111/ddi.13262