

## Manuscript Details

<b>Manuscript number</b>	EARTH_2018_550_R1
<b>Title</b>	Ancient DNA from marine sediments: precautions and considerations for seafloor coring, sample handling and data generation
<b>Article type</b>	Invited review article

### Abstract

The study of ancient DNA (aDNA) from sediments (sedaDNA) offers great potential for paleoclimate interpretation, and has recently been applied as a tool to characterise past marine life and environments from deep ocean sediments over geological timescales. Using sedaDNA, palaeo-communities have been detected, including prokaryotes and eukaryotes that do not fossilise, thereby revolutionising the scope of marine micropalaeontological research. However, many studies to date have not reported on the measures taken to prove the authenticity of sedaDNA-derived data from which conclusions are drawn. aDNA is highly fragmented and degraded and extremely sensitive to contamination by non-target environmental DNA. Contamination risks are particularly high on research vessels, drilling ships and platforms, where logistics and facilities do not yet allow for sterile sediment coring, and due consideration needs to be given to sample processing and analysis following aDNA guidelines. This review clarifies the use of aDNA terminology, discusses common pitfalls and highlights the urgency behind adopting new standards for marine sedaDNA research, with a focus on sampling optimisation to facilitate the incorporation of routine sedaDNA research into International Ocean Discovery Program (IODP) operations. Currently available installations aboard drilling ships and platforms are reviewed, improvements suggested, analytical approaches detailed, and the controls and documentation necessary to support the authenticity of aDNA retrieved from deep-sea sediment cores is outlined. Beyond practical considerations, concepts relevant to the study of past marine biodiversity based on aDNA, and the applicability of the new guidelines to the study of other contamination-susceptible environments (permafrost and outer space) are discussed.

**Keywords** ancient DNA; marine sediments; deep biosphere; phytoplankton; contamination; seafloor; IODP; biomarkers; Mars

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**Suggested reviewers** Fumio Inagaki, Mikkel Winther Pedersen, Chris McKay, Jill Banfield

## Submission Files Included in this PDF

### File Name [File Type]

Coverletter\_LA\_et\_al\_Marine\_aDNA\_review\_R1.pdf [Cover Letter]

Response\_to\_reviewers\_R1.docx [Response to Reviewers]

Armbrecht-et-al\_aDNA\_Review\_R1\_highlights.docx [Revised Manuscript with Changes Marked]

Abstract\_R1.docx [Abstract]

Armbrecht-et-al\_aDNA\_Review\_R1.docx [Manuscript File]

Fig.1.pdf [Figure]

Fig.2\_IODP\_CoringSystems.pdf [Figure]

## Submission Files Not Included in this PDF

### File Name [File Type]

Table1.xlsx [Table]

Table2.xlsx [Table]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

## **Research Data Related to this Submission**

There are no linked research data sets for this submission. The following reason is given:  
No data was used for the research described in the article



THE UNIVERSITY  
of ADELAIDE

Prof. Ian Candy  
Mr Timothy J. Horscroft  
Editorial Office  
Earth-Science Reviews

30.05.2019

Dear Prof. Candy and Mr Horscroft,

We herewith re-submit our invited review manuscript entitled "*Ancient DNA from marine sediments: precautions and considerations for seafloor coring, sample handling and data generation*" to Earth-Science Reviews (EARTH\_2018\_550).

The manuscript has been revised according to the reviewer's suggestions, and all changes are detailed in the "Response to Reviewers" document.

Our review now includes a 250-word abstract, 9,146 words of main text (including Acknowledgements and Funding Sources), two figures, two tables and 142 references. I declare that all co-authors have agreed to the submission.

Yours sincerely,

Dr Linda Armbrecht

Reviewer 1 - Comments	Author's response
<p>The paper by Ambrecht et al. "Ancient DNA from marine sediments: precautions and considerations for seafloor coring, sample handling and data generation" aims at presenting the review of state-of-the-art practices in ancient DNA studies of marine sediments, as well as providing general guidelines for sampling and lab protocols, which should be applied in future, in particular in IODP missions.</p>	
<p>In general, the topic of ancient DNA is of the highest interest. However, in the present form, it may be more suitable for a journal of narrower scope. The manuscript would benefit a lot if it would be focused not only on technical aspects of coring, sampling etc. but also if it would provide a review of aDNA applications in various marine environments, time ranges, taxon groups etc. There are many recent studies (many of them included in the reference list) showing the potential of ancient DNA in the progress of science. Although several valuable reviews have been published during the last several years (e.g. Torti et al. 2015, Rawlence et al. 2014, Pedersen et al. 2015), there are still many issues, which could be covered – including the specific character of the marine environment. DNA-related problems revealed by the recent studies incorporate the limits of applications, the challenges, not only related to sampling and contamination, but also to bioinformatics, identification of taxonomic units, qualitative vs quantitative approaches etc.</p>	<p>We disagree with the reviewer's comment. This review forms the base of any future study in the emerging discipline of marine sedimentary ancient DNA, which is interdisciplinary in its core, and thus highly applicable to earth, marine, geo- and climate scientists. As Reviewer 2 also acknowledges, commonly used modern marine genomics techniques have been mis-applied to this new field, demonstrating the urgency to raise awareness amongst earth and marine scientists that appropriate ancient DNA techniques must be used if the aim is to acquire authentic ancient DNA; therefore, this manuscript is ideally suited to ESR and its readership.</p> <p>Our focus is on contamination and best-practise techniques, and we specifically point out in this review that many studies to date fail to provide adequate records of negative controls. Therefore, a review of currently reported taxonomic groups and age estimates is impossible and we do not provide further details than already given.</p> <p>However, we agree with the reviewer that more information can be provided on bioinformatics, identification of taxonomic units and approaches, and have expanded our data-analysis section accordingly.</p>
<p>Moreover, some of the chapters related to planetary exploration, although intriguing, are so far away from the main topic of the paper that they should be removed or shortened.</p>	<p>We have shortened this section in the revised version. However, we would prefer to retain this section and not entirely remove it, as in our opinion the marine aDNA research guidelines are relevant to other low biomass environments such as permafrost and other planets.</p>
<p>I. 38 – 39 "The study of ancient DNA ... has recently been applied as a tool to characterize past and modern life in deep ocean sediments". First of all, the study of ancient DNA does not help in the characterization of modern life (we use modern DNA for that). Second, ancient DNA is studied and used for palaeoclimate etc. not only in oceanic sediments (see for instance numerous studies on ancient DNA in lake sediments).</p>	<p>We agree with the reviewer and have removed "modern life" from this sentence.</p> <p>We agree with the reviewer, however, this review focuses on the marine environment, which is introduced in this first sentence.</p>
<p>I. 89, I. 92-93 and elsewhere in the manuscript - "plankton" – here, and also later on in the manuscript, the authors focus only on plankton or deep biosphere. How about benthic organisms, which are also of importance and are used as indicatory species (e.g. foraminifera)? The aDNA of active swimmers (fish) may</p>	<p>We removed plankton in this context, and only refer to planktonic organisms where previous studies have focused on those in particular.</p>

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<p>also be preserved and should not be neglected completely.</p>	
<p>I.128-132 – the authors refer to a paper by Kirkpatrick et al 2016, reporting retrieval of 1.4 million years old DNA and underlying that the “origin of DNA must be carefully considered”. Later on in the text (I. 139-141), the authors list “to date, the oldest authenticated aDNA records” and do not take into account the one by Kirkpatrick et al (2016). It seems that the authors do not find the finding by Kirkpatrick et al. (2016) to be confirmed. I would expect much more detail information about this case – what was found in original paper and what was wrong in the opinion of the authors and why. Such a case is very interesting and important in terms of making progress in science, as the scientific community may learn from potential failures. Giving reasons for not recognizing the results obtained by Kirkpatrick et al. may give also a chance for them to address the constructive critics in the future correspondence or papers.</p>	<p>We acknowledge that Kirkpatrick et al. have taken utmost care that samples are not contaminated, certain precautions were not taken. For example, while sampling was undertaken immediately following core retrieval on the catwalk of the IODP research ship <i>RV Joides Resolution</i>, there is no mention of core liner decontamination before cutting, thus potential contamination of the inner core during cutting cannot be excluded. The authors also describe that PFTs (a chemical tracer) were run and below detection limit, however, it is unclear whether these low PFT concentrations were only measured at the centre of the core or also on the periphery (the latter would be a sign of unsuccessful tracer delivery to the core). All laboratory work was conducted in laminar flow hoods, these create air movement and are not as suitable as special ultra-low background DNA (ancient DNA) facilities; it is also not mentioned whether previous work on marine organisms has been performed in this hood. PCR was used to amplify the 16S V4 and V5 gene regions (each &gt;100bp, thus surprising as aDNA is typically &lt;100bp), then subtracting all but chloroplast derived sequences. We provide detailed information in this review on the biases of PCR and its unsuitability to study aDNA. We acknowledge that the decrease in cpDNA with depth measured by Kirkpatrick et al. is a good indicator for a realistic result, however, the possibility remains that the cpDNA signal might be derived from contaminating seawater DNA. The major diatom taxa detected, <i>Thalassiosira</i> and <i>Chaetoceros</i>, are indeed important contributors to the fossil record, but also highly represented in the water column. Better indicators for ancient DNA authenticity are, for example, DNA fragment size and degradation.</p>
<p>I.157 “2.1” is missing</p>	<p>Corrected.</p>
<p>I. 160 “where the DNA was not initially preserved for later analysis” – I am not sure what the authors mean. The same expression is used also in table 1. How could be DNA initially preserved for being analysed as ancient DNA?</p>	<p>We changed this to: ‘aDNA research involves the biomolecular study of non-modern genetic material preserved in a broad range of biological samples’.</p>
<p>I. 170 – 182 definitions. The authors try to make an order in a number of similar terms used in literature. I find it very useful. However, after reading this paragraph, I find some confusing statements. First of all, the authors contrast aDNA and PalEnDNA (I. 171-172), while they seem to overlap, as also stated by the authors later on (I. 179-180). I suggest presenting the ranges of application of particular terms in form of figure (see for instance somehow similar figure in Torti et al. 2015). I also think that it is not necessary to add a new term ‘marine aDNA’. This term is well covered by the existing term ‘sedimentary aDNA’. If the authors find it necessary to define a new term then they should provide a precise definition. Does this term refer only to DNA of marine organisms? The DNA pool may</p>	<p>Both Reviewer 1 and 2 commented on the terminology and definitions of PalEnDNA, aDNA, sedaDNA and marine aDNA. Reviewer 2 suggests PalEnDNA to be superfluous, while Reviewer 1 criticises the use of ‘marine aDNA’ as both marine and freshwater environments can be influenced by freshwater and marine DNA sources, respectively. We agree that in regions characterised by brackish waters our term marine aDNA might indeed be too narrow, as such, we have adjusted our terminology and use an extension of the existing term sedaDNA (‘marine sedaDNA’) for ancient DNA from marine environments, on which this review focuses. We consider our table of definitions appropriate and do not see the need to display the definitions in a figure, neither did Reviewer 2.</p>

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<p>contain also terrigenous DNA delivered with rivers etc (see for instance Torti et al. 2015 and references therein). On the other hand, some processes, e.g. tsunami, may deliver and deposit marine sediments containing DNA of marine organisms on land (e.g. Szczucinski et al 2016). Is the analysis of marine sediments so different from lake sediments to create a specific term? Please note that for instance in case of well-studied Black Sea, some of its older sediments were formed in lake conditions, not in marine. So, shall we use two separate terms in that case?</p>	
<p>I. 186 – chapter 2.21 – this chapter should be in my opinion much better illustrated (table/figure). It is one of the chapters, which potentially may attract attention also of non-aDNA specialists. Particularly interesting may be to show the limits. The authors have mentioned (I. 201-202), that in well-oxygenated deep-sea sediments aDNA was also preserved. However, it was also preserved in much less suitable settings as for instance coastal marshes (tsunami deposits mentioned above).</p>	<p>We have rewritten this paragraph to integrate the reviewers' comment on the retrieval of aDNA from oxygenated sediments, and outlining the limits of marine sedaDNA research with regard to environmental characteristics and age retrieval. However, we have not added a table or figure as neither would not add any information, and solely be a repetition of the text in the manuscript.</p>
<p>I. 195 – 'extremely small grain size ... offer a high adsorption surface' – I do not think that it is extremely small grain size that matters, it is the high surface area (ratio of surface to volume).</p>	<p>We agree and modified this sentence.</p>
<p>I. 259 – 'geological' – actually it is a biomechanical process</p>	<p>We replaced 'geological' with 'biomechanical'.</p>
<p>I.280 – chapter 2.26 – it is the next chapter worth to be extended. For instance issue of comparison of various records (micropaleontological and DNA). The problem of quantification of aDNA record. The mentioned results from the Black Sea could be represented by a combined figure showing an example of the application of various proxies.</p>	<p>We expanded this section according to the reviewer's suggestion. However, we are unsure what type of figure the reviewer is requesting here - a timeline of events in the Black Sea, or a hybrid of figure of already published Black Sea results? In either case, we believe that re-illustrating Black Sea results/data exceeds the scope of this review, and have therefore decided against adding another figure in this context. (Instead we focused on refining Figure 1 (sedaDNA workflow) and adding Figure 2 (coring systems)).</p>
<p>I. 314 – chapter 3.1 - I wonder if the specific chapter only about IODP is really necessary.</p>	<p>We shortened this chapter considerably, keeping only information on available coring platforms, which provides important context for the following descriptions of drilling strategies suitable for deep seafloor aDNA recovery.</p>
<p>I. 324 – table 2 is not necessary. It is much easier to include these three points in the text.</p>	<p>We removed this table.</p>
<p>I. 334- 348 – provide at least the project title.</p>	<p>We shortened this section and project titles are no longer applicable.</p>
<p>I. 350 – chapter 3.2 on drilling strategies. Various details of coring systems are discussed. However, the chapter may be difficult to follow for not specialists – consider representing the coring systems and the differences between them on a figure. The authors claim that the paper is to be used by researchers working also in permafrost and other planets – make it accessible for them.</p>	<p>We have included a new figure showing the differences in coring systems.</p>

178 179	I. 444 -447 - It is not clear why the authors expect the freezing to affect DNA leaching.	We added an explanation in the text.
180 181	I. 546 - 'quantitative' - it is not clear what do the authors mean. A number of sequences?	We removed 'quantitative' in this context.
182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198	I. 615 - chapter 5. This chapter is poorly linked with the main topic of the paper and in fact, could be shortened to a single paragraph. This paper introduces marine aDNA guidelines (which are not yet established), and in this chapter an extensive description of its potential applications are discussed for non-marine settings. In particular for planetary exploration - very attractive topic, however in situation, when we are not sure if there is any life on other planets, not to mention if it is DNA-based, I do not find useful to discuss if suggested coring techniques, contaminant treatments for marine settings etc. may be useful in planetary exploration on Mars and other planets and moons. In fact, the chapter 5.1 reveals more on applications of experience in studies of aDNA in permafrost for marine settings (630-635) than vice versa.	We have shortened this section in the revised version. However, we would prefer to retain this section and not entirely remove it, as in our opinion the marine aDNA research guidelines are relevant to other low biomass environments such as permafrost and other planets.
199 200 201	The manuscript could be also enriched in figures (there is only a single figure, so far). A good picture is worth a thousand words.	We added a figure showing coring systems.
202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236	<p>The references need to be rechecked throughout - many cited references are not in the reference list (over 20!) and vice versa. I have listed below some of the references cited in the text and not included in the reference list but it needs to be rechecked once more. Also, the alphabetic order in the references is not followed, in particular in cases of the same first author.</p> <p>The references cited in the text and missing in the references:</p> <p>I. 84. Ambrecht et al. 2018  I.86 Loucaides et al. 2011  I. 89 Castaneda et al. 2011 (do you mean Castaneda and Schouten?)  I. 134 Coolen et al. 2011  I.189 Boere et al. 1008 (should be 2009?)  I. 191 (should be Lyon?)  I.241 Levy-Booth et al. 2007  I. 291 Calvert et al. 1987; Hay 1988  I.292 Major et al. 2006  I.303 Lyra et al 2013  I. 338 Frueh-Green - be consistent in writing the name with the reference list  I. 512 Brotherton et al. 2012 (should be 2013?)  I. 547 Klappenback et al 2001  I. 622 Bossenkol et al 2012  I.625 Gittel et al. 2014  I. 631 Neghandhi et al 2016  I. 640 McConnell et al 2007  I.661 Grotzinger et al 2012  I. 1179 (Table 1) - van Everdingen 1998, Fry et al. 2003</p>	We corrected the reference list.

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I.1186 (Table 3) - Haile et al. 2012, Leite et al. 2014, Bidle et al 2007	
<b>Reviewer 2 - Comments</b>	<b>Author's response</b>
I think this is a nice and timely review in the field of ancient DNA (aDNA) research in marine sediments. Although I am not particularly expert in marine and freshwater sediments I have good knowledge of aDNA studies in terrestrial sediments and the two aDNA research fields suffer of similar contamination problems. As also the authors suggest, many aDNA studies recently have not succeeded in reporting exact measures taken to prove authenticity of results, particularly studies dealing with environmental DNA (eDNA) extracted from sediments and studies to investigate ancient microbiome communities. It is true that modern bacteria and other microorganism are present nearly in every part of our environments, from open research fields to modern clean laboratories. It is also true that in many cases common and standard molecular laboratories have been used in such studies for extracting DNA from ancient sediments and prepare samples for sequencing and that procedures for subsampling from cores have not been documented and reported carefully. This review is therefore very welcome and hopefully will encourage researchers dealing with aDNA data from marine environments to take all necessary precautions during sediment coring, sample handling and data generation.	
I have only some minor comments that hopefully the authors will take into consideration before publication.	
In general I agree with most of the suggestions provided by the authors and with most of their statements. However, I would give more importance to contamination that often occur in the laboratories during DNA extraction and PCR/library preparations rather than in the field during coring when is really hard to avoid it. Contamination is never possible to reduce to zero and will unfortunately always occurs. However it is possible to minimize and to monitor it during all steps. Therefore, rather than insisting on the importance of performing coring in sterilized conditions, which is indeed crucial but very hard to do especially on ships, I would stress much more the importance of avoiding contamination during subsampling and during analyses in the laboratories, as here it is indeed possible to work efficiently to minimize it. No matter how clean we work on the ship and during coring it is very likely that contamination will occur from the modern environment during sampling. What is crucial therefore is to clean samples as much as possible prior to analyses and especially during subsampling to remove the outer part of the samples using sterilized tools, wearing lab mask, lab coats, gloves etc. In order to sample the internal uncontaminated part of the core it is therefore preferable to use larger rather than smaller corers in order to get as much material as possible. A second	We refined section 3.5 "Marine aDNA sample processing and analysis" according to the reviewer's suggestion.



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<p>important step to minimize and monitor contamination is the use of negative controls during DNA extraction and PCR/library preparation. Both types of controls, as also mentioned by the authors, should always be processed in parallel with sediment samples from PCR, to DNA sequence and to bioinformatic analyses. It is not enough to measure the DNA amount and even if this is zero, controls must be analyzed all the way along all steps.</p>	
<p>There is one paper on which I have strong doubts about authenticity (Inagaki et al. 2005). I don't think it is possible that the authors have extracted and analyzed DNA from a continental core 108 million years old. Such results are very likely created by contaminants and therefore not authentic and should therefore not be used to support any statement in this paper. This is especially true since the authors say correctly that at the moment the oldest authenticated DNA sequences comes from remains that are ca 700 kyr (Orlando et al. 2013).</p>	<p>We agree with the reviewer and do not cite this paper.</p>
<p>Please notice that on line 225 the reference is not correct (Inagaki et al. 2015 should be 2005), therefore I would not call this as a 'recent' study.</p>	<p>There are two different publications, Inagaki et al. 2005 and Inagaki et al. 2015. The latter study reports on slow-growing live microbes in 2.5km deep ocean sediments, and is cited in our manuscript. There is no reference to Inagaki et al., 2005 (reporting on 100 Mio. years old ancient microbes).</p>
<p>Line 160. Maybe I miss something here but I don't understand the meaning of the sentence: 'where the DNA was not initially preserved for later analyses'.</p>	<p>We have changed this sentence to: "aDNA research involves the biomolecular study of non-modern genetic material preserved in a broad range of biological samples."</p>
<p>Line 170. The term PalEnDNA is in my opinion superfluous. In literature we have already several established acronyms (aDNA for ancient DNA, eDNA for environmental DNA, sedaDNA or sedDNA for sedimentary ancient DNA, see Pedersen et al. 2015, Ficetola et al. 2015, Parducci et al. 2017). My suggestion is to use only: aDNA, eDNA, sedaDNA and marine aDNA.</p>	<p>We agree with the reviewer that the term PalEnDNA is somewhat superfluous, and have re-written this paragraph to give this term less emphasis. However, as it has been used in the literature to describe ancient DNA from a variety of environmental samples, we decided to retain a brief explanation of this term in the text and Table 1. Additionally, in response to this comment and the comment made by reviewer 1 regarding the terminology, we now use the term 'marine sedaDNA' throughout the text.</p>
<p>Line 178. Some of these references are not correctly cited. Giguet Covex, Pansu and Alsos papers are about lake sediments and investigate mainly plants, but also animals growing around lakes and therefore in terrestrial environments.</p>	<p>We adjusted this sentence. ("Modern sequencing technologies and bioinformatic tools ease the analysis of these complex environmental aDNA samples and of the biological responses to human or climate change, with investigations having focussed on terrestrial settings (Jørgensen et al., 2012; Giguet-Covex et al., 2014; Willerslev et al., 2014; Alsos et al., 2015; Pansu et al., 2015).")</p>
<p>Line 191, I am a bit uncertain whether Lindhal paper suggests that hydrostatic pressure contribute to DNA preservation. Are the authors sure of this statement?</p>	<p>We removed this statement.</p>
<p>Line 201: well-oxygenated is misspelled.</p>	<p>Corrected.</p>
<p>Line 266: There is no leaching in lake sediments (Parducci et al. 2017 New Phyt). There should not be either leaching occurring in marine sediments in my opinion.</p>	<p>We agree with the reviewer and have mentioned this in the text (section 2.2.5). However, in terrestrial non-frozen sediments leaching has been found to be a factor (Haile et al., 2007), and as no studies exist that</p>

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	investigate potential leaching in marine environments, its possibility cannot be excluded to date.
Lines 296-298 I don't agree with this statement especially because based on Inagaki et al. 2005 on which I have doubts.	We have changed this sentence, however, as mentioned above we only refer to Inagaki et al. (2015), only (not Inagaki et al., 2005).
Line 358: I don't know what a drill-ship is exactly. Do the authors mean from a stable platform like MSP or from a ship? Maybe this can be explained for non-experts.	We have modified this sentence, to clarify that we are referring to a ship that is capable of performing drilling operations.
Lines 486-490: I am not sure about the statement that samples from the top part of the cores should be subsampled and processed in a non-aDNA laboratory. In these samples DNA even if more abundant is always fragmented and damaged and therefore ancient; contamination risk remains therefore high. If the authors means instead that this increase the risk for 'cross-contamination among samples then I only partially agree since cross-contamination must be always avoided regardless of the amount of DNA present in the ancient samples.	We removed this statement.
Lines 498-501: this depends also on the approach used: metabarcoding or shotgun sequencing. Using the latter in combination with capture technique may increase ability of detecting rare samples/species, particularly if these are present in the reference database.	We added this information to the text.
Lines 533-534: I think it is very good that this review brings up this problem, which is indeed serious. I would strength even more the importance of using strict aDNA methodologies and facilities in this field.	We added a sentence of the end of this paragraph to stress again the importance of strictly using aDNA facilities and methodologies as suggested by the reviewer.
In chapter 4.2 I would add one point here on the importance of negative controls and that these must be always processed and sequenced (and result shown) along with sediment samples.	We welcome this suggestion by the reviewer and have added this point to the 'future priorities' list.
Figure 1 is a too simplified and lacks important details. I suggest the authors to provide more details and improve the figure as well as the legend as this is an important figure for this review.	We updated this figure and added some more details on controls to be taken.

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3 **Highlights corresponding to changes indicated in 'Response to Reviewers'**  
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9 **Title:**

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11 *Ancient DNA from marine sediments: precautions and considerations for seafloor coring,*  
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13 *sample handling and data generation*  
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18 **Authors:**

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108 44 **Abstract**

109  
110 45 The study of ancient DNA (aDNA) from sediments (*sedaDNA*) offers great potential for  
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112 46 paleoclimate interpretation, and has recently been applied as a tool to characterise past  
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114 47 marine life and environments from deep ocean sediments over geological timescales. Using

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121 48 *sedaDNA*, palaeo-communities have been detected, including prokaryotes and eukaryotes  
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123 49 that do not fossilise, thereby revolutionising the scope of marine micropalaeontological  
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125 50 research. However, many studies to date have not reported on the measures taken to prove  
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127 51 the authenticity of *sedaDNA*-derived data from which conclusions are drawn. aDNA is highly  
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129 52 fragmented and degraded and extremely sensitive to contamination by non-target  
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131 53 environmental DNA. Contamination risks are particularly high on research vessels, drilling  
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133 54 ships and platforms, where logistics and facilities do not yet allow for sterile sediment coring,  
134  
135 55 and due consideration needs to be given to sample processing and analysis following aDNA  
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137 56 guidelines. This review clarifies the use of aDNA terminology, discusses common pitfalls and  
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139 57 highlights the urgency behind adopting new standards for marine *sedaDNA* research, with a  
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141 58 focus on sampling optimisation to facilitate the incorporation of routine *sedaDNA* research into  
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143 59 International Ocean Discovery Program (IODP) operations. Currently available installations  
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145 60 aboard drilling ships and platforms are reviewed, improvements suggested, analytical  
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147 61 approaches detailed, and the controls and documentation necessary to support the  
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149 62 authenticity of aDNA retrieved from deep-sea sediment cores is outlined. Beyond practical  
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151 63 considerations, concepts relevant to the study of past marine biodiversity based on aDNA,  
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153 64 and the applicability of the new guidelines to the study of other contamination-susceptible  
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155 65 environments (permafrost and outer space) are discussed.

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159 67 **Keywords:** ancient DNA; marine sediments; deep biosphere; phytoplankton; contamination;  
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161 68 seafloor; IODP; biomarkers; Mars

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165 70 **Abbreviations:** aDNA, ancient DNA; APC, Advanced Piston Corer; HLAPC, Half-Length  
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167 71 Advanced Piston Corer; IODP, International Ocean Discovery Program; mbsf, metres below  
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169 72 seafloor; MSP, Mission Specific Platforms; NGS, Next generation Sequencing; PCR,

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180 73 polymerase chain reaction; PFT, perfluorocarbon tracer; PMCH, perfluoromethylcyclohexane;  
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182 74 PFMD, perfluoromethyldecalin; *seda*DNA, sedimentary ancient DNA  
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## 186 75 1 Introduction

187  
188 76 Past marine environments have generally been investigated using a suite of methodological  
189 77 approaches and interdisciplinary research fields, such as geology, organic and inorganic  
190 78 geochemistry, paleoceanography and micropaleontology. Discoveries in all of these  
191 79 disciplines have contributed greatly to our understanding of the climatic history of Earth and  
192 80 the evolution and responses of its inhabitants. However, to date, it has not been possible to  
193 81 achieve a detailed picture of all living organisms that have occupied global oceans in the past,  
194 82 restricting estimates of past environmental conditions and climate. The techniques that have  
195 83 traditionally been applied to reconstruct marine palaeo-communities are limited, such as  
196 84 microscopy to investigate the microfossil record (e.g., Winter et al., 2010; Armbrecht et al.,  
197 85 2018). Due to dissolution and degradation of phytoplankton and microzooplankton while  
198 86 sinking to the seafloor post-mortem, only the most robust skeletons and shells are preserved  
199 87 within a complex geological record (Loucaides et al., 2011). Often, these microfossils are  
200 88 broken, altered by chemical processes and unrecognizable. In the absence of well-preserved  
201 89 diagnostic morphological features, lipid biomarkers can provide supplementary information on  
202 90 biological sources in sediment records (Volkman et al., 1998; Coolen et al., 2004; Sinninghe  
203 91 Damste et al., 2004; Brocks et al., 2011), however, the majority of plankton members do not  
204 92 possess highly diagnostic biomarkers.

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208 93 New marine metagenomic approaches have allowed the routine characterisation of the  
209 94 diversity of both living hard- and soft-bodied plankton communities in the water column and  
210 95 sub-seafloor. Large-scale “omics” studies, such as the Tara Oceans project (a global sampling  
211 96 program to characterise pro- and eukaryotes of the surface ocean), have shed a new light on  
212 97 our understanding of modern (present day) marine ecosystems and diversity (de Vargas et  
213 98 al., 2015; Sunagawa et al., 2015; Carradec et al., 2018). The deep sea and sub-seafloor have  
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99 also been targeted with high-resolution metagenomic surveys revealing new insights into the  
100 abundance and composition of organisms existing in these largely unexplored environments  
101 (e.g., Zinger et al., 2011; Bienhold et al., 2016; Inagaki et al., 2015; Morono and Inagaki, 2016;  
102 Orsi et al., 2017, respectively). Such comprehensive studies on living marine communities are  
103 continually improving genome reference databases for the hundreds of thousands of pro- and  
104 eukaryotic organisms present in the marine environment (Sunagawa et al., 2015; Klemetsen  
105 et al., 2017). As a consequence, modern marine metagenomics has not only inspired marine  
106 palaeo-research, but also created a means of identifying ancient taxa from marine sediments  
107 over geological timescales.

108 In the last decade, marine palaeo-research has been reinvigorated by genomic techniques  
109 that enable the analysis of ancient DNA (aDNA) molecules from long-dead organisms. Past  
110 prokaryotic and eukaryotic plankton communities have been reconstructed using aDNA  
111 sequencing approaches (e.g., Coolen and Overmann, 1998; 2007; Coolen et al., 2004; 2008;  
112 2013; Bissett et al., 2005; D'Andrea et al., 2006; Boere et al., 2009; Lejzerowicz et al., 2013;  
113 Hou et al., 2014; Randlett et al., 2014; More et al., 2018). These studies have confirmed that  
114 phyto- and zooplankton are good targets for aDNA-based studies, while also being particularly  
115 relevant for ecosystem-climate reconstructions. It is reasonable to assume that obligate  
116 photosynthetic plankton (phytoplankton) and/or zooplankton do not survive and reproduce  
117 after burial in deep sediments, and represent uncommon lab contaminants (e.g., Lejzerowicz  
118 et al., 2013; Hou et al., 2014; More et al., 2018). aDNA analysis has shown that even after  
119 their voyage through the water column plankton-derived particles that had settled on the  
120 seafloor still reflect the global biogeographic patterns of living species (Morard et al., 2017).  
121 Notably, the reconstruction of past marine communities using aDNA is possible using just a  
122 few grams of sediment, facilitating sediment sample collection, transport and storage for the  
123 purpose of aDNA analyses.

124 The marine aDNA archive extends back to the Pleistocene, as shown by studies of genomic,  
125 18S rRNA gene markers targeting various eukaryotic groups. For example, aDNA has been

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126 recovered from various eukaryotic plankton taxa in 43,000-year-old Arabian Sea sediments  
127 (More et al., 2018). Taxon-specific approaches targeting small, degraded DNA fragments  
128 allowed the retrieval of foraminiferal aDNA from ~800-year-old fjord sediments (Pawlowska et  
129 al., 2014) and ~30,000-year-old deep-sea sediments with the additional benefit of enabling  
130 the detection of rare taxa (Lejzerowicz et al., 2013). However, if a targeted approach is used,  
131 the origin and fate of the DNA in question must be carefully considered, especially for very old  
132 claims, such as the retrieval of 1.4 million years old DNA from chloroplasts (Kirkpatrick et al.,  
133 2016), which are subject to kleptoplasty (sequestration and maintenance of chloroplasts;  
134 Bernhard and Bowser, 1999). While Kirkpatrick et al. (2016) used thorough contamination  
135 control, the finding of >1 million years old DNA remains to be replicated using adapted control  
136 measures (e.g., sediment core decontamination and metagenomic sequencing, as outlined in  
137 this review). Most studies to date have involved well-dated sediment records and used a cross-  
138 validation through paired analysis of aDNA and diagnostic lipid biomarkers as well as  
139 geochemical proxies (e.g., Coolen et al., 2006; 2009). Yet, the absence of modern  
140 contaminants in analysed samples was not always verified through sequencing analysis of  
141 negative sampling and/or extraction controls, which is crucial for the interpretation of aDNA  
142 data even if DNA values measured following amplification (by polymerase chain reaction;  
143 PCR) are zero (as DNA may be present but simply be below detection limit). To date, the  
144 oldest authenticated aDNA records are from ~400,000-year-old cave sediments (Willerslev et  
145 al., 2003) and ~700,000-year-old permafrost mammal bones (Orlando et al., 2013).

146  
147 Despite technologies now being available to rapidly extract and sequence aDNA from marine  
148 sediments, and the enormous potential of aDNA research to improve palaeo-oceanographic,  
149 -ecosystem and -climate models, marine *seda*aDNA studies remain scarce. This is mainly due  
150 to the difficulties and high costs associated with deep-sea aDNA material, for which rarity and  
151 hence value justify the deployment of state-of-the-art practices. We review current problems  
152 and pitfalls incurred in ship-board sediment sampling, laboratory processing and  
153 computational analysis. We suggest solutions to improve sediment coring and sampling



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357 154 strategies so that aDNA research can become a well-established staple in marine  
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359 155 biogeosciences. The focus is on sampling protocols within the framework of the International  
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361 156 Ocean Discovery Program (IODP) “Biosphere Frontiers” theme, which is dedicated to  
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363 157 understanding sub-seafloor communities. Our guidelines for deep-ocean *seda*DNA isolation  
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365 158 are applicable to any low-biomass and setting, including permafrost regions or planet Mars.  
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## 369 159 **2 Definitions and pre-sampling considerations**

### 370 371 160 2.1 Ancient DNA (aDNA), sedimentary ancient DNA (sed aDNA), and palaeo-environmental 372 373 161 DNA (PalEnDNA)

374  
375 162 aDNA research involves the biomolecular study of non-modern genetic material preserved in  
376  
377 163 a broad range of biological samples (Shapiro und Hofreiter, 2012; Table 1). When an organism  
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379 164 dies, mechanisms that ensure DNA repair in the cell are no longer active, leaving the DNA to  
380  
381 165 degrade over time (Allentoft et al., 2012). Eventually, DNA from dead specimens becomes  
382  
383 166 ancient. aDNA is highly fragmented to an average length of less than 100 base pairs (bp), for  
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385 167 example, an average length of 48 bp has been determined in the oldest microbial genome  
386  
387 168 assembled to date - from a 48,000-year-old Neandertal (Weyrich et al., 2017). aDNA is  
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389 169 affected by post-mortem oxidative and deamination damage, such as thymine enrichment at  
390  
391 170 the end of DNA sequences (Briggs et al., 2007; Ginolhac et al., 2011). Both fragmentation and  
392  
393 171 damage patterns can be used to authenticate aDNA, and damage can even be used to predict  
394  
395 172 its age in certain scenarios (Kistler et al., 2017).  
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397

398 173 aDNA research mainly focuses on organismal DNA extracted from some tissue remnants of  
399  
400 174 a wide range of single specimen (e.g., tooth, bone, hair, eggshell, feather). In contrast,  
401  
402 175 environmental DNA (eDNA) focuses on disseminated genetic material found in environmental  
403  
404 176 samples such as soil, sediment, water and ice (Taberlet et al., 2012a). Such samples contain  
405  
406 177 complex mixtures of DNA from taxonomically diverse organisms (e.g., bacteria, archaea,  
407  
408 178 plants, animals). In addition to aDNA and eDNA, the term sedimentary aDNA (*seda*DNA) has  
409  
410 179 been coined to describe aDNA that is exclusively recovered from sediments (Willerslev et al.,  
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180 2003; Jørgensen et al., 2012). The term fossil DNA has also been used in pioneer studies  
181 where sedimentary plankton DNA and lipid biomarkers (i.e., “chemical fossils”) derived from  
182 the same historical source organisms were analysed in parallel to validate the ancient DNA  
183 results (e.g., Coolen and Overmann, 1998; 2007; Coolen et al., 2004). To a lesser degree,  
184 ‘palaeo-environmental DNA’ (PalEnDNA) has also been used to describe disseminated  
185 genetic material in a broad range of ancient environmental samples including sediments as  
186 well as soil, paleosols, coprolites, water and ice (Rawlence et al., 2014). Modern sequencing  
187 technologies and bioinformatic tools ease the analysis of these complex environmental aDNA  
188 samples and of the biological responses to human or climate change, with investigations  
189 having focussed on terrestrial settings (Jørgensen et al., 2012; Giguet-Covex et al., 2014;  
190 Willerslev et al., 2014; Alsos et al., 2015; Pansu et al., 2015). In this review, we use the term  
191 ‘marine *seda*aDNA’, which specifically refers to aDNA recovered from ocean sediments. A  
192 detailed list of terms frequently used in aDNA research and their definitions is given in Table  
193 1.

194  
195 2.2 Authenticity of marine aDNA  
196 2.2.1 Environments favourable for marine aDNA preservation  
197 Organic-rich sediments deposited in the deep, cold ocean under stratified and anoxic  
198 conditions present several favourable characteristics for the preservation of aDNA (e.g.,  
199 Coolen and Overmann, 1998; 2007; Coolen et al., 2004; 2013; Boere et al., 2011). Oxidative  
200 and deamination damage is reduced in the absence of oxygen (Lindahl, 1993). The absence  
201 of irradiation (Lyon et al. 2010), the generally low temperatures (Willerslev et al., 2004), and  
202 the high concentration of borate (Furukawa et al., 2013) further contribute to DNA  
203 preservation. Additionally, the typically high mud content of deep-sea sediment offers a  
204 particularly well-suited matrix for the preservation and accumulation of DNA (Torti et al., 2015).  
205 The high surface:volume ratio of extremely small clay minerals in clay-rich sediments offer a  
206 high adsorption surface onto which DNA molecules can bind and remain sheltered from the

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207 activity of nucleases (Dell'Anno et al., 2002; Corinaldesi et al., 2008, 2011, 2014, 2018).  
208 However, although the above listed properties have been reported to positively impact on DNA  
209 preservation, locations with other characteristics that seem less ideal might still be suitable for  
210 aDNA research. For example, well-oxygenated Atlantic deep-sea sediments and sand-rich  
211 coastal paleo-tsunami deposits have been used to extract and characterise aDNA from  
212 foraminifera (Lejzerowicz et al., 2013; Szczuciński et al., 2016, respectively). In conclusion,  
213 the preservation of aDNA in marine settings appears to be variable depending on regional  
214 environmental characteristics with less favourable to favourable conditions retaining aDNA  
215 between a few thousand to, at least, a few ten thousand years. More research is needed to  
216 estimate how far back in time authentic marine *seda*DNA can be detected, which could be  
217 achieved, for example, by investigating sediment records from various deep seafloor locations  
218 over geological timescales.

219

### 220 2.2.2 Marine *seda*DNA degradation and fragment length

221 18S rRNA gene fragments of past dinoflagellates, diatoms, and haptophytes as long as 500  
222 bp in length have been amplified and sequenced (e.g., Coolen et al., 2004), after DNA was  
223 isolated from sediments exhibiting characteristics favourable for aDNA preservation (Section  
224 2.2.1). Up to 20% of genomic DNA from haptophyte algae has been reported to still be of high  
225 molecular weight after 2,700 years of deposition in Black Sea sediments, and the ratio  
226 between 500 bp-long haptophyte 18S rDNA fragments and the concentration of haptophyte-  
227 diagnostic long-chain alkenones did not vary substantially for at least 7,500 years after  
228 deposition, indicative that both types of biomolecules from the same plankton source were  
229 equally well preserved (Coolen et al., 2006). This contradicts the generalised view that aDNA  
230 is characterised by fragment lengths of <100bp. Nevertheless, studies that report the recovery  
231 of exceedingly long aDNA fragments should be viewed with scepticism especially in the  
232 absence of sampling and extraction controls, where there is no indication on whether the data  
233 might reflect modern signals. However, to date, no data are available on average aDNA

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234 fragment length for deep-sea sediments, which could be obtained from metagenomic shotgun  
235 sequencing. Gaining insights into the latter should be the focus of future research as this  
236 information will ultimately help to choose the most suitable and efficient aDNA extraction and  
237 sequencing library preparation techniques for degraded *seda*DNA (see Section 3.5).

238

### 239 2.2.3 Contamination sources by modern DNA

240 Key to the viability of marine *seda*DNA studies is the capability to differentiate between true  
241 ancient signals (representative that lived at a particular time-period in the past) and modern  
242 contamination (introduced through the sampling and analysis process, or naturally by the  
243 environment). Microorganisms and their DNA coat nearly every part of this planet (Weyrich et  
244 al., 2015) and a recent study has shown that slow-growing microbes even occur in marine  
245 sediments up to 2.5 km deep (Inagaki et al., 2015). The DNA of active deep-biosphere  
246 organisms is likely to blur the aDNA signal, as would be the case for microorganisms  
247 introduced to ancient sediment samples through the drilling process (see Section 3.2).  
248 Moreover, microbial DNA is widely present in laboratory environments and reagents, including  
249 in those labelled DNA-free (Salter et al., 2014). If PCR is applied to amplify aDNA, the DNA  
250 from modern microorganisms may amplify preferentially over damaged, fragmented aDNA  
251 and obscure the true aDNA signals within the sample (Willerslev and Cooper, 2005).  
252 Therefore, utmost care must be taken to control and account for contaminants and background  
253 DNA throughout the whole process of collecting, processing and sequencing aDNA, e.g., by  
254 including negative controls in every step of the analysis process (Fig. 1).

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### 256 2.2.4 Intracellular vs. extracellular DNA

257 One approach to separating ancient from modern DNA in sediments has been to differentiate  
258 between intracellular and extracellular DNA. Intracellular DNA is defined as DNA contained  
259 within living cells, structurally intact dead cells and intact resting stages (e.g., bacterial spores,

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260 or other cyst-forming plankton). Extracellular DNA is defined as DNA that has been released  
261 from cells and preserved for substantial periods of time through mineral and/or microfossil  
262 adsorption or within clay aggregates (Levy-Booth et al., 2007). Extracellular DNA represent  
263 an archive of taxa that were autochthonous at the time of deposition (Cornaldesi et al., 2008;  
264 2011). DNA extraction methods have been developed to target either of these DNA fractions  
265 (Cornaldesi et al., 2005; Taberlet et al., 2012b; Alawi et al., 2014). However, it is difficult to  
266 prove at what time in the past the organism died, and its DNA became extracellular.  
267 Furthermore, the extra- and intracellular DNA pool may not always be clearly distinguishable  
268 as genetic material present in the environment might have been taken up by competent  
269 bacteria (Demanèche et al., 2001; Dell’Anno et al., 2004) and even by eukaryotes (Overballe-  
270 Petersen and Willerslev, 2014). It is also important to note that if only the extracellular pool  
271 was to be studied, the paleontological value of dormant yet ancient DNA (e.g., from cysts  
272 deposited far back in time) will be lost. Due to these issues, extraction techniques targeting  
273 only the extracellular portion are currently not recommended for marine *seDNA* studies.  
274 Alternatively, bioinformatics approaches that can clearly identify ancient signals (Ginolhac et  
275 al., 2011; Kistler et al., 2017) are preferred options for authenticating aDNA sequences  
276 (Jónsson et al., 2013).

277

### 278 2.2.5 Vertical DNA movement in marine sediment cores

279 Three major processes are associated with the vertical movement of DNA in sediment cores:  
280 DNA leaching, bioturbation and migration. Bioturbation is a biomechanical process that results  
281 in the multidirectional re-organisation of sediments primarily in the upper 10 cm of the sub-  
282 seafloor (Boudreau, 1998). DNA leaching is a passive process describing the downward  
283 movement of DNA across sediment layers (Haile et al., 2007), without a lowermost boundary.  
284 The mixing of sediment layers, and consequently of modern and ancient DNA, can lead to  
285 misinterpretations of genomic data. Experimental trials to assess DNA leaching through  
286 terrestrial sediments exist (Ceccherini et al., 2007; Poté et al., 2007), with initial results

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287 indicating that the extent of leaching depends on the taxonomic source (Haile et al., 2007). In  
288 Previous studies from lake sediments have shown that leaching is not a factor (Parducci et  
289 al., 2017), and in seafloor sediments DNA it seems to play a minor role as aDNA and lipid  
290 biomarkers derived from the same microbial source were found to co-exist or to be both below  
291 detection limit in marine sediments just centimetres apart (Boere et al., 2009; Coolen et al.,  
292 2006; 2009; 2013). In the latter studies it therefore appears that the pore size of the laminated  
293 sediments was too small for intracellular DNA to migrate, and that all extracellular plankton  
294 DNA was adsorbed to the mineral matrices. Recent studies showing *upwards* vertical pore  
295 fluid movement also demonstrate the potential for vertical migration of relict or intact DNA  
296 within sediments (Torres et al., 2015), and should likewise be considered. Vertical migration  
297 of relict or intact DNA is expected to be especially a concern in sediments with micron scale  
298 pore sizes and/or a low clay content and a poor capacity to adsorb extracellular DNA. Future  
299 experimental research is required to quantify DNA leaching and/or migration through marine  
300 sediments, acknowledging the challenge of replicating a complex environmental system  
301 varying widely in hydrodynamics and sediment type.

302  
303 2.2.6 Cross validation of marine aDNA and palaeo-environmental proxies

304 In addition to using proper contamination controls, downcore changes in past plankton  
305 compositions inferred from marine *seda*DNA can be validated through a complementary  
306 analysis of independent biological (e.g., microfossils, lipid biomarkers) and geochemical  
307 proxies (indicative of the prevailing paleoenvironmental conditions) (Boere et al., 2009; Coolen  
308 et al., 2004; 2006; 2013; Hou et al., 2014; More et al., 2018). The most detailed comparison  
309 between past ecosystem changes using marine *seda*DNA and the paleo-depositional  
310 environment to date has been performed on Holocene sediments from the permanently anoxic  
311 and sulfidic Black Sea (Coolen, 2011; Coolen et al., 2006; 2009; 2013; Giosan et al., 2012;  
312 Manske et al., 2008). The anoxic and laminated sediments of this semi-isolated sea are devoid  
313 of bioturbation and form high-resolution archives of climate-driven hydrological and

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711 314 environmental changes (Calvert et al., 1987; Hay, 1988). Episodes of postglacial sea-level  
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713 315 rise ~9,000 years ago (Major et al., 2006) and sea surface salinity increase ~5,200 years ago  
714  
715 316 (Giosan et al., 2012) have been associated based on *sedaDNA* with freshwater to  
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717 317 brackish/marine planktonic community transitions (Coolen et al., 2013). For example, the  
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719 318 gradual increase in sea surface salinity coincided with the arrival of marine copepods (*Calanus*  
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721 319 *euxinus*), which could only be identified through *sedaDNA* analysis (Coolen et al., 2013) as  
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723 320 these important zooplankton members generally do not leave other diagnostic remains in the  
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725 321 fossil record besides difficult to distinguish resting eggs (Marcus et al., 1996).

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727 322 *Vice versa*, paleoenvironmental conditions inferred from more traditional geochemical  
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729 323 and micropaleontological proxies have been verified from parallel *sedaDNA* analysis. By way  
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731 324 of example, Black Sea sediments deposited since the last 2,500 years contain coccoliths from  
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733 325 the calcified marine haptophyte *Emiliana huxleyi* whereas haptophyte-derived diagnostic long  
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735 326 chain alkenones in the absence of coccoliths were abundant in up to 7,500-year-old sediments  
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737 327 (Hay et al., 1991; Coolen et al., 2009). Paired analysis of long-chain alkenones and *sedaDNA*  
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739 328 analysis (18S rRNA) revealed that that the first haptophytes that colonized the Black Sea  
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741 329 ~7,500 years ago were initially a mixture of *E. huxleyi* and a highly diverse suite of previously  
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743 330 overlooked non-calcified haptophytes related to alkenone-producing brackish *Isochrysis*  
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745 331 species. *E. huxleyi* remained the only alkenone producer after 5,200 years BP when salinity  
746  
747 332 reached modern day levels (Coolen et al., 2009). It was concluded that while calcite dissolution  
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749 333 prevented the preservation of *E. huxleyi* coccoliths in sediments older than 2,500 years ago,  
750  
751 334 their molecular fossils (DNA fragments and long-chain alkenones) survived much longer and  
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753 335 showed that in reality this marine haptophyte entered the Black Sea already shortly after the  
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755 336 marine reconnection which occurred ~9,000 years ago (Coolen et al., 2009; 2013). Even more  
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757 337 detailed analyses of *E. huxleyi* (targeting 250-bp-long mitochondrial cytochrome oxidase  
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759 338 subunit I; mtCOI) indicate a series of transitions from possibly low-salinity to high-salinity  
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761 339 adapted strains of *E. huxleyi* in the Black Sea (7.5 – 5.2 ka BP), to a different suite of strains  
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763 340 during the most marine stage (5.2 – 2.5 ka BP), returning to low salinity strains after 2.5 ka  
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765 341 BP. The latter transition coincides with the onset of the cold and wet Subatlantic climate  
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770 342 (Coolen, 2011) when the Black Sea experienced re-freshening from 32 to 18 ppt (Van der  
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772 343 Meer et al., 2011; Giosan et al., 2012; Coolen et al., 2013). The analysis of similar length  
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774 344 preserved sequences of viral major capsid protein (mcp) genes revealed a continuous co-  
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776 345 existence of *E. huxleyi* and coccolithoviruses in the Black Sea since the last 7,000 years and  
777  
778 346 that the same *E. huxleyi* strains, which occurred shortly after the marine reconnection returned  
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780 347 with the same viral strains after the re-freshening during the Subatlantic climate thousands of  
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782 348 years later (Coolen, 2011). More recently, detailed sedimentary 18S rDNA profiling targeting  
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784 349 the shorter (130 bp) V9 region revealed that long-term expansion of past oxygen minimum  
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786 350 zones (OMZ) created isolated habitats for unicellular eukaryotes (protists) capable of  
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788 351 sustaining oxygen depletion either by adapting a parasitic life cycle (e.g., apicomplexans) or  
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790 352 by establishing mutualistic connections with others (e.g., radiolarians and mixotrophic  
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792 353 dinoflagellates). These examples show that *sed* aDNA can be used to identify biological  
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794 354 sources of lipid biomarkers, to verify the reliability of paleoenvironmental information inferred  
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796 355 from more traditional proxies, and to reconstruct past ecosystems at multiple trophic levels.

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798 356 The reconstruction of seafloor prokaryote communities is more complicated since the  
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800 357 DNA may be derived from living intact cells in the sediment (see Section 2.2.4). However, 16S  
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802 358 rRNA gene profiling from total (intracellular and extracellular) sedimentary DNA has revealed  
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804 359 useful insights into sub-seafloor microbial indicators of the palaeo-depositional environment.  
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806 360 For example, microbiomes in 20 million years-old coalbeds underlying 2 km of marine  
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808 361 sediments were shown to resemble forest soil communities (Inagaki et al., 2015). Variations  
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810 362 in bacterial communities found in Baltic Sea sediments have been linked to palaeo-salinity  
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812 363 changes (Lyra et al., 2013). Orsi et al. (2017) showed that the genomic potential for  
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814 364 denitrification correlated with past proxies for oxygen minimum zone strength in up to 43 ka-  
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816 365 old Arabian Sea sediments. The presence of fermentation pathways and their correlation with  
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818 366 the depth distribution of the same denitrifier groups, however, suggests that these microbes  
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820 367 were possibly alive upon burial, but low postdepositional selection criteria may explain why  
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822 368 they nevertheless formed a long-term genomic archive of past environmental conditions



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829 369 spanning the last glacial-interglacial cycle (Orsi et al., 2017). Further studies are required to  
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831 370 determine as to how far the persistence of this phenomenon extends with increased depth in  
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833 371 the biosphere. Nevertheless, these examples show that the complementary analysis of marine  
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835 372 *seda*DNA-inferred past plankton composition and biological and geochemical proxies is a  
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837 373 powerful tool to reconstruct palaeo-environments.  
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### 843 375 **3 aDNA research in the International Ocean Discovery Program (IODP) framework**

#### 845 376 3.1 IODP infrastructure

847 377 IODP is the global community's longest marine geoscience program, operating for 51 years.  
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849 378 Its scientific strategy has been to answer globally-significant research questions about the  
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851 379 Earth's structure, and the processes that have, and continue to, shape our planet and its  
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853 380 climatic history. More recently, additional focus has been cast on biological evolution and  
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855 381 limits, particularly in the sub-seafloor environment, under the new Biosphere Frontiers theme  
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857 382 (Bickle et al., 2011). This theme has been inspired by the rapidly evolving knowledge and  
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859 383 technical capabilities across the multiple merging fields of molecular biology, microbiology,  
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861 384 organic and inorganic geochemistry, and micropalaeontology and includes scope for the  
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863 385 integration of marine *seda*DNA research. IODP is currently serviced through three platforms,  
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865 386 the United States of America's research vessel *JOIDES Resolution*, Japan's *Chikyu* and by  
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867 387 the European consortium's Mission Specific Platforms (MSP). In recent years, the laboratories  
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869 388 and storage facilities on the ships were modified, or purpose built, to ensure addressing Deep  
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871 389 Biosphere questions was possible. As a result, the latest IODP decadal plan considered  
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873 390 options to enable access to uncontaminated samples, their processing and preservation on-  
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875 391 board. The latter has led to new coring technologies such as the Half-Length Advanced Piston  
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877 392 Corer (HLAPC) allowing a coring depth extension of the conventionally used Advanced Piston  
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879 393 Corer (APC), and the use of chemical contamination tracers such as perfluorocarbon tracers  
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881 394 (PFTs) (see Sections 3.2 and 3.3, respectively). Particularly useful to aDNA studies may be  
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395 the development of remotely controlled instruments allowing sediment sampling at ambient  
396 pressure (MeBo; Pape et al., 2017) and a rock-drilling device (RD2; Früh-Green et al., 2015).  
397 Notable achievements under the new Deep Biosphere theme include the finding of millions of  
398 years old active microbial community from coal beds buried at 2.5 km below the seafloor  
399 (Inagaki et al., 2015), and the preservation of an imprint of the Chicxulub impact catastrophe  
400 (Cockell et al., 2017). A lot remains to be understood before this theme and its challenges are  
401 satisfactorily addressed and it is clear that scientists engaging in Biosphere Frontiers will push  
402 methodological, technological and multidisciplinary studies.

403

### 404 3.2 Coring strategies suitable for marine *seda*DNA retrieval

405 Ideally, marine *seda*DNA sampling involves multiple spatial replicates to ensure that the  
406 biodiversity captured is representative of a particular site and time period. However, the ability  
407 to collect multiple deep ocean sediment cores to characterise palaeo-plankton is hindered by  
408 high costs and logistical issues associated with drilling operations. Thorough planning and  
409 collaboration to maximise the use of expensive expeditions and precious deep ocean  
410 sediment core material are indispensable in marine *seda*DNA research. To date, several  
411 coring strategies exist that differ in machinery as well as sub-seafloor depth that can be  
412 reached, and their application is largely dependent on which drilling platform is used (ship or  
413 MSP), and what type of sediment is to be cored/drilled (soft sediment or hard rock). This review  
414 concentrates on describing piston coring strategies, which are generally better suited to  
415 retrieve sediments for aDNA analysis due to relatively low contamination risks. Rotary core  
416 barrel systems are required to drill some sedimentary and most igneous rocks, and as they  
417 operate with drill-heads and drilling fluids (e.g., seawater) the risk of contamination is  
418 dramatically increased (see Section 3.3, Fig. 2).

419 Piston coring, referred to as Advanced Piston Coring (APC) or Hydraulic Piston Coring System  
420 (HPCS), is used to sample unconsolidated or poorly consolidated (i.e., softer) marine

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421 sediments. Briefly, these instruments are pushed into the sediment while a piston inside the  
422 core pipe creates a vacuum so that the collected sediment remains in the pipe during retrieval.  
423 Sediments obtained by piston coring preserve laminated sediments well, are associated with  
424 a relatively low risk of environmental contamination and the preferred method to obtain  
425 sediments for aDNA analysis (Lever et al., 2006; Smith et al., 2000; More et al., 2018; Fig. 2).  
426 Using the piston coring approach, a successive recovery of marine sediments has been  
427 achieved to a depth of ~490 m below seafloor (mbsf) (Tada et al., 2013). If only a few metres  
428 long (soft) sediment cores are required, gravity-based coring systems, such as a Kasten-, or  
429 a Multicorer provide a good alternative (Coolen et al., 2004; 2009). Progress has also been  
430 made towards modifying piston coring instruments so that contamination-free sampling is  
431 possible, at least for short (<4 m) sediments (Feek et al., 2011). For example, the 'Mk II  
432 sampler' uses an air and water-tight piston coring system with a pointed aluminium head,  
433 preventing contamination of the sampled sediment from smearing or water infiltration (Feek et  
434 al., 2011). However, to date this corer has only been used in shallow waters, thus it remains  
435 to be tested whether use of such an instrument would be feasible during coring operations in  
436 deeper waters and which modifications may be required.

437

### 438 3.3 Contamination tracing during coring

439 Deep ocean coring requires the lowering of coring instruments through hundreds to thousands  
440 of metres of seawater before the seafloor is reached, hence exposes the instruments to  
441 contamination by modern DNA (Fig. 1). This unavoidable issue has called for the development  
442 of methods for environmental DNA contaminant tracing during coring operations. One  
443 approach has been to compare biological material found in the contaminating source material  
444 (e.g., seawater, drilling fluid) to that of sub-seafloor communities, and to exclude all signals  
445 occurring in either from the final analyses (e.g., Expedition 330 Scientists, 2012; Cox et al.,  
446 2018). This approach can be implemented for either piston coring or rotary core barrel drilling,  
447 provided other sampling constraints associated with these coring systems can be

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1006 448 accommodated. However, this procedure does not account for potential “false negative” DNA  
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1008 449 signals that might indeed occur in both ancient sediments *and* modern contaminating material.  
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1010 450 However, in some cases, the microbial community structure of modern contamination (e.g.,  
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1012 451 drilling “mud”) can be resolved, particularly if functional genes are being targeted in sediment  
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1014 452 samples (Cox et al., 2018).

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1016 453 Another approach has been the introduction of fluorescent microspheres, which are particulate  
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1018 454 tracers of 0.2 - 1.0 mm in diameter physically mimicking contaminating organisms. The  
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1020 455 microspheres have been introduced near the coring head, i.e., where the sediment enters the  
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1022 456 corer and coring pipe, spreading across the outside of the core (inside the pipe) while drilling,  
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1024 457 simulating particle movement (Expedition 330 Scientists, 2012; Orcutt et al., 2017).  
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1026 458 Microscopy has been used to quantify the number of microspheres at the periphery and in the  
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1028 459 centre of the core to assess contamination (Expedition 330 Scientists, 2012; Orcutt et al.,  
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1030 460 2017). Similar methods using other perfluorocarbon tracers (PFT’s) including  
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1032 461 perfluoromethylcyclohexane (PMCH) have been developed for the USA drilling vessel  
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1034 462 *JOIDES Resolution* (Smith et al., 2000) already in the early phases of IODP. Later, PMCH-  
1035  
1036 463 based contamination tracing has also been applied during riser drilling on the *Chikyu* (Inagaki  
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1038 464 et al., 2015). During the IODP Expedition 357 (Atlantis Massif Serpentinization and Life), the  
1039  
1040 465 PMCH tracer delivery system was further developed to fit the seafloor-based drilling systems  
1041  
1042 466 MeBO (Pape et al., 2017) and RD2 (Früh-Green et al., 2015) (see Section 3.1). PMCH is  
1043  
1044 467 highly volatile which can lead to false positive measurements in uncontaminated samples,  
1045  
1046 468 therefore, more recent investigations during IODP expeditions have moved to the use of the  
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1048 469 heavier chemical tracer perfluoromethyldecalin (PFMD, 512.09 g mol<sup>-1</sup>) (e.g., Fryer et al.,  
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1050 470 2018).

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1055 472 3.4 Subsampling after core acquisition  
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473 Key to enable interdisciplinary sampling and correlations of independent measurements is a  
474 detailed sampling plan, specifying sample types as well as the sequence in which these  
475 samples are to be collected. Sampling for aDNA is time-sensitive (to avoid exposure to  
476 oxygen, high temperatures and contamination), thus should be conducted immediately after  
477 core retrieval on an untreated core-half (i.e., prior to any type of scanning such as by X-Ray).  
478 The laboratory in which subsampling for aDNA is carried out should be clean and  
479 workbenches and surfaces decontaminated with bleach (considered to be most efficient at  
480 removing contaminating DNA) and, if applicable, ethanol (to prevent corrosion of metal after  
481 bleach-treatment). Detailed records on whether molecular and amplification techniques (i.e.,  
482 PCR) have been employed in on-board laboratories and which organisms were targeted  
483 should be kept on record within IODP to ensure sampling for aDNA can be spatially separated  
484 from these laboratories. While most vessels are not currently equipped for complete DNA  
485 decontamination, such records may be invaluable for post-expedition aDNA data analyses.

486  
487 Two sampling approaches are the most feasible on board IODP ships and MSP's: cutting  
488 whole round cores or direct subsampling after core cutting into 1.5 m long sections. The choice  
489 of approach needs to be made on a case-by-case basis, and depends on the specific facilities,  
490 consumables, chemicals and researcher expertise available during each mission. It is  
491 recommended that cutting or subsampling are performed under filtered air, e.g., a portable  
492 type of a horizontal laminar flow clean air system as described in Morono and Inagaki (2016).  
493 Additionally, subsampling should be conducted from the bottom to the top of the core (ancient  
494 to modern), using clean (e.g., bleach and ethanol treated) sampling tools for each sample to  
495 avoid any form of cross-contamination. Most commonly, soft sediments acquired by piston  
496 coring are used for *seda*aDNA analyses, therefore, we focus on subsampling procedures of the  
497 latter here, subsequently briefly outlining sampling recommendations for hard rock material.

498  
499 If the sampling decision is in favour of whole round core samples, the newly acquired core  
500 sections are cut into 5 - 50 cm sections (preferably under cold conditions), which should be

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501 packed in sterile bags or wrap and transferred directly into a fridge or freezer. Although quick  
502 and providing a large amount of material for later sub-sampling, this approach has the  
503 disadvantage that a lot of freezer space is required, and post-expedition transport can be  
504 costly due to the high sample volume and weight.

505  
506 An alternative to whole round core cutting is direct subsampling immediately after core cutting,  
507 either directly from the centre of the top or bottom of each unsplit core section (usually 1.5 m  
508 long), or after or splitting the core sections into two halves. In any case the core liner should  
509 be cleaned with bleach to remove potential contamination from seawater, and core cutters  
510 and splitting-wires, usually metal and sensitive to bleach should be cleaned with ethanol. If  
511 sampling from uncut sections, surface material (~0.5 cm) should be removed with bleach and  
512 ethanol-treated scrapers before sampling, which is most easily done with sterile cut-tip  
513 syringes, placed into sterile plastic bags and stored frozen.

514  
515 If sampling is undertaken on split core halves, simultaneous visual sedimentological  
516 assessments are possible that enable more targeted sampling at specific depths of interest.  
517 Using DNA-clean tools, the top 0.5 cm of the core surface should be scraped off perpendicular  
518 to the core pipe using sterile scrapers (from bottom to top of the core). Alternatively, the core  
519 half to be sampled can be covered with plastic wrap, followed by powdered dry ice, which will  
520 result in the top 0.5 cm to become solid frozen. After 5 min, the frozen outer sediment layer  
521 can be lifted at one edge with a sterile scalpel creating a contaminant-free surface, from which  
522 subsamples can be taken (Coolen and Overmann, 1998). Then, subsampling should be  
523 undertaken using sterile (e.g., gamma-irradiated) plastic syringes or centrifuge tubes (e.g.,  
524 capacity of ~15 mL). Cut-tip syringes have the advantage that more sediment can be collected  
525 as no pressure builds up when pushing the syringe into the sediment (the filled syringe should  
526 be placed into a sterile plastic bag immediately, e.g., Whirl-Pak®). Alternatively, sterile  
527 centrifuge tubes can be used as is to collect 'plunge-samples', usually providing ~1 - 3 cc of  
528 sediment material. The outside of the 'mini-cores' should be cleaned with bleach and placed

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529 into sterile plastic bags to avoid cross-contamination between samples. For subsamples,  
530 storage at -20 °C or -80 °C is recommended as freezing has been shown to facilitate  
531 phytoplankton cell-lysis during DNA extractions (Armbrecht et al., *in prep.*). Sub-samples can  
532 also be collected by transferring a small amount of sediment into a sterile microcentrifuge tube  
533 using clean metal or disposable spatulas (particular care needs to be taken to avoid cross-  
534 contamination when using the same sampling tool for different samples). The latter approach  
535 may be a good solution when only a few small samples are required, e.g., to supplement other  
536 scientific questions of an ongoing expedition. For replication purposes it is recommended that  
537 duplicate samples are taken at each depth.

538  
539 **If the material is hard rock or similar, subsamples are most easily collected from whole round**  
540 **or split cores. The same decontamination procedures as outlined above should be considered**  
541 **throughout the subsampling procedure (i.e., decontamination of work-surfaces and sampling**  
542 **tools with bleach and ethanol, sampling under cold conditions and filtered or low air-flow,**  
543 **packing of samples into sterile bags before storage). A de-contaminated metal cutter or a**  
544 **hammer and chisel are best used to remove the outer layer of the exposed sediment, at least**  
545 **at those depths where subsampling is anticipated.**

### 3.5 Marine aDNA sample processing and analysis

549 Marine aDNA samples should be processed in a specialised aDNA laboratory to prevent  
550 contamination with modern DNA. Such a laboratory is generally characterised by creating a  
551 low-DNA environment, with a clear separation of no-DNA (e.g., buffer preparation) and DNA-  
552 containing (e.g., DNA extraction) workflows, regular and thorough sterilisation procedures,  
553 positive air pressure, and protective clothing of the analyst (lab coat/suit, gloves, facemask,  
554 visor). Details on optimised laboratory set-up, techniques and workflows have been reviewed  
555 before (Cooper and Poinar, 2000; Pedersen et al., 2015). The introduction of aDNA samples

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556 into such facilities is relatively straight-forward, as the outer packaging and surface of the  
557 sample can be easily sterilised (e.g., using bleach and/or UV).

558 As on-board subsampling, DNA extractions should be carried out from the most ancient to  
559 most recent samples, to prevent modern DNA inadvertently being carried to ancient samples.

560 The amount of sediment used in DNA extractions should capture a representative picture of  
561 the biota present in a sample. Despite suggestions that bulk DNA extractions from up to 10 g  
562 of material can improve detection of taxa and better represent the diversity of the area of  
563 interest (e.g., Taberlet et al., 2012b; Coolen et al., 2013), using such large volumes of  
564 sediment is often not practical and can be quite costly in this field where typically many  
565 samples are processed. Instead, numerous studies have used replicate extractions of a  
566 smaller sample size (e.g., 0.25 g; Table 2) to increase the likelihood of yielding aDNA from  
567 rare taxa, as well as successive DNA extractions from a single 0.25 g sediment sample (e.g.,  
568 Willerslev et al., 2003). Post-extraction, the use of RNA-probe based enrichment approaches  
569 coupled with shotgun sequencing, a common technique in aDNA research, may furthermore  
570 drastically improve the detection of rare taxa (Horn et al., 2012).

571 While it would be ideal to find one extraction method that will yield the best quality data and  
572 enable standardisation across ancient marine sediment studies, the type of sediment or target  
573 organisms may require some adjustments of standard protocols (Hermans et al., 2018).

574 Extraction methods can bias the diversity observed due to differential resilience of taxa to the  
575 cell-lysis method (Zhou et al., 1996; Young et al., 2015) and DNA binding capacities of  
576 different soil and sediment types (Lorenz and Wackernagel, 1994; Miller et al., 1999). As a  
577 result, the aDNA extraction efficiency can be poor and the detection of an aDNA signal lost.

578 To date, a variety of commercial kits have been successfully used to isolate aDNA from  
579 sediments (Table 3). To further increase the yield of very low amounts of highly fragmented  
580 aDNA several studies have been utilising extraction protocols that include a liquid-silica DNA  
581 binding step (e.g., Brotherton et al., 2013 and Weyrich et al., 2017 for non-sediment samples)  
582 or ethylenediaminetetraacetic acid (EDTA) cell-lysis step (Slon et al., 2017; utilising cave-



1299  
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1301 583 sediment samples). Other studies have replaced the Bead Solution in the DNeasy extraction  
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1303 584 kits (Qiagen; Table 2) by 1M sodium phosphate pH 9 - 10 and 15 vol% ethanol to efficiently  
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1305 585 release clay-adsorbed DNA, and to prevent DNA released from intact cells from adsorbing to  
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1307 586 clay minerals during the extraction (Direito et al., 2012; Orsi et al., 2017; More et al., 2018).  
1308  
1309 587 The latter is especially important when working with low organic, high carbonate rocks and  
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1311 588 sediments (Direito et al., 2012).

1312  
1313 589 Two points are particularly important to prevent contamination during extractions. Firstly, as  
1314  
1315 590 with the samples themselves, it is crucial that all tools and reagents undergo rigorous  
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1317 591 sterilisation procedures before utilisation, such as by bleach and UV treatment of any packing  
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1319 592 material before entering ancient DNA facilities. Secondly, blank controls should be included  
1320  
1321 593 for every step of the laboratory process, i.e., extraction/library preparation blank controls,  
1322  
1323 594 sequencing and bioinformatic analysis controls (Ficetola et al., 2016). Controlling and  
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1325 595 monitoring contamination is particularly important when analysing bacterial diversity due to  
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1327 596 their presence in all laboratory environments and reagents (Weyrich et al., 2015). Optimally,  
1328  
1329 597 extraction blanks are included in a 1:5 ratio (Willerslev and Cooper, 2005), with a bare  
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1331 598 minimum of one control with each set of extractions. Aside from bioinformatically removing  
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1333 599 any organisms determined in such extraction blanks from the investigated sample material,  
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1335 600 the contaminants should be tracked within a laboratory, and contaminant lists published  
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1337 601 alongside the data for reasons of data transparency and authenticity.

1338  
1339 602 Post-extraction, many marine aDNA studies have employed methods that are routinely used  
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1341 603 for modern marine DNA analysis. Although modern DNA work is not exempt from precautions,  
1342  
1343 604 there are several issues with aDNA work: (i) as outlined in Sections 2.1. and 2.2. aDNA is  
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1345 605 highly fragmented and degraded and any small amount of modern DNA present in the sample  
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1347 606 (from reagents, labs or living cells that were present in the sediment sample) will amplify over  
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1349 607 the aDNA; (ii) sampling and extraction controls are often not included in the sequencing  
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1351 608 sample; (iii) PCRs are often inhibited due to the co-extraction of humic substances, pigments  
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1353 609 and heavy metals along with DNA (Webster et al., 2003 and references therein), requiring  
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610 adequate removal of these impurities (e.g., Coolen et al., 2009); (iv) successful PCRs are  
611 prone to bias due to random amplification in reactions that contain very low amounts of DNA  
612 template, thus PCR drift (stochastic variation in the first PCR cycles) can occur (Wagner et al.,  
613 1994; Polz and Cavanaugh, 1998; Webster et al., 2003). More importantly, the number of,  
614 e.g., bacterial 16S and eukaryotic 18S rRNA operons can greatly vary between per genome  
615 and per cell and can cause a biased representation of the past community structure (e.g.,  
616 Klappenbach et al., 2001). The above biases can be reduced and the detection limit lowered  
617 when PCR approaches selectively, amplifying particular groups of organisms indicative of  
618 environmental changes, are paired with independent geochemical proxies (e.g., Coolen et al.,  
619 2004; 2006; 2009). However, we strongly advocate for the use of strict aDNA methodologies  
620 and facilities in order to achieve the generation of authentic marine *seda*DNA data, following  
621 the guidelines in this review.

622 Shotgun metagenomics are currently widely accepted and the least biased method to analyse  
623 the broad diversity of ancient environmental samples (e.g., Slon et al., 2017). Although only a  
624 small portion of the generated sequence data might be attributable to the ancient organism in  
625 question (Morard et al., 2017), next generation sequencing (NGS) generates large quantities  
626 of data that enable meaningful statistics, with the additional benefit of preserving the relative  
627 proportion of detected taxa. To analyse aDNA sequence data, robust bioinformatic pipelines  
628 (e.g., Paleomix, Schubert et al., 2014) have been developed and are available for the  
629 application to marine *seda*DNA, integrating damage detection algorithms (e.g., Ginolhac et al.  
630 2011; Kistler et al., 2017) that enable the distinction between ancient and modern signals.  
631 Determining the extent of cytosine residues deamination (C to T and G to A, Weyrich et al.  
632 2017) should also be considered to assess authenticity of aDNA sequences, especially when  
633 the data was generated from mixed communities, such as from marine *seda*DNA. It is  
634 furthermore crucial to carefully screen sequencing data for any low-complexity reads, which  
635 may get incorrectly assigned to taxa during alignments against genetic databases, as well as  
636 ensuring that taxonomic assignments in the database of choice are correct. Bioinformatic

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637 pipelines removing such misidentification-derived errors do not currently exist and should be  
638 the focus of future research, as well as the comparison of shotgun and amplicon marine  
639 *seda*DNA data to accurately determine biases and analysis strategies best suited to this new  
640 discipline.

642 **4 Future marine aDNA sampling considerations**

643 4.1 Equipment and installations required aboard IODP platforms

644 In addition to the recent upgrades and investments IODP has made to enable sediment  
645 sampling suitable for Biosphere Frontiers theme (Section 3.1) we suggest the following items  
646 to facilitate contamination-free sediment sampling and the tracing of contaminants.

647 (i) Laboratories in which sampling for aDNA is undertaken should be carefully chosen to  
648 minimise contamination. Rapid transport of the core from the deck to the lab, thorough  
649 decontamination measures (see Section 3.4), and easy access to fridges or freezers are  
650 crucial. While a positively air-pressured lab (standard for aDNA laboratories) may not be  
651 feasible, air-flow can be reduced by keeping all doors shut and fans off during aDNA sampling.  
652 Contamination by human DNA from analysts can be greatly reduced by wearing adequate  
653 protective clothing (gloves, facemask, freshly laundered/disposable lab coat/overall). A  
654 detailed record of any molecular work undertaken in ship-board labs should be maintained by  
655 IODP, and under no circumstances should aDNA sampling be conducted in labs used  
656 previously to run PCRs (see Section 3.4). Alternatively, the equipment of a shipping container  
657 exclusively dedicated to aDNA sampling could be a good solution to spatially separate aDNA  
658 sampling aboard drilling-platforms and installation could be as required during expeditions that  
659 involve aDNA sampling.

660 (ii) DNA is likely to behave quite different from chemical tracers and microspheres currently  
661 used to track contamination. **With constantly advancing technologies in the field of synthetic**

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662 biology, the possibility arises to develop 'non-biological DNA' with known sequences. Such  
663 non-viable DNA tags are already used in the oil industry, where a different tag is introduced  
664 into oil pipes monthly to monitor when and where leaks occur (Forecast Technology Ltd).  
665 Using such tags during seafloor coring operations instead of chemical tracers should enable  
666 a precise assessment of contamination by environmental DNA, where bioinformatics pipelines  
667 could be adjusted to detect and quantify the amount of tags present in the final sequencing  
668 data.

669  
670 4.2 Ground-truthing marine aDNA research and data

671 To ground-truth marine aDNA studies and to ensure the generation of authentic aDNA data  
672 we suggest future research in this field to focus on the following aspects:

673 (i) The establishment of a public record of common contaminants. This can be achieved, for  
674 example, through an inter-lab comparison focused on analysing the same samples and  
675 integrating extraction blanks to trace contaminants associated with particular coring  
676 equipment, ship- and land- based laboratories.

677 (ii) Investigation of factors that might considerably bias marine *seda*aDNA data. This might  
678 include information on sediment-type and environmental condition dependent aDNA  
679 preservation, taxon-specific DNA degradation rates, average aDNA fragment length, and  
680 shotgun and amplification-based aDNA data comparisons.

681 (iii) Ongoing enrichment of genetic reference databases for modern marine plankton, to enable  
682 taxonomic assignment of the hundreds of thousands of ancient sequences expected to be  
683 found in marine sediments.

684 (iv) The inclusion of negative controls during extractions, library preparations and in  
685 sequencing runs, and the publication of the results in the context of independent multiproxy  
686 biological and environmental metadata obtained from the same sediment interval.

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687 (iv) Once (i) - (iv) are addressed, the development of a dedicated aDNA coring proposal is  
688 encouraged, in which sediment cores are collected using the above outlined, best-suited  
689 coring strategies, sampling and analysis procedures. During such an expedition, basic  
690 questions such as optimal on-board contamination tracing techniques, feasible work-flows,  
691 spatial replication required to achieve representative community data, and age to which  
692 marine *seda*DNA can be determined should be addressed. Such baseline data is missing to  
693 date and remains the most important step towards the generation of authentic aDNA data from  
694 marine sediments.

695  
**5 Application of marine *seda*DNA research guidelines to other contamination  
susceptible environments**

698 5.1 Permafrost

699 Permafrost molecular biological studies provide the opportunity to study living organisms that  
700 have successfully adapted to extremely cold environments and comprise an analogous  
701 cryogenic environment to that found on other planets, such as Mars (Amato et al., 2010).  
702 Molecular investigations have focussed on humans (Rasmussen et al., 2010), plants  
703 (Willerslev et al., 2003), megafauna (Boessenkool et al. 2012), fungi (Bellemain et al., 2013)  
704 and microbes (Willerslev et al., 2004). Permafrost top layers are characterised by a more  
705 abundant and diverse microbial community compared to the deeper soil (Gittel et al. 2014).  
706 To overcome the hurdle of distinguishing between the modern and ancient DNA signal,  
707 metatranscriptomics have been applied to identify the active community only (e.g., Coolen and  
708 Orsi, 2015). Despite the challenges in experimental approaches, such as rapid community  
709 shifts after thawing even at nearly ambient conditions (Negandhi et al. 2016), studies of  
710 permafrost environments have advanced our understanding of feedback loops associated with  
711 the response of extremophiles to warming, ultimately informing modelling studies including  
712 marine palaeo-environments.

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713 Sampling for ice and permafrost in polar regions is challenging in terms of logistics and  
714 minimising contamination risks for both the sample and the sampled environment. For  
715 example, permafrost soil samples are, like marine sediment cores, retrieved through drilling,  
716 which can introduce microbial contaminants to the deeper permafrost soil layers as the drill  
717 head and liquid pass through the top active soil layer (Bang-Andreasen et al., 2017).  
718 Additionally, the cryosphere has been accumulating industrial chemicals and metals since the  
719 1850's (McConnell et al., 2007), so that the present-day microbial community is now capable  
720 of degrading industrial contaminants, thereby representing an anthropogenically-adapted  
721 rather than an original pristine community (Hauptmann et al., 2017). With both these newly  
722 adapted anthropogenic and drilling fluid communities containing characteristics for heavy  
723 metal degradation, distinguishing indigenous ice core or permafrost communities from drilling  
724 fluid communities will become more difficult in the future (Miteva et al., 2014). Therefore, the  
725 described guidelines in this review for distinguishing ancient from modern and contaminant  
726 signals, as well as the need for aseptic sampling procedures, are highly applicable to  
727 permafrost environments and, more generally, the cryosphere.

728

## 729 5.2 Planetary exploration

730 The methodologies advocated in this review that enable aDNA in marine sediments to be  
731 distinguished from modern DNA are also applicable to the search for life on other planets or  
732 moons. Astrobiologists are especially interested in the possibility of detection of Life 2.0, where  
733 the life has an independent genesis to that on Earth. The search for life beyond Earth has  
734 been potentially possible since the 1970s, with the two Viking lander missions to Mars, but  
735 there are other possible targets in our solar system, notably some of the moons around Jupiter  
736 and Saturn (e.g., Europa, Titan). Space technology has now reached the point where the  
737 detection of life, if it exists or existed elsewhere in the solar system, is becoming a realistic  
738 possibility in the next 50 years. There have been several rovers that have carried out  
739 successful exploration of the surface of Mars, including Curiosity, the Mars Science Laboratory

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740 that in 2018 is mid-way through its predicted mission (Grotzinger et al., 2014). The rover Mars  
741 2020 is being designed at present to test for evidence of life in the near-surface environment.  
742 It will drill, collect and cache samples from the Martian surface, which will then be returned to  
743 Earth for more detailed analysis (Beatty et al., 2015). Sample return from Mars to Earth is  
744 planned for the end of the 2020's (Foust, 2018). Active planning is also ongoing for possible  
745 missions to land and analyse materials from the surfaces of moons such as Europa and Titan,  
746 by both NASA and the European Space Agency. For example, Europa (a moon of Jupiter) is  
747 known to have a global saltwater ocean below its icy crust, as well as a rocky seafloor, so is  
748 one of the highest priority targets in the search for present-day life beyond Earth (Hand et al.,  
749 2017). A key concern with this solar system exploration is planetary protection, which is  
750 governed by the United Nations Outer Space Treaty (United Nations Office for Disarmament  
751 Affairs, 2015) and the Committee on Space Research (COSPAR) of the International  
752 Committee for Science. There are two important categories of planetary protection. The first  
753 is "forward contamination", where Earth-derived microbial life hitches a ride on spacecraft and  
754 contaminates parts of a planetary surface being explored. The second is "backward  
755 contamination", where life from an explored planet or moon is inadvertently returned to Earth,  
756 maybe in a spacecraft or within a rock sample. The relevance to aDNA analytical protocols is  
757 in forward contamination (i.e., the risk of contaminating sample material that could lead to data  
758 misinterpretations, and/or generally introducing Earth contaminants to other planets; Rummel  
759 and Conley, 2017). It should be noted that if indeed there is or was life on other planetary  
760 bodies, it may well not be based on a genetic code composed of DNA and RNA. Independently  
761 originated Life 2.0 would be highly unlikely to have evolved exactly the same nucleic acid  
762 genetic code as life on Earth (e.g., Rummel and Conley, 2017). Indeed, it has been postulated  
763 that an alternative biosphere could exist as a "shadow biosphere" on Earth (Davies et al.,  
764 2009). If DNA or RNA-based extant life is found on Mars, for example, then it is most likely  
765 that it would represent either past natural exchange of rocks between the two planets  
766 (panspermia), or anthropogenic forward contamination. Therefore, the procedures used for  
767 distinguishing indigenous life in planetary exploration will need broadening to include the

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768 possibility of life with a different genetic code. The protocols developed for aDNA sampling of  
769 marine sediments on Earth, including the ability to distinguish from modern DNA, have  
770 relevance for the designing of methods to look for past life on Mars or outer solar system  
771 moons using molecular biology techniques (Beaty et al., 2015; Hand et al., 2017).

773 **Conclusions**

774 Ancient DNA in marine deep-sea sediments holds the potential to open a new era of marine  
775 palaeo-environment and -climate reconstruction. However, anti-contamination measures  
776 central to all aDNA research have logistical constraints and are particularly poorly-suited to  
777 shipboard sediment sampling and processing. For example, sterile coring equipment and  
778 ultra-clean laboratories are usually not available on any type of drilling platform. Current and  
779 future IODP drilling vessels are aware of the increasing need for improved and innovative  
780 solutions to coring, non-contaminant drill fluids and appropriate laboratories and storage  
781 facilities. Such logistical advances should go hand-in-hand with the establishment of new  
782 criteria and standards to ensure the acquisition and preservation of sediment cores with  
783 minimal environmental contaminants. Complementary genetic and geochemical information  
784 currently available to date suggests that, realistically, environmental reconstructions based on  
785 marine *seda*DNA from past plankton can be achieved for at least the last glacial-interglacial  
786 cycle, and potentially back to ~400,000 years. These guidelines can be applied in other  
787 scientific areas to facilitate and optimise research conducted in extremely remote locations,  
788 contamination-susceptible environmental samples, and even during the future exploration of  
789 other planets.

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815 **Figures:**

816 **Figure 1:** Schematic showing the key steps involved in acquiring deep marine sediment  
817 cores, subsampling, DNA extraction, aDNA preparation for sequencing and data generation.  
818 Indicated are sources of potential contamination and reduction in data quality, as well as

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819 recommended precautions to be considered and/or controls to be taken. An impact score (1-  
820 3 stars) is given to indicate the severity of potential contamination or the impact that impaired  
821 data would have on the results at each step in the process. Schematic graphics are not to  
822 scale.

823  
824 **Figure 2:** Overview of IODP coring systems. A) Advanced piston coring system (APC), shown  
825 before and after stroking; only small volumes of drill fluid can enter the space between the  
826 core barrel and collar from above after stroking, greatly reducing the risk of contamination. B)  
827 Extended core barrel system (XCB) and C) Rotary core barrel system (RCB); both containing  
828 circulation jets at the bottom of the core barrel through which drill-fluid enters and removes  
829 coring debris by transporting it upwards within the drill hole to the surface. D) Comparison of  
830 rotary and piston cored sediments demonstrating the well-preserved lamination in Piston  
831 cored material. Figure adapted from Sun et al. (2018) and IODP  
832 ([iodp.tamu.edu/tools/index.html](http://iodp.tamu.edu/tools/index.html)).

833  
834 **Table 1:** Terms commonly used in marine aDNA research and their definition. aDNA terms  
835 are listed hierarchically, all other terms are listed alphabetically.

836  
837 **Table 2:** Commonly used DNA extraction kits in aDNA studies to date.

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## **Abstract**

The study of ancient DNA (aDNA) from sediments (*sedaDNA*) offers great potential for paleoclimate interpretation, and has recently been applied as a tool to characterise past marine life and environments from deep ocean sediments over geological timescales. Using *sedaDNA*, palaeo-communities have been detected, including prokaryotes and eukaryotes that do not fossilise, thereby revolutionising the scope of marine micropalaeontological research. However, many studies to date have not reported on the measures taken to prove the authenticity of *sedaDNA*-derived data from which conclusions are drawn. aDNA is highly fragmented and degraded and extremely sensitive to contamination by non-target environmental DNA. Contamination risks are particularly high on research vessels, drilling ships and platforms, where logistics and facilities do not yet allow for sterile sediment coring, and due consideration needs to be given to sample processing and analysis following aDNA guidelines. This review clarifies the use of aDNA terminology, discusses common pitfalls and highlights the urgency behind adopting new standards for marine *sedaDNA* research, with a focus on sampling optimisation to facilitate the incorporation of routine *sedaDNA* research into International Ocean Discovery Program (IODP) operations. Currently available installations aboard drilling ships and platforms are reviewed, improvements suggested, analytical approaches detailed, and the controls and documentation necessary to support the authenticity of aDNA retrieved from deep-sea sediment cores is outlined. Beyond practical considerations, concepts relevant to the study of past marine biodiversity based on aDNA, and the applicability of the new guidelines to the study of other contamination-susceptible environments (permafrost and outer space) are discussed.

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3 **1 Title:**  
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6 *2 Ancient DNA from marine sediments: precautions and considerations for seafloor coring,*  
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8 *3 sample handling and data generation*  
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103 42 **Abstract**  
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106 43 The study of ancient DNA (aDNA) from sediments (*sedaDNA*) offers great potential for  
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108 44 paleoclimate interpretation, and has recently been applied as a tool to characterise past  
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110 45 marine life and environments from deep ocean sediments over geological timescales. Using  
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112 46 *sedaDNA*, palaeo-communities have been detected, including prokaryotes and eukaryotes  
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125 50 fragmented and degraded and extremely sensitive to contamination by non-target  
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127 51 environmental DNA. Contamination risks are particularly high on research vessels, drilling  
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129 52 ships and platforms, where logistics and facilities do not yet allow for sterile sediment coring,  
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131 53 and due consideration needs to be given to sample processing and analysis following aDNA  
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133 54 guidelines. This review clarifies the use of aDNA terminology, discusses common pitfalls and  
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135 55 highlights the urgency behind adopting new standards for marine *seda*DNA research, with a  
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137 56 focus on sampling optimisation to facilitate the incorporation of routine *seda*DNA research into  
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139 57 International Ocean Discovery Program (IODP) operations. Currently available installations  
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141 58 aboard drilling ships and platforms are reviewed, improvements suggested, analytical  
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143 59 approaches detailed, and the controls and documentation necessary to support the  
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145 60 authenticity of aDNA retrieved from deep-sea sediment cores is outlined. Beyond practical  
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147 61 considerations, concepts relevant to the study of past marine biodiversity based on aDNA,  
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149 62 and the applicability of the new guidelines to the study of other contamination-susceptible  
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151 63 environments (permafrost and outer space) are discussed.

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156 65 **Keywords:** ancient DNA; marine sediments; deep biosphere; phytoplankton; contamination;  
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158 66 seafloor; IODP; biomarkers; Mars

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163 68 **Abbreviations:** aDNA, ancient DNA; APC, Advanced Piston Corer; HLAPC, Half-Length  
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165 69 Advanced Piston Corer; IODP, International Ocean Discovery Program; mbsf, metres below  
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167 70 seafloor; MSP, Mission Specific Platforms; NGS, Next generation Sequencing; PCR,  
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169 71 polymerase chain reaction; PFT, perfluorocarbon tracer; PMCH, perfluoromethylcyclohexane;  
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171 72 PFMD, perfluoromethyldecalin; *seda*DNA, sedimentary ancient DNA

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180 73 1 **Introduction**  
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182 74 Past marine environments have generally been investigated using a suite of methodological  
183 75 approaches and interdisciplinary research fields, such as geology, organic and inorganic  
184 76 geochemistry, paleoceanography and micropaleontology. Discoveries in all of these  
185 77 disciplines have contributed greatly to our understanding of the climatic history of Earth and  
186 78 the evolution and responses of its inhabitants. However, to date, it has not been possible to  
187 79 achieve a detailed picture of all living organisms that have occupied global oceans in the past,  
188 80 restricting estimates of past environmental conditions and climate. The techniques that have  
189 81 traditionally been applied to reconstruct marine palaeo-communities are limited, such as  
190 82 microscopy to investigate the microfossil record (e.g., Winter et al., 2010; Armbrecht et al.,  
191 83 2018). Due to dissolution and degradation of phytoplankton and microzooplankton while  
192 84 sinking to the seafloor post-mortem, only the most robust skeletons and shells are preserved  
193 85 within a complex geological record (Loucaides et al., 2011). Often, these microfossils are  
194 86 broken, altered by chemical processes and unrecognizable. In the absence of well-preserved  
195 87 diagnostic morphological features, lipid biomarkers can provide supplementary information on  
196 88 biological sources in sediment records (Volkman et al., 1998; Coolen et al., 2004; Sinninghe  
197 89 Damste et al., 2004; Brocks et al., 2011), however, the majority of plankton members do not  
200 90 possess highly diagnostic biomarkers.

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216 91 New marine metagenomic approaches have allowed the routine characterisation of the  
217 92 diversity of both living hard- and soft-bodied plankton communities in the water column and  
218 93 sub-seafloor. Large-scale “omics” studies, such as the Tara Oceans project (a global sampling  
219 94 program to characterise pro- and eukaryotes of the surface ocean), have shed a new light on  
220 95 our understanding of modern (present day) marine ecosystems and diversity (de Vargas et  
221 96 al., 2015; Sunagawa et al., 2015; Carradec et al., 2018). The deep sea and sub-seafloor have  
222 97 also been targeted with high-resolution metagenomic surveys revealing new insights into the  
223 98 abundance and composition of organisms existing in these largely unexplored environments  
224 99 (e.g., Zinger et al., 2011; Bienhold et al., 2016; Inagaki et al., 2015; Morono and Inagaki, 2016;  
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100 Orsi et al., 2017, respectively). Such comprehensive studies on living marine communities are  
101 continually improving genome reference databases for the hundreds of thousands of pro- and  
102 eukaryotic organisms present in the marine environment (Sunagawa et al., 2015; Klemetsen  
103 et al., 2017). As a consequence, modern marine metagenomics has not only inspired marine  
104 palaeo-research, but also created a means of identifying ancient taxa from marine sediments  
105 over geological timescales.

106 In the last decade, marine palaeo-research has been reinvigorated by genomic techniques  
107 that enable the analysis of ancient DNA (aDNA) molecules from long-dead organisms. Past  
108 prokaryotic and eukaryotic plankton communities have been reconstructed using aDNA  
109 sequencing approaches (e.g., Coolen and Overmann, 1998; 2007; Coolen et al., 2004; 2008;  
110 2013; Bissett et al., 2005; D'Andrea et al., 2006; Boere et al., 2009; Lejzerowicz et al., 2013;  
111 Hou et al., 2014; Randlett et al., 2014; More et al., 2018). These studies have confirmed that  
112 phyto- and zooplankton are good targets for aDNA-based studies, while also being particularly  
113 relevant for ecosystem-climate reconstructions. It is reasonable to assume that obligate  
114 photosynthetic plankton (phytoplankton) and/or zooplankton do not survive and reproduce  
115 after burial in deep sediments, and represent uncommon lab contaminants (e.g., Lejzerowicz  
116 et al., 2013; Hou et al., 2014; More et al., 2018). aDNA analysis has shown that even after  
117 their voyage through the water column plankton-derived particles that had settled on the  
118 seafloor still reflect the global biogeographic patterns of living species (Morard et al., 2017).  
119 Notably, the reconstruction of past marine communities using aDNA is possible using just a  
120 few grams of sediment, facilitating sediment sample collection, transport and storage for the  
121 purpose of aDNA analyses.

122 The marine aDNA archive extends back to the Pleistocene, as shown by studies of genomic,  
123 18S rRNA gene markers targeting various eukaryotic groups. For example, aDNA has been  
124 recovered from various eukaryotic plankton taxa in 43,000-year-old Arabian Sea sediments  
125 (More et al., 2018). Taxon-specific approaches targeting small, degraded DNA fragments  
126 allowed the retrieval of foraminiferal aDNA from ~800-year-old fjord sediments (Pawlowska et

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127 al., 2014) and ~30,000-year-old deep-sea sediments with the additional benefit of enabling  
128 the detection of rare taxa (Lejzerowicz et al., 2013). However, if a targeted approach is used,  
129 the origin and fate of the DNA in question must be carefully considered, especially for very old  
130 claims, such as the retrieval of 1.4 million years old DNA from chloroplasts (Kirkpatrick et al.,  
131 2016), which are subject to kleptoplasty (sequestration and maintenance of chloroplasts;  
132 Bernhard and Bowser, 1999). While Kirkpatrick et al. (2016) used thorough contamination  
133 control, the finding of >1 million years old DNA remains to be replicated using adapted control  
134 measures (e.g., sediment core decontamination and metagenomic sequencing, as outlined in  
135 this review). Most studies to date have involved well-dated sediment records and used a cross-  
136 validation through paired analysis of aDNA and diagnostic lipid biomarkers as well as  
137 geochemical proxies (e.g., Coolen et al., 2006; 2009). Yet, the absence of modern  
138 contaminants in analysed samples was not always verified through sequencing analysis of  
139 negative sampling and/or extraction controls, which is crucial for the interpretation of aDNA  
140 data even if DNA values measured following amplification (by polymerase chain reaction;  
141 PCR) are zero (as DNA may be present but simply be below detection limit). To date, the  
142 oldest authenticated aDNA records are from ~400,000-year-old cave sediments (Willerslev et  
143 al., 2003) and ~700,000-year-old permafrost mammal bones (Orlando et al., 2013).

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145 Despite technologies now being available to rapidly extract and sequence aDNA from marine  
146 sediments, and the enormous potential of aDNA research to improve palaeo-oceanographic,  
147 -ecosystem and -climate models, marine *sedaDNA* studies remain scarce. This is mainly due  
148 to the difficulties and high costs associated with deep-sea aDNA material, for which rarity and  
149 hence value justify the deployment of state-of-the-art practices. We review current problems  
150 and pitfalls incurred in ship-board sediment sampling, laboratory processing and  
151 computational analysis. We suggest solutions to improve sediment coring and sampling  
152 strategies so that aDNA research can become a well-established staple in marine  
153 biogeosciences. The focus is on sampling protocols within the framework of the International  
154 Ocean Discovery Program (IODP) “Biosphere Frontiers” theme, which is dedicated to

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155 understanding sub-seafloor communities. Our guidelines for deep-ocean *sedaDNA* isolation  
156 are applicable to any low-biomass and setting, including permafrost regions or planet Mars.

157 **2 Definitions and pre-sampling considerations**

158 2.1 Ancient DNA (aDNA), sedimentary ancient DNA (sed aDNA), and palaeo-environmental  
159 DNA (PalEnDNA)

160 aDNA research involves the biomolecular study of non-modern genetic material preserved in  
161 a broad range of biological samples (Shapiro und Hofreiter, 2012; Table 1). When an organism  
162 dies, mechanisms that ensure DNA repair in the cell are no longer active, leaving the DNA to  
163 degrade over time (Allentoft et al., 2012). Eventually, DNA from dead specimens becomes  
164 ancient. aDNA is highly fragmented to an average length of less than 100 base pairs (bp), for  
165 example, an average length of 48 bp has been determined in the oldest microbial genome  
166 assembled to date - from a 48,000-year-old Neandertal (Weyrich et al., 2017). aDNA is  
167 affected by post-mortem oxidative and deamination damage, such as thymine enrichment at  
168 the end of DNA sequences (Briggs et al., 2007; Ginolhac et al., 2011). Both fragmentation and  
169 damage patterns can be used to authenticate aDNA, and damage can even be used to predict  
170 its age in certain scenarios (Kistler et al., 2017).

171 aDNA research mainly focuses on organismal DNA extracted from some tissue remnants of  
172 a wide range of single specimen (e.g., tooth, bone, hair, eggshell, feather). In contrast,  
173 environmental DNA (eDNA) focuses on disseminated genetic material found in environmental  
174 samples such as soil, sediment, water and ice (Taberlet et al., 2012a). Such samples contain  
175 complex mixtures of DNA from taxonomically diverse organisms (e.g., bacteria, archaea,  
176 plants, animals). In addition to aDNA and eDNA, the term sedimentary aDNA (*sedaDNA*) has  
177 been coined to describe aDNA that is exclusively recovered from sediments (Willerslev et al.,  
178 2003; Jørgensen et al., 2012). The term fossil DNA has also been used in pioneer studies  
179 where sedimentary plankton DNA and lipid biomarkers (i.e., “chemical fossils”) derived from  
180 the same historical source organisms were analysed in parallel to validate the ancient DNA

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181 results (e.g., Coolen and Overmann, 1998; 2007; Coolen et al., 2004). To a lesser degree,  
182 ‘palaeo-environmental DNA’ (PalEnDNA) has also been used to describe disseminated  
183 genetic material in a broad range of ancient environmental samples including sediments as  
184 well as soil, paleosols, coprolites, water and ice (Rawlence et al., 2014). Modern sequencing  
185 technologies and bioinformatic tools ease the analysis of these complex environmental aDNA  
186 samples and of the biological responses to human or climate change, with investigations  
187 having focussed on terrestrial settings (Jørgensen et al., 2012; Giguët-Covex et al., 2014;  
188 Willerslev et al., 2014; Alsos et al., 2015; Pansu et al., 2015). In this review, we use the term  
189 ‘marine *seda*aDNA’, which specifically refers to aDNA recovered from ocean sediments. A  
190 detailed list of terms frequently used in aDNA research and their definitions is given in Table  
191 1.

192

## 193 2.2 Authenticity of marine aDNA

### 194 2.2.1 Environments favourable for marine aDNA preservation

195 Organic-rich sediments deposited in the deep, cold ocean under stratified and anoxic  
196 conditions present several favourable characteristics for the preservation of aDNA (e.g.,  
197 Coolen and Overmann, 1998; 2007; Coolen et al., 2004; 2013; Boere et al., 2011). Oxidative  
198 and deamination damage is reduced in the absence of oxygen (Lindahl, 1993). The absence  
199 of irradiation (Lyon et al. 2010), the generally low temperatures (Willerslev et al., 2004), and  
200 the high concentration of borate (Furukawa et al., 2013) further contribute to DNA  
201 preservation. Additionally, the typically high mud content of deep-sea sediment offers a  
202 particularly well-suited matrix for the preservation and accumulation of DNA (Torti et al., 2015).  
203 The high surface:volume ratio of extremely small clay minerals in clay-rich sediments offer a  
204 high adsorption surface onto which DNA molecules can bind and remain sheltered from the  
205 activity of nucleases (Dell’Anno et al., 2002; Corinaldesi et al., 2008, 2011, 2014, 2018).  
206 However, although the above listed properties have been reported to positively impact on DNA  
207 preservation, locations with other characteristics that seem less ideal might still be suitable for

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208 aDNA research. For example, well-oxygenated Atlantic deep-sea sediments and sand-rich  
209 coastal paleo-tsunami deposits have been used to extract and characterise aDNA from  
210 foraminifera (Lejzerowicz et al., 2013; Szczuciński et al., 2016, respectively). In conclusion,  
211 the preservation of aDNA in marine settings appears to be variable depending on regional  
212 environmental characteristics with less favourable to favourable conditions retaining aDNA  
213 between a few thousand to, at least, a few ten thousand years. More research is needed to  
214 estimate how far back in time authentic marine *seda*aDNA can be detected, which could be  
215 achieved, for example, by investigating sediment records from various deep seafloor locations  
216 over geological timescales.

217

#### 218 2.2.2 Marine *seda*aDNA degradation and fragment length

219 18S rRNA gene fragments of past dinoflagellates, diatoms, and haptophytes as long as 500  
220 bp in length have been amplified and sequenced (e.g., Coolen et al., 2004), after DNA was  
221 isolated from sediments exhibiting characteristics favourable for aDNA preservation (Section  
222 2.2.1). Up to 20% of genomic DNA from haptophyte algae has been reported to still be of high  
223 molecular weight after 2,700 years of deposition in Black Sea sediments, and the ratio  
224 between 500 bp-long haptophyte 18S rDNA fragments and the concentration of haptophyte-  
225 diagnostic long-chain alkenones did not vary substantially for at least 7,500 years after  
226 deposition, indicative that both types of biomolecules from the same plankton source were  
227 equally well preserved (Coolen et al., 2006). This contradicts the generalised view that aDNA  
228 is characterised by fragment lengths of <100bp. Nevertheless, studies that report the recovery  
229 of exceedingly long aDNA fragments should be viewed with scepticism especially in the  
230 absence of sampling and extraction controls, where there is no indication on whether the data  
231 might reflect modern signals. However, to date, no data are available on average aDNA  
232 fragment length for deep-sea sediments, which could be obtained from metagenomic shotgun  
233 sequencing. Gaining insights into the latter should be the focus of future research as this

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234 information will ultimately help to choose the most suitable and efficient aDNA extraction and  
235 sequencing library preparation techniques for degraded *seda*DNA (see Section 3.5).

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### 237 2.2.3 Contamination sources by modern DNA

238 Key to the viability of marine *seda*DNA studies is the capability to differentiate between true  
239 ancient signals (representative that lived at a particular time-period in the past) and modern  
240 contamination (introduced through the sampling and analysis process, or naturally by the  
241 environment). Microorganisms and their DNA coat nearly every part of this planet (Weyrich et  
242 al., 2015) and a recent study has shown that slow-growing microbes even occur in marine  
243 sediments up to 2.5 km deep (Inagaki et al., 2015). The DNA of active deep-biosphere  
244 organisms is likely to blur the aDNA signal, as would be the case for microorganisms  
245 introduced to ancient sediment samples through the drilling process (see Section 3.2).  
246 Moreover, microbial DNA is widely present in laboratory environments and reagents, including  
247 in those labelled DNA-free (Salter et al., 2014). If PCR is applied to amplify aDNA, the DNA  
248 from modern microorganisms may amplify preferentially over damaged, fragmented aDNA  
249 and obscure the true aDNA signals within the sample (Willerslev and Cooper, 2005).  
250 Therefore, utmost care must be taken to control and account for contaminants and background  
251 DNA throughout the whole process of collecting, processing and sequencing aDNA, e.g., by  
252 including negative controls in every step of the analysis process (Fig. 1).

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### 254 2.2.4 Intracellular vs. extracellular DNA

255 One approach to separating ancient from modern DNA in sediments has been to differentiate  
256 between intracellular and extracellular DNA. Intracellular DNA is defined as DNA contained  
257 within living cells, structurally intact dead cells and intact resting stages (e.g., bacterial spores,  
258 or other cyst-forming plankton). Extracellular DNA is defined as DNA that has been released  
259 from cells and preserved for substantial periods of time through mineral and/or microfossil



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260 adsorption or within clay aggregates (Levy-Booth et al., 2007). Extracellular DNA represent  
261 an archive of taxa that were autochthonous at the time of deposition (Cornaldesi et al., 2008;  
262 2011). DNA extraction methods have been developed to target either of these DNA fractions  
263 (Cornaldesi et al., 2005; Taberlet et al., 2012b; Alawi et al., 2014). However, it is difficult to  
264 prove at what time in the past the organism died, and its DNA became extracellular.  
265 Furthermore, the extra- and intracellular DNA pool may not always be clearly distinguishable  
266 as genetic material present in the environment might have been taken up by competent  
267 bacteria (Demanèche et al., 2001; Dell'Anno et al., 2004) and even by eukaryotes (Overballe-  
268 Petersen and Willerslev, 2014). It is also important to note that if only the extracellular pool  
269 was to be studied, the paleontological value of dormant yet ancient DNA (e.g., from cysts  
270 deposited far back in time) will be lost. Due to these issues, extraction techniques targeting  
271 only the extracellular portion are currently not recommended for marine *seda*DNA studies.  
272 Alternatively, bioinformatics approaches that can clearly identify ancient signals (Ginolhac et  
273 al., 2011; Kistler et al., 2017) are preferred options for authenticating aDNA sequences  
274 (Jónsson et al., 2013).

### 275 276 2.2.5 Vertical DNA movement in marine sediment cores

277 Three major processes are associated with the vertical movement of DNA in sediment cores:  
278 DNA leaching, bioturbation and migration. Bioturbation is a biomechanical process that results  
279 in the multidirectional re-organisation of sediments primarily in the upper 10 cm of the sub-  
280 seafloor (Boudreau, 1998). DNA leaching is a passive process describing the downward  
281 movement of DNA across sediment layers (Haile et al., 2007), without a lowermost boundary.  
282 The mixing of sediment layers, and consequently of modern and ancient DNA, can lead to  
283 misinterpretations of genomic data. Experimental trials to assess DNA leaching through  
284 terrestrial sediments exist (Ceccherini et al., 2007; Poté et al., 2007), with initial results  
285 indicating that the extent of leaching depends on the taxonomic source (Haile et al., 2007). In  
286 Previous studies from lake sediments have shown that leaching is not a factor (Parducci et

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287 al., 2017), and in seafloor sediments DNA it seems to play a minor role as aDNA and lipid  
288 biomarkers derived from the same microbial source were found to co-exist or to be both below  
289 detection limit in marine sediments just centimetres apart (Boere et al., 2009; Coolen et al.,  
290 2006; 2009; 2013). In the latter studies it therefore appears that the pore size of the laminated  
291 sediments was too small for intracellular DNA to migrate, and that all extracellular plankton  
292 DNA was adsorbed to the mineral matrices. Recent studies showing *upwards* vertical pore  
293 fluid movement also demonstrate the potential for vertical migration of relict or intact DNA  
294 within sediments (Torres et al., 2015), and should likewise be considered. Vertical migration  
295 of relict or intact DNA is expected to be especially a concern in sediments with micron scale  
296 pore sizes and/or a low clay content and a poor capacity to adsorb extracellular DNA. Future  
297 experimental research is required to quantify DNA leaching and/or migration through marine  
298 sediments, acknowledging the challenge of replicating a complex environmental system  
299 varying widely in hydrodynamics and sediment type.

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301 2.2.6 Cross validation of marine aDNA and palaeo-environmental proxies

302 In addition to using proper contamination controls, downcore changes in past plankton  
303 compositions inferred from marine *seda*DNA can be validated through a complementary  
304 analysis of independent biological (e.g., microfossils, lipid biomarkers) and geochemical  
305 proxies (indicative of the prevailing paleoenvironmental conditions) (Boere et al., 2009; Coolen  
306 et al., 2004; 2006; 2013; Hou et al., 2014; More et al., 2018). The most detailed comparison  
307 between past ecosystem changes using marine *seda*DNA and the paleo-depositional  
308 environment to date has been performed on Holocene sediments from the permanently anoxic  
309 and sulfidic Black Sea (Coolen, 2011; Coolen et al., 2006; 2009; 2013; Giosan et al., 2012;  
310 Manske et al., 2008). The anoxic and laminated sediments of this semi-isolated sea are devoid  
311 of bioturbation and form high-resolution archives of climate-driven hydrological and  
312 environmental changes (Calvert et al., 1987; Hay, 1988). Episodes of postglacial sea-level  
313 rise ~9,000 years ago (Major et al., 2006) and sea surface salinity increase ~5,200 years ago

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711 314 (Giosan et al., 2012) have been associated based on *sedaDNA* with freshwater to  
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713 315 brackish/marine planktonic community transitions (Coolen et al., 2013). For example, the  
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715 316 gradual increase in sea surface salinity coincided with the arrival of marine copepods (*Calanus*  
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717 317 *euxinus*), which could only be identified through *sedaDNA* analysis (Coolen et al., 2013) as  
718  
719 318 these important zooplankton members generally do not leave other diagnostic remains in the  
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721 319 fossil record besides difficult to distinguish resting eggs (Marcus et al., 1996).  
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723 320 *Vice versa*, paleoenvironmental conditions inferred from more traditional geochemical  
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725 321 and micropaleontological proxies have been verified from parallel *sedaDNA* analysis. By way  
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727 322 of example, Black Sea sediments deposited since the last 2,500 years contain coccoliths from  
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729 323 the calcified marine haptophyte *Emiliana huxleyi* whereas haptophyte-derived diagnostic long  
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731 324 chain alkenones in the absence of coccoliths were abundant in up to 7,500-year-old sediments  
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733 325 (Hay et al., 1991; Coolen et al., 2009). Paired analysis of long-chain alkenones and *sedaDNA*  
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735 326 analysis (18S rRNA) revealed that that the first haptophytes that colonized the Black Sea  
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737 327 ~7,500 years ago were initially a mixture of *E. huxleyi* and a highly diverse suite of previously  
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739 328 overlooked non-calcified haptophytes related to alkenone-producing brackish *Isochrysis*  
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741 329 species. *E. huxleyi* remained the only alkenone producer after 5,200 years BP when salinity  
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743 330 reached modern day levels (Coolen et al., 2009). It was concluded that while calcite dissolution  
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745 331 prevented the preservation of *E. huxleyi* coccoliths in sediments older than 2,500 years ago,  
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747 332 their molecular fossils (DNA fragments and long-chain alkenones) survived much longer and  
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749 333 showed that in reality this marine haptophyte entered the Black Sea already shortly after the  
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751 334 marine reconnection which occurred ~9,000 years ago (Coolen et al., 2009; 2013). Even more  
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753 335 detailed analyses of *E. huxleyi* (targeting 250-bp-long mitochondrial cytochrome oxidase  
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755 336 subunit I; mtCOI) indicate a series of transitions from possibly low-salinity to high-salinity  
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757 337 adapted strains of *E. huxleyi* in the Black Sea (7.5 – 5.2 ka BP), to a different suite of strains  
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759 338 during the most marine stage (5.2 – 2.5 ka BP), returning to low salinity strains after 2.5 ka  
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761 339 BP. The latter transition coincides with the onset of the cold and wet Subatlantic climate  
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763 340 (Coolen, 2011) when the Black Sea experienced re-freshening from 32 to 18 ppt (Van der  
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765 341 Meer et al., 2011; Giosan et al., 2012; Coolen et al., 2013). The analysis of similar length  
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342 preserved sequences of viral major capsid protein (mcp) genes revealed a continuous co-  
343 existence of *E. huxleyi* and coccolithoviruses in the Black Sea since the last 7,000 years and  
344 that the same *E. huxleyi* strains, which occurred shortly after the marine reconnection returned  
345 with the same viral strains after the re-freshening during the Subatlantic climate thousands of  
346 years later (Coolen, 2011). More recently, detailed sedimentary 18S rDNA profiling targeting  
347 the shorter (130 bp) V9 region revealed that long-term expansion of past oxygen minimum  
348 zones (OMZ) created isolated habitats for unicellular eukaryotes (protists) capable of  
349 sustaining oxygen depletion either by adapting a parasitic life cycle (e.g., apicomplexans) or  
350 by establishing mutualistic connections with others (e.g., radiolarians and mixotrophic  
351 dinoflagellates). These examples show that *sed* aDNA can be used to identify biological  
352 sources of lipid biomarkers, to verify the reliability of paleoenvironmental information inferred  
353 from more traditional proxies, and to reconstruct past ecosystems at multiple trophic levels.

354 The reconstruction of subseafloor prokaryote communities is more complicated since the  
355 DNA may be derived from living intact cells in the sediment (see Section 2.2.4). However, 16S  
356 rRNA gene profiling from total (intracellular and extracellular) sedimentary DNA has revealed  
357 useful insights into sub-seafloor microbial indicators of the palaeo-depositional environment.  
358 For example, microbiomes in 20 million years-old coalbeds underlying 2 km of marine  
359 sediments were shown to resemble forest soil communities (Inagaki et al., 2015). Variations  
360 in bacterial communities found in Baltic Sea sediments have been linked to palaeo-salinity  
361 changes (Lyra et al., 2013). Orsi et al. (2017) showed that the genomic potential for  
362 denitrification correlated with past proxies for oxygen minimum zone strength in up to 43 ka-  
363 old Arabian Sea sediments. The presence of fermentation pathways and their correlation with  
364 the depth distribution of the same denitrifier groups, however, suggests that these microbes  
365 were possibly alive upon burial, but low postdepositional selection criteria may explain why  
366 they nevertheless formed a long-term genomic archive of past environmental conditions  
367 spanning the last glacial-interglacial cycle (Orsi et al., 2017). Further studies are required to  
368 determine as to how far the persistence of this phenomenon extends with increased depth in

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369 the biosphere. Nevertheless, these examples show that the complementary analysis of marine  
370 *sedaDNA*-inferred past plankton composition and biological and geochemical proxies is a  
371 powerful tool to reconstruct palaeo-environments.

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### 373 **3 aDNA research in the International Ocean Discovery Program (IODP) framework**

#### 374 3.1 IODP infrastructure

375 IODP is the global community's longest marine geoscience program, operating for 51 years.

376 Its scientific strategy has been to answer globally-significant research questions about the

377 Earth's structure, and the processes that have, and continue to, shape our planet and its

378 climatic history. More recently, additional focus has been cast on biological evolution and

379 limits, particularly in the sub-seafloor environment, under the new Biosphere Frontiers theme

380 (Bickle et al., 2011). This theme has been inspired by the rapidly evolving knowledge and

381 technical capabilities across the multiple merging fields of molecular biology, microbiology,

382 organic and inorganic geochemistry, and micropalaeontology and includes scope for the

383 integration of marine *sedaDNA* research. IODP is currently serviced through three platforms,

384 the United States of America's research vessel *JOIDES Resolution*, Japan's *Chikyu* and by

385 the European consortium's Mission Specific Platforms (MSP). In recent years, the laboratories

386 and storage facilities on the ships were modified, or purpose built, to ensure addressing Deep

387 Biosphere questions was possible. As a result, the latest IODP decadal plan considered

388 options to enable access to uncontaminated samples, their processing and preservation on-

389 board. The latter has led to new coring technologies such as the Half-Length Advanced Piston

390 Corer (HLAPC) allowing a coring depth extension of the conventionally used Advanced Piston

391 Corer (APC), and the use of chemical contamination tracers such as perfluorocarbon tracers

392 (PFTs) (see Sections 3.2 and 3.3, respectively). Particularly useful to aDNA studies may be

393 the development of remotely controlled instruments allowing sediment sampling at ambient

394 pressure (MeBo; Pape et al., 2017) and a rock-drilling device (RD2; Früh-Green et al., 2015).

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395 Notable achievements under the new Deep Biosphere theme include the finding of millions of  
396 years old active microbial community from coal beds buried at 2.5 km below the seafloor  
397 (Inagaki et al., 2015), and the preservation of an imprint of the Chicxulub impact catastrophe  
398 (Cockell et al., 2017). A lot remains to be understood before this theme and its challenges are  
399 satisfactorily addressed and it is clear that scientists engaging in Biosphere Frontiers will push  
400 methodological, technological and multidisciplinary studies.

401

### 402 3.2 Coring strategies suitable for marine *seda*DNA retrieval

403 Ideally, marine *seda*DNA sampling involves multiple spatial replicates to ensure that the  
404 biodiversity captured is representative of a particular site and time period. However, the ability  
405 to collect multiple deep ocean sediment cores to characterise palaeo-plankton is hindered by  
406 high costs and logistical issues associated with drilling operations. Thorough planning and  
407 collaboration to maximise the use of expensive expeditions and precious deep ocean  
408 sediment core material are indispensable in marine *seda*DNA research. To date, several  
409 coring strategies exist that differ in machinery as well as sub-seafloor depth that can be  
410 reached, and their application is largely dependent on which drilling platform is used (ship or  
411 MSP), and what type of sediment is to be cored/drilled (soft sediment or hard rock). This review  
412 concentrates on describing piston coring strategies, which are generally better suited to  
413 retrieve sediments for aDNA analysis due to relatively low contamination risks. Rotary core  
414 barrel systems are required to drill some sedimentary and most igneous rocks, and as they  
415 operate with drill-heads and drilling fluids (e.g., seawater) the risk of contamination is  
416 dramatically increased (see Section 3.3, Fig. 2).

417 Piston coring, referred to as Advanced Piston Coring (APC) or Hydraulic Piston Coring System  
418 (HPCS), is used to sample unconsolidated or poorly consolidated (i.e., softer) marine  
419 sediments. Briefly, these instruments are pushed into the sediment while a piston inside the  
420 core pipe creates a vacuum so that the collected sediment remains in the pipe during retrieval.

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421 Sediments obtained by piston coring preserve laminated sediments well, are associated with  
422 a relatively low risk of environmental contamination and the preferred method to obtain  
423 sediments for aDNA analysis (Lever et al., 2006; Smith et al., 2000; More et al., 2018; Fig. 2).  
424 Using the piston coring approach, a successive recovery of marine sediments has been  
425 achieved to a depth of ~490 m below seafloor (mbsf) (Tada et al., 2013). If only a few metres  
426 long (soft) sediment cores are required, gravity-based coring systems, such as a Kasten-, or  
427 a Multicorer provide a good alternative (Coolen et al., 2004; 2009). Progress has also been  
428 made towards modifying piston coring instruments so that contamination-free sampling is  
429 possible, at least for short (<4 m) sediments (Feek et al., 2011). For example, the 'Mk II  
430 sampler' uses an air and water-tight piston coring system with a pointed aluminium head,  
431 preventing contamination of the sampled sediment from smearing or water infiltration (Feek et  
432 al., 2011). However, to date this corer has only been used in shallow waters, thus it remains  
433 to be tested whether use of such an instrument would be feasible during coring operations in  
434 deeper waters and which modifications may be required.

435

### 436 3.3 Contamination tracing during coring

437 Deep ocean coring requires the lowering of coring instruments through hundreds to thousands  
438 of metres of seawater before the seafloor is reached, hence exposes the instruments to  
439 contamination by modern DNA (Fig. 1). This unavoidable issue has called for the development  
440 of methods for environmental DNA contaminant tracing during coring operations. One  
441 approach has been to compare biological material found in the contaminating source material  
442 (e.g., seawater, drilling fluid) to that of sub-seafloor communities, and to exclude all signals  
443 occurring in either from the final analyses (e.g., Expedition 330 Scientists, 2012; Cox et al.,  
444 2018). This approach can be implemented for either piston coring or rotary core barrel drilling,  
445 provided other sampling constraints associated with these coring systems can be  
446 accommodated. However, this procedure does not account for potential "false negative" DNA  
447 signals that might indeed occur in both ancient sediments *and* modern contaminating material.

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1006 448 However, in some cases, the microbial community structure of modern contamination (e.g.,  
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1008 449 drilling “mud”) can be resolved, particularly if functional genes are being targeted in sediment  
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1010 450 samples (Cox et al., 2018).  
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1012  
1013 451 Another approach has been the introduction of fluorescent microspheres, which are particulate  
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1015 452 tracers of 0.2 - 1.0 mm in diameter physically mimicking contaminating organisms. The  
1016  
1017 453 microspheres have been introduced near the coring head, i.e., where the sediment enters the  
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1019 454 corer and coring pipe, spreading across the outside of the core (inside the pipe) while drilling,  
1020  
1021 455 simulating particle movement (Expedition 330 Scientists, 2012; Orcutt et al., 2017).  
1022  
1023 456 Microscopy has been used to quantify the number of microspheres at the periphery and in the  
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1025 457 centre of the core to assess contamination (Expedition 330 Scientists, 2012; Orcutt et al.,  
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1027 458 2017). Similar methods using other perfluorocarbon tracers (PFT’s) including  
1028  
1029 459 perfluoromethylcyclohexane (PMCH) have been developed for the USA drilling vessel  
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1031 460 *JOIDES Resolution* (Smith et al., 2000) already in the early phases of IODP. Later, PMCH-  
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1033 461 based contamination tracing has also been applied during riser drilling on the *Chikyu* (Inagaki  
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1035 462 et al., 2015). During the IODP Expedition 357 (Atlantis Massif Serpentinization and Life), the  
1036  
1037 463 PMCH tracer delivery system was further developed to fit the seafloor-based drilling systems  
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1039 464 MeBO (Pape et al., 2017) and RD2 (Früh-Green et al., 2015) (see Section 3.1). PMCH is  
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1041 465 highly volatile which can lead to false positive measurements in uncontaminated samples,  
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1043 466 therefore, more recent investigations during IODP expeditions have moved to the use of the  
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1045 467 heavier chemical tracer perfluoromethyldecalin (PFMD, 512.09 g mol<sup>-1</sup>) (e.g., Fryer et al.,  
1046  
1047 468 2018).  
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#### 1049 469 1050 1051 470 3.4 Subsampling after core acquisition 1052

1053 471 Key to enable interdisciplinary sampling and correlations of independent measurements is a  
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1055 472 detailed sampling plan, specifying sample types as well as the sequence in which these  
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1057 473 samples are to be collected. Sampling for aDNA is time-sensitive (to avoid exposure to  
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474 oxygen, high temperatures and contamination), thus should be conducted immediately after  
475 core retrieval on an untreated core-half (i.e., prior to any type of scanning such as by X-Ray).  
476 The laboratory in which subsampling for aDNA is carried out should be clean and  
477 workbenches and surfaces decontaminated with bleach (considered to be most efficient at  
478 removing contaminating DNA) and, if applicable, ethanol (to prevent corrosion of metal after  
479 bleach-treatment). Detailed records on whether molecular and amplification techniques (i.e.,  
480 PCR) have been employed in on-board laboratories and which organisms were targeted  
481 should be kept on record within IODP to ensure sampling for aDNA can be spatially separated  
482 from these laboratories. While most vessels are not currently equipped for complete DNA  
483 decontamination, such records may be invaluable for post-expedition aDNA data analyses.

484  
485 Two sampling approaches are the most feasible on board IODP ships and MSP's: cutting  
486 whole round cores or direct subsampling after core cutting into 1.5 m long sections. The choice  
487 of approach needs to be made on a case-by-case basis, and depends on the specific facilities,  
488 consumables, chemicals and researcher expertise available during each mission. It is  
489 recommended that cutting or subsampling are performed under filtered air, e.g., a portable  
490 type of a horizontal laminar flow clean air system as described in Morono and Inagaki (2016).  
491 Additionally, subsampling should be conducted from the bottom to the top of the core (ancient  
492 to modern), using clean (e.g., bleach and ethanol treated) sampling tools for each sample to  
493 avoid any form of cross-contamination. Most commonly, soft sediments acquired by piston  
494 coring are used for *sedaDNA* analyses, therefore, we focus on subsampling procedures of the  
495 latter here, subsequently briefly outlining sampling recommendations for hard rock material.

496  
497 If the sampling decision is in favour of whole round core samples, the newly acquired core  
498 sections are cut into 5 - 50 cm sections (preferably under cold conditions), which should be  
499 packed in sterile bags or wrap and transferred directly into a fridge or freezer. Although quick  
500 and providing a large amount of material for later sub-sampling, this approach has the

1122  
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1124 501 disadvantage that a lot of freezer space is required, and post-expedition transport can be  
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1126 502 costly due to the high sample volume and weight.  
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1128 503  
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1130 504 An alternative to whole round core cutting is direct subsampling immediately after core cutting,  
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1132 505 either directly from the centre of the top or bottom of each unsplit core section (usually 1.5 m  
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1134 506 long), or after or splitting the core sections into two halves. In any case the core liner should  
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1136 507 be cleaned with bleach to remove potential contamination from seawater, and core cutters  
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1138 508 and splitting-wires, usually metal and sensitive to bleach should be cleaned with ethanol. If  
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1140 509 sampling from uncut sections, surface material (~0.5 cm) should be removed with bleach and  
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1142 510 ethanol-treated scrapers before sampling, which is most easily done with sterile cut-tip  
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1144 511 syringes, placed into sterile plastic bags and stored frozen.  
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1148 513 If sampling is undertaken on split core halves, simultaneous visual sedimentological  
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1150 514 assessments are possible that enable more targeted sampling at specific depths of interest.  
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1152 515 Using DNA-clean tools, the top 0.5 cm of the core surface should be scraped off perpendicular  
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1154 516 to the core pipe using sterile scrapers (from bottom to top of the core). Alternatively, the core  
1155  
1156 517 half to be sampled can be covered with plastic wrap, followed by powdered dry ice, which will  
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1158 518 result in the top 0.5 cm to become solid frozen. After 5 min, the frozen outer sediment layer  
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1160 519 can be lifted at one edge with a sterile scalpel creating a contaminant-free surface, from which  
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1162 520 subsamples can be taken (Coolen and Overmann, 1998). Then, subsampling should be  
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1164 521 undertaken using sterile (e.g., gamma-irradiated) plastic syringes or centrifuge tubes (e.g.,  
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1166 522 capacity of ~15 mL). Cut-tip syringes have the advantage that more sediment can be collected  
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1168 523 as no pressure builds up when pushing the syringe into the sediment (the filled syringe should  
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1170 524 be placed into a sterile plastic bag immediately, e.g., Whirl-Pak®). Alternatively, sterile  
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1172 525 centrifuge tubes can be used as is to collect 'plunge-samples', usually providing ~1 - 3 cc of  
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1174 526 sediment material. The outside of the 'mini-cores' should be cleaned with bleach and placed  
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1176 527 into sterile plastic bags to avoid cross-contamination between samples. For subsamples,  
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1178 528 storage at -20 °C or -80 °C is recommended as freezing has been shown to facilitate  
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1183 529 phytoplankton cell-lysis during DNA extractions (Armbrecht et al., *in prep.*). Sub-samples can  
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1185 530 also be collected by transferring a small amount of sediment into a sterile microcentrifuge tube  
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1187 531 using clean metal or disposable spatulas (particular care needs to be taken to avoid cross-  
1188  
1189 532 contamination when using the same sampling tool for different samples). The latter approach  
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1191 533 may be a good solution when only a few small samples are required, e.g., to supplement other  
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1193 534 scientific questions of an ongoing expedition. For replication purposes it is recommended that  
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1195 535 duplicate samples are taken at each depth.

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1199 537 If the material is hard rock or similar, subsamples are most easily collected from whole round  
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1201 538 or split cores. The same decontamination procedures as outlined above should be considered  
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1203 539 throughout the subsampling procedure (i.e., decontamination of work-surfaces and sampling  
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1205 540 tools with bleach and ethanol, sampling under cold conditions and filtered or low air-flow,  
1206  
1207 541 packing of samples into sterile bags before storage). A de-contaminated metal cutter or a  
1208  
1209 542 hammer and chisel are best used to remove the outer layer of the exposed sediment, at least  
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1211 543 at those depths where subsampling is anticipated.

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### 1217 546 3.5 Marine aDNA sample processing and analysis

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1219 547 Marine aDNA samples should be processed in a specialised aDNA laboratory to prevent  
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1221 548 contamination with modern DNA. Such a laboratory is generally characterised by creating a  
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1223 549 low-DNA environment, with a clear separation of no-DNA (e.g., buffer preparation) and DNA-  
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1225 550 containing (e.g., DNA extraction) workflows, regular and thorough sterilisation procedures,  
1226  
1227 551 positive air pressure, and protective clothing of the analyst (lab coat/suit, gloves, facemask,  
1228  
1229 552 visor). Details on optimised laboratory set-up, techniques and workflows have been reviewed  
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1231 553 before (Cooper and Poinar, 2000; Pedersen et al., 2015). The introduction of aDNA samples  
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1233 554 into such facilities is relatively straight-forward, as the outer packaging and surface of the  
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1235 555 sample can be easily sterilised (e.g., using bleach and/or UV).

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556 As on-board subsampling, DNA extractions should be carried out from the most ancient to  
557 most recent samples, to prevent modern DNA inadvertently being carried to ancient samples.  
558 The amount of sediment used in DNA extractions should capture a representative picture of  
559 the biota present in a sample. Despite suggestions that bulk DNA extractions from up to 10 g  
560 of material can improve detection of taxa and better represent the diversity of the area of  
561 interest (e.g., Taberlet et al., 2012b; Coolen et al., 2013), using such large volumes of  
562 sediment is often not practical and can be quite costly in this field where typically many  
563 samples are processed. Instead, numerous studies have used replicate extractions of a  
564 smaller sample size (e.g., 0.25 g; Table 2) to increase the likelihood of yielding aDNA from  
565 rare taxa, as well as successive DNA extractions from a single 0.25 g sediment sample (e.g.,  
566 Willerslev et al., 2003). Post-extraction, the use of RNA-probe based enrichment approaches  
567 coupled with shotgun sequencing, a common technique in aDNA research, may furthermore  
568 drastically improve the detection of rare taxa (Horn et al., 2012).

569 While it would be ideal to find one extraction method that will yield the best quality data and  
570 enable standardisation across ancient marine sediment studies, the type of sediment or target  
571 organisms may require some adjustments of standard protocols (Hermans et al., 2018).  
572 Extraction methods can bias the diversity observed due to differential resilience of taxa to the  
573 cell-lysis method (Zhou et al., 1996; Young et al., 2015) and DNA binding capacities of  
574 different soil and sediment types (Lorenz and Wackernagel, 1994; Miller et al., 1999). As a  
575 result, the aDNA extraction efficiency can be poor and the detection of an aDNA signal lost.  
576 To date, a variety of commercial kits have been successfully used to isolate aDNA from  
577 sediments (Table 3). To further increase the yield of very low amounts of highly fragmented  
578 aDNA several studies have been utilising extraction protocols that include a liquid-silica DNA  
579 binding step (e.g., Brotherton et al., 2013 and Weyrich et al., 2017 for non-sediment samples)  
580 or ethylenediaminetetraacetic acid (EDTA) cell-lysis step (Slon et al., 2017; utilising cave-  
581 sediment samples). Other studies have replaced the Bead Solution in the DNeasy extraction  
582 kits (Qiagen; Table 2) by 1M sodium phosphate pH 9 - 10 and 15 vol% ethanol to efficiently

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1301 583 release clay-adsorbed DNA, and to prevent DNA released from intact cells from adsorbing to  
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1303 584 clay minerals during the extraction (Direito et al., 2012; Orsi et al., 2017; More et al., 2018).  
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1305 585 The latter is especially important when working with low organic, high carbonate rocks and  
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1307 586 sediments (Direito et al., 2012).  
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1310 587 Two points are particularly important to prevent contamination during extractions. Firstly, as  
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1312 588 with the samples themselves, it is crucial that all tools and reagents undergo rigorous  
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1314 589 sterilisation procedures before utilisation, such as by bleach and UV treatment of any packing  
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1316 590 material before entering ancient DNA facilities. Secondly, blank controls should be included  
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1318 591 for every step of the laboratory process, i.e., extraction/library preparation blank controls,  
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1320 592 sequencing and bioinformatic analysis controls (Ficetola et al., 2016). Controlling and  
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1322 593 monitoring contamination is particularly important when analysing bacterial diversity due to  
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1324 594 their presence in all laboratory environments and reagents (Weyrich et al., 2015). Optimally,  
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1326 595 extraction blanks are included in a 1:5 ratio (Willerslev and Cooper, 2005), with a bare  
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1328 596 minimum of one control with each set of extractions. Aside from bioinformatically removing  
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1330 597 any organisms determined in such extraction blanks from the investigated sample material,  
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1332 598 the contaminants should be tracked within a laboratory, and contaminant lists published  
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1334 599 alongside the data for reasons of data transparency and authenticity.

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1336 600 Post-extraction, many marine aDNA studies have employed methods that are routinely used  
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1338 601 for modern marine DNA analysis. Although modern DNA work is not exempt from precautions,  
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1340 602 there are several issues with aDNA work: (i) as outlined in Sections 2.1. and 2.2. aDNA is  
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1342 603 highly fragmented and degraded and any small amount of modern DNA present in the sample  
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1344 604 (from reagents, labs or living cells that were present in the sediment sample) will amplify over  
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1346 605 the aDNA; (ii) sampling and extraction controls are often not included in the sequencing  
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1348 606 sample; (iii) PCRs are often inhibited due to the co-extraction of humic substances, pigments  
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1350 607 and heavy metals along with DNA (Webster et al., 2003 and references therein), requiring  
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1352 608 adequate removal of these impurities (e.g., Coolen et al., 2009); (iv) successful PCRs are  
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1354 609 prone to bias due to random amplification in reactions that contain very low amounts of DNA  
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610 template, thus PCR drift (stochastic variation in the first PCR cycles) can occur (Wagner et al.,  
611 1994; Polz and Cavanaugh, 1998; Webster et al., 2003). More importantly, the number of,  
612 e.g., bacterial 16S and eukaryotic 18S rRNA operons can greatly vary between per genome  
613 and per cell and can cause a biased representation of the past community structure (e.g.,  
614 Klappenbach et al., 2001). The above biases can be reduced and the detection limit lowered  
615 when PCR approaches selectively, amplifying particular groups of organisms indicative of  
616 environmental changes, are paired with independent geochemical proxies (e.g., Coolen et al.,  
617 2004; 2006; 2009). However, we strongly advocate for the use of strict aDNA methodologies  
618 and facilities in order to achieve the generation of authentic marine *seda*DNA data, following  
619 the guidelines in this review.

620 Shotgun metagenomics are currently widely accepted and the least biased method to analyse  
621 the broad diversity of ancient environmental samples (e.g., Slon et al., 2017). Although only a  
622 small portion of the generated sequence data might be attributable to the ancient organism in  
623 question (Morard et al., 2017), next generation sequencing (NGS) generates large quantities  
624 of data that enable meaningful statistics, with the additional benefit of preserving the relative  
625 proportion of detected taxa. To analyse aDNA sequence data, robust bioinformatic pipelines  
626 (e.g., Paleomix, Schubert et al., 2014) have been developed and are available for the  
627 application to marine *seda*DNA, integrating damage detection algorithms (e.g., Ginolhac et al.  
628 2011; Kistler et al., 2017) that enable the distinction between ancient and modern signals.  
629 Determining the extent of cytosine residues deamination (C to T and G to A, Weyrich et al.  
630 2017) should also be considered to assess authenticity of aDNA sequences, especially when  
631 the data was generated from mixed communities, such as from marine *seda*DNA. It is  
632 furthermore crucial to carefully screen sequencing data for any low-complexity reads, which  
633 may get incorrectly assigned to taxa during alignments against genetic databases, as well as  
634 ensuring that taxonomic assignments in the database of choice are correct. Bioinformatic  
635 pipelines removing such misidentification-derived errors do not currently exist and should be  
636 the focus of future research, as well as the comparison of shotgun and amplicon marine

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637 *se*dDNA data to accurately determine biases and analysis strategies best suited to this new  
638 discipline.

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#### 640 **4 Future marine aDNA sampling considerations**

##### 641 4.1 Equipment and installations required aboard IODP platforms

642 In addition to the recent upgrades and investments IODP has made to enable sediment  
643 sampling suitable for Biosphere Frontiers theme (Section 3.1) we suggest the following items  
644 to facilitate contamination-free sediment sampling and the tracing of contaminants.

645 (i) Laboratories in which sampling for aDNA is undertaken should be carefully chosen to  
646 minimise contamination. Rapid transport of the core from the deck to the lab, thorough  
647 decontamination measures (see Section 3.4), and easy access to fridges or freezers are  
648 crucial. While a positively air-pressured lab (standard for aDNA laboratories) may not be  
649 feasible, air-flow can be reduced by keeping all doors shut and fans off during aDNA sampling.  
650 Contamination by human DNA from analysts can be greatly reduced by wearing adequate  
651 protective clothing (gloves, facemask, freshly laundered/disposable lab coat/overall). A  
652 detailed record of any molecular work undertaken in ship-board labs should be maintained by  
653 IODP, and under no circumstances should aDNA sampling be conducted in labs used  
654 previously to run PCRs (see Section 3.4). Alternatively, the equipment of a shipping container  
655 exclusively dedicated to aDNA sampling could be a good solution to spatially separate aDNA  
656 sampling aboard drilling-platforms and installation could be as required during expeditions that  
657 involve aDNA sampling.

658 (ii) DNA is likely to behave quite different from chemical tracers and microspheres currently  
659 used to track contamination. With constantly advancing technologies in the field of synthetic  
660 biology, the possibility arises to develop 'non-biological DNA' with known sequences. Such  
661 non-viable DNA tags are already used in the oil industry, where a different tag is introduced

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662 into oil pipes monthly to monitor when and where leaks occur (Forecast Technology Ltd).  
663 Using such tags during seafloor coring operations instead of chemical tracers should enable  
664 a precise assessment of contamination by environmental DNA, where bioinformatics pipelines  
665 could be adjusted to detect and quantify the amount of tags present in the final sequencing  
666 data.

667  
668 4.2 Ground-truthing marine aDNA research and data

669 To ground-truth marine aDNA studies and to ensure the generation of authentic aDNA data  
670 we suggest future research in this field to focus on the following aspects:

671 (i) The establishment of a public record of common contaminants. This can be achieved, for  
672 example, through an inter-lab comparison focused on analysing the same samples and  
673 integrating extraction blanks to trace contaminants associated with particular coring  
674 equipment, ship- and land- based laboratories.

675 (ii) Investigation of factors that might considerably bias marine *seda*aDNA data. This might  
676 include information on sediment-type and environmental condition dependent aDNA  
677 preservation, taxon-specific DNA degradation rates, average aDNA fragment length, and  
678 shotgun and amplification-based aDNA data comparisons.

679 (iii) Ongoing enrichment of genetic reference databases for modern marine plankton, to enable  
680 taxonomic assignment of the hundreds of thousands of ancient sequences expected to be  
681 found in marine sediments.

682 (iv) The inclusion of negative controls during extractions, library preparations and in  
683 sequencing runs, and the publication of the results in the context of independent multiproxy  
684 biological and environmental metadata obtained from the same sediment interval.

685 (iv) Once (i) - (iv) are addressed, the development of a dedicated aDNA coring proposal is  
686 encouraged, in which sediment cores are collected using the above outlined, best-suited



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687 coring strategies, sampling and analysis procedures. During such an expedition, basic  
688 questions such as optimal on-board contamination tracing techniques, feasible work-flows,  
689 spatial replication required to achieve representative community data, and age to which  
690 marine *seda*DNA can be determined should be addressed. Such baseline data is missing to  
691 date and remains the most important step towards the generation of authentic aDNA data from  
692 marine sediments.

693  
694 **5 Application of marine *seda*DNA research guidelines to other contamination**  
695 **susceptible environments**

696 5.1 Permafrost

697 Permafrost molecular biological studies provide the opportunity to study living organisms that  
698 have successfully adapted to extremely cold environments and comprise an analogous  
699 cryogenic environment to that found on other planets, such as Mars (Amato et al., 2010).  
700 Molecular investigations have focussed on humans (Rasmussen et al., 2010), plants  
701 (Willerslev et al., 2003), megafauna (Boessenkool et al. 2012), fungi (Bellemain et al., 2013)  
702 and microbes (Willerslev et al., 2004). Permafrost top layers are characterised by a more  
703 abundant and diverse microbial community compared to the deeper soil (Gittel et al. 2014).  
704 To overcome the hurdle of distinguishing between the modern and ancient DNA signal,  
705 metatranscriptomics have been applied to identify the active community only (e.g., Coolen and  
706 Orsi, 2015). Despite the challenges in experimental approaches, such as rapid community  
707 shifts after thawing even at nearly ambient conditions (Negandhi et al. 2016), studies of  
708 permafrost environments have advanced our understanding of feedback loops associated with  
709 the response of extremophiles to warming, ultimately informing modelling studies including  
710 marine palaeo-environments.

711 Sampling for ice and permafrost in polar regions is challenging in terms of logistics and  
712 minimising contamination risks for both the sample and the sampled environment. For

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1596 713 example, permafrost soil samples are, like marine sediment cores, retrieved through drilling,  
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1598 714 which can introduce microbial contaminants to the deeper permafrost soil layers as the drill  
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1600 715 head and liquid pass through the top active soil layer (Bang-Andreasen et al., 2017).  
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1602 716 Additionally, the cryosphere has been accumulating industrial chemicals and metals since the  
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1604 717 1850's (McConnell et al., 2007), so that the present-day microbial community is now capable  
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1606 718 of degrading industrial contaminants, thereby representing an anthropogenically-adapted  
1607  
1608 719 rather than an original pristine community (Hauptmann et al., 2017). With both these newly  
1609  
1610 720 adapted anthropogenic and drilling fluid communities containing characteristics for heavy  
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1612 721 metal degradation, distinguishing indigenous ice core or permafrost communities from drilling  
1613  
1614 722 fluid communities will become more difficult in the future (Miteva et al., 2014). Therefore, the  
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1616 723 described guidelines in this review for distinguishing ancient from modern and contaminant  
1617  
1618 724 signals, as well as the need for aseptic sampling procedures, are highly applicable to  
1619  
1620 725 permafrost environments and, more generally, the cryosphere.

## 1622 726 1623 1624 1625 727 5.2 Planetary exploration 1626

1627 728 The methodologies advocated in this review that enable aDNA in marine sediments to be  
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1629 729 distinguished from modern DNA are also applicable to the search for life on other planets or  
1630  
1631 730 moons. Astrobiologists are especially interested in the possibility of detection of Life 2.0, where  
1632  
1633 731 the life has an independent genesis to that on Earth. The search for life beyond Earth has  
1634  
1635 732 been potentially possible since the 1970s, with the two Viking lander missions to Mars, but  
1636  
1637 733 there are other possible targets in our solar system, notably some of the moons around Jupiter  
1638  
1639 734 and Saturn (e.g., Europa, Titan). Space technology has now reached the point where the  
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1641 735 detection of life, if it exists or existed elsewhere in the solar system, is becoming a realistic  
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1643 736 possibility in the next 50 years. There have been several rovers that have carried out  
1644  
1645 737 successful exploration of the surface of Mars, including Curiosity, the Mars Science Laboratory  
1646  
1647 738 that in 2018 is mid-way through its predicted mission (Grotzinger et al., 2014). The rover Mars  
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1649 739 2020 is being designed at present to test for evidence of life in the near-surface environment.  
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740 It will drill, collect and cache samples from the Martian surface, which will then be returned to  
741 Earth for more detailed analysis (Beatty et al., 2015). Sample return from Mars to Earth is  
742 planned for the end of the 2020's (Foust, 2018). Active planning is also ongoing for possible  
743 missions to land and analyse materials from the surfaces of moons such as Europa and Titan,  
744 by both NASA and the European Space Agency. For example, Europa (a moon of Jupiter) is  
745 known to have a global saltwater ocean below its icy crust, as well as a rocky seafloor, so is  
746 one of the highest priority targets in the search for present-day life beyond Earth (Hand et al.,  
747 2017). A key concern with this solar system exploration is planetary protection, which is  
748 governed by the United Nations Outer Space Treaty (United Nations Office for Disarmament  
749 Affairs, 2015) and the Committee on Space Research (COSPAR) of the International  
750 Committee for Science. There are two important categories of planetary protection. The first  
751 is "forward contamination", where Earth-derived microbial life hitches a ride on spacecraft and  
752 contaminates parts of a planetary surface being explored. The second is "backward  
753 contamination", where life from an explored planet or moon is inadvertently returned to Earth,  
754 maybe in a spacecraft or within a rock sample. The relevance to aDNA analytical protocols is  
755 in forward contamination (i.e., the risk of contaminating sample material that could lead to data  
756 misinterpretations, and/or generally introducing Earth contaminants to other planets; Rummel  
757 and Conley, 2017). It should be noted that if indeed there is or was life on other planetary  
758 bodies, it may well not be based on a genetic code composed of DNA and RNA. Independently  
759 originated Life 2.0 would be highly unlikely to have evolved exactly the same nucleic acid  
760 genetic code as life on Earth (e.g., Rummel and Conley, 2017). Indeed, it has been postulated  
761 that an alternative biosphere could exist as a "shadow biosphere" on Earth (Davies et al.,  
762 2009). If DNA or RNA-based extant life is found on Mars, for example, then it is most likely  
763 that it would represent either past natural exchange of rocks between the two planets  
764 (panspermia), or anthropogenic forward contamination. Therefore, the procedures used for  
765 distinguishing indigenous life in planetary exploration will need broadening to include the  
766 possibility of life with a different genetic code. The protocols developed for aDNA sampling of  
767 marine sediments on Earth, including the ability to distinguish from modern DNA, have

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768 relevance for the designing of methods to look for past life on Mars or outer solar system  
769 moons using molecular biology techniques (Beatty et al., 2015; Hand et al., 2017).

770

771 **Conclusions**

772 Ancient DNA in marine deep-sea sediments holds the potential to open a new era of marine  
773 palaeo-environment and -climate reconstruction. However, anti-contamination measures  
774 central to all aDNA research have logistical constraints and are particularly poorly-suited to  
775 shipboard sediment sampling and processing. For example, sterile coring equipment and  
776 ultra-clean laboratories are usually not available on any type of drilling platform. Current and  
777 future IODP drilling vessels are aware of the increasing need for improved and innovative  
778 solutions to coring, non-contaminant drill fluids and appropriate laboratories and storage  
779 facilities. Such logistical advances should go hand-in-hand with the establishment of new  
780 criteria and standards to ensure the acquisition and preservation of sediment cores with  
781 minimal environmental contaminants. Complementary genetic and geochemical information  
782 currently available to date suggests that, realistically, environmental reconstructions based on  
783 marine *seda*DNA from past plankton can be achieved for at least the last glacial-interglacial  
784 cycle, and potentially back to ~400,000 years. These guidelines can be applied in other  
785 scientific areas to facilitate and optimise research conducted in extremely remote locations,  
786 contamination-susceptible environmental samples, and even during the future exploration of  
787 other planets.

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789

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813 **Figures:**

814 **Figure 1:** Schematic showing the key steps involved in acquiring deep marine sediment  
815 cores, subsampling, DNA extraction, aDNA preparation for sequencing and data generation.  
816 Indicated are sources of potential contamination and reduction in data quality, as well as  
817 recommended precautions to be considered and/or controls to be taken. An impact score (1-  
818 3 stars) is given to indicate the severity of potential contamination or the impact that impaired

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819 data would have on the results at each step in the process. Schematic graphics are not to  
820 scale.

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822 **Figure 2:** Overview of IODP coring systems. A) Advanced piston coring system (APC), shown  
823 before and after stroking; only small volumes of drill fluid can enter the space between the  
824 core barrel and collar from above after stroking, greatly reducing the risk of contamination. B)  
825 Extended core barrel system (XCB) and C) Rotary core barrel system (RCB); both containing  
826 circulation jets at the bottom of the core barrel through which drill-fluid enters and removes  
827 coring debris by transporting it upwards within the drill hole to the surface. D) Comparison of  
828 rotary and piston cored sediments demonstrating the well-preserved lamination in Piston  
829 cored material. Figure adapted from Sun et al. (2018) and IODP  
830 ([iodp.tamu.edu/tools/index.html](http://iodp.tamu.edu/tools/index.html)).

831  
832 **Table 1:** Terms commonly used in marine aDNA research and their definition. aDNA terms  
833 are listed hierarchically, all other terms are listed alphabetically.

834  
835 **Table 2:** Commonly used DNA extraction kits in aDNA studies to date.

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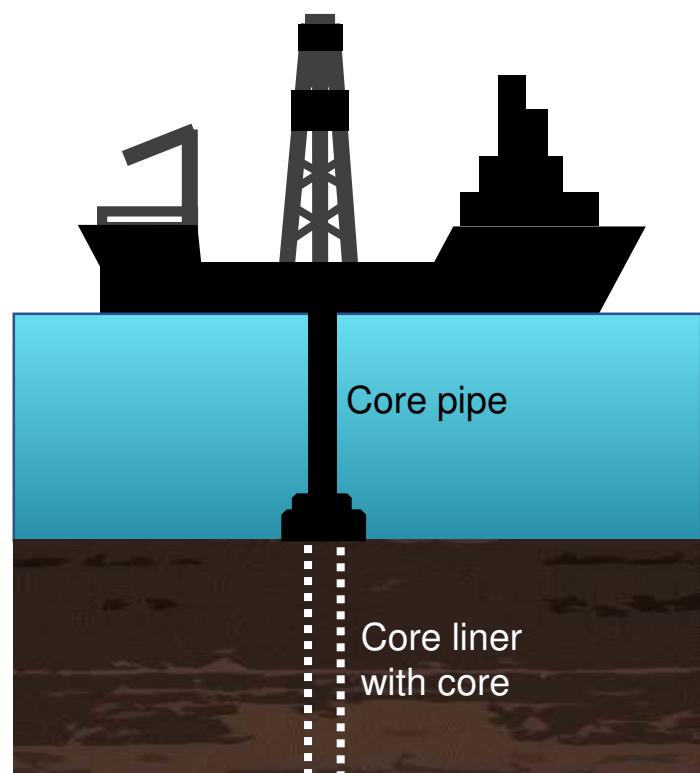


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## Impact subject

## Impact score

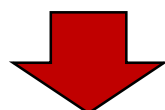
### Sediment coring



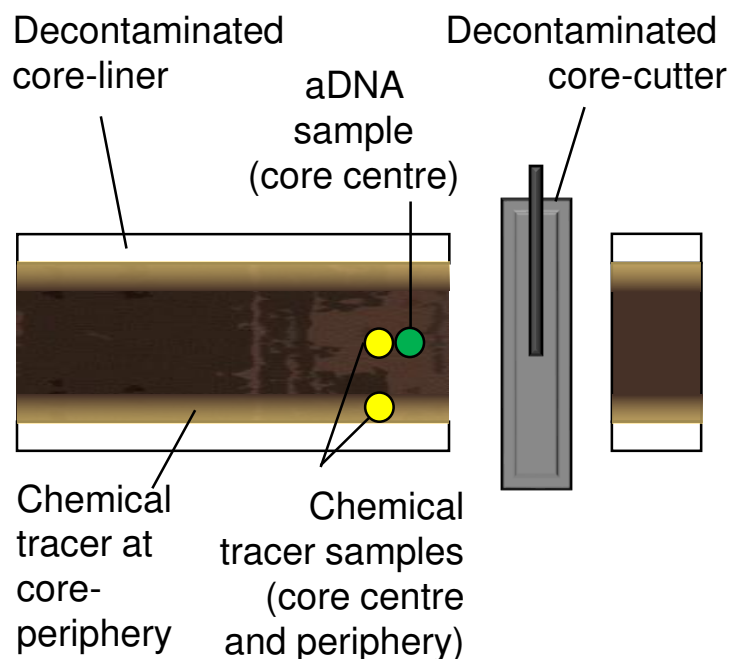
- Modern DNA in seawater
- Contaminant DNA in drilling fluid
- Surface sediment DNA pushed downwards during coring

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*Recommended contamination control measures: seawater control sample, application of chemical tracers*



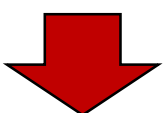
### Sediment core subsampling



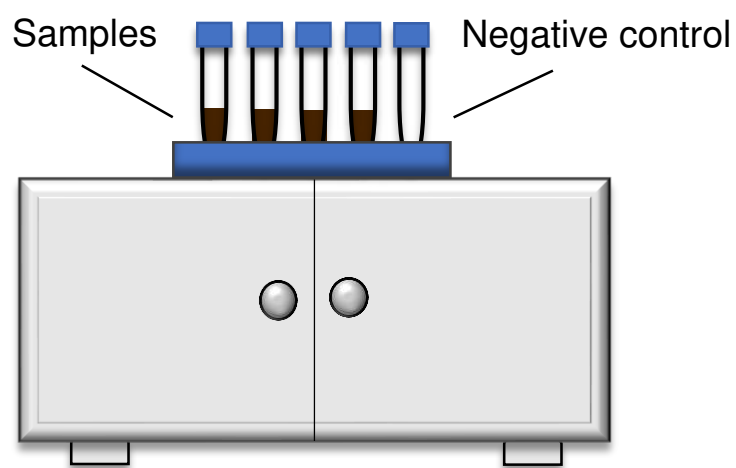
- Human DNA from analysts
- Contaminant DNA present in working area
- Cross-contamination

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*Recommended contamination control measures: reduction of airflow, sampling under cold conditions, decontamination of core-liners, removal of exposed surface sediments, air controls and lab swabs*



### aDNA extraction



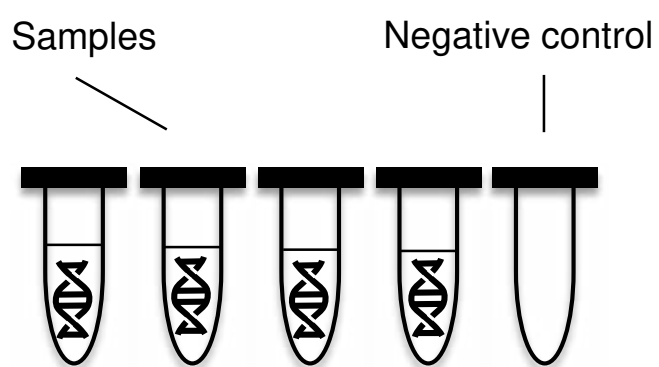
- Contaminant DNA present in reagents
- Contaminant DNA present in laboratory
- Cross-contamination

\*

*Recommended contamination control measures: working on low-DNA environment, personal protective equipment, negative controls*



### aDNA preparation for sequencing



- Metagenomic library preparation (reagent contaminants)
- PCR and amplicon library preparation (PCR bias)

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*Recommended contamination control measures: working on low-DNA environment, personal protective equipment, negative controls*



### aDNA data analysis

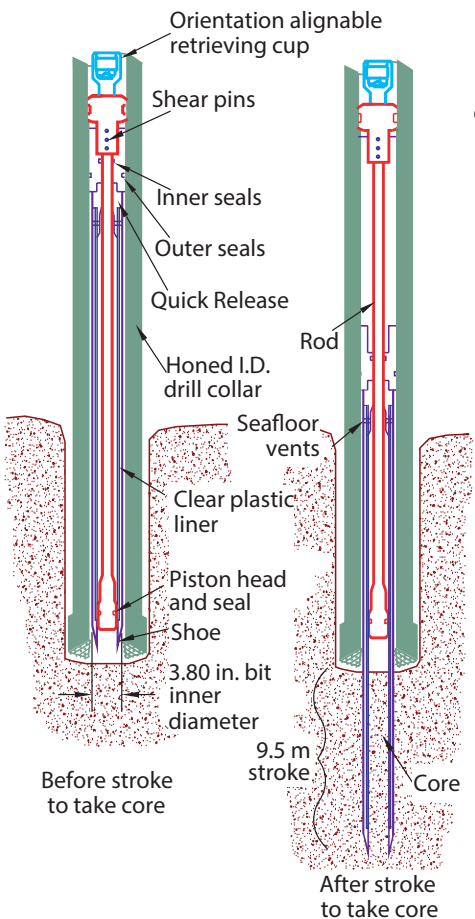


- Vigorous data filtering and quality control

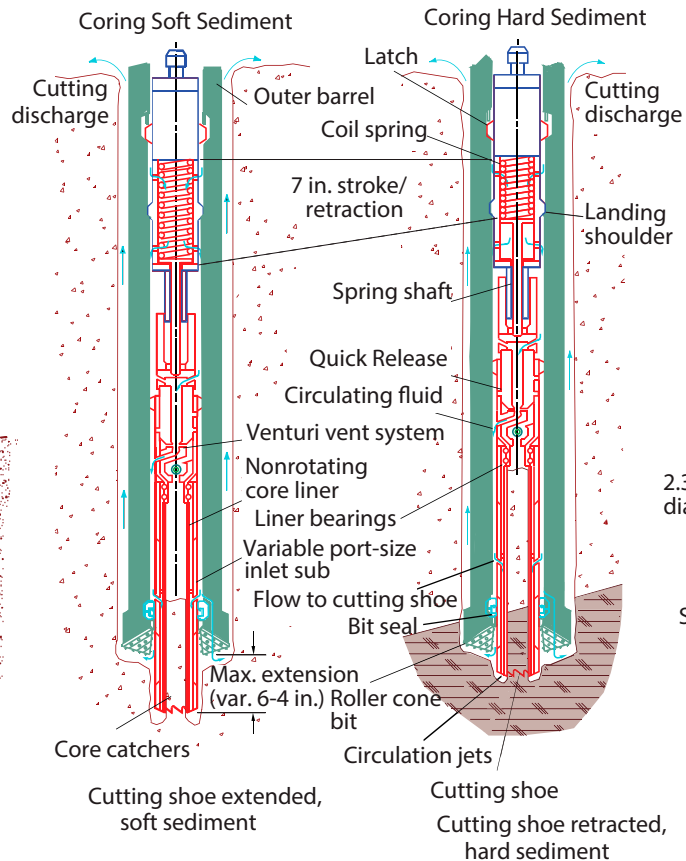
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*Recommended contamination control measures: analysis of negative controls alongside samples*

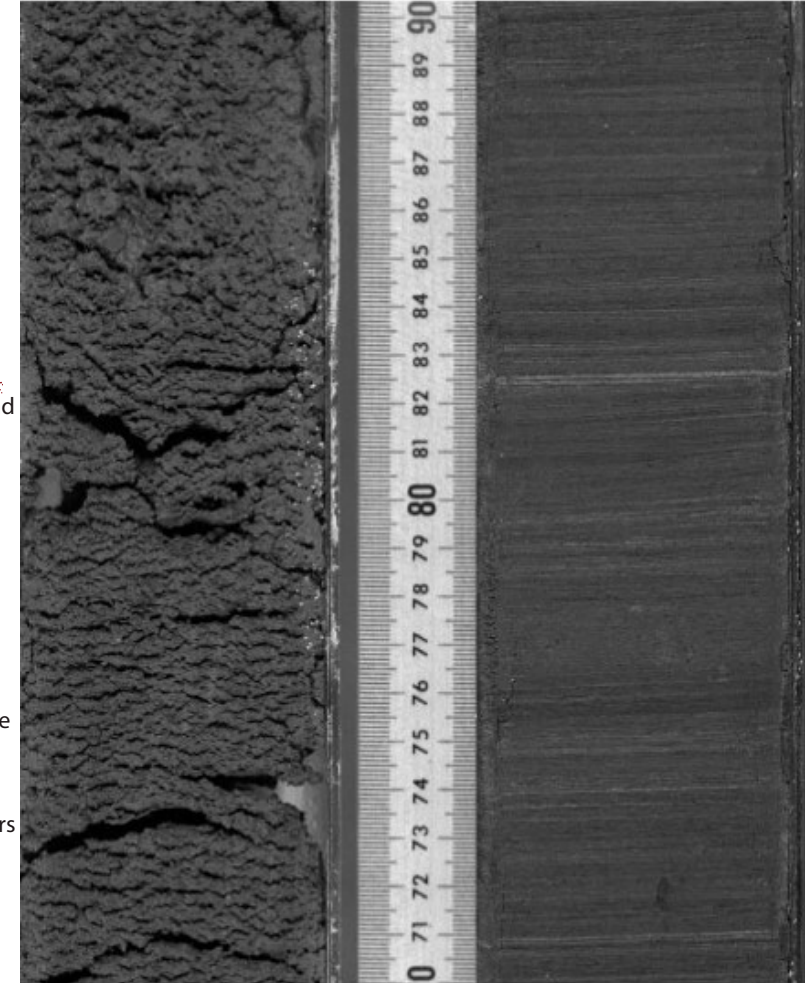
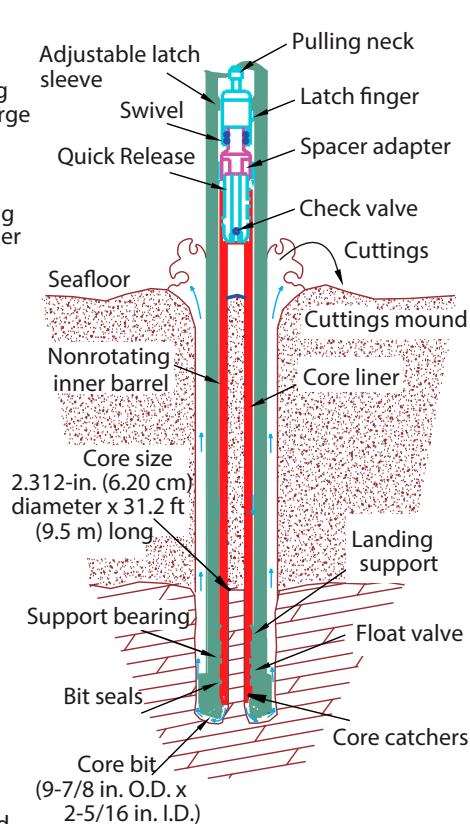
A) APC coring system



B) XCB coring system



C) RCB coring system



D) Rotary (left) and Piston (right) cored sediment core