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1 # The preservation of ancient DNA in
2 # archaeological fish bone

3

4 Running title: High-throughput sequencing of fish bone

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38 Keywords

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40 # Abstract

41 The field of ancient DNA is taxonomically dominated by studies focusing on mammals. This
42 taxonomic bias limits our understanding of endogenous DNA preservation for vertebrate taxa
43 with different bone physiology, such as teleost fish. In contrast to most mammalian bone, teleost
44 bone is typically brittle, porous, lightweight and is characterized by a lack of bone remodeling
45 during growth. Using high-throughput shotgun sequencing, we here investigate the preservation
46 of DNA in a range of different bone elements from over 200 archaeological Atlantic cod (*Gadus*
47 *morhua*) specimens from 38 sites in northern Europe, dating up to 8000 years before present. We
48 observe that the majority of archaeological sites (79%) yield endogenous DNA, with 40% of sites
49 providing samples that contain high levels (> 20%). Library preparation success and levels of
50 endogenous DNA depend mainly on excavation site and pre-extraction laboratory treatment. The
51 use of pre-extraction treatments lowers the rate of library success, although — if successful — the
52 fraction of endogenous DNA can be improved by several orders of magnitude. This trade-off
53 between library preparation success and levels of endogenous DNA allows for alternative
54 extraction strategies depending on the requirements of down-stream analyses and research
55 questions. Finally, we find that — in contrast to mammalian bones — different fish bone elements
56 yield similar levels of endogenous DNA. Our results highlight the overall suitability of
57 archaeological fish bone as a source for ancient DNA and provide novel evidence for a possible
58 role of bone remodeling in the preservation of endogenous DNA across different classes of
59 vertebrates.

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61 # Introduction

62 Driven by revolutionary advances in laboratory methods, sequencing technologies and
63 downstream analyses, an increasing number of (non-)model species have been investigated using
64 ancient DNA (aDNA). Such studies have addressed a wide range of questions related to archaic
65 human history, animal domestication, or extinct megafauna (e.g., Hofreiter et al., 2015; Ollivier et
66 al., 2018; Palkopoulou et al., 2018; Skoglund & Mathieson, 2018) and have yielded fundamental
67 methodological insights. For instance, a seminal discovery revealed that the petrous bone, i.e., the
68 *pars petrosa* of the temporal bone, which is the hardest and densest bone in mammals (Frisch et
69 al., 1998), has an increased potential of containing high levels of endogenous DNA (Gamba et al.,
70 2014; Pinhasi et al., 2015). Knowing which type of bone element may yield the best results for
71 aDNA research is crucial for a variety of reasons. First, focusing on sampling bone elements with
72 high endogenous DNA greatly improves the economy of high-throughput sequencing studies
73 (Rizzi et al., 2012) and helps avoid costly analyses for samples that are likely suboptimal. Second,
74 sampling for aDNA is most often destructive. Knowing how to select the right elements helps
75 minimize the destruction of unique archaeological materials that represent a finite resource
76 (Pálsdóttir et al., 2019). Third, such knowledge may further aid archaeologists in making informed
77 choices when collecting and preserving zooarchaeological material in the field, maximizing the
78 research potential for a variety of studies. This insight has therefore transformed the field of
79 aDNA, allowing the cost-efficient, genome-wide analysis of hundreds of individual ancient
80 specimens (e.g., Damgaard et al., 2018; Fages et al., 2019; Mathieson et al., 2018; Olalde et al.,
81 2018).

82 In mammals, low bone density is usually associated with poor DNA preservation (Geigl & Grange,
83 2018). Archaeological fish bone (Figure 1A) is typically lightweight, porous, brittle and
84 susceptible to taphonomic damage (Szpak, 2011) and such bone could thus be considered a

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85 suboptimal source of aDNA from a mammalian preservation perspective. In contrast to mammals,
86 however, fish bone does not serve as a calcium reservoir under normal conditions (Moss, 1961;
87 Witten & Huysseune, 2009). Most higher teleosts, including Atlantic cod (*Gadus morhua*), lack
88 osteocytes (Kranenbarg et al., 2005; Moss, 1961; Shahar & Dean, 2013; Witten & Villwock, 1997).
89 In acellular fish bone, bone remodeling takes place to a lesser extent and through different cellular
90 and physiological processes (Harland & Van Neer, 2018; Kranenbarg et al., 2005; Witten &
91 Villwock, 1997). An absence of bone remodeling may be important for DNA preservation for
92 several reasons. For example, it has been suggested that an absence of cell *lacunae* improves the
93 resistance of acellular fish bone to microbial degradation (Szpak, 2011). Moreover, recent
94 evidence indicates that an absence of bone remodeling may aid DNA preservation in specific
95 mammalian bone elements (Kontopoulos et al., 2019). It is therefore possible that the
96 fundamental differences between mammalian and fish skeletal physiology, and especially the lack
97 of bone remodeling in most fish, affects the aDNA preservation potential of archaeological fish
98 bone.

99 Interestingly, multiple studies have reported the successful retrieval of aDNA from archaeological
100 fish bone for a variety of species, locations and age (Oosting et al., 2019). aDNA has been
101 consistently amplified from herring (Speller et al., 2012), Pacific salmon (Grier et al., 2013;
102 Johnson et al., 2018; Royle et al., 2018; Speller et al., 2005; Yang et al., 2004), Atlantic cod
103 (Hutchinson et al., 2015; Ólafsdóttir et al., 2014), sturgeon (Ludwig et al., 2009; Nikulina &
104 Schmölcke, 2016; Pagès et al., 2009), Mediterranean trout (Splendiani et al., 2016), Northern pike
105 (Wooller et al., 2015), and other fish taxa (Arndt et al., 2003; Ciesielski & Makowiecki, 2005;
106 Živaljević et al., 2017), in some cases from bones up to 6,000 yBP or older (Johnson et al., 2018;
107 Nikulina & Schmölcke, 2016; Speller et al., 2012; Splendiani et al., 2016; Wooller et al., 2015; Yang
108 et al., 2004). Fish aDNA has also been successfully amplified in metagenomic analyses using bulk
109 bone approaches, even from warm tropical climates (Grealy et al., 2016). Finally, high-throughput
110 sequencing (HTS) approaches have yielded high levels (15-50%) of endogenous DNA from a

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111 limited number of sites up to one thousand years old (Boessenkool et al., 2017; Star et al., 2017).
112 Despite the clear potential for aDNA preservation in archaeological fish remains, however, no
113 studies have yet investigated the factors that underlie this preservation and it is unclear if the
114 expectation of intra-skeletal variability in DNA preservation observed for mammals is applicable
115 to other vertebrate taxa such as fish.

116 Here, we investigate the preservation of aDNA in archaeological Atlantic cod bones (n = 204)
117 obtained from 38 excavations in northern Europe, dating from 6500 BCE to c.1650 CE (spanning
118 the Mesolithic to early modern periods, Figure 1B, Tables 1 and S1). We use a HTS approach to
119 investigate whether bone element, archaeological site, DNA extraction method, and/or
120 sequencing library preparation protocol can be used to predict library success (i.e., the successful
121 retrieval and amplification of aDNA) and the relative proportion of endogenous DNA. We interpret
122 our results in light of down-stream analytical requirements and provide practical
123 recommendations in order to maximize throughput and inference of whole genome sequencing
124 (WGS) data from ancient fish bone.

125 Materials and Methods

126 Sample processing and DNA extraction

127 A total of 204 Atlantic cod bones originating from 38 sites (Figure 1B, Tables 1 and S1) were
128 processed following one of three DNA extraction protocols – standard extraction (adapted from
129 Dabney et al., 2013), with the inclusion of a pre-digestion step (DD, Damgaard et al., 2015), or with
130 the addition of a mild bleach treatment and pre-digestion step (BLEDD, Boessenkool et al., 2017).
131 All laboratory protocols were carried out in a dedicated aDNA clean laboratory at the University
132 of Oslo following standard anti-contamination and authentication protocols (e.g., Cooper &
133 Poinar, 2000; Gilbert et al., 2005; Llamas et al., 2017). Bones were UV-treated for 10 minutes per

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134 side and pulverized using a stainless-steel mortar (Gondek et al., 2018) or a Retsch MM400 mixer
135 mill. Up to two times 150-200 mg of bone powder was digested for 18-24 hours in 0.5 M EDTA,
136 0.5 mg/ml proteinase K and 0.5% N-Laurylsarcosine. Digests were combined and DNA was
137 extracted with 9 × volumes of PB buffer (QIAGEN) or a 3:2 mixture of QG buffer (QIAGEN) and
138 isopropanol. MinElute purification was carried out using the QIAvac 24 Plus vacuum manifold
139 system (QIAGEN). Parallel non-template controls were included. A subset of 73 samples was
140 subjected to multiple treatments (Table S1).

141 Library preparation, sequencing and read processing

142 Single- or double-indexed blunt-end sequencing libraries were built from 15-16 µl of DNA extract
143 or non-template extraction blank, following either the single-tube (BEST) protocol (Carøe et al.,
144 2018) with the modifications described in (Mak et al., 2017) or following the Meyer-Kircher
145 protocol (Kircher et al., 2012; Meyer & Kircher, 2010) with the modifications listed in (Schroeder
146 et al., 2015). Blunt-end repair, adapter ligation and set up of indexing PCRs were performed in the
147 aDNA clean laboratory. Library quality and concentration were inspected with a High Sensitivity
148 DNA Assay on the Bioanalyzer 2100 (Agilent) or with a High Sensitivity NGS Fragment Analysis
149 Kit on the Fragment Analyzer™ (Advanced Analytical). Libraries were sequenced on the Illumina
150 HiSeq 2500 or HiSeq 4000 platforms at the Norwegian Sequencing Centre with paired-end 125
151 bp (HiSeq 2500) or 150 bp (HiSeq 4000) reads and demultiplexed allowing zero mismatches in
152 the index tag. Reads were downsampled (n = 100,000) for each library and processed using
153 PALEOMIX v.1.2.13 (Schubert et al., 2014). Paired-end reads were trimmed, filtered, and collapsed
154 with AdapterRemoval v.2.1.7 (Lindgreen, 2012), discarding reads shorter than 25 bp. Collapsed
155 reads were aligned to the Atlantic cod GadMor3 reference genome (Star et al., 2011; Tørresen et
156 al., 2017) with BWA v.0.7.12 (Li & Durbin, 2009), using the aln algorithm with disabled seeding
157 and a minimum quality score of 25. aDNA deamination patterns were assessed with mapDamage
158 v.2.0.6 (Jónsson et al., 2013).

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159 ## Statistical analysis

160 Samples that underwent multiple treatments (*n = 73,* 146 treatments) were used to fit a
161 Generalized Linear Mixed Effect Model (GLM, family = binomial, using sample ID as random effect
162 to account for paired data) to test the effect of DNA extraction protocol, library preparation
163 protocol, site, and bone element on failure or success of library preparation (library outcome ~
164 extraction protocol + library protocol + site + bone element + (1 | Sample)). Outcome of library
165 preparation was also assessed using all generated libraries, excluding sites with less than three
166 samples (*n = 191*) and controlling for multiple treatments by randomly subsampling one
167 treatment per sample. Subsampling was performed 100 times generating (*i = 100*) resampled
168 datasets. A GLM (library outcome ~ extraction protocol + library protocol + site + bone element)
169 was run on all resampled datasets. A sensitivity analysis was run to evaluate the consistency of
170 the results recording significant factors for each iteration. In order to test the effect of DNA
171 extraction protocol, library preparation protocol, site, and bone element on endogenous DNA
172 content successfully sequenced libraries (defined as libraries that yielded more than 10,000
173 sequencing reads), excluding sites with less than three successful libraries (*n = 124* from 19 sites),
174 were used to fit a Generalized Linear Regression (GLR, endogenous DNA fraction ~ extraction
175 protocol + library protocol + site + bone element). Normality of the data for endogenous DNA
176 content was tested by levels in each of the factors using a Shapiro-Wilk Normality Test. For the
177 GLM and GLR described above several models were run discarding factors that did not show
178 significance in more complex models. Akaike (AIC) and Bayesian Information Criterion (BIC) were
179 used to select the best fitting models.

180 # Results

181 A total of 277 sequencing libraries were generated from 204 Atlantic cod bones collected at 38
182 archaeological sites (Figure 1B, Table S1). Of these, 140 libraries from 29 sites had a minimum

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183 concentration of 0.1 ng/µl and were sequenced (Figure S1, Figure 3A). All libraries showed
184 patterns of DNA fragmentation, fragment length, and deamination rates that were consistent with
185 those of authentic aDNA (Jónsson et al., 2013; Figures S1 and S2, Table S1). Most samples (*n =*
186 *131*) were processed once, but a subset of samples (*n = 73*) was processed using two or more
187 treatment combinations, either using different extraction or library preparation protocols (Figure
188 3B, Table S1). Bone elements were categorized into three major groups – cranial, postcranial, and
189 pectoral girdle bones (Figure 2A). The representation of these major groups differs across sites
190 (Figure 2B, Table S1), which is driven by the availability of elements at the different locations or
191 by post-excavation sample selection (Box 1).

192 Library preparation following the standard extraction protocol generated more successful
193 sequencing libraries (70 out of 84 libraries yielded more than 10,000 sequencing reads)
194 compared to the double digestion (DD) or combined double digestion and bleach (BLEDD)
195 protocols (7 of 26 libraries for DD and 62 of 167 libraries for BLEDD, Figure 3A). The Meyer-
196 Kircher (MK) library preparation protocol yielded a higher success rate (107 out of 150 libraries)
197 than the single tube library protocol (BEST) (32 out of 127 libraries, Figure 3A). A number of
198 library preparations (*n = 62*) for sites with initial high failure rates were repeated using the
199 standard extraction protocol without pre-extraction washes (Figure 3B) resulting in greater
200 success rates. For example, library preparations after DNA extraction using the DD (*n = 4*) or
201 BLEDD (*n = 5*) protocols for samples from the site of Ørland Main Air Base (site 4) failed, while
202 library preparation following the standard DNA extraction protocol was more successful (14 out
203 of 17 libraries, Table S1).

204 To statistically infer the most important factors explaining library success we applied two models.
205 First, we focused on the samples that were processed with multiple treatments (*n* = 73, Figure
206 3B). Second, we incorporated all samples generated from sites with more than three samples (*n =*
207 *191* samples from 27 sites), correcting for multiple treatments by randomly downsampling a

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208 single treatment per sample iteratively (*i* = 100, Figure 3C). The GLM focusing on the samples with
209 multiple treatments (library outcome ~ extraction protocol + library protocol + site + bone
210 element + (1 | Sample)) shows that the outcome is significantly dependent on DNA extraction and
211 library preparation protocols (Table S2). The sensitivity analysis with the 100 iterations of GLMs
212 incorporating all samples (library outcome ~ extraction protocol + library protocol + site + bone
213 element) shows similar results, with site and DNA extraction protocol as the most prevalent
214 significant factors (presenting mean estimates across iterations of 4.50 and 3.79 respectively,
215 Figure 3C), followed by library preparation protocol (mean estimate = 2.90). Bone element has no
216 significant effect on library preparation outcome and after excluding it from the model the latter
217 shows a better fit to the data (Table S3).

218 We further assessed whether the same factors affect levels of endogenous DNA for samples (*n* =
219 124) from 19 locations for which three or more specimens were successfully sequenced by fitting
220 a GLR (endogenous DNA fraction ~ extraction protocol + library protocol + site + bone element).
221 Significantly higher endogenous DNA contents are observed in samples that underwent the DD or
222 BLEDD pre-treatments, compared to a standard DNA extraction (Figure 4A, Table S4). Given that
223 a number of samples for which DD or BLEDD extraction failed (*n = 62*) were re-extracted using
224 the standard protocol (figure 3B), such samples may *a priori* be suspected to have relatively poor
225 DNA preservation. In contrast, library preparation protocol had no significant effect on
226 endogenous DNA content (Table S4). Although postcranial bones tend to have lower levels of
227 endogenous DNA, these differences are not significant, and especially bones from the cranial and
228 pectoral girdle yield comparable levels of endogenous DNA, independent of DNA extraction
229 protocol (Figure 4B, Table S4). Finally, we observe significant differences in endogenous DNA
230 between sites (Figure 4C, Table S4) with 8 out of 19 sites yielding samples with high levels (>
231 20%) of endogenous DNA, which includes the oldest excavation (Sævarhelleren, site 7, dated to
232 ca. 6500-6200 BCE). When excluding the non-significant factors from the GLR (bone element and

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233 library preparation protocol, Table S5), DNA extraction protocol and site remain significant. The
234 most complex model including all factors shows the best fit to the data (Table S5).

235 Discussion

236 Here, we present the largest study on DNA preservation in ancient fish bones to date, assessing
237 the effects of bone element, archaeological site, DNA extraction and sequencing library
238 preparation protocols on library success and levels of endogenous DNA. We obtain several
239 conclusions.

240 First, although we did not exhaustively sample all different elements possible, our findings imply
241 that most fish bone elements of sufficient size may be suitable for high-throughput shotgun aDNA
242 analyses. We observe no significant differences in either library preparation success or
243 endogenous DNA levels amongst different bone element groups (i.e., cranial, pectoral girdle or
244 postcranial) in archaeological fish bones. This observation is strikingly different from ancient DNA
245 results obtained from mammalian bones, where high endogenous DNA preservation is localized,
246 either in the petrous bone (Gamba et al., 2014; Pinhasi et al., 2015) or in the dense, recently
247 deposited circumferential lamellae of long bones (Alberti et al., 2018). Moreover, our findings
248 support a recent alternative hypothesis for DNA preservation in vertebrate bone. The localized
249 DNA preservation in mammals has usually been explained by the observed high density of bones
250 or bone regions (Bollongino et al., 2008; Geigl & Grange, 2018; Kendall et al., 2018; Alberti et al.,
251 2018) that may be more resistant to exogenous microbial colonization or taphonomic degradation
252 (Campos et al., 2012; Gamba et al., 2014). Recently, however, it has been suggested that it is the
253 absence of bone remodeling in these bones that helps promote DNA preservation (Kontopoulos
254 et al., 2019; Sirak et al., 2020), following observations that the petrous bone (Kontopoulos et al.,
255 2019), the auditory ossicle (Sirak et al., 2020) and the circumferential lamellae of long bones
256 (Treuting et al., 2017) experience little or no bone remodeling. This absence is analogous to the

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257 lack of bone remodeling during growth in acellular fish bone (Kranenbarg et al., 2005; Witten &
258 Villwock, 1997). Here, we provide novel comparative data supporting a hypothesis that distinct
259 bone developmental characteristics, specifically bone remodeling (e.g., Kontopoulos et al., 2019;
260 Sirak et al., 2020), may contribute to increased levels of endogenous DNA preservation in a wide
261 range of vertebrates.

262 Second, depending on the down-stream computational requirements, sample sizes consisting of
263 poor-quality DNA specimens can be increased in an economical way by avoiding pre-extraction
264 digestion or bleach wash treatments. We observed a distinct trade-off between levels of
265 endogenous DNA and library success when using bleach wash and pre-digestion treatments. As
266 previously reported, bleach wash and pre-digestion treatments increase levels of endogenous
267 DNA (e.g., (Boessenkool et al., 2017; Damgaard et al., 2015; Korlević et al., 2015), yet this increase
268 is coupled to higher failure rates during library creation. Presumably, when samples have
269 relatively poor DNA preservation, this DNA can be lost using pre-extracting wash steps, resulting
270 in the failure of a sample that could otherwise yield a sequencing library with low levels of
271 endogenous DNA. This results in a trade-off where the number of investigated individuals can be
272 maximized at the cost of sequencing depth or vice-versa. Such a trade-off can be exploited in
273 situations where low sequencing coverage data can yield meaningful archaeological or biological
274 information. For instance, genetic sex can be easily obtained for mammals using low numbers
275 (e.g., < 10,000) of sequencing reads, even in samples with low levels (< 0.5%) of endogenous DNA
276 (e.g., Barrett et al., 2020; Nistelberger et al., 2019; Pečnerová et al., 2017). Analogously, in Atlantic
277 cod there are several large, ecologically important chromosomal inversions (e.g., Berg et al., 2016;
278 Berg et al., 2017) that can easily be determined using low coverage sequencing data (Star et al.,
279 2017).

280 Third, we recommend utilizing library protocols with intermediate purification procedures when
281 targeting archaeological fish bones in order to maximize the potential of successful library

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282 creation. It is advantageous to minimize hands-on-time and laboratory costs while simultaneously
283 increasing sample throughput. For this reason, we implemented the single-tube (BEST) protocol
284 (Carøe et al., 2018; Mak et al., 2017), which is an economically efficient protocol with a reduced
285 number of purification steps compared to the Meyer-Kircher protocol (Kircher et al., 2012; Meyer
286 & Kircher, 2010). Both protocols yield similar levels of endogenous DNA and can therefore be used
287 to retrieve high-quality aDNA libraries from archaeological fish bone. Nonetheless, we did observe
288 significantly increased library amplification failure rates when following BEST (Carøe et al., 2018;
289 Mak et al., 2017), which reduces the efficiency of this method in overall sample throughput. During
290 the BEST protocol, multiple enzymatic reactions occur successively in the same tube, and we
291 suspect it is possible that this protocol is more sensitive to contaminants than protocols with
292 intermediate purification steps such as the Meyer-Kircher protocol. It is further possible that
293 archaeological fish bone contains more such contaminants than mammalian bone. Fish bone may
294 therefore be less suited for single-tube library preparation protocols than mammalian bone,
295 which can be more efficiently cleaned.

296 Finally, we conclude that a wide range of preservation and excavation conditions can yield high
297 endogenous aDNA preservation in archaeological fish bone. We observe site-specific differences
298 in aDNA preservation, with some sites yielding consistently high rates of library success and levels
299 of endogenous DNA whereas others do not. These site-dependent results make it difficult to
300 predict specific factors underlying sufficient aDNA preservation, as samples from each site are
301 associated with a wide range of different, potentially unknown, *pre-* and *post*-excavation
302 taphonomic processes. However, our results confirm that cave sites typically offer ideal
303 conditions for DNA preservation (Bollongino et al., 2008; Hardy et al., 1995), thanks to stable low
304 temperatures and lack of precipitation (Hedges & Millard, 1995). Here, we report the oldest WGS
305 results for archaeological fish bone from the cave site of Sævarhelleren (site 7, Bergsvik et al.,
306 2016), which is one of the sites with better DNA preservation despite being up to 8500 years old.
307 In addition to this, we have obtained excellent DNA of bones obtained from dry shell middens (e.g.,

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308 Orkney Quoygrew, site 12, Harland & Barrett, 2012), as well as bones from waterlogged sediments
309 that were excavated decades ago (e.g., Haithabu Harbour, site 14, Heinrich, 2006).

310 Only a limited number of fish aDNA studies have been published, despite their environmental and
311 economic importance, and the abundance of archaeological fish bone (Barrett, 2019; Oosting et
312 al., 2019). Especially whole genome HTS approaches (e.g., Star et al., 2017) focusing on fish
313 remains are rare. Here we show that, despite high variability in DNA preservation across
314 archaeological sites, high endogenous aDNA can consistently be recovered from archaeological
315 fish bone. Overall, we obtained successful sequencing libraries from 50% of all fish bone samples
316 analyzed, retrieving samples with more than 20% endogenous DNA from 40% of sites. Our results
317 provide insights for the study design and laboratory processing of archaeological fish bone
318 remains, highlight the suitability of this material as a source for aDNA, and provide novel evidence
319 for the role of bone remodeling in the preservation of DNA in vertebrate bone.

320

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321 ## Data availability

322 All ancient read data are available at the European Nucleotide Archive, www.ebi.ac.uk/ena,
323 (accession no. PRJEB37681).

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334 ## Author contributions

335 B.S., G.F. and J.H.B designed research; O.K., A.H.P., A.T.G. and G.F. carried out laboratory work. A.C.
336 and G.F. analyzed data. A.K.H., I.Y., I.J., S.W., G.F.B., J.H., R.N., D.O., B.C., R.B. and J.H.B. provided
337 samples and archaeological context information. R.B. selected UK specimens. B.S. and G.F. wrote
338 the manuscript with input from all authors. B.S., J.H.B. and S.B. provided funding and consumables.
339

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340 ## Box 1

341 As part of the conservation process prior to long-distance transport, Atlantic cod were typically
342 decapitated (Barrett, 1997) and thus archaeological sites can differ significantly in their bone
343 element distribution (Orton et al., 2014). Specifically, if cod was caught locally, cranial bones may
344 be observed in high abundance, whereas if cod was imported, postcranial bones are likely
345 overrepresented. Bones from the pectoral girdle are anatomically close to the point of
346 decapitation and their presence at import sites may therefore vary (Barrett, 1997; Orton et al.,
347 2014). This variation is clear in the distribution of skeletal elements at different sites in this study
348 (Figure 2B). For example, cod bones found at sites in Norway and Orkney are likely to originate
349 from local catches where cranial bones are abundant. In contrast, sites in England and in the
350 Netherlands are characterized by a lower availability of cranial bones. Moreover, by consistently
351 sampling the same bone element, cranial bones also offer the opportunity to easily avoid
352 resampling the same individual.
353

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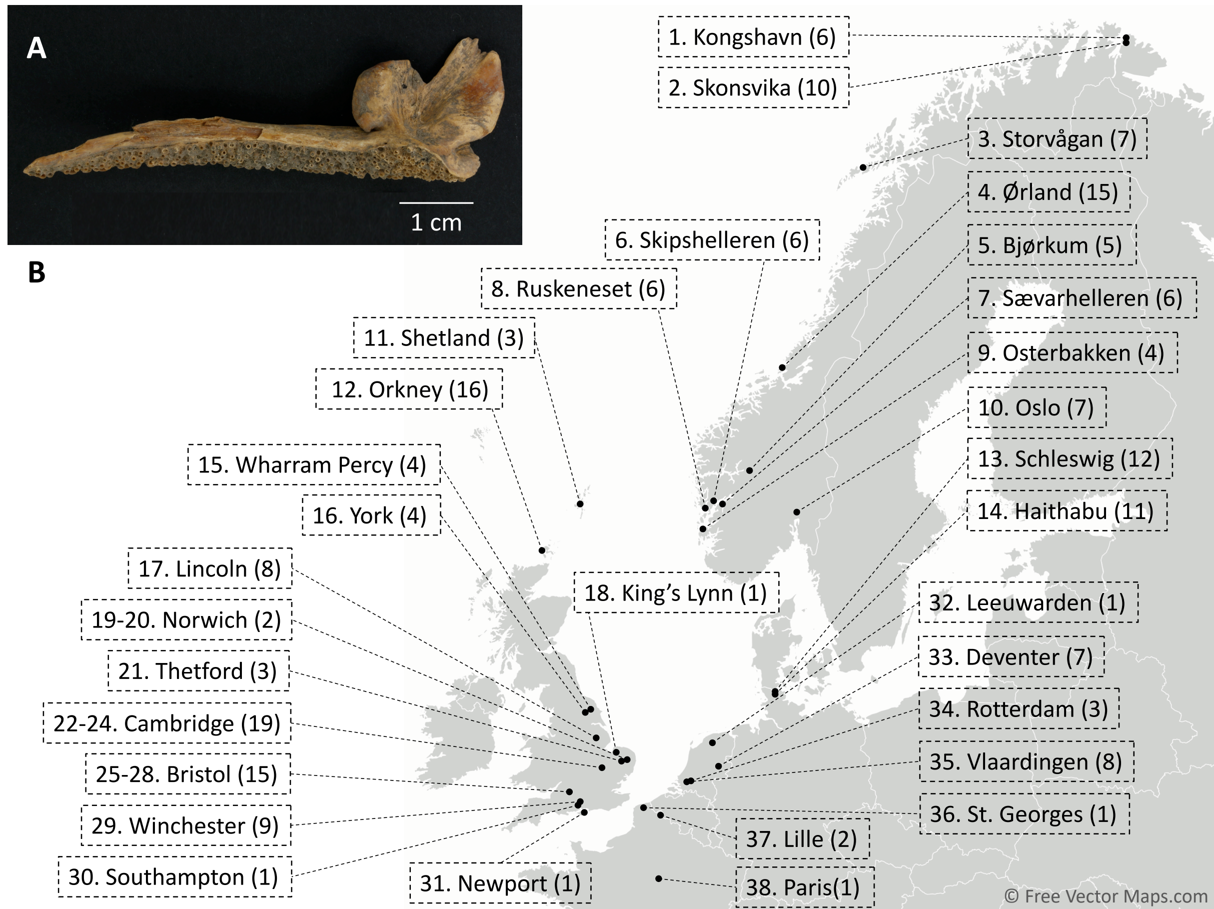
354 Table 1: Archaeological sites ($n = 38$) in northwest Europe from which archaeological Atlantic cod
355 bones were obtained. For each site, the country, date and number of bones (n) are provided.
356 Dating is based on archaeological context. For locations see also Figure 1B. NO = Norway, UK =
357 United Kingdom, DE = Germany, NL = The Netherlands, F = France.
358

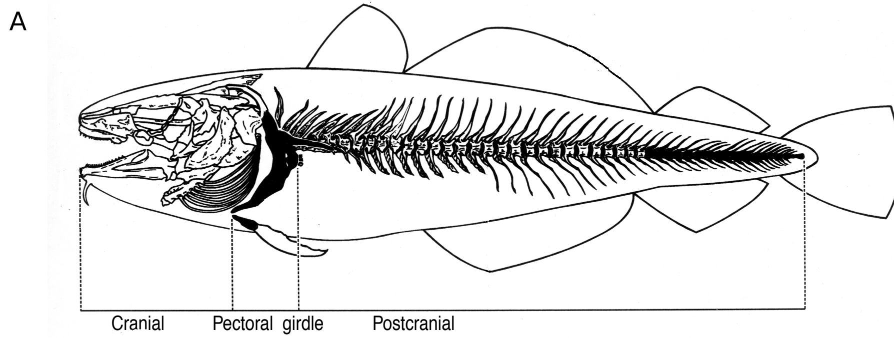
ID	Site name	Country	Date (BCE/CE)	Samples (n)	References
1	Kongshavn	NO	1300-1400 CE	6	(Amundsen, 2011)
2	Skonsvika	NO	1240-1390 CE	10	(Amundsen, 2011)
3	Storvågan	NO	1150-1270 CE	7	(Wickler, 2013)
4	Ørland Main Air Base	NO	200-400 CE	15	(Storå et al., 2019)
5	Bjørkum	NO	700-1000 CE	5	(Barrett et al., 2015); M. Ramstad, pers. comm)
6	Skipshelleren	NO	6000 BCE - 1000 CE	6	(Hjelle et al., 2006; Rosvold et al., 2013)
7	Sævarhelleren	NO	6500-6200 BCE	6	(Bergsvik et al., 2016)
8	Ruskeneset	NO	2000 BCE - 1000 CE	6	(Hufthammer, 2015)
9	Osterbakken	NO	2000 BCE - 1000 CE	4	(Hufthammer, 2015)
10	Oslo Mindets tomt	NO	pre-1175-1350 CE	7	(Lie, 1988)
11	Shetland Sandwick South	UK	1100-1350 CE	3	(Bigelow, 1989)
12	Orkney Quoygrew	UK	1000-1200 CE	16	(Harland & Barrett, 2012)
13	Schleswig Schild	DE	c.1050-1280 CE	12	(Heinrich, 1987)
14	Haithabu Harbour	DE	c.800-1050 CE	11	(Heinrich, 2006)
15	Wharram Percy	UK	1250-1400 CE	4	(Barrett, 2005)
16	York Coppergate 16-22	UK	1040-1375 CE	4	(Harland et al., 2016)
17	Lincoln Castle	UK	1150-1200 CE	8	(Barrett, unpublished)
18	King's Lynn Raynham House	UK	1250-1350 CE	1	(Locker, 2000)
19	Norwich Castle Mall	UK	1050-1100 CE	1	(Locker, 2009)
20	Norwich Fishergate	UK	1000-1150 CE	1	(Locker, 1994)
21	Thetford St. Barnabas' Hospital	UK	c.1000-1100 CE	3	(Jones, 1984)
22	Cambridge Corpus Christi College	UK	1500-1600 CE	4	(Harland, 2007)
23	Cambridge Grand Arcade	UK	1300-1600 CE	12	(Harland, 2019)
24	Cambridge St. John's Triangle	UK	1550-1650 CE	3	(Harland, 2009)
25	Bristol Broad Quay	UK	c.1000-1200 CE	1	(Russ, 2011)
26	Bristol Dundas Wharf	UK	c.1225-1400 CE	2	(Jones & Watson, 1987)
27	Bristol Finzel's Reach	UK	1125-1500 CE	9	(Nicholson, 2017)
28	Bristol Redcliff Street 82-90	UK	c.1125-1375 CE	3	(Nicholson, 2000)
29	Winchester Brook Street	UK	1000-1350 CE	9	(Barrett, unpublished)
30	Southampton French Quarter	UK	1250-1350 CE	1	(Nicholson, 2011)
31	Newport Ship	UK	c.1469 CE	1	(Russ, 2012)
32	Leeuwarden Oldehoofsterkerkhof	NL	725-900 CE	1	(Thilderkvist, 2013)
33	Deventer Burseplein 434	NL	1100-1250 CE	7	(Beerenhout, 2015)
34	Rotterdam Hoogstraat	NL	1300-1400 CE	3	(Carmiggelt et al., 1997)
35	Vlaardingen Gat in de Markt	NL	1200-1350 CE	8	(Buitenhuis et al., 2006)
36	St. Georges sur l'Aa	F	800-1000 CE	1	(Clavel et al., 2015)
37	Lille Chateau de Courtrai	F	1300-1400	2	(Clavel, 2001)
38	Paris St. Michel	F	1400-1500 CE	1	(Clavel, 2001)

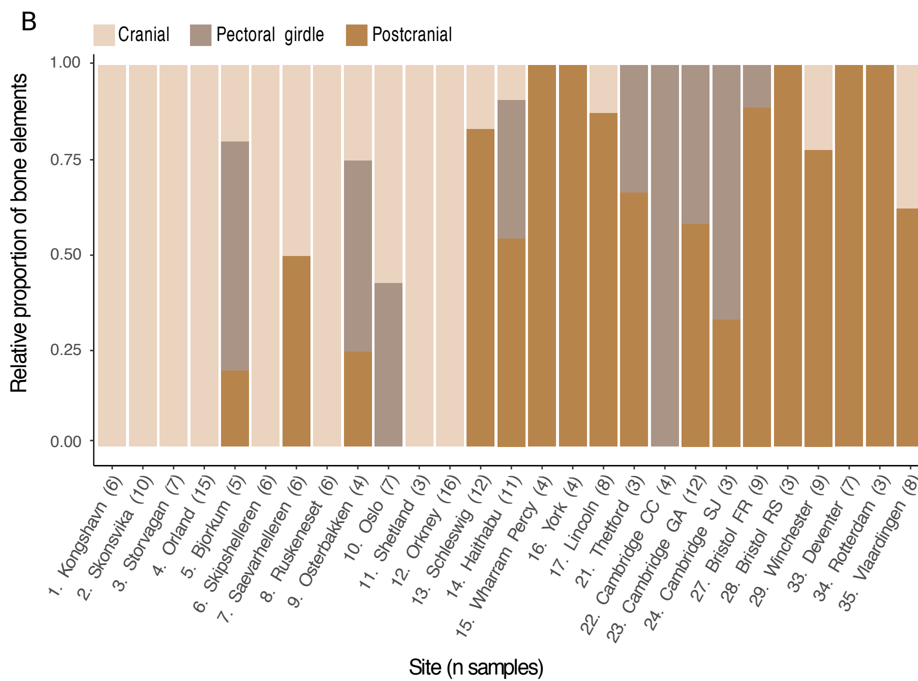
359

360



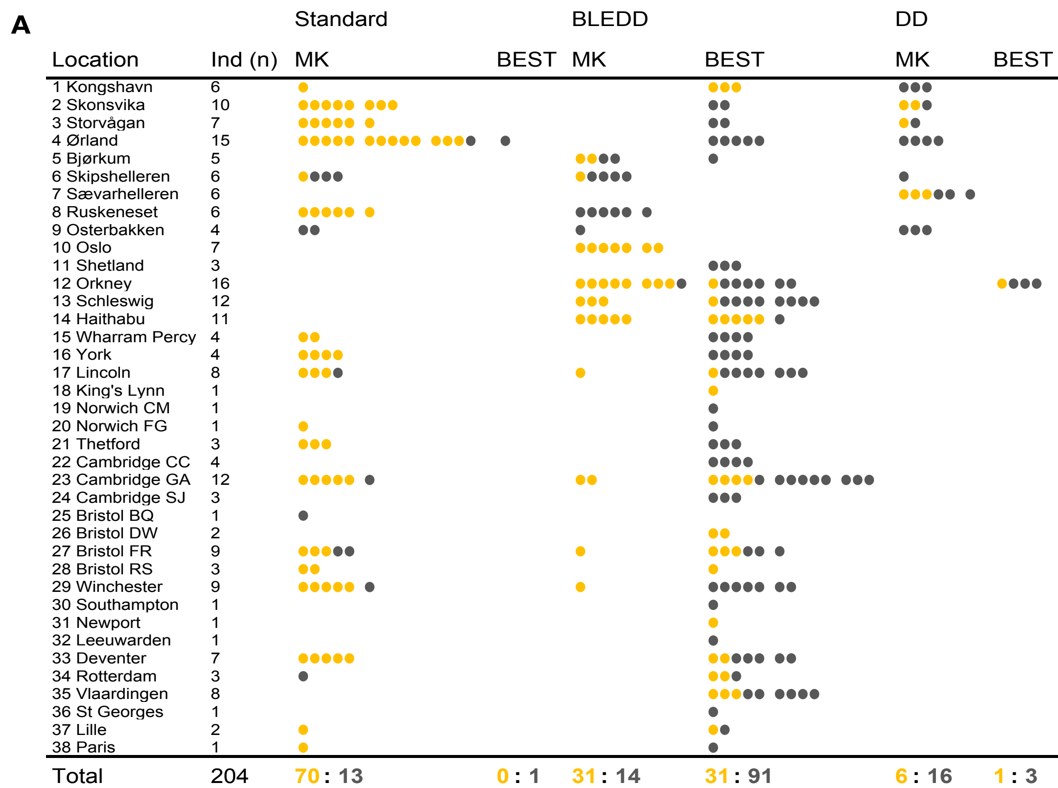
361 Figure 1: Archaeological Atlantic cod bones. (A) Archaeological Atlantic cod jawbone (premaxilla)
362 from the site of Orkney Quoygrew (1000-1200 CE). (B) Locations of fish bone specimens (n = 204)
363 from 38 archaeological sites. Bones from Norwich, Cambridge and Bristol were obtained from
364 distinct, individually numbered archaeological sites (see Table 1).

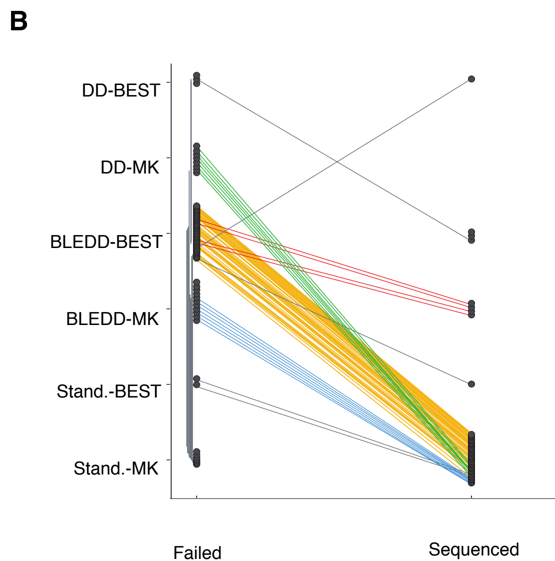


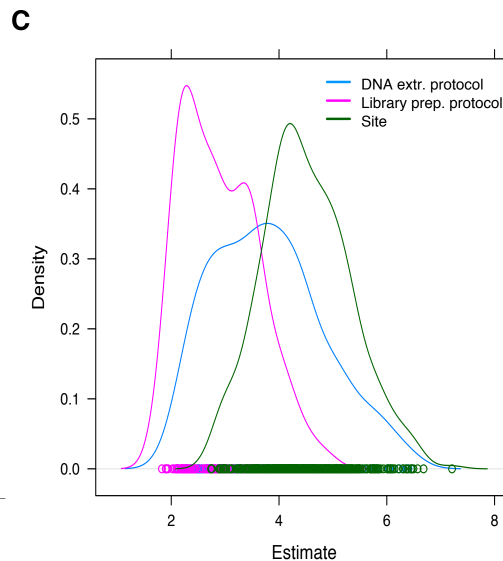


365

366 Figure 2: Distribution of Atlantic cod bone elements. (A) Classification of fish bone element
367 groups. Adapted from (Barrett et al., 1999). (B) Distribution of bone elements groups per site.
368 Only sites with three or more samples (n) are shown. Note that the distribution of selected bone
369 elements is not necessarily representative of their relative rate of retrieval at specific sites.



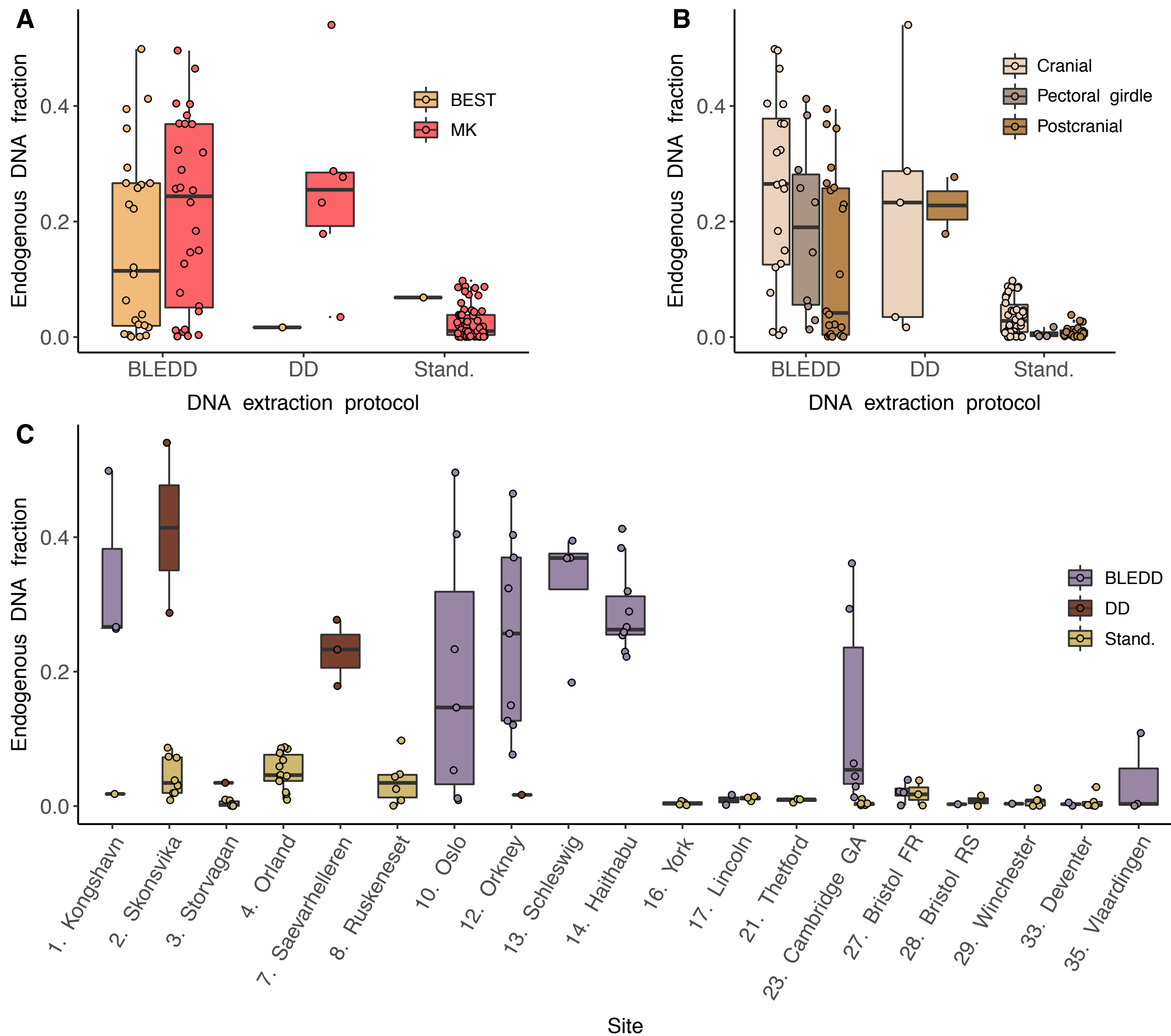




370

371 Figure 3: Success rates of high-throughput library preparation from Atlantic cod bones. (A)
372 Schematic of libraries generated for all archaeological sites (yellow, sequenced; gray, processed
373 but not sequenced library) divided into groups according to DNA extraction and library
374 preparation protocols utilized. Note that the number of libraries can be higher than the number
375 of specimens (Ind) due to multiple treatments. DD = double digestion extraction protocol, BLEDD
376 = bleach treatment combined with double digestion extraction protocol, MK = Meyer-Kircher
377 library preparation protocol, BEST = single tube library preparation protocol. (B) Treatment
378 overview and library outcomes (fail or success) for samples processed using multiple treatment
379 combinations (n = *73*). Different treatment combinations per individual are indicated by

380 connecting lines and colored according to treatment combination. (C) Sensitivity analysis: density
381 distribution of significant factors (site, DNA extraction and library preparation protocols)
382 following iterative ($i = 100$) logistic regression (library outcome ~ extraction protocol + library
383 protocol + site + bone element + (1 | Sample)), using randomly resampled data.
384



385
386 Figure 4: Endogenous DNA fraction. (A) Endogenous DNA per extraction and library preparation
387 protocols. Double digestion and mild bleach wash pre-treatments result in higher endogenous
388 DNA, independently from library preparation protocols. (B) Endogenous DNA per skeletal
389 element. No significant differences in endogenous DNA content can be observed between cranial,
390 postcranial and pectoral girdle bones. (C) Endogenous DNA per site. Significant differences in DNA
391 preservation can be observed between sites. DD = double digestion extraction protocol, BLEDD =
392 bleach treatment and double digestion extraction protocol, MK = Meyer-Kircher library
393 preparation protocol, BEST = single tube library preparation protocol. Only sites for which three
394 or more libraries were successfully sequenced are plotted.
395

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