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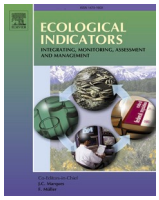

Review

eDNA-based approaches advance ecotoxicology: Insights and best practices from eDNA metabarcoding studies in evaluating stress-induced aquatic (macro-) invertebrate community composition

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ABSTRACT

Aquatic ecosystems are confronted with increasing levels of anthropogenic stress, prompting the need for rapid and reliable biomonitoring methods to allow ecological risk assessment and start science-based mitigation activities. Morphology-based sampling techniques have been the cornerstone of such evaluations and can be utilized to assess the impacts of anthropogenic stress on aquatic systems. However, environmental DNA (eDNA) has emerged as a promising alternative tool for biomonitoring. Macroinvertebrate species observations are pivotal in ecotoxicological studies and water quality assessment, nonetheless, few studies have implemented eDNA methods for stress-induced macroinvertebrate community composition assessment. To this end, we performed a systematic literature review, focusing on studies that analyzed the effects of anthropogenic stressors on macroinvertebrate community composition through eDNA metabarcoding. Our study aimed to 1) assess the relation between eDNA and morphology-based data for the assessment of stress-induced macroinvertebrate community composition; 2) evaluate the current quality of stress-induced macroinvertebrate community composition eDNA studies, and 3) formulate a minimum reporting and best practices guide for future studies. Our findings reveal that eDNA-derived beta diversity serves as a robust and sensitive indicator, outperforming morphology-based observations for determining beta diversity, making it a strong tool for invertebrate community assessment within ecotoxicology. However, we observed little consistency in applied methodology and reporting among the included studies, even though standardization is desired to increase the reproducibility and reliability of scientific research. To this end, we propose minimum reporting standards and a best practice guide for future studies, which will allow a wider and more systematic integration of eDNA metabarcoding to assess stress-induced (macro-) invertebrate community composition.

1. Introduction

Aquatic ecosystems face notable challenges, with rivers and coasts serving as hotspots for human civilizations (Best, 2019; He and Silliman, 2019). Anthropogenic influences on the environment, including land-use change, agricultural intensification or the introduction of materials like pesticides, plastics or engineered nano-materials are posing a threat to these ecosystems worldwide (Häder et al., 2020; IPBES, 2019; Sumudumali and Jayawardana, 2021). Understanding the intricacies of aquatic ecosystems under the influence of these anthropogenic stressors is essential for effective environmental management (Jaiswal et al., 2021; Weiskopf et al., 2020). One way to assess the effects of stress on ecosystems is through diversity metrics. Alpha and beta diversity have been key metrics in ecological studies for decades, as they allow the quantification of diversity and subsequently can be used to assess changes in space and time between communities, as reviewed by Daly et al. (2018). Diversity metrics provide insights into the overall health and resilience of (aquatic) ecosystems, aiding in the development of targeted conservation and restoration strategies (Rowland et al., 2020). Alternatively, it is also possible to assess ecosystem health or status in

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aquatic environments through the use of biotic indices (Sumudumali and Jayawardana, 2021). These are metrics that serve as proxies for water quality based on the tolerance of certain taxa to varying water chemistry, often based on macroinvertebrate community composition (Abbasi and Abbasi, 2011; Sumudumali and Jayawardana, 2021). Some well-known examples are the Hilsenhoff Biotic Index (HBI), which estimates water quality by measuring the relative abundance of certain stream invertebrate taxa which are assigned and weighted by a pollution tolerance score (Hilsenhoff, 1977), or the Ephemeroptera, Plecoptera and Trichoptera (EPT) index, which expresses the relative abundance of three sensitive orders (corresponding to the name) against other stream invertebrate taxa (Lenat, 1988). This type of monitoring has been standard practice in ecology and ecotoxicology research to assess the state of an ecosystem for decades and is generally based on morphology-based data (Abbasi and Abbasi, 2011; Brantschen et al., 2021; Pawlowski et al., 2018).

While these metrics are useful tools for the quantification of diversity or pollution, they are also prone to errors, such as underestimating taxa richness, due to incomplete or incorrect sampling efforts (Flotemersch et al., 2017; Haase et al., 2006; Keck et al., 2022a). Additionally, morphological identification requires thorough knowledge of the studied taxa to prevent misidentification (Tahir et al., 2018). Moreover, sampling and especially morphological identification are a time-consuming activity and are therefore costly, limiting the sample size (Ntislidou et al., 2021; Ramos-Merchante and Prenda, 2017). As a result, the potential understanding of a studied system's status is often based on sparse point measurements. Despite these challenges, quantifying whole community responses to the plethora of chemicals and other stressors is crucial for advancing ecotoxicological research, offering ecosystem-wide insights into the direct and indirect effects of anthropogenic stressors (Barmentlo et al., 2018; Saaristo et al., 2018; Vijver, 2019; Zhang, 2019).

One approach is environmental DNA (eDNA), a powerful tool used to track the presence or absence of a species in almost any given environment, which is now regularly seen and utilized as a good alternative to conventional surveys and monitoring programs (Barnes and Turner, 2016; Beng and Corlett, 2020; Thomsen and Willerslev, 2015). Protocols using eDNA allow for rapid, accurate, non-invasive, and cost-effective collection of data on species distribution and their relative abundance, and can be used to confirm the presence of rare and cryptic species (Beng and Corlett, 2020; Bohmann et al., 2014). Nowadays, additional applications have been developed for eDNA techniques including community composition assessment through eDNA metabarcoding (Miya, 2022; Ruppert et al., 2019; Zhang, 2019). Increasing evidence demonstrates cases where eDNA techniques outperform traditional ones, for example in species detection and catch-per-unit effort with eDNA compared to traditional surveying methods (Fediajevaite et al., 2021; Gehri et al., 2021; Rees et al., 2014b). As a result, it has become an important *go-to* technique in ecological surveys (for instance, see Matthias et al., 2021; Rees et al., 2014a; Uchida et al., 2020; Valentin et al., 2020; Yang and Zhang, 2020).

As many of the shortcomings related to morphology-based field surveying can be overcome by the eDNA toolkit, it has high potential for use in ecotoxicological studies (e.g., see Tahmid et al., 2024; Zhang, 2019; Zhang et al., 2018). However, most of the current ecotoxicological studies have focused on taxa that are otherwise hard to study, such as bacteria and fungi (for instance, Guo et al., 2017; Hemmat-Jou et al., 2018; Liu et al., 2020; W. Yang et al., 2021; Zhao et al., 2019). To date, only limited ecotoxicology studies on macroinvertebrate community composition have been published that successfully incorporated eDNA to assess stress-induced community responses (for instance, the mesocosm studies by Beentjes et al., 2021 and Yang et al., 2018).

The goal of the current study is to investigate the potential and compatibility of eDNA in ecotoxicology as a tool to assess stress-induced effects on macroinvertebrate community composition and to provide a clear path forward for better integration of eDNA-based community assessment within ecotoxicology. To this end, we performed a systematic literature search to obtain and review the literature on macroinvertebrate community composition that compared communities exposed to different (anthropogenic) stressors through eDNA metabarcoding. As this approximates stress-induced community composition assessment as seen in ecotoxicological research, we think the information obtained here can be extrapolated to macroinvertebrate community composition assessment in ecotoxicology studies. Within this review, we aim to assess 1) the relation between eDNA and morphology-based data for the assessment of stress-induced macroinvertebrate community composition, as well as 2) the current reporting standards of stress-induced macroinvertebrate community composition eDNA studies. Subsequently, to facilitate reproducibility and reliability 3) we propose a minimum reporting standard and best practice guide.

2. Methods

For this review, we followed the guidelines from Siddaway et al. (2019). The literature search was conducted at the beginning of 2022, following the PRISMA approach (Moher et al., 2009), followed by the snowballing approach to maximize our search effort (Wohlin, 2014). A final update was made on July 31, 2023. Web of Science and PubMed were searched for peer-reviewed research papers, and subsequent snowballing was repeated until no new relevant records were found.

2.1. Search strings and screening process

The search string we used was:

(communit* OR "community composition" OR "species composition")
AND
(eDNA OR "environmental DNA" OR environmental-DNA OR metabarcoding OR ddPCR OR NGS OR "next-gen* seq*" OR "high-throughput seq*" OR metagenomic* OR ecogenomic*)
NOT
(RNA OR epigenom* OR metabolom* OR proteom* OR transcriptom* OR bacteri* OR fungus OR fungi OR prokary*)

No inclusion terms for macroinvertebrates were added, as this drastically decreased the number of returned hits, resulting in the exclusion of relevant papers. Instead, the inclusion of the correct taxa was confirmed during the screening process.

Returned records were first screened for inclusion factors based on the title and abstract. Subsequently, the full text was assessed and data were extracted from eligible papers. The following criteria were applied for the screening process:

1. Papers had to include a macroinvertebrate community composition assessment based on eDNA metabarcoding data. Since many of the papers that included macroinvertebrates targeted a broader taxonomic spectrum, papers on overarching taxa were also included, providing that macroinvertebrate taxa were specifically stated in the data analyses allowing us to filter out and exclude papers exclusively on micro- or meio-eukaryotes.
2. Papers had to include a quantified stressor and link community composition to this stressor.
3. Only papers on aquatic communities were included to stay within the scope of this article.
4. Only peer-reviewed research papers were used for data extraction.

2.2. Data extraction

We extracted information on the experimental set-up, including study type, ecosystem, the studied taxa and stressor(s), other community composition assessment methods, sample size, sampled substrate and sampling methods. We also extracted information on eDNA-related

methods, bioinformatics, data analysis and the main findings (Table 1).

2.3. Data synthesis

Using the extracted information, we first analyzed the relation between community composition data obtained via eDNA and via morphology-based observations. We focused on the sensitivity of both techniques in detecting stress-induced changes in community composition. Next, we conducted an in-depth analysis of the applied methods of the studies to assess the current quality, reproducibility and reliability of the studies and subsequently to create the minimum reporting and best practices guides.

2.3.1. Comparing traditional techniques with eDNA

To determine the suitability of eDNA for the assessment of stress-induced macroinvertebrate community composition, we compared the sensitivity of eDNA community composition data with that of morphology-based data. We collected the papers that used both techniques side-by-side to analyze the relation between the studied stressor and community composition. Per paper, we scored whether a significant correlation was observed between the studied stressor and community composition for both techniques and used this to assess how eDNA relates to morphology-based data. As alpha and beta diversity are widely used measures to quantify biodiversity and can be used to analyze similarities and differences between communities (Daly et al., 2018), these were selected to compare the sensitivity of the different sampling techniques primarily. Additionally, we extracted information on the use of biotic indices as well as morphologically identified taxa and assigned operational taxonomic units (OTU; genetic sequence cluster representing a single species) from studies that incorporated these elements for both data types. Subsequently, we conducted a comparison between eDNA and traditional methods based on these elements, aiming to provide insights into their respective performances in assessing stress-induced macroinvertebrate community composition.

2.3.2. Quality control and reproducibility

To provide a path forward for better integration of eDNA metabarcoding within ecotoxicology – and in general, a standardized approach is desired to ensure the quality of studies, promoting reproducibility and comparability (Bunholi et al., 2023; Dickie et al., 2018; Johnson, 2002; Pawlowski et al., 2018). Recognizing the need for standardization, we focused specifically on assessing the current state of reporting and methodological practices in eDNA-based stress-induced community composition studies. To achieve this, we conducted an in-depth analysis of the applied methods of the collected studies. In developing our workflow, we drew inspiration from the methodology applied by Shea et al. (2023).

From each of the obtained studies, we assessed the content and extracted all elements that could affect the quality (i.e., reliability and reproducibility) of the study. We determined the relevance of each element through subsequent literature analysis and created an overview of all elements that should be reported into a minimum reporting guide. This minimum reporting guide comprises a step-by-step checklist, stipulating the essential elements that should be included in the reported study. For improved ease of following the minimum reporting guide, we divided the elements into six main steps that can be considered the main steps in any (e)DNA metabarcoding study (Taberlet et al., 2018a), being: *1) Experimental set-up; 2) Sample collection; 3) DNA extraction; 4) Library preparation (including all amplification steps); 5) Bioinformatics* and *6) Data analysis*. Each of the above-mentioned steps was divided into one or two levels of sub-categories. Per sub-category, a list of elements was given that should be included in the reported study, either in the paper or in the Supplementary Data. The minimum reporting guide is designed to enhance reproducibility and comparability across studies.

Building upon our minimum reporting guide, we conducted a comprehensive re-evaluation of the selected papers to assess the current state of reporting quality. This involved scoring the inclusion or exclusion of each element outlined in the guide, calculating and visualizing the percentage of inclusion for individual elements as well as groups of elements across all studies. To this end, we categorized the elements into distinct groups, slightly different from those in the minimum reporting guide. The groups were as follows: *1) General information, set-up, and sample size; 2) Sample collection and storage; 3) DNA extraction; 4) Genetic markers; 5) PCR and library preparation; 6) Bioinformatics; 7) Raw data;* and *8) Data analysis*. These were selected to allow us to pinpoint more precisely which sections within studies are reported consistently and which require attention. Furthermore, we analyzed the overall percentage of included elements per study, seeking patterns that could illuminate trends over time (De Ruijter et al., 2020). Insights gained from this evaluation contribute to our understanding of the broader consistency in reporting practices within stress-induced eDNA metabarcoding studies on (macro-) invertebrates and are crucial for delineating the necessary steps towards better integrating eDNA in ecotoxicological research.

Subsequently, a comprehensive analysis of the applied methods across all studies was conducted to ascertain the level of consistency in applied methods. This investigation aimed to elucidate whether existing methodologies could seamlessly pave the way forward or if additional tools and best practices are warranted. Informed by our findings, a best practices guide for eDNA-based stress-induced (macro-) invertebrate community composition assessment studies was crafted, aligning with the consensus of the 24 included studies and drawing from existing literature and best practices. For this, we used the same categorization of the steps as in the minimum reporting guide, with the exception of the *Experimental set-up*, as this depends on the goal of each individual study. For the remaining five steps we formulated a set of guidelines, aimed to increase sample yield, reduce sample degradation, account for contamination, improve reliability and improve reproducibility. The proposed best practices provide guidance for future eDNA-based stress-induced (macro-) invertebrate community composition assessment studies and accommodate the diverse requirements of different studies (e.g., different environments, substrates, taxa, etc.).

Table 1
Key information extracted from 24 papers on eDNA-based stress-induced (macro-) invertebrate community composition included in this review.

Characteristic	Description/categories
Study type*	Field study; Meso-/microcosm experiment
Ecosystem*	Marine; Freshwater
Studied taxa*	Macroinvertebrates, overarching taxa; Other taxa
Studied stressor(s)*	Description of studied stressor(s)
Other community composition assessment techniques	Traditional sampling; Bulk metabarcoding
Sample size	Description including number of samples, time points, replicates.
Sample substrate*	Water; Sediment
Sampling methods	Sample volume/weight; collection methods, storage conditions; quality control steps
eDNA related methods	eDNA extraction methods; storage conditions; PCR and library preparation steps; quality control steps
Bioinformatics	Quality scores; bioinformatics steps; reference library; taxonomic resolution (OTU/ASV)
Data analysis	Obtained taxa; diversity indices; biotic indices; statistical analysis; statistical output
Main findings*	Effects of stressor(s) on community composition

* Indicates minimum required information for an article to be included in this review.

3. Results and discussion

A total of 7 922 unique records were screened for inclusion, of which 7 195 were obtained using the search terms through Web of Science and PubMed and 727 through subsequent snowballing. After title and

abstract screening, 104 papers remained. From these, 80 papers were excluded based on a full assessment for not fitting the criteria (Fig. 1, Table S1). A total of 24 papers remained and were used for data extraction (Fig. 1). Eleven studies applied a different method for community composition assessment besides eDNA sampling, being morphology-based sampling techniques (n = 9), bulk metabarcoding (i.e., the metabarcoding of processed organisms rather than an environmental sample, n = 1) and historical data (n = 1). Those that used morphology-based methods were used for the comparison between eDNA and morphology-based data. The low number of retained papers as well as the reoccurrence of the same authors indicate once more that eDNA has not been widely implemented in ecotoxicology as a tool for macroinvertebrate community composition assessment (see Supplementary Fig. S1 for a citation network).

A summary of the key characteristics of the selected papers can be found in Fig. 2 (a complete overview is presented in Table S2). From the 24 obtained papers, 22 studies were conducted in the field, while two were micro- or mesocosm studies. Sixteen studies focused exclusively on freshwater communities, seven on marine communities and one on both. Five studies were exclusively on (macro-) invertebrates, 19 were on overarching taxa (Metazoans/Eukaryotes). Eleven studies also included other kingdoms, such as Bacteria and Fungi. Based on the quantification method, the studied stressors could be divided into three main categories, being geo- and physiochemical parameters (n = 12), land-use types (n = 5) and (distance to/effect of) offshore (drilling) station (n = 4). Four studies fell outside these categories; one on unnatural barriers, one on anthropogenic disturbance, one on thiacloprid and one on copper. The genetic marker used most to target invertebrates and overarching taxa – from here on out referred to as invertebrates – was COI (n = 17), followed by 18S (n = 8).

The majority of studies used alpha as well as beta diversity to quantify diversity (n = 16), while one study only used alpha diversity and seven studies only used beta diversity. The diversity metrics that were used most frequently were taxa richness (n = 17) and the Shannon diversity index (n = 16) for alpha diversity and Bray-Curtis dissimilarity (n = 10) and the Jaccard index (n = 6) for beta diversity. Most studies quantified alpha diversity through two or more metrics, while beta diversity was always quantified using a single metric. While all studies included alpha or beta diversity to quantify diversity, some did not statistically analyze the differences between communities through the obtained diversity scores. Sixteen studies statistically analyzed community differences based on both alpha and beta diversity indices, while one study only did so based on alpha diversity and seven only based on beta diversity. From those, 11 found an effect of the stressor on at least one of the included alpha diversity metrics (n = 16) and 20 on the included beta diversity metric (n = 22; Table S3 and S4). Besides diversity metrics, six studies also implemented biotic indices to analyze the differences between communities (Fig. 2). Among the six studies that applied biotic indices to analyze the data, two used the AZTI's Marine Biotic Index (AMBI), one the Family-level Biotic Index (FBI) and one the Norwegian Sensitivity Index (NSI). One study used the EPT, Diptera and Chironomid index and one study used the Benthic Pollution Index (BPI) and Biodiversity Index (BI). All six studies observed an effect of the stressor based on the applied biotic index (Table S5). Besides the analysis of stress-induced community composition, eleven studies also used the obtained metabarcoding data to investigate species networks and ten used the data to identify indicator taxa.

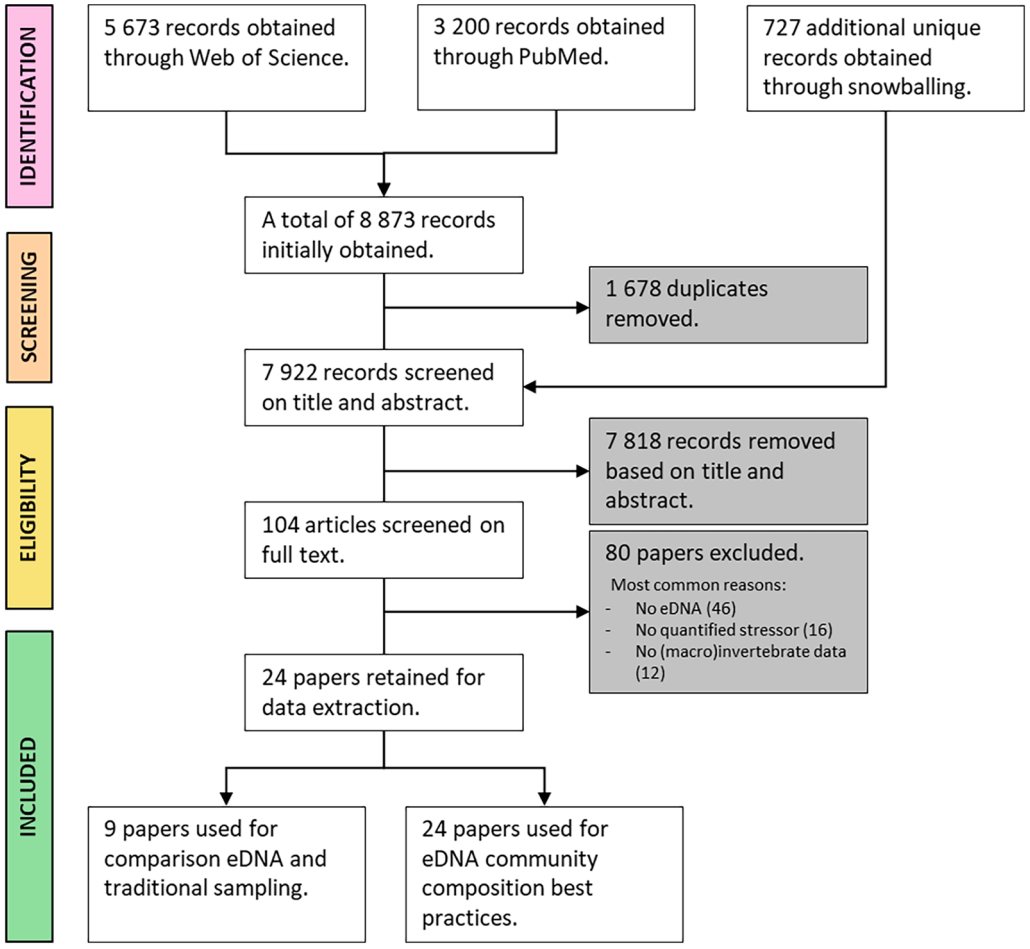

Fig. 1. Scheme showing the selection process used to obtain relevant articles for this review.

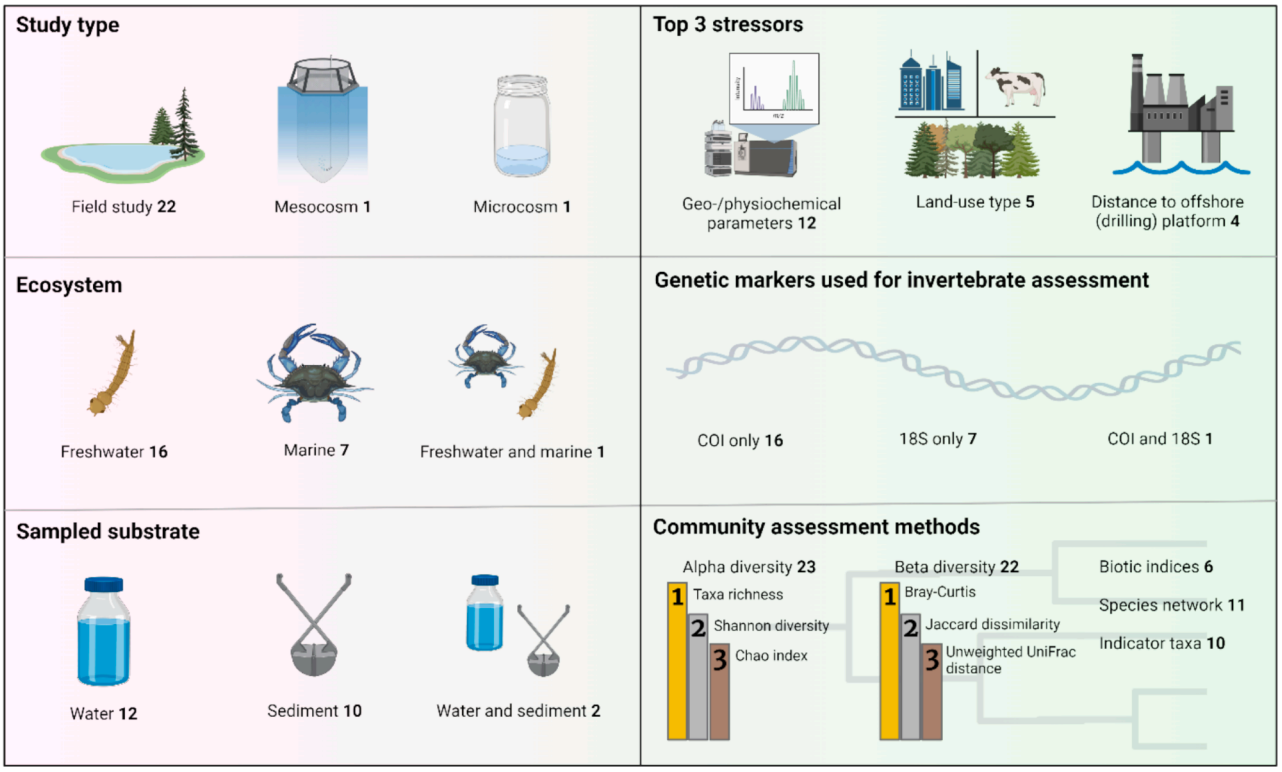

Fig. 2. General summary infographic on the set-up and methods of 24 studies that used eDNA metabarcoding to assess stress-induced (macro-) invertebrate community composition. The numbers refer to the amount of studies that were used per category.

3.1. Comparing morphology-based data with eDNA

To assess the suitability of eDNA as a tool for evaluating stress-induced macroinvertebrate community composition in ecotoxicology, we compared the studies that used eDNA as well as morphology-based methods to analyze the effects of the studied stressor(s) on community composition. From the nine studies that included morphology-based macro-invertebrate data next to eDNA data, four analyzed the difference between communities based on both alpha and beta diversity, while the other five only analyzed the effects on beta diversity. Four studies compared morphologically identified taxa with assigned OTUs and four studies examined biotic indices based on both sampling methods.

Based on the four studies that analyzed alpha diversity, it seems both eDNA and morphology-based sampling methods perform equally in detecting stress-induced changes in invertebrate communities based on alpha diversity (Table 2). Two studies found a significant effect of the stressor on both eDNA and morphology-based community data, while one study only did so based on eDNA and one only on morphology data. In contrast, based on the studies that compared beta diversity derived from both data types, eDNA appeared to be more sensitive in detecting stress-induced changes in invertebrate communities than morphology-based data (Table 2). From the nine studies that included beta diversity, eight observed a significant correlation between beta diversity and the studied stressor based on eDNA data, while only five did so based on morphology data. One of the studies observed no correlation between the studied stressor and beta diversity for both techniques.

Alpha and beta diversity each have different implications for

Table 2
eDNA vs. morphology for stress-induced invertebrate community assessment. This table presents the results from nine studies that used eDNA-metabarcoding as well as morphology-based survey methods to assess stress-induced (macro-) invertebrate community composition through alpha and beta diversity, as well as biotic indices.

Ecosystem	Substrate	Reference	Stressor	Alpha		Beta		Biotic index	
				eDNA	*Trad.*	*eDNA*	*Trad.*	*eDNA*	*Trad.*
Freshwater	Sediment	(Ji et al., 2022)	Land-use			*	ns	*[c]	*
		(Zhou et al., 2022)	Waste water discharge			ns	ns		
	Water	(Beentjes et al., 2021)	Thiacloprid (pesticide)			*	*		
		(Seymour et al., 2021)	Land-use			*	ns		
		(Uchida et al., 2020)	Water quality			*	ns	*	ns
Marine	Sediment	(Mauffrey et al., 2021)	Offshore drilling activities	*	ns	*	*	*[cd]	*[d]
		(Lanzén et al., 2021)	Offshore drilling activities	*	*	*	*	*[d]	*[d]
		(Klunder et al., 2020)	Offshore drilling activities	ns	*[a]	*	*		
		(Laroche et al., 2018)	Offshore drilling activities	*[b]	*[b]	*	*		

a: Three components of the stressor were tested, of which one correlated with traditional data and non with eDNA data.
b: Both methods correlated with different components of the stressor.
c: Both methods identified the pollution status of the system; however, eDNA metabarcoding showed that the pollution level increased from upstream to downstream, which was not observed by the traditional survey.
d: The biotic index scores were not linked to the stressor per method but BI scores per method were directly compared. In both papers BI scores of both methods correlated.

ecological status (Piazzi and Ceccherelli, 2020; Rombouts et al., 2019) and are often used in concert in studies focusing on stress-induced macroinvertebrate community composition (e.g., see the studies included in this review). Alpha diversity quantifies the number or evenness of species in a community while beta diversity quantifies the variation between communities (Daly et al., 2018). Hence, alpha diversity only reveals changes in community composition when there is an effect causing significant changes in species numbers or evenness. Species turnover is not incorporated in alpha diversity, while this is an important indicator of community composition change. Moreover, while only based on four studies, the inconsistency we observed between eDNA and morphology-based data could indicate that alpha diversity is not a reliably sensitive metric for stress-induced (macro-) invertebrate community composition assessment. Beta diversity on the other hand does include species turnover (Legendre et al., 2005), and therefore could be a more sensitive metric for stress-induced community composition assessment. Moreover, based on our data, it appears that eDNA-derived beta diversity is even more sensitive to stress-induced community change compared to morphology-based beta diversity (Table 2). A possible explanation for this could be a difference in sample size between the methods. Among the three papers that exclusively observed a significant effect of the stressor on beta diversity based on eDNA, two collected more eDNA samples than morphological samples (Table S6). A key advantage of using eDNA is that increasing the sample size does not substantially increase the workload, unlike morphological samples where each additional sample adds to the workload relatively uniformly. As a result, when using eDNA, increasing sample size is less time-constrained, allowing researchers to collect more samples through time and space (Keck et al., 2017). A related benefit of eDNA is that with limited extra effort, different taxonomic groups can be studied, using the same samples (Beng and Corlett, 2020). Within the included papers, 11 papers did so (Table S2). For example, Laroche et al. (2018) included bacteria and foraminifera in their analyses. They found bacteria to be most sensitive to the stressor, followed by foraminifera, and invertebrates – both through eDNA and traditional sampling. Similar trends were observed by Beentjes et al. (2021), Li et al. (2022) and Xie et al. (2017), who all found that micro-organisms were the most sensitive group. Expanding the range of target taxa could thus improve the efficiency of (early) detection of stress.

Another explanation for the difference between eDNA and morphology-based beta diversity could be the higher sensitivity of eDNA data compared to traditional surveying methods. This is one of the main advantages of eDNA, as it allows for the detection of rare or elusive species that might be missed by traditional methods, creating a more accurate and comprehensive understanding of the studied community (Beng and Corlett, 2020). Moreover, the higher number of taxa or OTUs likely increases the chance of detecting sensitive taxa. In turn, these affect beta diversity as they are affected by the stressor (Pound et al., 2019). From the nine studies that assessed community composition through eDNA as well as morphology-based data, four studies directly compared the number of morphologically identified organisms with the number of assigned OTUs from the eDNA dataset (Table S6). In each study, eDNA consistently revealed higher taxonomic richness, with differences ranging from 2 to almost 50-fold compared to traditional data. Among these studies, three observed no effect of the stressor on beta diversity based on morphology-based data, while they did find a significant effect on beta diversity based on eDNA data. The remaining paper observed no effect on both datasets. While this observation is only based on four studies, a comprehensive *meta*-study conducted by Fediajevaite et al. (2021), encompassing over 200 papers, demonstrated the same trend. Their *meta*-analysis revealed that eDNA consistently outperformed traditional surveys in terms of sensitivity, detecting a greater species diversity.

However, despite the high sensitivity of the technique, eDNA sometimes fails to detect certain taxa, as was the case in three out of four studies (Table S6). This is a potential limitation of eDNA, which can be caused by multiple reasons, such as varying DNA shedding rates between different species (Trimbos et al., 2021), sample quality (Barnes and Turner, 2016), primer bias (i.e., preferential amplification of certain species; Beng and Corlett, 2020) or reference database bias (i.e., incomplete or biased databases; Piper et al., 2019). Moreover, the high sensitivity also increases the chance of false positives, something that is less prevalent in morphology-based surveys where the presence of a species is definitive – albeit prone to misidentification (Fediajevaite et al., 2021). However, advancements in eDNA methods such as the development of better primers and databases likely will further increase the sensitivity and quality of eDNA data (Keck et al., 2022b; Piper et al., 2019). Moreover, based on our findings potential false positives and false negatives do not appear to be problematic for stress-induced community composition assessment, as almost all studies could link beta diversity to the stressor (Table S4), even better so than morphology-based methods (Table 2).

While for stress-induced beta-diversity assessment potential false positives and negatives might not be problematic (that is, within reason), traditional water quality assessment is often based on biotic indices, which in turn are based on specific families, genera or species observations (Chessman, 1995; Hilsenhoff, 1977). In this case, it is important to know exactly which species are present or absent. Among the collected papers, four assessed community data using biotic indices based on eDNA and morphology-based data (Table 2; Table S6). One directly analyzed the correlation between the two datasets (Lanzén et al., 2021), observing a significant correlation between eDNA and traditionally derived BI scores (Table 2). Two other studies, while not directly assessing the correlation between the two datasets, assessed the correlation between the analyzed stressor and each dataset based on a biotic index (Ji et al., 2022; Uchida et al., 2020). They found that eDNA outperformed morphology-based data, being more sensitive in detecting effects of the stressor (Table 2). The last study, by Mauffrey et al. (2021), did both. While observing a significant correlation between the two datasets, they also observed a more outspoken trend in the eDNA data compared to the morphology-based data, indicating higher sensitivity of the eDNA data. It appears that, despite the potential for false negatives, eDNA is a reliable data source for biotic indices and in some cases may even outperform traditional methods in assessing stress-induced community composition. While this observation is only based on four papers – as this review only includes papers that linked community composition directly to a stressor, Pawlowski et al. (2018) reviewed studies comparing biotic index scores derived from aquatic eDNA and bulk metabarcoding with traditionally derived community data and found that there was a relatively good correlation between the metabarcoding and morphology-based data, supporting our observation. Furthermore, due to the high sensitivity, eDNA may contribute to the discovery of additional indicator taxa that elude detection through conventional surveying methods but hold potential significance for assessing ecosystem health, facilitating optimization of biotic indices (Pawlowski et al., 2018). In one of the obtained papers that incorporated morphology-based methods besides eDNA an indicator taxa analysis was included (Laroche et al., 2018). Based on the morphology data they identified 19 species indicative of the studied stressor. Based on Eukaryote eDNA data, they again found 19 assigned species to be indicative; however, they also found 121 unassigned indicative Eukaryote OTUs. Moreover, based on bacterial eDNA data, they found 374 indicator OTUs. Another case highlighting the potential benefits of the higher sensitivity of eDNA is the study by Ji et al. (2022). Although not specifically assessing indicator taxa, the authors did specify differences in obtained taxa numbers between the eDNA and morphology-based datasets. They found 379 species with eDNA and 40 with traditional methods. After analyzing the ecological status of a river system using a biotic index based on eDNA and traditional data, a correlation between water quality and the biotic index based on both methods was observed. However, the eDNA dataset revealed a pollution gradient from upstream to downstream, which was not the case based on the

morphology data. The higher resolution (i.e., almost 10-fold higher number of species) and subsequently potentially higher number of included indicator taxa could be the reason behind the difference between the datasets.

While it seems that eDNA could improve ecological or ecotoxicological stress-assessment through (macro-) invertebrate community composition assessment, there is one factor that should be considered before incorporating eDNA as a standard surveying method. As mentioned, traditional water quality assessments are often based on biotic indices, which in turn are designed based on morphology-based datasets. The higher sensitivity of eDNA compared to morphology-based methods could therefore result in the misalignment of datasets. Proper calibration is needed before switching to a different, more sensitive method (Pawlowski et al., 2021), for instance by increasing the number of studies that simultaneously collect eDNA and morphology-based data within the same study for subsequent comparison and calibration, either within the study or through future *meta*-studies.

3.2. Quality control and reproducibility

Based on the 24 included studies and subsequent literature analysis, around 60 elements were extracted that are relevant for the reproducibility and reliability of a study. We visualized these into a diagram that can be used as a minimum reporting guide (Fig. 3). Additional information on the relevance of each element is given in the Supplementary Data (Table S7). Based on the inclusion of the elements in the overarching categories, *General information, set-up and sample size* demonstrated the most robust reporting, achieving an overall rate of 98 %. In contrast, other groups exhibited varying degrees of reporting consistency, ranging between 88 % and 39 %. Ordered by descending percentage of reporting, *Data analysis* emerged as the second most consistently reported category at 88 %, succeeded by *Genetic markers*

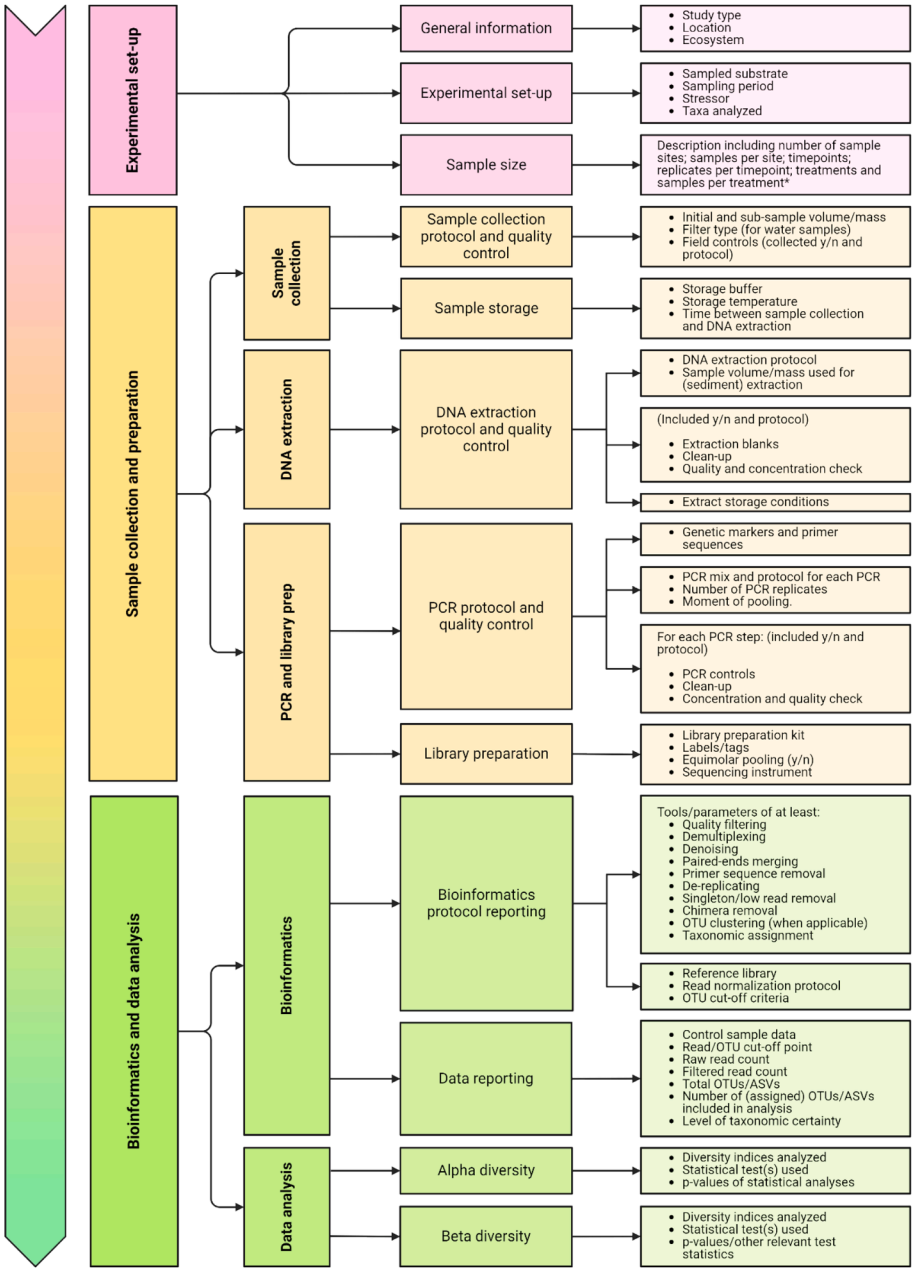

Fig. 3. A step-by-step reporting checklist for stress-induced invertebrate eDNA community composition studies, based on the consensus of 24 papers. Note: Elements marked with an asterisk can only be included when applicable.

(78 %), *Sample collection and storage* (66 %), *Bioinformatics* (63 %), *Raw data* (61 %) and *PCR and library preparation* (60 %). The category with the lowest overall reporting rate was *DNA extraction*, at 39 % (Fig. 4). Below, we discuss the elements reported infrequently (<50 %) and a selection of other relevant components in the order of the experimental workflow.

3.2.1. Sample collection and storage

The first step in the workflow of an eDNA study is the collection of environmental samples. This generally takes place in an uncontrolled environment and some contamination is likely to occur. Negative control samples should be collected in order to assess potential contamination of the collected samples and subsequently to subtract read data from the actual samples in order to account for the contamination (Dickie et al., 2018; Forstchen, 2020). Within the 14 analyzed papers that collected water samples, about half reported to have collected negative controls (57 %) and one specifically reported that they did not. While there are steps downstream that allow for false-positive control (e.g., singleton removal), these steps are not based on actual detected reads or taxa and are therefore not as secure as implementing negative field controls in the sampling effort. Moreover, the collection of field controls for water samples is a simple step – DNA-free water is filtered in the same manner as other samples are – and should be possible in almost all cases. For sediment samples, it is more complicated to collect a field control sample. One option is to swab field materials during sampling (Dickie et al., 2018). Another possible option would be to bring sterile sediment into the field that is subsequently processed in the same manner as the field samples. However, to our knowledge, there is no common practice yet on how to handle negative sediment field controls.

Next, collected samples should be stored appropriately to maintain the quality of the genetic material. Within the included studies, the storage buffer in which samples were kept was reported in 25 % of the papers and the time between sample collection and DNA extraction (i.e. storage time) was reported only in two studies (8 %). The choice of storage buffer – or lack thereof – can affect the quality of genetic material in a sample (Majaneva et al., 2018), and while studies on the effect of storage time are scarce, it is recommended to keep storage time to a minimum to prevent degradation of genetic material (Lear et al., 2018). A lack of comprehensive reporting on contamination control (through control samples) and storage conditions in studies undermines the ability to verify the sample quality.

3.2.2. DNA extraction

Overall, elements related to DNA extraction were reported most inconsistently (39 %). Like field controls, including negative controls during DNA extraction (i.e., an extraction with no genetic material) improves the reliability of a study as it allows for contamination control (Lear et al., 2018; Tedersoo et al., 2022). This can be done for water as well as sediment samples and should be included and reported. However, only 29 % of the included studies reported on the inclusion of negative extraction controls.

Another poorly reported element in the DNA extraction was the extract clean-up, which was reported in only 13 % of the studies. While a clean-up is not always required, it does affect the quality of the extract and can improve downstream processing steps (Goldberg et al., 2015). Reporting whether this was included or not, e.g., based on quality control, could increase the reliability of metabarcoding studies. Quality control as well as concentration checks of extracted samples enable the assessment of whether a sample is fit for downstream processing. About half of the studies reported checking PCR success through gel electrophoresis or the application of a more quantitative method to assess quality or concentration (e.g., Invitrogen Qubit system). The final element within the DNA extraction step that was reported poorly were the storage conditions, with only 25 % reporting. As with environmental samples, storage conditions of the DNA extract can affect the degradation of genetic material and should preferably be reported in order to validate the quality of the samples within a study (Coudy et al., 2021; Goldberg et al., 2015; Röder et al., 2010). Strengthening adherence to these quality control measures across studies is crucial for enhancing the robustness of findings and ensuring the validity of scientific conclusions.

3.2.3. PCR and library preparation

Following DNA extraction, PCR and other library preparation steps are performed to prepare samples for sequencing. During these steps samples are handled a lot and quality and contamination should be monitored to maintain high-quality samples (Alberdi et al., 2018; Piper

Category (average across elements)	Element described/included	% Inclusion		
General info, set-up and sample size (98%)	Study type	100		
	Location	100		
	Ecosystem	100		
	Year of sampling	96		
	Month of sampling (n=23)	91		
	Stressor	100		
	Taxa analyzed	100		
	Complete description of sampling effort	100		
Sample collection and storage (66%)	Initial sample volume/weight	92		
	Subsample volume/weight	96		
	Filter type and pore size (water) (n=14)	93		
	Field controls collected (water) (n=14)	64		
	Storage buffer filter/sediment sample	25		
	Storage temperature filter/sediment sample	92		
	Time between sample collection and DNA extraction	8		
DNA extraction (39%)	DNA extraction method	100		
	Extraction controls (y/n)	29		
	Clean-up (y/n)	13		
	Quality/concentration control (y/n)	29		
	DNA extract storage conditions	25		
Genetic markers (78%)	Genetic markers reported	100		
	Primer names reported	83		
	Primer sequence given	50		
PCR and library prep. (60%)	PCR mix (n=24 | n=7 | n=1)	50	29	0
	PCR conditions (n=24 | n=7 | n=1)	83	57	100
	PCR clean-ups (y/n; n=23 | n=6 | n=1)	52	50	0
	PCR quality/concentration checks (y/n; n=23 | n=6 | n=1)	74	67	100
	PCR controls (y/n)	63		
	Replicate PCRs	71		
	Moment of pooling replicates (n=17)	53		
	Equimolar pooling (y/n)	79		
	Final clean-up (y/n)	33		

Category (average across elements)	Element described/included	% Inclusion	
PCR and library prep. (60%)	Final quality/concentration check (y/n)	33	
	Library preparation kit	38	
	Sequencing instrument	100	
Bioinformatics (63%)	Quality filtering (y/n | p, n=23)	96	91
	Demultiplexing (y/n | p, n=8)	33	50
	Denoising (y/n | p, n=1)	4	100
	Paired-ends merging (y/n | p, n=16)	67	88
	Removing primer sequences (y/n | p, n=7)	29	71
	Dereplicating sequences (y/n | p, n=13)	54	54
	Singelton/low read filtering (y/n | p, n=13)	54	23
	Removing chimeras (y/n | p, n=16)	67	69
	OTU/ASV clustering (y/n | p, n=22)	92	100
	Taxa assignment (y/n | p, n=15)	63	100
	Taxonomic resolution (OTU/ASV)	100	
	Reference library	96	
	Clustering percentage	71	
	Read normalization	63	
	Read/OTU cut-off criteria	63	
Raw data (61%)	Read data control samples (n = 18)	28	
	Read/OTU cut-off data	38	
	Raw read count	67	
	Filtered read count	63	
	Total OTUs	79	
	Assigned OTUs	83	
	Level of taxonomic certainty	79	
Data analysis (88%)	Alpha diversity analysis included	96	
	Alpha div. statistically analyzed (n=23)	74	
	Alpha div. statistical output reported (n=14)	76	
	Beta diversity analysis included	92	
	Beta div. statistically analyzed (n=22)	100	
	Beta div. statistical output reported (n=22)	91	

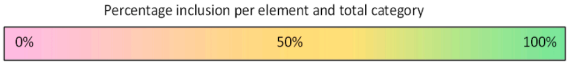

Fig. 4. Overview of reported elements, percentage inclusion per element and average percentage of inclusion per group. N = 24, unless stated otherwise. Some elements within the section PCR and library prep show three different values, indicating the percentage of included elements within PCR1, PCR2 and PCR3 (when applicable). y/n indicates whether a study mentioned the element was included. p indicates whether the tool/parameters were reported. The average percentage from bioinformatics is based on the y/n percentage from the elements that show two values. Average percentage was based on inclusion of elements, weighted by n.

et al., 2019; Tedersoo et al., 2022). Within the included studies, many quality-related steps – such as the inclusion of (negative) PCR controls, PCR clean-up, quality and concentration control – were only reported in about half of the studies. Steps related to the finalization of the metabarcoding library were reported even less, with library clean-up as well as library quality/concentration checks being reported in 33 % of the studies. These steps can affect the quality of the generated data (Taberlet et al., 2018b) and should be performed and reported adequately in order to guarantee reliability and reproducibility. Moreover, to maximize reproducibility, complete documentation of PCR protocols (mix, conditions, replicates, product pooling, etc.) should be provided.

3.2.4. Bioinformatics

After PCR and library preparation, samples are sequenced and data is generated for subsequent analysis. However, before analysis, some steps are needed to prepare the data. During this process, data is cleaned and structured (e.g. removing primer sequences or assigning taxa to OTUs), following a bioinformatics pipeline. As bioinformatic steps can affect the generated data, it is important to properly report these (Alberdi et al., 2018; Bokulich et al., 2013; Creedy et al., 2022; Piper et al., 2019). We extracted a number of common bioinformatics steps (see Fig. 4, Bioinformatics: Quality filtering – OTU/ASV clustering), of which most were reported in roughly half of the studies, with some being reported less (i.e., demultiplexing and denoising). Moreover, for many of the steps, incomplete information was given on the applied tools or parameters, making reproduction problematic.

A similar deficiency was observed in the reporting of negative control results, normalization procedures, and the selection of OTU cut-off points across the included studies, which are pivotal aspects in the data preparation process, substantially impacting the final dataset. As mentioned before, negative controls should be collected in order to account for contamination during sampling or subsequent processes. From the seventeen studies that included negative controls at least once (during sample collection, DNA extraction or PCR), five reported the generated read or OTU data from these samples. This data is indicative of the quality of the samples (Taberlet et al., 2018b, 2018c) and ideally should be reported. Oftentimes, samples have different sequencing depths (i.e., varying numbers of reads per sample). To make sure samples within a study are comparable, data is normalized, for instance by rarefaction (Mbareche et al., 2020). Sixty-three percent of the studies reported on this process, the other papers did not specify on this. Proper normalization is crucial for accurate comparisons between samples, ensuring that observed differences in community composition are not solely influenced by variations in sequencing depth. Related to this step is the selection of an OTU cut-off point. It is common practice to remove singletons (OTUs represented by a single read) during bioinformatics (Alberdi et al., 2018). However, often, different parameters are selected for this step, leading to diverse cut-off points. As there is no consensus on the selection of a cut-off point in metabarcoding studies, various methods are employed. For example, a certain minimum number of reads higher than two is selected (e.g., see Cordier et al., 2019 or Klunder et al., 2020), multiple numbers are selected to create multiple datasets (e.g. Frontalini et al., 2018), only OTUs that occur in multiple replicates are retained (e.g. Li et al., 2020; Li et al., 2023), or a minimum percentage rather than a number of reads is selected (e.g. Lanzén et al., 2021). Consequently, taxa are excluded from the dataset under the assumption of being false positives based on the selected criteria. Given that different filtering criteria yield different datasets (as demonstrated by Frontalini et al., 2018), it is crucial to be transparent about the applied parameters and thoroughly report all information pertaining to the chosen cut-off point. Within the included studies, about half reported the OTU cut-off point, either by indicating singletons were removed or by reporting a different cut-off point. Reporting negative control data as well as subsequent steps taken to account for contamination (when needed), data normalization and OTU-cut-off criteria should be standard practice in order to facilitate full transparency.

Another consideration is the assignment of taxa to OTUs. One of the advantages of eDNA over morphology-based surveys is that no thorough taxonomic knowledge is needed in order to obtain community data since (online) reference databases are used for the identification process (Keck et al., 2017). In the paper by Uchida et al. (2020), a significant correlation was observed between the stressor and two biotic indices at the genus level, but not at the family level. Since based on morphology, taxa could only be identified up to the family level, no correlation was observed through morphology-based methods, only through eDNA methods. This showcases the advantage of eDNA, as the data could be analyzed without the need for thorough taxonomic expertise. However, as mentioned earlier, one of the drawbacks of eDNA-based methods is the incompleteness of reference databases. If a species is not yet included in any database, it cannot be assigned to the corresponding OTU. While this can be problematic for the assessment of effects on actual species, indicator species or biotic indices, effects on alpha and beta diversity can still be assessed, even without exact species information. Assuming each OTU represents a different species allows for data analysis based on unique OTUs, mitigating the impact of incomplete databases (Keck et al., 2017). Alternatively, assigning taxa to a level higher than species ensures the inclusion of OTUs relevant to the target taxa, while also providing confidence that the OTUs included in the dataset genuinely belong to the intended taxonomic group. This approach allows for the incorporation of those OTUs that cannot be confidently identified at the species level, while preserving their status as unique and relevant species within the analysis.

3.2.5. Data analysis

The last step in the workflow is the data analysis. Elements within data analysis were reported quite consistently with an overall reporting score of 88 %. However, of the 23 studies that included alpha diversity, nine did not statistically analyze differences between communities based on alpha diversity. Moreover, of the 17 studies that did, four did not report statistical output. While we argue that alpha diversity potentially might be a less relevant indicator of stress-induced community composition compared to other diversity metrics, we still think it is important to fully report data on this to allow future, comprehensive comparisons between data types and assessment methods.

3.2.6. Harmonized reporting

When considering the reporting quality of each individual study, we observed an average reporting rate of 70 % across all elements, ranging between 53 % and 86 % (Fig. 5). Remarkably, none of the papers approximates 100 % reporting of elements that potentially affect the outcome of the study. Moreover, based on the publication year, it appears there is no current upward trend in reporting quality (Fig. 5). It is clear that there is room for improvement in reporting standards of eDNA-based stress-induced (macro-) invertebrate community composition studies. All of the above-mentioned steps can significantly impact the outcomes of an eDNA metabarcoding study and should be properly reported in order to ensure reliable and comparable research. Moreover, transparency is key in enabling the reproduction of research and harmonization of reporting standards should be upheld for eDNA-based stress-induced macroinvertebrate community composition assessment studies – and eDNA studies in general (Dickie et al., 2018; Fediajevaite et al., 2021; Johnson, 2002). We advise future studies to make use of the proposed minimum reporting guide.

An in-depth examination of the methodologies employed in the included studies revealed a large variation in applied methods (Table S9). This has been a known issue in eDNA (metabarcoding) studies and there have been multiple calls for reporting and methodology standardization (Bunholi et al., 2023; Dickie et al., 2018; Fediajevaite et al., 2021; Pawlowski et al., 2018; Shea et al., 2023). A possible explanation for why to date this has not yet been achieved is the complexity of standardizing a method that is applicable across diverse environments, sample substrates, and targeted taxa. While discrepancies

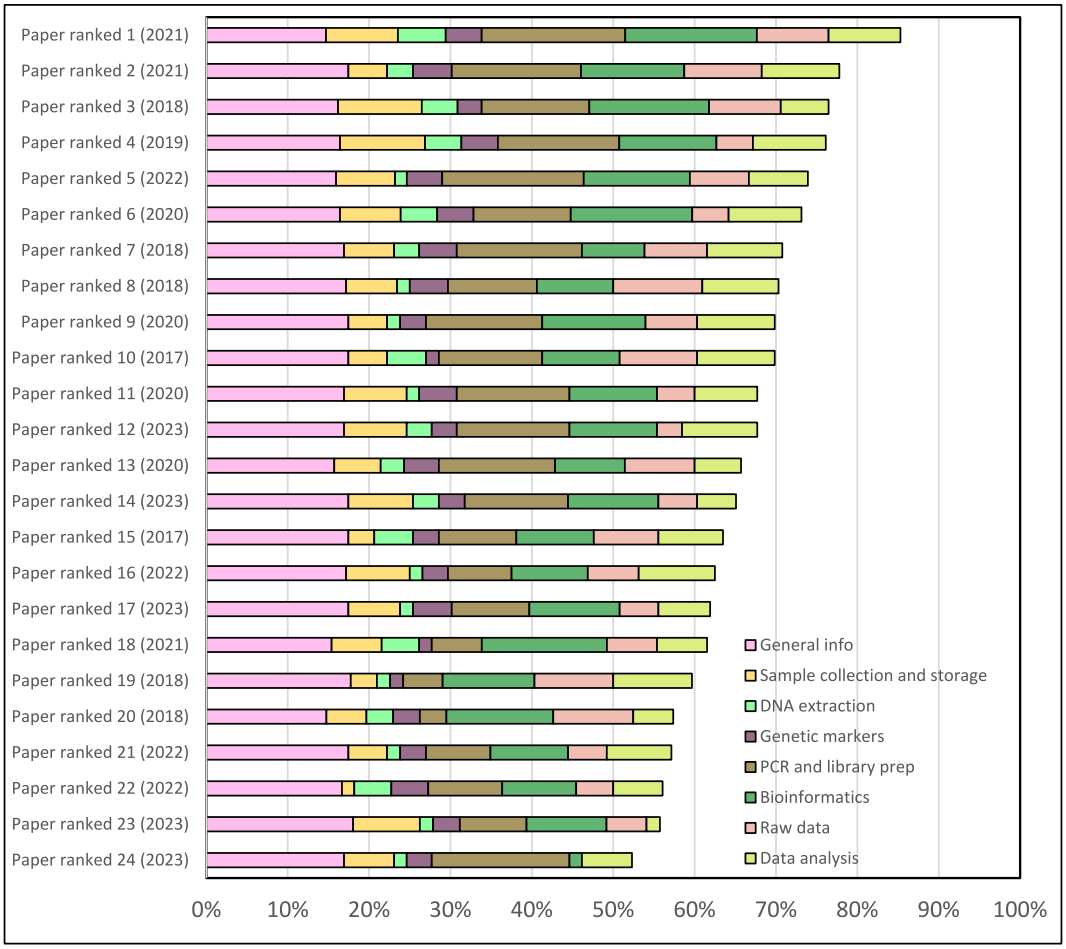

Fig. 5. List of 24 anonymized eDNA papers with publication year on stress-induced (macro-) invertebrate community composition, organized from high to low based on the reporting percentages of elements related to the study set up, methods and results. Different colors indicate different sections of the reported elements.

in the applied methods of eDNA-based stress-induced macroinvertebrate community composition studies are evident, the sensitivity of eDNA in detecting stress-induced changes – at least in beta diversity – does appear to be quite robust as discussed in this review (Table 2; Table S4). This robustness, demonstrated by the consistent beta diversity outcomes despite variations in methodology, aligns with the findings observed in a bulk metabarcoding study conducted by Van den Bulcke et al. (2023), who compared alpha and beta diversity scores between samples prepared in different labs using different protocols and found that bulk DNA metabarcoding yielded consistent, nearly identical beta diversity patterns, even when different protocols were used. Nevertheless, it is crucial to acknowledge that different methodological steps can yield varying results. Moreover, the same study also found that alpha diversity patterns were in fact susceptible to changes in the protocol, showing the need for standardized protocols to enhance comparability in alpha diversity between different studies. To address these challenges and bolster the integration of eDNA within ecotoxicology while moving towards a more standardized approach, we propose a best practices guide (Fig. 6). A comprehensive overview of the steps included in the best practices can be found in the Supplementary Information (Table S7). It should be noted that the proposed best practices are guiding principles rather than rigid protocols, due to the inherent variability in methodologies across the broad spectrum of eDNA-based community composition studies.

4. Conclusions

Within this study, the potential and compatibility of eDNA in ecotoxicology as a tool to assess community composition for macroinvertebrate communities is given as well as a clear path forward is provided for better integration of eDNA within ecotoxicology. By reviewing twenty-four papers that used eDNA metabarcoding to analyze stress-induced invertebrate community composition, we found that eDNA-derived beta diversity was a robust, sensitive indicator, outperforming traditionally obtained beta diversity data, making it a strong tool for invertebrate community assessment within ecotoxicology. Additionally, our investigation suggests that eDNA holds promise for water quality assessment through biotic indices, although further research is essential for validation. Moreover, the inclusion of taxa beyond (macro-) invertebrates, such as micro-organisms, could be a useful addition to the ecotoxicology toolkit for monitoring practices, especially since it is only a small extra effort and likely enhances the resolution and early detection of environmental changes. Despite occasional limitations in detecting certain taxa and potential false positives, eDNA appears a reliable source for stress-induced community composition assessment. Moreover, the non-invasive nature of eDNA techniques aligns with the 3Rs (Replacement, Reduction, Refinement) principle in animal research, contributing to the reduction of the need for traditional sampling methods that may involve invasive procedures, thus promoting ethical and responsible research practices. Nonetheless, our findings prompt consideration of potential pitfalls in the transition from morphology-based to eDNA monitoring within established standards.

Currently, there is little consistency in reporting and applied methodology within eDNA-based stress-induced macroinvertebrate community composition studies, even though this is desired to increase the

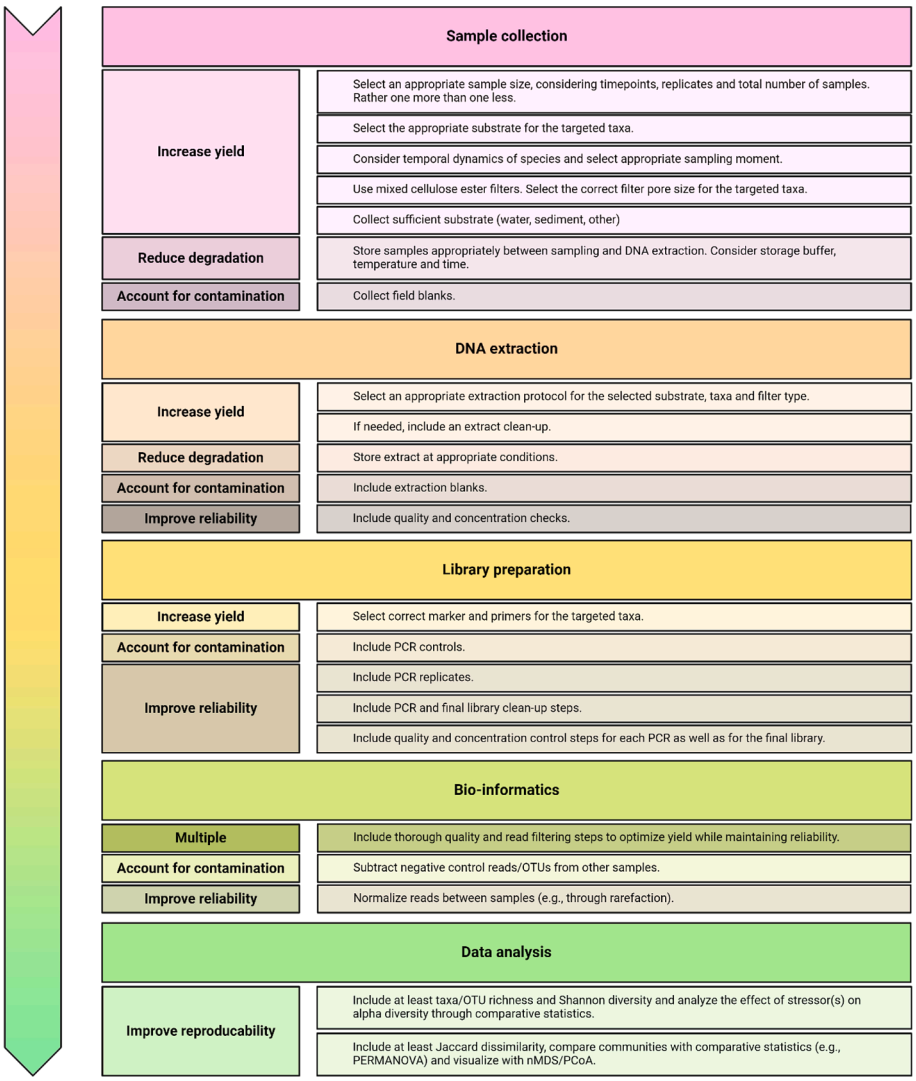

Fig. 6. Guidelines to improve stress-induced (macro-) invertebrate eDNA community composition studies, designed based on the applied methodologies of 24 relevant studies and relating literature.

reproducibility and reliability of studies. Predominantly elements related to quality control such as the implementation of negative controls throughout the workflow and other elements involved in DNA extraction, PCR and library preparation and bioinformatics we reported inconsistently. In order to validate and reproduce research, the quality of reporting has to improve. Moreover, to increase comparability between studies, a more standardized approach is desired. We proposed a minimum reporting, harmonization and best practice guide for future studies that aim to assess stress-induced (invertebrate) community composition through eDNA-metabarcoding.

CRediT authorship contribution statement

Martin van der Plas: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Krijn B. Trimbos:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Thijs Bosker:** Conceptualization, Writing – review & editing. **Martina G. Vijver:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Martina G. Vijver reports financial support was provided by European Union. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2025.113269.

Data availability

All data is included in the supplementary tables.

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