



Taxonomic and geographical representation of freshwater environmental DNA research in aquatic conservation

Christina C. Belle | Bernhard C. Stoeckle | Juergen Geist

Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management, Technical University of Munich, Freising, Germany

Correspondence

Juergen Geist, Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management, Technical University of Munich, Muehlenweg 22, D-85354 Freising, Germany. Email: geist@wzw.tum.de

Abstract

1. Freshwater macro-organismic environmental DNA (eDNA) is gaining increasing popularity in detecting invasive species, assessing community assemblages, and in mapping the distribution of taxa that are rare or otherwise difficult to monitor. The objectives of this article are to review the targets of published freshwater eDNA research in relation to aquatic conservation with a focus on geographic regions covered, as well as the habitats and species investigated.
2. The analysis of 272 peer-reviewed articles published between 2005 and 2018 revealed that 57% of the 238 primary research papers have a focus on conservation science, mostly addressing invasive and endangered species, followed by 23% papers investigating methodological developments and 11% biodiversity surveys also using eDNA metabarcoding. A strong geographical pattern emerged, with Africa, South America, and the tropics being under-represented. Taxonomic coverage was dominated by 123 fish species, followed by 29 amphibian and 28 mollusc species. Freshwater arthropods (27 taxa) were under-represented in relation to their estimated species richness.
3. Taxonomic bias towards certain species such as fishes observed in freshwater eDNA research is pervasive in biodiversity research and conservation sciences, and thus is not surprising. Geographical representation was biased, with a few industrialized countries from the Northern Hemisphere contributing 72% of the studies. Both findings parallel biases known from other research areas, such as marine eDNA analysis, taxonomy, or invasion biology.
4. The application of eDNA in freshwater conservation will benefit from the development of general standards and guidelines that are necessary to integrate freshwater microbial eDNA techniques in existing monitoring frameworks. To aid future freshwater conservation, our suggestions are to harmonize eDNA methods for comparable and easier implementation worldwide, and to increase international cooperation and funding for under-represented geographical regions and neglected taxa. This is especially crucial for the known biodiversity hotspots in developing countries where rapid changes occur to freshwater habitats and biodiversity.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2019 The Authors. Aquatic Conservation: Marine and Freshwater Ecosystems published by John Wiley & Sons Ltd

KEYWORDS

eDNA, fishes, freshwater conservation, geographical bias, macroinvertebrates, methods standardization, monitoring, taxonomic representation

1 | INTRODUCTION

Freshwater ecosystems are considered the most imperilled habitat type worldwide (Boon & Pringle, 2009; Geist, 2011; Reid et al., 2019; Strayer & Dudgeon, 2010; Vörösmarty et al., 2010). In temperate zones, they are also one of the most intensively monitored habitats owing to legal requirements such as the European Water Framework Directive (WFD; Council of the European Communities, 2000) or the Habitats Directive (Council of the European Communities, 1992). Most of the monitoring approaches apply classical field-based techniques (Geist, 2015; Hering, Moog, Sandin, & Verdonshot, 2004; reviewed by Birk et al., 2012). However, classical monitoring methods for biodiversity assessment and freshwater conservation, such as electrofishing, gill netting, or kick sampling, often include expensive and labour-intensive capturing (Mueller, Pander, Knott, & Geist, 2017) that can adversely affect the target organisms that are being surveyed, either by direct impact on these species through sampling or by disturbance of their habitats (Hering et al., 2018).

Environmental DNA (eDNA) techniques are gaining increasing importance in ecological and conservation sciences (Bohmann et al., 2014; Corlett, 2017; Lawson Handley, 2015; Leese et al., 2016; Rees, Maddison, Middleditch, Patmore, & Gough, 2014; Thomsen & Willerslev, 2015). They provide a fast, non-destructive, and economic alternative to classical surveys of metazoan biodiversity patterns (Hänfling et al., 2016), species presence-absence (Dejean et al., 2012; Jerde, Mahon, Chadderton, & Lodge, 2011; Pilliod, Goldberg, Arkle, & Waits, 2013), and for biodiversity or species distribution monitoring (Thomsen et al., 2012).

'Microbial eDNA' techniques refer to various molecular approaches in which shed intra- or extracellular metazoan DNA (eDNA) is extracted from soil, air, or water. Subsequently, the genetic material obtained is amplified and then analysed, or analysed directly. Two different approaches are applied: either detection of specific species using specific primers ('target species approach'; Ficetola, Miaud, Pompanon, & Taberlet, 2008); or a complete assessment of the species or taxon diversity within a given ecosystem or habitat using either non-specific or taxonomic group-specific primers, or direct DNA metabarcoding ('eDNA metabarcoding'; Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012).

eDNA techniques provide non-destructive alternatives to classical survey methods, being most frequently assayed or implemented in aquatic environments (Deiner et al., 2017; Evans & Lamberti, 2018; Roussel, Paillisson, Tréguier, & Petit, 2015). For species presence-absence data, eDNA methods have been shown to outperform traditional biodiversity surveys (Hänfling et al., 2016; Pilliod et al., 2013). Analogously, for detecting species richness, with its associated sampling costs and survey time, eDNA metabarcoding can be superior over

classical methods, sometimes by several orders of magnitude (Boussarie et al., 2018; Sigsgaard et al., 2017). Part of the reason is that only water samples need to be collected for analyses instead of directly sampling or observing the target species. Owing to the sensitivity of eDNA detection and the greater chance of overlooking rare species in their habitat by classical surveys, it also typically results in greater species richness estimates than conventional surveys. eDNA methods as an addition to classical sampling help to obtain more accurate estimates relevant for species conservation; for example, by elucidating 'dark' diversity (or 'hidden species richness'), microhabitat use, or the influence of human disturbance on dispersal (Boussarie et al., 2018). Some even suggest entirely replacing classical monitoring approaches by the sole use of eDNA techniques for biomonitoring (Bourlat et al., 2013; Leese et al., 2016). This approach led to a highly controversial discussion, especially concerning quantitative estimates derived from eDNA techniques to supplement or to replace classical monitoring (Leese et al., 2018; Rees, Gough, Middleditch, Patmore, & Maddison, 2015; Rees, Maddison, et al., 2014; Roussel et al., 2015). The strong and increasing interest in eDNA is also reflected in the large number of reviews, opinion papers, and letters published in the field of freshwater eDNA (collated by Coble et al., 2019). To date, most of these papers address how eDNA can be used to detect biodiversity throughout different environments (Bohmann et al., 2014; Pedersen et al., 2015; Rees, Maddison, et al., 2014). There is a wealth of systematic reviews that summarize and focus on the methodology of eDNA techniques in fresh water (Goldberg, Strickler, & Pilliod, 2015), eDNA persistence and transport in aquatic environments (Barnes et al., 2014), the possible use of eDNA techniques in detecting aquatic invasive species (Darling, 2015; Darling & Mahon, 2011), the potential of eDNA techniques in ecology and wildlife monitoring (Barnes & Turner, 2016; Bohmann et al., 2014), in palaeolimnology (Domaizon, Winegardner, Capo, Gauthier, & Gregory-Eaves, 2017), in the use of eDNA metabarcoding in surveying plant and animal communities (Deiner et al., 2017), as well as in determining the environmental factors that affect the success of eDNA detection (Stoeckle et al., 2017). Other pieces of work outline the potential use of eDNA metabarcoding in biological assessments or biomonitoring of aquatic ecosystems (Macher et al., 2018; Mächler et al., 2019; Pawlowski et al., 2018), and possibilities for its application to ecological status assessment under the WFD (Hering et al., 2018). Fewer reviews or comments specifically address critical issues concerning the implementation of eDNA methods in general (Roussel et al., 2015), and particularly the standardization of sampling designs (Dickie et al., 2018; Wilcox et al., 2018), as well as downstream laboratory and data analysis methods (Goldberg et al., 2016).

Considering the many reviews already published about the applicability of eDNA techniques, it is most surprising that, to the best of our knowledge, the aspect of the general applicability of eDNA techniques

in the context of freshwater conservation has rarely been addressed (but see Harper, Buxton et al., 2019; Harper, Lawson et al., 2019). Knowledge of the current application and implementation of eDNA techniques in fresh water is crucial to assess potential gaps and biases, and to recommend future development in the context of biological conservation. Obtaining important knowledge and insights in assessing the representation of specific research output (taxonomically and in terms of geographical representation) has been shown already in other areas—for example, invasion biology (Jeschke et al., 2012; Pysek et al., 2008), biodiversity sciences and taxonomy (Cardoso, Erwin, Borges, & New, 2011; Clark & May, 2002; Troudet, Grandcolas, Blin, Vignes-Lebbe, & Legendre, 2017), or for other specific molecular techniques (e.g. conservation genetics; Pérez-Espona, 2017).

This article aims to guide the future direction of eDNA research in freshwater conservation by (a) briefly reviewing the research development over time by systematically analysing the objectives of published eDNA research focusing on freshwater environments, (b) assessing the representation of different habitat types and the geographical regions covered, and (c) using two approaches to investigate the representation of taxonomic groups and potential taxonomic biases.

2 | METHODS

To obtain an overview of the history and the current use of eDNA techniques in the context of freshwater conservation, a literature database search was conducted, covering the period 2005–2018. The online publication databases Web of Science Core Collection (www.webofknowledge.com), Scopus (www.scopus.com), and PubMed (www.ncbi.nlm.nih.gov/pubmed) were initially searched using the keywords and search strings given in Supporting information Table S1. Publications solely considering microbial organisms or prokaryotic biodiversity were subsequently excluded, as well as those articles on marine and terrestrial environments. Other papers not covered by the database queries were identified from screening the reference lists of publications found through the keyword literature search (Supporting information Table S2). Some studies may not have been identified because of the use of different discipline-specific keywords—for example, 'ecogenomics' for elucidating micro- and microbial communities under stress (Xie et al., 2017; Xie et al., 2017), 'surface water contamination' (Schill & Mathes, 2008), or 'molecular scatology' (Thalinger et al., 2016). Some new studies may also have been missed owing to the time lag in uploading to literature databases.

The following information was extracted from each publication: authors, title, year of publication, journal, type of article (e.g. review or primary research), objective(s), geographical region, and taxonomic group(s) investigated. A trend analysis of the annual increase in publication numbers and their focus (taxonomic group and target [i.e. original paper or review]) was conducted using the R package *bibliometrix* version 1.9.3. (Aria & Cuccurullo, 2017). Mann–Kendall trend tests, in the R package 'trend' (Pohlert, 2018), were used to test for significance.

Subsequently, the collated articles were read in full and initially classified as 'primary research articles' or 'non-primary research

articles'. 'Non-primary research articles' comprised reviews, letters, comments, opinions, views, and introductions to special journal issues that summarized, discussed, or analysed data from studies already published on aquatic macro-organismic eDNA research. To avoid data duplication, 'non-primary research articles' were therefore excluded from further analysis.

For evaluating the research content, the primary research publications were categorized according to their general aim addressing: (a) biodiversity assessments using the 'eDNA metabarcoding' approach (*sensu* Taberlet et al., 2012; Deiner et al., 2017) with universal or group-specific primers targeting the expected overall metazoan biodiversity or a specific taxonomic group in the respective habitat; (b) 'endangered species' or (c) 'invasive species' research; or (d) 'methodological development', referring to publications focused on methodological aspects of eDNA sampling, eDNA persistence, laboratory methods including inhibition, and further downstream analysis without direct application to biodiversity conservation; and (e) 'other articles'. 'Other articles' comprised work indirectly relevant either for freshwater biodiversity or freshwater species using eDNA methods, but without directly probing the freshwater environment (e.g. analyses of ethanol preservative for aquatic macroinvertebrate specimens by DNA metabarcoding (Erdozain et al., 2019; Hajibabaei, Spall, Shokralla, & van Konynenburg, 2012; Zika, Leese, Peinert, & Geiger, 2019), or slightly violating the rule of not directly capturing any organisms for eDNA analysis (e.g. incorporating aquatic organism disease monitoring on captured specimens, not water samples).

The habitats investigated were categorized according to: (a) lentic; (b) lotic; (c) sediments; (d) artificial ecosystems, such as mesocosms, aquaria, or research ponds; and (e) 'others'—for example, ballast water and bait shop tanks, drinking water holes, and preservatives from aquatic macro-organism bulk samples (Supporting information Table S3).

To assess the taxonomic groups investigated, and whether there is any taxonomic bias in the eDNA methods applied to freshwater conservation and research, the major taxonomic groups targeted in the current eDNA literature were classified according to species/order/class/phylum. To elucidate the taxonomic bias in eDNA primary research, the freshwater species in research papers using a target species approach were recorded. Subsequently, the number of eDNA studies per taxonomic group and the proportion of the number of freshwater species (species richness) per taxonomic group covered by eDNA research were compared with the freshwater species richness described for the respective taxonomic group and the groups' relative abundances. Both values were extracted from Balian et al. (2010).

3 | RESULTS

3.1 | Number of publications and trends

The literature database keyword search resulted in 1,480 (Web of Sciences Core Collection), 1,214 (Scopus), and 864 (PubMed) articles that indexed 'environmental DNA' or 'eDNA' either in the title,

abstracts, or keywords. Overall, 13% of the articles retrieved were published in concurrence with the keyword 'conservation', and, on average, 400 articles (34%) of all publications targeted or included aquatic environments (14% 'freshwater', 16% 'marine'; Supporting information Table S1). Surprisingly, only one publication (Harper, Buxton, et al., 2019) was returned from Scopus and none from either Web of Sciences or PubMed when querying 'environmental DNA' (eDNA) AND 'aquatic conservation'. Likewise, only one publication (Bellemain et al., 2016) was returned from Scopus and Web of Sciences, and none from PubMed, when querying 'environmental DNA' (eDNA) AND 'freshwater conservation'. After skimming all abstracts, 272 publications were collated on metazoan freshwater eDNA from 2005 to 2018 that met the selected criteria, covering primary research articles, reviews, letters, opinions, notes, and comments (Figure 1, Supporting information Tables S2, S3).

The total number of publications since 2005 increased exponentially ($y = 0.3367 \cdot e^{0.5118x}$; Pearson $R^2 = 0.98$), with a maximum of 77 papers in 2017 on eDNA in freshwater research, and an average increase in the annual publication rate of 51% between 2005 and 2018 (Figure 1a, b). This pattern was even more pronounced for freshwater fishes, the taxonomic group most studied. The research output on freshwater fish eDNA also increased exponentially ($y = 1.0866 \cdot e^{0.7185x}$; Pearson $R^2 = 0.99$) with an average increase in the annual publication rate of 72% (Figure 1b). For the two other major subgroups— amphibians (annual average increase 45%) and 'non-primary research articles' (56%)—the pattern was less pronounced, but the scientific output on these also increased significantly over the years (all Mann–Kendall tests $P < 0.001$).

By far the majority (87%) of all publications were primary research articles, whereas non-primary research articles (reviews, letters, comments, opinions, views, and introductions) comprised only 13% of the dataset (Supporting information Table S3).

3.2 | Objectives of the studies

The most common objective of all primary research publications in the dataset (57%) was conservation science, in terms of (a) invasive species (86 articles) and (b) endangered or rare species (67 articles) (Figure 2a, Supporting information Table S3). Primary research output with a focus on advancing or elucidating freshwater eDNA methodological development comprised 61 studies (23% of the total). Methods included the development of filtering, storage, and extraction methods (Minamoto, Naka, Moji, & Maruyama, 2016), or identifying possible sources of field cross-contamination (Merkes, McCalla, Jensen, Gaikowski, & Amberg, 2014). Of the primary research articles, more than 80% (203 studies) used a target species detection approach, whereas a small but annually increasing proportion of studies used eDNA metabarcoding (30 studies, 11%; Figure 2a). The objectives of the remaining 24 articles comprised eDNA techniques as diverse as food web analyses ('molecular scatology', Koizumi et al., 2016; Thalinger et al., 2016), or the detection of 'surface water contamination' (Martellini, Payment, & Villemur, 2005; Schill & Mathes, 2008).

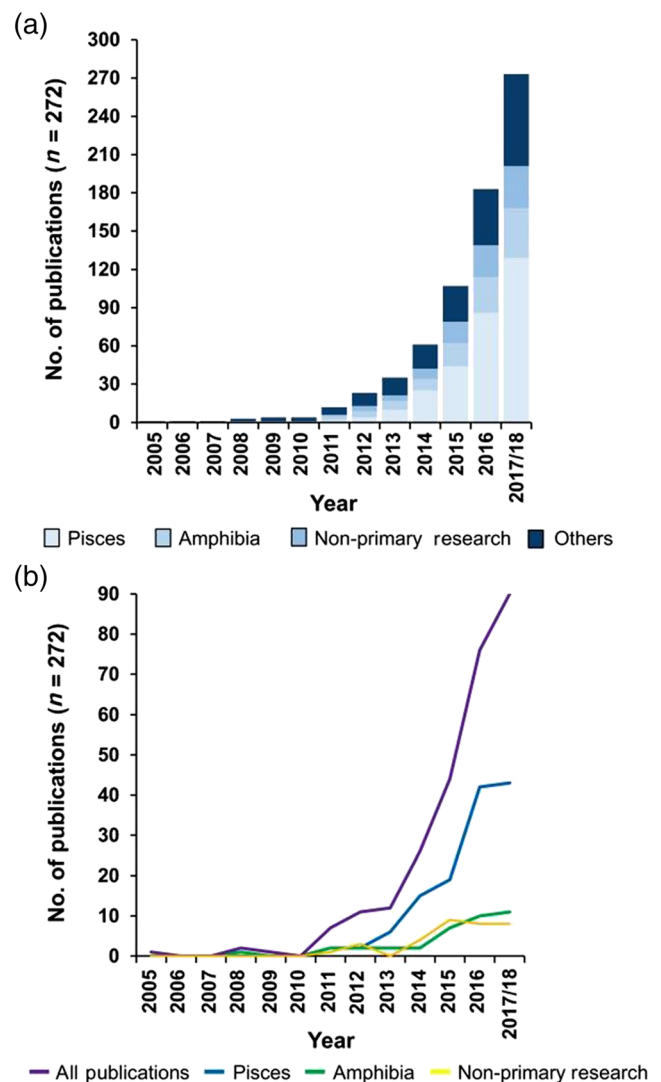


FIGURE 1 (a) Cumulative number and (b) annual trend curves of freshwater environmental DNA (eDNA) studies using metazoan organisms ('macrobial freshwater eDNA') from 2005 to 2018

3.3 | Representation of habitats

Of the 238 field studies, 77% were performed in lentic (35%) and lotic (42%) habitats (Figure 2b, Supporting information Table S3). The lotic environments investigated included habitats as diverse as small headwater streams (Baldigo, Sporn, George, & Ball, 2017) and small brooklets (Katano, Harada, Doi, Souma, & Minamoto, 2017), up to complete river catchments, such as the Cuyahoga River in Ohio, USA (Cannon et al., 2016), the River Glatt catchment in Switzerland (Deiner, Fronhofer, Machler, Walser, & Altermatt, 2016), or the Mekong River in South East Asia (Bellemain et al., 2016). The lentic habitat diversity ranged from phytothelmata (Brozio et al., 2017) and small ponds (Mauvisseau et al., 2018) to the North American Great Lakes in the USA and Canada (Jerde et al., 2013), and artificial and highly impaired freshwater reservoirs in Singapore (Lim et al., 2016). Artificial ecosystems like mesocosms were used in 45 studies (16%). Various sediments as an additional 'habitat' for eDNA in mesocosms

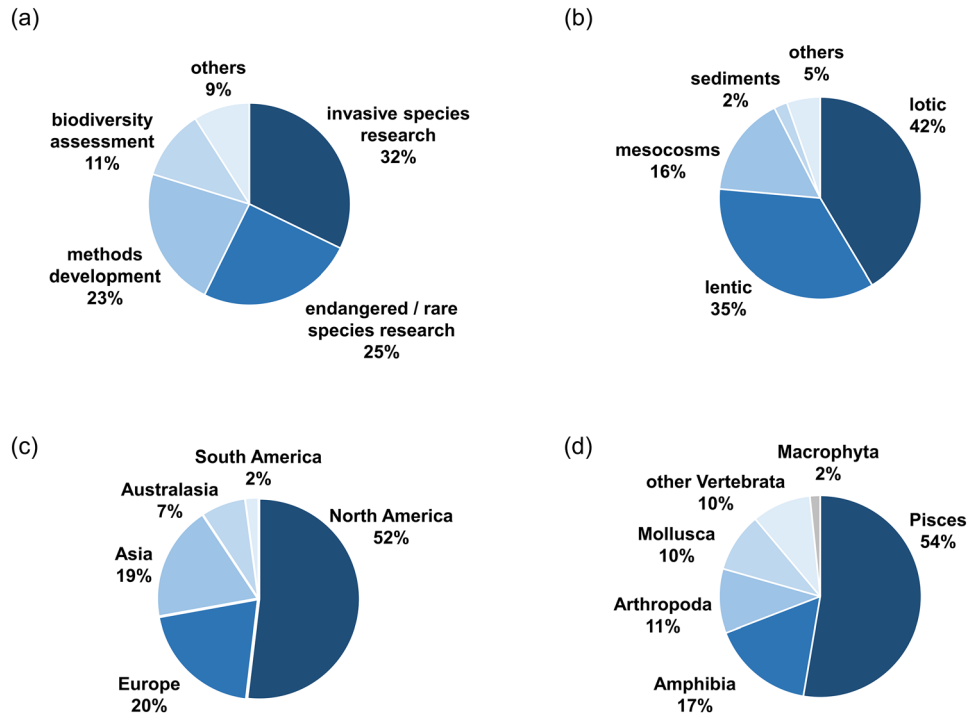


FIGURE 2 The percentage of primary research articles using macrobial environmental DNA to detect the presence of freshwater species according to (a) objective, (b) habitats (ecosystem types), (c) geographical region (geographical distribution of ecological studies per continent), and (d) target species (order/class/phylum level) used. If more than one objective, continent, freshwater ecosystem type, or target species was included in an article, the respective variable was counted as many times as it occurred to produce these charts. For the true number of articles, see Supporting information Table S3

and lentic or lotic systems were investigated in seven (2%) of the studies (Figure 2b, Supporting information Table S3). Subterranean or groundwater habitats (the overall predominant freshwater habitats worldwide) were investigated in two studies in the dataset (Niemiller et al., 2018; Vörös, Marton, Schmidt, Gal, & Jelic, 2017; see also Supporting information Table S2).

3.4 | Representation of geographic regions

More than 90% of all primary research papers described work undertaken in North America (52%, 123 studies), Europe (20%, 48 studies), and Asia (19%, 44 studies) (Figure 2c, Supporting information Table S2). With almost 70% (30 studies) of the Asian studies performed, Japan is the most investigated geographical area of this continent (Table S2). Australasia (17 studies) and South America (six studies) contributed 9% to the research output (e.g. Brozio et al., 2017; Lopes et al., 2017; Robson et al., 2016; Simpfendorfer et al., 2016) (Figure 2c), but there were no contributions from Africa (Figure 2c).

There is great potential for further expansion of eDNA studies in the tropics, as more than 75% (179 studies) of the research papers concerned temperate to subtropical zones either in the Northern or Southern Hemisphere (Table S2). The exceptions are the studies conducted in northern Australia (e.g. Robson et al., 2016), as well

as a few in South East Asia (Bellemain et al., 2016; Wilson, Sing, Chen, & Zieritz, 2018), and the first example of eDNA metabarcoding in monitoring tropical freshwater reservoirs in Singapore (Lim et al., 2016).

3.5 | Taxonomic group composition and potential taxonomic bias

Fish species were the most frequently investigated taxa (54%, 128 studies; Figure 2d). Studies were focused either on detecting particular species (Boothroyd, Mandrak, Fox, & Wilson, 2016; Clusa & García-Vázquez, 2018) or on analysing taxon richness (Hänfling et al., 2016), or they used fish as model organisms to address methodological issues (Barnes et al., 2014; Stoeckle et al., 2017). Amphibians (40 articles; e.g. Biggs et al., 2015; Ficetola et al., 2008) and freshwater arthropods (25 studies; e.g. Doi et al., 2017; Mächler, Deiner, Steinmann, & Altermatt, 2014) were the second- and third-most investigated taxa, with a surprisingly high percentage of the studies (10%) targeting freshwater molluscs (e.g. Currier, Morris, Wilson, & Freeland, 2018; Stoeckle, Kuehn, & Geist, 2016) (Figure 2d, Supporting information Table S3). Each of the other groups contributed less than 10% to the research output (Figure 2d).

The primary research articles using a target species approach investigated 123 freshwater fish species, 29 amphibian species, and

28 mollusc species. Freshwater mammals (four species), trematodes (two species), and fungi (two disease species) were the least investigated groups (Supporting information Table S2). With 23 articles published on each of the invasive common and Asian carp species, *Cyprinus carpio* Linnaeus, 1758 and *Hypophthalmichthys molitrix* (Valenciennes, 1844)/*Hypophthalmichthys nobilis* (Richardson, 1845) (e.g. Mahon et al., 2013; Wilson et al., 2014), these were the best investigated research organisms, followed by the bluegill sunfish (*Lepomis macrochirus* Rafinesque, 1819; e.g. Evans et al., 2016; Takahara, Minamoto, & Doi, 2013). Apart from amphibians, where the red-listed great crested newt, *Triturus cristatus* (Laurenti, 1768), was investigated in six studies (e.g. Rees et al., 2014), invasive species were the research organisms most frequently targeted in all other major taxonomic groups: for molluscs, the zebra mussel, *Dreissena polymorpha* (Pallas, 1771) (eight studies), quagga mussel, *Dreissena bugensis* (Andrusov, 1897) (seven studies), and Asian clam, *Corbicula fluminea* (O.F.Müller, 1774) (three studies); and for arthropods, red swamp crayfish, *Procambarus clarkii* (Girard, 1852) (six studies), signal crayfish, *Pacifastacus leniusculus* (Dana, 1852) (four studies), and the amphipod *Gammarus pulex* (Linnaeus, 1758) (three studies) (e.g. Ardura, Zaiko, Borrell, Samuiloviene, & Garcia-Vazquez, 2017; Clusa, Miralles, Basanta, Escot, & Garcia-Vazquez, 2017; Deiner, Walser, Machler, & Altermatt, 2015; Harper, Turnbull, Bean, & Leaver, 2018; Mauvisseau et al., 2018).

Taking into account the relative estimated number of freshwater species in relation to the number of eDNA studies (Figure 3a), and the investigated number of species in each taxonomic group (Figure 3b), eDNA in different vertebrates—especially reptiles, mammals, and fish species—was more often investigated than in all other taxonomic groups (Figure 3a, b). For invertebrate species, molluscs were the most investigated group in relation to their estimated freshwater species richness, whereas aquatic insect species were the least represented and investigated taxonomic group (Figure 3a, b). The number of eDNA studies of freshwater crustaceans was more balanced compared with their relative species richness in freshwater ecosystems (Figure 3a). In contrast, the number of species investigated in relation to the total estimated crustacean species richness was low (Figure 3b). Freshwater macrophytes showed the same investigated ratio of species/total species number as the amphibian species investigated (Figure 3b).

4 | DISCUSSION

The increasing popularity of eDNA in biodiversity monitoring demonstrated in this study calls for a critical review on the trends, applications, and geographical and taxonomic representation of this technique in the context of freshwater conservation. The study showed a strong bias towards specific species and taxonomic groups, such as freshwater fish, both monitoring rare and endangered species as well as invasive alien species, but a more balanced representation of studies from lentic and lotic habitats. The relatively large number of studies addressing methodological issues and 'artificial ecosystems', such as mesocosms,

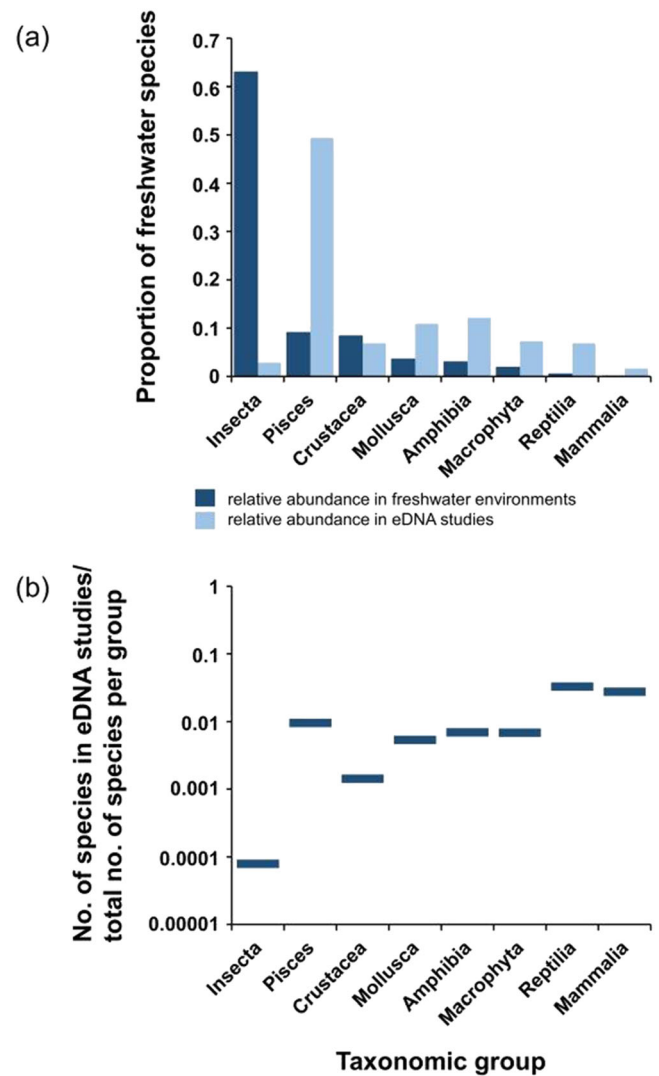


FIGURE 3 (a) Taxonomic representation of environmental DNA (eDNA) studies (light blue bars) in different taxonomic groups in relation to their relative abundance in freshwater environments (dark blue bars). The overall freshwater species richness and relative abundance follow Balian et al. (2010). The relative abundance in eDNA studies considers the number of species investigated in target-species freshwater eDNA studies per taxonomic group. (b) Ratio of the number of species considered in target-species freshwater eDNA studies to the overall number of known species for different taxonomic groups. If the ratio approaches 1, the relative abundance of species studied equals the relative abundance of the respective taxonomic group. Note that the y-axis is log-transformed. Only two species of freshwater fungi and two species of parasitic trematodes were investigated using eDNA techniques. Both were excluded from the diagram owing to uncertain freshwater species estimates in those two taxonomic groups

reveals the initial need for tackling fundamental challenges such as inhibition and contamination before reaching more harmonized and standardized approaches. The results showed a distinct gap in studies in the tropics in general, and on the African continent in particular, suggesting that regions with exceptionally high freshwater biodiversity have not yet been sufficiently targeted by eDNA monitoring.

The finding of only a single paper for both search terms 'environmental DNA' and 'freshwater conservation' (Bellemain et al., 2016) indicates that researchers publishing papers on eDNA may fail to recognize the significance of their work to freshwater conservation. In addition, some of the topics reviewed, such as the methodological development in invasive species research, may not always be carried out with regard to their impact on native species and their conservation as the primary motivation. However, there are a few studies where the use of eDNA was clearly related to meeting a conservation objective (Bellemain et al., 2016; Harper, Buxton, et al., 2019). Harper, Buxton, et al. (2019) recommended a broad standardization of eDNA workflows in order to ensure more robust, comparable, and ecologically meaningful data for pond biodiversity. The development of standard protocols is essential for including eDNA in effective management and conservation yet is also needed across habitat types.

eDNA methods have been used most frequently for invasive species research, followed by endangered or rare species monitoring. The observed focus on endangered and rare species can be explained by the fundamental interest in mapping and understanding distribution patterns as basic information in conservation sciences (Stewart, Ma, Zheng, & Zhao, 2017; Tucker et al., 2005; Yoccoz, Nichols, & Boulinier, 2001). Knowledge about the distribution and abundance of a species is a prerequisite for conservation management (Gaston, 2010; Heywood, 2011), yet endangered species are often rare, with small population sizes and patchy distribution patterns. They may also react sensitively to human disturbance, and therefore are difficult to detect or monitor with classical survey methods (Schill & Galbraith, 2019; Sigsgaard et al., 2017; Sigsgaard, Carl, Møller, & Thomsen, 2015). Given the sensitivity of species detection using eDNA, this technique can be an important supplementary tool for endangered species monitoring (Biggs et al., 2015; Itakura et al., 2019; Stoeckle et al., 2016; Thomsen et al., 2012), especially in deep and turbid aquatic habitats (Bellemain et al., 2016).

The value of eDNA techniques in endangered species and freshwater conservation can be illustrated using the example of freshwater mussels, as these belong to the most threatened groups in fresh water (Lopes-Lima et al., 2017) and are considered target species for conservation (Geist, 2010, 2015). Even monitoring the adults in populations of endangered European freshwater pearl mussel, *Margaritifera margaritifera* (Linnaeus, 1758), can be extremely difficult, as this species also occurs in large and fast-flowing rivers where access is difficult (Boon et al., 2019). With many captive breeding programmes for the species being in place in Europe (Gum, Lange, & Geist, 2011) checks are needed to determine whether the release of juvenile mussels into stream sections previously without mussels has been successful. As juvenile mussels burrow into the substrate, their detectability with classical habitat survey methods is low and in certain countries such as Sweden is not permitted owing to the potential destruction of their habitat by monitoring (Boon et al., 2019). eDNA protocols are available for this species that can be particularly useful in screening larger rivers for the occurrence of mussels as well as for the detection of juveniles (Stoeckle et al., 2016), both providing useful information for conservation. On the other hand, limitations of eDNA

methodology must be considered, such as the lack of information that it provides on population size and demography (Stoeckle et al., 2016) as well as the possible release of DNA from dead shell material (Geist, Wunderlich, & Kuehn, 2008). Consequently, a combination of eDNA as a screening tool with detailed field survey work can contribute to more effective monitoring and conservation in freshwater mussels.

There are several possible reasons for the large number of eDNA studies focusing on invasive species research. First, as well as the known ecosystem impacts, invasive species can cause severe economic damage, as evident, for example, from work on zebra mussels and quagga mussels (Connelly, O'Neill, Knuth, & Brown, 2007; Vanderploeg et al., 2002) and on the Asian carp species in the Mississippi and Laurentian Great Lakes (Pimentel, 2005; Pimentel, Zuniga, & Morrison, 2005). Second, the control and mitigation of biological invasions are most effective during the early stages of invasion, especially before establishment, during transport, introduction, and naturalization (Hulme, 2006; Simberloff et al., 2013). In these early stages, the abundance of invasive species is low; and because of small population sizes, these species are often not detectable with classical methods. Because of the high sensitivity, and subsequently the possibility to detect low individual abundances, or even single individuals, eDNA techniques are increasingly suggested and used as an important tool to monitor biological invasions (Darling & Mahon, 2011; Dejean et al., 2012; Egan et al., 2015).

Both lentic and lotic systems are the surface freshwater habitats that are most frequently biomonitoring in regions where legal frameworks, such as the WFD and Habitats Directive in Europe (Council of the European Communities, 1992, 2000) and the Endangered Species Act and National Invasive Species Act in North America (Department of the Interior US Fish and Wildlife Service, 1973; US Congress, 1990), make regular biological monitoring a necessity. Environmental agencies and government bodies often rely on well-established monitoring methods and, to date, mostly use and accept eDNA approaches for specific targets such as the monitoring of invasives only (Laramie, Pilliod, Goldberg, & Strickler, 2015). The relatively large proportion of studies addressing methodological development highlights a continuing need for further adjustment, validation, and optimization of eDNA techniques, from sampling through to laboratory detection, in the great variety of lentic and lotic habitats that all differ in their environmental conditions, such as water chemistry. Although eDNA provides an efficient tool for detecting species composition, it has its limitations, such as the lack of reliable abundance assessments (Leese et al., 2018; Stoeckle et al., 2016). In the context of most monitoring programmes, such as the European WFD (Council of the European Communities, 2000), both species composition and abundance need to be recorded, limiting the universal applicability of using eDNA assessments exclusively (Leese et al., 2018). An additional challenge is the use of eDNA in rivers as opposed to lakes, where it is more difficult to know the location from which the detected eDNA originated (Deiner et al., 2016; Macher & Leese, 2017; Song, Small, & Casman, 2017; Wilcox et al., 2018).

The observed geographic bias towards Northern Hemisphere temperate zones can be partly explained by the greater number and

execution of existing legal frameworks in these regions requiring aquatic biomonitoring programmes. The eDNA research need in the tropics and African countries probably results from socioeconomic constraints and the lack of the required infrastructure in many of these countries, which makes it difficult to implement eDNA studies, or biodiversity studies in general (Di Marco et al., 2017; Leese et al., 2018; Sodhi, Koh, Brook, & Ng, 2004; Waldron et al., 2013). So far, most of the eDNA research conducted in the tropics is restricted to areas where the necessary infrastructure is in place—for example, northern Australia (Robson et al., 2016) and Singapore (Lim et al., 2016). The lack of taxonomic resolution (Gaston, 1994) or expertise (Gaston & May, 1992), and therefore incomplete species or DNA reference databases, also hampers the development of eDNA techniques in most biodiversity-rich tropical countries in Africa and South America. The under-representation of eDNA studies from areas that are likely hotspots of aquatic biodiversity (and at the same time of aquatic habitat change and biodiversity decline) is alarming and should be given higher priority in future international research and development programmes. In addition, a systematic assessment of diversity between morphotaxonomists and DNA barcoders is needed here (Leese et al., 2018).

In line with the geographical bias, the taxonomic group representation and the research bias towards iconic vertebrate species evident from this study is pervasive in conservation sciences (Clark & May, 2002; Di Marco et al., 2017), and thus not surprising. It has previously been described by several other workers in associated research fields—for example, conservation genetics (Pérez-Espona, 2017) and invasion biology (Pysek et al., 2008). Following this general bias, freshwater fish are the taxa investigated most, as they represent iconic and socioeconomically important flagship species, many globally invasive species, and keystone species for ecosystem services (Geist, 2015). Given the importance of macroinvertebrates in biomonitoring (Hering et al., 2004), as invasive species (Connelly et al., 2007; Vanderploeg et al., 2002), and as emerging infectious disease vectors in a changing world climate (Conn, 2014; Schneider et al., 2016), it is surprising that more macroinvertebrate species are not investigated using eDNA methods. Biodiverse groups such as aquatic insects appear severely under-represented, also in regions where the legal framework requires monitoring of macroinvertebrates. For example, the saprobic system is based on tolerance values of certain known macroinvertebrate species (Rolauuffs, Hering, Sommerhäuser, Rödiger, & Jähnig, 2003), and, not just in Europe, freshwater macroinvertebrates are very important indicators in assessing water quality (e.g. as biological quality elements in the WFD; Council of the European Communities, 2000). Given the great richness of aquatic insect species, for many groups there is still the need to resolve the classical, integrative, or molecular taxonomy (e.g. aquatic Diptera/Psychodidae; Kvitte, 2018), and to enhance the respective DNA barcode reference databases (Elbrecht, Vamos, Meissner, Aroviita, & Leese, 2017; Leese et al., 2018). Furthermore, invertebrate species complexes that underwent rapid radiations resulting in incomplete genetic lineage sorting pose challenges to eDNA detection if using traditional mitochondrial DNA barcodes (Meier, Shiyang,

Vaidya, & Ng, 2006). In general, cryptic species, a lack of alpha taxonomy, and insufficient taxonomic knowledge may hamper the current progress, as well as the fact that genetic reference databases are exhaustive only for certain aquatic invertebrate groups—for example, freshwater bivalves or European Ephemeroptera, Plecoptera, and Trichoptera taxa (Hendrich et al., 2015; Moriniere et al., 2017; Zhou et al., 2016). The example of 85% coverage of North American freshwater invertebrate genera (biological quality elements) in public barcoding databases (Curry, Gibson, Shokralla, Hajibabaei, & Baird, 2018) shows the great potential for next-generation biomonitoring if public barcode libraries are exhaustive enough. In addition, the high genetic resolution obtained offers great potential. If standardized molecular operational taxonomic units (*sensu* Blaxter et al., 2005) are used for analysis, the effects of single or multiple stressors can be detected even for cryptic or undescribed species (Beermann et al., 2018; Macher et al., 2016).

5 | CONCLUSIONS

Analyses of eDNA are of increasing importance and relevance for freshwater monitoring and conservation. To date, there is no universally applicable methodology for freshwater eDNA techniques. Many taxonomic groups and large geographical areas have not yet been investigated or assayed with eDNA techniques. Nevertheless, in conservation, biodiversity assessment, and management programmes, the application of 'traditional' genetic methods is already widely accepted, and genetic information is explicitly included in conservation decision making (Geist, 2011), as suggested by the Convention on Biological Diversity, Article 2 (United Nations, 1992). The increasing development and recent technical progress in the field of eDNA research is likely to lead to the increasing use of eDNA techniques in biomonitoring, invasion biology, and aquatic conservation. Harmonization of eDNA techniques and data analyses are needed if this method is to be applied routinely and reproducibly for the different taxonomic groups in different geographical regions, and in the various habitats encountered in freshwater environments worldwide. This becomes most important if eDNA methods are integrated within existing legal monitoring frameworks. In regions that are considered biodiversity hotspots and where rapid habitat change and biodiversity loss are likely, biological monitoring may benefit from using eDNA approaches as an initial and comparatively rapid step for determining species occurrences and distributions instead of laborious and expensive classical monitoring (Bálint et al., 2018). To help with this, the development of new European standards produced by the European Committee for Standardization for the emerging field of eDNA-based biomonitoring have been proposed and are currently in preparation (F. Leese, personal communication, April 2019).

Equally important, and also neglected in many other research areas, negative results or failure of certain eDNA methods should also be published to provide a realistic picture of the possibilities and pitfalls of this technique in freshwater conservation.

ACKNOWLEDGEMENTS

We would like to thank the Chief Editor, Professor Philip J Boon, as well as Dr Bernd Hänfling and Professor Florian Leese for their helpful comments on an earlier version of this paper.

ORCID

Christina C. Belle  <https://orcid.org/0000-0001-8811-6283>

Bernhard C. Stoeckle  <https://orcid.org/0000-0001-7109-0048>

Juergen Geist  <https://orcid.org/0000-0001-7698-3443>

REFERENCES

- Ardura, A., Zaiko, A., Borrell, Y. J., Samuiloviene, A., & Garcia-Vazquez, E. (2017). Novel tools for early detection of a global aquatic invasive, the zebra mussel *Dreissena polymorpha*. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 27, 165–176. <https://doi.org/10.1002/aqc.2655>
- Aria, M., & Cuccurullo, C. (2017). bibliometrix: An R-tool for comprehensive science mapping analysis. *Journal of Informetrics*, 11, 959–975. <https://doi.org/10.1016/j.joi.2017.08.007>
- Baldigo, B. P., Sporn, L. A., George, S. D., & Ball, J. A. (2017). Efficacy of environmental DNA to detect and quantify brook trout populations in headwater streams of the Adirondack Mountains, New York. *Transactions of the American Fisheries Society*, 146, 99–111. <https://doi.org/10.1080/00028487.2016.1243578>
- Balian, H. I., Butchart, S., Chambers, P., Cordeiro, J., Cumberlidge, N., de Moor, F., ... Yeo, D. (2010). A wealth of life: Species diversity in freshwater systems. In R. A. Mittermeier, T. Farrell, I. J. Harrison, A. J. Upgren, & T. Brooks (Eds.), *Freshwater: The essence of life* (pp. 50–89). China: Earth in Focus Editions.
- Bálint, M., Nowak, C., Márton, O., Pauls, S. U., Wittwer, C., Aramayo, J. L., ... Jansen, M. (2018). Accuracy, limitations and cost efficiency of eDNA-based community survey in tropical frogs. *Molecular Ecology Resources*, 18, 1415–1426. <https://doi.org/10.1111/1755-0998.12934>
- 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>
- Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, 48, 1819–1827. <https://doi.org/10.1021/es404734p>
- Beermann, A. J., Elbrecht, V., Karnatz, S., Ma, L., Matthaei, C. D., Piggott, J. J., & Leese, F. (2018). Multiple-stressor effects on stream macroinvertebrate communities: A mesocosm experiment manipulating salinity, fine sediment and flow velocity. *Science of the Total Environment*, 610–611, 961–971. <https://doi.org/10.1016/j.scitotenv.2017.08.084>
- Belleman, E., Patricio, H., Gray, T., Guegan, F., Valentini, A., Miaud, C., & Dejean, T. (2016). Trails of river monsters: Detecting critically endangered Mekong giant catfish *Pangasianodon gigas* using environmental DNA. *Global Ecology and Conservation*, 7, 148–156. <https://doi.org/10.1016/j.gecco.2016.06.007>
- Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R. A., ... Dunn, F. (2015). Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation*, 183, 19–28. <https://doi.org/10.1016/j.biocon.2014.11.029>
- Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., ... Hering, D. (2012). Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. *Ecological Indicators*, 18, 31–41. <https://doi.org/10.1016/j.ecolind.2011.10.009>
- Blaxter, M., Mann, J., Chapman, T., Thomas, F., Whitton, C., Floyd, R., & Abebe, E. (2005). Defining operational taxonomic units using DNA barcode data. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360, 1935–1943. <https://doi.org/10.1098/rstb.2005.1725>
- 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 & Evolution*, 29, 358–367. <https://doi.org/10.1016/j.tree.2014.04.003>
- Boon, P. J., Cooksley, S. L., Geist, J., Killeen, I. J., Moorkens, E. A., & Sime, I. (2019). Developing a standard approach for monitoring freshwater pearl mussel (*Margaritifera margaritifera*) populations in European rivers. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29, 1365–1379. <https://doi.org/10.1002/aqc.3016>
- Boon, P. J., & Pringle, C. M. (Eds.) (2009). *Assessing the conservation value of fresh waters: An international perspective*. Cambridge, UK: Cambridge University Press.
- Boothroyd, M., Mandrak, N. E., Fox, M., & Wilson, C. C. (2016). Environmental DNA (eDNA) detection and habitat occupancy of threatened spotted gar (*Lepisosteus oculatus*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 26, 1107–1119. <https://doi.org/10.1002/aqc.2617>
- Bourlat, S. J., Borja, A., Gilbert, J., Taylor, M. I., Davies, N., Weisberg, S. B., ... Obst, M. (2013). Genomics in marine monitoring: New opportunities for assessing marine health status. *Marine Pollution Bulletin*, 74, 19–31. <https://doi.org/10.1016/j.marpolbul.2013.05.042>
- Boussarie, G., Bakker, J., Wangenstein, O. S., Mariani, S., Bonnin, L., Juhel, J.-B., ... Mouillot, D. (2018). Environmental DNA illuminates the dark diversity of sharks. *Science Advances*, 4, eaap9661. <https://doi.org/10.1126/sciadv.aap9661>
- Brozio, S., Manson, C., Gourevitch, E., Burns, T. J., Greener, M. S., Downie, J. R., & Hoskisson, P. A. (2017). Development and application of an eDNA method to detect the critically endangered Trinidad golden tree frog (*Phytotriades auratus*) in bromeliad phytotelmata. *PLoS ONE*, 12, e0170619. <https://doi.org/10.1371/journal.pone.0170619>
- Cannon, M. V., Hester, J., Shalkhauser, A., Chan, E. R., Logue, K., Small, S. T., & Serre, D. (2016). In silico assessment of primers for eDNA studies using PrimerTree and application to characterize the biodiversity surrounding the Cuyahoga River. *Scientific Reports*, 6, 22908. <https://doi.org/10.1038/srep22908>
- Cardoso, P., Erwin, T. L., Borges, P. A. V., & New, T. R. (2011). The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation*, 144, 2647–2655. <https://doi.org/10.1016/j.biocon.2011.07.024>
- Clark, J. A., & May, R. M. (2002). Taxonomic bias in conservation research. *Science*, 297, 191–192. <https://doi.org/10.1126/science.297.5579.191b>
- Clusa, L., & García-Vázquez, E. (2018). A simple, rapid method for detecting seven common invasive fish species in Europe from environmental DNA. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28, 619–629. <https://doi.org/10.1002/aqc.2890>
- Clusa, L., Miralles, L., Basanta, A., Escot, C., & Garcia-Vazquez, E. (2017). eDNA for detection of five highly invasive molluscs. A case study in urban rivers from the Iberian Peninsula. *PLoS ONE*, 12, e0188126. <https://doi.org/10.1371/journal.pone.0188126>
- Coble, A. A., Flinders, C. A., Homyack, J. A., Penaluna, B. E., Cronn, R. C., & Weitemier, K. (2019). eDNA as a tool for identifying freshwater species in sustainable forestry: A critical review and potential future applications. *Science of the Total Environment*, 649, 1157–1170. <https://doi.org/10.1016/j.scitotenv.2018.08.370>

- US Congress (1990). Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990. Public Law 101-646.
- Conn, D. B. (2014). Aquatic invasive species and emerging infectious disease threats: A One Health perspective. *Aquatic Invasions*, 9, 383–390. <https://doi.org/10.3391/ai.2014.9.3.12>
- Connelly, N. A., O'Neill, C. R., Knuth, B. A., & Brown, T. L. (2007). Economic impacts of zebra mussels on drinking water treatment and electric power generation facilities. *Environmental Management*, 40, 105–112. <https://doi.org/10.1007/s00267-006-0296-5>
- Corlett, R. T. (2017). A bigger toolbox: Biotechnology in biodiversity conservation. *Trends in Biotechnology*, 35, 55–65. <https://doi.org/10.1016/j.tibtech.2016.06.009>
- Council of the European Communities (1992). Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora. *Official Journal of the European Communities: Legislation*, L206, 7–50.
- Council of the European Communities (2000). Directive 2000/60/EC on establishing a framework for community action in the field of water policy. *Official Journal of the European Communities: Legislation*, L327, 1–73.
- 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, 545–558. <https://doi.org/10.1002/aqc.2869>
- Curry, C. J., Gibson, J. F., Shokralla, S., Hajibabaei, M., & Baird, D. J. (2018). Identifying North American freshwater invertebrates using DNA barcodes: Are existing COI sequence libraries fit for purpose? *Freshwater Science*, 37, 178–189. <https://doi.org/10.1086/696613>
- Darling, J. A. (2015). Genetic studies of aquatic biological invasions: Closing the gap between research and management. *Biological Invasions*, 17, 951–971. <https://doi.org/10.1007/s10530-014-0726-x>
- Darling, J. A., & Mahon, A. R. (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>
- Deiner, K., Bik, H. M., Machler, E., Seymour, M., Lacoursiere-Roussel, A., Altermatt, F., ... Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, 26, 5872–5895. <https://doi.org/10.1111/mec.14350>
- Deiner, K., Fronhofer, E. A., Machler, E., Walser, J. C., & Altermatt, F. (2016). Environmental DNA reveals that rivers are conveyor belts of biodiversity information. *Nature Communications*, 7, 12544. <https://doi.org/10.1038/ncomms12544>
- Deiner, K., Walser, J. C., Machler, E., & Altermatt, F. (2015). Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation*, 183, 53–63. <https://doi.org/10.1016/j.biocon.2014.11.018>
- Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., & Miaud, C. (2012). Improved detection of an alien invasive species through environmental DNA barcoding: The example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology*, 49, 953–959. <https://doi.org/10.1111/j.1365-2664.2012.02171.x>
- Department of the Interior US Fish and Wildlife Service (1973). Endangered Species Act, 16 USC §§ 1531–1544.
- Di Marco, M., Chapman, S., Althor, G., Kearney, S., Besancon, C., Butt, N., ... Watson, J. E. M. (2017). Changing trends and persisting biases in three decades of conservation science. *Global Ecology and Conservation*, 10, 32–42. <https://doi.org/10.1016/j.gecco.2017.01.008>
- Dickie, I. A., Boyer, S., Buckley, H. L., Duncan, R. P., Gardner, P. P., Hogg, I. D., ... Weaver, L. (2018). Towards robust and repeatable sampling methods in eDNA-based studies. *Molecular Ecology Resources*, 18, 940–952. <https://doi.org/10.1111/1755-0998.12907>
- Doi, H., Katano, I., Sakata, Y., Souma, R., Kosuge, T., Nagano, M., ... Tojo, K. (2017). Detection of an endangered aquatic heteropteran using environmental DNA in a wetland ecosystem. *Royal Society Open Science*, 4, 170568. <https://doi.org/10.1098/rsos.170568>
- Domaizon, I., Winegardner, A., Capo, E., Gauthier, J., & Gregory-Eaves, I. (2017). DNA-based methods in paleolimnology: New opportunities for investigating long-term dynamics of lacustrine biodiversity. *Journal of Paleolimnology*, 58, 1–21. <https://doi.org/10.1007/s10933-017-9958-y>
- Egan, S. P., Grey, E., Olds, B., Feder, J. L., Ruggiero, S. T., Tanner, C. E., & Lodge, D. M. (2015). Rapid molecular detection of invasive species in ballast and harbor water by integrating environmental DNA and light transmission spectroscopy. *Environmental Science & Technology*, 49, 4113–4121. <https://doi.org/10.1021/es5058659>
- Elbrecht, V., Vamos, E. E., Meissner, K., Aroviita, J., & Leese, F. (2017). Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. *Methods in Ecology and Evolution*, 8, 1265–1275. <https://doi.org/10.1111/2041-210X.12789>
- Erdozain, M., Thompson, D. G., Porter, T. M., Kidd, K. A., Kreutzweiser, D. P., Sibley, P. K., ... Hajibabaei, M. (2019). Metabarcoding of storage ethanol vs. conventional morphometric identification in relation to the use of stream macroinvertebrates as ecological indicators in forest management. *Ecological Indicators*, 101, 173–184. <https://doi.org/10.1016/j.ecolind.2019.01.014>
- Evans, N. T., & Lamberti, G. A. (2018). Freshwater fisheries assessment using environmental DNA: A primer on the method, its potential, and shortcomings as a conservation tool. *Fisheries Research*, 197, 60–66. <https://doi.org/10.1016/j.fishres.2017.09.013>
- Evans, N. T., Olds, B. P., Renshaw, M. A., Turner, C. R., Li, Y. Y., Jerde, C. L., ... Lodge, D. M. (2016). Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. *Molecular Ecology Resources*, 16, 29–41. <https://doi.org/10.1111/1755-0998.12433>
- 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>
- Gaston, K. J. (1994). Spatial patterns of species description: How is our knowledge of the global insect fauna growing? *Biological Conservation*, 67, 37–40. [https://doi.org/10.1016/0006-3207\(94\)90006-X](https://doi.org/10.1016/0006-3207(94)90006-X)
- Gaston, K. J. (2010). Biodiversity. In N. S. Sodhi, & P. R. Ehrlich (Eds.), *Conservation biology for all* (pp. 27–44). Oxford, UK: Oxford University Press.
- Gaston, K. J., & May, R. M. (1992). Taxonomy of taxonomists. *Nature*, 356, 281–282. <https://doi.org/10.1038/356281a0>
- Geist, J. (2010). Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera* L.): A synthesis of conservation genetics and ecology. *Hydrobiologia*, 644, 69–88. <https://doi.org/10.1007/s10750-010-0190-2>
- Geist, J. (2011). Integrative freshwater ecology and biodiversity conservation. *Ecological Indicators*, 11, 1507–1516. <https://doi.org/10.1016/j.ecolind.2011.04.002>
- Geist, J. (2015). Seven steps towards improving freshwater conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 25, 447–453. <https://doi.org/10.1002/aqc.2576>
- Geist, J., Wunderlich, H., & Kuehn, R. (2008). Use of mollusc shells for DNA-based molecular analyses. *Journal of Molluscan Studies*, 74, 337–343. <https://doi.org/10.1093/mollus/eyn025>

- Goldberg, C. S., Strickler, K. M., & Pilliod, D. S. (2015). 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., ... Taberlet, P. (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>
- Gum, B., Lange, M., & Geist, J. (2011). A critical reflection on the success of rearing and culturing juvenile freshwater mussels with a focus on the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 21, 743–751. <https://doi.org/10.1002/aqc.1222>
- Hajibabaei, M., Spall, J. L., Shokralla, S., & van Konynenburg, S. (2012). Assessing biodiversity of a freshwater benthic macroinvertebrate community through non-destructive environmental barcoding of DNA from preservative ethanol. *BMC Ecology*, 12, 28. <https://doi.org/10.1186/1472-6785-12-28>
- Hänfling, B., Lawson Handley, L., Read, D. S., Hahn, C., Li, J. L., Nichols, P., ... Winfield, I. J. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology*, 25, 3101–3119. <https://doi.org/10.1111/mec.13660>
- Harper, K. J., Turnbull, J. F., Bean, C. W., & Leaver, M. J. (2018). Searching for a signal: Environmental DNA (eDNA) for the detection of invasive signal crayfish, *Pacifastacus leniusculus* (Dana, 1852). *Management of Biological Invasions*, 9, 137–148. <https://doi.org/10.3391/mbi.2018.9.2.07>
- Harper, L. R., Buxton, A. S., Rees, H. C., Bruce, K., Brys, R., Halfmaerten, D., ... 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>
- Harper, L. R., Lawson Handley, L., Hahn, C., Boonham, N., Rees, H. C., Lewis, E., Adams IP, Brotherton P, Phillips S, Hänfling, B. (2019). Testing ecological hypotheses at the pondscape with environmental DNA metabarcoding: A case study on a threatened amphibian. [bioRxiv, 278309](https://doi.org/10.1101/278309). <https://doi.org/10.1101/278309>
- Hendrich, L., Moriniere, J., Haszprunar, G., Hebert, P. D., Hausmann, A., Kohler, F., & Balke, M. (2015). A comprehensive DNA barcode database for central European beetles with a focus on Germany: Adding more than 3500 identified species to BOLD. *Molecular Ecology Resources*, 15, 795–818. <https://doi.org/10.1111/1755-0998.12354>
- Hering, D., Borja, A., Jones, J. I., Pont, D., Boets, P., Bouchez, A., ... Kelly, M. (2018). Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. *Water Research*, 138, 192–205. <https://doi.org/10.1016/j.watres.2018.03.003>
- Hering, D., Moog, O., Sandin, L., & Verdonschot, P. F. M. (2004). Overview and application of the AQEM assessment system. *Hydrobiologia*, 516, 1–20. <https://doi.org/10.1023/B:HYDR.0000025255.70009.a5>
- Heywood, V. (2011). Monitoring of areas and species/populations to assess effectiveness of conservation/management actions. In D. Hunter, & V. Heywood (Eds.), *Crop wild relatives: A manual of in situ conservation* (pp. 295–313). London, UK: Earthscan.
- Hulme, P. E. (2006). Beyond control: Wider implications for the management of biological invasions. *Journal of Applied Ecology*, 43, 835–847. <https://doi.org/10.1111/j.1365-2664.2006.01227.x>
- Itakura, H., Wakiya, R., Yamamoto, S., Kaifu, K., Sato, T., & Minamoto, T. (2019). Environmental DNA analysis reveals the spatial distribution, abundance, and biomass of Japanese eels at the river-basin scale. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29, 361–373. <https://doi.org/10.1002/aqc.3058>
- Jerde, C. L., Chadderton, W. L., Mahon, A. R., Renshaw, M. A., Corush, J., Budny, M. L., ... Lodge, D. M. (2013). Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program. *Canadian Journal of Fisheries and Aquatic Sciences*, 70, 522–526. <https://doi.org/10.1139/cjfas-2012-0478>
- 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>
- Jeschke, J. M., Aparicio, L. G., Haider, S., Heger, T., Lortie, C. J., Pyšek, P., & Strayer, D. L. (2012). Taxonomic bias and lack of cross-taxonomic studies in invasion biology. *Frontiers in Ecology and the Environment*, 10, 349–350. <https://doi.org/10.1890/12.WB.016>
- Katano, I., Harada, K., Doi, H., Souma, R., & Minamoto, T. (2017). Environmental DNA method for estimating salamander distribution in headwater streams, and a comparison of water sampling methods. *PLoS ONE*, 12, e0176541. <https://doi.org/10.1371/journal.pone.0176541>
- Koizumi, N., Mori, A., Mineta, T., Sawad, E., Watabe, K., & Takemura, T. (2016). Exploratory environmental DNA analysis for investigating plant-feeding habit of the redeared turtle using their feces samples. *Jurnal Teknologi*, 78, 9–13. <https://doi.org/10.11113/jt.v78.7253>
- Kvifte, G. M. (2018). Molecular phylogeny of moth flies (Diptera, Psychodidae, Psychodinae) revisited, with a revised tribal classification. *Systematic Entomology*, 43, 596–605. <https://doi.org/10.1111/syen.12288>
- Laramie, M. B., Pilliod, D. S., Goldberg, C. S., & Strickler, K. M. (2015). *Environmental DNA sampling protocol—Filtering water to capture DNA from aquatic organisms. Techniques and Methods*, 2–A13. Reston, VA: US Geological Survey. <https://doi.org/10.3133/tm2A1>
- Lawson Handley, L. (2015). How will the 'molecular revolution' contribute to biological recording? *Biological Journal of the Linnean Society*, 115, 750–766. <https://doi.org/10.1111/bij.12516>
- Leese, F., Altermatt, F., Bouchez, A., Ekrem, T., Hering, D., Meissner, K., ... Zimmermann, J. (2016). DNAqua-Net: Developing new genetic tools for bioassessment and monitoring of aquatic ecosystems in Europe. *Research Ideas and Outcomes*, 2, e11321. <https://doi.org/10.3897/rio.2.e11321>
- Leese, F., Bouchez, A., Abarenkov, K., Altermatt, F., Borja, Á., Bruce, K., ... Weigand, A. M. (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. In D. A. Bohan, A. J. Dumbrell, G. Woodward, & M. Jackson (Eds.), *Next generation biomonitoring: Part 1* (Vol. 58). *Advances in ecological research*. (pp. 63–99). Kidlington, UK: Academic Press.
- Lim, N. K. M., Tay, Y. C., Srivathsan, A., Tan, J. W. T., Kwik, J. T. B., Baloglu, B., ... Yeo, D. C. J. (2016). Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. *Royal Society Open Science*, 3, 160635. <https://doi.org/10.1098/rsos.160635>
- Lopes, C. M., Sasso, T., Valentini, A., Dejean, T., Martins, M., Zamudio, K. R., & Haddad, C. F. B. (2017). eDNA metabarcoding: A promising method for anuran surveys in highly diverse tropical forests. *Molecular Ecology Resources*, 17, 904–914. <https://doi.org/10.1111/1755-0998.12643>
- Lopes-Lima, M., Sousa, R., Geist, J., Aldridge, D. C., Araujo, R., Bergengren, J., ... Zogaris, S. (2017). Conservation status of freshwater mussels in Europe: State of the art and future challenges. *Biological Reviews*, 92, 572–607. <https://doi.org/10.1111/brv.12244>

- Macher, J.-N., & Leese, F. (2017). Environmental DNA metabarcoding of rivers: Not all eDNA is everywhere, and not all the time. 164046. <https://doi.org/10.1101/164046>
- Macher, J. N., Salis, R. K., Blakemore, K. S., Tollrian, R., Matthaei, C. D., & Leese, F. (2016). Multiple-stressor effects on stream invertebrates: DNA barcoding reveals contrasting responses of cryptic mayfly species. *Ecological Indicators*, 61, 159–169. <https://doi.org/10.1016/j.ecolind.2015.08.024>
- Macher, J.-N., Vivancos, A., Piggott, J. J., Centeno, F. C., Matthaei, C. D., & Leese, F. (2018). Comparison of environmental DNA and bulk-sample metabarcoding using highly degenerate cytochrome c oxidase I primers. *Molecular Ecology Resources*, 18, 1456–1468. <https://doi.org/10.1111/1755-0998.12940>
- Mächler, E., Deiner, K., Steinmann, P., & Altermatt, F. (2014). Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. *Freshwater Science*, 33, 1174–1183. <https://doi.org/10.1086/678128>
- Mächler, E., Little, C. J., Wuethrich, R., Alther, R., Fronhofer, E. A., Gounand, I., ... Altermatt, F. (2019). Assessing different components of biodiversity across a river network using eDNA. *bioRxiv*, 546549. <https://doi.org/10.1101/546549>
- Mahon, A. R., Jerde, C. L., Galaska, M., Bergner, J. L., Chadderton, W. L., Lodge, D. M., ... Nico, L. G. (2013). Validation of eDNA surveillance sensitivity for detection of Asian carps in controlled and field experiments. *PLoS ONE*, 8, 6. <https://doi.org/10.1371/journal.pone.0058316>
- Martellini, A., Payment, P., & Villemur, R. (2005). Use of eukaryotic mitochondrial DNA to differentiate human, bovine, porcine and ovine sources in fecally contaminated surface water. *Water Research*, 39, 541–548. <https://doi.org/10.1016/j.watres.2004.11.012>
- Mauvisseau, Q., Coignet, A., Delaunay, C., Pinet, F., Bouchon, D., & Souty-Grosset, C. (2018). Environmental DNA as an efficient tool for detecting invasive crayfishes in freshwater ponds. *Hydrobiologia*, 805, 163–175. <https://doi.org/10.1007/s10750-017-3288-y>
- Meier, R., Shiyang, K., Vaidya, G., & Ng, P. K. L. (2006). DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. *Systematic Biology*, 55, 715–728. <https://doi.org/10.1080/10635150600969864>
- Merkes, C. M., McCalla, S. G., Jensen, N. R., Gaikowski, M. P., & Amberg, J. J. (2014). Persistence of DNA in carcasses, slime and avian feces may affect interpretation of environmental DNA data. *PLoS ONE*, 9, e113346. <https://doi.org/10.1371/journal.pone.0113346>
- Minamoto, T., Naka, T., Moji, K., & Maruyama, A. (2016). Techniques for the practical collection of environmental DNA: Filter selection, preservation, and extraction. *Limnology*, 17, 23–32. <https://doi.org/10.1007/s10201-015-0457-4>
- Moriniere, J., Hendrich, L., Balke, M., Beermann, A. J., König, T., Hess, M., ... Haszprunar, G. (2017). A DNA barcode library for Germany's mayflies, stoneflies and caddisflies (Ephemeroptera, Plecoptera and Trichoptera). *Molecular Ecology Resources*, 17, 1293–1307. <https://doi.org/10.1111/1755-0998.12683>
- Mueller, M., Pander, J., Knott, J., & Geist, J. (2017). Comparison of nine different methods to assess fish communities in lentic flood-plain habitats. *Journal of Fish Biology*, 91, 144–174. <https://doi.org/10.1111/jfb.13333>
- Niemiller, M. L., Porter, M. L., Keany, J., Gilbert, H., Fong, D. W., Culver, D. C., ... Taylor, S. J. (2018). Evaluation of eDNA for groundwater invertebrate detection and monitoring: A case study with endangered *Stygobromus* (Amphipoda: Crangonyctidae). *Conservation Genetics Resources*, 10, 247–257. <https://doi.org/10.1007/s12686-017-0785-2>
- Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apotheloz-Perret-Gentil, L., Beja, P., Boggero, A., ... Kahlert, M. (2018). The future of biotic indices in the ecogenomic era: Integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems. *Science of the Total Environment*, 637, 1295–1310. <https://doi.org/10.1016/j.scitotenv.2018.05.002>
- Pedersen, M. W., Overballe-Petersen, S., Ermini, L., Sarkissian, C. D., Haile, J., Hellstrom, M., ... Willerslev, E. (2015). Ancient and modern environmental DNA. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370, 20130383. <https://doi.org/10.1098/rstb.2013.0383>
- Pérez-Espona, S. (2017). Conservation genetics in the European Union—Biases, gaps and future directions. *Biological Conservation*, 209, 130–136. <https://doi.org/10.1016/j.biocon.2017.01.020>
- Pilliod, D. S., Goldberg, C. S., Arkle, R. S., & Waits, L. P. (2013). Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences*, 70, 1123–1130. <https://doi.org/10.1139/cjfas-2013-0047>
- Pimentel, D. (2005). Aquatic nuisance species in the New York State Canal and Hudson River systems and the Great Lakes Basin: An economic and environmental assessment. *Environmental Management*, 35, 692–702. <https://doi.org/10.1007/s00267-004-0214-7>
- Pimentel, D., Zuniga, R., & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, 52, 273–288. <https://doi.org/10.1016/j.ecolecon.2004.10.002>
- Pohlert, T. (2018). Trend v1.1.1: Non-parametric trend tests and change-point detection. Version 1.1.1.
- Pysek, P., Richardson, D. M., Pergl, J., Jarosik, V., Sixtova, Z., & Weber, E. (2008). Geographical and taxonomic biases in invasion ecology. *Trends in Ecology & Evolution*, 23, 237–244. <https://doi.org/10.1016/j.tree.2008.02.002>
- Rees, H. C., Bishop, K., Middleditch, D. J., Patmore, J. R. M., Maddison, B. C., & Gough, K. C. (2014). The application of eDNA for monitoring of the great crested newt in the UK. *Ecology and Evolution*, 4, 4023–4032. <https://doi.org/10.1002/ece3.1272>
- 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>
- Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., ... Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, 94, 849–873. <https://doi.org/10.1111/brv.12480>
- Robson, H. L. A., Noble, T. H., Saunders, R. J., Robson, S. K. A., Burrows, D. W., & Jerry, D. R. (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>
- Rolauffs, P., Hering, D., Sommerhäuser, M., Rödiger, S., & Jähnig, S. (2003). Entwicklung eines leitbildorientierten Saprobienindex für die biologische Fließgewässerbewertung. Umweltforschungsplan des Bundesministeriums für Umwelt, Naturschutz und Reaktorsicherheit, Forschungsbericht 200 24 227. Retrieved from <https://www.umweltbundesamt.de/sites/default/files/medien/publikation/long/2253.pdf>
- 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. <https://doi.org/10.1111/1365-2664.12428>
- Schill, W. B., & Galbraith, H. S. (2019). Detecting the undetectable: Characterization, optimization, and validation of an eDNA detection assay for the federally endangered dwarf wedgemussel, *Alasmidonta heterodon* (Bivalvia: Unionoida). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29, 603–611. <https://doi.org/10.1002/aqc.3069>
- Schill, W. B., & Mathes, M. V. (2008). Real-time PCR detection and quantification of nine potential sources of fecal contamination by analysis of mitochondrial cytochrome b targets. *Environmental Science and Technology*, 42, 5229–5234. <https://doi.org/10.1021/es800051z>
- Schneider, J., Valentini, A., Dejean, T., Montarsi, F., Taberlet, P., Glairot, O., & Fumagalli, L. (2016). Detection of invasive mosquito vectors using environmental DNA (eDNA) from water samples. *PLoS ONE*, 11, e0162493. <https://doi.org/10.1371/journal.pone.0162493>
- Sigsgaard, E. E., Carl, H., Møller, P. R., & Thomsen, P. F. (2015). Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. *Biological Conservation*, 183, 46–52. <https://doi.org/10.1016/j.biocon.2014.11.023>
- Sigsgaard, E. E., Nielsen, I. B., Bach, S. S., Lorenzen, E. D., Robinson, D. P., Knudsen, S. W., ... Thomsen, P. F. (2017). Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. *Nature Ecology & Evolution*, 1, 0004. <https://doi.org/10.1038/s41559-016-0004>
- Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., ... Vilà, M. (2013). Impacts of biological invasions: What's what and the way forward. *Trends in Ecology & Evolution*, 28, 58–66. <https://doi.org/10.1016/j.tree.2012.07.013>
- Simpfendorfer, C. A., Kyne, P. M., Noble, T. H., Goldsbury, J., Basiita, R. K., Lindsay, R., ... Jerry, D. R. (2016). Environmental DNA detects Critically Endangered largemouth sawfish in the wild. *Endangered Species Research*, 30, 109–116. <https://doi.org/10.3354/esr00731>
- Sodhi, N. S., Koh, L. P., Brook, B. W., & Ng, P. K. (2004). Southeast Asian biodiversity: An impending disaster. *Trends in Ecology & Evolution*, 19, 654–660. <https://doi.org/10.1016/j.tree.2004.09.006>
- Song, J. W., Small, M. J., & Casman, E. A. (2017). Making sense of the noise: The effect of hydrology on silver carp eDNA detection in the Chicago Area Waterway System. *Science of the Total Environment*, 605–606, 713–720. <https://doi.org/10.1016/j.scitotenv.2017.06.255>
- Stewart, K., Ma, H., Zheng, J., & Zhao, J. (2017). Using environmental DNA to assess population-wide spatiotemporal reserve use. *Conservation Biology*, 31, 1173–1182. <https://doi.org/10.1111/cobi.12910>
- Stoeckle, B. C., Beggel, S., Cerwenka, A. F., Motivans, E., Kuehn, R., & Geist, J. (2017). A systematic approach to evaluate the influence of environmental conditions on eDNA detection success in aquatic ecosystems. *PLoS ONE*, 12, e0189119. <https://doi.org/10.1371/journal.pone.0189119>
- Stoeckle, B. C., Kuehn, R., & Geist, J. (2016). Environmental DNA as a monitoring tool for the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.): A substitute for classical monitoring approaches? *Aquatic Conservation: Marine and Freshwater Ecosystems*, 26, 1120–1129. <https://doi.org/10.1002/aqc.2611>
- Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: Recent progress and future challenges. *Journal of the North American Benthological Society*, 29, 344–358. <https://doi.org/10.1899/08-171.1>
- Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, 21, 2045–2050. <https://doi.org/10.1111/j.1365-294X.2012.05470.x>
- Takahara, T., Minamoto, T., & Doi, H. (2013). Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PLoS ONE*, 8, e56584. <https://doi.org/10.1371/journal.pone.0056584>
- Thalinger, B., Oehm, J., Mayr, H., Obwexer, A., Zeisler, C., & Traugott, M. (2016). Molecular prey identification in central European piscivores. *Molecular Ecology Resources*, 16, 123–137. <https://doi.org/10.1111/1755-0998.12436>
- Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., ... 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>
- 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>
- Troudet, J., Grandcolas, P., Blin, A., Vignes-Lebbe, R., & Legendre, F. (2017). Taxonomic bias in biodiversity data and societal preferences. *Scientific Reports*, 7, 9132. <https://doi.org/10.1038/s41598-017-09084-6>
- Tucker, G., Bubb, P., de Heer, M., Miles, L., Lawrence, A., Bajracharya, S. B., ... Chapagain, N. R. (2005). *Guidelines for biodiversity assessment and monitoring for protected areas*. 2005. Kathmandu, Nepal: KMTNC.
- United Nations. (1992). Convention on Biological Diversity. Retrieved from <https://www.cbd.int/doc/legal/cbd-en.pdf>
- Vanderploeg, H. A., Nalepa, T. F., Jude, D. J., Mills, E. L., Holeck, K. T., Liebig, J. R., ... Ojaveer, H. (2002). Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 59, 1209–1228. <https://doi.org/10.1139/f02-087>
- Vörös, J., Marton, O., Schmidt, B. R., Gal, J. T., & Jelic, D. (2017). Surveying Europe's only cave-dwelling chordate species (*Proteus anguinus*) using environmental DNA. *PLoS ONE*, 12, e0170945. <https://doi.org/10.1371/journal.pone.0170945>
- Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., ... Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature*, 467, 555–561. <https://doi.org/10.1038/nature09440>
- Waldron, A., Mooers, A. O., Miller, D. C., Nibbelink, N., Redding, D., & Kuhn, T. S. (2013). Targeting global conservation funding to limit immediate biodiversity declines. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 12144–12148. <https://doi.org/10.1073/pnas.1221370110>
- Wilcox, T. M., Carim, K. J., Young, M. K., McKelvey, K. S., Franklin, T. W., & Schwartz, M. K. (2018). The importance of sound methodology in environmental DNA sampling. *North American Journal of Fisheries Management*, 38, 592–596. <https://doi.org/10.1002/nafm.10055>
- Wilson, C., Wright, E., Bronnenhuber, J., MacDonald, F., Belore, M., & Locke, B. (2014). Tracking ghosts: Combined electrofishing and environmental DNA surveillance efforts for Asian carps in Ontario waters of Lake Erie. *Management of Biological Invasions*, 5, 225–231. <https://doi.org/10.3391/mbi.2014.5.3.05>
- Wilson, J. J., Sing, K. W., Chen, P. N., & Zieritz, A. (2018). Tracking the southern river terrapin (*Batagur affinis*) through environmental DNA: Prospects and challenges. *Mitochondrial DNA Part A, DNA Mapping, Sequencing, and Analysis*, 29, 862–866. <https://doi.org/10.1080/24701394.2017.1373109>
- Xie, Y., Wang, J., Yang, J., Giesy, J. P., Yu, H., & Zhang, X. (2017). Environmental DNA metabarcoding reveals primary chemical contaminants in freshwater sediments from different land-use types. *Chemosphere*, 172, 201–209. <https://doi.org/10.1016/j.chemosphere.2016.12.117>

- Xie, Y. W., Hong, S., Kim, S., Zhang, X. W., Yang, J. H., Giesy, J. P., ... Khim, J. S. (2017). Ecogenomic responses of benthic communities under multiple stressors along the marine and adjacent riverine areas of northern Bohai Sea, China. *Chemosphere*, 172, 166–174. <https://doi.org/10.1016/j.chemosphere.2016.12.121>
- Yoccoz, N. G., Nichols, J. D., & Boulinier, T. (2001). Monitoring of biological diversity in space and time. *Trends in Ecology & Evolution*, 16, 446–453. [https://doi.org/10.1016/S0169-5347\(01\)02205-4](https://doi.org/10.1016/S0169-5347(01)02205-4)
- Zhou, X., Frandsen, P. B., Holzenthal, R. W., Beet, C. R., Bennett, K. R., Blahnik, R. J., ... Kjer, K. M. (2016). The Trichoptera barcode initiative: A strategy for generating a species-level Tree of Life. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371, 20160025. <https://doi.org/10.1098/rstb.2016.0025>
- Zika, V. M. A., Leese, F., Peinert, B., & Geiger, M. F. (2019). DNA metabarcoding from sample fixative as a quick and voucher-

preserving biodiversity assessment method. *Genome*, 62, 122–136. <https://doi.org/10.1139/gen-2018-0048>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Belle CC, Stoeckle BC, Geist J. Taxonomic and geographical representation of freshwater environmental DNA research in aquatic conservation. *Aquatic Conserv: Mar Freshw Ecosyst*. 2019;29:1996–2009. <https://doi.org/10.1002/aqc.3208>