ORIGINAL ARTICLE

Performance of extraction methods for extracellular DNA from sediments across marine habitats

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Abstract

Background: Using environmental DNA (eDNA) to detect taxa is quickly becoming an important technique to determine the distribution and diversity of macroorganisms. Assessing differences in communities based on eDNA extracted from sediment could enhance our understanding of marine communities among the many different marine habitats both from the present and from the past using sediment cores. Although the effect of extraction methods on metabarcoding results has been tested for both water and sediment samples, less is known about how sediment types may affect the extraction and sequencing of eDNA.

Methods and Results: We used two extraction methods to assess if the amount of extracted DNA was different among three marine habitats: coral reefs, mangrove forests, and seagrass meadows. First, an equal amount of DNA was added to sterilized sediment and there was no difference in the DNA extracted from the different habitats. Second, an equal amount of DNA from a species that did not occur naturally was added to the sediment and the number of sequenced reads of this species was not different among habitats. A concomitant test found that a commercial extraction kit, the MO BIO PowerSoil DNA isolation kit, and published extraction protocol from Lever et al. (2015, Frontiers in Microbiology, 6, 476) had similar results.

Conclusions: Although different environmental conditions within the habitats will likely affect the degradation of eDNA, these findings suggest that diverse characteristic of the sediments from different habitats does not affect the extraction of DNA. This is an important step in determining whether eDNA within sediment can be utilized to compare the communities from different marine habitats.

KEYWORDS
18S rRNA, community ecology, conservation genetics, environmental DNA, sediment

1 | INTRODUCTION

Determining the distribution of organisms is the foundation to many ecological studies and theories, but sampling frequency in space and time often limits our ability to measure broad-scale patterns. To overcome these limitations, ecologists strive to create more efficient methods to enhance sampling coverage. Using environmental DNA (eDNA) to detect macroorganisms within aquatic environments is a promising technique that could broaden the scale of ecological studies measuring the distribution of animals, as it is more cost effective

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than many traditional techniques (Civade et al., 2016; Yamamoto et al., 2017). This has contributed to rapid growth in the use of eDNA to detect the presence (DiBattista et al., 2019; Jeunen et al., 2019; Willerslev et al., 2003) and possibly estimate the relative abundance of organisms (Port et al., 2016; Thomsen et al., 2012).

Environmental DNA is the genetic material left by organisms in their environment. Depending on the goals of a study, environmental DNA can be extracted from many environmental matrices, including water or sediment. DNA can persist for months to millennia in sediment (Turner, Uy, & Everhart, 2015; Willerslev et al., 2014), so that extracting eDNA from sediment potentially offers an unprecedented potential to detect organisms in the ecosystem currently and in the past. The persistence of DNA in sediment results from adsorption to sediment particles (Deere et al., 1996; Lorenz & Wackernagel, 1987). However, sediment characteristics likely affect DNA adsorption (Ogram, Sayler, Gustin, & Lewis, 1988), which in turn can affect the extraction of eDNA. Moreover, the extracted solution of DNA may also include PCR inhibitors, such as humic substances present in silt and clay, which have been shown to inhibit amplification of DNA (Buxton et al., 2017; Lekang, Thompson, & Troedsson, 2015; Schrader, Schielke, Ellerbroek, & Johne, 2012). Thus, sediment characteristics could alter metabarcoding results and may limit the feasibility to compare communities detected by eDNA from different environments.

Studies have looked at how different sediments affect the quality and quantity of extracted DNA, as well as the diversity of organisms identified by metabarcoding. Buxton (Buxton et al., 2017) found that top soil, with high organic content, reduced detection of a species because of PCR inhibition, as compared to sand. Other studies have found that both sediment type and extraction protocol can affect results from eDNA. For example, the amount of extracted DNA was consistent among extraction protocols for sand but not clay, while the community from cloning was not affected by extraction protocol in sand but was affected in clay (Lekang et al., 2015). In addition, sediment type (soil or stream sediment) and commercial extraction kits used affected the quality and quantity of extracted DNA (Hermans, Buckley, & Lear, 2018). Thus, both extraction protocol and sediment type can affect results from eDNA, potentially hindering the use of sediment eDNA as a source of information for species richness across ecosystems.

Our goal was to provide robust procedures for using sediment eDNA to quantify diversity across marine habitats and more specifically to assess how results from eDNA of macroorganisms are affected by sediment type sampled from three tropical marine habitats, coral reefs, seagrass meadows, and mangrove forests. These habitats are important for eDNA studies as they have important ecological and economic roles (Barbier et al., 2011; Costanza et al., 1997), and together account for much of the biodiversity in tropical marine habitats (Gratwicke & Speight, 2005; Gray, 1997). Unlike former studies, we standardized the DNA within sediment as opposed to measuring the DNA within natural sediments, given that the amount of DNA may vary within and among habitats. Specifically, we conducted two experiments to achieve this goal: (a) measured

the amount of extracellular DNA extracted from sediments that had DNA removed prior to a controlled DNA addition; (b) quantified the number of sequenced reads in sediments after we added an equal amount of DNA of a species not occurring naturally in the habitats. In addition, we also tested three extraction methods during the second experiment to see if extraction method could interact with sediment type to affect the amount of extracted DNA.

2 | MATERIALS AND METHODS

2.1 | Sample collection

At each sampling, five subsamples were taken at each location by inserting 10 ml modified plastic syringes, with the tips removed (21 mm diameter opening), 5 cm into the sediment. Subsamples were collected haphazardly within a 100 m^2 area, each separated by at least 2 m. The subsamples were kept on ice, returned to the laboratory, and the top 8 mm of the sediment from each subsample was collected by pushing the core with a plunger until the correct amount was above the syringe and "cut" with a sterile plastic knife. The five subsamples were pooled to make one sample (2.8 cm^3 of sediment), which was then kept in 50-ml falcon tubes at −80°C until DNA extraction. Nitrile gloves were worn during collection and processing to reduce contamination, and all sampling materials were sterilized before use by soaking them in 10% bleach for at least 30 min. Samples were collected from three different habitats: coral reefs, seagrass meadows, and mangrove forests. Mangrove sediment was collected from moist sediment just above the water level. Collecting samples within the coral reef and seagrass meadow necessitated snorkeling because subtidal sampling was not feasible from the boat because of damaging the habitat-forming species. Sampling protocol with syringe was chosen to minimize contamination from the snorkeler and all precautions were taken to reduce contamination, which included sampling from down current, and quickly collecting and capping the sample. In addition, steps were taken to reduce sample contamination including conducting extraction and pre- and post-PCR preparation in separate areas where surfaces and instruments were deeply decontaminated with bleach and UV light.

2.2 | DNA extraction

We used a commercial extraction method for extracting DNA from sediments with DNA removed and added two additional extraction methods for testing the extraction of novel DNA. All protocols targeted extracellular DNA. The first protocol (referred to here as MO BIO) utilized a phosphate buffer solution which was added to the sediment; then, this buffer was purified using the MO BIO, PowerSoil DNA isolation kit (Taberlet et al., 2012) (MoBio Laboratories, Inc.). First, a phosphate buffer solution (1.97 g of NaH_2PO_4 and 14.7 g of Na_2HPO_4 per liter of sterile water) was added to sediment in a 50-ml falcon tube for a 1:1 volume ratio (Taberlet et al., 2012) and gently mixed for 30 min using a HulaMixer (Life Technologies). The mixed samples were then centrifuged at 10,000 g for 10 min, and

TABLE 1 Details of samples collected for experiments on eDNA recovery

Experiment	Habitat	Latitude	Longitude	Depth	Date collected
Sediments with DNA removed	Coral reef	22.38980	30.13550	0.5 m	Sept. 2016
	Seagrass meadow	22.25290	38.96120	0.5 m	Sept. 2016
	Mangrove forest	22.31100	39.09150	intertidal	Sept. 2016
Sequencing experiment	Coral reef	22.26990	35.64570	10 m	July 2017
	Coral reef	25.29460	36.95160	10 m	July 2017
	Coral reef	20.15940	40.21540	10 m	July 2017
	Seagrass meadow	25.15730	37.16270	4 m	July 2017
	Seagrass meadow	22.24870	39.07160	2 m	July 2017
	Seagrass meadow	20.15940	40.21540	5 m	July 2017
	Mangrove forest	22.31100	39.09150	intertidal	May 2018
	Mangrove forest	22.32150	39.10240	intertidal	May 2018
	Mangrove forest	22.34050	39.08970	intertidal	May 2018

the supernatant was transferred into a 15 ml falcon tube and stored at −80°C until further processing. Then, 750 μl of the phosphate buffer containing extracellular DNA was processed using a MO BIO, PowerSoil DNA isolation kit skipping the cell lysis steps (skipped steps 1–12). The second extraction protocol was based on Lever et al. (2015) and now referred to as the Lever extraction. The published protocol was followed using the extracellular extraction with the carbonate removal treatment. This protocol was chosen because it has a carbonate removal step which is not present in the MO BIO protocol. The third protocol was a combination of the two protocols previously described, adding the extracted DNA from the MO BIO protocol back to the sediment (sediment was already treated with phosphate buffer for the MO BIO protocol) before conducting the Lever extraction. This protocol, which extracted DNA twice from the sediment, was accessed to see if combining both protocols improved DNA extraction. An extraction blank (nuclease-free water) for both extraction methods was included through all steps.

2.3 | Experiment using sediments with DNA removed

Sediment samples were collected from a seagrass meadow a coral reef and a mangrove forest along the central Saudi Arabian Red Sea coast (see Table 1 for details). Basic characteristics of the sediment were noted (preponderance of mud or sand), and organic and inorganic matter were measured from sediment (not used for the DNA extraction) by the loss-on-ignition method (Luczak, Janquin, & Kupka, 1997). Briefly, the loss-on-ignition method consisted of heating 3 g of the sediment to 375°C for 3 hr, and the lost mass was measured as organic material. The sediment was then heated to 800°C for 12 hr and the lost mass was measured as inorganic material.

To remove existing DNA in the sediment samples, we autoclaved and bleached the samples before we added a known amount of DNA. First, 20 g of a sediment sample from each habitat was autoclaved at 121°C for 80 min. Three samples from each habitat (0.5 ml)

from the autoclaved sample were then put into a sterile 2-ml tube. Second, the sediment was washed with 0.5 ml of bleach two times. To remove the bleach, the sediment was then washed with nuclease-free water three times. A separate sample of sediment was then run through the same extraction steps as described for extracellular DNA and analyzed with a Qubit 2.0 fluorometer (Quant-IT dsDNA High Sensitivity Assay kit; Invitrogen) to ensure that the DNA concentration was below detection limit. In agreement with a past study (Otte et al., 2018), initial tests found that DNA was still present after autoclaving (tested with Qubit 2.0 fluorometer), so the bleach step was added. Ultrapure Salmon Sperm (*Oncorhynchus keta*; 0.5 ml) with a concentration of 100 ng/μl (Invitrogen, USA) was added to the DNA-free sediment samples and mixed for two hours in a HulaMixer. After mixing, tubes were centrifuged and the supernatant was separated into a different 2 ml tube. The DNA in both the sediment (0.5 ml) and supernatant (0.5 ml added to phosphate buffer) were extracted using the MO BIO protocol previously described and analyzed with Qubit 2.0 fluorometer to determine DNA concentrations.

2.4 | Sequencing experiment

Sediment samples were collected from three coral reefs: three seagrass meadows, and three mangrove forests along the Saudi Arabian coast of the Red Sea (Table 1). Sites were chosen to broaden the breadth of our findings with different sediment sources both within and among the different habitats. Salmon sperm DNA at a concentration of 10 ng/μl and volume of 1 ml was added per 1 ml of sediment sample. Three grams of sediment was mixed with the salmon DNA for 2 hr using a HulaMixer. The sediment was then divided into individual 1 g aliquots, and DNA was extracted from these aliquots using each of the three different extraction protocols.

For DNA amplification and sequencing, we used primers targeting a 130 base pairs region of the18S rRNA gene (F 5′-TTGTACACACCGCCC -3′; R 5′- CCTTCYGCAGGTTCACCTAC -3′) (Amaral-Zettler et al., 2009). The primer included an Illumina adapter (F 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG;

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R 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′). This primer set was chosen because it is commonly used to detect eukaryotes and amplified salmon DNA based on initial PCR tests, with the goal of making our findings relevant to metabarcoding studies. PCR reactions were conducted using Amplitaq DNA Polymerase kit (Thermo Fisher), using 5 µl of buffer II, 0.2 µl of Taq polymerase, 1 µl of both 10 mM forward and reverse primer, 5 µl of sample, 7.8 µl of PCR grade water, and 1 µl of DNA. Thermal conditions for the PCR were 95°C for 5 min followed by 35 cycles of 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s, which was followed by a final stage of 72°C for 2 min. Five replicate PCR assays for each sample were run and then pooled to decrease PCR bias. An extraction, a PCR and positive blank were included in all procedures. The positive blanks consisted of four sequentially diluted samples of salmon DNA (1 µl of 1.0, 0.1, 0.01, and 0.001 ng/µl) to measure if DNA concentration was related with the number of reads. PCR products were visualized using gel electrophoresis (1.5%) and cleaned by AMPure XP magnetic bead-based purification (Beckman Coulter) according to the MiSeq library preparation guide.

Dual indices and Illumina sequencing adapters from the Illumina Nextera XT Index Kits v2 (Illumina, Inc.) were added to the target amplicon in a second PCR step using Kapa HotStart HiFi 2× ReadyMix DNA polymerase (Kapa Biosystems Ltd.) following MiSeq library preparation guide (12.5 µl of polymerase, 2.5 µl of each index, 5 µl of sample, and 5 µl of PCR grade water; 95°C for 3 min followed by eight cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, which was followed by a final stage of 72°C for 5 min). Libraries were again visualized by gel electrophoresis and purified using AMPure XP beads. DNA concentrations were quantified using TapeStation system (Agilent Technologies) following the manufacturers protocol and then pooled using equimolar amounts based on TapeStation results. If a sample had less than 15 ng of DNA, the entire sample was added to the pool. Finally, the library pool was purified by Wizard SV Gel and PCR Clean-Up System (Promega) to remove any remaining contaminants and PCR artifacts and quantified using KAPA SYBR FAST Universal qPCR kit with Illumina Primer Premix (Kapa Biosystems Ltd.) and the average DNA strand size was assessed using a Bioanalyzer (Agilent Technologies). Six pM of the 18S rRNA gene amplicon libraries were sequenced on one lane of the Illumina MiSeq platform with 25% PhiX control at the KAUST sequencing facility. The libraries were sequenced using 2 × 300 bp overlapping paired-end reads following the MiSeq library preparation guide.

2.5 | Sequences analysis

The DADA2 workflow was used for sequence analysis to model and correct substitution errors, and filter and cluster the amplicons (Callahan et al., 2016). The DADA pipeline was more accurate and resulted in fewer spurious reads than other commonly used pipelines based on clustering similar reads (Callahan et al., 2016), and unique reads are called sequence variants (SV). The entire workflow was conducted in R version 3.5.1 (R Development Core Team, 2012) using the *DADA2* package (Callahan et al., 2016),

except for primer removal. First, the primers were removed from the sequences using CUTADAPT allowing one error for every 10 base pairs in the primer sequence (Martin, 2011). During the DADA2 pipeline, the forward and reverse reads were trimmed to 115 and 95 bp based on visual inspection of the error rates using the fastqPairedFilter function with two expected errors per read. The reads were then de-replicated (derepfastq function), and the SVs were determined using the dada function based on the reads from all samples. Paired reads that matched perfectly were merged (mergePairs function), and chimera reads were removed using the removeBimeraDenovo function. Taxonomy was assigned to the SVs using the RDP classifier (Lan, Wang, Cole, & Rosen, 2012; Wang, Garrity, Tiedje, & Cole, 2007), which implements a naïve Bayesian classifier method to assign taxonomic classification to each SV at successively broader taxonomic levels. The assignTaxonomy function was used to assign taxonomy with the default values, except for minboot which was set at a more conservative value of 70 instead of the default of 50. A reference library was created from the SILVA database (version 132 SSU Nr99), and sequences were filtered based on in silico amplification using virtualPCR function from the insect package (Wilkinson, Stat, Bunce, & Davy, 2018). Code used is available for both the pipeline (https://github.com/ngeraldi/dada2-and-insect-pipeline) and creation of the reference library (https://github.com/ngeraldi/Training_datasets).

Additional filters were used to reduce the likelihood of false positives. SVs were removed if they were present in less than two samples to reduce PCR and sequencing errors. To remove errors from contamination and sequencing, SVs were removed if the maximum number of reads from any control (extraction and PCR blanks) was greater than 0.1% of the sum of the number of reads for the SV for all samples, or if the total number of reads was less than 0.1% of the total reads in samples. This balanced the false positives from contamination in the laboratory and sequencing, but allowed a small amount of cross-contamination to reduce false negatives. After filtering, the number reads per sample were rarefied to the smallest number of reads within a sample to standardize the number of reads among samples. The salmon DNA was identified as *Oncorhynchus mykiss* (>90% of the Salmonidae Family), and we included the reads for all SVs identified as Salmonidae Family. After filtering, the majority of reads in samples was salmon and analysis of the other eukaryotes identified was not conducted because this community was likely not representative of the natural community given the low read counts.

For statistical analysis of the first extraction test using sediment with the DNA removed, the amount of extracted DNA from the three different habitats was tested for heterogeneity with the Levene's test and for differences among the means with a linear model (LM). For the second extraction test, the difference in organic and inorganic matter was tested with a LM. The difference in salmon reads among sediment from the three habitats and extraction protocol was tested with a 2-way LM with the fixed factors and the interaction between them. Significant differences among the levels within factors were determined from nonoverlapping standard

errors from the model output. Summary statistics were calculated using the ANOVA function from the car package (Fox & Weisberg, 2011) for all LMs.

3 | RESULTS

### 3.1 | Experiment using sediments with DNA removed

The seagrass sediments were muddy, and the mangrove sediments were primarily mud with some sand, while the coral sediment was almost exclusively coarse carbonate sand. The organic content of the sediment was 2.3% dry weight (DW) from the coral reef and the seagrass meadow, and 1.2% DW from the mangrove forest. The inorganic content of sediment, largely composed of carbonate minerals, was 45% DW from the coral reef, 43% DW from the seagrass meadow, and 25% DW from the mangrove forest. The amount of DNA extracted from the different habitats had homogeneous variance (Levene's Test, $F_{2,6}$ = 2.874, p = .133). The extraction of extracellular DNA did not differ among sediment sources (ANOVA, $F_{2,9}$ = 0.710, p = .529). The amount of DNA recovered from the sediment was of 10,300 ± 363 ng. A similar amount of DNA was extracted from the liquid surrounding the sediment, 10,181 ± 746 ng.

3.2 | Sequencing experiment

For the sequencing experiment, samples were collected throughout the Red Sea, with both coral reef and seagrass meadow sediments being dominated by carbonate sand while the mangrove sediments were primarily mud with some carbonate sand. The organic carbon did not differ among habitats, 2.3 ± 0.3% DW organic carbon (ANOVA, $F_{2,6}$ = 0.094, p = .912), while the inorganic carbon was lower in the mangrove forest (19.0 ± 3.2) compared to the other two habitats (44.0 ± 1.1; ANOVA, $F_{2,6}$ = 46.51, p < .001). After filtering, the raw number of reads and rarefied reads had a similar pattern (Figure 1). Samples had 2006 ± 163 salmon reads with a range of 44 to 3,004 reads. The number of salmon reads in positive blanks did directly rank with the concentration of salmon DNA (3,096, 2,941, 1,817, and 1,405 reads for samples of 1.0, 0.1, 0.01, and 0.001 ng/μl, respectively; Figure 2b). Habitat did not affect the number of salmon reads (ANOVA, $F_{2,18}$ = 1.641, p = .221), but the extraction method did affect the number of salmon reads (ANOVA, $F_{2,18}$ = 5.057, p = .018: Figure 2b), with the combination protocol having fewer salmon reads than the MO BIO protocol. There was no interaction between the two fixed factors (ANOVA, $F_{4,18}$ = 0.504, p = .733).

4 | DISCUSSION

Using eDNA to monitor the presence of animals is an emerging technique that can enhance our understanding of the distribution of organisms, particularly when using DNA in marine sediments, that contain a fingerprint of animals in the ecosystems integrated over
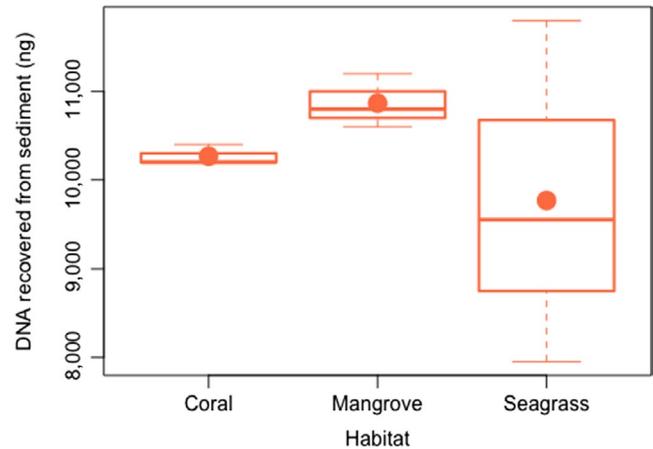

FIGURE 1 The amount of salmon extracellular DNA extracted from sediment (MO BIO protocol) after being treated to remove existing DNA and adding 5 μl of salmon DNA. There were no differences in variances (Leven's Test, p = .133) or means (ANOVA, p = .529) among the sediment collected from different habitats. Boxplots show the median with the upper and lower quartiles, while the whiskers extend to the extreme data point but no more than 1.5 times the respective quartile. The mean is indicated by the circle

time. We found that the amount of extracellular DNA extracted from sediments that had DNA removed prior to a controlled DNA addition and that the number of reads from an exogenous species did not differ among sediments from different important tropical habitats. These findings suggest that the extraction of eDNA from sediment was not affected by sediment characteristics associated with these habitats. Although other variables such as eDNA degradation could affect cross-habitat comparisons, such as more rapid DNA degradation in coral reefs compared to that in seagrass meadows because of higher UV radiation (Strickler, Fremier, & Goldberg, 2015), these findings suggest that eDNA could be a useful tool in detecting communities among different marine habitats.

Sediment characteristics can affect the extraction of DNA and PCR products. For example, DNA adsorbs to sediment which reduces degradation but could also affect extraction (Lekang et al., 2015; Lorenz & Wackernagel, 1987). The use of heat and bleach to remove DNA from the sediment prior to adding a known amount of DNA could have altered the properties of the sediment that are known to affect adsorption, such as ionic strength and pH (Ogram et al., 1988), and the results could be different to what would be found with "untreated" sediment. The second experiment was designed to negate the potential artifact of DNA removal, and this experiment sequenced DNA after an exogenous DNA spike to the sediment. This addition experiment corroborated the findings of the first and found that the number of reads of the added DNA did not differ across sediments from different marine habitats.

Extraction protocols can also affect the quality and quantity of DNA extracted from sediment(Hermans et al., 2018; Lekang et al., 2015; Lever et al., 2015). Extraction methods are developed to both isolate DNA, which can be affected by adsorption to sediments, and remove contaminants that inhibit PCR such as
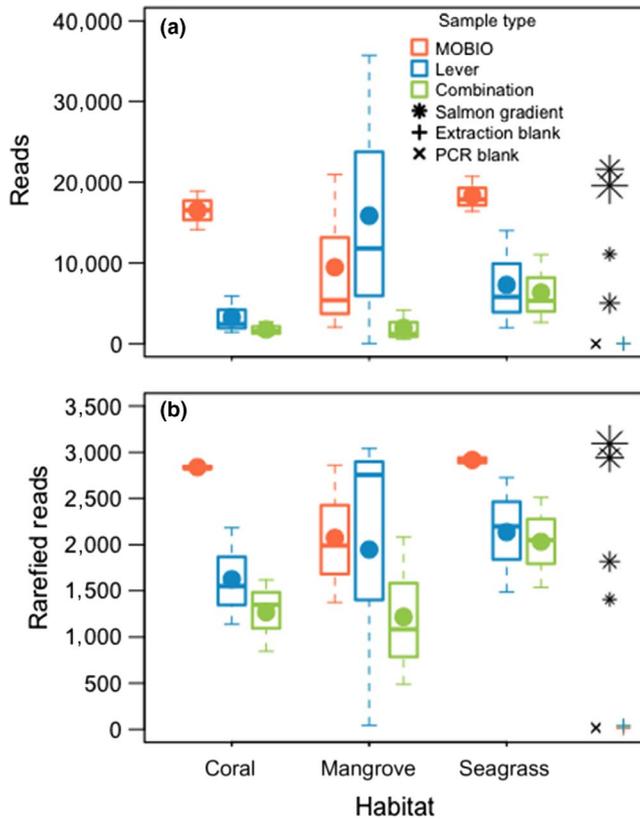
FIGURE 2 The number of reads (a) and reads after rarefaction (b) of salmon sequenced from DNA that was extracted using 3 different protocols from sediment from three different habitats. Boxplots show the median with the upper and lower quartiles, while the whiskers extend to the extreme data point but no more than 1.5 times the respective quartile. The mean is indicated by the circle. Extraction and PCR blanks are shown, as well as positive blanks with four different concentrations of salmon DNA (0.001, 0.01, 0.1, and 1.0 ng/µl) as shown by size of asterisks

humic substances present in organic-rich sediment (Buxton et al., 2017). We found that there was no difference in the number of salmon reads between the Lever extraction and the other two methods, but that the MO BIO extraction had more salmon reads than the combination extraction. In the study that developed the extraction protocol, the Lever extraction had greater DNA yields than the MO BIO when tested on different sediment types, which was attributed to high PO_4 concentrations and making the pH > 9 during initial protocol steps (Lever et al., 2015). In this study, the reduced reads in the combination protocol likely resulted from the samples being over-processed and losing DNA with the additional steps resulting in a reduced DNA yield as opposed to the extra processing resulting in more purified DNA.

Our experiments were designed to build upon previous experiments that compared DNA extracted from sediments with naturally occurring DNA which varies among and within types of sediment. Our findings are based on equal DNA additions to determine the effect of sediment type and extraction on DNA yield. Although this gives us more insight into how sediment type affects DNA extraction, it may differ from naturally occurring eDNA. We

simulated DNA addition to the sediment by adding salmon sperm DNA to sediment and mixing it, but this may differ from how DNA is added and adsorbed to the sediment in nature. For example, the exogenous DNA spike may not have been absorbed to the sediment, perhaps because it was added at too high of a concentration, resulting in equal amounts of extracted DNA. Future studies could add a known amount of exogenous DNA to sediment in natural conditions. However, such a study would not be able to isolate the effect of sediment from habitat-specific DNA degradation. A second consideration with regard to comparing eDNA in different habitats is that in most metabarcoding experiments, equimolar samples are used for sequencing and the number of reads per sample is standardized (rarefaction), which likely minimize potential eDNA artifacts resulting from differences among habitats. However, eDNA studies do have a detection limit, such as a minimum number of reads at which a taxa is determined to be present and not just type II error. Thus, determining how habitat-specific characteristics affect DNA extraction and subsequent metabarcoding results become more important as species become less abundant. We suggest future studies add sequentially lower concentrations of the exogenous DNA spike to sediment, in addition to testing different habitats to improve our understanding of whether sediment types may affect eDNA detection.

Detecting organisms and assessing diversity within different habitats, as well as measuring species overlap among habitats, is a key aspect of ecology. Yet, the majority of survey techniques do not have perfect detection. Findings from sampling methods can find different taxa assemblages among habitats because of survey artifacts and not actual differences in the communities. For example, visual surveys quantified the greatest lobster abundance on structurally complex habitat while traps measured the greatest lobster abundance on structure-less substrate (Geraldi, Wahle, & Dunnington, 2009). In addition, some fish species were not consistently detected when comparing surveys by visual census, traps, and video camera, which may impart be from biases associated with habitat characteristics (Bacheler et al., 2017). There is a clear need to develop and test survey techniques that have consistent species detections among different locations regardless of abiotic and biotic factors. This is particularly pertinent in complex marine habitats, such as coral reefs, mangrove forests, and seagrass meadows, which support high biodiversity including many cryptic species that take shelter within the structure. Our findings suggest that eDNA offers a new tool that could improve ecologists' ability to quantify and compare biodiversity among marine habitats.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

Sequences from this study have been deposited at NCBI's SRA under project no. PRJNA579138 (https://www.ncbi.nlm.nih.gov/sra/PRJNA579138).

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REFERENCES

Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLOS ONE*, *4*(7), e6372. https://doi.org/10.1371/journal.pone.0006372

Bacheler, N., Geraldi, N., Burton, M., Muñoz, R., & Kellison, G. (2017). Comparing relative abundance, lengths, and habitat of temperate reef fishes using simultaneous underwater visual census, video, and trap sampling. *Marine Ecology Progress Series*, *574*, 127–140. https://doi.org/10.3354/meps12172

Barbier, E., Hacker, S., Kennedy, C., Koch, E., Stier, A., & Silliman, B. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, *81*, 169–193. https://doi.org/10.1890/10-1510.1

Buxton, A. S., Groombridge, J. J., & Griffiths, R. A. (2017). Is the detection of aquatic environmental DNA influenced by substrate type? *PLOS ONE*, *12*(8), e0183371. https://doi.org/10.1371/journal.pone.0183371

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. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Callahan, B. J., Sankaran, K., Fukuyama, J. A., McMurdie, P. J., & Holmes, S. P. (2016). Bioconductor workflow for microbiome data analysis: From raw reads to community analyses. *F1000Research*, *5*, 1492. https://doi.org/10.12688/f1000research.8986.2

Civade, R., Dejean, T., Valentini, A., Roset, N., Raymond, J.-C., Bonin, A., … Pont, D. (2016). Spatial representativeness of environmental DNA metabarcoding signal for fish biodiversity assessment in a natural freshwater system. *PLOS ONE*, *11*(6), e0157366. https://doi.org/10.1371/journal.pone.0157366

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., … van den Belt, M. (1997). The value of the world's ecosystem services and natural capital. *Nature*, *387*(6630), 253–260. https://doi.org/10.1038/387253a0

Deere, D., Porter, J., Pickup, R., & Edwards, C. (1996). Survival of cells and DNA of *Aeromonas salmonicida* released into aquatic microcosms. *Journal of Applied Bacteriology*, *81*(3), 309–318. https://doi.org/10.1111/j.1365-2672.1996.tb04333.x

DiBattista, J. D., Reimer, J. D., Stat, M., Masucci, G. D., Biondi, P., Brauwer, M. D., & Bunce, M. (2019). Digging for DNA at depth: Rapid universal metabarcoding surveys (RUMS) as a tool to detect coral reef biodiversity across a depth gradient. *PeerJ*, *7*, e6379. https://doi.org/10.7717/peerj.6379

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression*, 2nd ed. Thousand Oaks, CA: Sage.

Geraldi, N. R., Wahle, R. A., & Dunnington, M. J. (2009). Habitat effects on American lobster (*Homarus americanus*) movement and density: Insights from georeferenced trap arrays, seabed mapping, and tagging. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 460–470.

Gratwicke, B., & Speight, M. R. (2005). The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. *Journal of Fish Biology*, *66*(3), 650–667. https://doi.org/10.1111/j.0022-1112.2005.00629.x

Gray, J. S. (1997). Marine biodiversity: Patterns, threats and conservation needs. *Biodiversity & Conservation*, *6*(1), 153–175. https://doi.org/10.1023/A:1018335901847

Hermans, S. M., Buckley, H. L., & Lear, G. (2018). Optimal extraction methods for the simultaneous analysis of DNA from diverse organisms and sample types. *Molecular Ecology Resources*, *18*(3), 557–569. https://doi.org/10.1111/1755-0998.12762

Jeunen, G.-J., Knapp, M., Spencer, H. G., Lamare, M. D., Taylor, H. R., Stat, M., … Gemmell, N. J. (2019). Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. *Molecular Ecology Resources*, *19*(2), 426–438. https://doi.org/10.1111/1755-0998.12982

Lan, Y., Wang, Q., Cole, J. R., & Rosen, G. L. (2012). Using the RDP classifier to predict taxonomic novelty and reduce the search space for finding novel organisms. *PLoS ONE*, *7*(3), e32491. https://doi.org/10.1371/journal.pone.0032491

Lekang, K., Thompson, E. M., & Troedsson, C. (2015). A comparison of DNA extraction methods for biodiversity studies of eukaryotes in marine sediments. *Aquatic Microbial Ecology*, *75*(1), 15–25. https://doi.org/10.3354/ame01741

Lever, M. A., Torti, A., Eickenbusch, P., Michaud, A. B., Šantl-Temkiv, T., & Jørgensen, B. B. (2015). A modular method for the extraction of DNA and RNA, and the separation of DNA pools from diverse environmental sample types. *Frontiers in Microbiology*, *6*, 476. https://doi.org/10.3389/fmicb.2015.00476

Lorenz, M. G., & Wackernagel, W. (1987). Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. *Applied and Environmental Microbiology*, *53*(12), 2948–2952.

Luczak, C., Janquin, M. A., & Kupka, A. (1997). Simple standard procedure for the routine determination of organic matter in marine sediment. *Hydrobiologia*, *345*, 87–94. https://doi.org/10.1023/A:1002926626798

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *Embnet Journal*, *17*(1), 10–12. https://doi.org/10.14806/ej.17.1.200

Ogram, A., Sayler, G. S., Gustin, D., & Lewis, R. J. (1988). DNA adsorption to soils and sediments. *Environmental Science & Technology*, *22*(8), 982–984. https://doi.org/10.1021/es00173a020

Otte, J. M., Blackwell, N., Soos, V., Rughöft, S., Maisch, M., Kappler, A., … Schmidt, C. (2018). Sterilization impacts on marine sediment––Are we able to inactivate microorganisms in environmental samples? *FEMS Microbiology Ecology*, *94*(12), https://doi.org/10.1093/femsec/fiy189

Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J., … Kelly, R. P. (2016). Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Molecular Ecology*, *25*(2), 527–541. https://doi.org/10.1111/mec.13481

R Development Core (2012). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Schrader, C., Schielke, A., Ellerbroek, L., & Johne, R. (2012). PCR inhibitors – occurrence, properties and removal. *Journal of Applied Microbiology*, *113*(5), 1014–1026. https://doi.org/10.1111/j.1365-2672.2012.05384.x

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Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, *183*, 85–92. https://doi.org/10.1016/j.biocon.2014.11.038

Taberlet, P., Prud'homme, S. M., Campione, E., Roy, J., Miquel, C., Shehzad, W., … Coissac, E. (2012). Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. *Molecular Ecology*, *21*(8), 1816–1820. https://doi.org/10.1111/j.1365-294X.2011.05317.x

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS ONE*, *7*(8), e41732. https://doi.org/10.1371/journal.pone.0041732

Turner, C. R., Uy, K. L., & Everhart, R. C. (2015). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation*, *183*, 93–102. https://doi.org/10.1016/j.biocon.2014.11.017

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wilkinson, S. P., Stat, M., Bunce, M., & Davy, S. K. (2018). Taxonomic identification of environmental DNA with informatic sequence classification trees. *PeerJ Preprints*, *6*, e26812v1. https://doi.org/10.7287/peerj.preprints.26812v1

Willerslev, E., Davison, J., Moora, M., Zobel, M., Coissac, E., Edwards, M. E., … Taberlet, P. (2014). Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature*, *506*(7486), 47–51. https://doi.org/10.1038/nature12921

Willerslev, E., Hansen, A. J., Binladen, J., Brand, T. B., Gilbert, M. T. P., Shapiro, B., … Cooper, A. (2003). Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science*, *300*(5620), 791–795. https://doi.org/10.1126/science.1084114

Yamamoto, S., Masuda, R., Sato, Y., Sado, T., Araki, H., Kondoh, M., … Miya, M. (2017). Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Scientific Reports*, *7*, 40368. https://doi.org/10.1038/srep40368