Short Technical Research Report

Date: April 2020

ANCIENT DNA OF TUNA BONES FROM THE ALVEBERGET EXCAVATION IN ARENDAL, NORWAY

Giada Ferrari[1], Lourdes Martinez Garcia[1], Inger M. Berg-Hansen[2*], Anja Mansrud[2], Bastiaan Star[1#]

Contact information

[1]Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo, Blindernveien 31, NO-0371 Oslo, Norway

[2]Kulturhistorisk Museum, University of Oslo, St. Olavsgt. 29, NO-0371 Oslo, Norway

Email:
* i.m.berg-hansen@khm.uio.no
# bastiaan.star@ibv.uio.no

Background

Multiple studies have reported the successful amplification of ancient DNA (aDNA) from archaeological fish bone for a variety of species, locations and age (Oosting et al. 2019). High-throughput sequencing (HTS) approaches have also been successful and have yielded high rates (15-50%) of endogenous DNA from fish bones up to thousands of years old (Star et al. 2017). Such approaches provide novel opportunities to study past marine ecosystems (Barrett 2019; Oosting et al. 2019). Nonetheless, we have observed significant variation in the retrieval of ancient DNA from fish bones depending on archaeological context (Ferrari et al. 2020). Here, we investigate the preservation of aDNA in archaeological tuna bones (n = 9) obtained from Alveberget excavation in Arendal, Norway dating from c. 5300 to 4400 calBP based on C14-dating of 10 charcoal samples. The site was located at the shores of a small bay on the island Tromøya, with good conditions for fishing and sea-mammal hunting. Several finds of bones of marine fish and sea-mammals confirmed the exploitation of such species. We use a HTS approach to investigate whether the archaeological tuna bones from this location yield any ancient DNA.

Material and Methods

Sample processing and DNA extraction

All laboratory protocols were carried out in a dedicated aDNA clean laboratory at the University of Oslo following standard anti-contamination and authentication protocols (Poinar et al. 2000; Gilbert et al. 2005). We processed nine tuna bones (Figure 1, Table 1) from Alveberget. Bones were UV-treated for 10 minutes per side and the outer surface was removed using a dental sandblaster (Renfert basic quattro IS) with 50 µm (270 mesh) sand. UV-treatment was repeated and bones were pulverized using a stainless-steel mortar (Gondek et al. 2018). DNA was extracted from four samples following a short (30 min) pre-digestion step (Damgaard et al. 2015; Boessenkool et al. 2017) using to two times 200mg of bone powder. Bone powder was digested for 18-24 hours in 0.5M EDTA, 0.5mg/ml proteinase K and 0.5% N-Laurylsarcosine. DNA was extracted with 9× volumes of PB buffer (QIAGEN) before MinElute purification using the QIAvac 24 Plus vacuum manifold system (QIAGEN). Parallel non-template controls were included.

Table 1. *Tuna bones from Alveberget, Norway. The IBV-aDNA laboratory ID, excavation ID, bone element type, amount of material for aDNA extraction and remaining bone power (if any) are provided. Four extracted samples were used to prepare high-throughput sequencing libraries.*

Lab_ID	Excavation ID	Bone element	Milled for extraction	Bone powder left (g)	Extracted
TUN009	254x 339y lag 3; 76:6	Articular dx	2x200mg	0.31	No
TUN010	254x 339y lag 2; 74:3	Maxillare	2x200mg	0.69	Yes
TUN011	255x 340y lag 3; 79:1	Precaudal vertebra	2x200mg	0.82	Yes
TUN013	254x 339y lag 2; 74:4	Vertebra	2x200mg	0.57	Yes
TUN014	254x 339y lag 3; 76:4	Vertebra	2x200mg	0.55	Yes
TUN016	255x 340y lag 5; 87:1	Vertebra	2x200mg	0	No
TUN017	254x 339y lag 3; 76:3	Precaudal vertebra	2x200mg	1	No
TUN021	254x 340y lag 4; 81:1	Vertebra	2x200mg	0.18	No
TUN022	254x 340y lag 3; 77:2	Vertebra	2x200mg	0.69	No

Figure 1. *Archaeological tuna bones from Alveberget, Norway. We processed a total of nine samples of which four (TUN010, TUN011, TUN013 and TUN014) were subsequently used for aDNA extraction and high-throughput sequencing library preparation.*

Library preparation, sequencing and read processing

15µl of DNA extract or non-template extraction blank were converted into double-indexed sequencing libraries following Meyer and Kircher (Meyer et al. 2010), with the modifications listed in (Schroeder et al. 2015) as described in (Star et al 2016; Star et al. 2017). Blunt-end repair, adapter ligation and set up of indexing PCRs were performed in the aDNA clean laboratory. PCRs were done in 15 µL [2.5 U PfuTurbo Cx Hotstart DNA Polymerase (Agilent Technologies), 1× buffer, 0.2 mM per dNTP, 0.2 µM P7 index primer, 0.2 µM P5 IS4 primer, and 0.4 mg/mL BSA] for 12 cycles (2 min at 95 °C, 13 cycles of 30 s at 95 °C, 30 s at 60 °C, and 70 s at 72 °C with a final extension of 10 min at 72 °C). Amplified products were cleaned by using Agencourt AMPure XP beads at a 1:1.7 ratio, eluted in 30 µL EB buffer (QIAGEN). Library quality and concentration were inspected with a High Sensitivity NGS Fragment Analysis Kit on the Fragment AnalyzerTM (Advanced Analytical).

Results and Discussion

During extraction of the four samples, practical problems were encountered. MinElute columns were clogging up, and contaminants remained present in the DNA extracts being visible as a "rusty" color (*not shown*). Such samples usually contain significant amounts of contaminating chemicals that will interact with DNA extraction and further processing. Following library preparation, we analyzed the four samples on the Fragment Analyzer (Figure 2). These four samples did not yield sufficient quality DNA. Given the difficulties experienced during DNA extraction, suspected presence of contaminants, and absence of DNA based on Fragment Analyses plots for all samples, we conclude that the Alverberget site yields insufficient preservation for HTS library preparation.

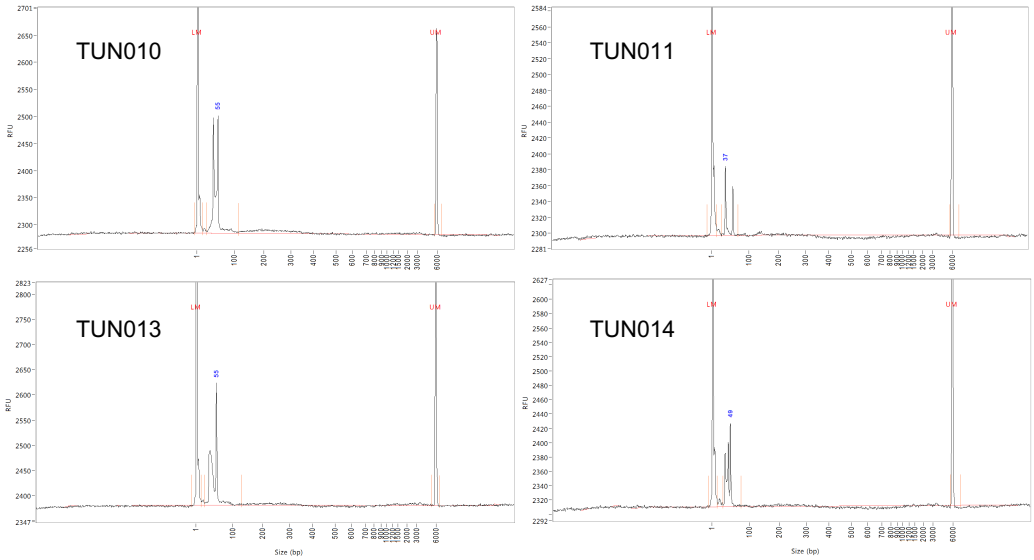

Figure 2. *Fragment analyzer plots of four aDNA libraries from archaeological tuna bones. We observe upper and lower markers peaks (indicated with red letters). Primer-dimer peaks (indicated with blue letters) are present just to the right of the lower marker peak. No sufficient aDNA for high-throughput sequencing is present.*

Acknowledgments

This work was supported by Research Council of Norway Projects “Catching the Past: Discovering the legacy of Atlantic cod exploitation using ancient DNA” (262777).

Author contributions

B.S. and G.F. designed research; G.F. and L.M.G. took photos, extracted DNA and created libraries. G.F. analyzed data. I.M.B. and A.M. provided ancient samples and archaeological context information. B.S. provided funding and consumables. B.S. and G.F. wrote the report with input from all authors.

References

Barrett, JH. **2019**. An environmental (pre) history of European fishing: past and future archaeological contributions to sustainable fisheries, *Journal of Fish Biology*

Boessenkool, S*, et al.* 2017. Combining bleach and mild pre-digestion improves ancient DNA recovery from bones, *Molecular Ecology Resources*, 17: 742-51.

Damgaard, PB*, et al.* 2015. Improving access to endogenous DNA in ancient bones and teeth, *Scientific reports*, 5

Gilbert, M*, et al.* 2005. Assessing ancient DNA studies, *Trends in Ecology & Evolution*, 20: 541 - 44.

Gondek, AT*, et al.* 2018. A stainless-steel mortar, pestle and sleeve design for the efficient fragmentation of ancient bone, *BioTechniques*, 64: 266-69.

Giada, F, *et al.* 2020 The preservation of ancient DNA in archaeological fish bone. *BioRxiv*. doi: 10.1101/2020.04.27.063677

Meyer, M*, et al.* 2010. Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing, *Cold Spring Harbor Protocols*, 2010: pdb.prot5448.

Oosting, T*, et al.* 2019. Unlocking the potential of ancient fish DNA in the genomic era, *Evolutionary Applications*

Poinar, HN*, et al.* 2000. Ancient DNA: do it right or not at all, *Science*, 5482: 1139.

Schroeder, H*, et al.* 2015. Genome-wide ancestry of 17th-century enslaved Africans from the Caribbean, *PNAS*, 112: 3669-73.

Star, B. *et al.* 2016 Preferential amplification of repetitive DNA during whole genome sequencing library creation from historic sample**s**. *STAR: Science & Technology of Archaeological Research* 2.1 (2016): 36-45

Star, B*, et al.* 2017. Ancient DNA reveals the Arctic origin of Viking Age cod from Haithabu, Germany, *Proceedings of the National Academy of Sciences*, 114: 9152-57.