Science of the Total Environment 923 (2024) 171413

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

From DNA to diagnostics: A case study using macroinvertebrate metabarcoding to assess the effectiveness of restoration measures in a Dutch stream

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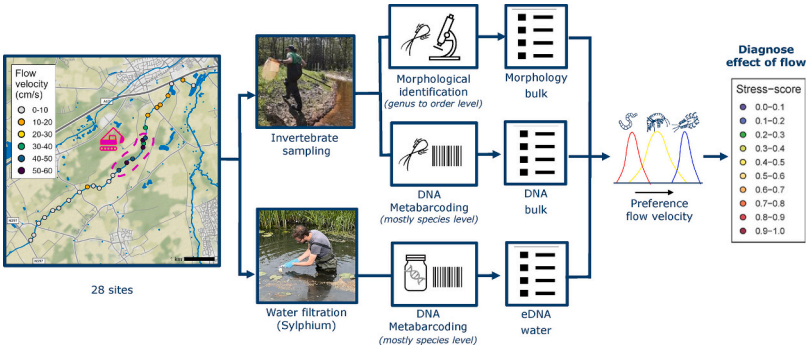
HIGHLIGHTS

- This study evaluated the potential of invertebrate DNA-metabarcoding for diagnosis.
- We computed a flow index using morphological identification, DNA and eDNA.
- eDNA could not discern differences in flow, as indicator taxa were not identified.
- Morphology and DNA identified similar communities, and indicated improved flow.
- DNA of bulk specimens is suitable to diagnose stress and assess restoration projects.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Sergi Sabater

Keywords:
DNA
Macroinvertebrates
Diagnosis
Restoration
Flow

ABSTRACT

Stream ecosystems are under pressure due to multiple stressors. Restoration measures can halt further degradation and improve their ecological status. However, assessment of the effectiveness of the implemented measures is often insufficient because of logistic and financial constraints. DNA-metabarcoding has been proposed to scale up sample processing, although its application as a diagnostic tool has received less attention. The aim of our study was to evaluate if DNA-metabarcoding of stream macroinvertebrates can be used to compute a stressor-specific index to assess the effectiveness of a stream restoration project. For this purpose, we sampled the upstream, restored, and downstream section of a recently restored lowland stream in the Netherlands. At each site, we applied three different methods of macroinvertebrate identification: morphological identification of bulk samples (morphology), DNA-metabarcoding of the same bulk samples (DNA) and metabarcoding of eDNA extracted from the water (eDNA). First, we compared the community composition identified by each method. The communities identified by morphology and DNA were highly similar, whereas the communities generated by the eDNA differed. Second, we analysed whether the identification methods could be used to assess the effectiveness of the restoration project, focussing on a stressor-specific index for flow as the restoration measures aimed at improving flow conditions. Both the morphology and bulk DNA samples indicated improved flow

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https://doi.org/10.1016/j.scitotenv.2024.171413
Received 14 December 2023; Received in revised form 12 February 2024; Accepted 29 February 2024
Available online 4 March 2024

conditions in the restored section of the stream (i.e., less stress from the reduction or absence of flow than in the unrestored sections). Contrary, the eDNA-water samples did not differentiate the amount of stress throughout the catchment, although applying recent developments in eDNA sampling could lead to more robust results. In conclusion, this study forms proof of concept that DNA from bulk samples can be utilized to assess the effectiveness of restoration measures, showing the added value of this approach for water managers.

1. Introduction

Streams and rivers are under pressure due to anthropogenic stressors, such as altered hydro-morphology, nutrient enrichment, and toxic substances (Birk et al., 2020; Lemm et al., 2021). To halt further degradation of these ecosystems and to improve their ecological status, many restoration projects have been implemented (e.g., reviews by Bernhardt et al., 2005, Griffith and McManus, 2020). Yet, evidence for positive effects of these restoration efforts on the biota remains limited (meta-analysis by Al-Zankana et al., 2020). To increase the success rate of restoration projects, there is a need to diagnose the most important stressors affecting the ecological status of the water body at relevant spatiotemporal scales (Dos Reis Oliveira et al., 2020). Moreover, the effectiveness of the implemented measures needs to be monitored more adequately (Friberg et al., 2016).

Traditionally, aquatic macroinvertebrates have been most widely used for monitoring the ecological status of streams and rivers, because of their ubiquitous occurrence and wide degree of known ecological preferences between species (Bonada et al., 2006). However, the morphological identification of macroinvertebrates to species-level requires taxonomic expertise and can also be time consuming which therefore can make it costly to process many samples (Jones, 2008). These logistical and financial constraints have often limited the monitoring of restoration projects at relevant spatiotemporal scales. To increase the number of sampling sites at the same cost, identification may be done at a coarser taxonomic resolution (e.g., genus or family level), which in turn leads to a loss of precision, as species within the same genus or family may vary in their environmental preference (Jones, 2008; Lenat and Resh, 2001). Alternatively, it has been advocated that molecular identification through high-throughput sequencing (DNA-metabarcoding) may be employed to overcome this trade-off between taxonomic resolution and the number of samples that can be processed in a certain timeframe (Bush et al., 2019; Vitecek et al., 2021).

As DNA-metabarcoding does not require taxonomic expertise and as it is less time consuming than morphological identification, it allows for higher spatial and/or temporal frequency of sampling in monitoring schemes (Elbrecht et al., 2017; Duarte et al., 2021). Moreover, it allows for finer taxonomic resolution, including identification of immature and damaged individuals that can often not be reliable identified to species-level based on morphological characteristics (Gleason et al., 2021). However, the accuracy of DNA-based methods is affected by the quality and completeness of reference databases (Weigand et al., 2019). Another limitation of DNA-metabarcoding is that estimation of species abundance is not possible (Pawlowski et al., 2018; Duarte et al., 2021; Turunen et al., 2021). Even though many of the biotic indices computed rely on abundance data, the samples can also be classified to the same categories using presence/absence data (e.g., Ecological Quality Ratio, Beentjes et al., 2018), and hence DNA-metabarcoding can be used to assess the ecological status of streams (Elbrecht et al., 2017; Kuntke et al., 2020).

Besides the identification of samples, sorting of macroinvertebrate individuals from the conventional debris-filled bulk samples is also time-consuming, and prone to errors (Haase et al., 2006). Hence, there is need to examine DNA-based methods that allow faster sample processing. Potentially, environmental DNA (eDNA) may be used as rapid, cost-effective, and non-invasive approach to characterize communities (Thomsen and Willerslev, 2015). For this, genetic material (e.g., cells or tissue) is collected from the environment, for example by filtering stream water, and subsequently analysed through DNA-metabarcoding (Deiner et al., 2016). Various studies have suggested that eDNA-metabarcoding may successfully be used to calculate macroinvertebrate-based water quality indices (e.g., Fernández et al., 2019; Ji et al., 2022), whilst others have argued that eDNA does not adequately represent local aquatic macroinvertebrate communities (e.g., Hajibabaei et al., 2019; Gleason et al., 2021). Thus, it remains disputed whether eDNA samples reflect the local macroinvertebrate community captured by bulk samples (Blackman et al., 2019).

To date, both bulk DNA (i.e., genetic material derived from the macrofauna specimens directly) and eDNA water samples have predominantly been used to compute indices designed to integrate the impact of multiple stressors into a single assessment of the ecological status, ranging for example from 'high' to 'bad' (EU Water Framework Directive) (e.g., Elbrecht et al., 2017; Fernández et al., 2019; Kuntke et al., 2020; Ji et al., 2022). These generic assessment metrics do, however, not allow for diagnosing the potential causes (i.e., stressors) of the ecological degradation (Pawlowski et al., 2018; Feld et al., 2020). One alternative is to compute stressor-specific indices based on the environmental preferences of macroinvertebrate species (Lemm et al., 2019; Poikane et al., 2020). Computing such stressor-specific indices at the appropriate spatial and temporal coverage would allow water managers to better select appropriate restoration measures to improve the ecological quality, and subsequently assess the effectiveness of the implemented measures on the specific stressors (Dos Reis Oliveira et al., 2020).

To date, assessment of the effectiveness of restoration measures is often insufficient because of logistic and financial constraints. DNA-metabarcoding has been proposed to scale up sample processing, although its application as a diagnostic tool has received less attention. Therefore, the aim of our study was to evaluate if DNA-metabarcoding of macroinvertebrates can be used to compute a stressor-specific index to assess the effectiveness of a restoration project. To this end, we sampled 28 sites in the upstream, restored, and downstream section of a recently restored lowland stream in the Netherlands. The study area was selected as it entails a typical Dutch lowland stream restoration project aiming at improving instream flow conditions, comprising of channel re-profiling and re-meandering (Dos Reis Oliveira et al., 2020). At each site, we applied three different methods of macroinvertebrate identification: 1. Morphological identification of bulk macroinvertebrate samples (morphology), 2. DNA-metabarcoding tissue from bulk macroinvertebrate samples (DNA) and 3. Metabarcoding of eDNA extracted from the water using filtration (eDNA). First, we compared the macroinvertebrate community composition identified by each method. We hypothesized that morphological identification, DNA of the bulk samples and eDNA of the water would identify a similar macroinvertebrate community. Second, we analysed whether the different identification methods could be used to assess the effectiveness of the restoration project, focussing on a stressor-specific index for flow as the restoration measures aimed at improving flow conditions. We hypothesized that the stressor-specific index computed for each identification method would indicate less stress by the (temporary) reduction or absence of flow in the restored section of the stream compared to the unrestored sections.

2. Material and methods

2.1. Study area

The present study was performed in the upper course of the lowland stream the Run, located in the south of the Netherlands (Fig. 1A, Supplement Table S1). The studied section comprised 9.5 km (slope of 0.9 m/km) of the stream between the provincial road N397 upstream (51°21′12.0″N 5°20′35.2″E) to the mouth of the stream into the stream the Dommel near the village Veldhoven (51°23′57.4″N 5°25′14.3″E) (Fig. 1B). In total, 28 sites were selected with an inter-site distance of 0.3 ± 0.1 km (± standard deviation). Until the mid-20th century, the catchment of the stream the Run consisted of bogs and marshlands. Land use changes, increased drainage and hydromorphological modifications resulted in a channelized and normalized stream embedded in an agricultural landscape. The intensification of agricultural activities in the catchment led to deterioration of the water quality of the stream. Discharge varies between approximately 400 l/s in the winter to 100 l/s in the summer, with values dropping below 50 l/s during periods of prolonged drought (Duursma, 2017). In 2021, the local water authorities restored a stream section of 3 km near the nature area Grootgoor to improve the ecological water quality and to increase the resistance of the catchment to climatic extremes (Fig. 1C). Restoration measures applied included decreasing the width and depth of the channel, re-meandering and adding dead wood. Additionally, the stream valley has been modified to retain and store water better during heavy rainfall events, reducing peak discharges downstream and to extend the period of water release during long periods without precipitation.

2.2. Field sampling

On the 10th and 11th of May 2022, macroinvertebrate samples were collected and eDNA was extracted from the water at the 28 sites. The macroinvertebrates were collected by sweeping a pond net over 1.5 m length of the dominant (micro-)habitats at each site (Supplement Table S2). The samples were transported to the laboratory and stored for maximum two days at 4 °C until further processing. The eDNA was extracted by taking a 750 ml water sample using the Sylphium eDNA Dual Filter Capsule (0.8 μm pore size; Sylphium) targeting the same (micro-)habitats as the macroinvertebrate samples at each site. For macro-organisms, it is commonly recommended to use a smaller pore size than we used in this study (e.g., Goldberg et al., 2018; Turner et al., 2014), yet since sections of the stream were heavily populated by filamentous green algae, we opted for a somewhat bigger pore size. However, it was still not always possible to reach the target volume due to filter clogging (Supplement Table S2). The filters were transported back to the laboratory in a cooler and were subsequently stored at −20 °C until further analysis. Additionally, various abiotic parameters were measured, including the dimensions of the stream, flow velocity, electrical conductivity and pH using a hand meter.

2.3. Macroinvertebrate sorting and identification

The macroinvertebrate samples were washed over 1 mm and 250 μm sieves, the full sample was sorted alive, and preserved in 50 ml tube with 96 % ethanol and kept at −20 °C until identification and subsequent DNA extraction. The macroinvertebrates were identified to genus- (Ephemeroptera, Plecoptera, Trichoptera and Coleoptera), subfamily- (Chironomidae), order- (Hydracarina, Oligochaeta), and in all other cases family level.

2.4. DNA extraction and amplification

The DNA extraction and amplification was performed at the dedicated DNA laboratory of Wageningen Environmental Research. The eDNA collected using the Sylphium Filter Capsules was extracted using the Environmental DNA isolation kit (Sylphium) following the manufacturers' protocol. A negative extraction control consisting of an unused Sylphium filter was included in this run. For the bulk samples, we use both DNA of the organisms as well as DNA extracted from the preservative ethanol and added these together for each sample, since several studies have shown that DNA extracted from this preservative ethanol in which specimens are collected can significantly increase the recovered diversity compared to only extracting DNA from specimens (Kirse et al., 2023; Marquina et al., 2019). DNA from preservative ethanol was first precipitated at −20 °C for one night by the addition of 1/33 (*v*/v) NaAc (PH 5.2, 3 M) and mixed by shaking and vortexing, followed by a centrifugation of 60 min at 4643*g* at 4 °C. Ethanol was then removed by pouring or using a pipet if the pellet was too fragile to pour. DNA from whole bodies of macrofauna was extracted using the Dneasy PowerMax

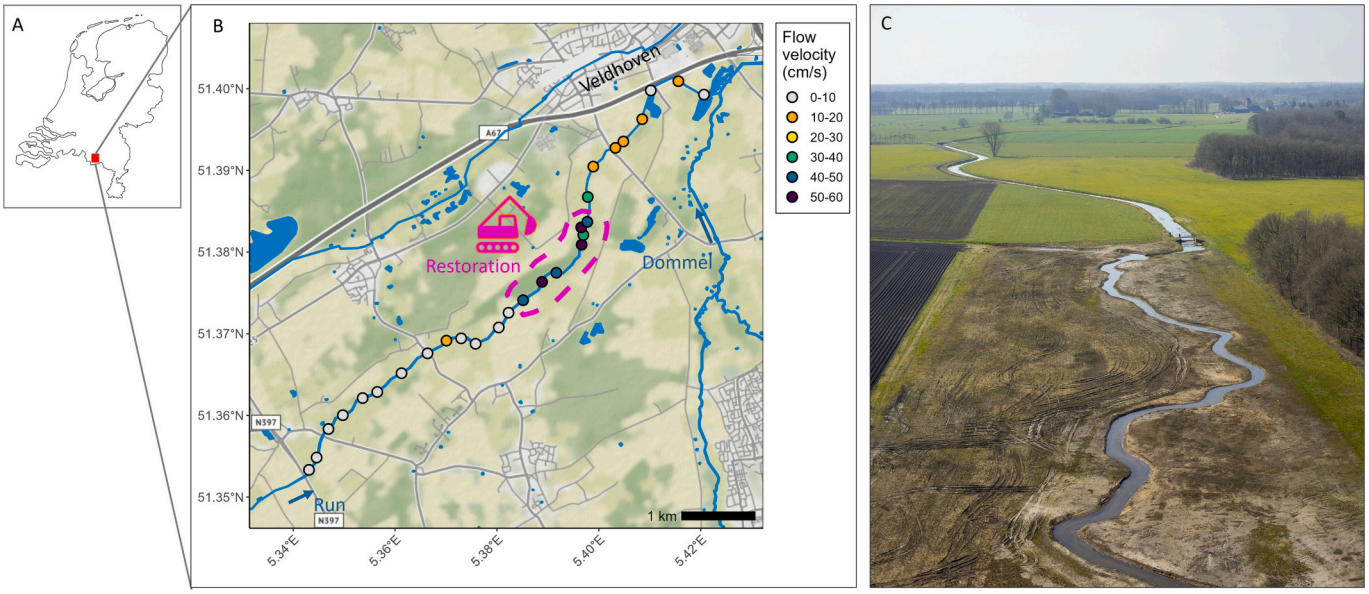

Fig. 1. Overview of the study site. A) location of the stream the Run in the Netherlands (indicated by a red square), B) location of the sampling sites (indicated by a dot with the colour indicating the flow velocity during sampling) and the location of the restoration measures (indicated by a pink dashed line), C) arial photograph of the restoration works with the unrestored upstream section in the background (picture: Matthijs de Vos).

Soil DNA Isolation kit (Qiagen) according to the manufacturer's protocol with an overnight incubation step with proteinase K at 56 °C and including negative extraction controls in each of the two extraction batches of samples.

For next-generation sequencing, a two-step PCR protocol was used to create a dual index amplicon library using primers mlCOIintF – HC02198 to target a 313 bp subset of the Folmer region of the mitochondrial COI marker (Leray et al., 2013). All primers were flanked by Fluidigm CS1/CS2 adapters at their 5′ ends. PCRs were performed in duplicate which were combined after amplification to take stochasticity in the reaction into account. Each reaction consisted of 12,5 μl, including 1 U Platinum Taq (Fisher Scientific), 1× PCR buffer, 2.5 mM MgCl2, 5 % (m/m) Trehalose, 200 ng/μl BSA, 200 μM dNTP, 250 μM water and 2.0 μl of sample DNA extract. The program consisted of 2 min activation at 94 °C, followed by 15 cycli of 30 s denaturation at 94 °C and annealing for 3 min using a touchdown program starting at 56 °C and decreasing by 1 °C each cycle (touchdown PCR), 1 min elongation at 72 °C, followed by 20 cycli of 30 s at 94 °C, 3 min at 39 °C and 10 min final extension at 72 °C. Two negative extraction blanks, two PCR negative and one positive control (*Eupoecila australasiae*, an Australian scarab beetle known not to occur in Europe) were included as well. Library preparation and addition of sample-specific index sequences ligated onto all PCR products was performed by Centre d'expertise et de services Génome Québec, Canada before sequencing on an Illumina MiSeq 2 × 250 bp paired end, v3 kit.

2.5. DNA bioinformatics and filtering

Raw fastq files were demultiplexed by Genome Quebec, who provided R1 and R2 files for each sample. All files were renamed into the QIIME2 format and imported into the QIIME2 platform version 2021.11 (Bolyen et al., 2019). The cutadapt plugin (Martin, 2011) was used to delete forward and reverse primers from both the R1 and R2 sequences, using a minimum sequence length of 200 bp and a maximum error rate of 0.2, discarding any reads that were untrimmed. The sequences were subsequently joined, denoised into ASVs (Amplicon Sequence Variants) and chimeras removed using the DADA2 plugin (Callahan et al., 2016). After visual inspection, the forward and reverse reads were truncated during this step at 200 bp with a read quality truncation of 20. After this step, only ASVs with a length of 3 bp shorter and longer than the expected 313 bp amplicon size were kept. The VSEARCH tool was used to cluster the resulting ASVs into 98 % clusters (OTUs; Operational Taxonomic Units) to reduce the number of sequences with identical taxonomic identifications (Rognes et al., 2016). Finally, to determine whether an ASV represented an actual biological sequence or a potential sequencing or PCR error, the LULU algorithm was used for curation using a minimum ratio setting of 10 (Frøslev et al., 2017).

Taxonomy assignment was performed using the BOLDigger package version 2.1.0 (Buchner and Leese, 2020) that makes it possible to access all records on the Barcode Of Life Data (BOLD) system, including early access and private records. The option digger_hit from the JAMP pipeline was used to obtain a last common ancestor of the top 20 hits returned from the BOLD website and all flagged hits were manually curated. The following thresholds were used: at least 98 % sequence similarity for species level identification, 95 % for genus, 90 % for family and anything lower is classified to the order level. Taxa with identical identifications were merged before filtering. The resulting sequences were further filtered to remove any I that was not identified as an aquatic macroinvertebrate according to the Dutch taxonomic water management list (TWN 06-04-2020), removing e.g., fungi, amoeba, fish, rotifers, and protists. The maximum number of reads from the positive control (*Eupoecila australasiae*) in any of the other samples was used as a filtering threshold to correct for potential leakage. This resulted in filtering out identifications that did not exceed 0,001 % of the total sum of reads pIOTU. After these filtering steps, the negative PCR control, eDNA extraction controls and one of the extraction negative controls of the bulk DNA samples were found to be clean. One extraction control of the bulk samples still contained more reads of the freshwater beetle *Haliplus lineatocollis* than in any of the samples, and this taxon was removed from the final dataset.

2.6. Calculation of the stressor-specific index for flow

The stressor-specific index for was calculated using the macroinvertebrate bottleneck analysis tool (https://knelpuntanalyse.containers.wur.nl/). The index applies the environmental preferences of the macroinvertebrate species determined by Verberk et al. (2012). Specifically, for flow it applies the combined preference score for medium (16–25 m/s) and fast flow (> 25 m/s). For the calculation of the stressor-specific index, the best available taxonomic resolution was used. As the morphology samples were identified to genus to order level, the mean preference was calculated for all the species within the higher taxonomic level that occur in upper course lowland streams in the Netherlands (Dutch Water Framework Directive water type R4a; Van der Molen et al., 2018). For morphology the index was calculated with abundance $\log_{10}(x + 1)$ transformed to reduce the effect of high densities, as well as set to 1 (present) and 0 (absent). For the DNA and eDNA samples, the abundance was set to 1 (present) and 0 (absent) at species level, as quantification of abundance through metabarcoding is not possible (Duarte et al., 2021). Next, the stress-score for flow was calculated as:

$$Stress - score_{flow} = \frac{\sum Abundance_{taxon} * Preference\ score\ flow_{taxon}}{\sum Abundance_{all\ taxa\ with\ preference\ score\ flow}}$$

The index then ranges the scores between 0 and 1 to the 5 % worst and 95 % best stress-scores for flow based on all macroinvertebrate data in upper course lowland streams obtained from the Dutch water authorities since 2005 (total 671 samples of water type R4a). Last, the index inverts the scores so that 0 indicates absence of stress and 1 indicates high stress by the (temporary) reduction or absence of flow.

2.7. Statistical analyses

We performed all data manipulation and statistical analyses using R version 4.2.1 (R Core Team, 2022). To test our first hypothesis stating that the three methods identified a similar macroinvertebrate community, we calculated Jaccard similarity scores between each different identification method (i.e., morphology vs. DNA, morphology vs. eDNA, and DNA vs. eDNA) for each site (*vegdist* function in *vegan* package, Oksanen et al., 2007). For calculation of the Jaccard similarity scores, we used presence/absence data and scaled back the taxonomic resolution of the DNA and eDNA samples to the coarser taxonomic resolution of the morphological identifications (i.e. at genus to order level). Next, we tested whether there was a significant difference in the Jaccard similarity scores between the different identification methods using a generalized linear mixed model (GLMM) with a random effect of intercept among sites, which accounts for correlation between measurements taken at the same site (*lmer* function in *lme4* package, Bates et al., 2015). The GLMM model was fitted using the restricted maximum likelihood (REML) approach and the *p*-values were derived using the Satterthwaite approximations to degrees of fre10um (*summ* function in *jtools* package, Long, 2022). Visual inspection of the quantile-quantile plot (qq-plot) showed that the residuals of the model approximated normal distribution (*qqmath* function in *lattice* package, Sarkar, 2008). To determine which Jaccard similarity scores differed, we conducted a pairwise analysis of the estimated marginal means with Holm correction for multiple testing (*emmeans* function in *emmeans* package, Lenth, 2023).

To test our second hypothesis stating that the stressor-specific index computed for each identification method would indicate less stress by the (temporary) reduction or absence of flow in the restored section compared to the unrestored sections, we visually assessed the spatial

distribution of the different stress-scores in relation to location of the restoration project. Next, we tested the relation between the stress-score for flow and the flow velocity measured for each identification method using linear regression models (*lm* function in *stats* package, R Core Team, 2022). Visual inspection of the qq-plots indicated that the residuals of the model approximated normal distribution.

3. Results

3.1. Comparison of the macroinvertebrate community composition

In the bulk samples of the 28 sites, we collected a total of 6505 individuals with a mean number per sample of 232 individuals (range: 61–817). The morphological identification of these bulk samples resulted in 75 taxa (mean: 18 taxa/site, range: 10–32, identified at genus to order level), whilst the DNA-metabarcoding of the same samples resulted in 240 taxa (mean: 41 taxa/site, range: 19–64, mostly identified at species level). The eDNA analysis failed at 2 sites due to filter clogging. Analysis of the remaining 26 sites resulted in 160 taxa (mean: 19 taxa/site, range: 20–38, mostly identified at species level). Excluding the 2 failed sites for the other two methods slightly reduced the total number of taxa observed in the morphology (72) and DNA samples (230) but did not change the mean and range of taxa per site.

In total, 106 taxa were identified by both DNA and eDNA, with Diptera and Oligochaeta as dominant groups (Fig. 2). The 54 taxa that were only identified by eDNA belonged also primarily to these groups. Another 124 taxa were uniquely identified by DNA, including taxa from various other groups, such as Coleoptera, Hydrachnidae, Odonata, Plecoptera and Trichoptera (details in Supplement Table S3).

To compare the community captured by all three methods, we scaled back the DNA and eDNA samples to the coarser taxonomic resolution of the morphological identification (i.e. at genus to order level) and then calculated the similarity between the communities per site. The Jaccard similarity scores differed significantly between comparisons (Fig. 3, Supplement Table S4). The communities identified by morphology and DNA samples were highly similar (mean ± sd: 75 ± 10 %), whilst the communities generated by the eDNA were significantly less like both the morphology (31 ± 7 %) and the DNA samples (32 ± 8 %).

3.2. Stressor-specific index for flow

We calculated a stressor-specific index for flow based on the ecological preference scores of the taxa found at each site with each method of identification. For this, the best available taxonomic resolution was used, i.e., morphology at genus to order level and both DNA and eDNA mostly at species level. A high stress-score indicates the (temporary) reduction or absence of flow. Both the morphology and DNA samples indicated a lower level of stress (i.e., improved flow conditions) in the restored section compared to the unrestored sections (Fig. 4A). Overall, the DNA samples provided a more positive assessment over a longer trajectory than the morphology based on abundance, as well as presence. Contrary to the bulk-samples, the eDNA samples did not differentiate the amount of stress throughout the catchment. Accordingly, the flow velocity in the stream related significantly to the stress-score for flow computed for the morphology samples (abundance Adj. $R^2 = 0.51$, *p*-value <0.001; presence Adj. $R^2 = 0.48$, p-value <0.001) and the DNA samples (Adj. $R^2 = 0.67$, p-value <0.001), but not to the eDNA samples (Adj. $R^2 = 0.03$, p-value = 0.19) (Fig. 4B). The higher adjusted R^2 of the DNA suggest that this method provided a more detailed assessment of the flow conditions in the catchment than the morphological identification.

4. Discussion

In line with our first hypothesis, the macroinvertebrate communities identified by morphology and DNA samples were highly similar. However, the eDNA samples captured a different subset of the macroinvertebrate community, including mostly Diptera and Oligochaeta taxa, whilst taxa from various other groups, such as Coleoptera, Hydrachnidae, Odonata, Plecoptera and Trichoptera were not detected. The latter macroinvertebrate taxa are often sensitive to environmental change (Schmidt-Kloiber and Hering, 2015), and thus crucial for computing stressor-specific indices. As such, the eDNA samples could not differentiate the amount of stress from (temporary) reduction or absence of flow throughout the catchment, contrary to our second hypothesis. In line with our second hypothesis, DNA-metabarcoding and morphological identification of bulk samples could both be used to assess the improved flow conditions in the restored section of the stream compared to the unrestored sections. The stronger relation of flow velocity to the stressor-specific score for flow computed for the DNA samples suggest that this method provides a more detailed assessment of the flow conditions than the morphological identification.

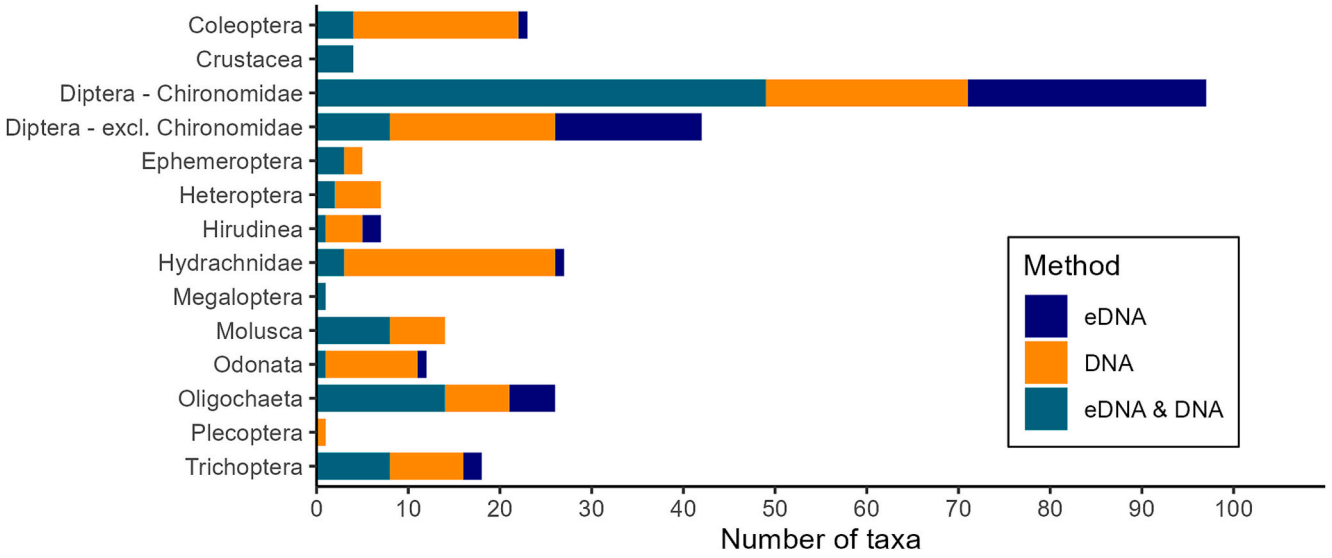

Fig. 2. Number of unique and shared macroinvertebrate taxa (identified mostly at species level) between the metabarcoding-based identification methods (DNA and eDNA) for different organism groups ($N = 26$ sites in the Run that were successfully identified by both methods).

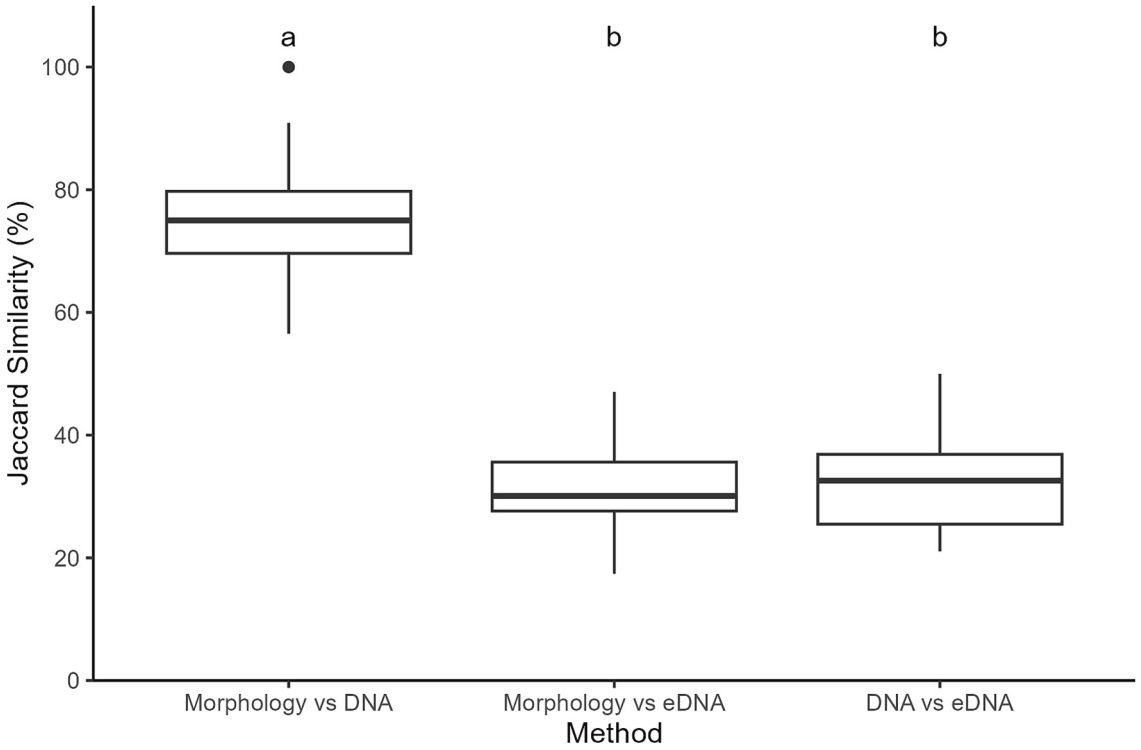

Fig. 3. Boxplot of the Jaccard Similarity between macroinvertebrate communities generated by three different identification methods. For comparison, the identifications of the DNA and eDNA samples were scaled back to the taxonomic resolution of the morphological identification (i.e. at genus to order level). Displayed are the central tendency, spread (whiskers: 1.5 IQR), and presence of outliers (dots). A different letter above a box plot represents a significant difference (N = 26 sites that were successfully identified by all three methods; details of the GLMM model in Supplement Table S4).

4.1. Lack of indicator taxa in eDNA samples

The low overlap between the macroinvertebrate community detected with eDNA filtered from the water compared to the DNA-metabarcoding and morphological identification of bulk samples at each site corresponds with the recent findings by Gleason et al. (2021) who found that eDNA did not represent the local community. Although several studies have shown that eDNA can detect a high macroinvertebrate species richness (Deiner et al., 2016; Mächler et al., 2019), there is evidence in line with our study that many indicator taxa are lacking when using eDNA (Macher et al., 2018; Hajibabaei et al., 2019; Keck et al., 2022). The effectiveness of detecting taxa with eDNA seems dependent on species traits and environmental characteristics (Doi et al., 2023). Potentially, the indicator taxa were not detected by eDNA because they were less abundant than the more ubiquitous Diptera and Oligochaeta or their hard exoskeleton results in less epithelial shedding (Barnes and Turner, 2016; Gleason et al., 2021). Moreover, it could be due to methodological issues, such as an inherent primer bias, the used water filter, or because large amounts of non-targeted taxa are detected with eDNA compared to DNA (Gleason et al., 2021). Accordingly, the number of taxa detected by eDNA may be improved by sampling, laboratory or bioinformatic processing (see Blackman et al., 2019). Notably, a recent study by Altermatt et al. (2023) suggests that to gain a full representation of the aquatic community much larger volumes of water need to be sampled than is currently done in most eDNA studies, including this study.

4.2. Potential of using DNA for diagnostics

Here, we give a proof of concept that DNA from macroinvertebrate bulk samples can be utilized to diagnose the variation in stress by the (temporary) reduction or absence of flow in the stream. Specifically, it was shown that the computed stressor-specific score for flow related to the flow velocity. It should be emphasized that water column flow velocity measurement used in our study are a simplified proxy for the improved hydrological conditions in the stream. Flow naturally fluctuates over time and is spatially complex, making it difficult to measure hydrological parameters relevant for the ecology (Verdonschot and van den Hoorn, 2010). Hence, stressor-specific indices based on DNA-metabarcoding offer the important advantage that the invertebrates integrate the environmental conditions over longer periods of time and relevant spatial scales even when the flow is temporarily lower or halted. The scope of this study was, however, restricted to diagnostics of one type of stressor, namely flow. Given that stressor-specific indices are rare and predominantly concentrate on eutrophication (Lemm et al., 2019; Poikane et al., 2020), we recommend that future research should focus on developing and testing indices for other stressors, such as drought, pesticides, siltation, and extreme water temperature fluctuations. To achieve this, the wide array of data on environmental preferences available on, for example, freshwaterecology.info could be drawn upon (Schmidt-Kloiber and Hering, 2015).

4.3. Implications for water management

Continued advancement of DNA-metabarcoding as diagnostic tool could assist water managers in assessing the effectiveness of restoration projects at multiple sampling points in the catchment. Our finding that flow velocity related stronger to the stressor-specific score for flow based on the DNA samples than the morphology samples implies that the finer taxonomic resolution obtained by DNA-metabarcoding seems to outweigh the lack of abundance data when calculating stressor-specific scores. Herewith, DNA-metabarcoding accounts for the varying habitat requirements and environmental preferences that occur at species-level (Jones, 2008). We should note that more detailed morphological identification would likely have given similar results, but this would have required a considerably greater time (and thus monetary) investment.

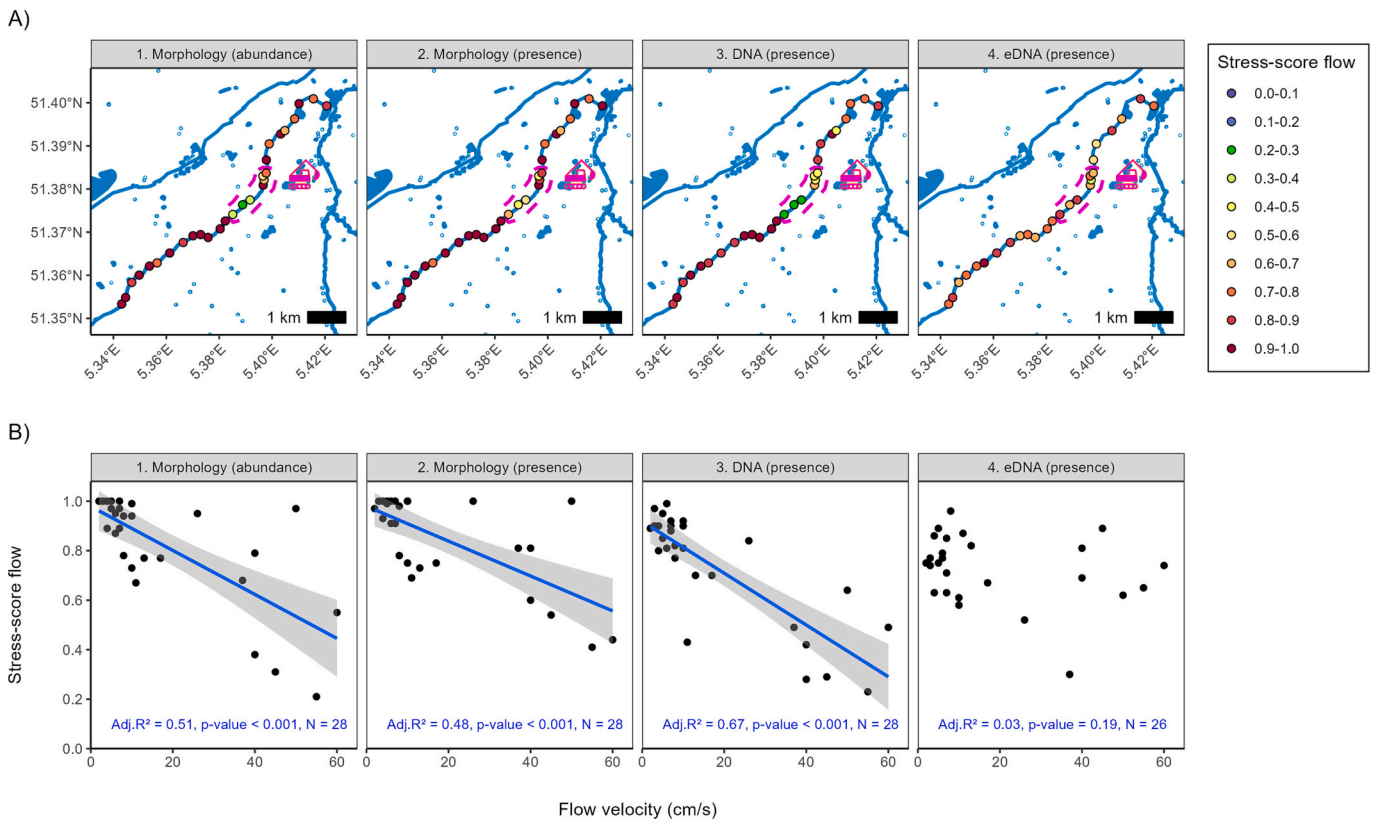

Fig. 4. Stress-score for macroinvertebrate flow preference shown A) spatially, with an indication of the restored section, and B) in relation to the flow velocity measured at each site tested for significance using a linear regression model. The stress-score for flow was computed based on: 1. Morphology (abundance, at genus to order level), 2. Morphology (presence, at genus to order level), 3. DNA (presence, at species level), and eDNA (presence, at species level). A stress-score of 0 indicates absence of stress and 1 indicates high stress by the (temporary) reduction or absence of flow.

Moreover, DNA-metabarcoding allows the identification of specimens which are in immature life stages during the sampling, which reduces the impact of seasonality in monitoring (i.e., reduce to need to visit a site in two different seasons; Orlofske and Baird, 2013). Although this study was only focussed on the spatial variation in the stream, the use of DNA-metabarcoding also opens the possibility for water managers to increase the number of samples over time. Adopting a Before-After-Control-Impact (BACI) design over longer time periods is one of the best ways to evaluate restoration measures and could strongly enhance the process of 'learning-by-doing' in stream restoration (Smokorowski and Randall, 2017; Dos Reis Oliveira et al., 2020).

5. Conclusions

To date, various studies have demonstrated that both bulk DNA and eDNA of water of macroinvertebrates can be applied to gain a generic assessment of the ecological status from "high" to "bad". In this study, we expanded on these findings by assessing the utility of these identification methods as diagnostic tool. Specifically, we evaluated whether DNA-metabarcoding of macroinvertebrates could be used to assess the effectiveness of a stream restoration project in the Netherlands by computing a stressor-specific index for flow. Both the morphology and bulk DNA samples indicated improved flow conditions in the restored section of the stream (i.e., less stress from the reduction or absence of flow than in the unrestored sections). Contrary, the eDNA-water samples did not differentiate the amount of stress throughout the catchment, although applying recent developments in eDNA sampling could lead to more robust results. In conclusion, this study forms proof of concept that DNA from bulk samples can be utilized to assess the effectiveness of restoration measures, showing the added value of this approach for water managers.

CRediT authorship contribution statement

Gea H. van der Lee: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Marcel Polling:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Iris van der Laan:** Writing – review & editing, Conceptualization. **Linda Kodde:** Writing – review & editing, Investigation. **Ralf C.M. Verdonschot:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gea H. van der Lee reports financial support was provided by Dutch Ministry of Agriculture, Nature and Food Quality. 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.

Data availability

Data will be made available on request.

Acknowledgements

This research was supported by the Dutch Ministry of Agriculture, Nature and Food Quality (BO-43-123-003). The research was initiated in collaboration with Aquon and MicroLan. We thank Mieke Moeleker for taking the macroinvertebrate samples, Anne-Marie van Noord for helping sorting and identifying the samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171413.

References

Altermatt, F., Carraro, L., Antonetti, M., Albouy, C., Zhang, Y., Lyet, A., Pellissier, L., 2023. Quantifying Biodiversity Using eDNA From Water Bodies: General Principles and Recommendations for Sampling Designs. Environmental DNA.

Al-Zankana, A.F.A., Matheson, T., Harper, D.M., 2020. How strong is the evidence–based on macroinvertebrate community responses–that river restoration works? Ecohydrol. Hydrobiol. 20 (2), 196–214.

Barnes, M.A., Turner, C.R., 2016. The ecology of environmental DNA and implications for conservation genetics. Conserv. Genet. 17 (1), 1–17.

Bates, B., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48.

Beentjes, K.K., Speksnijder, A.G., Schilthuizen, M., Schaub, B.E., van der Hoorn, B.B., 2018. The influence of macroinvertebrate abundance on the assessment of freshwater quality in the Netherlands. Metabarcoding Metagenomics 2, e26744.

Bernhardt, E.S., Palmer, M.A., Allan, J.D., Alexander, G., Barnas, K., Brooks, S., Sudduth, E., 2005. Synthesizing US river restoration efforts. Science 308 (5722), 636–637.

Birk, S., Chapman, D., Carvalho, L., Spears, B.M., Andersen, H.E., Argillier, C., Hering, D., 2020. Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. Nat. Ecol. Evol. 4 (8), 1060–1068.

Blackman, R.C., Mächler, E., Altermatt, F., Arnold, A., Beja, P., Boets, P., Deiner, K., 2019. Advancing the use of molecular methods for routine freshwater macroinvertebrate biomonitoring–the need for calibration experiments. Metabarcoding Metagenomics 3, 49–57.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37 (8), 852–857.

Bonada, N., Prat, N., Resh, V.H., Statzner, B., 2006. Developments in aquatic insect biomoni- toring: a comparative analysis of recent approaches. Annu. Rev. Entomol. 51, 495–523.

Buchner, D., Leese, F., 2020. BOLDigger–a Python package to identify and organise sequences with the barcode of life data systems. Metabarcoding Metagenomics 4, e53535.

Bush, A., Compson, Z.G., Monk, W.A., Porter, T.M., Steeves, R., Emilson, E., Baird, D.J., 2019. Studying ecosystems with DNA-metabarcoding: lessons from biomonitoring of aquatic macroinvertebrates. Front. Ecol. Evol. 7, 434.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13 (7), 581–583.

Deiner, K., Fronhofer, E.A., Mächler, E., Walser, J.C., Altermatt, F., 2016. Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nat. Commun. 7 (1), 12544.

Doi, H., Matsuoka, S., Matsuzaki, S.I.S., Nagano, M., Sato, H., Yamanaka, H., Miya, M., 2023. Species traits and ecosystem characteristics affect species detection by eDNA metabar- coding in lake fish communities. Freshw. Biol. 68 (8), 1346–1358.

Dos Reis Oliveira, P.C., van der Geest, H.G., Kraak, M.H., Westveer, J.J., Verdonschot, R. C., Verdonschot, P.F., 2020. Over forty years of lowland stream restoration: lessons learned? J. Environ. Manage. 264, 110417.

Duarte, S., Leite, B.R., Feio, M.J., Costa, F.O., Filipe, A.F., 2021. Integration of DNA-based approaches in aquatic ecological assessment using benthic macroinvertebrates. Water 13 (3), 331.

Duursma, S., 2017. Projectplan Run Grootgoor. HaskoningDHV.

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 Ecol. Evol. 8 (10), 1265–1275.

Feld, C.K., Saeedghalati, M., Hering, D., 2020. A framework to diagnose the causes of river ecosystem deterioration using biological symptoms. J. Appl. Ecol. 57 (11), 2271–2284.

Fernández, S., Rodríguez-Martínez, S., Martínez, J.L., Garcia-Vazquez, E., Ardura, A., 2019. How can eDNA contribute in riverine macroinvertebrate assessment? A metabarcoding approach in the Nalón River (Asturias, Northern Spain). Environ. DNA 1 (4), 385–401.

Friberg, N., Angelopoulos, N.V., Buijse, A.D., Cowx, I.G., Kail, J., Moe, T.F., Wolter, C., 2016. Effective river restoration in the 21st century: from trial and error to novel evidence-based approaches. Adv. Ecol. Res. 55, 535–611.

Frøslev, T.G., Kjøller, R., Bruun, H.H., Ejrnæs, R., Brunbjerg, A.K., Pietroni, C., Hansen, A. J., 2017. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nat. Commun. 8 (1), 1188.

Gleason, J.E., Elbrecht, V., Braukmann, T.W., Hanner, R.H., Cottenie, K., 2021. Assessment of stream macroinvertebrate communities with eDNA is not congruent with tissue-based metabarcoding. Mol. Ecol. 30 (13), 3239–3251.

Goldberg, C.S., Strickler, K.M., Fremier, A.K., 2018. Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: increasing efficacy of sampling designs. Sci. Total Environ. 633, 695–703.

Griffith, M.B., McManus, M.G., 2020. Consideration of spatial and temporal scales in stream restorations and biotic monitoring to assess restoration outcomes: a literature review. River Res. Appl. 36 (8), 1385–1415.

Haase, P., Murray-Bligh, J., Lohse, S., Pauls, S., Sundermann, A., Gunn, R., Clarke, R., 2006. Assessing the impact of errors in sorting and identifying macroinvertebrate samples. In: The Ecological Status of European Rivers: Evaluation and Intercalibration of Assessment Methods, pp. 505–521.

Hajibabaei, M., Porter, T.M., Robinson, C.V., Baird, D.J., Shokralla, S., Wright, M.T., 2019. Watered-down biodiversity? A comparison of metabarcoding results from DNA extracted from matched water and bulk tissue biomonitoring samples. PloS One 14 (12), e0225409.

Ji, F., Han, D., Yan, L., Yan, S., Zha, J., Shen, J., 2022. Assessment of benthic invertebrate diversity and river ecological status along an urbanized gradient using environmental DNA-metabarcoding and a traditional survey method. Sci. Total Environ. 806, 150587.

Jones, F.C., 2008. Taxonomic sufficiency: the influence of taxonomic resolution on freshwater bioassessments using benthic macroinvertebrates. Environ. Rev. 16 (NA), 45–69.

Keck, F., Hürlemann, S., Locher, N., Stamm, C., Deiner, K., Altermatt, F., 2022. A triad of kicknet sampling, eDNA-metabarcoding, and predictive modeling to assess aquatic macroinverte- brate biodiversity. bioRxiv 2022-01.

Kirse, A., Bourlat, S.J., Langen, K., Zapke, B., Zizka, V.M., 2023. Comparison of destructive and nondestructive DNA extraction methods for the metabarcoding of arthropod bulk samples. Mol. Ecol. Resour. 23, 92–105.

Kuntke, F., de Jonge, N., Hesselsøe, M., Nielsen, J.L., 2020. Stream water quality assessment by metabarcoding of invertebrates. Ecol. Indic. 111, 105982.

Lemm, J.U., Feld, C.K., Birk, S., 2019. Diagnosing the causes of river deterioration using stressor-specific metrics. Sci. Total Environ. 651, 1105–1113.

Lemm, J.U., Venohr, M., Globevnik, L., Stefanidis, K., Panagopoulos, Y., van Gils, J., Birk, S., 2021. Multiple stressors determine river ecological status at the European scale: towards an integrated understanding of river status deterioration. Glob. Chang. Biol. 27 (9), 1962–1975.

Lenat, D.R., Resh, V.H., 2001. Taxonomy and stream ecology—the benefits of genus-and species-level identifications. J. North Am. Benthol. Soc. 20 (2), 287–298.

Lenth, R., 2023. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.7.

Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Machida, R.J., 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Front. Zool. 10 (1), 1–14.

Long, J.A., 2022. jtools: Analysis and Presentation of Social Scientific Data. R package version 2.2.0.

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. Mol. Ecol. Resour. 18 (6), 1456–1468.

Mächler, E., Little, C.J., Wüthrich, R., Alther, R., Fronhofer, E.A., Gounand, I., Altermatt, F., 2019. Assessing different components of diversity across a river network using eDNA. Environ. DNA 1 (3), 290–301.

Marquina, D., Esparza-Salas, R., Roslin, T., Ronquist, F., 2019. Establishing arthropod community composition using metabarcoding: surprising inconsistencies between soil samples and preservative ethanol and homogenate from Malaise trap catches. Mol. Ecol. Resour. 19, 1516–1530.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. J. 17 (1), 10–12.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J., Suggests, M.A.S.S., 2007. The Vegan Package. Community Ecology Package, 10 (631–637), 719.

Orlofske, J.M., Baird, D.J., 2013. The tiny mayfly in the room: implications of size-dependent invertebrate taxonomic identification for biomonitoring data properties. Aquat. Ecol. 47, 481–494.

Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apothéloz-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. Sci. Total Environ. 637, 1295–1310.

Poikane, S., Herrero, F.S., Kelly, M.G., Borja, A., Birk, S., van de Bund, W., 2020. European aquatic ecological assessment methods: a critical review of their sensitivity to key pressures. Sci. Total Environ. 740, 140075.

R Core Team, R, 2022. R: A Language and Environment for Statistical Computing.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584.

Sarkar, D., 2008. Lattice: Multivariate Data Visualization with R. Springer, New York.

Schmidt-Kloiber, A., Hering, D., 2015. www. freshwaterecology. Info–an online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. Ecol. Indic. 53, 271–282.

Smokorowski, K.E., Randall, R.G., 2017. Cautions on using the before-after-control-impact design in environmental effects monitoring programs. Facets 2 (1), 212–232.

Thomsen, P.F., Willerslev, E., 2015. Environmental DNA–an emerging tool in conservation for monitoring past and present biodiversity. Biol. Conserv. 183, 4–18.

Turner, C.R., Barnes, M.A., Xu, C.C., Jones, S.E., Jerde, C.L., Lodge, D.M., 2014. Particle size distribution and optimal capture of aqueous macrobial eDNA. Methods Ecol. Evol. 5 (7), 676–684.

Turunen, J., Mykrä, H., Elbrecht, V., Steinke, D., Braukmann, T., Aroviita, J., 2021. The power of metabarcoding: can we improve bioassessment and biodiversity surveys of stream macroinvertebrate communities? Metabarcoding Metagenomics 5, e68938.

Van der Molen, D.T., Pot, R., Evers, C.H.M., Van Herpen, F.C.J., Van Nieuwerburgh, L.L. J., 2018. Referenties en maatlatten voor natuurlijke watertypen voor de Kaderrichtlijn Water 2021–2027. STOWA report 2018-49, Amersfoort.

Verberk, W.C.E.P., Verdonschot, P.F.M., Van Haaren, T., Van Maanen, B., 2012. Milieu-en habitatpreferenties van Nederlandse zoetwater-macrofauna. STOWA.

Verdonschot, P.F., van den Hoorn, M., 2010. Using discharge dynamics characteristics to predict the effects of climate change on macroinvertebrates in lowland streams. J. N. Am. Benthol. Soc. 29 (4), 1491–1509.
Vitecek, S., Johnson, R.K., Poikane, S., 2021. Assessing the ecological status of European rivers and lakes using benthic invertebrate communities: a practical catalogue of metrics and methods. Water 13 (3), 346.
Weigand, H., Beermann, A.J., Ćiampor, F., Costa, F.O., Csabai, Z., Duarte, S., Ekrem, T., 2019. DNA barcode reference libraries for the monitoring of aquatic biota in Europe: gap-analysis and recommendations for future work. Sci. Total Environ. 678, 499–524.