Received: 22 November 2019 | Accepted: 18 July 2020

DOI: 10.1111/mec.15597

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Assessment of stream macroinvertebrate communities with eDNA is not congruent with tissue-based metabarcoding

Jennifer Erin Gleason[1] | Vasco Elbrecht[2,3] | Thomas W. A. Braukmann[3] | Robert H. Hanner[1] | Karl Cottenie[1]

[1]Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

[2]Centre for Biodiversity Monitoring, Zoological Research Museum Alexander Koenig, Bonn, Germany

[3]Centre for Biodiversity Genomics, University of Guelph, Guelph, ON, Canada

Correspondence
Jennifer Erin Gleason, Department of Integrative Biology, University of Guelph, Guelph, ON, Canada.
Emails: jgleason@uoguelph.ca; jen.gleason13@gmail.com

Funding information
Canada First Research Excellence Fund; Natural Sciences and Engineering Research Council of Canada

Abstract

Freshwater biomonitoring programmes routinely sample aquatic macroinvertebrates. These samples are time-consuming to collect, as well as challenging and costly to identify reliably genus or species. Environmental DNA (eDNA) metabarcoding has emerged as a surrogate to traditional collection techniques and has been used in whole-community approaches across several taxa and ecosystems. However, the usefulness of eDNA-based detection of freshwater macroinvertebrates has not been extensively explored. Few studies have directly compared bulk sample and eDNA metabarcoding at a local scale to assess how effective each method is at characterizing aquatic macroinvertebrate communities. Here, we collected both eDNA and kick-net samples at the same sample transect locations across nine different streams in southern Ontario, Canada. We observed minimal overlap in community composition between these paired samples. Bulk tissue metabarcoding resulted in a greater proportion of sequences belonging to metazoan taxa (over 99%) than eDNA (12%) and had higher OTU richness for macroinvertebrate taxa. We suggest that degenerate primers are not effective for eDNA metabarcoding due to the high degree of nontarget amplification and subsequently low yield of target DNA. While both bulk sample and eDNA metabarcoding had the power to detect differences between stream communities, eDNA did not represent local communities. Bulk tissue metabarcoding thus provides a more accurate representation of local stream macroinvertebrate communities and is the preferred method if smaller-scale spatial resolution is an important factor in data analyses.

KEYWORDS
biomonitoring, eDNA, macroinvertebrates, metabarcoding, streams

1 | INTRODUCTION

Freshwater habitats are particularly sensitive to changes caused by anthropogenic stress, including habitat degradation, pollution and alteration of water inputs and flow (Dudgeon et al., 2006). Bioindicators are species or taxonomic groups that respond predictably to environmental changes (Cairns & Pratt, 1993) and are often used to evaluate the degree of disturbance or habitat impairment (Norris & Hawkins, 2000). Aquatic macroinvertebrates are commonly used bioindicators in both lentic and lotic systems (Bonada, Prat, Resh, & Statzner, 2006; Cairns & Pratt, 1993); however, they present challenges to traditional morphological identification as some immature stages cannot be reliably identified. The difficulty of identifying juvenile or damaged specimens can result in a significant

amount of human error due to misidentification (Haase et al., 2006). Additionally, the vast diversity of macroinvertebrates compared to other groups (e.g. vertebrates and plants) and the large number of individuals per sample can make sorting samples challenging, and obtaining species-level taxonomic resolution requires taxonomic expertise and is both time-consuming and costly (Marshall, Steward, & Harch, 2006). Routine biomonitoring typically involves processing numerous samples, and due to the above constraints, environmental assessments based on morphological identifications often use coarse taxonomic resolution (e.g. family level) as a surrogate (Buss et al., 2015). However, benthic macroinvertebrate families can be very diverse and contain genera and species across several different feeding guilds with specific habitat preferences or tolerances (Beermann, Zizka, Elbrecht, Baranov, & Leese, 2018; Macher et al., 2016; Resh & Unzicker, 1975). Lumping species together using coarse-level taxonomic identification can mask species-level turnover within a genus or family, or not prove sensitive enough to detect impairment or other ecological patterns (e.g. Gleason & Rooney, 2017).

Due to the challenges imposed by morphological approaches, the use of molecular identification through high-throughput sequencing (HTS; e.g. DNA metabarcoding, Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012) has become increasingly popular for aquatic macroinvertebrate communities (Carew, Pettigrove, Metzeling, & Hoffmann, 2013; Elbrecht, Vamos, Meissner, Aroviita, & Leese, 2017; Hajibabaei, Shokralla, Zhou, Singer, & Baird, 2011; Keck, Vasselon, Tapolczai, Rimet, & Bouchez, 2017). The use of molecular identification can yield higher taxonomic resolution than morphological approaches (e.g. Serrana, Miyake, Gamboa, & Watanabe, 2019; Sweeney, Battle, Jackson, & Dapkey, 2011), and HTS platforms can efficiently and cost-effectively sequence entire bulk specimen samples (Shokralla et al., 2014). Several metabarcoding studies (Elbrecht et al., 2017; Shokralla et al., 2014) have already demonstrated the ability of DNA-based taxonomy to resolve difficult-to-identify macroinvertebrate taxa. For example, Beermann, Zizka, et al. (2018) detected 183 operational taxonomic units (OTUs) in the family Chironomidae (a diverse and ubiquitous family of Diptera) in a single-stream mesocosm experiment. Chironomid larvae are often the most abundant family in macroinvertebrate samples (Beermann, Elbrecht, et al., 2018; Gleason & Rooney, 2018), but are difficult to identify and are often lumped together as one group. Using metabarcoding, Beermann, Zizka, et al. (2018) not only unveiled the vast diversity of chironomids within a single mesocosm, but also demonstrated that different groups displayed unique sensitivities to environmental stressors. Using DNA metabarcoding in lieu of coarse-level identification can reveal species-level responses that were previously hidden by taxonomic surrogacy and provide valuable information on the response patterns of organisms to changes in environmental conditions.

Despite the continued cost-effectiveness of HTS platforms and utility of metabarcoding for biomonitoring applications, this approach still requires sorting hundreds or thousands of individual specimens from often debris-filled benthic samples. While sorting benthic macroinvertebrate samples does not require taxonomic expertise, it is still a time-consuming process and can bottleneck freshwater monitoring research. In some cases, specimens can be ground without sorting (Pereira-da-Conceicoa et al., 2020), but it is not clear if that is a possibility for all stream conditions (e.g. samples with a lot of organic debris or sand). A potential solution is to bypass benthic samples all together and collect environmental DNA (eDNA) directly from water samples to characterize macroinvertebrate communities (Deiner, Fronhofer, Mächler, Walser, & Altermatt, 2016; Hajibabaei et al., 2019; Macher et al., 2018; Mächler et al., 2019). This is appealing because it avoids the time-limiting step of physically collecting and sorting through bulk samples. In addition to saving costs and person-hours, eDNA field sampling avoids disturbance to the stream bed and minimizes the risk of transferring aquatic pathogens between study sites, making it appealing from a biomonitoring perspective. The interest in the potential of eDNA to sample entire communities (e.g. through metabarcoding) has consequently increased (Creer et al., 2016; Deiner et al., 2017; Valentini et al., 2016). Deiner et al. (2016) demonstrated the power of eDNA methods to sample diverse communities by detecting 296 families of eukaryotic metazoans in a Swiss river. They compared these results to benthic kicknet sampling and observed greater family-level richness using eDNA, suggesting that eDNA may be a more effective method for monitoring taxa richness (Deiner et al., 2016). The promise of a sensitive and cost-effective tool has made eDNA appealing for biomonitoring and biodiversity science (Thomsen & Willerslev, 2015) and has prompted research exploring its applications across many different taxa and habitat types (Deiner et al., 2017).

However, there are currently many unknowns surrounding the origin, fate and longevity of DNA in aquatic habitats (Barnes & Turner, 2016). The transport of eDNA is particularly important in lotic systems. Genetic material should flow downstream (Deiner & Altermatt, 2014; Jane et al., 2015), but the distance it can travel and how long it persists in the environment can vary. For example, Wacker et al. (2019) observed no decrease in eDNA signal from freshwater pearl mussels (*Margaritifera margaritifera*) along a 1.7 km stretch of river, whereas Nukazawa, Hamasuna, and Suzuki (2018) were able to detect common carp (*Cyprinus carpio)* as far as 3 km downstream from its source while the amount of target DNA copies/L decreased with distance. When sampling for another species of mussel (*Unio tumidus*), Deiner and Altermatt (2014) discovered that the amount of eDNA decreased with distance but could still be detected up to 9.1 km from the source population. The potential of eDNA to travel far from its point of origin in rivers and streams means that eDNA presence may not reflect local communities but rather a landscape-scale integration of taxa present across the entire watershed (Deiner et al., 2016).

The comparison of eDNA-generated community inventories collected via water samples to those collected or surveyed using standard methods like kick sampling is an essential validation step to determine the reliability of using eDNA for a given taxon and habitat type. For aquatic vertebrates, the comparison of eDNA to traditional collection techniques has been promising. For both amphibians and

fish, Valentini et al. (2016) detected an equal or greater number of species using eDNA across several different aquatic habitats, and Fuji et al. (2019) observed a 70% overlap in species composition when comparing eDNA metabarcoding to traditional sampling techniques for fish. However, the effectiveness of eDNA for sampling benthic macroinvertebrate communities has been mixed. In a targeted approach, Mächler, Deiner, Steinmann, and Altermatt (2014) compared benthic kicknet samples and eDNA samples for six macroinvertebrate species (including gastropods, isopods and insects) and observed medium to high concordance between detections for each sampling method. However, Hajibabaei et al. (2019) reported that overall arthropod richness in Canadian wetlands was three times higher using bulk sample metabarcoding than eDNA metabarcoding and that there was only an 18% overlap in ESVs (exact sequence variants) between all pooled samples. While more taxa were detected using eDNA metabarcoding compared to bulk sample metabarcoding in New Zealand streams by Macher et al. (2018), more metazoan (and classical bioindicator taxa) OTUs were detected using bulk sample metabarcoding. Since environmental sampling contains all the microorganisms present in the water sample, it is possible that eDNA metabarcoding results in a higher amount of nonspecific amplification than bulk sample metabarcoding (Pereira-da-Conceicoa et al., 2020). While Macher et al. (2018) reported the proportions of OTUs belonging to major taxonomic groups detected using each method using presence–absence data, it is also informative to compare how targeted groups are represented by total sequence reads to determine which method best samples the community of interest.

Few studies have directly compared the community composition of aquatic macroinvertebrate samples collected using eDNA and kicknets and those that have focused on overall community dissimilarities between the two methods (Deiner et al., 2016; Macher et al., 2018; Pereira-da-Conceicoa et al., 2020). By pooling all samples and comparing them, it is not possible to observe variations in community composition that occur at the site or stream level. For example, eDNA samples taken along the same transect in a river may not detect the same species because eDNA is not necessarily homogeneously distributed in the water column. Macher and Leese (2017) collected water samples for eDNA metabarcoding at four proximate sample points in three different rivers and in some streams, and observed differences in community composition based on sample location (right or left riverbank) or depth (top or bottom of water column). At small sample scales, this patchy distribution of eDNA can be detrimental to the goals of biodiversity monitoring and it is therefore important to compare paired bulk samples and eDNA samples directly.

How closely eDNA sampling reflects the community composition locally present in the stream benthos remains unclear. Given that previous comparative studies have reported taxa unique to each sampling method, it is likely that eDNA sampled from the water column and bulk samples collected from the benthos at the same location detect different species. Here, we compared paired eDNA and bulk samples using three methods of aquatic macroinvertebrate identification: (a) coarse-level taxonomic resolution based on morphological identification of bulk macroinvertebrate samples, (b) metabarcoding tissue from bulk macroinvertebrate samples and (c) metabarcoding eDNA samples collected via water filtration. Based on previous studies and the uncertainties surrounding eDNA transport and persistence, we predicted that the communities generated using eDNA metabarcoding will differ from those collected using traditional kicknet collection (both morphological and HTS identification) and also contain substantially more nontarget reads than metabarcoding of bulk invertebrate samples. While we expect there to be differences in community composition between these two collection methods, we aim to quantify this difference. A large difference in community composition (e.g. minimal overlap in OTUs) would be important to consider for river assessments. To test these predictions, we determined the overall composition between major groups at both the OTU and sequence read level and compared dissimilarities between methods. Additionally, we addressed an existing knowledge gap by comparing the "overlap" in OTU identity between paired bulk and eDNA samples rather than the overall difference between pooled samples. Previous studies have combined entire eDNA data sets to compare against similarly pooled bulk tissue samples, and this does not accurately reflect whether or not an OTU was detected by each method at the same site. We further hypothesized that both molecular approaches will provide higher taxonomic resolution and thus have a stronger stream-level signal than coarse-level morphological identification. Finally, between the two metabarcoding approaches (bulk sample and eDNA), we expected that bulk samples will be better able to characterize aquatic macroinvertebrate communities at the stream level because kicknet collecting is sampling from the local benthos, whereas eDNA sampling likely incorporates community information from larger spatial scales.

2 | METHODS

2.1 | Fieldwork

2.1.1 | Site selection

We selected three streams in three adjacent watersheds that drain into Lake Erie for a total of nine stream sites in southern Ontario, Canada (Figure 1a). The sites were located either on Conservation Authority properties or on privately owned land with farms in the catchment and thus ranged across a gradient of local and landscape-level agricultural activity. At each stream, we selected a downstream and upstream transect location approximately 50 m apart. Six sites were sampled once at each upstream and downstream transects (2 samples total), and three sites were sampled more intensively (5–6 samples total, split between upstream and downstream sampling locations) for both eDNA and benthic macroinvertebrate sampling (Figure 1b).

2.1.2 | eDNA collection

We collected eDNA by filtering 1.5 L of water through a 5-μm nitrocellulose filter on site using the ANDE backpack system (Thomas,

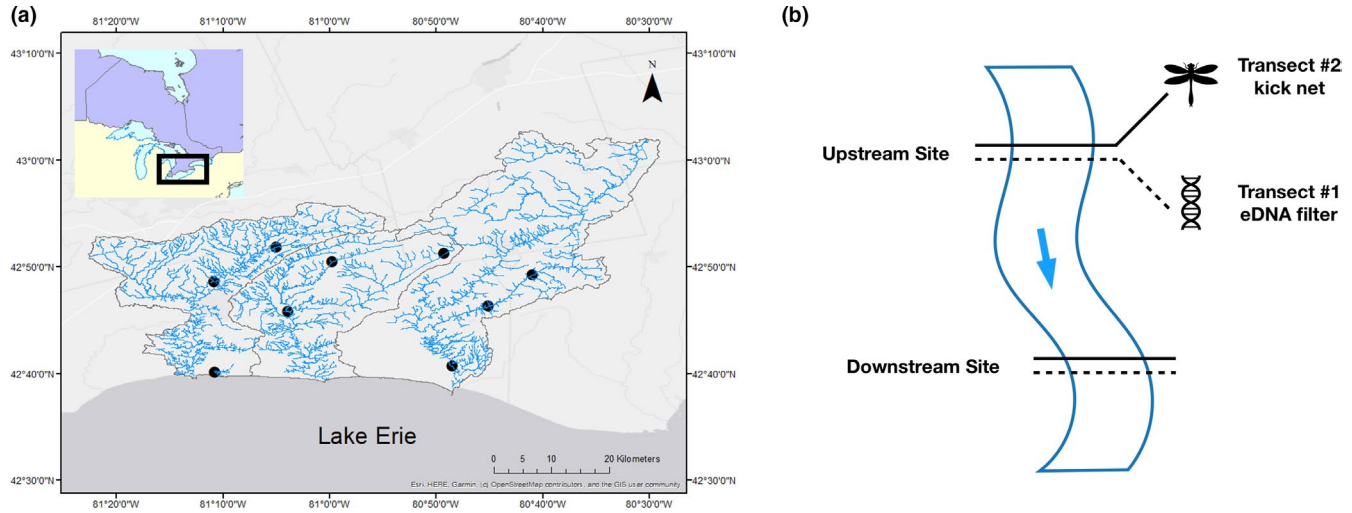

FIGURE 1 (a) A map of nine stream sites in three subwatersheds in southern Ontario, Canada, and (b) a diagram of our paired eDNA and benthic kicknet sampling design

Howard, Nguyen, Seimon, & Goldberg, 2018). We selected a 5-μm filter over a smaller pore size as our stream sites were turbid, and we chose to prioritize total volume filtered over a smaller pore size, as suggested by Thomas et al. (2018). At each site, we also filtered 1.5 L of de-ionized water through a 5-μm filter as a negative field control to assess the amount of field contamination that occurs during eDNA sampling. We preserved each eDNA filter (or negative control) in a 2-mL vial filled with 95% ethanol. The samples were stored in a cooler during transit from the field site and then stored in a −80°C freezer until DNA extraction.

2.1.3 | Benthic macroinvertebrate collection

After eDNA sampling, we collected benthic macroinvertebrates using a standard 3-min travelling kick-and-sweep transect using a 500-μm mesh D-net based on the Ontario Stream Assessment Protocol (Stanfield, 2017). All collected material was sorted using a 500-μm sieve, and benthic macroinvertebrates samples were preserved on site in 95% ethanol. We transported the samples back to the laboratory in a cooler and stored them at 4°C until further processing.

2.2 | Laboratory work

2.2.1 | Benthic sorting and DNA extraction

Benthic macroinvertebrate samples were sorted and identified to family-level resolution for arthropods and phylum-level resolution for molluscs and annelids (taxon list and abundance available in Supplementary information S5). We air-dried the benthic macroinvertebrate samples on a clean laboratory bench in grinding tubes covered with Kimwipes to prevent infall. Once dry, we ground the tissue samples at 4,000 rpm for 30 min using an IKA Tube Mill with 20-ml tubes and ten 4-mm-diameter steel beads (IKA, Staufen, Germany). Smaller samples were ground in 2-ml reaction tubes with two steel beads using a TissueLyser II (Qiagen, Hilden, Germany) set to a speed of 30 hz (or 1,800 oscillations a minute) for one minute. After homogenization, we transferred an average of 18.89 mg (± 7.95 *SD*) of each sample to a sterile 2-mL tube. We extracted DNA from ground tissue powder using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) and visualized the products using agarose gel electrophoresis.

2.2.2 | eDNA extraction

We used a CTAB extraction protocol for DNA extraction from the eDNA filters (see Turner, Miller, Coyne, & Corush, 2014). We cut each filter into eighths and placed two filter pieces into a 2-ml vial containing approximately 100 μl of sterilized glass beads and 500 μl of prewarmed (65°C) CTAB lysis buffer (VWR International, Radnor, PA, USA) for a total of four vials per filter. We homogenized the samples using a TissueLyser II (Qiagen, Hilden, Germany) at 30 hz for two rounds of thirty seconds each followed by an incubation period of one hour at 65°C with periodic inversion. We added 500 μl of 24:1 chloroform:isoamyl alcohol (Sigma Aldrich, St. Lewis, MO, USA) to each sample, mixed briefly using a vortex and then centrifuged at 13,000 g for 15 min. After centrifugation, we transferred the supernatant to a new tube and repeated the addition of 24:1 chloroform:isoamyl, mixing and centrifugation. We transferred the resulting supernatant to a new tube and added an equal volume of isopropyl alcohol (Thermo Fisher Scientific, Inc., Waltham, MD, USA) and half this volume of 5M NaCL solution (Sigma Aldrich, St. Lewis, MO, USA). DNA was precipitated overnight at −20°C. The following day, we centrifuged samples at 13,000 g for 15 min and discarded the supernatant. We washed the remaining pellets with 70% ethanol, allowed them to air-dry and then resuspended them in 25 μl of low TE buffer (Sigma Aldrich, St. Lewis, MO, USA). We briefly warmed the samples to 65°C and recombined each quarter

sample into a single tube (for a total volume of 100 μl DNA extract for sample). We visualized the extracts using agarose gel electrophoresis and quantified them using a NanoDrop 8,000 Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MD, USA).

2.2.3 | PCR amplification, library preparation and sequencing

We amplified a 421-base-pair region of the mitochondrial CO1 gene (the animal DNA barcode region, Hebert, Cywinska, Ball, & deWaard, 2003) using primers designed and validated for freshwater macroinvertebrates (BF2 + BR2; Elbrecht & Leese, 2017). We prepared the PCR using a Qiagen Multiplex PCR Kit (Qiagen, Hilden, Germany) with a total volume of 25 μl for each reaction. Each reaction contained 1 μl DNA extract (bulk sample concentration = 327.22 ng/μl ± 275.52 *SD*; eDNA average concentration = 56.61 ng/μl ± 35.26 *SD*), 12.5 μl Qiagen master mix, 10.5 μl molecular grade water and 0.5 μl (concentration: 0.2 μM) of each primer (BF2 + BR2). Twelve negative controls were included containing 1 μl of molecular grade water in place of DNA. Our thermocycling profile was a 95°C initial denaturation for five minutes, 25 cycles (35 for eDNA) of 95°C for 30 s, 50°C for 30 s and 72°C for 50 s, and a final extension at 72°C for five minutes. The size of the PCR products was verified using agarose gel electrophoresis. For one paired sample set (e.g. one bulk tissue and one eDNA sample from the same site), we included a technical replicate to assess PCR stochasticity.

We used a two-step PCR approach, amplifying COI using BF2 + BR2 primers (Elbrecht & Leese, 2017) in the first step, and fusion primers for sample tagging in the second step (Elbrecht & Steinke, 2018). For the second PCR, we followed the same PCR protocol as above with the following changes: 1 μl of PCR product from the first reaction, 12.5 μl Qiagen master mix, 9 μl molecular grade water and 1.25 μl each of a forward and reverse fusion primer pair (concentration: 0.2 μM). Our thermocycling protocol included an initial denaturation at 95°C for five minutes, 15 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 2 min.

After this second PCR, we normalized the DNA concentration using the SequalPrep Normalization Plate (96) Kit (Thermo Fisher Scientific Inc., Waltham, MD, USA). We then pooled the library, separated it into eight aliquots and used SPRIselect clean-up (0.76 ratio; Beckman Coulter Life Sciences) to purify the amplicons and remove primer dimers from the normalized library. The aliquots were then pooled again and quantified using a Qubit HS Kit (Thermo Fisher Scientific Inc., Waltham, MD, USA). We submitted the pooled library to the Advanced Analysis Centre, University of Guelph, Ontario, for sequencing using the Illumina MiSeq platform with 300 paired-end sequencing and 5% PhiX spike-in.

2.2.4 | Bio-informatics pipeline

We assessed the raw sequence data quality with FASTQC v.0.11.8 (Andrews, 2010) and used the bio-informatics platform JAMP version 0.67 (http://github.com/VascoElbrecht/JAMP). After demultiplexing with JAMP, we used USEARCH (v. 11.0.667; Edgar, 2010) to merge paired-end reads and CUTADAPT (v. 1.15; Martin, 2011) to remove primers from sequence reads. We trimmed the primers from the sequences, resulting in the 421-bp target region (COI region amplified by BF2 + BR2) and filtered out low-quality sequences (maximum expected error = 1). We clustered OTUs using USEARCH (v. 11.0.667; Edgar, 2010) with a minimum match criterion of 97% similarity. After clustering, we removed singletons (OTUs present in one sample) and OTUs with very low sequence reads (minimum 0.01% abundance in at least one sample to retain an OTU). We used the Barcode of Life Database (BOLD; Ratnasingham & Hebert, 2007) reference library to assign taxonomic information to OTUs. The JAMP script we used is available as Supplementary information S6.

2.2.5 | Data analyses

We performed all data manipulation and statistical analyses using R version 3.5.3 statistical software (R Core Team, 2019), and our R code is available as Supplementary information S7. Before any analysis, we subtracted the maximum read number of OTUs present in the negative controls from the rest of the samples. For eDNA samples, we also subtracted the maximum read number of OTUs present in the corresponding field-negative control (i.e. eDNA had both field- and laboratory-negative controls). To compare the diversity between bulk sample tissue metabarcoding and eDNA metabarcoding, we calculated the total number of OTUs belonging to major taxonomic groups (invertebrates, algae, bacteria and others). We repeated this using the number of sequence reads (instead of OTUs) to better address the degree of unspecific amplification between each method. We then removed any nontarget taxa from the data set for subsequent analyses (e.g. we focused only on arthropods, molluscs and annelids). Finally, we did not include OTUs that had less than a 90% similarity match to the BOLD reference database (e.g. identification not reliable beyond family-level resolution) in further analyses. We thus obtained two OTU tables of high-quality presence/absence taxa based on (1) bulk sample tissue metabarcoding and (2) eDNA metabarcoding, in addition to a coarse-level taxa table based on morphological identification of bulk sample macroinvertebrates.

To test our prediction that bulk samples and eDNA do not sample the same community, we compared OTU presence/absence in each transect by calculating the number of OTUs shared by each paired eDNA and benthic sample (e.g. the overlap) and the number of OTUs that were unique to each sampling method. Next, we scaled back identifications to match those used in the coarse-level morphological data set (family level for arthropods, phylum for Annelida and Mollusca) and calculated Jaccard dissimilarity scores between each different identification method (bulk sample morphology vs. bulk sample tissue metabarcoding, bulk sample morphology vs. eDNA metabarcoding, and bulk sample tissue metabarcoding vs. eDNA metabarcoding) for each sample. We used a repeated-measures ANOVA to determine whether there was a statistically significant

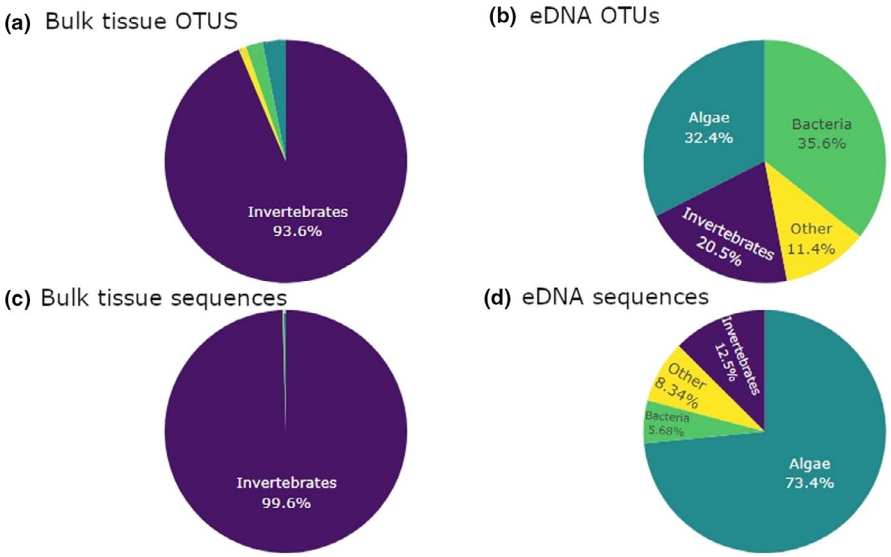

FIGURE 2 Pie charts representing the major groups detected by bulk sample metabarcoding and eDNA metabarcoding (invertebrates, algae, bacteria and others). Panels (a) and (b) represent the number of OTUs and their identity in bulk tissue samples (713) and eDNA samples (5,543 total). Panels (c) and (d) represent the total number of sequence reads and their identity in bulk tissue samples (3,099,384) and eDNA samples (3,750,834)

difference between dissimilarities, followed by post hoc repeated-measures ANOVAs to determine which groups differed. The repeated-measures ANOVA allows us to directly compare differences in Jaccard dissimilarity scores between paired samples, rather than overall differences between the methods, and therefore controls for variation due to site and watershed-level differences.

To evaluate which sampling and identification method had the strongest stream-level signal, we used a distance-based redundancy analysis (RDA) with stream as the constraining factor. We calculated Jaccard dissimilarity indices based on presence/absence data for our three identification methods (coarse level for morphology and OTU level for both bulk sample and eDNA metabarcoding) and performed three ordinations using the "capscale" function in the R package *vegan* (Oksanen et al., 2017). The sample sites were symbolized by stream, and samples from the same stream were joined by a line for visualization. The differences in community composition within a site can also be visualized and assessed based on the RDA plots. To assess the significance of the ordination constraint (stream), we performed an ANOVA with 999 permutations and calculated the adjusted R^2 (Legendre & Legendre, 2012).

3 | RESULTS

The MiSeq run resulted in 18,142,288 sequence reads, and subsequent quality filtration retained 8,042,910 reads. Raw sequence data are available under the NCBI SRA ID: PRJNA575063. The OTU tables and taxonomic identifications used in our analyses, along with all metadata, are available as Supplementary information S1-S4. The negative field controls for eDNA (9 samples, one per stream site) contained 582,181 sequences, or approximately 7% of total sequence reads postfiltering (average of 64,686.78 sequences ± 53,587.61 *SD* and 113.22 OTUs ± 69.02 *SD* per sample). The negative laboratory controls (12) contained 230,532 sequences, which was approximately 3% of all sequences. Per sample, the negative controls contained an average of 19,221 sequences (± 25,080.71 *SD*) and 97.58 OTUs (± 49.74 *SD*). We had a total 6,983 OTUs based on raw sequences. This was reduced to 6,076 OTUs after subtracting the maximum read number present in the negative controls (laboratory controls for all samples and field negatives for associated eDNA samples). Prior to accounting for the negative controls, tissue samples contained a total of 3,296,721 sequences (average of 109,890.7 ± 60,496.24 *SD* sequences per sample) and 812 OTUs. The raw eDNA samples contained 3,933,476 sequences (average of 126,886.3 ± 56,854,44 sequences per sample) and 5,671 OTUs.

3.1 | Sequence reads vs. OTU counts

After controlling for the reads present in negative controls, we compared the total number of both OTUs and raw sequence counts belonging to major groups between bulk tissue metabarcoding and eDNA metabarcoding. We observed a much higher amount of OTUs in eDNA samples (5,543 OTUs) compared to bulk tissue samples (713 OTUs); however, the taxonomic composition of these OTUs varied considerably. In bulk tissue samples, the majority of the OTUs were arthropods, molluscs or annelids (94%), followed by algae (3%) and bacteria (2%; Figure 2a). However, the majority of OTUs in eDNA samples were either bacteria (36%) or algae (32%; Figure 2b). More OTUs belonged to our three target phyla (Arthropoda, Annelida and Mollusca) in tissue metabarcoding samples compared to eDNA metabarcoding samples (21%).

While there were more OTUs present in eDNA samples, the total number of sequences between tissue and eDNA samples were similar (3,099,384 and 3,750,834 sequence reads, respectively). The majority of the sequences in the bulk tissue samples belonged to Arthropoda (87%), and over 99% of sequences (a total of 3,085,602) belonged to our target phyla (Figure 2c). For eDNA, the sequences were represented by algae (73%), while sequence reads belonging to target phyla only made up 12% of total eDNA sequences (a total of 456,770 sequences; Figure 2d).

3.2 | Community dissimilarity between sampling methods

After eliminating any nontarget phyla from the data set, we observed very little overlap in OTU identity between paired bulk and eDNA samples (Figure 3). Paired transects shared between 0 and 11 OTUs (or 0%–18.03% of total OTUs) with an average shared percentage of 5.43% ± 6.34 (*SD*). Most OTUs present in the overlap were unique (occurring in 1–3 samples), but OTUs matching Chironomidae (specifically *Hydrobaenus* sp. and *Orthocladius oliveri* at 17 and 11, respectively) and *Tubifex tubifex* (in 9 paired samples) were the three most common OTUs shared between tissue and eDNA samples.

To include an appropriate comparison with the morphological identification, we calculated community dissimilarity for all three identification methods scaled to family-level resolution (for Arthropoda) and to phylum for Annelida and Mollusca. The communities generated by eDNA metabarcoding were highly dissimilar to those generated by both morphological identification and bulk tissue metabarcoding (Figure 4). The Jaccard dissimilarity scores were significantly different between comparisons (repeated-measures ANOVA: $F_{2,56} = 74.73$; $p < .001$; Figure 4). Post hoc tests revealed morphology and tissue had significantly lower dissimilarity between communities than the other sampling methods (Figure 4, Table 1).

3.3 | Community composition and stream

Stream identity was a significant predictor of community composition based on family-level morphological identification ($F_{8,20} = 2.1841$, $p = .001$) and had an adjusted R^2 of 26.21%. The stream constraint also explained a significant portion of the variation in stream communities for both bulk tissue ($F_{8,20} = 1.8153$, $p = .001$; Figure 5b) and eDNA ($F_{8,20} = 1.7154$, $p = .001$; Figure 5c). The explained variation for bulk sampling was slightly higher for bulk tissue (adjusted $R^2 = 18.89\%$) than eDNA (adjusted $R^2 = 16.97\%$). While morphology had the highest explained variation, the RDA plot also had the most overlap in community composition between streams (Figure 5a).

4 | DISCUSSION

Our study compared paired bulk macroinvertebrate samples collected via benthic kicknet sampling to eDNA samples collected via water filtration. The bulk samples were identified both by coarse-level morphological identifications and via metabarcoding. Our goal was to evaluate the suitability of eDNA metabarcoding for local stream biomonitoring using aquatic macroinvertebrates as our focal community. We compared the collection methods by contrasting the overall richness and taxonomic overlap of paired samples collected via kicknet and aqueous eDNA sampling using a metabarcoding pipeline and determined how effective each method was at characterizing stream communities. Previous work exploring community composition in streams generated by eDNA has reported differences in community composition based on pooled samples at coarse taxonomic resolution (Deiner et al., 2017; Macher et al., 2018; Mächler et al., 2019). Here, we are interested in exploring differences at the local (rather than landscape) scale to ascertain which collection and identification methods are most effective for aquatic macroinvertebrate biomonitoring.

4.1 | Sequence reads and OTU identity

We observed greater overall OTU richness in eDNA vs. bulk samples, which is consistent with the conclusions of previous eDNA metabarcoding studies in streams (Deiner et al., 2016; Macher et al., 2018). However, bulk sample metabarcoding yielded more OTUs belonging to macroinvertebrate phyla (94% of bulk sample OTUs were macroinvertebrates compared to 21% of eDNA OTUs). Our eDNA OTUs match the numbers described in Macher et al. (2018), who reported that 49.9% of OTUs were Metazoa in bulk samples compared to

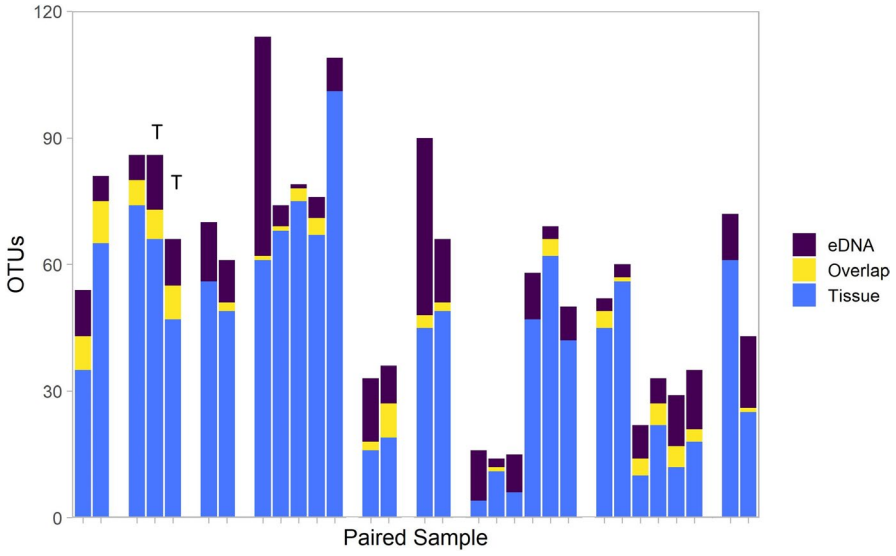

FIGURE 3 The number of OTUs unique and shared to each paired sample between sampling methods (bulk tissue metabarcoding and eDNA metabarcoding). Each stack bar represents one sample pair. A larger space between bars indicates that samples were taken from a different stream site. The T above two of the bars indicates that these are technical replicates

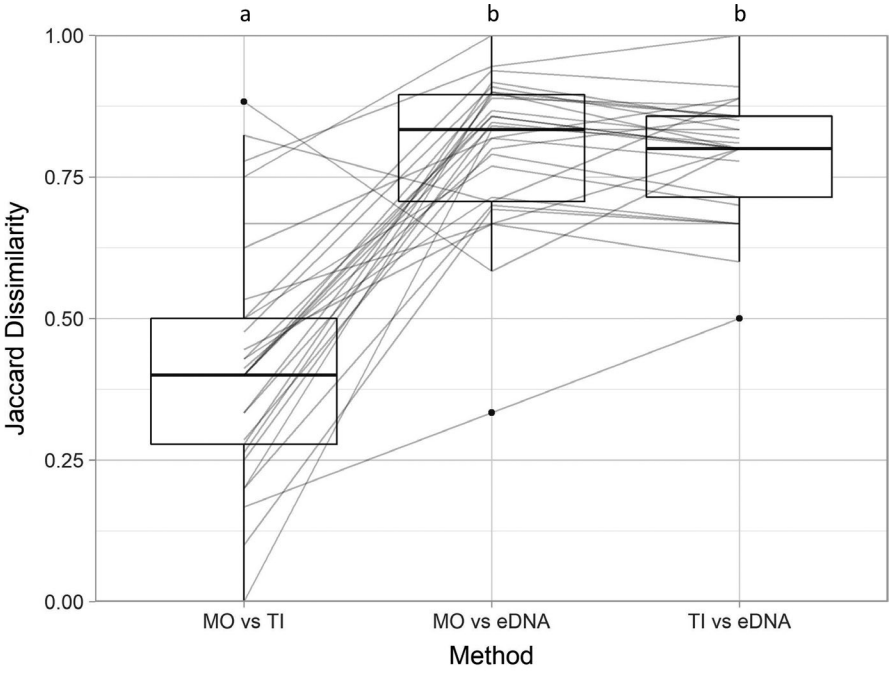

FIGURE 4 Jaccard dissimilarity scores for comparisons between communities generated by three different identification methods (MO, morphology identification from bulk samples; TI, tissue metabarcoding from bulk samples, eDNA, eDNA metabarcoding from water samples; Repeated-measures ANOVA: $F_{2,56} = 74.73$; $p < .001$). The lines represent the same paired sample set (bulk sample and eDNA) across each comparison. A different letter above a box plot represents a statistically significant difference

Methods	$F_{1,28}$	p
MO vs. TI & MO vs. eDNA	65.19	<.0001
MO vs. TI & TI vs. eDNA	103.5	<.0001
MO vs. eDNA & TI vs. eDNA	0.032	.86

Abbreviations: MO, family-level morpholog; TI, metabarcoding tissue; eDNA, metabarcoding eDNA.

TABLE 1 Post hoc repeated-measures ANOVA values for Jaccard dissimilarity scores between each identification method

21.2% in eDNA samples; however, our bulk samples contained proportionally more macroinvertebrate OTUs. In addition to calculating the number of OTUs belonging to our target groups, we also determined what proportion of the sequence reads were macroinvertebrates for both bulk samples and eDNA. We observed that over 99% of all sequences obtained via metabarcoding bulk tissue samples were invertebrates. Surprisingly, invertebrate taxa only made up a small percentage (12%) of eDNA sequences, which were instead dominated by algal sequences. This large discrepancy in sequence read numbers between bulk sample and eDNA metabarcoding for macroinvertebrates is surprising, and we suggest that read number is an important component to include in the results of eDNA metabarcoding studies. Our results here indicate that bulk tissue metabarcoding is more effective at characterizing aquatic macroinvertebrate communities.

In our eDNA samples, the low proportion of non-macroinvertebrate sequences compared to algal sequences suggests a high amount of nontarget amplification is occurring. In contrast, we observed very few non-macroinvertebrate sequences from bulk sample metabarcoding. In an in vitro test of marker choice (COI and 12S) and primer pairs for sequencing fish eDNA, Collins et al. (2019) determined that 12S performed better than COI due to the high occurrence of nonspecific amplification when using COI. It is possible that COI may not be an ideal marker for eDNA metabarcoding due to low reproducibility caused by nonspecific amplification (e.g. Collins et al., 2019). Even if a degenerate primer mainly targets arthropod DNA, it may still amplify nontarget DNA. Since eDNA sampling captures a large amount of nontarget DNA (e.g. algae and bacteria) relative to arthropod DNA (as we demonstrate here), the detection of target taxa will be reduced when using a degenerate COI primer. Using ribosomal markers and primers with conserved binding sites can help (Collins et al., 2019; Elbrecht et al., 2016), but these markers lack the reference library that the standard barcode marker COI has. A robust reference library is particularly important when sequencing very diverse groups such as arthropods and other invertebrates. The choice of molecular marker and primer pair is of paramount importance for a metabarcoding study, and Collins et al. (2019) suggest that an ideal metabarcoding primer must successfully amplify the taxonomic group while also avoiding nonspecific amplification. This dichotomy of a universal yet specific primer set may prove challenging to achieve for eDNA samples collected via water filtration, where the large proportion of prokaryotic groups present in the water column may overwhelm the target community, as seen in our results. However, recent studies have managed to reduce nontarget amplification using a COI primer with limited degeneracy (Leese et al., 2020). While decreasing amplification efficiency for certain groups,

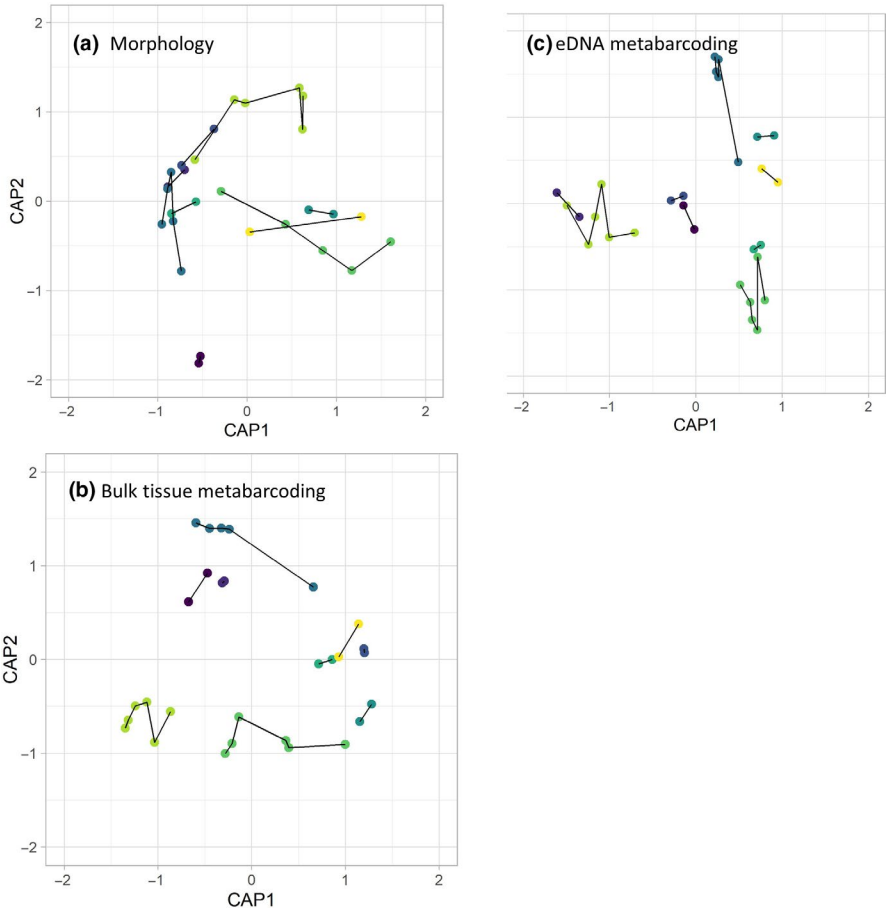

FIGURE 5 Redundancy analysis (RDA) ordinations based on presence/absence Jaccard dissimilarity indices of aquatic macroinvertebrate community composition for (a) morphological identification (coarse-level identification), (b) bulk sample tissue metabarcoding (OTUs) and (c) eDNA metabarcoding (OTUs). Each symbol represents an individual sample (benthic kicknet or water filtration for eDNA), and lines connect samples collected from the same stream (different streams are also represented by different colours)

most of the reads recovered from eDNA samples were target macroinvertebrate reads (Leese et al. 2020). While many degenerate primers designed for bulk metabarcoding are likely not suitable for eDNA samples, COI metabarcoding for eDNA samples can be effective if appropriate primers are designed, but this is not a trivial process.

4.2 | Community composition and taxonomic overlap

We observed a very low overlap in taxonomic identity between paired bulk and eDNA samples using metabarcoding approaches to taxon identification. In some paired transects, there were no shared taxa between bulk samples and eDNA samples. To our knowledge, our numbers are the lowest reported overlap in OTU identity for stream macroinvertebrates. In Swiss streams, Deiner et al. (2016) report a 73% overlap between eDNA and kicknet samples identified morphologically across all the samples pooled and at family-level resolution. Similarly, Macher et al. (2018) found a 64% overlap for EPTs (Ephemeroptera, Trichoptera, Plecoptera) for all samples pooled, and Mächler et al. (2019) observed a 62% overlap between bulk samples and eDNA at the genus level. Contrary to these previous stream studies, Hajibabaei et al. (2019) compared bulk sample and eDNA metabarcoding at the individual site level in wetlands using exact sequence variants (ESVs) and observed that the highest proportion of shared aquatic macroinvertebrate ESVs between eDNA and bulk sample metabarcoding was only 14%. Hajibabaei et al. (2019) clearly demonstrate the importance of taxonomic resolution when comparing similarities in community composition. Despite the difference in our study systems (lenthic vs. lotic), our results are most similar to Hajibabaei et al., (2019), and we observed very little taxonomic overlap between sample methods. It is likely that calculating the number of shared taxa between bulk sample and eDNA-generated community data sets after pooling all the samples does not accurately reflect the variation in community composition that occurs at the site level, which would explain why our overlap percentages are much lower than previous studies (Deiner et al., 2016; Macher et al., 2018; Mächler et al., 2019). Additionally, comparing community composition at the family, or even genus, level is likely masking turnover within a group and artificially inflating overlap percentages.

We expected there to be unique aquatic macroinvertebrate OTUs collected by each method, in part because eDNA effectively encompasses a larger sampling region (e.g. upstream and terrestrial run-off); however, we found it surprising that our results were so extreme. For example, nearly one third of our paired samples did not share any OTUs. In addition to this lack of overlap, we were also surprised that bulk samples contained more unique macroinvertebrate taxa than eDNA samples. Previous literature has suggested that eDNA metabarcoding results in higher taxonomic richness than bulk samples (Deiner et al., 2016), likely due to the fact that eDNA

is incorporating community information from upstream reaches. However, when considering only macroinvertebrate groups, bulk samples collected more unique taxa than eDNA. The higher richness of our bulk samples, combined with the minimal taxonomic overlap between the two methods, suggests that eDNA is not effectively characterizing the local aquatic macroinvertebrate communities in our study system.

It is not clear why the overlap in our study is so low between bulk samples and eDNA, but several possibilities exist. The OTUs that were most commonly detected in both methods all belong to ubiquitous groups (Chironomidae, Elmidae, Oligochaeta). It is possible that aqueous eDNA sampling is not as effective at detecting less abundant benthic taxa or that macroinvertebrates are shedding less eDNA into the water column than larger, more taxa (e.g. fish and amphibians). Alternatively, the DNA shed by benthic-dwelling macroinvertebrates could be retained in the sediment and is not being released into the water at high enough quantities to reliably detect. The fate of eDNA is not well understood, particularly for aquatic macroinvertebrates. Many of these groups are not active swimmers and spend much of their aquatic life cycle on or within the substrate, and it is unclear how much DNA is being shed by these organisms, let alone whether it is released into the water column or settles into the sediment. Most of the research on the eDNA shedding rates of aquatic taxa focus on vertebrates (e.g. Wilcox et al. 2016), and perhaps, the exoskeleton of arthropods results in less epithelial shedding. It is also possible that DNA is being moved downstream and the signal is being lost, but Hajibabaei et al. (2019) also observed low taxonomic overlap in wetlands where, theoretically, eDNA should be more concentrated. This discrepancy between bulk tissue and eDNA-generated community composition suggests that the two sampling methods are actually sampling different microhabitats within a stream (e.g. the water column vs. the benthic substrate).

For other freshwater groups, eDNA has been more successful at characterizing local communities. For example, visual and eDNA surveys were very concordant (between 73% and 93% overlap) for fish communities in large rivers (Pont et al., 2018). However, in marine environments, eDNA metabarcoding was more likely to detect algae and small-bodied invertebrates and visual surveys primarily detected larger arthropods, such as kelp crabs (Shum, Barney, Leary, & Palumbi, 2019). This suggests that visual or physical surveys are biased towards larger-bodied organisms; however, our kicknet sampling was more effective at surveying macroinvertebrate biodiversity than eDNA.

4.3 | Stream signal and implications for biomonitoring

All three identification methods (family-level morphological identification, bulk tissue metabarcoding and eDNA metabarcoding) had a significant stream-level signal, meaning that stream had an influence on the community composition of aquatic macroinvertebrates for all sampling and identification types. Surprisingly, coarse-level morphological identifications had the highest explained variation when we expected that molecular identifications would yield further insight into community patterns. While some studies suggest that little information is lost by using coarse-level taxonomy (Marshall et al., 2006), others demonstrated the power of using molecular identification to detect complex patterns within a family (Beermann, Zizka, et al., 2018; Macher et al., 2016). When comparing approaches to identifying stream macroinvertebrates, Sweeney et al., (2011) found that identification method and resolution changed the total number of observed taxa from 26 (coarse family-level identification) to 150 (DNA barcoding). Likewise, the number of OTUs in our metabarcoding data sets greatly exceeded the number of families in the morphology data set. The smaller amount of possible variation likely contributed to the higher adjusted R^2 value for our morphological data set. When observing the RDA plots, the morphological data set (Figure 5a) had the most overlap in community composition between sites whereas both bulk sample and eDNA metabarcoding had more unique community compositions between streams (Figure 5b and c). Based on this and the increased diversity, we still suggest that species-level resolution is preferred to detect patterns that occur within a family (Beermann, Zizka, et al., 2018), and given the costs and challenges associated with morphological identification, bulk sample metabarcoding is an effective way to characterize macroinvertebrate biodiversity (Elbrecht & Steinke, 2018; Elbrecht et al., 2017; Gibson et al., 2015).

Metabarcoding both bulk tissue and eDNA samples allowed us to detect differences in community composition between streams, indicating that each method is collecting communities that are unique to each stream. However, we determined that bulk tissue and eDNA are not sampling the same communities (e.g. sampling different stream microhabitats) as there was very little overlap in community composition between paired local samples. Our eDNA samples were lower in OTU richness than the bulk samples and perhaps did not capture sufficient aquatic macroinvertebrate eDNA to accurately characterize local stream communities. While increasing the number of biological and technical replicates could increase our detection success with eDNA (Alberdi, Aizpurua, Gilbert, & Bohmann, 2018), our current sampling method was robust enough to detect patterns using bulk samples. While we did not test for inhibition in the eDNA samples, partial inhibition could be occurring and could result in low-abundance OTUs not amplifying during PCR. Currently, inhibition testing is not common in the eDNA metabarcoding literature, but we suggest that future work explores the affect of inhibition in field samples, particularly for low-abundance taxa. Another possibility for the lack of overlap in our study, and the low proportion of macroinvertebrate DNA in the water samples, could be that macroinvertebrate eDNA fragments are too small to be collected with 5-μm filters (Moushomi, Wilgar, Carvalho, Creer, & Seymour, 2019), but decreasing our filter pore size would have reduced the volume of water we were able to filter on site. Finally, despite precautions being taken during all sample processing and laboratory work, the sequences present in our negative controls indicate that contamination is still occurring and may increase the presence of false positives

in our data set. Morey, Bartley, and Hanner (2020) detected and reported evidence of contamination in an eDNA metabarcoding study of a tropical fish aquarium and commented on the lack of standardization the field has for treatment of negative controls. Indeed, while most literature report that negative controls have been included in the data set and somehow accounted for, there is little discussion of what is present in these controls or how the data were treated, indicating the need for transparency in future studies in order to develop appropriate thresholds (see Morey et al., 2020). While there is promising literature on the use of eDNA in routine freshwater assessments, such as its effectiveness for difficult-to-detect organisms (salamanders in Spear, Groves, Williams, & Waits, 2015), there remain many methodological choices than can influence its success. Here, we demonstrate that metabarcoding bulk samples are more effective at representing local communities of aquatic macroinvertebrates than metabarcoding eDNA, but it is important to consider the influence of primer sets and marker choice on the results of such comparisons as degenerate COI primers may not be an appropriate choice for eDNA metabarcoding studies based on our results.

4.4 | Synthesis

We observed that bulk sample and eDNA metabarcoding differ in community composition for stream macroinvertebrates and that there is very little overlap in OTU identity at the site level. We demonstrated that morphological identification (at family-level resolution), bulk sample metabarcoding and eDNA metabarcoding are all successful at characterizing stream communities. However, bulk tissue metabarcoding captured the highest aquatic macroinvertebrate diversity in addition to detecting differences in community composition between streams. We suggest that eDNA does not adequately sample local aquatic macroinvertebrate DNA as it is not reflecting what we collected with kicknet sampling. While eDNA may be an effective tool for detecting physically larger and less diverse groups (e.g. fish and amphibians), there are still challenges associated with sampling macroinvertebrate eDNA. Future studies could further explore the differences in shedding rates between macroinvertebrates and vertebrates, or the fate of macroinvertebrate eDNA (e.g. water column vs. substrate sampling; Westfall, Therriault, & Abbott, 2019), in order to inform better sampling strategies. It is possible that aquatic macroinvertebrate DNA is being retained in the benthic sediments, and we suggest that future work comparing paired kicknet samples to both sediment and water eDNA samples would be a valuable contribution to the eDNA literature. We also acknowledge that methodological decisions in the field (pore size and volume of water filtered), laboratory (choice of polymerase, primer set and inhibition testing) and during bio-informatic processing of data (e.g. clustering thresholds) can all influence the results. We suggest that detailed methodological studies are important to address the challenges of eDNA, including mesocosm work which explores the influence of water quality parameters on eDNA success rates. Finally, eDNA detected more OTUs from nonarthropod groups (e.g. algae and bacteria) and there is the potential that our reliance community composition of large-bodied invertebrates for bioassessments may need to be revisited in the era of eDNA. However, here we conclude that eDNA does not match local aquatic macroinvertebrate communities, and if small-scale spatial resolution is important to a study design, metabarcoding bulk samples is a more accurate representation of community composition. This work has important implications for biomonitoring programmes, many of which already use aquatic macroinvertebrates as indicators. Incorporating metabarcoding pipelines into routine biomonitoring can provide greater estimates of biodiversity and thus provide better tools to detect ecological patterns and offer greater insight into stream condition.

ACKNOWLEDGEMENTS

We would like to thank Emily Champagne, Christine Dulal-Whiteway, Marie Gutgesell and Dr. Kevin McCann for their assistance in locating study sites and carrying out fieldwork. Ian Thompson assisted in sorting and identifying the aquatic macroinvertebrate samples. This research was funded by a Canada First Research Excellence Fund (Food from Thought) to KC/RHH, an NSERC Discovery Grant to KC and an NSERC CGS-D scholarship to JEG.

AUTHOR CONTRIBUTIONS

J.E.G., R.H.H. and K.C. designed the study. J.E.G. conducted the fieldwork. J.E.G., V.E. and T.B. conducted the laboratory work. J.E.G. performed the analyses. J.E.G. wrote the first draft of the manuscript, and all authors edited and cowrote the final version.

DATA AVAILABILITY STATEMENT

MiSeq raw sequence data have been deposited in NCBI's Short Read Archive and will be available upon publication (Accession no: PRJNA575063).

ORCID

Jennifer Erin Gleason https://orcid.org/0000-0001-9774-859X

REFERENCES

Alberdi, A., Aizpurua, O., Gilbert, M. T. P., & Bohmann, K. (2018). Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution*, *9*, 134–147. https://doi.org/10.1111/2041-210X.12849

Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data*. Retrieved from http://www.bioinformatics.babraham.ac.uk/projects/fastqc

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

Beermann, A. J., Elbrecht, V., Karnatz, S., Ma, L., Matthaei, C. D., Piggott, J. J., & Leese, F. (2018). Multiple-stressor effects on stream macroinvertebrate communities: A mesocosm experiment manipulating salinity, fine sediment and flow velocity. *Science of the Total Environment*, *610*, 961–971. https://doi.org/10.1016/j.scitotenv.2017.08.084

Beermann, A. J., Zizka, V. M. A., Elbrecht, V., Baranov, V., & Leese, F. (2018). DNA metabarcoding reveals the complex and hidden responses of

chironomids to multiple stressors. *Environmental Sciences Europe*, *30*, 26. https://doi.org/10.1186/s12302-018-0157-x

Bonada, N., Prat, N., Resh, V. H., & Statzner, B. (2006). Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. *Annual Review of Entomology*, *51*, 495–523. https://doi.org/10.1146/annurev.ento.51.110104.151124

Buss, D. F., Carlisle, D. M., Chon, T.-S., Culp, J., Harding, J. S., Keizer-Vlek, H. E., … Hughes, R. M. (2015). Stream biomonitoring using macroinvertebrates around the globe: A comparison of large-scale programs. *Environmental Monitoring and Assessment*, *187*, 4132. https://doi.org/10.1007/s10661-014-4132-8

Cairns, J., & Pratt, J. R. (1993). A History of Biological Monitoring Using Benthic Macroinvertebrates. In D. M. Rosenberg, & V. H. Resh (Eds.), *Freshwater Biomonitoring and Benthic Macroinvertebrates* (pp. 10–27). New York, NY: Chapman & Hall.

Carew, M. E., Pettigrove, V. J., Metzeling, L., & Hoffmann, A. A. (2013). Environmental monitoring using next generation sequencing: Rapid identification of macroinvertebrate bioindicator species. *Frontiers in Zoology*, *10*, 45. https://doi.org/10.1186/1742-9994-10-45

Collins, R. A., Bakker, J., Wangensteen, O. S., Soto, A. Z., Corrigan, L., Sims, D. W., … Mariani, S. (2019). Non-specific amplification compromises environmental DNA metabarcoding with COI. *Methods in Ecology and Evolution*, *10*, 1985–2001. https://doi.org/10.1111/2041-210X.13276

Creer, S., Deiner, K., Frey, S., Porazinska, D., Taberlet, P., Thomas, W. K., … Bik, H. M. (2016). The ecologist's field guide to sequence-based identification of biodiversity. *Methods in Ecology and Evolution*, *7*, 1008–1018. https://doi.org/10.1111/2041-210X.12574

Deiner, K., & Altermatt, F. (2014). Transport Distance of Invertebrate Environmental DNA in a Natural River. *PLoS One*, *9*, e88786. https://doi.org/10.1371/journal.pone.0088786

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

Deiner, K., Fronhofer, E. A., Mächler, E., Walser, J.-C., & Altermatt, F. (2016). Environmental DNA reveals that rivers are conveyer belts of biodiversity information. *Nature Communications*, *7*, 12544. https://doi.org/10.1038/ncomms12544

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., … Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews*, *81*, 163–182. https://doi.org/10.1017/S1464793105006950

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Elbrecht, V., & Leese, F. (2017). Validation and Development of COI Metabarcoding Primers for Freshwater Macroinvertebrate Bioassessment. *Frontiers in Environmental Science*, *5*, 1–17. https://doi.org/10.3389/fenvs.2017.00011

Elbrecht, V., & Steinke, D. (2018). Scaling up DNA metabarcoding for freshwater macrozoobenthos monitoring. *Freshwater Biology*, *64*, 380–387. https://doi.org/10.7287/peerj.preprints.3456v3

Elbrecht, V., Taberlet, P., Dejean, T., Valentini, A., Usseglio-Polatera, P., Beisel, J.-N., … Leese, F. (2016). Testing the potential of a ribosomal 16S marker for DNA metabarcoding of insects. *PeerJ*, *4*, e1966. https://doi.org/10.7717/peerj.1966

Elbrecht, V., Vamos, E. E., Meissner, K., Aroviita, J., & Leese, F. (2017). Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. *Methods in Ecology and Evolution*, *8*, 1265–1275. https://doi.org/10.1111/2041-210X.12789

Fuji, K., Doi, H., Matsuoka, S., Nagano, M., Sato, H., & Yamanaka, H. (2019). Environmental DNA metabarcoding for fish community analysis in backwater lakes: A comparison of capture methods. *PLoS One*, *14*, e0210357. https://doi.org/10.1371/journal.pone.0210357

Gibson, J. F., Shokralla, S., Curry, C., Baird, D. J., Monk, W. A., King, I., & Hajibabaei, M. (2015). Large-scale biomonitoring of remote and threatened ecosystems via high- throughput sequencing. *PLoS One*, *10*, e0138432. https://doi.org/10.1371/journal.pone.0138432

Gleason, J. E., & Rooney, R. C. (2017). Aquatic macroinvertebrates are poor indicators of agricultural activity in northern prairie pothole wetlands. *Ecological Indicators*, *81*, 333–339. https://doi.org/10.1016/j.ecolind.2017.06.013

Gleason, J. E., & Rooney, R. C. (2018). Pond permanence is a key determinant of aquatic macroinvertebrate community structure in wetlands. *Freshwater Biology*, *63*, 264–277. https://doi.org/10.1111/fwb.13057

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. *Hydrobiologia*, *566*, 505–521. https://doi.org/10.1007/s10750-006-0075-6

Hajibabaei, M., Porter, T., Robinson, C., Baird, D., Shokralla, S., & Wright, M. (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. https://doi.org/10.1371/journal.pone.0225409

Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G. A. C., & Baird, D. J. (2011). Environmental barcoding: a next-generation sequencing approach for biomonitoring applications using river benthos. *PLoS One*, *6*, e17497. https://doi.org/10.1371/journal.pone.0017497

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*, 313–321. https://doi.org/10.1098/rspb.2002.2218

Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., … Whiteley, A. R. (2015). Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology*, *15*, 216–227. https://doi.org/10.1111/1755-0998.12285

Keck, F., Vasselon, V., Tapolczai, K., Rimet, F., & Bouchez, A. (2017). Freshwater biomonitoring in the Information Age. *Frontiers in Ecology and the Environment*, *15*, 266–274. https://doi.org/10.1002/fee.1490

Leese, F., Sander, M., Buchner, D., Elbrecht, V., Haase, P., & Zizka, V. M. A. (2020). Improved freshwater macroinvertebrate detection from eDNA through minimized non-target amplification. *bioRxiv*, 1–42. https://doi.org/10.1101/2020.04.27.063545

Legendre, P., & Legendre, L. (2012). *Numerical ecology* (p. 1006). Amsterdam: Elsevier Science BV.

Macher, J.-N., & Leese, F. (2017). Environmental DNA metabarcoding of rivers: Not all eDNA is everywhere, and not all the time. *BioRxiv*, 1–26. https://doi.org/10.1101/164046

Macher, J.-N., Salis, R. K., Blakemore, K. S., Tollrian, R., Matthaei, C. D., & Leese, F. (2016). Multiple-stressor effects on stream invertebrates: DNA barcoding reveals contrasting responses of cryptic mayfly species. *Ecological Indicators*, *61*, 159–169. https://doi.org/10.1016/j.ecolind.2015.08.024

Macher, J.-N., Vivancos, A., James Piggott, J., Centeno, F. C., David Matthaei, C., & Leese, F. (2018). Comparison of environmental DNA and bulk-sample metabarcoding using highly degenerate COI primers. *Molecular Ecology Resources*, *18*, 1456–1468. https://doi.org/10.1111/1755-0998.12940

Mächler, E., Deiner, K., Steinmann, P., & Altermatt, F. (2014). Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. *Freshwater Science*, *33*, 1174–1183. https://doi.org/10.1086/678128

Mächler, E., Little, C. J., Wüthrich, R., Alther, R., Fronhofer, E. A., Gounand, I., … Altermatt, F. (2019). Assessing different components of diversity across a river network using eDNA. *Environmental DNA*, *1*, 290–301. https://doi.org/10.1002/edn3.33

Marshall, J. C., Steward, A. L., & Harch, B. D. (2006). Taxonomic resolution and quantification of freshwater macroinvertebrate samples from an Australian dryland river: The benefits and costs of using species abundance data. *Hydrobiologia*, *572*, 171–194. https://doi.org/10.1007/s10750-005-9007-0

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *Embnet Journal*, *17*, 10–12. https://doi.org/10.14806/ej.17.1.200

Morey, K., Bartley, T., & Hanner, R. H. (2020). Validating environmental DNA metabarcoding for marine fishes in diverse ecosystems using a public aquarium. *Environmental DNA*, *2*(3), 330–342. https://doi.org/10.1002/edn3.76

Moushomi, R., Wilgar, G., Carvalho, G., Creer, S., & Seymour, M. (2019). Environmental DNA size sorting and degradation experiment indicates the state of *Daphnia magna* mitochondrial and nuclear eDNA is subcellular. *Scientific Reports*, *9*, 12500. https://doi.org/10.1038/s41598-019-48984-7

Norris, R. H., & Hawkins, C. P. (2000). Monitoring river health. *Hydrobiologia*, *435*, 5–17.

Nukazawa, K., Hamasuna, Y., & Suzuki, Y. (2018). Simulating the advection and degradation of the environmental DNA of common carp along a river. *Environmental Science & Technology*, *52*, 10562–10570. https://doi.org/10.1021/acs.est.8b02293

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., O'Hara, R. B., Simpson, G. L., Wagner, H. (2017). *vegan: Community Ecology Package*, Vol. 1. R Package Version 2.4-2. https://cran.r-project.org/web/packages/vegan/

Pereira-da-Conceicoa, L., Elbrecht, V., Hall, A., Briscoe, A., Barber-James, H., & Price, B. (2020). Metabarcoding unsorted kick-samples facilitates macroinvertebrate-based biomonitoring with increased taxonomic resolution, while outperforming environmental DNA. *Environmental DNA*. https://doi.org/10.1002/edn3.116

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., … Dejean, T. (2018). Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Scientific Reports*, *8*, 10361. https://doi.org/10.1038/s41598-018-28424-8

R Core Team (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes*, *7*, 355–364. https://doi.org/10.1111/j.1471-8286.2006.01678.x

Resh, V. H., & Unzicker, J. D. (1975). Water quality monitoring and aquatic organisms: the importance of species identification. *Journal of Water Pollution Control Federation*, *47*, 9–19.

Serrana, J. M., Miyake, Y., Gamboa, M., & Watanabe, K. (2019). Comparison of DNA metabarcoding and morphological identification for stream macroinvertebrate biodiversity assessment and monitoring. *Ecological Indicators*, *101*, 963–972. https://doi.org/10.1016/j.ecolind.2019.02.008

Shokralla, S., Gibson, J. F., Nikbakht, H., Janzen, D. H., Hallwachs, W., & Hajibabaei, M. (2014). Next-generation DNA barcoding: Using next-generation sequencing to enhance and accelerate DNA barcode capture from single specimens. *Molecular Ecology Resources*, *14*, 892–901. https://doi.org/10.1111/1755-0998.12236

Shum, P., Barney, B. T., Leary, J. K. O., & Palumbi, S. R. (2019). Cobble community DNA as a tool to monitor patterns of biodiversity within kelp forest ecosystems. *Molecular Ecology Resources*, *19*, 1470–1485. https://doi.org/10.1111/1755-0998.13067

Spear, S. F., Groves, J. D., Williams, L. A., & Waits, L. P. (2015). Using environmental DNA methods to improve detectability in a hellbender (*Cryptobranchus alleganiensis*) monitoring program. *Biological Conservation*, *183*, 38–45. https://doi.org/10.1016/j.biocon.2014.11.016

Stanfield, L. (2017). *Ontario stream assessment protocol. Version 9.0* (p. 505). Peterborough, ON: Fisheries Policy Section. Ontario Ministry of Natural Resources.

Sweeney, B. W., Battle, J. M., Jackson, J. K., & Dapkey, T. (2011). Can DNA barcodes of stream macroinvertebrates improve descriptions of community structure and water quality? *Freshwater Science*, *30*, 195–216. https://doi.org/10.1899/10-016.1

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next- generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*, 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

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

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

Turner, R., Miller, D. J., Coyne, K. J., & Corush, J. (2014). Improved methods for capture, extraction, and quantitative assay of environmental DNA from Asian Bigheaded Carp (*Hypophthalmichthys* spp.). *PLoS One*, *9*, e11439. https://doi.org/10.1371/journal.pone.0114329

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

Wacker, S., Fossøy, F., Mejdell, B., Hege, L., Sivertsgård, R., & Karlsson, S. (2019). Downstream transport and seasonal variation in freshwater pearl mussel (*Margaritifera margaritifera*) eDNA concentration. *Environmental DNA*, *1*, 64–73. https://doi.org/10.1002/edn3.10

Westfall, K. M., Therriault, T. W., & Abbott, C. L. (2019). A new approach to molecular biosurveillance of invasive species using DNA metabarcoding. *Global Change Biology*, *26*(2), 1012–1022. https://doi.org/10.1111/gcb.14886

Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. J., Shepard, B. B., Jane, S. F., … Schwartz, M. K. (2016). Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biological Conservation*, *194*, 209–216. https://doi.org/10.1016/j.biocon.2015.12.023

SUPPORTING INFORMATION

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

How to cite this article: Gleason JE, Elbrecht V, Braukmann TWA, Hanner RH, Cottenie K. Assessment of stream macroinvertebrate communities with eDNA is not congruent with tissue-based metabarcoding. *Mol Ecol*. 2020;00:1–13. https://doi.org/10.1111/mec.15597