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An Outlook for the Acquisition of Marine Sedimentary Ancient DNA (*sed*aDNA) From North Atlantic Ocean Archive Material

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Key Points:

- We show that, generally, sedimentary ancient DNA (*sed*aDNA) can be extracted from North Atlantic archived sediment samples
- Issues exist around contamination deriving from previous sample handling and non-ideal storage conditions
- Bioinformatics can help disentangle ancient and contaminant DNA, but key to *sed*aDNA analyses are clean sampling and optimal storage conditions

Supporting Information:

Supporting Information may be found in the online version of this article.

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Abstract Studies incorporating sedimentary ancient DNA (*sed*aDNA) analyses to investigate paleo-environments have increased considerably over the last few years, and the possibility of utilizing archived sediment cores from previous field campaigns could unlock an immense resource of sampling material for such paleo-investigations. However, *sed*aDNA research is at a high risk of contamination by modern environmental DNA, as sub-optimal sediment storage conditions may allow for contaminants (e.g., fungi) to grow and become dominant over preserved *sed*aDNA in the sample. Here, we test the feasibility of *sed*aDNA analysis applied to archive sediment material from five sites in the North Atlantic, collected between 1994 and 2013. We analyzed two samples (one younger and one older) per site using a metagenomic shotgun approach and were able to recover eukaryotic *sed*aDNA from all samples. We characterized the authenticity of each sample through *sed*aDNA fragment size and damage analyses, which allowed us to disentangle *sed*aDNA and contaminant DNA. Although we determined that contaminant sequences originated mainly from Ascomycota (fungi), most samples were dominated by *Emiliania huxleyi*, a haptophyte species that commonly blooms in the study region. We attribute the presence of contaminants to non-ideal sampling and sample storage conditions of the investigated samples. Therefore, while we demonstrate that *sed*aDNA analysis of archival North Atlantic seafloor sediment samples are generally achievable, we stress the importance of best-practice ship-board sampling techniques and storage conditions to minimize contamination. We highly recommend the application of robust bioinformatic tools that help distinguish ancient genetic signals from modern contaminants, especially when working with archive material.

Plain Language Summary The seafloor is a natural archive of marine organisms that have lived in the past. When marine organisms die, they sink to the bottom of the ocean, and over thousands of years, they form layers as part of the sedimentary record. Although some organisms do not fossilize, they can still leave traces of their DNA behind. To date, most research used to reconstruct the marine life of thousands of years ago makes use of fossilized organisms, but obviously lack information about the non-fossilizing organisms. The study of ancient DNA preserved in the sedimentary record can be analyzed to aid a more comprehensive reconstructions of marine life. We studied North Atlantic sediment cores that were archived in core repositories to determine if this type of long-term stored material was suitable for sedimentary ancient DNA analyses. While we observed possible modern contamination from fungi, likely due to non-optimal storage conditions, other eukaryotes, such as the coccolithophore *Emiliania huxleyi*, which is widely distributed in the modern ocean, were also observed. Although we show that analysis of sediment material from the North Atlantic seafloor is generally achievable, we stress the importance of ensuring steps are taken to mitigate contamination from sample collection and storage.

1. Introduction

The study of marine sedimentary ancient DNA (*sed*aDNA) involves the analysis of DNA from organisms that have inhabited the ocean in the past, and that have since sunk to the seafloor and become preserved in the sediment record. Sedimentary ancient DNA (*sed*aDNA) has recently become increasingly applied to study paleo-environments, as this new paleo-proxy can detect signatures of organisms that do and do not leave a fossil-trace in the sediment record. Traditionally, variations in oceanographic conditions and marine biological communities have been derived from the analyses of microfossils preserved in sediments (Yasuhara et al., 2020). For example,

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microscopy analyses of fossilized eukaryotes, such as diatoms, foraminifera, dinoflagellate cysts, radiolaria, and coccolithophores (all belonging to the large group of single-celled protists) have been the gold standard to reconstruct paleoenvironments, paleoproductivity and palaeoceanographic conditions (Mudie et al., 2006; O'Brien et al., 2021; Oksman et al., 2019; Weckström et al., 2020; Yasuhara et al., 2020). However, such microfossil-based reconstructions are limited, as only the more robust and fossilized species are preserved in seafloor sediments, meaning that the vast number of soft-bodied organisms that have also thrived in the past ocean are not accounted for (e.g., many flagellates, chlorophytes, haptophytes, ciliates, zooplankton) (Ellegaard et al., 2020; Witkowski et al., 2016). The study of sedimentary ancient DNA (*sed*aDNA) has the potential to fill this gap and achieve a more complete picture of past marine ecosystems across the whole food web, which will be useful to help improve our understanding of possible future ecosystem responses to climate and ocean changes.

A caveat of *sed*aDNA research is that the DNA that has been preserved in seafloor records for thousands of years is highly fragmented and degraded. Additionally, the *sed*aDNA of important environmental indicator organisms, such as many eukaryotes (phytoplankton, zooplankton, and higher organisms) only occur in trace amounts within marine sediments. For example, *sed*aDNA fragments from Tasmanian coastal and Antarctic deep ocean sediments have been shown to be only ∼69 and ∼40–50 base pairs (bp) long, respectively (Armbrecht, 2020; Armbrecht et al., 2020), compared to much larger DNA fragments (hundreds to thousands of bp long) that are used to characterize living organisms in the modern ocean (Carradec et al., 2018; Obiol et al., 2020). This scarcity and degraded nature of *sed*aDNA requires sampling, extraction, laboratory and bioinformatic techniques to be optimized (Armbrecht et al., 2019; Briggs, 2020). Paying utmost attention to avoid contamination throughout all sampling and experimental steps is crucial, as even small amounts of modern environmental DNA contamination can override the weak *sed*aDNA signal (Armbrecht, 2020).

Recent studies have focused on the optimisation of *sed*aDNA techniques specifically to maximize the yield of ancient genetic material stemming from marine eukaryotes (Armbrecht et al., 2020; Foster et al., 2020). These include ship-based sampling procedures to minimize contamination, extraction of *sed*aDNA to maximize the yield of marine eukaryote DNA, and bioinformatic pipelines to analyze and authenticate *sed*aDNA (Armbrecht et al., 2019). A key method to analyze ancient DNA is the use of metagenomics techniques, such as shotgun sequencing, where the DNA from all organisms (i.e., "total" DNA) in a sample is analyzed. As only a small fraction of the total DNA is from eukaryotes, metagenomic approaches are computationally intense, and can be considered costly. However, these approaches are key to obtain the small *sed*aDNA fragments typical for ancient DNA, including their characteristic DNA damage patterns (Capo & Monchamp et al., 2022). Both fragment-size variability and damage are required to assess the authenticity of the DNA (*i.e.*, ensuring that the genetic signal is truly ancient).

Due to the extensive risk of contamination, it is recommended that samples for *sed*aDNA research are purpose-collected. That is, best-practise techniques should be followed during sample acquisition, storage, the laboratory, and analysis process (Armbrecht et al., 2019; Capo et al., 2021). This limits the availability of samples for *sed*aDNA analysis to a relatively small pool of available sediment cores recently collected following extensive de-contamination procedures. In contrast, thousands of cores from field campaigns carried out over the last few decades are currently stored as "archive material" at various core repositories around the globe. Being able to utilize this archive material would unlock an immense resource to the field of marine *sed*aDNA research. However, archive cores are usually stored at 4°C and under oxic conditions, which poses the question of whether these cores are suitable for *sed*aDNA analyses. Increased temperatures and exposure to oxygen are factors known to contribute to rapid DNA degradation and microbial contamination, which may lead to contaminants overriding the ancient DNA signal of rare taxa, including eukaryotes (Llamas et al., 2017).

In this study, several archived sediment cores that were taken from the North Atlantic have been analyzed for *sed*aDNA. This basin was chosen because the North Atlantic is a key region for the global climate system that is also undergoing well-documented, dramatic changes in its oceanography that are associated with anthropogenic climate change and for which there are corresponding changes in its marine ecosystem. For example, increases in greenhouse gases have contributed to rising ocean and sea surface temperatures (Hartmann et al., 2013; Shi et al., 2018), sea-levels (Zickfeld et al., 2017), and an acceleration of Greenland's ice melting (Bamber et al., 2018; Briner et al., 2020; Trusel et al., 2018). Likely in response to these forcing factors, building evidence suggests that the circulation systems of the North Atlantic are changing. These alterations have included a 20th century weakening of the Florida Current (Piecuch, 2020), anomalous industrial-era, centennial-scale, changes in the subpolar

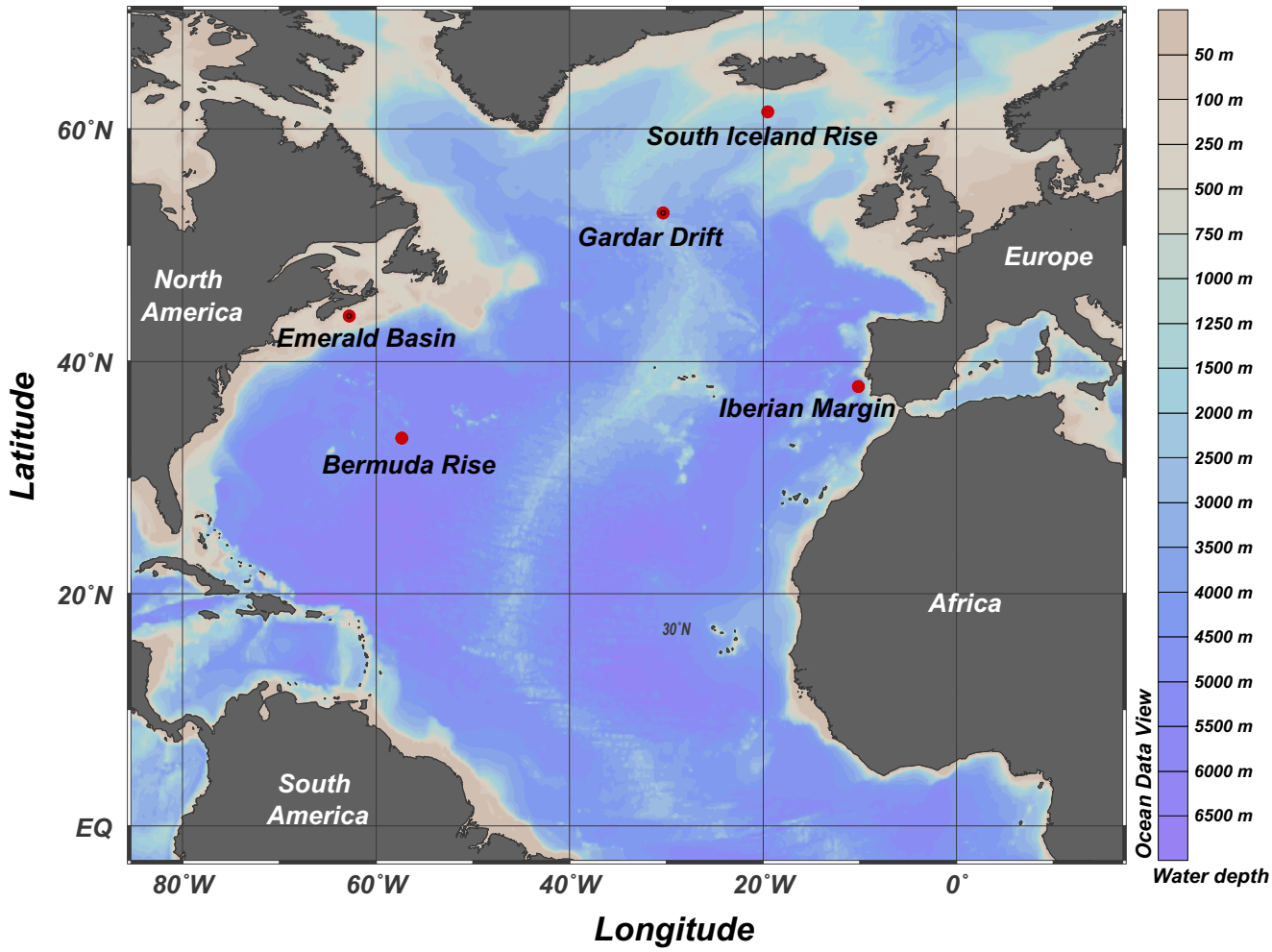

Figure 1. Map of coring sites. Samples were collected at five sites across the North Atlantic Ocean: Emerald Basin, Bermuda Rise, South Iceland Rise, Gardar Drift and, Iberian Margin. Map was created in ODV (Schlitzer, 2018).

gyre (SPG) (Spooner et al., 2020), and a proposed weakening of the Atlantic meridional overturning circulation (AMOC) (Caesar et al., 2018, 2021; Thornalley et al., 2018). Subsequently, changes in oceanographic conditions have been linked to alterations in biological communities of the North Atlantic. Measurements and reconstruction of salinity, nitrates, wind, water circulation and available nutrients have been associated with variability in marine biota (Boyd & Hutchins, 2012), including both planktonic and benthic species (Barton et al., 2016; Hátún et al., 2016, 2009; Henson et al., 2009; O'Brien et al., 2021; Osman et al., 2019). Predictions on future Atlantic ocean conditions indicate that temperature redistributions and slowing of the AMOC will likely cause diatom and dinoflagellate communities (important planktonic primary producers) to shift northwards (Barton et al., 2016). Additionally, it is forecasted that climate change will result in phytoplankton overgrowth in the North Atlantic region, which might deplete oxygen levels in the water column and near the sea bed, thereby severely disrupting ecosystem functioning (Holt et al., 2018). Thus, there is strong motivation to explore and improve methods that can be applied in the North Atlantic region to better reconstruct the ecosystem response to past changes in climate and ocean circulation, and improve future projections.

The aim of this study is to test whether eukaryotic *sed*aDNA is preserved and can be extracted from previously collected archived sediment material, examining five well-studied palaeoceanographic sites in the North Atlantic Ocean (Figure 1; Emerald Basin, Bermuda Rise, Gardar drift, South Iceland Rise, and Iberian Margin). We apply shotgun sequencing to investigate *sed*aDNA characteristics, such as *sed*aDNA fragment length and damage

Table 1
North Atlantic Sediment Sample Details

Sample name	Site	Core name	Year collected & storage condition	Sampling equipment	Latitude (N)	Longitude (W)	Water depth (m)	Core sample depth (cmbsf)	Approx. age (kyr BP)
EB79	Emerald Basin[1]	OCE-326-30GGC	1998 Cold store	Spatula into sample bag	43.89	62.80	240	79	3 (a)
EB244	Emerald Basin[1]	OCE-326-30GGC	1998 Cold store	Spatula into sample bag	43.89	62.80	240	244	8 (a)
BR2	Bermuda Rise[2]	KNR-191-3-19CDH	2010 Bagged cold store	Spatula into sample bag	33.41	57.35	4,541	2	0.5 (a)
BR951	Bermuda Rise[2]	KNR-191-3-19CDH	2010 Bagged cold store	Spatula into sample bag	33.41	57.35	4,541	951	23.5 (a)
SI300	South Iceland Rise[3]	RAPiD-17-5P	2004 Cold store	Centrifuge tube	61.48	19.54	2,303	300	3.5 (a)
SI750	South Iceland Rise[3]	RAPiD-17-5P	2004 Cold store	Centrifuge tube	61.48	19.54	2,303	750	9.5 (a)
GD5	Gardar Drift[4]	NEAP-18K	1994 Bagged cold store	Centrifuge tube	52.77	30.34	3,275	5	0.5 (b)
GD79	Gardar Drift[4]	NEAP-18K	1994 Bagged cold store	Centrifuge tube	52.77	30.34	3,275	79	19.5 (b)
IM60	Iberian Margin[5]	JC89-7-4P	2013 Cold store	Centrifuge tube	37.85	10.15	3,081	60	6 (b)
IM350	Iberian Margin[5]	JC89-7-4P	2013 Cold store	Centrifuge tube	37.85	10.15	3,081	350	20 (b)

Note. Listed are sample names and locations, site characteristics, storage conditions (cold store = ~4°C), and estimated age (N = north; W = west; *m* = meter; cmbsf = centimeters below seafloor; kyr BP = thousand years before present). Site and chronological information provided in: 1, Keigwin et al. (2003); 2, Henry et al. (2016); 3, Spooner et al. (2020): 4, Bianchi and McCave (2000) and Chapman and Shackleton (1999); 5, Hodell et al. (2013), approximate ages provided by comparison of color reflectance data to nearby sites; (a) indicates radiocarbon based age models, with uncertainties of approx. +/−500 years, whereas (b) indicates stratigraphic based ages with larger uncertainties, approx. +/−2000 years.

patterns, and paleo-eukaryote composition, to assess the feasibility of future *sed*aDNA analyses across the North Atlantic Ocean and through time.

2. Methods

2.1. Sites and Sampling

Samples were obtained from existing sediment cores across five sites in the North Atlantic Ocean, collected during different cruises: Emerald Basin (EB; Oceanus, OCE326), Bermuda Rise (BR; Knorr, KNR 191), Gardar Drift (GD; RRS Charles Darwin, cruise 88), South Iceland Rise (SI; Charles Darwin, CD159), and Iberian Margin (IM; RSS James Cook, JC089) (Figure 1, Table 1). These sites were chosen on the basis that they encompass several well-studied palaeoceanographic sites, but they also allow testing of the presence of *sed*aDNA in cores collected at different times, and at sites spanning a range of water depths, from shelf (EB) to abyssal depths (BR), with broad geographic coverage from the subtropical to subpolar North Atlantic, and from continental margins (EB, SI, IM) as well as the ocean interior (BR, GD). Although detailed downcore analysis is not conducted here, these sites are also from regions that are sensitive to several important oceanic circulation features, such as the extension of the subpolar gyre (SPG), changes in the Gulf Stream position, as well as upwelling and oligotrophic sites, and thus have the potential to be of particular interest for future studies, if authentic *sed*aDNA is present and detectable.

The samples selected were all obtained from cores that had been split into two halves and then stored in marine sediment cold storage facilities, that is, in the dark at ~4°C, in sealed plastic containers. Samples from Bermuda Rise (BR) and Gardar Drift (GD) were obtained from cores that, shortly after splitting, had been sliced into 1 cm intervals and stored, in cold storage, in sealed plastic bags. For each core, two samples (a younger and an older sample) were collected, targeting samples of mostly Holocene age but also including a few older samples (with sample ranging from ~0.5 to ~24 thousand years before present (kyr BP), based on existing ^{14}C and stratigraphic chronologies (Table 1). To minimize contamination, a face covering and sterile nitrile gloves were worn for sampling. Work surfaces and equipment were sterilized using ethanol. Samples were taken by either inserting a DNA-free 1 cm diameter centrifuge tube into the mud, or by using a freshly sterilized spatula (see Table 1), to obtain ~1–3 cc of sediment. For the samples obtained from intact core-halves (EB, SI, IM), the upper few mm of surface sediment was scraped away using a sterilized metal scraper prior to obtaining the sample; this was

not possible for the samples obtained from the pre-sampled bagged material (BR, GD). All subsamples were taken in December 2017 and stored at 4°C before being sent to the Australian Centre for Ancient DNA (ACAD), Australia, in January 2018, where they were stored at 4°C until analysis in September 2018.

2.2. *Seda*DNA Extraction and Shotgun Library Preparation

Subsampling, *sed*aDNA extraction, and shotgun library preparation were performed in an ultraclean ancient DNA laboratory at the ACAD, following strict decontamination standards (Willerslev & Cooper, 2005). Prior to entering the laboratory, sample bags were wiped with bleach and the outside of the bag exposed to UV for 5 min. DNA was extracted following the "Si4" method previously described (Armbrecht et al., 2020), which combines *sed*aDNA extraction from 0.25 g of refrigerated (4°C) sediment material using the DNeasy PowerLyzer PowerSoil Kit (Qiagen) including bead-beating (3 × 20 sec with 5 min breaks, FastPrep FP120, Thermo Savant, USA) with a DNA binding step in silica-containing QG Buffer (Qiagen). *sed*aDNA was eluted in 100 µL TLE buffer (50 µl 1 M Tris HCL, 10 µl 0.5 M EDTA, 5 mL H_2O). To monitor laboratory contamination, two extraction blank controls were processed alongside the samples by treating an empty bead-tube with the same extraction protocol.

Shotgun libraries of samples and extractions blank controls were prepared following the "Si4" method (Armbrecht et al., 2020), except that we used 20 µL *sed*aDNA template as input volume. This protocol involves a repair step to enable ligation with truncated Illumina-adapter sequences containing two unique 7 bp barcodes (Meyer & Kircher, 2010) a first amplification with primers IS7/IS8 (eight replicates/sample) including 13 cycles followed by *sed*aDNA purification using Axyprep magnetic beads (Axygen Biosciences, 1:1.8 library:beads). Subsequent indexing primer (IS4/GaII) amplifications (8 replicates/sample) also included 13 cycles, followed by *sed*aDNA purification using Axyprep magnetic beads (1:1.1 library:beads) with one to two clean-ups depending on the sample to ensure removal of all primer-dimer. We quantified our *sed*aDNA libraries using Tapestation (Agilent Technologies), prepared an equimolar pool of 1.87 nM (0.265 ng/µL) and sent it for Illumina NextSeq sequencing (2 × 75 bp cycle) to the Australian Cancer Research Foundation (ACRF) Genomics Facility & Centre for Cancer Biology (CCB), Adelaide, Australia.

2.3. Bioinformatic *sed*aDNA Data Processing and Authentication

We processed and filtered the sequencing data following (Armbrecht et al., 2021), which included demultiplexing and adapter removal (Schubert et al., 2016), as well as low-complexity (--threshold 0.55, Clarke et al., 2019) and duplicate read removal (BBMap v37.36). FastQC and MultiQC quality controls were performed on the raw sequencing data and after each step of data filtering (FastQC v0.11.8 (Andrews, 2010); MultiQC6 v1.0.dev0, Ewels et al., 2016). We used the NCBI Nucleotide database (ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nt.gz, downloaded November 2019) as the reference database to build a step-3 MALT index and aligned our *sed*aDNA sequences (MALT version 0.4.0; semiglobal alignment, minimum percent identity = 95%, LCA = 80, Herbig et al., 2016). Following conversion of .blastn to .rma6 format using the Blast2RMA tool in MEGAN6CE (v6.18.9; Huson et al., 2016), we imported our sequences into MEGAN6CE (v6.19.8; Huson et al., 2016) and removed all species detected in extraction blank controls (contaminant species; see Table S1) from samples. Additionally, mammalian sequences, except for Cetacea sequences (aquatic mammals), were also removed due to possible contamination. For the complete computer code see (Armbrecht et al., 2020; Armbrecht et al., 2021).

Post-filtering, eukaryotic sequences were imported into Geneious 2020.2 (https://www.geneious.com) to determine *sed*aDNA fragment length distribution. Further, eukaryotic read counts at the phylum taxonomic level were imported into *R* (v3.6.1) (R Core Team, 2019), and a heatmap was created using the ggplot2 package (Wickham, 2016). Species level information was collected to (a) obtain species richness (i.e., the number of species) per sample, and (b) identify more abundant taxa (i.e., phyla >0.1% average relative abundance and removing any species with less than 10 total reads).

Authenticity of *sed*aDNA can be characterized by deamination, or "damage," on either side of the DNA fragment, termed the 5' and 3' ends, which is tracked by C to *T* substitutions at the 5' end of DNA sequences, and *G* to A substitutions at the 3' end of DNA sequences. To assess *sed*aDNA authenticity we ran the "MALTExtract" and "Postprocessing" tools of the HOPS v0.33-2 pipeline using the rma6-files as input and default configurations except topMaltEx = 0.10, minPIdent = 95, meganSummary = 1, and destackingOff = 1 (Hübler et al., 2019).

Table 2
Sequencing Information and Characteristics of Eukaryotic sedaDNA

Sample	Total sequences (million)	Total assigned sequences	Total filtered eukaryotic sequences	Eukaryotic *sed*aDNA fragment size (bp; mean ± SD)	% Eukaryotic *sed*aDNA damage	% Fungi *sed*aDNA damage
EB79	6.2	333,713	1,035	70 (±21)	7.2	1.6
EB244	4.5	292,831	649	81 (±21)	3.5	3.5
BR2	7.2	1,211,478	5,429	52 (±16)	5.9	4.6
BR951	5.4	1,650,565	3,218	69 (±28)	1.6	0.4
SI300	9.0	394,825	8,296	58 (±20)	10.3	5.7
SI750	5.4	185,881	2,423	61 (±19)	16.8	5.6
GD5	9.5	1,332,906	12,173	47 (±14)	7.9	1.4
GD79	2.6	548,374	6,826	74 (±21)	17.6	0
IM60	7.4	786,730	1,111	52 (±19)	7.3	2.9
IM350	4.8	305,864	1,785	64 (±23)	8.6	4.3
Total/Mean	62.0	7,043,167	42,945	63	8.7	3.0

Note. Listed are sample names, total number of sequences acquired before and after filtering, as well as *sed*aDNA fragment size (base pairs; bp), "% eukaryotic *sed*aDNA damage" and "% fungi *sed*aDNA damage". Totals are provided for the number of sequences prior to fand after filtering, and averages for *sed*aDNA fragment sizes (with standard deviation; SD) and percentage of eukaryotic or fungi damaged sequences per sample.

HOPS determines DNA damage patterns per taxon (by comparing *sed*aDNA sequences to modern references), that is, a list of input taxa to search for is required. We ran the program using two different species lists: (a) a list that contained only the term "Eukaryota" (providing results for all eukaryotic taxa detected), and (b) "Fungi" (as we detected high abundance of taxa belonging to this group in some of our samples, requiring closer investigation as to whether these were ancient or possibly modern contaminant sequences; see Results). Processing in "def_anc" mode categorized our reads into reads that passed our stringent filtering criteria ("default"; D) and reads that had at least one damage lesion in their first 5 bases from either the 5' or 3' end ("ancient"; A). Using the read counts from our "Eukaryota" run (a), we calculated the sum of ancient and default reads per sample, and calculated the "% eukaryotic *sed*aDNA damage", that is, proportion of ancient Eukaryota reads per sample (Armbrecht et al., 2021). Applying the same procedure to our output from the Fungi run (b), we also determined the "% fungi *sed*aDNA damage". We compared this to the "% eukaryotic *sed*aDNA damage", where less damage of the Fungi reads may signal modern contamination. A correlation analysis (Pearson) was performed to test the relationship between storage time and percentage of Ascomyota (identified as the most prominent group of Fungi, see Results) sequences. Finally, we used the MaltExtract Interactive Plotting Application (MEx-IPA) (Fellows Yates et al., 2021) to visualize *sed*aDNA damage profiles for those taxa that had >50 sequences assigned (Hübler et al., 2019).

3. Results

3.1. Sequence Counts and *sed*aDNA Authenticity

We recovered a total of 62 million filtered sequences from 10 marine sediment samples across the North Atlantic (Table 2; Figure S1 in Supporting Information S1). Post-filtering, a total of 42,945 sequences (0.61%) were assigned to Eukaryota (Table 2; Figure S1 in Supporting Information S1). In most cases, the younger samples retained more sequences than the older samples, with Gardar Drift (GD) retaining the most, and Emerald Basin (EB) retaining the least number of sequences (18,999 and 1,684, respectively). On average, sequences assigned to Eukaryota were 63 bp long (range: 47–81 bp) (Table 2; Figure S1 in Supporting Information S1). Surprisingly, older samples contained longer *sed*aDNA fragments than the younger samples at each site. Additionally, a lower number of smaller fragments were observed in the older samples (Figure 2a).

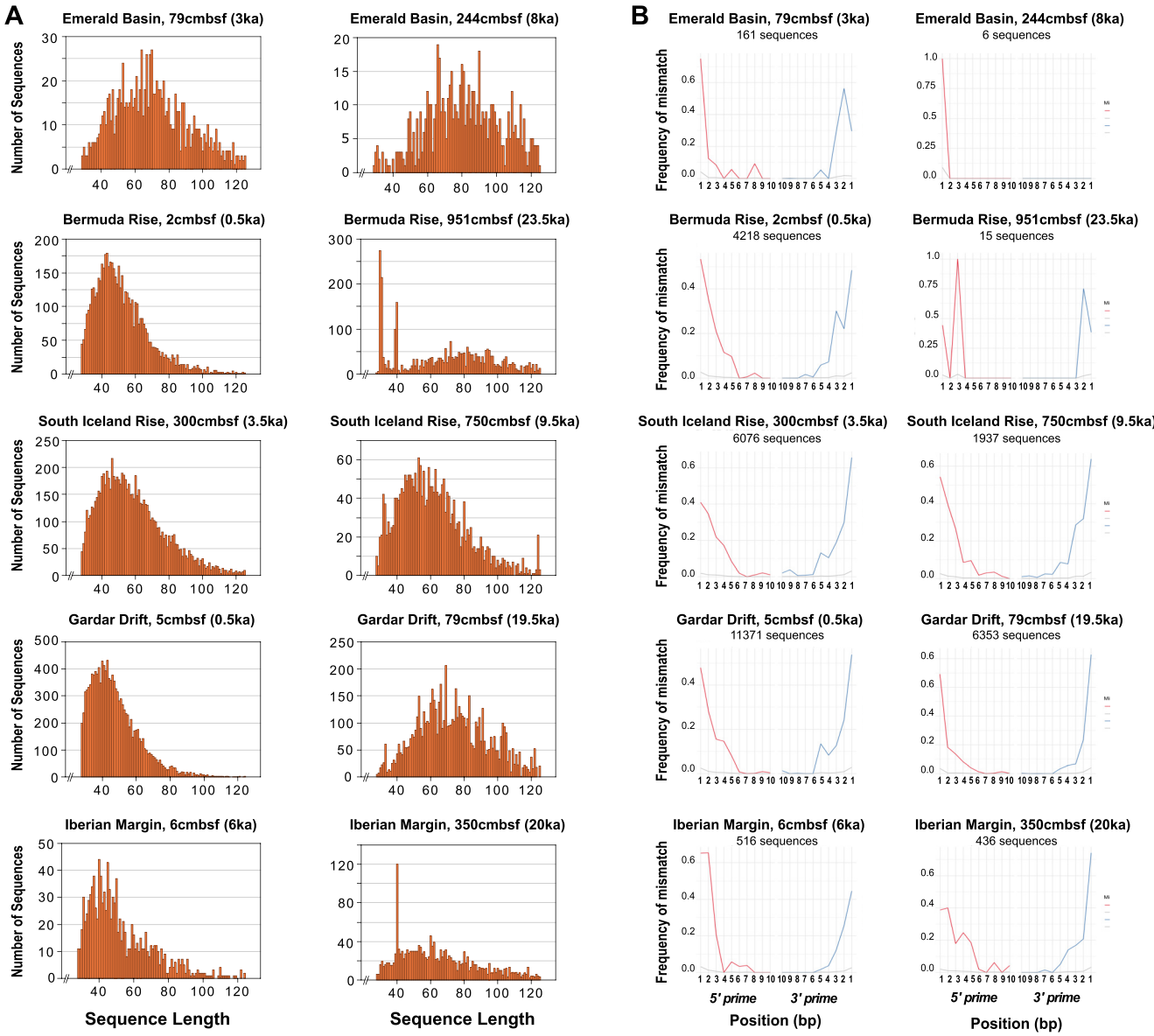

Figure 2. DNA fragments characteristic of sedimentary ancient DNA (*sed*aDNA). (a) DNA fragment distribution of filtered eukaryotic *sed*aDNA sequences. The DNA fragment distribution histograms were created in Geneious (v2020.2) by extracting eukaryotic sequences from MEGAN6CE (v6.19.8) and importing into Geneious (v2020.2). DNA fragment length and the number of sequences for each fragment length was recorded. Note the difference in y-axis scales across samples. (b) Deamination profiles of *Emiliania huxleyi*. Deamination of fragment ends were captured for 10 bp on both the 5' end (cytosine to thymine substitution; red) and 3' end (guanidine to adenine substitution; blue). Typically, a higher rate of deamination occurs on the outmost base and this decreases with base position. The number of *Emiliania huxleyi* sequences used to generate each damage plot is specified under each site header. Damage profiles were visualized in the MaltExtract Interactive Plotting Application (MEx-IPA, by J. Fellows Yates; https://github.com/jfy133/MEx-IPA).

The "% eukaryotic *sed*aDNA damage" averaged 8.7% across all samples (Table 2; Figure S1 in Supporting Information S1). The "% fungi *sed*aDNA damage' was lower than the overall "% eukaryotic *sed*aDNA damage" for all samples except for EB244, which maintained the same percentage of damaged sequences in both tests. A weak negative correlation between storage time and percentage of Ascomyota sequences (Pearson correlation coefficient = −0.58, p-value = 0.08) was observed. The highest rate of *sed*aDNA damage is likely to occur on the outmost bases of a DNA fragment, decreasing to minimal damage by approximately the 5th to 10th base within a DNA sequence (Figure 2b). Due to the relatively low number of reads recovered for most eukaryotes, *sed*aDNA damage could only be tracked for one haptophyte species for which we received a sufficient number of reads, namely *Emiliania huxleyi* (Figure 2b). Apart from two samples, EB244 and BR951, which contained <50

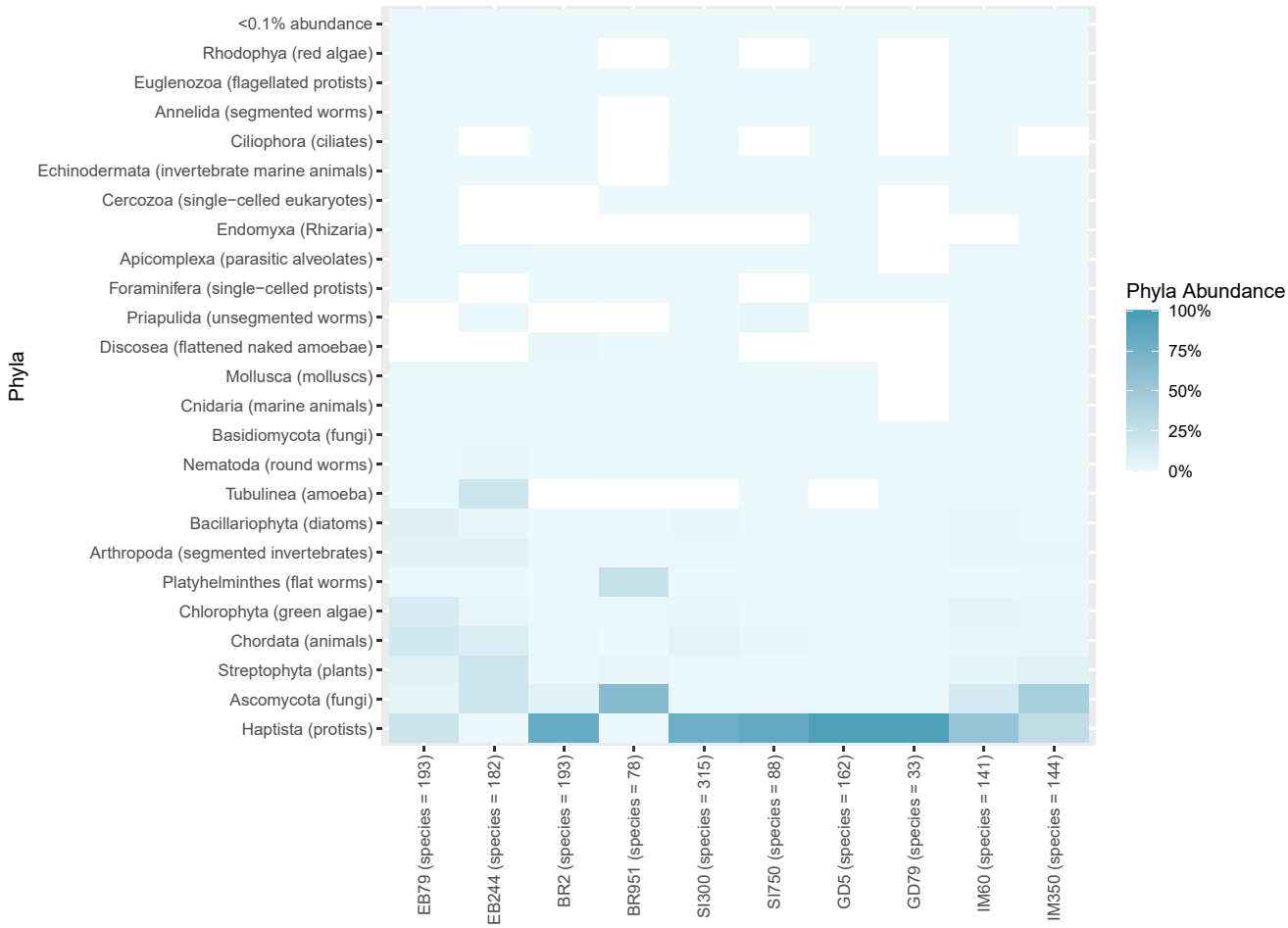

Figure 3. Composition of eukaryotic sedimentary ancient DNA at the phylum level for core samples collected in the North Atlantic Ocean. Filtered Eukaryota sequences were collapsed at the phylum taxonomic level and are displayed by relative abundance per sample using a heatmap. All zero values, that is, no detected phyla signal for a sample, are displayed in white. For each sample, the species richness (i.e., number of species in a sample) was recorded and is shown next to the sample name.

E. huxleyi sequences (Table S2), all remaining samples contained "damaged" sequences, which indicated their *sed*aDNA authenticity (Figure 2b).

3.2. Eukaryota Detected Through *sed*aDNA

At all sites, we determined a higher species richness for all younger samples (>141 species), except for IM60 (Figure 3). Two locations, Emerald Basin (EB) and Iberian Margin (IM), contained a similar species richness for both younger and older samples (EB:193 and 182 species; IM: 141 and 144 species, respectively) (Figure 3).

At each site, the composition of eukaryotes (phylum level) was proportional between younger and older samples, except for BR (Figure 3; see Table S3 for raw phyla sequence counts). EB samples contained a large number of highly abundant taxa (>0.5% relative abundance), including Haptista (21% relative abundance in EB79 and 1% in EB244), Ascomycota (5% and 20%), Streptophyta (8% and 21%), Chordata (18% and 12%), Chlorophyta (14% and 4%), Platyhelminthes (1% and 2%), Arthropoda (6% and 7%), Bacillariophyta (9% and 3%), Basidiomycota (2% each), Nematoda (2% each), Cnidaria (2% and 1%), and Mollusca (1% each), as well as Tubulinea contributing 20% to Eukaryota in EB244 (Figures 3a and 3b). At BR, the younger sample, BR2, was characterized by a large proportion of Haptista (82%) and Ascomycota (7%), whereas the older, glacial age sample, BR951, was composed of Ascomycota (64%), Platyhelminthes (25%), Streptophyta (4%), and Chlorophyta (2%) (Figures 3c and 3d), with only few sequences assigned to Haptista (Table S3). SI samples were mainly composed of Haptista (79% in SI300 and 86% in SI750), with Chordata, Chlorophyta, Arthropoda, Bacillariophyta, and Streptophyta

also represented (Figures 3e and 3f). Like SI samples, Haptista dominated samples from GD, contributing 96% to Eukaryota in both the young and old samples. Iberian Margin (IM) samples were characterized by similar proportions of Streptophyta (4% in IM60 and 8% in IM300), Chordata (3% in each), Chlorophyta (5% and 3%), Arthropoda (4% and 3%), Bacillariophyta (2% in each), Tubulinea (1% in each), and Nematoda (1% in each) in both younger and older samples (Figures 3g and 3h); however, Haptista (56%) was the most dominant phylum in the younger sample that also contained 17% of Ascomycota, whereas Ascomycota (43%) was most dominant in the older sample (glacial age), which contained 29% of Haptista (Figures 3i and 3j).

Analyzing the dominant phyla at the species level showed that Haptista was primarily composed of sequences assigned to the coccolithophore *Emiliania huxleyi* (Table S2). Within Ascomycota, different species dominated different samples; for example, *Exophiala xenobiotica* (black yeast) dominated BR951 (88% relative abundance), while *Pseudogymnoascus* spp. (cold-adapted fungi) were determined in EB244 (19% relative abundance), BR2 (3%) and both Iberian Margin samples (12% in IM60 and 38% in IM350) at high relative abundance (Table S2). The Atlantic cod, *Gadus morhua*, was present in all samples, except BR951 and IM60. *Bathycoccus prasinos*, a picoplankton species, was consistently observed across all samples. Lastly, we also detected both fossilizing and non-fossilizing phyto- and zooplankton species at low abundance, including the foraminifer *Globorotalia inflata*; the diatoms *Pseudo-nitzschia multiseries*, *Phaeodactylum tricornutum*, *Chaetoceros simplex* and *Thalassiosira pseudonana*; and the copepod *Calanus finmarchicus* (Table S2).

4. Discussion

In this study, we obtained short DNA fragments using shotgun sequencing, which allowed us to provide a first assessment of the feasibility for *sed*aDNA analysis applied to archive sediment core samples. Using archive sediment material from previous coring sites across the North Atlantic, we identified authentic *sed*aDNA via fragment length, deamination of fragment ends, and *sed*aDNA damage analyses. Our analyses also revealed new insights into issues around contamination, confirming that adequate sample storage and handling conditions are of utmost importance to achieve a high quality *sed*aDNA signal. Below, we discuss the challenges of working with archive sediment material that was not initially collected for *sed*aDNA analyses, outline possible future improvements for this area of research, and, in the context of the identified technical limitations, provide insights into the compositional data acquired from *sed*aDNA at different geographical locations within the North Atlantic.

4.1. Challenges of Using Archive Material

Considering the vast number of sediment samples that are stored at globally distributed core repositories, there is strong motivation for testing the feasibility of *sed*aDNA analyses using such archive material. While contamination introduced during sampling on board ships and research vessels is now being paid high attention to, contamination is normally explored and discussed in relation to laboratory contamination and is commonly detected through extraction blank controls (Warinner et al., 2017; Weyrich et al., 2019). However, contamination that is introduced and/or enhanced by keeping sediment cores at sub-optimal conditions over long periods of time can easily be overlooked and more difficult to detect. The samples analyzed within this study were collected between the years 1994 and 2013 and were stored at 4°C for a minimum of 5 years, rather than the recommended freezer storage conditions for individual ancient samples, especially those that are wet (Llamas et al., 2017). As these sediment samples were not purpose-collected for *sed*aDNA analyses, and hence, to a large degree lacking the required precautions to avoid contamination by modern DNA, extensive and stringent bioinformatic filtering approaches were required to remove mammalian (e.g., human, primate, bovid, deer, carnivore, and rodent) sequences, in addition to routine filtering of species detected from the laboratory environment.

We observed a lower percentage of damaged fungi sequences compared to the percentage of damaged eukaryote sequences, suggesting that the fungi sequences are most likely (modern) contaminants (rather than growth of preserved fungal spores). The lowest damage percentages of fungi sequences were detected in samples taken from material bagged straight after core cutting without further decontamination control (i.e., BR and GD samples). Most notably, sample BR951 had both a low damage percentage of fungi sequences and a large proportion of *Exophiala xenobiotica*, an opportunistic black yeast. Indeed, *E. xenobiotica* has been previously reported to grow in wet indoor environments (Wang et al., 2018); therefore, it is possible that *E. xenobiotica* was introduced during sampling or during storage. With continued growth of this species over time, the genetic signals of the original

communities may have become overridden. Despite the evidence suggesting contamination being the source of the fungal sequences, we cannot exclude that some fungal spores may have been preserved and grown while the cores were in refrigerated storage. Therefore, it is crucial to follow rigorous contamination mitigation standards throughout sampling and appropriate sample storage conditions, including at low humidity, to recover and detect the minuscule amounts of eukaryote *sed*aDNA preserved in marine sediments.

4.2. Authenticity Assessments of Eukaryote *sed*aDNA in North Atlantic Archive Samples

An assessment of DNA fragment size can be used as a first proxy of *sed*aDNA authenticity. In this study, the average fragment size of *sed*aDNA was between 47 and 81 bp, which is in agreement with typical ancient DNA fragment size (i.e., <100 bp; Warinner et al., 2017), and is comparable to marine eukaryotic *sed*aDNA fragment sizes from Tasmania, Australia (~69 bp; Armbrecht et al., 2020) and the Southern Ocean (~40–50 bp; Armbrecht, 2020). In this study, however, we observe longer fragment size for older samples, which suggests that using fragment size as a proxy for *sed*aDNA authenticity is more complicated. Possibly, the smaller DNA fragments in our samples were more susceptible to degradation than longer ones or were too short to pass our bioinformatics filtering criteria (all fragments <25 bp were removed during data processing); however, this is speculative and only considers the eukaryotic sequences rather than the whole sample. Fragment size is a relatively simple measure for the authenticity of *sed*aDNA as many factors can influence fragmentation, including chemical and physical properties, taphonomy, and diagenesis (Ellegaard et al., 2020; Giguet-Covex et al., 2019). In ancient bone samples, sample age does not necessarily correlate with increased fragmentation; however, sample age does correlate with increased DNA deamination frequency (Kistler et al., 2017), thus the latter is a more robust approach to assess DNA authenticity.

In this study, approximately 8.7% of eukaryote sequences in a sample showed damaged fragments. This proportion of damage is lower compared to eukaryote *sed*aDNA damage determined in sediment cores collected offshore Tasmania, Australia (~25% in an ~9,000-year-old core; Armbrecht et al., 2021), noting that the latter study is the only published record of marine eukaryote *sed*aDNA damage to date and thus offers only limited grounds for comparisons. However, the authors extracted and analyzed *sed*aDNA 1 year after sampling, whereas in this study, up to 20 years had passed between these steps. Unfortunately, we are unable to provide an estimate of the fraction of DNA damage that might be attributable to 20 years of storage, but a future study could explore this question by comparing *sed*aDNA damage patterns of duplicate samples, where one replicate is extracted immediately after coring and the other multiple years after being in storage. Such a study could be combined with experimental investigations into how chemical, physical, and/or biological parameters might influence *sed*aDNA preservation, relationships about which little is known but which are of high importance to improve the accuracy of paleo-community reconstructions.

4.3. Compositional Changes in *sed*aDNA Between Sites

Given the relatively small number of samples analyzed across five sites, we focus our species composition discussion on a few general observations. First, we determined lower species richness in the older samples at three of sites (BR, SI, GD), which may be due to poorer preservation of older *sed*aDNA. However, two sites (EB and IM) maintained a high similarity in species richness in both old and young samples. This could reflect similar actual in situ species richness in marine eukaryote community at both timepoints. Alternatively, the expected (but not observed) decline in *sed*aDNA species richness at greater depths, as seen at our other three sites, could have been offset at EB and IM by counteracting taphonomic and diagenetic controls; for example, the input of distil, allochthonous material because of ice-rafting occurring during the deposition of the older (glacial) IM sample could have contributed additional, diverse, *sed*aDNA.

Second, samples from the open ocean sites of BR, SI, and GD were dominated with *E. huxleyi*—a ubiquitous coccolithophore. Seasonal blooms of *E. huxleyi* are frequently observed within the surface waters of the northeast Atlantic Ocean (Tyrrell & Taylor, 1996) and near Bermuda (Haidar & Thierstein, 2001), which supports the detection of *E. huxleyi* in these central sites. Lower relative abundances of Haptista in the older, glacial age samples from BR and IM may be due to changes in the sources of sedimentary material deposited at these sites during the glacial period. An alternative cause might be enhanced dissolution of carbonate (including coccoliths) that occurred in the deep glacial North Atlantic—with the greatest influence of corrosive Antarctic Bottom

Water likely occurring at the abyssal BR site and the deep eastern margin site of the IM (Sarnthein et al., 1994; Thornalley et al., 2013). Future work analyzing transects of cores in close regional proximity that span a range of water depths could test the role of carbonate dissolution in *sed*aDNA preservation. Lower abundances of Haptista are also recorded in the older EB sample, which may be related to altered sedimentary sources and routing of material in the early-mid Holocene, or the altered oceanographic conditions at this site during the early-mid Holocene (Yang & Piper, 2021).

Many unknowns remain surrounding how possible taphonomic or diagenetic biases—including sediment composition, sedimentation rate, and advection and redistribution of sediment by ocean currents (e.g., McCave, 2002)—may alter the delivery and preservation of *sed*aDNA in the seafloor. To resolve this important issue, future research could focus on comprehensive seafloor surface sediment analyses of *sed*aDNA across the North Atlantic basin, comparing results obtained from different sediment types, as well as regional paleo- and modern ocean communities (e.g., by making use of modern ocean datasets such available through Tara Oceans (Sunagawa et al., 2020) and bioGEOTRACES (Biller et al., 2018)). A similarly comprehensive study of modern environmental DNA has recently been undertaken by Cordier et al. (2022), analyzing 1,685 surficial seafloor samples from around the globe to disentangle planktonic versus benthic inputs to the seafloor DNA pool.

4.4. Optimisations for the Improved Recovery of Marine Eukaryotic *sed*aDNA

While we were able to retain a sufficient number of eukaryotic *sed*aDNA sequences for our analyses, we suggest the following optimisations that may be useful to the analysis of North Atlantic samples in the future. First, this could include the application of *sed*aDNA extraction methods specifically developed to achieve a high yield of *sed*aDNA from fragile soft-bodied, as well as more robust marine eukaryotes (Armbrecht et al., 2020; Shapiro & Hofreiter, 2012). Although costly, deeper shotgun sequencing (whereby more sequencing resources are provided to each sample) could also be used to obtain even more eukaryotic *sed*aDNA sequences. Third, to better focus the sequencing effort, the application of RNA baits designed to target and enrich certain taxonomic groups (e.g., phytoplankton and other microalgae (Armbrecht et al., 2021; or plants and animals; Murchie et al., 2020), using a multi-loci approach (Foster et al., 2020; Horn, 2012) could be applied, acknowledging that this method usually requires ∼100 ng of input DNA, which was not available for this data set. Finally, through de novo assembly of short DNA fragments that are compiled into metagenomic assembled genomes, it is possible that more unassigned sequences can be identified. While this method is relatively new, it has been utilized for describing unassigned modern eukaryotic species (Olm et al., 2019, p. 20; Saary et al., 2020) and bacterial species from historical samples (Brealey et al., 2020).

5. Conclusions and an Outlook for *sed*aDNA Research in Deep Marine Settings

This study is the first application of a metagenomic shotgun sequencing approach to acquire *sed*aDNA from archive marine sediment samples from the North Atlantic deep seafloor. We were able to extract *sed*aDNA from all samples and observed characteristic *sed*aDNA damage patterns and DNA fragment lengths in the eukaryotic fraction of sequenced reads, suggesting that these DNA fragments are ancient. Overall, we determined that it is possible to detect *sed*aDNA in archived material; however, we also detected strong evidence for contamination. Therefore, this study provides strong support for the need of best-practice techniques and protocols throughout sampling and sample storage to minimize contamination and acquire high-quality *sed*aDNA for the study of palaeoceanographic conditions. Furthermore, future studies should be carried out to gain a more complete understanding of this ecological proxy. Although this study has focused on sediments taken from North Atlantic cores, our results are likely relevant for the potential use of *sed*aDNA in the other major ocean basins. We outline a number of suggestions for methodological improvements, as well as highlighting the need for investigation of taphonomic and diagenetic biases that may affect *sed*aDNA preservation in seafloor sediments, which might help to further refine marine *sed*aDNA analyses in the future and enable a more accurate reconstruction of marine ecosystems through time and with changing climate conditions.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

Complete code for data processing can be found preserved at Armbrecht et al., 2020, https://doi.org/10.1111/1755-0998.13162 and Armbrecht et al., 2021, https://doi.org/10.1038/s41598-021-82578-6. Eukaryotic data and *R* scripts used for the correlation analysis and heatmap figure can be found at https://doi.org/10.5281/zenodo.6342810 on the Zenodo database. Sequences were assigned using the NCBI nucleotide database (ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nt.gz), downloaded from https://www.ncbi.nlm.nih.gov/home/download/ in November 2019, as a reference. Data filtering was performed using: Adapter Removal (v2) available via https://adapterremoval.readthedocs.io/en/stable/index.html and preserved at https://doi.org/10.1186/s13104-016-1900-2; Komplexity available via https://github.com/eclarke/komplexity and preserved at https://doi.org/10.1186/s40168-019-0658-x; and, he duplicate read removal function (Dedupe) as part of BBMap (v37.36) available via https://sourceforge.net/projects/bbmap/ and preserved at https://jgi.doe.gov/data-and-tools/software-tools/bbtools/. Quality control of the data was checked using both FastQC (v0.11.8), available via https://www.bioinformatics.babraham.ac.uk/projects/fastqc/, and MultiQC (v1.0.dev0), available at https://github.com/ewels/MultiQC and preserved at https://doi.org/10.1093/bioinformatics/btw354. MALT (v0.4.0) was used to index and align reads, which is available via https://github.com/husonlab/malt and preserved at https://doi.org/10.1101/050559. The blast2rma tool in MEGAN6 was used to convert.blastn to rma6 files for import into MEGAN6CE (v6.19.8), which was used to filtered contaminant sequences, which is available via https://doi.org/10.1371/journal.pcbi.1004957 and is preserved at https://software-ab.informatik.uni-tuebingen.de/download/megan6/welcome.html. HOPS (v0.33.2) was used to determine DNA damage patterns, which is available via https://github.com/rhuebler/HOPS and preserved at https://doi.org/10.5281/zenodo.3362248 Figures were generated using: Ocean Data View available at https://odv.awi.de/; Geneious Prime (v2020.2) available with a license and can be found https://www.geneious.com; *R* (v3.6.1) with the ggplot2 package, available via https://ggplot2.tidyverse.org/ and preserved at ISBN 978-3-319-24277-4; and, the MaltExtract Interactive Plotting Application (MEx-IPA), available via https://github.com/jfy133/MEx-IPA and preserved at https://doi.org/10.5281/zenodo.4771942.

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