Hornsea
Offshore Wind Farm Project One

REPORT

Hornsea Project One Offshore Wind Farm

Stage 3 Geoarchaeology Assessment Report

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1. Non-Technical Summary

This report summarises the geoarchaeological assessments undertaken on the material collected at Hornsea Project One Offshore Wind Farm. This report presents the results of the Stage 3 Archaeological Sub-sampling and Assessment and incudes specialist's reports as appendices. The staged approach is further detailed in Section **Error! Reference source not found.** and in Historic Environment Analysis guidance (COWRIE, 2011).

The key aim of this work was to offset the impact of development, as identified in the Environmental Statement, on sites or deposits of archaeological or palaeoenvironmental significance by conducting a staged archaeological assessment of geotechnical cores in order to develop our understanding of the palaeoenvironment of the region.

The work undertaken on the available cores included in the geoarchaeological study is in accordance with, and is as specified in, the Archaeological Written Scheme of Investigation (WSI) (Maritime Archaeology Ltd, 2015). All cores and core material was collected by the geotechnical contractors, Fugro UK, for geotechnical engineering purposes and cores collected during 2015 were documented by geotechnical technicians provided with archaeological training.

The findings from this archaeological and palaeoenvironmental assessment are integrated with the results and known archaeological potential derived from offshore cores collected during all previous geotechnical campaigns. The geoarchaeological work to date has been summarised in Table 1. The deposits of archaeological interest recovered and assessed in the study area (**Error! Reference source not found.**) are discussed in Section 6.

This Stage 3 assessment report concludes that the study has made a useful contribution to the overall knowledge and understanding of the pre-historic deposits present within the study area, particularly once integrated with the results from the other project subzones, when available. Based on the evidence derived from this study, and also due to the overarching limitation of the available sub-samples and long-term storage conditions, the recommendation is that no further benefit is expected to be realised from continued analysis of these samples collected from the offshore area at Hornsea Project One Offshore Wind Farm.

However, in order to ensure that the collective knowledge derived from these geoarchaeological studies undertaken to fulfil the various mitigation strategies achieve the maximum benefit in the public domain, it is recommended that these results, including those from the intertidal studies and the adjoining subzones, are collated and presented as a whole in a peer-reviewed article and published in an appropriate academic journal.

2. Introduction

Maritime Archaeology has been commissioned by Orsted A/S to provide a Stage 3 assessment of sub-samples recovered from geotechnical boreholes from the offshore zone relating to the Hornsea Project One Offshore Wind Farm (the Project) (see Figure 1).

The Stage 3 geoarchaeological assessment correlates to the third element in the archaeological assessment of geotechnical data as defined in:

- *Offshore Geotechnical Investigations and Historic Environment Analysis: Guidance for the Renewable Energy Sector* (COWRIE, 2011).

This work forms part of Work Package 1 as outlined within the agreed WSI for the development (Maritime Archaeology, 2015).

- [Work Package 1] Geotechnical assessment of offshore boreholes and vibrocores and integration with geophysical survey results to develop a deposit model for the development area.

This Stage 3 geoarchaeological report refines the results gained from micro and macro assessments undertaken on soils and deposits recovered from offshore cores collected at Hornsea Project One Offshore Wind Farm. The samples assessed were submitted following the recommendations made in the Stage 1 and Stage 2 geoarchaeological assessment (Maritime Archaeology, 2016).

2.1 Brief Scheme Background

This geoarchaeological assessment covers both the generation and offshore transmission assets. This document does not consider any area of the development above mean low water (MLW).

The Written Schemes of Investigation (WSIs) for the Project identify the known and potential archaeology within the development area, review potential impacts and present agreed mitigation strategies (DONG Energy, 2016a, 2016b). Two WSIs have been prepared, one to cover the offshore transmission assets and one in relation to the generation assets, of the Project.

2.2 Project Design Details

The Project will have a total generating capacity of 1,200 MW with 174 7 MW wind turbines. Two types of foundation for the turbines are being considered (monopiles, and suction bucket jacket foundations). The current proposed layout has been agreed in principle with Trinity House (TH) and The Maritime and Coastguard Agency (MCA).

Three offshore export cables will connect the wind farm to the onshore landfall where the onshore cables will connect to the onshore substation at North Killingholme. Offshore export cable installation will likely involve one, or possibly a combination, of the following methods: ploughing, trenching, jetting, rock-cutting, dredging, surface laying with post lay burial, or surface laying. The maximum target burial depth is anticipated as 1.1 m to top of product for most of the three export cable routes except for inshore of KP10.5 (Kilometre Point measured from the transition joint bay which is immediately behind the sea defence) where the maximum burial depth will be 0.6 m to top of product unless deeper burial is required to lower the cables below areas of high seabed mobility. Installation of offshore export cables has the potential to impact upon any archaeology that is located on the seafloor or buried within the anticipated 1.1 m of the seabed.

2.3 Previous Geoarchaeological Work

The previous assessment of offshore geotechnical and geophysical survey data collected in the Project One area revealed the presence of Pleistocene fluvial and estuarine sediments with the potential to contain hominid remains beneath the Devensian glacial till (generally at depths of 15 m or more below the seafloor). Closer to the seabed surface, this work identified Early Holocene 'Upper Botney Cut' channels, generally up to 15 m deep and 80 m wide, which are cut into larger late Glacial channels of considerably greater size containing reworked glacial till (Wessex Archaeology, 2013:16).

The cable corridor crosses some Late Pleistocene and Early Holocene channels on its way to shore lying at variable depth below the surface. The most significant feature to the west of Inner Silver Pit is a large palaeochannel that extends 4 km from landfall and appears to be a segment of the palaeo-Humber (SMart Wind, 2013).

The likelihood of survival of the remains of Mesolithic activity and settlement in and particularly on the side of these later channels is high (Coles, 1998; Flemming, 2004 and Boomer *et al.*, 2007), although there are no known prehistoric terrestrial sites within the Project area. Sampling undertaken during the Humber Regional Environmental Characterisation (REC) study (Tappin *et al.,* 2011) has shown that these deposits generally lie close to the surface of the seabed. It is therefore likely that the general area contains important prehistoric archaeological sites and finds and palaeo-environmental evidence.

The Stage 1 and 2 review of offshore samples collected in 2016 concluded that the small amount of samples recovered could not enhance the initial interpretation of the Project area. It was therefore recommended that further Stage 3 assessment should be undertaken with samples from all previous geoarchaeological campaigns. Table 1 summarises the geoarchaeological campaigns undertaken to date.

Year	Samples acquired	Archaeological report	Report summary
2011	• 28 Near shore zone vibrocores • Offshore bagged samples	Palaeoenvironmental assessment of near shore and offshore cores from the Hornsea Zone (Krawiec et al., 2011).	28 VC logs examined, 6 cores assessed together with bagged samples from the offshore zone. The samples yielded mixed results, the pollen concentrations were extremely low in some of the samples and assessment counts were not always possible. The accuracy of the radiocarbon dates were questioned and further work was recommended.
2012	• 12 boreholes • 129 vibrocores	Round 3 Hornsea Offshore Wind Farm Subzone 1 and export cable route Stage 1 and 2 Geoarchaeological Assessment, (Wessex Archaeology, 2013).	12 borehole locations and 27 vibrocore samples from the export cable route were assessed. Glacial, fluvial, estuarine and coastal sediments relating to former potentially inhabited landscapes were identified Stage 3 samples were recommended to further understand the sequence.

2014-2016	• 3BHs • 5 Wireline Push Samples • Downhole push CPT's (drilled)	Hornsea Project One Offshore Wind Farm Stage 1-2 Updated Geoarchaeological Assessment Report. (Maritime Archaeology, 2016)	Three boreholes and five Wireline Push Samples collected in 2014-2015 were assessed in terms of their archaeological and palaeoenvironmental potential. The small amount of samples recovered could not enhance the initial interpretation of the project area. It was recommended that further Stage 3 assessment should be undertaken with samples from all previous geoarchaeological campaigns.
2017	• 6 intertidal boreholes • 7 intertidal trial pits	Hornsea Project One Offshore Wind Farm Stage 1 Intertidal Geoarchaeological Assessment (Maritime Archaeology, 2017a)	Logs and photographs were reviewed to establish the potential for further geoarchaeological recording, assessment and analysis. The Stage 1 review showed that it was potentially possible to recover material containing preserved macro and micro fauna of archaeological interest from the retained samples and therefore a Stage 2 recording and sampling programme was recommended.
2017	• 6 intertidal boreholes • 7 intertidal trial pits	Hornsea Project One Offshore Wind Farm Stage 2 Intertidal Geoarchaeological Assessment (Maritime Archaeology, 2017b)	Further assessment of the material concluded that there was some potential in the sediments to contain preserved macro and micro fauna of archaeological interest resulting in Stage 3 recommendations being made.
2017	• Sub-samples from 4 intertidal cores.	Hornsea Project One Offshore Wind Farm Stage 3 Intertidal Geoarchaeological Assessment (Maritime Archaeology, 2017c)	The results from the assessment of the environmental Indicators proved that very few and poorly preserved species had survived. The results from the dating sequence indicated that there has been significant re-working in the intertidal area. No further assessment was therefore recommended.

Table 1 Previously undertaken geoarchaeological campaigns.

3. Project aims and objectives

The aim of this study is to inform the Project and provide continuity of geoarchaeological assessment regarding the archaeological potential of the offshore development area. This has been achieved by sub-sampling and assessing offshore sub-samples collected from cores retained from previous geotechnical campaigns.

The objectives of the archaeological Stage 3 recording and sub-sampling of the recommended deposits are to:

- Answering questions regarding the occurrence of Holocene deposits in the study area to map potential channels;
- Understand and date the organic material that has been identified in the Swarte Bank formation; and,
- Identify datable material from Yarmouth Roads formation, and Egmond Ground, identified in previous studies. If datable material is located in these units, it can be used for comparison to previously dated material from on-shore areas.

4. Methodology

The assessment of potential archaeological deposits follows the staged approach described in *Model Clauses for Archaeological Written Schemes of Investigation: Offshore Renewables Projects* (The Crown Estate, 2010), COWRIE's *Offshore Geotechnical Investigations and Historic Environment Analysis: Guidance for the Renewable Energy Sector* (COWRIE, 2011), and *Environmental Archaeology: a guide to the theory and practice of methods, from sampling to post excavation* (English Heritage, 2011). This comprises the following elements:

- Stage 1 – Desk-based Assessment: archaeological review of geotechnical logs (Maritime Archaeology, 2017a);
- Stage 2 – Splitting, recording geotechnical cores and sub-sampling (Maritime Archaeology, 2017b);
- Stage 3 – Assessment (this report); and
- Stage 4 – Analysis and dating.

The staged approach is designed to flow sequentially with each stage leading to the subsequent stage of work, or representing the end of the assessment if the findings of any stage show that no further work is beneficial.

4.1 Stage 3 Assessment

Sub-samples from the cores listed in Table 2 below were selected for the Stage 3 assessment based previous recommendations (Maritime Archaeology, 2016, Wessex Archaeology, 2013), as well as on their potential to contain environmental indicators relevant for the Project and their likely preservation condition after long-term storage. Sub-samples of between 1-250 g were collected from the units of interest for archaeological laboratory assessment and analysis. The sub-samples were thereafter sent for further review by the environmental specialists. The results from the Stage 3 assessment have been compared with the earlier studies to clarify and strengthen our understanding of the units present and the nature of the pre-historic environment.

Core ID	Depth BSB	Deposit	Assessment focus
OSS1_BH	15.05	Swarte Bank	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds
OSS1_BH	23.9	Swarte Bank	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds, AAR dating
OSS1_BH	30	Yarmouth Roads	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds
OSS2_BH	22.2	Swarte Bank	Ostracods, Foraminifera, Diatoms, Plant/Peat/ Seeds
OSS3_BH	14.9	Swarte Bank	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds
OSS3_BH	27.5	Swarte Bank	AAR dating
OSS3_BH	57.5	Yarmouth Roads	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds
AC1a-CS	19.3	Swarte Bank	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds
AC2-CS	43.75	Swarte Bank	Ostracods, Foraminifera, Diatoms, Plant/Peat/ Seeds

AC2-CS	52.75	Yarmouth Roads	Ostracods, Foraminifera, Diatoms, Plant/Peat/ Seeds
AC3-CS	25.75	Swarte Bank	Ostracods, Foraminifera, Diatoms, Plant/Peat/ Seeds
DC1_CS	52.7	Yarmouth Roads	Ostracods, Foraminifera, Diatoms, Plant/Peat/ Seeds, AAR dating
RS-CS	12.35	Egmond Ground	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds
RS-CS	15.3	Egmond Ground	Ostracods, Foraminifera, Diatoms, Plant/Peat/ Seeds
RS-CS	17.5	Egmond Ground	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds
VC85a	1.4	Bolders Bank and Lower Botney Cut	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds
VC91	0.42	Bolders Bank and Lower Botney Cut	C14 dating
VC91	1.08	Bolders Bank and Lower Botney Cut	C14 dating
VC91	1.17	Bolders Bank and Lower Botney Cut	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds
VC92	0.47	Bolders Bank and Lower Botney Cut	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds
VC92	0.51	Bolders Bank and Lower Botney Cut	AAR dating
VC92	0.68	Bolders Bank and Lower Botney Cut	AAR dating
VC93	0.55	Bolders Bank and Lower Botney Cut	AAR dating
VC93	1.41	Bolders Bank and Lower Botney Cut	Ostracods, Foraminifera, Pollen, Diatoms, Plant/Peat/ Seeds

Table 2 Sub-samples assessed for environmental indicator and dating potential.

4.1.1 Pollen

Pollen is a valuable tool for reconstructing past environments. It can aid us in understanding the environmental landscape, economy and prehistoric human culture (English Heritage, 2011). Pollen are produced by higher plants (Vascular plants) and can, with the help of wind and/or water, travel relatively far. Pollen should therefore ideally be analysed with other environmental proxy evidence in order to gain a comprehensive understanding of the deposits (English Heritage, 2011).

The study was undertaken to determine presence or absence of sub-fossil pollen and spores and, if present, to provide a preliminary picture of the palaeo-vegetation and environment of the Project site and local environs during the timespan represented by the sedimentary units.

Standard techniques for pollen concentration of the sub-fossil pollen and spores were used on sub-samples of 1.5 ml volume (Moore and Webb, 1978; Moore *et al*., 1992), with the addition of micromesh sieving to aid removal of silica (clay/fine silt). Pollen counts of between c. 50 and 100 grains per level were made depending on the absolute numbers of pollen present. However, only minimal numbers (<10) were obtained from the lower levels of BH-16.

These procedures were carried out by Visiting Professor of Paleoecology, Dr Rob Scaife, in the Paleoecology Laboratory of the Department of Geography, University of Southampton. The results from the pollen assessment are presented in Section 5 and Appendix I.

4.1.2 Diatoms

Diatoms are freshwater and marine algae and, as the species are habitat-specific, they can be used to indicate water quality, temperature and salinity, nutrient and mineral levels, acidity and degree of oxygenation. Diatoms are most useful when investigating coastal and estuarine sites, providing data on marine influence and phases of sea-level change (Historic England, 2011).

The diatom analysis forms part of a wider palaeoenvironmental investigation at the site. The purpose of carrying out a diatom assessment is to test for the presence or absence of diatoms and the potential of the sediments for further diatom analysis. The diatom assessment of each sample takes into account the numbers of diatoms, the state of preservation of the diatom assemblages, species diversity and their environmental preferences. Of particular interest here are the salinity conditions represented by diatom assemblages.

The diatom preparation followed standard techniques (Battarbee *et al*., 2001). Two coverslips were made from each sample and fixed in Naphrax for diatom microscopy. A large area of the coverslips on each slide was scanned for diatoms at magnifications of x400 and x1000 under phase contrast illumination.

Diatom floras and taxonomic publications were consulted to assist with diatom identification. These salinity groups are summarised as follows:

1. Polyhalobian: >30 g l-1;
2. Mesohalobian: 0.2-30 g l-1;
3. Oligohalobian - Halophilous: optimum in slightly brackish water;
4. Oligohalobian - Indifferent: optimum in freshwater but tolerant of slightly brackish water;
5. Halophobous: exclusively freshwater, and;
6. Unknown: taxa of unknown salinity preference.

The diatom assessment was carried out by Dr Nigel Cameron of the Environmental Change Research Centre Department of Geography, University College London. The results from the assessment are presented in Section 5 and Appendix II.

4.1.3 Foraminifera and Ostracods

Foraminifera are a group of marine shell-bearing protozoans and their position in the sediment can be used for palaeoenvironmental study. Planktonic foraminifera live in marine waters of normal salinity and are very rare in brackish waters. These benthonic forms live at or near the sediment-water interface and occur in brackish to normal marine habitats, and at all depths. They are ideal for palaeoenvironmental analysis as many species have narrowly defined niches (Murray, 1991).

Ostracods are small bivalve crustaceans with calcareous shells that grow by shedding their old shell and secreting a new one. Ostracods inhabit nearly all types of aquatic environment from freshwater to marine, making them good indicators of changes in environment (Historic England, 2011).

A total of eighteen samples from twelve boreholes/vibrocores were weighed and then broken into small pieces by hand, placed in ceramic bowls and dried in an oven. Boiling-hot water was then poured over them, with a little sodium carbonate added to help disaggregate the clay fraction, and then left to

soak overnight. Washing was undertaken with hand-hot water through a 75 micron sieve, the remaining residue being returned to the ceramic bowl for final drying in the oven. The organic rich silts were, if required, processed twice and then the residues were stored in labelled plastic bags.

For examination, each sample was placed in a nest of sieves (>50, >250, >150 microns, and base pan) and thoroughly shaken. Each grade was then sprinkled onto a picking tray, a little at a time, and viewed under a binocular microscope. Organic remains were logged on a presence(x)/absence basis. The abundance of each species of foraminifera and ostracod was estimated semi-quantitatively (one specimen, several specimens, common and abundant/superabundant) by experience and by eye. For archive purposes, a representative sample of fauna of foraminifera and ostracods was also placed in 3x1" faunal slides.

The foraminifera and ostracods were assessed and analysed by Dr John E. Whittaker, Honorary Associate (Micropalaeontology), Department of Earth Sciences, The Natural History Museum, London. The results from the assessment are presented in Section 5 and Appendix III.

4.1.4 Radiocarbon Dating

Organic samples collected during sub-sampling that had the potential to yield reliable dates were analysed by the Scottish Universities Environmental Research Centre (SUERC) C14 laboratory in Glasgow.

The C14 age is quoted in conventional years BP (before 1950 AD). The error, which is expressed at the one sigma level of confidence, includes components from the counting statistics on the sample, modern reference standard and blank, and the random machine error. The carbon isotope ratios have been measured against Vienna Pee Dee Belemnite (VPDB).

The calibrated age ranges are determined from the University of Oxford Radiocarbon Accelerator Unit calibration program (OxCal v4). The marine calibration curve is based on Reimer *et al*. (2013).

The full specialist report from the C14 dating is presented in Appendix IV.

4.1.5 AAR dating

Amino acids are the building blocks of proteins. Proteins are found in all living tissues and can be preserved in fossil biominerals shells or coral. The rate of the breakdown of the amino acids within the protein can be analysed to indicate the date when the racemization (a spontaneous process starting after death) started to occur. The dating method is applicable to the whole of the Quaternary Period as it covers the date range from 10 years ago up to as long ago as 2.5 Ma. In the British context it is however most useful for dating Palaeolithic sites and Pleistocene deposits older than c. 40 ka.

The material examined for this project was examined under a microscope, sonicated and rinsed in HPLC-grade water and the shells were then crushed to <100 µm. 50 µL of 12% solution of sodium hypochlorite was added to each milligram of powdered sample and powders were bleached for 48 hours. It was then rinsed in HPLC-grade water and HPLC-grade methanol and dried. To release the protein bound amino acids an excess of 7 M HCl was added to the bleached powder and hydrolysing at 110oC for 24 hours (H*). 20 µL per milligram of sample of 7 M hydrochloric acid (HCl) was added to each hydrolysis ("Hyd", H*, THAA). The samples were flushed with nitrogen for 20 seconds to prevent oxidation of the amino acids, and were then placed in an oven at 110°C for 24 hours. Free amino-acid samples ("Free", F, FAA) were demineralised in cold 2 M HCl, which dissolves the

carbonate but minimises the hydrolysis of peptide bonds, and then dried in the centrifugal evaporator overnight.

When completely dry, samples were rehydrated and checked visually for undissolved particles. Approximately 15 µL of rehydrated sample was then placed in a sterile, labelled 2 mL autosampler vial and then placed on the autosampler tray of the HPLC. Amino acid enantiomers were separated by reverse phase high pressure liquid chromatography (RP-HPLC). Samples (2 µL) were derivitised with 2.2 µL o-phthaldialdehyde and thiol N-isobutyryl-L-cysteine automatically prior to injection. The resulting diastereomeric derivatives were then separated on Hypersil C18 BDS column (sphere d. 5 µm; 250 x 3 mm) using a linear gradient of a sodium acetate buffer (23 mM sodium acetate, 1.3 mM Na2EDTA; pH 6), methanol, and acetonitrile on an integrated 1100 liquid chromatograph (Agilent, USA).

The fluorescence intensity of derivitised amino acids was measured (Ex = 230 nm, Em = 445 nm) in each sample and normalised to the internal standard. All samples were run in duplicate alongside blank extracts that had been subjected to identical preparation procedures. Quantification of individual amino acids was achieved by comparison with the standard amino acid mixture. External standards containing a variety of D- and L- amino acids were analysed at the beginning and end of every run, and every ten samples.

The full specialist report from the AAR dating is presented in Appendix IV.

5. Results

The detailed results from the micro and macrofossil are presented below in Tables 2-14. The results have been included in the tables where samples where successful; for full sampling methodology, refer to Section 4. The results are discussed in their context in Section 6. The reports carried out by the external specialists, as outlined in Section 4, are included in Appendices I to V.

Core (Depth BSB)	Pollen	Foraminifera
OSS1_BH (15.05)	Pinus (pine) along with small numbers of Picea. Betula (birch), Alnus (alder), Quercus (oak) are present in notable numbers also with occasional Ulmus (elm), Salix (willow) and Corylus avellana type (hazel and/or bog myrtle). The herb pollen assemblages are dominated by Poaceae along with Cyperaceae (sedges) from the marsh/fen group. Single occurrences of Armeria (thrift or sea lavender) and Plantago maritima (sea plantain) along with important numbers of Chenopodiaceae (goosefoots, orache and samphire)	Marine foraminifera including *Elphidium* spp. and *Ammonia.*
OSS1_BH (23.90)	Pinus (pine) along with small numbers of Picea. Betula (birch), Alnus (alder), Quercus (oak) are present in notable numbers also with occasional Ulmus (elm), Salix (willow) and Corylus avellana type (hazel and/or bog myrtle). Calluna (ling) is present. The herb pollen assemblages are dominated by Poaceae along with Cyperaceae (sedges) from the marsh/fen group. Single occurrences of Armeria (thrift or sea lavender) and Plantago maritima (sea plantain) along with important numbers of Chenopodiaceae (goosefoots, orache and samphire)	Marine foraminifera including *Elphidium* spp. and *Ammonia*
OSS1_BH (30.00)	Pinus (pine) along with small numbers of Picea. Betula (birch), Alnus (alder), Quercus (oak) are present in notable numbers also with occasional Ulmus (elm), Salix (willow) and Corylus avellana type (hazel and/or bog myrtle). The herb pollen assemblages are dominated by Poaceae along with Cyperaceae (sedges) from the marsh/fen group. Single occurrences of Armeria (thrift or sea lavender) and Plantago maritima (sea plantain) along with important numbers of Chenopodiaceae (goosefoots, orache and samphire). Spores of Dryopteris type (typical frond ferns) and Sphagnum (bog moss) are of note.	Marine foraminifera including *Elphidium* spp. and *Ammonia*

Table 3 Micro and macro fossil assessment results from OSS1_BH

Core (Depth BSB)	Foraminifera
OSS1_BH (22.20)	Rare marine foraminifera of little diagnostic potential.

Table 4 Micro and macro fossil assessment results from OSS2_BH

Core (Depth BSB)	Pollen	Foraminifera
OSS3_BH (14.90)	Contains Picea, Pinus (Dominant) Betula, Alnus, very degraded and possible derived Tilia (lime) and occasional Quercus are present with Alnus being most important. Small numbers of dwarf shrub Calluna (ling) and Empetrum (crowberry) are present. The herb pollen assemblages are dominated by Poaceae (grasses) with only sporadic occurrences of other taxa. Marsh and aquatic plants comprise largely Cyperaceae and Pediastrum with Alisma plantago-aquatica, Typha/Sparganium type and Sphagnum.	Marine foraminifera including *Elphidium* spp. and *Ammonia*
OSS3_BH (57.5)	Contains Picea, Pinus (Dominant) Betula, Alnus, very degraded and possible derived Tilia (lime) and occasional Quercus are present with Alnus being most important. Small numbers of dwarf shrub Calluna (ling) and Empetrum (crowberry) are present. The herb pollen assemblages are dominated by Poaceae (grasses) with only sporadic occurrences of other taxa. Marsh and aquatic plants comprise largely Cyperaceae and Pediastrum with Alisma plantago-aquatica, Typha/Sparganium type and Sphagnum.	Marine foraminifera including *Elphidium* spp. and *Ammonia*

Table 5 Micro and macro fossil assessment results from OSS3_BH

Core (Depth BSB)	Pollen	Foraminifera
AC1a-CS (19.30)	Small numbers of reworked, pre-Quaternary palynomorphs (pollen, spores and Hystrichospheres). Quaternary pollen was absent apart from very occasional occurrences and as such, no pollen counts were obtained.	Rare marine foraminifera including Ammonia which is confined to temperate (interglacial) stages in the North Sea Basin.

Table 6 Micro and macro fossil assessment results from AC1a_CS

Core (Depth BSB)	Ostracods	Foraminifera	Plant/ Peat/ Seeds
AC2-CS (52.75)	Diverse micro fauna of ostracods, including *Sarscytheridea brad.*	Diverse micro fauna of marine foraminifera including *Elphidiella hannai* and *Elphidium spp.*	Plant debris (some “vitrified”) and megaspores. Remains of *Azolla filiculoides.*

Table 7 Micro and macro fossil assessment results from AC2-CS

Core (Depth BSB)	Plant/ Peat/ Seeds
AC3-CS (25.75)	Small black fragments, possibly vitrified plant debris and megaspores.

Table 8 Micro and macro fossil assessment results from AC3-CS

Core (Depth BSB)	Ostracods	Foraminifera	Plant/ Peat/ Seeds
DC1_CS (52.70)	Cold northern ostracods (*Sarscytheridea bradii* and *Robertsonites tuberculatus*	*Many Elphidiella hannai*	Shell and barnacle plate fragments

Table 9 Micro and macro fossil assessment results from DC1_CS

Core (Depth BSB)	Pollen	Foraminifera	Plant/ Peat/ Seeds
RS-CS (12.35)	Small numbers of reworked, pre-Quaternary *palynomorphs* (pollen, spores and *Hystrichospheres*). Very occasional occurrences of Quaternary pollen.	n/a	Contains lignite/peat fragments, megaspores, plant debris + seeds, insect remains, and *charophyte oogonia*
RS-CS (15.30)	n/a	n/a	Contains lignite/peat fragments, megaspores, plant debris + seeds, insect remains, and *charophyte oogonia*
RS-CS (17.50)	Small numbers of reworked, pre-Quaternary *palynomorphs* (pollen, spores and *Hystrichospheres*). Very occasional occurrences of Quaternary pollen.	Few possibly reworked marine foraminifera *Elphidium spp*	Contains lignite/peat fragments, megaspores, plant debris + seeds, insect remains, and *charophyte oogonia*

Table 10 Micro and macro fossil assessment results from RS_CS

Core (Depth BSB)	Pollen	Foraminifera	Plant/ Peat/ Seeds
VC85a (1.40)	Small numbers of comprised *Poaceae, Brassicaceae* (charlock family) and *Cyperaceae* (sedges). One individual cyst of freshwater algal *Pediastrum*.	Few marine foraminifera (*Miliolids* and *Ammonia)*	Marine molluscs (mainly fragments)

Table 11 Micro and macro fossil assessment results from VC85a

Core (Depth BSB)	Date (C14)	Diatoms	Ostracods	Pollen	Plant macros
VC91 (0.42)	43,586-42,389 cal BC				
VC91 (1.08)	46,058 cal BC				
VC91 (1.17)		Non-planktonic, marine diatoms Plagiogramma staurophorum, Opephora cf. marina and Grammatophora sp. The marine-brackish species include Cocconeis scutellum. The marine planktonic diatoms Paralia sulcata, Buddulphia sp. and Odontella cf. aurita The brackish-marine diatoms Achnanthes delicatula, Nitzschia punctata and cf. Campylodiscus echeneis	Brackish and freshwater ostracods	Dominated by grasses (*Poaceae)* also Containing (*autochthonous*) *Cyperaceae* (sedges) and *Typha angustifolia* type (bull rush and/or bur reed). Tree pollen include *Betula*, *Betula nana* (dwarf birch) and *Juniperus* (juniper) Abundant ferns spores of *Dryopteris* type (typical frond ferns) and cysts of freshwater algal *Pediastrum*.	Plant debris and seeds, insect remains, few *Mytilus juveniles.*

Table 12 Micro and macro fossil assessment results from VC91

Core (Depth BSB)	Date (AAR)	Ostracod	Plant/ Peat/ Seeds
VC92 (0.47)	n/a	Marine ostracods including *Sarsicytheridea bradii.*	Worn barnacle plates and mollusc fragments.
VC92 (0.68)	1,470 ± 40 BP (1,520-1,340 cal BP)		

Table 13 Micro and macro fossil assessment results from VC92

Core (Depth BSB)	Ostracods	Pollen	Foraminifera
VC93 (1.41)	Diverse micro fauna with marine ostracods, including *S. bradii*	Dominated by pollen of *coniferale* with few herbs. Substantial numbers of reworked/derived pre-Quaternary microfossils including spores, *saccate* pollen and *Hystrichospheres* (dinoflagellates).	Diverse micro fauna with marine foraminifera (*Elphidium spp*. and very abundant *Bulimina marginata*

Table 14 Micro and macro fossil assessment results from VC93

6. Discussion

It is clear from the amalgamated results that the study area is of an environmentally complex nature. The various factors that have led to the creation of what is now the sub-surface and sea floor of the North Sea have over thousands of years reformed and reworked the landscape. Periods of cold and hot climate have also caused environmental fluctuations over millennia, resulting in a highly multifaceted picture on which to base our understanding of the submerged landscape once widely inhabited by terrestrial people, flora and fauna.

The results of the assessments of environmental indicators in the form of micro and macro fossils and datable material, as presented in Section 5 and further detailed in Appendices I-V, provides some evidence for the nature of the deposits of archaeological potential as discussed below.

The aim of this Stage 3 assessment has been to inform the Project and provide continuity of geoarchaeological assessment regarding the archaeological potential of the offshore development area by aiming to answer the research questions presented in Section 3 and further discussed below.

1. Understanding the occurrence of Holocene deposits in the study area to map potential channels.

Samples recovered and assessed from what was assumed to be Upper Botney Cut and Holocene alluvium from vibrocores VC85a, VC91, VC92 and VC93 show that the sediments assessed from VC85a at 1.40 m BSB are most likely to contain Holocene material deriving from a possibly freshwater fen environment. This is derived from the pollen assemblage, with potential marine influxes based on the few marine foraminifera and molluscs that were recovered. The sample from VC92 was at 0.47 m BSB assumed to be an interglacial period, possibly MIS5, but was dated by AAR at 0.68 to 1,470 ± 40 BP (,1520-1,340 cal BP), indicating that the Holocene deposits recovered originated from a marine environment. Furthermore the ostracods and foraminifera recovered from VC91 at 1.17 m BSB are interpreted as being of Holocene origin. The C14 dates from the same core at 0.42 m and 1.08 m BSB, however, date the deposit to 43,586-42,389 calBC, 46,058calBC, respectively showing that the sediment from the top 1.5 m are most likely reworked and part of a much older deposit. The results from the sub-samples from 1.41 m BSB at VC93 indicate, from the identified pollen and foraminifera species, that the deposits are older than the Holocene period, deriving from the middle or earlier Pleistocene ages.

As noted in previous geoarchaeological assessments, it is apparent from the results of this Stage 3 assessment that the offshore study area does not support abundant Holocene deposits and it is therefore challenging to map potential channels using the current knowledge of the area. However, as seen in the Stage 3 assessment for sub-samples collected in the intertidal area (Maritime Archaeology, 2017), there is a potential for Holocene deposits located closer to the shore where a tidal mudflat would have been the primary landscape feature during the Holocene and until the sea levels settled at the current height around 7,000- 6,000 BP.

2. Understanding and dating the organic material that has been identified in the Swarte Bank formation.

The Swarte Bank Formation infills an array of palaeovalleys that are incised into the Early to Mid-Pleistocene deltaic and fluviatile formations (Southern North Sea Deltaic Group and Dunwich Group) and is in places intermittently present. The age of the infilling deposits spans from late Anglian to

earliest Hoxnian. The valleys are believed to have been cut by subglacial processes during Anglian times when the ice advanced into the southern North Sea area. The infilling of the valleys began with stiff glacial diamictons and glaciofluvial sand, overlain by glaciolacustrine muds and finishing with marine interglacial sediments of the Sand Hole and Egmond Ground formations.

Seven samples from six cores focused on understanding the Swarte Bank formation identified in the area during previous geoarchaeological assessments. The results, however, show that preservation of microfossils in the deposits has not been ideal which is why no material could be used for further dating of this unit. The assessment of the microfossils available show that the samples from OSS1_BH at 15.05 m and 23.90 m, as well as the sample from OSS3_BH at 14.90 m, contained some plant debris and seeds as well as megaspores and also some reworked shell fragments and rare marine foraminifera including *Elphidium spp.* and *Ammonia,* pointing towards a post-Anglian date (MIS 11 or MIS 9). The pollen samples from the samples in OSS1_BH suggest a grass-sedge fen, with indications of occasional alder growing along its fringes with possible local freshwater or salt march head of a marine transgression. The pollen from the two samples in OSS1_BH also insinuate a vegetation of very late boreal character, but this has not been confirmed though independent dating.

Some interesting results were contained in the sub-sample from OSS2_BH at 14.90 m where the pollen sample contained large numbers of derived/reworked pre-Quaternary microfossils (pollen, spores and hystrichospheres) which is indicative of a phase of fluvial erosion and alluvial deposition expected in the Swarte Bank deposit. The environment of deposition was a freshwater fen with standing freshwater with occasional local growth of Alder. Core AC1a_CS contained small numbers of reworked, pre-Quaternary palynomorphs (pollen, spores and Hystrichospheres) and rare marine foraminifera including *Ammonia* which is confined to temperate (interglacial) stages in the North Sea Basin. AC2-CS at 43.75 m contained a few marine foraminifera and some diverse micro fauna ostracods, which indicates a re-worked freshwater and marine of pre-Anglian or possibly Cromerian age. Based on this evidence, it can be said that the while no dates were obtained from the Swarte Bank deposit, it is apparent from the assessment of the microfossils that it is a reworked infill deposit of a sub-glacial valley system.

3. Identifying datable material from Unit 1, Yarmouth Roads formation, and Egmond Ground.

Due to the nature of the environment, poor preservation and lack of suitable organic material, no sub-samples from the Yarmouth Roads or Egmond ground deposits were successfully dated by C14 or AAR. However, in terms of further understanding the deposits using the results from the micro fossils, the most interesting evidence was found within core RS-CS, originally expected to contain the Egmond Ground deposit. The noteworthy result strongly indicated a freshwater environment with some evidence of re-worked marine deposits.

The sub-samples containing what was expected to be the Yarmouth Roads deposit presented mixed results with pollen indicating a boreal assemblage in OSS1_BH at 30.00 m BSB, mixed with taxon from earlier interglacial periods in OSS3BH_ at 57.50 m BSB. Samples from cores AC-CS and DC1_CS attest to the diverse micro fauna of ostracods and foraminifera which suggest a re-worked freshwater and marine, pre-Anglian or Cromerian age deposit. The results highlight the apparent glacial reworking of the area which has been noted during all of the geoarchaeological assessments conducted across the entirety of the subzone to date.

7. Recommendations

This Stage 3 assessment intended to identify and understand the environmental indicators within the sub-samples collected from the available cores, the results from which have shown that very few species have survived and that those detected within the deposits are poorly preserved. Further, the results from the dating sequence indicate that significant re-working has taken place across project

The overall condition of the sub-samples and the restricted results gained from this latest assessment stage, demonstrate that no further benefit is expected to be realised from continued analysis of the samples collected from the offshore area at Hornsea Project One Offshore Wind Farm. If further geotechnical work is undertaken in the intertidal or the offshore areas, geoarchaeological input should be sought early in the process to ensure that deposits located in areas with high archaeological potential are being retained and correctly stored to facilitate sub-sampling.

In order to ensure that the collective knowledge derived from the geoarchaeological studies undertaken to-date for the fulfilment of the various mitigation strategies achieves the maximum benefit in the public domain, it is recommended that these results are integrated with those from the intertidal studies and the adjoining subzones. Once collated, they are likely to make an important and beneficial contribution to our understanding of the palaeoenvironment and geography of the North Sea basin and should be presented as a whole in a peer-reviewed article and published in an appropriate academic journal.

8. References

Coles, B., 1998, Doggerland: a speculative survey, *Proceedings of the Prehistoric Society* 64: 45-81.

Battarbee, R.W., Jones, V.J., Flower, R.J., Cameron, N.G., Bennion, H.B., Carvalho, L. & Juggins, S.2001. Diatoms. In (J.P. Smol and H.J.B. Birks eds.), *Tracking Environmental Change Using Lake Sediments Volume 3: Terrestrial, Algal, and Siliceous Indicators*, 155-202. Dordrecht: Kluwer academic Publishers.

Boomer, I., Waddington, C., Stevenson, T., and Hamilton, D. 2007 "Holocene coastal change and geoarchaeology at Howick, Northumberland, UK" *The Holocene* 17, 89-104.

COWRIE, 2011. Gribble, J. and Leather, S. for EMU Ltd., 2011. *Offshore Geotechnical Investigations and Historic Environment Analysis: Guidance for the Renewable Energy Sector*. Commissioned by COWRIE Ltd (project reference GEOARCH-09). 2011.

DONG Energy, 2016a. *HOW01 Revised Archaeological Written Scheme of Investigation* –OFTO. June 2016.

DONG Energy, 2016b. *HOW01 Revised Archaeological Written Scheme of Investigation* – non-OFTO. June 2016.

DONG Energy, 2016c. *Hornsea Project One Written Scheme of Investigation (WSI) for Intertidal Archaeology*. September 2016.

DONG Energy, 2016d. *Hornsea Project One Offshore Windfarm Scope of Work Stage 1 Intertidal Archaeology.* December 2016.

English Heritage, 2011. *Environmental Archaeology: a guide to the theory and practice of methods, from sampling to post excavation*.

Krawiec, K., Hopla, E.and Gearey, B.R., 2011. *Palaeoenvironmental Assessment of near shore and offshore cores from the Hornsea Zone*. Birmingham ArchaeoEnvironmental.

Maritime Archaeology Ltd., 2015. *Hornsea Offshore Wind Farm Project One Revised Archaeological Written Scheme of Investigation*. September, 2015.

Maritime Archaeology Ltd., 2016. *Hornsea Offshore Wind Farm Project One Stage 1-2 Updated Geoarchaeological Assessment Report*. May, 2016.

Maritime Archaeology Ltd., 2017a. *Hornsea Offshore Wind Farm Project One Stage 1 Intertidal Geoarchaeological Assessment*. February, 2017.

Maritime Archaeology Ltd., 2017b. *Hornsea Offshore Wind Farm Project One Stage 2 Intertidal Geoarchaeological Assessment.* May, 2017.

Maritime Archaeology Ltd., 2017c. *Hornsea Project One Offshore Wind Farm Stage 3 Intertidal Geoarchaeological Assessment.* October, 2017.

Murray, J.W. *(eds) in Funnell, B.M. 1991. Chapter 12. Quaternary. In: Jenkins, D.G. & Murray, J.W. (eds), Stratigraphic Atlas of Fossil Foraminifera, Second Edition. British Micropalaeontological Society Series, John Wiley & Sons, London, pp. 563-569.*

Tappin, D R, Pearce, B, Fitch, S, Dove, D, Geary, B, Hill, J M, Chambers, C, Bates, R, Pinnion, J, Diaz Doce, D, Green, M, Gallyot, J, Georgiou, L, Brutto, D, Marzialetti, S, Hopla, E, Ramsay, E, and Fielding, H., 2011, *The Humber Regional Environmental Characterisation*. British Geological Survey Open Report OR/10/54. 357pp

The Crown Estate, 2014*. Protocol for Archaeological Discoveries: Offshore Renewables Projects.*

Wessex Archaeology, 2013. *Round 3 Hornsea Offshore Windfarm Subzone 1 and Export Cable Route Stage 1 and 2 Geoarchaeological Assessment*. April, 2013.

9. Figures

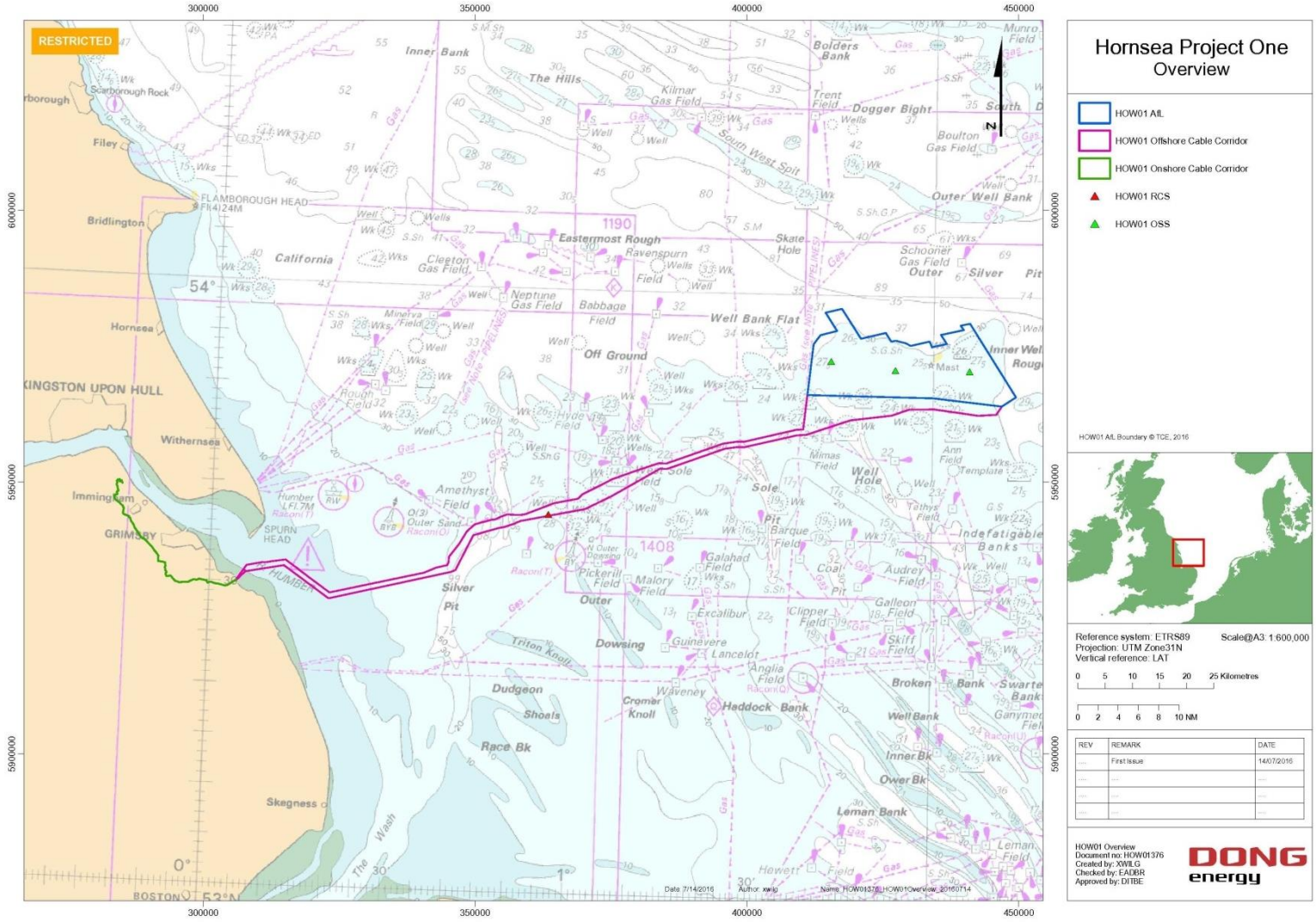

Figure 1 The location of Hornsea Project One Offshore Wind Farm (blue) and the consented export cable route corridor (magenta).

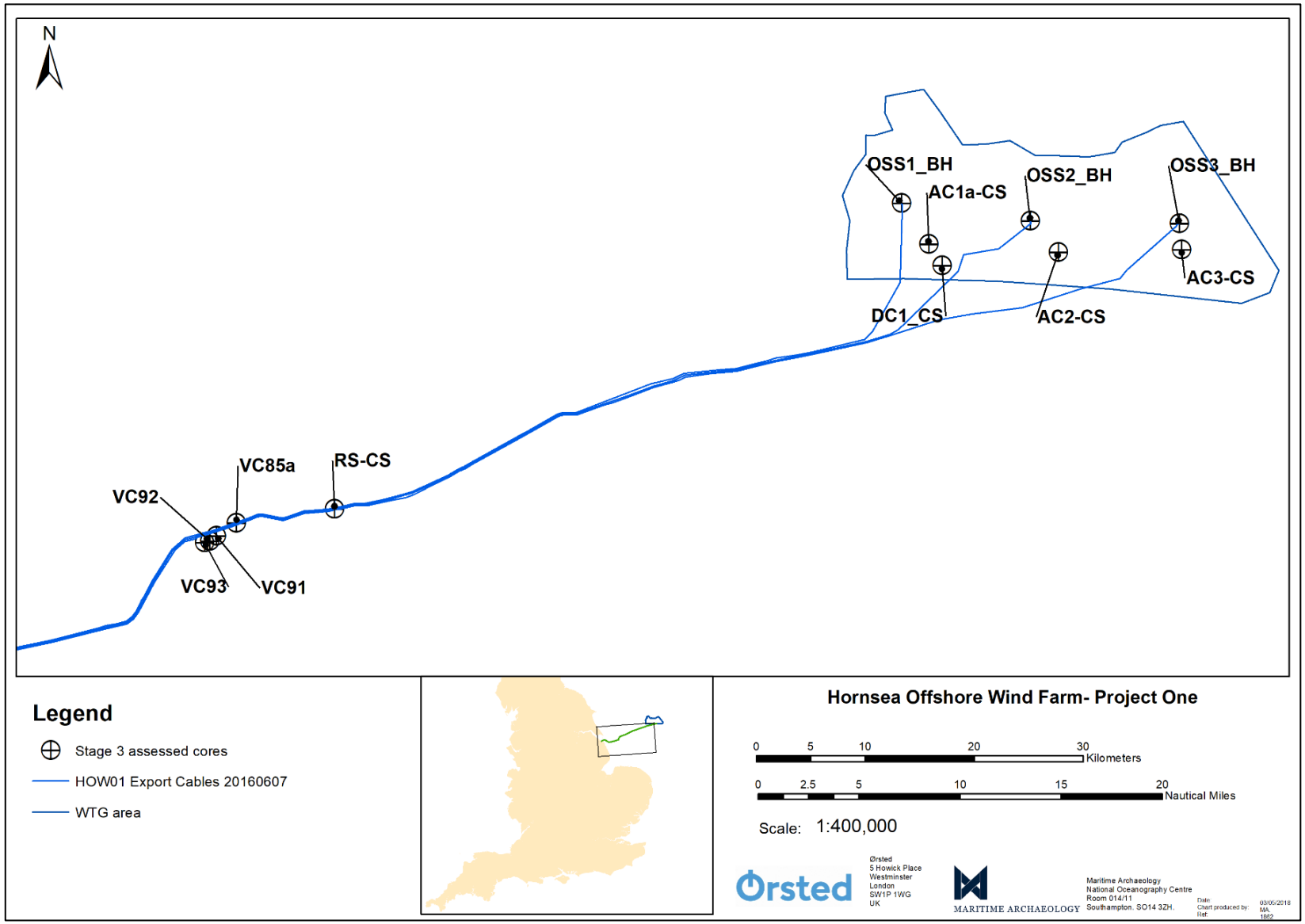

Figure 2 Locations of cores sub-sampled during the Stage 3 assessment.

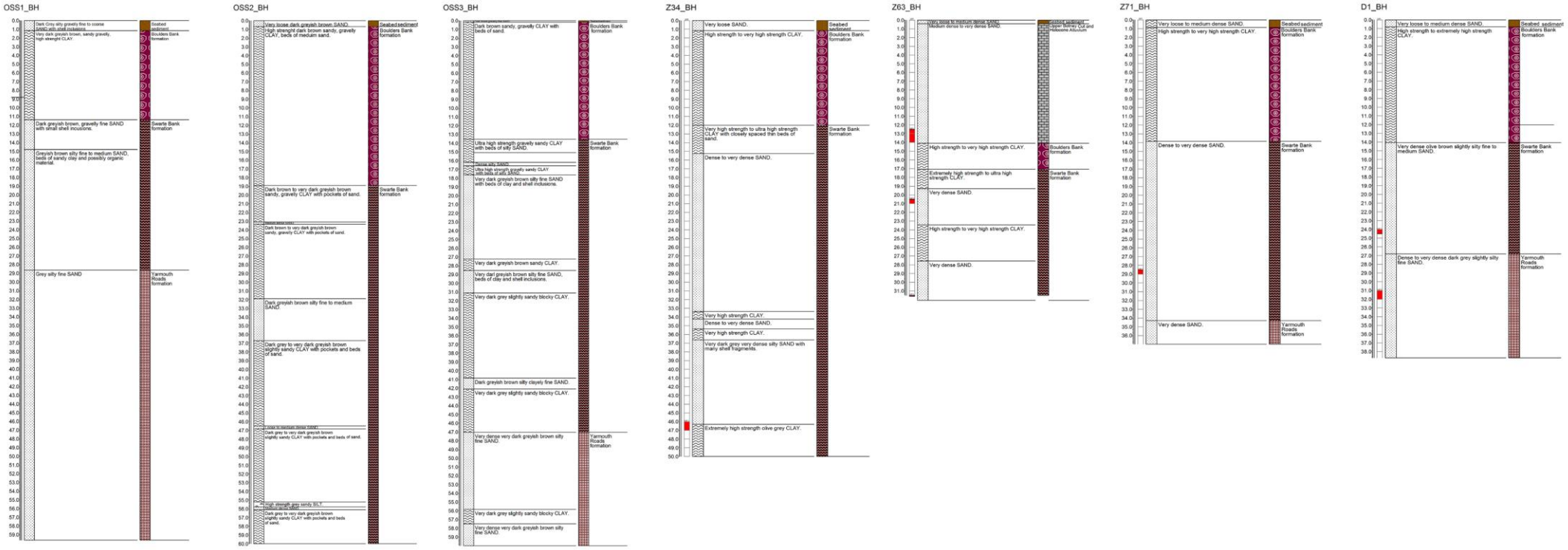

Figure 3 Deposit model based on the results of the Stage 3 assessment.

10. Appendix I Pollen Assessment

Hornsea (HOW 01) offshore sediment: an assessment of the sub-fossil pollen and spore content.

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1.) Introduction

Previous palynological studies (Langdon and Scaife 2017) demonstrated that some pollen and spores are preserved in this sediment archive. A further analysis has been undertaken in an attempt to find sediment with higher absolute pollen numbers. This would facilitate a more detailed and useful study of the past vegetation and environment and any change through time of the site and its surrounding zone. A series of pollen sub-samples were examined from a number of different bore-hole/core samples. As with the earlier analyses, preservation was found to be poor throughout. Some useful data have, however, been obtained from a number of samples which enhances the earlier/preliminary analysis.

2.) Pollen method

Samples of 5ml were prepared using techniques detailed in Moore and Webb (1978) and Moore *et al* (1982). Of the eleven samples examined, eight contained sub-fossil pollen and spores in sufficient numbers for identification and counting. Results obtained are presented in the following table 1.

Borehole	***VC91***	***VC93***	***OSS1_ BHSA***	***OSS1_ BHSA***	***OSS1- BHSA***	***OSS3_ BHSA***	***OSS3_ BHSA***	***VC85a***
Depth	***1.17***	***1.41***	***15.05***	***23.90***	***30.00***	***14.90***	***57.50***	***1.40***
Trees and shrubs								
Betula	18		1	3	24	4	19	
Betula cf nana	1							
Abies *		5						
Picea *		16	1	2	1	7	1	
Pinus		28	28	19	21	56	25	
cf Juniperus	1							
Tilia (degraded) *						7	1	
Ulmus					1			
Quercus				2	11		1	
Alnus		1	1	7	19	2	16	

Corylus avellana type					3			
Salix	1				1			
Calluna				2			3	
Empetrum						1	1	
Erica								
Herbs								
Poaceae	106		2	26	45	15	35	8
Ranunculus type							1	
Caltha type			1					
Brassicaceae								4
Chenopodiaceae	2			1	9			
Fabaceae indet.								1
Lotus type					1			
Caryophyllaceae indet.			1	1			1	
Spergularia						1		
Rosaceae		1			1			
Armeria 'B' line					2			
Pedicularis	1							
Plantago indet.				1				
Plantago lanceolata								
Plantago maritima					1			
Valeriana officinalis						1		
Bidens type							2	
Anthemis type			1					
Artemisia				1				
Lactucoideae			1		1	1	1	2
Marsh taxa								
Cyperaceae	15		6	6	7	8	17	3
Alisma plantago-aquatica						4		
Typha angustifolia type	21		1			4		
Sphagnum			5	15	38	5	14	
Pediastrum	94		1	32	31	130	17	1
Unident./ degraded pollen	1			2			2	
Ferns								
Dryopteris type	156	4	1	45	80	23	10	3
Osmunda regalis				1				
Equisetum	1							

Miscellaneous								
Pre-Quaternary	15	520	3	23	26	132	17	26
Carya								
Cuppressaceae								
Hystrichospheres	8	138	28	4		60	8	24

***Table** 1:* Pollen and spore counts obtained from samples taken from VC85A, VC91, VC93, OSS1_BHSA and OSS3_BHSA *(* taxa which may have been reworked earlier Pleistocene deposits)*

Sediment sub-samples from VC91, VC93, OSS1_BHSA and OSS3_BHSA produced enough preserved pollen and spores to enable pollen counts to be obtained. Samples were also examined from RS-CS; VC85A at 1.40; BH5 and AC1a-CS. These contained only small numbers of reworked, pre-Quaternary palynomorphs (pollen, spores and Hystrichospheres). Quaternary pollen was absent apart from very occasional occurrences and as such, no pollen counts were obtained.

3.) The pollen data
Each of the samples or sample sequences is described below in terms of the habitat of deposition, the surrounding vegetation and possible date. With regard to the latter, it should be noted that pollen is *not* a dating technique and suggestion are made only on the basis of what, in general, is known about sequences of vegetation change throughout the Pleistocene and Holocene periods.

***3.a.) VC91*:** A single sample at 1.41m. The pollen assemblage is dominated by herbs with Poaceae (grasses) being the dominant taxon. Other herb pollen taxa consist largely of the on-site (autochthonous) component comprising Cyperaceae (sedges) and *Typha angustifolia* type (bull rush and/or bur reed). There is relatively little tree pollen with *Betula* (birch) being most abundant with possible *Betula nana* (dwarf birch) and *Juniperus* (juniper) also present. There are abundant ferns spores of *Dryopteris* type (typical frond ferns) and cysts of freshwater algal *Pediastrum*.

Environment and suggested age: The environment of deposition was clearly freshwater as indicated by the large numbers of *Pediastrum*. This was probably fringed by typical grass-sedge fen with other taxa such as reedmace or bur reed and occasional willow.

The presence of birch, dwarf birch and juniper, the absence of other trees and the dominance of herbs suggest that this assemblage may be cold stage, probably Devensian

3.b.) VC93: A single sample at 1.41m. This sample is dominated by pollen of coniferale with few herbs. There are substantial numbers of reworked/derived pre-Quaternary microfossils including spores, saccate pollen and Hystrichospheres (Hystrichospheres).

There is little evidence for the character of the depositional habitat in terms of the on-site vegetation. However, given the large numbers of reworked pollen and spores, it is probable that this was an active fluvial floodplain environment with continuous sedimentation. The dominance of conifer pollen is interesting because, although *Pinus* (pine) appears dominant, the assemblage also includes *Picea* (spruce) and *Abies* (fir). Although there are substantial numbers of reworked saccate (i.e. conifer) grains of pre-Pleistocene age (based on the morphology of the saccae), we feel that these latter taxa are indeed attributable to spruce and fir. However, neither of these is native to Britain during the Holocene but were constituents of earlier interglacial phases. It is, therefore, possible that this sediment sample may be of middle Pleistocene age; from a late temperate zone. Alternatively, given the substantial numbers of reworked, pre-Quaternary microfossils, there is also the possibility that they may also be reworked but from earlier Pleistocene sequences which are also present in the North Sea Basin.
If contemporaneous with the sediment deposition, a boreal coniferous forest type of late temperate or immediate post temperate age is inferred by pollen of *Abies*.

From a single sample, it is obviously not possible to observe vegetation through a time-period. Accepting that there may have been some reworking of older, material,
***3c.)* VC85a:** This sample contained only very small numbers of pollen and little useful information can be gleaned. No tree or shrub pollen was seen and the few herb pollen taxa, in small numbers, comprised Poaceae, Brassicaceae (charlock family) and Cyperaceae (sedges). An individual cyst of freshwater algal *Pediastrum* was found.

If any interpretation can be made, it is that the sediment was probably lain down in a freshwater fen habitat.

3.d.) OSS1_BHSA): Three samples were taken at depths of 15.05m, 23.90m and 30.0m. *Pinus* (pine) is important in all samples along with small numbers of *Picea*. The basal sample assemblage at 30.0m contains more pollen of broad leaved/deciduous trees than other samples discussed. *Betula* (birch), *Alnus* (alder), *Quercus* (oak) are present in notable numbers also with occasional *Ulmus* (elm), *Salix* (willow) and *Corylus avellana* type (hazel and/or bog myrtle). *Calluna* (ling) is present at 23.90m.

The herb pollen assemblages are dominated by Poaceae along with Cyperaceae (sedges) from the marsh/fen group. Single occurrences of *Armeria* (thrift or sea lavender) and *Plantago maritima* (sea plantain) along with important numbers of Chenopodiaceae (goosefoots, orache and samphire) are of particular note indicating possible mud flat and salt marsh habitats. Spores of *Dryopteris* type (typical frond ferns) and *Sphagnum* (bog moss) are of note in the basal sample.

The environment of deposition was a grass-sedge fen, possibly with occasional alder growing along its fringes; especially in the lower profile. The presence of cysts of algal *Pediastrum* suggests local freshwater although fluvial transport may also be a factor. The notable numbers of *Sphagnum* spores in the basal level indicate the possibility of some acid conditions within the catchment.

Although only a single pollen occurrences, the presence of thrift or sea lavender (*Armeria/Limonium*) and sea plantain (*Plantago maritima*) along with the substantial numbers of Chenopodiaceae, as noted, are highly indicative of salt marsh halophytic plant communities. This was probably a prelude to marine transgression with initial brackish water incursions transporting pollen.

Overall, the pollen assemblage suggests vegetation of very late boreal character. That is, with importance of deciduous woodland but also with remaining pine. The latter, however, may to some extent be over represented due to the possible marine/brackish water conditions and the propensity of pine for over-representation in fluvial habitats. This would be in accord with a late Holocene Boreal age at *c.*7,500BP immediately prior to marine transgression. The presence of spruce (*Picea*) pollen remains enigmatic.

3.e.) OSS3_BHSA: Two samples were taken from 57.50m and 14.9m. This long sediment sequence does not contain *Abies* but does have *Picea* and *Pinus.* The latter is dominant, especially at 14.90m. There is also a range of other tree pollen of which *Betula*, *Alnus,* very degraded and possible derived *Tilia* (lime) and occasional *Quercus* are present with *Alnus* being most important. Small numbers of dwarf shrub *Calluna* (ling) and *Empetrum* (crowberry) are present. The herb pollen assemblages are dominated by Poaceae (grasses) with only sporadic occurrences of other taxa. Marsh and aquatic plants comprise largely Cyperaceae and *Pediastrum* with *Alisma plantago-aquatica*, *Typha/Sparganium* type and *Sphagnum*.

The environment of deposition was a freshwater fen as indicated by Cyperaceae (sedges), *Alisma plantago-aquatica* (water plantain) and *Typha angustifolia/Sparganium* (reed mace and/or bur reed) with standing freshwater as indicated by high numbers of algal *Pediastrum* in the upper sample at 14.90m (possibly fluvially transported). A proportion of the Poaceae may also be attributed to this fen habitat. The small numbers of *Alnus* (alder) may suggest occasional local growth perhaps surrounding the wetter fen habitat. More acid conditions are also indicated by Ericaceae (*Calluna* and *Empetrum*) of heathland affinity and ay also be associated with *Sphagnum*. The upper analysed sample contains large numbers of derived/reworked pre-Quaternary microfossils (pollen, spores and hystrichospheres) which is indicative of a phase of fluvial erosion and alluvial deposition.

The terrestrial vegetation probably comprised a largely boreal habitat with coniferous pine and spruce. As with other samples described, the presence of the former is enigmatic being a taxon of earlier interglacial periods and not of the Holocene. As also noted, taphonomic factors may play a role with the possibility of reworking of earlier sediment. Although many of the pre-Quaternary palynomorphs are of saccate form, those attributed here to *Picea* were considered after careful examination, to be of more recent origin. *Betula* (birch) is also of importance and is in accord with a boreal habitat of pre-temperate type.

4.) Discussion

It is unfortunate that pollen preservation is poor with small absolute pollen numbers present as some enigmatic questions have been raised about the age/date of the sediment. Palynologically, this relates to the presence of spruce (*Picea*) in most of the samples and of fir (*Abies*) in one (VC93). Neither trees are native to Britain during the Holocene but, were important woodland elements in previous interglacial and in some cases, interstadial periods into the early Devensian. Thus, it may be construed that the sediments are of middle or

earlier Pleistocene age. Such sedimentary sequences are relatively abundant in Eastern England having provided type sequences such as that for the Cromerian, Hoxnian and Ipswichian temperate stages. However, the striking numbers of reworked/derived pollen from earlier sediment may also suggest that these robust conifer pollen grains may similarly have been reworked from earlier Pleistocene sequences. Whilst there are many pre-Quaternary saccate coniferales pollen in some samples, we are (fairly!) confident that identifications were not of the pre-Quaternary palynomorph (*Piceapollenites* and *Abietopollenites*) and are Pleistocene.

Pine (*Pinus*) pollen in most of the samples with differing levels of birch (*Betula*) suggests that the samples date to an early (pre-temperate) stage of an interglacial. If this were Holocene, containing reworked spruce and fir, this would equate with the early Holocene pre-Boreal or Boreal periods (Flandrian chronozone 1a-b). Thus dating to sedimentation prior to late-Boreal and early middle Holocene marine transgression in the North Sea basin. If the sediment is of earlier interglacial age, a pre-temperate, similar boreal type habitat of conifers similarly applies. However, where sample *VC93* has fir, this would be attributable to a late or immediate post temperate phase Radiocarbon dating of suitable organic material might solve this problem, as a sample of an interglacial age would produce an infinite date.

The depositional shown by all of the samples was a freshwater grass-sedge fen with typical plants of that habitat. That is, grasses, sedges, reed mace, bur reed, water plantain, marsh marigold.

Some saline/brackish water conditions are indicated in one of the sequences (OSS1_BHSA) and as noted, may be a prelude to more extensive marine transgression.

5.) Summary and conclusions
The following principal points have been made in this analysis

- Pollen is sparse, poorly preserved and absent in some samples examined. However, some useful palaeoecological data have been obtained.

- The pollen assemblages contain predominantly conifer pollen which includes pine, spruce and fir suggesting boreal type vegetation habitats.

- The presence of spruce and fir pollen may indicate that the samples are of Pleistocene age. However, it is possible that, along with substantial numbers of geological microfossils, these may have also been eroded from earlier sediment. This emphasises the complexity of the pollen and sediment taphonomy.

- The depositional habitat of all of the samples appears to have been freshwater, grass-sedge fen, possibly with some alder in drier zones fringing wetter fen.

- The lower section of OSSQ_BHSA contains pollen of salt marsh and possible mud-flat halophytes. These may be pre-cursors to marine transgression.

Further analyses: Because of the poor pollen preserving conditions, it is unlikely that any further examination would produce significantly more information.

References

Bennett, K.D., Whittington, G. and Edwards, K.J. 1994 'Recent plant nomenclatural changes and pollen morphology in the British Isles'. *Quaternary Newsletter* 73,1-6

Moore, P.D. and Webb, J.A. 1978 *An Illustrated Guide to Pollen Analysis*. London: Hodder and Stoughton.

Moore, P.D., Webb, J.A. and Collinson, M.E. 1991 *Pollen Analysis.* Second edition. Oxford: Blackwell Scientific.

11. Appendix II Diatom Assessment

Diatom assessment of samples from Hornsea Offshore Wind farm (Offshore cores)

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Introduction

Following an earlier assessment of diatoms from four borehole sequences at the Hornsea Offshore Windfarm site inshore area at Horseshoe Point, Lincolnshire (Cameron 2017) a diatom assessment has been carried out on eighteen samples taken from offshore cores at the Hornsea Offshore Windfarm site. The offshore boreholes were sampled from the following seabed areas: Swarte Bank; Yarmouth Roads; Egmond Ground; Botney Cut and Holocene Alluvium.

Diatom analysis forms part of a wider palaeoenvironmental investigation at the site. The purpose of carrying out the diatom assessment is to test for the presence or absence of diatoms and the potential of the sediments for further diatom analysis. The diatom assessment of each sample takes into account the numbers of diatoms, the state of preservation of the diatom assemblages, species diversity and diatom species environmental preferences. Of particular interest here are the salinity conditions represented by diatom assemblages.

Methods

Diatom preparation followed standard techniques (Battarbee *et al.* 2001). Two coverslips were made from each sample and fixed in Naphrax for diatom microscopy. A large area of the coverslips on each slide was scanned for diatoms at magnifications of x400 and x1000 under phase contrast illumination.

Diatom floras and taxonomic publications were consulted to assist with diatom identification; these include Hendey (1964), Werff & Huls (1957-1974), Hartley *et al.* (1996), Krammer & Lange-Bertalot (1986-1991), Camburn & Charles (2000) and Witkowski *et al.* (2000). Diatom species' salinity preferences are indicated using the halobian groups of Hustedt (1953, 1957: 199), these salinity groups are summarised as follows:

1. Polyhalobian: >30 g l-1
2. Mesohalobian: 0.2-30 g l-1
3. Oligohalobian - Halophilous: optimum in slightly brackish water
4. Oligohalobian - Indifferent: optimum in freshwater but tolerant of slightly brackish water
5. Halophobous: exclusively freshwater
6. Unknown: taxa of unknown salinity preference.

Results & Discussion

The diatom sample identification numbers (Nos.D1-D18), the names of the seabed sampling areas, borehole numbers and sample depths are shown in Table 1. The results of the diatom evaluation are summarised in Table 2 below, and diatom species data are presented in Table 3 where the diatom taxa are grouped by salinity preferences.

Table 1. Outline of cores, depths and sample ID referenced in the report.

Core	Depth	Diatom sample ID
OSS1_BH	15.05	D1
OSS2_BH	22.20	D2
OSS3_BH	14.90	D3
AC1a-CS	19.30	D4
AC2-CS	43.75	D5
AC3-CS	25.75	D6
OSS1_BH	23.90	D7
OSS1_BH	30.00	D8
OSS3_BH	57.5	D9
AC2-CS	52.75	D10
DC1_CS	52.7	D11
VC92	0.47	D17
VC85a	1.40	D18
VC91	1.17	D12
VC93	1.41	D13
RS-CS	12.35	D14
RS-CS	15.3	D15
RS-CS	17.5	D16
VC92	0.47	D17
VC85a	1.40	D18

Table 2. Summary of diatom evaluation results for Hornsea Offshore Windfarm offshore borehole sequences (+ present; - absent; mar-bk marine-brackish; mar marine). See Table 1 for details of sites and cores.

Diatom Sample No./BH	Diatoms	Diatom Numbers	Quality of Preservation	Diversity	Assemblage type	Potential for % count
SWBK						
D1	-	-	-	-	-	none
D2	-	-	-	-	-	none
D3	-	-	-	-	-	none
D4	-	-	-	-	-	none
D5	-	-	-	-	-	none
D6	-	-	-	-	-	none
D7	-	-	-	-	-	none
YR						

D8	-	-	-	-	-	none
D9	-	-	-	-	-	none
D10	-	-	-	-	-	none
D11	-	-	-	-	-	none
uBCT/HA						
D12	+	v low	v poor	low	mar, mar-bk	none
D13	-	-	-	-	-	none
EG						
D14	-	-	-	-	-	none
D15	-	-	-	-	-	none
D16	-	-	-	-	-	none
uBCT/HA						
D17	-	-	-	-	-	none
D18	-	-	-	-	-	none

Swarte Bank (samples D1-D7)

Seven samples from six cores taken at the Swarte Bank site were assessed for diatoms. Diatoms are absent from all seven samples. There is no further potential for diatom analysis of these samples.

Yarmouth Roads (samples D8-D11)

Four samples from four cores taken at the Yarmouth Roads site were assessed for diatoms. Diatoms are absent from all four samples. There is no further potential for diatom analysis of these samples.

Egmond Ground (samples D14-D16)

Three samples from three cores taken at the Egmond Ground site were assessed for diatoms. Diatoms are absent from all three samples. There is no further potential for diatom analysis of these samples.

Upper Botney Cut and Holocene Alluvium (samples D12-D13, D17-D18)

Four samples from four cores taken at the Upper Botney Cut and Holocene Alluvium site were assessed for diatoms. Diatoms are absent from three samples. A very poorly-preserved diatom assemblage with low species diversity is present in sample D12. There is no further potential for diatom analysis of any of these samples. However, the diatoms present in D12 represent marine and marine-brackish habitats; no freshwater diatoms were identified.

Table 3 Assessment of diatoms present in sample D12 (1 - present; 2 - relatively common)

Diatom Taxon/Laboratory Sample Number	**D12**
Polyhalobous	
Biddulphia sp.	1
Grammatophora sp.	1
Paralia sulcata	1

Odontella cf. aurita	1
Opephora cf. marina	2
Plagiogramma staurophorum	2
Polyhalobous to Mesohalobous	
Cocconeis scutellum	1
Mesohalobous	
Achnanthes delicatula	1
cf..Campylodiscus echeneis	1
Nitzschia punctata	1
Unknown Salinity Group	
Amphora sp.	1
Diploneis sp.	1
Inderminate centric sp.	1
Unknown diatom fragment	2

The most common diatoms in D12 are the non-planktonic, marine diatoms *Plagiogramma staurophorum* and *Opephora* cf. *marina*. Other non-planktonic, marine diatoms in D12 include *Grammatophora* sp. and the marine-brackish species *Cocconeis scutellum*. The marine planktonic diatoms *Paralia sulcata*, *Buddulphia* sp. and *Odontella* cf. *aurita* are present. The brackish-marine diatoms *Achnanthes delicatula*, *Nitzschia punctata* and cf. *Campylodiscus echeneis* are also present in sample D12. The first of these diatoms is an attached, non-planktonic species, and the latter two are benthic taxa. Overall in D12 the relatively high number of non-planktonic diatoms suggests that the sedimentary environment was in shallow water.

The absence of diatoms from all but one, poorly preserved sample, taken from the Hornsea Offshore Windfarm offshore boreholes can be attributed to taphonomic processes (Flower 1993, Ryves *et al.* 2001). This loss of diatoms may be the result of diatom silica dissolution and breakage caused by factors such as extremes of sediment alkalinity or acidity, the under-saturation of sediment pore water with dissolved silica, cycles of prolonged drying and rehydration, movement of water, or physical damage to diatom valves from abrasion or wave action.

Conclusions

1. Eighteen samples were assessed for diatoms. The samples were taken from four areas (Swarte Bank, Yarmouth Roads, Egmond Ground, Upper Botney Cut and Holocene Alluvium) at the Hornsea Offshore Windfarm offshore site.
2. Diatoms are absent from these samples with the exception of sample D12 from VC91 taken from the Upper Botney Cut and Holocene Alluvium. Sample D12 contains a poorly-preserved marine and brackish-marine diatom assemblage. Freshwater diatoms are absent from the diatom assemblage. There are proportionately more non-planktonic diatom taxa and this suggests that the sedimentary environment was in shallow water.
3. The absence of diatoms from seventeen samples and the poor preservation of a single diatom assemblage in the Hornsea Offshore Windfarm offshore boreholes can be attributed to taphonomic processes. There is no further potential for diatom analysis of these samples.

Acknowledgements

Thanks to Christin Heamagi of The Maritime Archaeology Trust, Department of Oceanography, University of Southampton for providing the samples for diatom assessment and for information about the Hornsea Offshore Windfarm site.

References

Battarbee, R.W., Jones, V.J., Flower, R.J., Cameron, N.G., Bennion, H.B., Carvalho, L. & Juggins, S. 2001. Diatoms. In (J.P. Smol and H.J.B. Birks eds.), Tracking Environmental Change Using Lake Sediments Volume 3: Terrestrial, Algal, and Siliceous Indicators, 155-202. Dordrecht: Kluwer Academic Publishers.

Cameron, N.G. 2017. Diatom assessment of samples from Hornsea Offshore Windfarm (inshore area at Horseshoe Point, Lincolnshire). Unpublished Report to The Maritime Archaeology Trust, Department of Oceanography, University of Southampton. pp 6

Flower, R.J. 1993. Diatom preservation: experiments and observations on dissolution and breakage in modern and fossil material. *Hydrobiologia 269/270*: 473-484.

Ryves, D. B., Juggins, S., Fritz, S. C. & Battarbee, R. W. 2001.
Experimental diatom dissolution and the quantification of microfossil preservation in sediments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 172, 99-113

Hartley, B., H.G. Barber, J.R. Carter & P.A. Sims. 1996. *An Atlas of British Diatoms.* Biopress Limited. Bristol. pp. 601.

Hendey, N.I. 1964 An Introductory Account of the Smaller Algae of British Coastal Waters. Part V. Bacillariophyceae (Diatoms). Ministry of Agriculture Fisheries and Food, Series IV. pp. 317.

Hustedt, F. 1953. Die Systematik der Diatomeen in ihren Beziehungen zur Geologie und Okologie nebst einer Revision des Halobien-systems. *Sv. Bot. Tidskr.*, 47: 509-519.

Hustedt, F. 1957. Die Diatomeenflora des Fluss-systems der Weser im Gebiet der Hansestadt Bremen. *Ab. naturw. Ver. Bremen* 34, 181-440.

Krammer, K. & H. Lange-Bertalot, 1986-1991. *Bacillariophyceae.* Gustav Fisher Verlag, Stuttgart.

Werff, A. Van Der & H. Huls. 1957-1974 *Diatomeenflora van Nederland*, 10 volumes

Witkowski, A, H. Lange-Bertalot & D. Metzeltin 2000. *Diatom Flora of Marine Coasts I.* Iconographia Diatomologica. Annotated Diatom Micrographs Ed. by H. Lange-Bertalot Vol. 7. A.R.G. Gantner Verlag. Koeltz Scientific Books. Königstein, Germany pp 925

12. Appendix III Microfaunal assessment

Hornsea Offshore Windfarm, North Sea (How 01): microfossil assessment of samples from 12 boreholes/vibrocores

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INTRODUCTION

A total of 18 samples from twelve boreholes/vibrocores were received on 13th November 2017 from Christin Heamagi (Maritime Archaeology, Southampton). They are from the Hornsea Project One Offshore Windfarm, some 120-160km due east of Hornsea, on the Yorkshire coast, one of the largest North Sea windfarm arrays. All the cores except RS-C5 are from the main windfarm site, whereas the vibrocores and core RS-C5 are from the offshore cable route. Previous analyses on these and a great many other cores had concentrated on the sediments and their properties for the purposes of the windfarm construction. Attribution to several North Sea sedimentary formations, apparently extracted from confidential reports by contractors and the British Geological Survey, was provided in figures (via Wessex Archaeology) kindly provided by Christin Heamagi, but she suggests they are based on little, if any, real dating. The purpose of the present assessment, therefore, was not only to indicate the depositional environment of the samples examined, but also to provide, if possible, actual age determinations through their contained microfossils (foraminifera and ostracods) and, additionally, to suggest any suitable samples for AAR dating using shell material.

MATERIALS & METHODS

Core	Depth	Weight processed
AC1a-CS	19.30m	40g
AC2-CS	43.75m	45g
AC2-CS	52.75m	60g
AC3-CS	25.75m	60g
DC1-CS	52.70m	35g
OSS1-BH5A	15.05m	25g
OSS1-BH5A	23.90m	25g
OSS1-BH5A	30.00m	25g
OSS2-BH5A	22.20m	15g
OSS3-BH5A	14.90m	40g
OSS3-BH5A	57.50m	35g
RS-C5	12.35m	150g
RS-C5	15.30m	35g

RS-C5	17.50m	50g
VC85A	1.40m	90g
VC91	1.17m	75g
VC92	0.47m	50g
VC93	1.41m	50g

The samples were first broken up into small pieces and put into bowls to be dried in an oven. Then a teaspoon of sodium carbonate was added (to help remove any clay fraction) and hot water was poured over them. They were left to soak overnight. Each was then washed trough a 75 micron sieve, using hand-hot water and the resultant residue returned to the bowl. All the samples broke down readily. The residues were returned to the oven again and thoroughly dried, before being placed in labelled plastic bags for storage. Before picking, each dry sample was put through a nest of sieves (>500, >250,>150 microns and pan) and a little of each residue at a time sprinkled on a picking tray. A representative number of foraminifera and ostracods were picked out of each sample and transferred to a 32-square 3"x1" faunal slide for future reference.

RESULTS

The samples, in documents provided by Christin Heamagi (Maritime Archaeology), have been assigned to a number of North Sea Quaternary (and Holocene) formations as stated in the **Introduction** and below, but these are based, mainly it seems, on lithological characteristics unsupported by little if any real dating. Moreover, included in one document is a cryptic note which states … "most of the samples have been stored in bags and are therefore a mixed depth of 5cm to 25cm". So contamination cannot be ruled out either. Microfossils, nevertheless, found during the present assessment must provide a better indication of age and/or ecology than heretofore, imperfect though that might be. Palynological analysis is being undertaken on some of these samples and it is hoped AAR dating using shell material when available, may also be able to produce important results.

CORES

AC1a-CS and AC3-CS

Said to belong to the Swarte Bank Formation (samples from 19.30m 25.75m, respectively). This, Cameron & Holmes (*in* Bowen, 1999) state infills Anglian glacial palaeovalleys; commonly the sediments are glaciolacustrine muds but there are latterly also warm-temperate marine sediments. Thus the age is thought to be MIS 12 extending into MIS 11.

AC1a-CS (19.30m) is a white silty sand containing very rare marine foraminifera, but includes *Ammonia* which according to Funnell (1989) is... "confined to temperate (interglacial) stages in the North Sea Basin". That is all that can be said. There is very little residue available after processing the other sample from AC3-CS (25.75m). All it contains are small black fragments (?vitrified plant debris) and megaspores. Palynology may verify at least the ecology in this case.

AC2-CS

Said to be Swarte Bank Formation (sample from 43.75m) above Yarmouth Roads Formation (sample from 52.75m). This latter is strongly diachronous in the southern North Sea and was deposited before MIS 12, according to Cameron & Holmes (*in* Bowen, 1999).

The upper sample, an orange silty sand is completely barren. The sample from 52.75m, a grey silty sand, has potential. It contains plant debris (some "vitrified") and megaspores. In addition, are the "ice-cream-cone-like" remains of *Azolla filiculoides* which is immediately significant. This, the so-called "duckweed fern", is found only in (near)-freshwater locales. Preservation here was very good and the spores were still attached. If the *Azolla* was indeed *in situ,* as seems the case, then it has important age significance, because according to Watts (1988), restated by Turner (*in* Wenban-Smith *et al*., 2006)….. "it seems to have become extinct in Britain after MIS9". A further component comprised quite a diverse micro fauna of marine foraminifera and ostracods, including *Elphidiella hannai* and *Elphidium s*pp. (foraminifera), and the "northern" ostracod *Sarscytheridea bradii,* which today is confined to North Britain and Scandinavia. Moreover, *E. hannai*... "is not known from the British Isles area after the Aanglian stage", according to Funnell, 1989. It remains to reconcile both freshwater and marine components in the same sample (?reworking), but at least part of the micro fauna is pre-Anglian and possibly of Cromerian age.

DC1-CS

Said to belong to the Yarmouth Roads Formation. The single sample provided here from 62.70m contains shell and barnacle plate fragments, ?lignite, a great many *Elphidiella hannai* (foraminifera both in brown and white preservation) and "cool/cold northern" ostracods (*Sarscytheridea bradii* and *Robertsonites tuberculatus*, for example). *E. hannai* is not know in post-Anglian deposits in the North Sea (Funnell, 1989). This is further evidence that the Yarmouth Roads Formation is pre-Anglian.

OSS1-BH and OSS3-BH

Said to be Swarte Bank Formation (samples at 15.05, 23.90m in OSS1-BH, and 14.90m in OSS3-BH, respectively). The lower samples, at 30.00m and 57.50m, from the same respective cores are said to belong to the Yarmouth Roads Formation.

The samples said to be Swarte Bank Formation contains plant debris + seeds, ?lignite, and magaspores. They also contain shell fragments (?reworked) and rare marine foraminifera including *Elphidium* spp. and *Ammonia.* The fact that these residues are stony probably derived from Anglian till, contain *Ammonia* and lack *Elphidiella hannai* (see comments on these taxa above), all point to a post-Anglian date (MIS 11 or MIS 9). The supposed Yarmouth Roads samples, below, contain plant debris, seeds and spores for which palynology may prove useful.

OSS2-BH

Said to be Swarte Bank Formation (sample from 22.20m). Again the residue is stony. Contains only rare marine foraminifera of little diagnostic potential except to suggest they are post-Anglian.

RS-CS

Situated on the cable route. Said to be Egmond Ground Formation (samples from 12.35, 15.30 and 17.50m). According to Cameron & Holmes (*in* Bowen, 1999) it is said to be of interglacial aspect; equivalent sediments in the Dutch sector of the North Sea are thought to be of MIS9 age

Interval 12.35-17.50m. Contains mainly lignite/peat fragments, megaspores, plant debris + seeds, insect remains, and charophyte oogonia. Would appear to be freshwater in origin for which a palynological analysis may prove useful. In the sample from 17.50m, however, there are also a few possibly reworked marine foraminifera (poorly preserved *Elphidium* spp.).

Conclusions: There are probably not enough samples from each borehole to study properly the ecology and biostratigraphy of these sequences. It is true there are components in the samples which appear to be definitely pre-Anglian (containing *Elphidiella hannai*) and others which are post-Anglian (containing *Ammonia*). However, both freshwater and marine microfauna/flora are often present in the same sample and it is therefore contentious deciding which aspect is *in situ* and which is reworked. A palynological analysis of the samples, especially those containing peat/lignite, plant debris, seeds and spores, may serve to elucidate this problem.

VIBROCORES

All the vibrocores are situated on the cable route to the south and west of the main windfarm site. According to a map provide by Christin Heamagi the ones dealt with here all come from the vicinify of a lake (presumably at the time of Doggerland, in the early Holocene). However, it must be remembered that only four are dealt with here from approximately one hundred vibrocores taken along the cable route and shown on the map provided by Christin Heamagi.

VC85a

Said to be Upper Botney Cut and Holocene Alluvium. The Botney Cut, according to Cameron & Holmes *in* Bowen, 1999, occurs within Late Devensian palaeovalleys and comprises glaciolacustrine and glaciomarine clays.

The single sample from 1.40m depth might therefore be expected to be latest Devensian or Early Holocene in age. It contains high-energy pebbly shelly sands with marine molluscs (mainly fragments) and a few marine foraminifera (miliolids and *Ammonia* only) and would seem to be Holocene.

VC91

Said to be Upper Botney Cut and Holocene Alluvium.

The lithology is totally different to that seen in the other vibrocores examined here. The sample from 1.17m depth is an organic silt containing plant debris and seeds, insect remains, a few *Mytilus* juveniles, and a rich microfossil assemblage of brackish ostracods and foraminifera (plus subsidiary freshwater ostracods). It is estuarine and probably early Holocene in age and appears to relate to the time of Doggerland..

VC92

Again, said to be Upper Botney Cut and Holocene Alluvium.

The residue from 0.47m is that of polished silty sands. It contains worn barnacle plates and mollusc fragments plus a marine micro fauna including the ostracod *Sarsicytheridea bradii.* A probable interglacial deposit (?MIS 5e); a candidate for AAR dating.

VC93

Again attributed to the Upper Botney Cut and Holocene Alluvium.

Single sample from 1.41m depth contains iron tubes and an extremely diverse micro fauna with marine ostracods (including *S. bradii*) and foraminifera (*Elphidium* spp. and very abundant *Bulimina marginata*). Clearly an interglacial deposit (?MIS 5e or older), and another candidate for AAR dating.

Conclusions: None of these vibrocore samples would appear to be Devensian in age. Those from VC92 and VC93 belong to an interglacial but their microfaunas, though diverse, do not contain anything that is demonstrably indicative of any particular interglacial, neither do they match the microfaunas (thought to be of last interglacial age) described from the East Anglia One Windfarm site further to the south in the North Sea (Whittaker, 2017). The sample from VC91 will offer scope for a palynological analysis, at least to elucidate further the environment of deposition. From the evidence presented already it would seem, nevertheless, to be associated with an estuarine situation in Doggerland and is thus probably early Holocene. VC85A is problematical. It is a high-energy deposit of Holocene age, and could be quite modern. At least two of the four samples, as indicated above, might be AAR-dated using shell but is is necessary to find shell/molluscs that are clearly part of the fauna and not reworked. Radiocarbon dating ought to be possible in the case of the sample from VC91.

REFERENCES

Cameron, T.D.J. & Holmes, R. 1999. The Continental Shelf. *In:* Bowen, D.Q. (ed.) A revised correlation of Quaternary deposits of the British Isles. *Geological Society Special Report* no. 23, pp. 125- 139.

Funnell, B.M. 1989. Chapter 12. Quaternary. *In:* Jenkins, D.G. & Murray, J.W. (eds), *Stratigraphic Atlas of Fossil Foraminifera, Second Edition*. British Micropalaeontological Society Series, John Wiley & Sons, London, pp. 563-569.

Watts, W.A. 1988. Europe. *In:* Huntley, B. & Webb T. (eds.), *Vegetation History*. Kluwer Academic Publishers, Dordrecht, 155-192.

Wenban-Smith, F.F., Allen, P., Bates, M.R., Parfitt, S.A., Preece, R.C., Stewart, J.R., Turner, C. & Whittaker, J.E. 2006. The Clactonian elephant butchery site at *Southfleet Road, Ebbsfleet (UK). Journal of Quaternary Science,* **21**, 471-483.

Whittaker, J.E. 2017. *East Anglia One Windfarm, Southern North Sea: Microfossil Report on Four Vibrocores (52, 62, 72 and 74), and Three Boreholes (B21, F29 And G28).* Report for Maritime Archaeology, Southampton. The Natural History Museum, London [15th February 2017], 8pp.

13. Appendix IV Radiocarbon dating certificate

Scottish Universities Environmental Research Centre
Rankine Avenue, Scottish Enterprise Technology Park, East Kilbride, Glasgow G75 0QF, Scotland, UK
Director: Professor F M Stuart Tel: +44 (0)1355 223332 Fax: +44 (0)1355 229898 www.glasgow.ac.uk/suerc

RADIOCARBON DATING CERTIFICATE
05 April 2018

Laboratory Code	SUERC-78242 (GU47015)
Submitter	Christin Heamagi Maritime Archaeology Ltd. Room 014/11 National Oceanography Centre Empress Dock Southampton SO14 3ZH
Site Reference	Hornsea offshore
Context Reference	VC91 0.42
Sample Reference	MA001
Material	Shell : n/a
$\delta^{13}C$ relative to VPDB	0.1 ‰
Radiocarbon Age BP	41876 ± 307

N.B. The above ^{14}C age is quoted in conventional years BP (before 1950 AD) and requires calibration to the calendar timescale. The error, expressed at the one sigma level of confidence, includes components from the counting statistics on the sample, modern reference standard and blank and the random machine error.

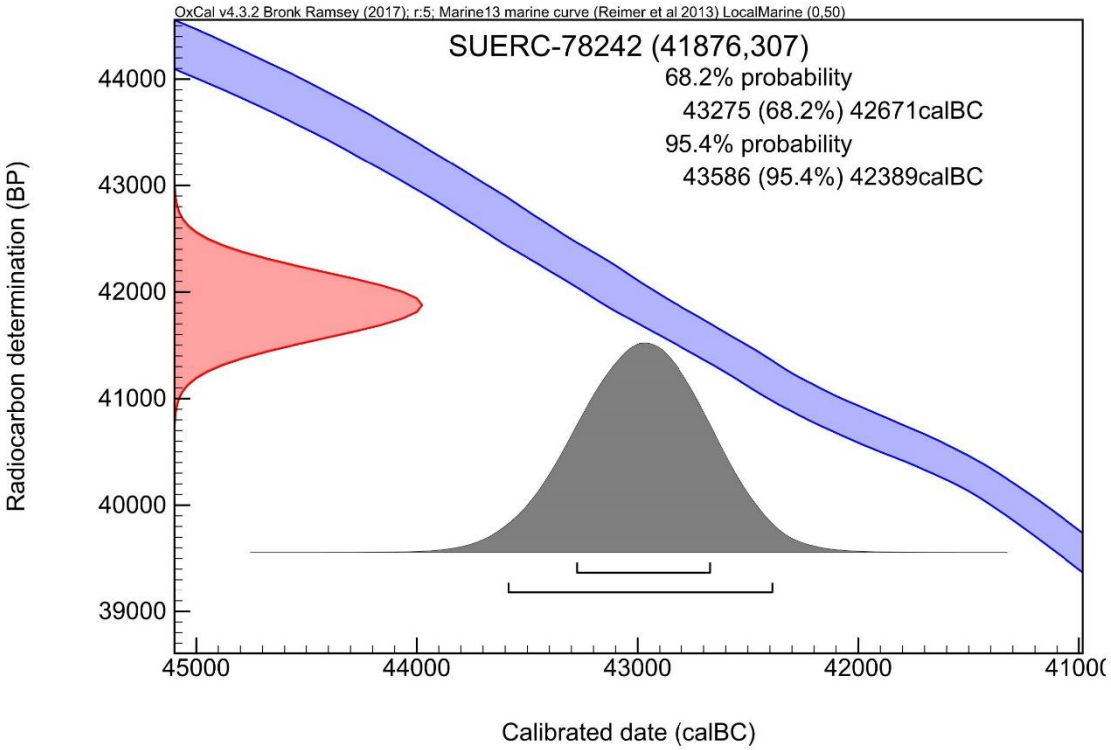
Samples with a SUERC coding are measured at the Scottish Universities Environmental Research Centre AMS Facility and should be quoted as such in any reports within the scientific literature. The laboratory GU coding should also be given in parentheses after the SUERC code.

Detailed descriptions of the methods employed by the SUERC Radiocarbon Laboratory can be found in Dunbar et al. (2016) *Radiocarbon 58(1) pp.9-23.*

For any queries relating to this certificate, the laboratory can be contacted at suerc-c14lab@glasgow.ac.uk.

Conventional age and calibration age ranges calculated by : E. Dunbar

Checked and signed off by : P. Naysmith



The radiocarbon age given overleaf is calibrated to the calendar timescale using the Oxford Radiocarbon Accelerator Unit calibration program OxCal 4.*

The above date ranges have been calibrated using the Marine13 calibration curve†

A regional marine offset (ΔR) of 0 ± 50 years has been used in the calibration.

Please contact the laboratory if you wish to discuss this further.

* Bronk Ramsey (2009) *Radiocarbon 51(1) pp.337-60*
† Reimer et al. (2013) *Radiocarbon 55(4) pp.1869-87*

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RADIOCARBON DATING CERTIFICATE
05 April 2018

Laboratory Code	SUERC-78243 (GU47016)
Submitter	Christin Heamagi Maritime Archaeology Ltd. Room 014/11 National Oceanography Centre Empress Dock Southampton SO14 3ZH
Site Reference	Hornsea offshore
Context Reference	VC91 1.08
Sample Reference	MA002
Material	Shell : n/a
$\delta^{13}C$ relative to VPDB	-6.2 ‰
Radiocarbon Age BP	45992 ± 485

N.B. The above ^{14}C age is quoted in conventional years BP (before 1950 AD) and requires calibration to the calendar timescale. The error, expressed at the one sigma level of confidence, includes components from the counting statistics on the sample, modern reference standard and blank and the random machine error.

Samples with a SUERC coding are measured at the Scottish Universities Environmental Research Centre AMS Facility and should be quoted as such in any reports within the scientific literature. The laboratory GU coding should also be given in parentheses after the SUERC code.

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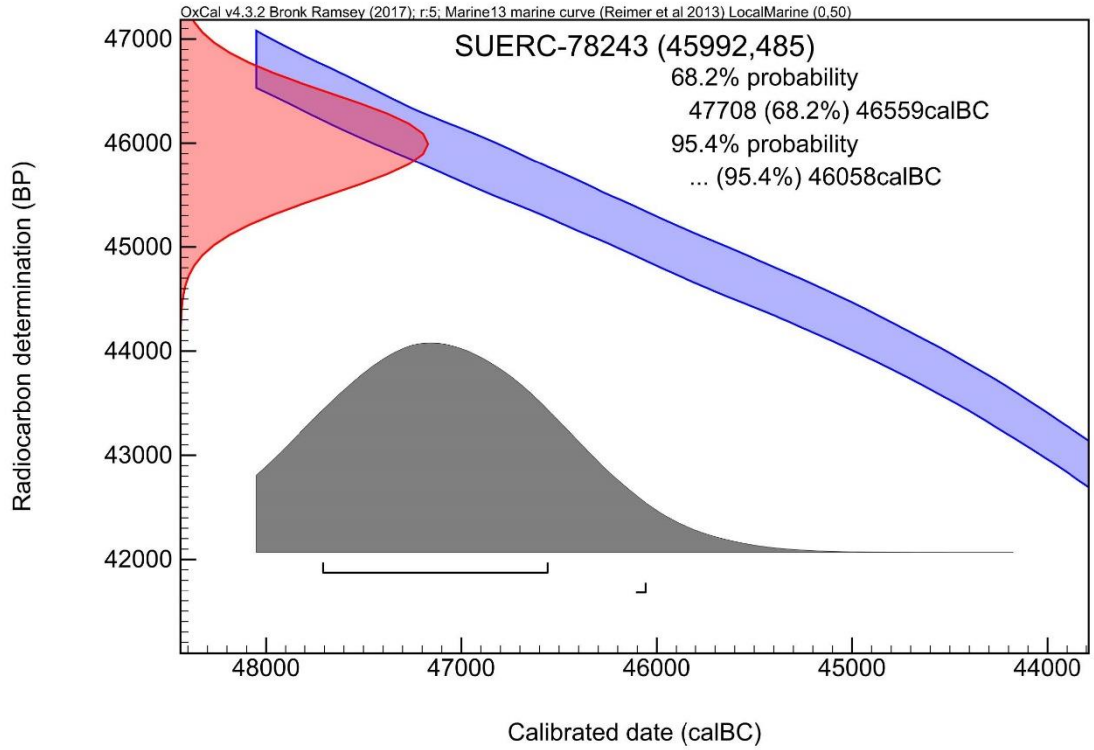
For any queries relating to this certificate, the laboratory can be contacted at suerc-c14lab@glasgow.ac.uk.

Conventional age and calibration age ranges calculated by : E. Dunbar

Checked and signed off by : P. Nayont



The radiocarbon age given overleaf is calibrated to the calendar timescale using the Oxford Radiocarbon Accelerator Unit calibration program OxCal 4.*

The above date ranges have been calibrated using the Marine13 calibration curve†

A regional marine offset (ΔR) of 0 ± 50 years has been used in the calibration.

Please contact the laboratory if you wish to discuss this further.

* Bronk Ramsey (2009) *Radiocarbon 51(1) pp.337-60*
† Reimer et al. (2013) *Radiocarbon 55(4) pp.1869-87*

14. Appendix V Pectinidae shell AAR report

Hornsea Offshore project Interim Pectinidae shell AAR report: Apr 2018

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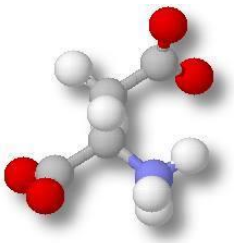
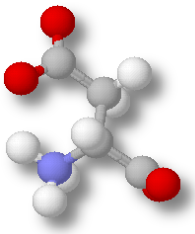


L-Asp



D-Asp

Summary

This report documents the attempts to conduct amino acid racemization analysis for age estimation on the intra-crystalline protein fraction of Pectinidae shells as part of the Hornsea Offshore project. It is concluded that the data from the *Pectenidae* shells indicate a Holocene age for the VC92 core at 0.68 depth.

Keywords
Amino acid racemization
Geochronology

Introduction

The project is part of the Hornsea Offshore windfarm project undertaken by Maritime Archaeology. This report details attempts to obtain age estimates on fossil shell material using amino acid racemization (AAR) at the NEaar laboratory at the University of York. This involves isolating the intra-crystalline protein fraction of the mollusc shells of Pectinidae, for which a growing database of protein degradation data has recently been assembled. This laboratory has been developing an improved methodology for the technique, and studies are ongoing to calibrate the amino acid data with reference to other dating techniques.

Amino Acid Racemization Geochronology

The presence of proteins in archaeological remains has been known for some time. Nearly fifty years ago Abelson (1954) separated amino acids from subfossil shell, and suggested the possibility of using the kinetics of the degradation of amino acids as the basis for a dating method (Abelson, 1955). Early methods of chemical separation, using ion-exchange liquid chromatography, are able to separate the enantiomers of one amino acid found in proteins, L-isoleucine (L-Ile, I), from its most stable diastereoisomer alloisoleucine (D-aile, A). By analysing the total whole shell protein content within non-marine mollusc shells from UK interglacial sites, an amino acid geochronology was developed using the increase in A/I, with correlations made with the marine oxygen isotope warm stages (Bowen *et al.,* 1989).

A revised technique of amino acid analysis has been developed for geochronological purposes (Penkman *et al*., 2007; 2008; 2011; 2013), combining a new reverse-phase high pressure liquid chromatography method of analysis (Kaufman & Manley, 1998) with the isolation of an “intra-crystalline” fraction of amino acids by bleach treatment (Sykes *et al.,* 1995). This combination of techniques results in the analysis of D/L values of multiple amino acids from the chemically-protected protein within the biomineral; enabling both decreased sample sizes and increased reliability of the analysis.

Theory

Amino acids, the building blocks of proteins, occur as two isomers that are chemically identical, but optically different. These isomers are designated as either D (dextrorotary) or L (laevorotary) depending upon whether they rotate plane polarised light to the right or left respectively (Fig. 1). In living organisms the amino acids in protein are almost exclusively L and the D/L value approaches zero[1]. The potential application to geochronology arises

[1] D-amino acids are synthesised by some organisms; they are found free in invertebrate body fluids where they play a role in osmoregulation and can occur peptide bound in bacterial peptidoglycan, where part of their function is resistance to proteases.

because after death amino acid isomers start to interconvert, a process commonly termed racemization. In time the D/L value approaches one. The proportion of D to L amino acids is therefore an estimate of the extent of protein degradation, and if this is assumed to be predictable over time can be used to estimate the age of a sample. Other indications of protein decomposition, such as the degradation of unstable amino acids, can also be used to estimate age.

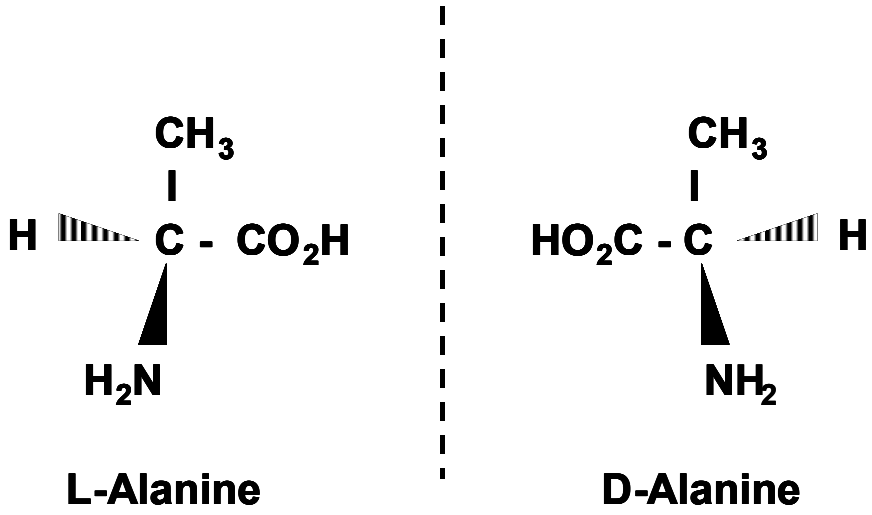


Figure 1: L- and D- amino acid structure

Mechanisms of racemization

The rate of racemization is governed by a variety of factors, most of which have been studied in detail only for free amino acids. North East amino acid racemization (NEaar) analyse the intra-crystalline amino acid fraction and in this closed system in which other factors (water content, concentration of cations, pH) are constant, the extent of racemization is a function of time and temperature. Over a small geographical area, such as that represented in this study, it can be assumed that the integrated temperature histories are effectively the same. Any differences in the extent of decomposition of protein within the sample are therefore age-dependent.

Intra-crystalline protein decomposition

The organic matter existing within individual crystals (intra-crystalline fraction) is believed to be a more reliable substrate for analysis than the whole shell (Sykes *et al.*, 1995; Penkman *et al.*, 2008). The initial bleaching step in the recovery of the intra-crystalline fraction removes both secondary contamination and the organic matrix of the shell. This organic matrix degrades and leaches at an unpredictable rate over time, leading to variation in the concentration and D/L of the amino acids. The molluscan racemisation data reported therefore contrasts with some previous work that examined D/L values from whole mollusc shells, containing both intra- and inter-crystalline material.

This isolation of the intra-crystalline fraction is believed to provide a closed system repository for the amino acids during the burial history of the shell. Only the amino acids within this fraction are protected from the action of external rate-affecting factors (except temperature), contamination by exogenous amino acids and leaching. Amino acids within the whole shell are not protected and can be leached out into the environment. Figure 2 shows a schematic of the intra-crystalline fraction with respect to the whole shell. The low level of free amino acids

observed in the inter-crystalline fraction of unbleached samples (Penkman *et al*., 2008) indicates that these have been lost through diagenesis, and as these tend to be more highly racemised than the total fraction, this loss would lead to a lower than expected D/L for the total fraction of the whole shell.

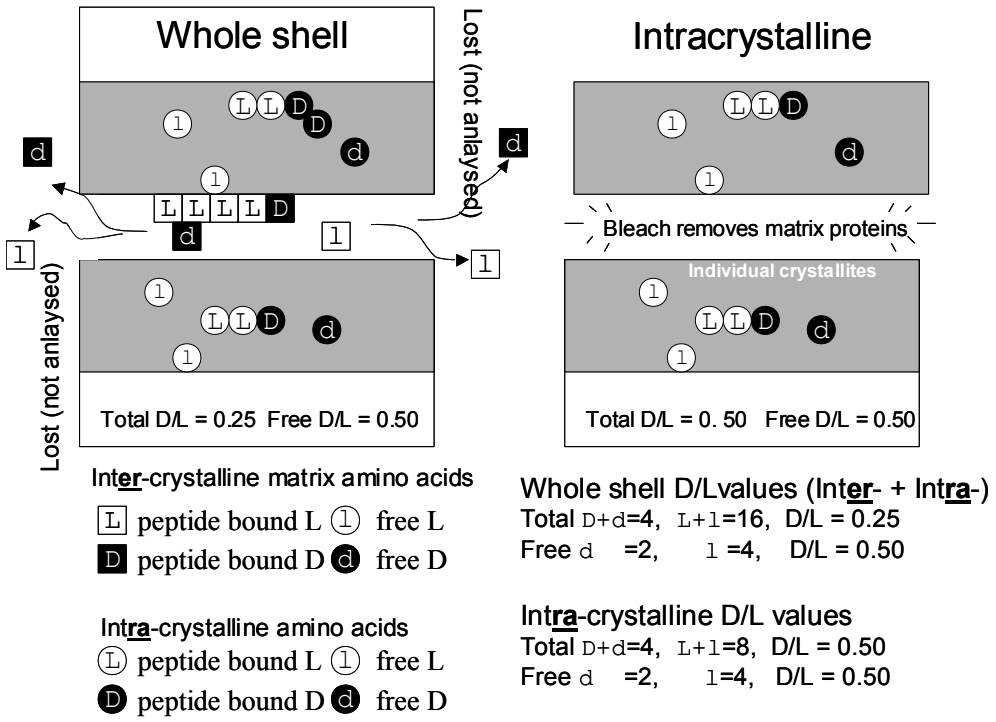


Figure 2: Schematic of intra-crystalline amino acids entrapped within carbonate crystallites. Unlike the proteins of the organic matrix between the crystallites, which leach from the shell with time, in a closed "intra-crystalline" system the amino acids are entrapped. Thus the relationship between the DL ratios of different amino acids and between free (non-protein bound) and total (both free and originally protein-bound amino acids, released by acid hydrolysis) amino acids is predictable. Analysis of the whole shell would result in lower than expected D/L for the total fraction, due to the loss of the more highly racemised frees.

Traditionally AAR studies targeted a single amino acid racemisation reaction, that of L-isoleucine to D-alloisoleucine (A/I), due to the technical ease of separation and its slow rate of racemisation. The approach used in this study diverges from this, as dates are derived from the analysis of multiple amino acids. Whilst racemisation rates differ between individual amino acids, they should be highly correlated in a closed system. The pattern of decomposition appears to be different between mollusc genera, requiring separate models for each genus or species studied.

In a closed system, it should be possible to predict the relationship between geological time and intra-crystalline protein decomposition, using not just racemisation but other measures of protein diagenesis, such as extent of hydrolysis and amino acid composition. This leads to the concept of age estimation using the extent of overall intra-crystalline protein degradation (IcPD), which links the hydrolysis, racemisation and decomposition of all the amino acids isolated by this method. The concept behind the IcPD is to combine multiple sources of information from a single sample to derive an overall measure of the extent of diagenesis of the protein in that fossil (Penkman et al., 2013). Subsequently palaeotemperature information can be included

and estimates made of the link between degradation and absolute age in environments with fluctuating temperatures. If an accurate temperature model is used, then age estimates can be derived directly from the IcPD data, although the results presented here do not incorporate any palaeotemperature information and are presented simply as a relative dating tool.

Materials and Method

Materials

Shell material was recovered from vibrocores and boreholes from the Southern North Sea as a part of a windfarm development. Subsamples of the sediments (Table 1) were sent to the NEaar laboratory, wet-sieved and examined under the microscope for potential dating material.

MA_ID	Core	Depth	Material	Identification after wet sieving
MA003	VC92	0.51	shell	bivalves
MA004	VC92	0.68	shell	bivalves, Pectinidae
MA005	VC93	0.55	shell	gastropods, bivalves, Quinqueloculina? foram
MA006	OSS1-BHA	23.9	shell	bivalves
MA007	DC1_CS	52.7	shell	bivalves, forams, gastropods
MA008	OSS3_BH	27.5	shell	bivalves, ostracod

Table 1: Material identified after wet-sieving of sediment subsamples

The only material for which an aminostratigraphic framework for the area had been developed was the Pectinidae. Amino acid racemization (AAR) analyses were therefore undertaken on 3 individual fragments (separated into 2-3 subsamples each) of Pectinidae shell from core VC92, 0.68 depth (NEaar 12158-64; MA004Pe1.1-2, 2.1-2, 3.1-3)

Sample Preparation

Shells were examined under a low powered microscope, sonicated and rinsed several times in HPLC-grade water. The shells were then crushed to <100 µm

Bleaching

50 µL of 12% solution of sodium hypochlorite at room temperature was added to each milligram of powdered sample and the caps retightened. The powders were bleached for 48 hours with a shake at 24 hours. The bleach was pipetted off and the powders were then rinsed five times in HPLC-grade water and a final rinse in HPLC-grade methanol (MeOH) to destroy any residual oxidant by reaction with the MeOH. The bulk of the MeOH was pippetted off and the remainder left to evaporate to dryness.

Hydrolysis

Protein bound amino acids are released by adding an excess of 7 M HCl to the bleached powder and hydrolysing at 110^{o}C for 24 hours (H*).

20 µL per milligram of sample of 7 M hydrochloric acid (HCl) was added to each hydrolysis (“Hyd”, H*, THAA) sample in sterile 2 ml glass vials, were flushed with nitrogen for 20 seconds

to prevent oxidation of the amino acids, and were then placed in an oven at 110°C for 24 hours. After 10 minutes in the oven, the caps of the 2 mL vials were re-tightened to prevent the escape of vapour.

After 24 hours, the samples were dried in a centrifugal evaporator overnight.

Demineralisation
Free amino-acid samples ("Free", F, FAA) were demineralised in cold 2 M HCl, which dissolves the carbonate but minimises the hydrolysis of peptide bonds, and then dried in the centrifugal evaporator overnight.

Rehydration
When completely dry, samples were rehydrated a rehydration fluid solution containing 0.01 mM HCl, 0.01 mM L-homo arginine internal standard, and 0.77 mM sodium azide at a pH of 2. Each vial was vortexed for 20 seconds to ensure complete dissolution, and checked visually for undissolved particles.

Approximately 15 µL of rehydrated sample was then placed in a sterile, labelled 2 mL autosampler vial containing a glass insert, capped and then placed on the autosampler tray of the HPLC.

For each set of sub-samples a blank vial was included at each stage to account for any background interference from the bleach, acid, or rehydration fluid added to the samples.

Analysis of Free and Hydrolysed Amino Acids

Amino acid enantiomers were separated by reverse phase high pressure liquid chromatography (RP-HPLC). NEaar uses a modified method of Kaufman and Manley (1998) using an automated RP-HPLC system. This method achieves separation and detection of L and D isomers in the sub- picomole range.

Samples (2 µL) were derivitised with 2.2 µL *o*-phthaldialdehyde and thiol *N*-isobutyryl-L-cysteine automatically prior to injection. The resulting diastereomeric derivatives were then separated on Hypersil C_{18} BDS column (sphere d. 5 µm; 250 x 3 mm) using a linear gradient of a sodium acetate buffer (23 mM sodium acetate, 1.3 mM Na_2EDTA; pH 6), methanol, and acetonitrile on an integrated 1100 liquid chromatograph (Agilent, USA).

Individual amino-acids are separated on a non-polar stationary phase according to their varied retention times: a function of their mass, structure, and hydrophobicity. A fluorescence detector is used to determine the concentrations of each amino-acid and record them as separate peaks on a chromatogram. A gradient elution programme was used to keep the retention time to below 120 minutes.

The fluorescence intensity of derivitised amino acids was measured (Ex = 230 nm, Em = 445 nm) in each sample and normalised to the internal standard. All samples were run in duplicate alongside blank extracts that had been subjected to identical preparation procedures. Quantification of individual amino acids was achieved by comparison with the standard amino acid mixture. External standards containing a variety of D- and L- amino acids were analyzed at the beginning and end of every run, and every ten samples.

The L and D isomers of 10 amino acids were routinely analysed. During preparative hydrolysis both asparagine and glutamine undergo rapid irreversible deamination to aspartic acid and glutamic acid respectively (Hill, 1965). It is therefore not possible to distinguish between the acidic amino acids and their derivatives and they are reported together as Asx and Glx.

Results and Discussion

The extent of racemization in five amino acids (D/L of Asx, Glx, Ser, Ala and Val), along with the ratio of the concentration of Ser to Ala ([Ser]/[Ala]), are reported for both the FAA and THAA fractions (Appendix 3). These indicators of protein decomposition have been selected as their peaks are cleanly eluted with baseline separation and they cover a wide range of rates of reaction. It is expected that with increasing age, the extent of racemization (D/L) will increase whilst the [Ser]/[Ala] value will decrease, due to the decomposition of the unstable serine.

The data obtained from Asx, Glx, serine (Ser), alanine (Ala) and valine (Val) are discussed in detail below. If the amino acids were contained within a closed system, the relationship between the FAA and the THAA fractions should be highly correlated, with non-concordance enabling the recognition of compromised samples (Preece & Penkman, 2005). The plot of FAA to THAA data from each sample can also be used as a relative timescale, with younger samples falling towards the bottom left corner of the graph and older samples falling towards the upper right corner, along the line of expected decomposition. The data from the Hornsea Offshore windfarm samples have been plotted in this way below for each of the amino acids, alongside data obtained from a Holocene horizon and a mid-Pleistocene horizon of the same material from the North Sea (see Dix & Sturt, 2011 for details).

Aspartic acid / asparagine (Asx)

Asx is one of the fastest racemizing of the amino acids discussed here (due to the fact that it can racemize whilst still peptide bound; Collins *et al.,* 1999). This enables good levels of resolution at younger age sites, but decreased resolution beyond MIS 7. The D/L Asx data from core VC92 are very similar to those from the Holocene material from the North Sea, radiocarbon dated to 1470 ± 40 BP (1520-1340 cal BP) (Fig. 3). The VC92 samples show much lower levels of Asx D/L than the Middle Pleistocene Pectenidae material, although this cannot be constrained further due to the lack of comparable material from this species.

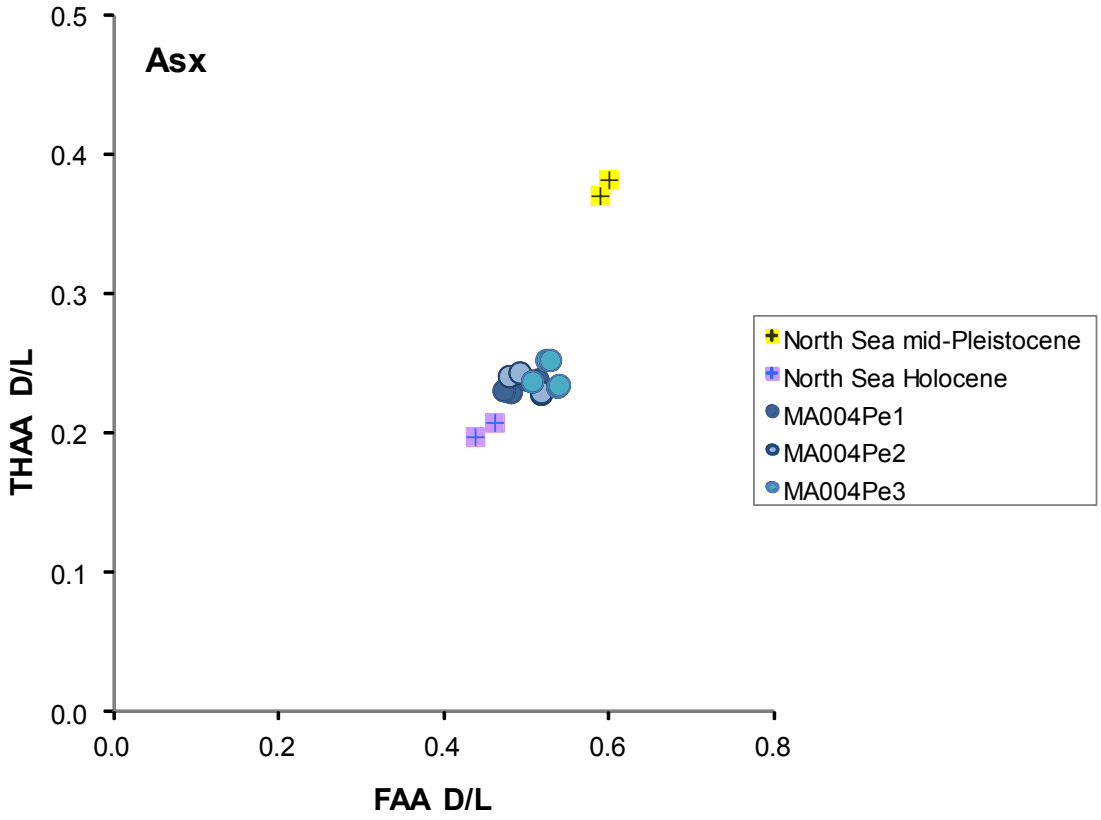


Figure 3: THAA D/L vs FAA D/L for Asx in Pectinidae shells from the Hornsea Offshore windfarm site, core VC92, compared to data obtained from shell from other North Sea sites.

Glutamic Acid / Glutamine (Glx)

Glx is one of the slower racemizing amino acids discussed here and so the level of resolution from young sites is less than that seen with faster racemizing amino acids such as Asx. It is noteworthy that Glx has a slightly unusual pattern of racemization in the free form, due to the formation of a lactam (see Walton, 1998). This results in difficulties in measuring Glx in the Free form, as the lactam cannot be derivitized and is therefore unavailable for analysis.

The Glx D/L values from core VC92 show values within the range of those expected from sites Holocene in age (Fig. 4).

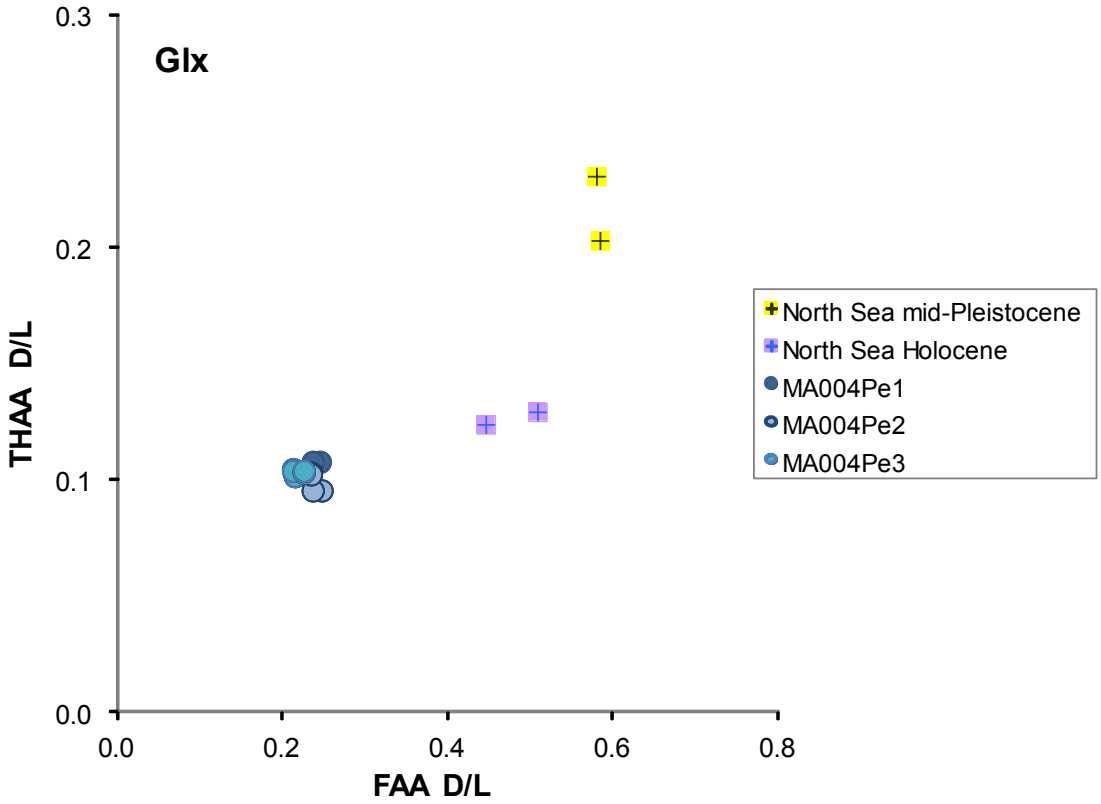


Figure 4: THAA D/L vs FAA D/L for Glx in Pectinidae shells from the Hornsea Offshore windfarm site, core VC92, compared to data obtained from shell from other North Sea sites.

Alanine

Alanine (Ala) is a hydrophobic amino acid, whose concentration is partly contributed from the decomposition of other amino acids (notably serine). Ala racemises at an intermediate rate, so is one of the amino acids that may help distinguish samples at these timescales. The results for Ala are broadly similar to that seen in the other amino acids (Fig. 5), supporting the interpretation that the VC92 core samples are Holocene in age.

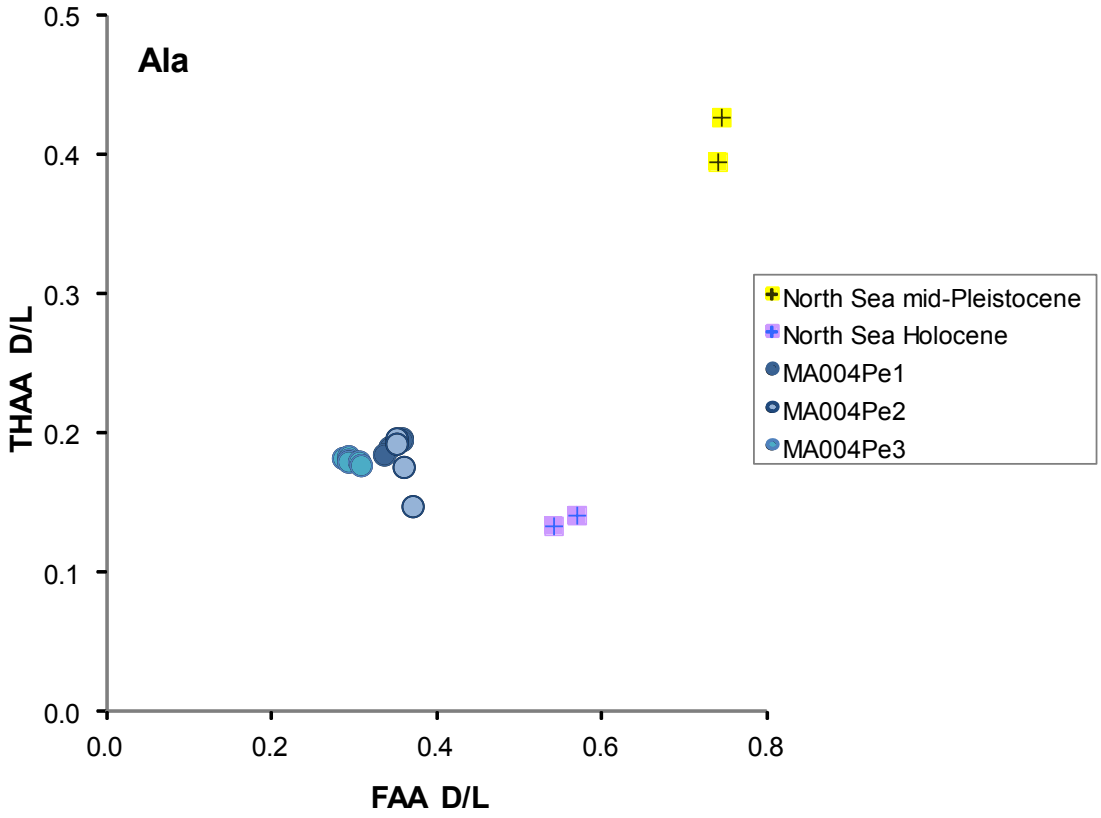


Figure 5: THAA D/L vs FAA D/L for Ala in Pectinidae shells from the Hornsea Offshore windfarm site, core VC92, compared to data obtained from shell from other North Sea sites.

Valine (Val)

Valine has extremely low rates of racemisation, and as the concentration of Val is quite low, the difficulty of measuring the D/L accurately results in higher variability. It does however still prove useful for age discrimination within material of Middle Pleistocene age. The Val D/L in the FAA and the THAA fractions again support the other amino acids (Fig. 6), with the data clustering in the Holocene region.

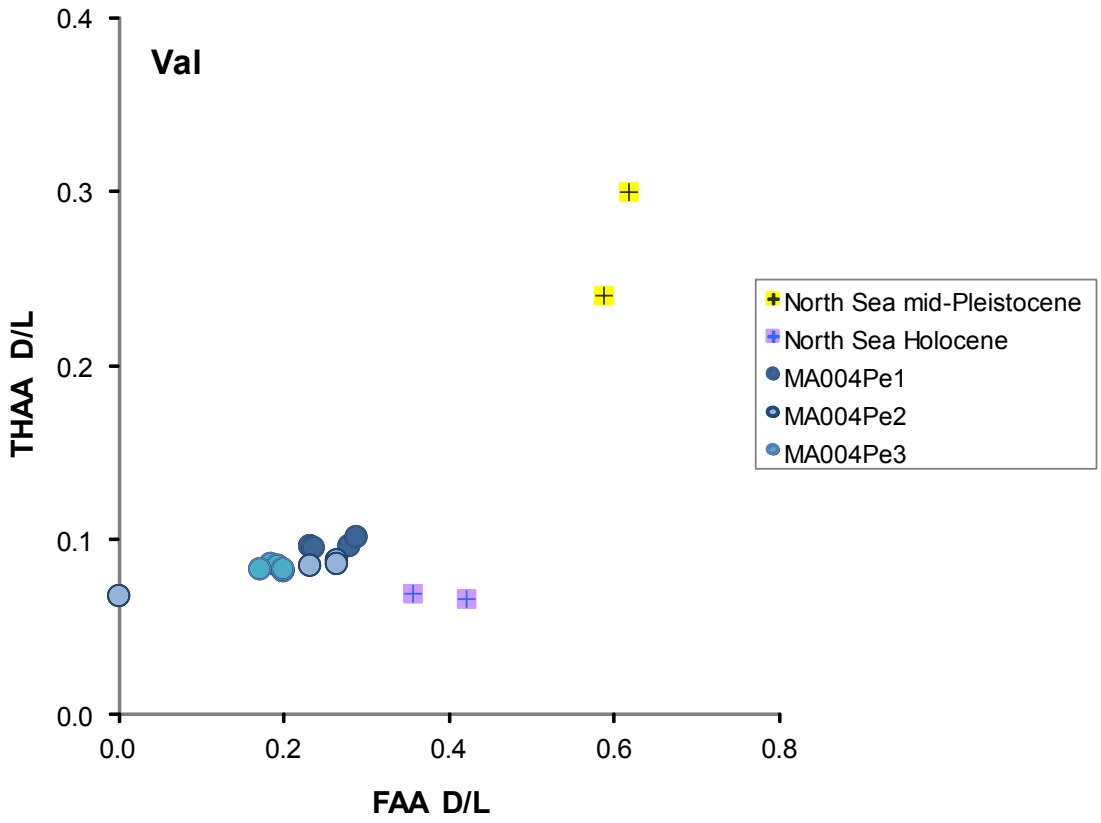


Figure 6: THAA D/L vs FAA D/L for Val in Pectinidae shells from the Hornsea Offshore windfarm site, core VC92, compared to data obtained from shell from other North Sea sites.

Conclusions

The analysis of the closed system of protein within shells (the intra-crystalline protein degradation; IcPD) allows a new concept of age estimation to be developed, which incorporates multiple amino acid data to give a single measure of the overall extent of protein breakdown within a sample. The amino acid data from the Pectinidae shell from the VC92 core from the Hornsea Offshore windfarm indicate deposition relatively late within the Holocene. It is likely that radiocarbon dating will be useful to provide higher age resolution.

Acknowledgements

Funding from NERC, English Heritage, the Marine Aggregates Levy Fund, the Wellcome Trust and the Leverhulme Trust enabled the master dataset of shell and opercula intra-crystalline protein degradation to be developed.

Appendix 1: Glossary

18MΩ water: The water has a resistivity of 18MΩ/cm, indicating a lack of ions.

HPLC grade water: In addition to low ion content, HPLC grade water has a low organic content (typically < 2 ppb).

Amino acids: the building blocks of proteins and consist of an alpha carbon atom (C_α) which has four different groups bonded to it: an amino group ($-NH_2$), a carboxyl group (-COOH), a hydrogen atom (-H), and a side chain, (often called an R group). About 20 amino acids normally occur in nature and some of these can undergo further modification (eg the hydroxylation of proline to hydroxyproline). The amino acids are commonly known by three letter codes (see Appendix 2: Abbreviations). They exist free in the cell, but are more commonly linked together by **peptide bonds** to form proteins, peptides, and sub-components of some other macromolecules (e.g. bacterial peptidoglycan).

Amino acid isomers: amino acids occur as two stereoisomers that are chemically identical, but optically different. These isomers are designated as either D (dextro-rotary) or L (laevo-rotary) depending upon whether they rotate plane polarised light to the right or left respectively. In living organisms the amino acids in protein are almost exclusively L and the D/L value approaches zero. Two amino acids, isoleucine and threonine, have two chiral carbon atoms and therefore have four stereoisomers each. As well as racemization, these two amino acids can undergo a process known as epimerization. The detection of the L-alloisoleucine epimer (derived from L-isoleucine) is possible by conventional ion-exchange chromatography, and was thus the most commonly used reaction pathway in geochronology before 1998.

Asx: Measurements of aspartic acid following hydrolysis also include asparagines, which decomposes to Asx. This combined signal of aspartic acid plus asparagine (Asp +Asn) is referred to as Asx.

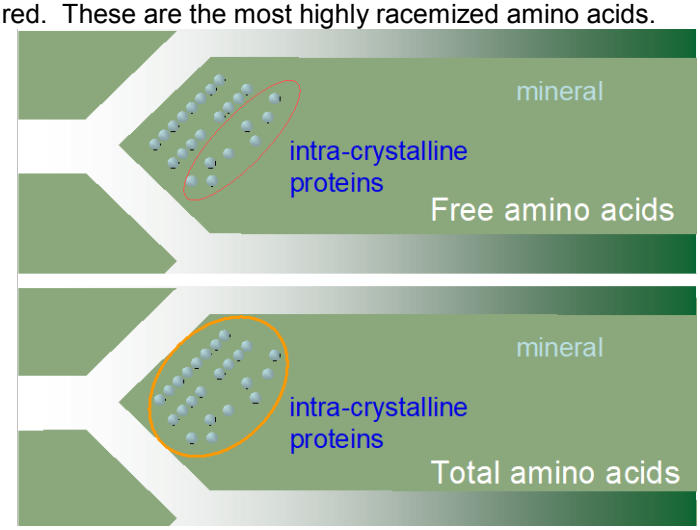
D-amino acid: dextrorotary amino acid, formed following synthesis of the protein as it degrades over time (remember as “dead amino acid”).

IcPD: Conventional racemization analysis tends to report an allosioleucine / isoleucine (A/I or D/L value). This amino acid ratio has the advantage of being relative easy to measure and also sufficiently slow to be used to “date” sediments in the European Quaternary. Our IcPD approach utilises multiple amino acids, which increases the level of resolution and allows a cross-check on the sample’s behaviour. If an amino acid has an unusually low ratio (e.g. due to modern contamination) or unusually high racemization (e.g. due to inclusion of bacterial cell wall contaminants) either some or all of the amino acids will no longer fit to the idealized degradation model, indicating a compromised sample.

Enantiomers / optical isomers: mirror image forms of the same compound that cannot be superimposed on one another.

Epimerisation: the inversion of the chiral α-carbon atom.

Free amino acid fraction (FAA): The fraction of amino acids directly amenable to racemization analysis. Only amino acids which have already been naturally hydrolysed (over time) are measured. These are the most highly racemized amino acids.



Hydrolysis: A chemical reaction involving water leading to the breaking apart of a compound (in this case the breaking of peptide bonds to release amino acids).

L-amino acid: levorotary amino acid, the constituent form of proteins (remember as “living amino acid”).

Peptide bond: an amide linkage between the carboxyl group of one amino acid and the amino group of another.

Racemization: the inversion of all chiral carbon atoms, leading to the decrease in specific optical rotation. When the optical rotation is reduced to zero, the mixture is said to be racemized.

Stereoisomers: molecules of the same compound that have their atoms arranged differently in space.

Total hydrolysable amino acid fraction (THAA): The extent of racemization of all amino acids in a sample, determined following aggressive high temperature hydrolysis with strong mineral acid, which has the effect of breaking apart all peptide bonds so that the total extent of racemization in all amino acids (both free and peptide bound) are measured.

Zwitterion: A dipolar ion containing ionic groups of opposite charge. At neutral pH the ionic form of amino acids which predominates is the zwitterion.

Appendix 2: Abbreviations used in this report

Abbreviation		AA 1-letter code	Number of chiral centres
AA	amino acid		
Ala	alanine	A	1
Arg	arginine	R	1
Asn	asparagine	N	1
Asp	aspartic acid	D	1
Asx	asparagine + aspartic acid		
FAA	free amino acids		
Gln	glutamine	Q	1
Glu	glutamic acid	E	1
Glx	glutamine + glutamic acid		
Gly	glycine	G	0
His	histidine	H	1
IBD(L)C	N-isobutyryl-D(L)-cysteine		
IcPD	intra-crystalline protein decomposition		
Ile	isoleucine	I	2
Leu	leucine	L	1
MeOH	methanol		
Met	methionine	M	1
OPA	ortho-phthaldialdehyde		
Phe	phenylalanine	P	1
RP-HPLC	reverse-phase high pressure liquid chromatography		
Ser	serine	S	1
THAA	total hydrolysable amino acids		
Thr	threonine	T	2
Tyr	tyrosine	Y	1
Val	valine	V	1

Appendix 3: Pectinidae shell data from Hornsea offshore windfarm, core VC92

		Concentration (picomoles per mg)																					
Neaar no.	Sample name	[Asx]	[Glx]	[Ser]	[L-Thr]	[L-His]	[Gly]	[L-Arg]	[Ala]	[Tyr]	[Val]	[Phe]	[Leu]	[Ile]	Asx D/L	Glx D/L	Ser D/L	Ala D/L	Tyr D/L	Val D/L	[S]/[A]	Family	materials	location
12158bF	MA004Pe1.1bF	97	38	86	25	15	131	18	119	3	27	29	39	11	0.483	0.220	0.904	0.346	0.147	0.232	0.718	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12158bF	MA004Pe1.1bF	92	37	91	24	4	140	18	119	2	27	30	45	12	0.476	0.218	0.912	0.339	0.000	0.281	0.767	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12158bH*	MA004Pe1.1bH*	882	360	215	66	23	516	59	293	2	116	85	125	45	0.229	0.102	0.353	0.190	0.000	0.097	0.733	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12158bH*	MA004Pe1.1bH*	865	352	222	67	25	560	60	295	2	115	84	122	46	0.231	0.102	0.356	0.185	0.000	0.096	0.753	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12159bF	MA004Pe1.2bF	94	37	86	24	13	129	16	118	3	27	29	40	12	0.516	0.245	0.898	0.359	0.269	0.288	0.728	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12159bF	MA004Pe1.2bF	95	37	85	23	0	108	15	121	2	27	27	43	11	0.502	0.237	0.883	0.361	0.000	0.236	0.702	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12159bH*	MA004Pe1.2bH*	1032	398	216	71	0	311	49	331	3	138	74	134	53	0.238	0.108	0.362	0.196	0.000	0.102	0.653	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12159bH*	MA004Pe1.2bH*	1064	405	216	72	0	294	47	336	3	140	70	118	50	0.238	0.108	0.364	0.194	0.000	0.095	0.641	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12160bF	MA004Pe2.1bF	88	35	80	19	10	157	14	99	2	21	1	44	10	0.480	0.248	0.862	0.373	0.000	0.232	0.817	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12160bF	MA004Pe2.1bF	87	35	87	19	8	178	15	99	2	17	2	46	11	0.495	0.237	0.859	0.361	0.000	0.000	0.878	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12160bH*	MA004Pe2.1bH*	1174	471	274	80	0	883	76	366	4	153	7	160	72	0.241	0.095	0.358	0.147	0.000	0.085	0.748	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12160bH*	MA004Pe2.1bH*	755	303	185	53	0	608	51	250	2	97	3	96	44	0.243	0.095	0.362	0.175	0.000	0.068	0.739	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12161bF	MA004Pe2.2bF	115	42	98	28	12	138	17	126	4	34	5	54	18	0.519	0.234	0.897	0.354	0.195	0.265	0.776	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12161bH*	MA004Pe2.2bH*	817	341	197	65	0	280	41	293	4	129	9	128	58	0.227	0.103	0.344	0.196	0.217	0.088	0.673	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12161bH*	MA004Pe2.2bH*	843	347	199	66	0	290	41	301	2	132	9	124	55	0.229	0.102	0.350	0.192	0.000	0.086	0.662	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12162bF	MA004Pe3.1bF	130	54	116	33	19	167	29	164	3	36	38	55	16	0.540	0.215	0.899	0.289	0.000	0.184	0.708	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12162bF	MA004Pe3.1bF	117	50	116	32	19	192	30	160	3	35	40	55	16	0.541	0.216	0.903	0.295	0.000	0.193	0.727	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12162bH*	MA004Pe3.1bH*	951	380	203	69	16	312	54	301	2	129	75	128	52	0.234	0.101	0.371	0.182	0.000	0.087	0.675	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12162bH*	MA004Pe3.1bH*	937	376	205	70	16	314	54	302	2	131	76	129	52	0.234	0.102	0.370	0.183	0.000	0.085	0.680	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12163bF	MA004Pe3.2bF	107	46	111	30	15	174	26	143	4	30	34	48	14	0.510	0.213	0.875	0.295	0.311	0.200	0.776	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12163bH*	MA004Pe3.2bH*	1048	411	209	80	0	254	51	334	4	157	75	128	59	0.237	0.104	0.380	0.181	0.000	0.082	0.625	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12163bH*	MA004Pe3.2bH*	1008	398	207	78	0	274	51	332	3	152	73	125	57	0.236	0.104	0.377	0.179	0.000	0.082	0.624	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12164bF	MA004Pe3.3bF	124	51	103	30	16	139	22	149	4	34	29	49	15	0.527	0.225	0.892	0.309	0.246	0.201	0.688	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12164bF	MA004Pe3.3bF	129	52	102	31	0	101	21	153	2	35	28	46	16	0.530	0.226	0.896	0.310	0.000	0.171	0.664	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12164bH*	MA004Pe3.3bH*	1294	485	239	93	0	313	61	386	4	186	89	149	68	0.252	0.103	0.382	0.179	0.088	0.083	0.620	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68
12164bH*	MA004Pe3.3bH*	1254	470	236	90	0	333	61	385	3	182	87	144	66	0.252	0.103	0.383	0.177	0.000	0.083	0.614	Pectinidae sp.	shell	Horsea Offshore windfarm, MA004, core VC92, depth 0.68

Works Cited in Text

Abelson, P.H. 1954. Amino acids in fossils, *Science,* **119**, 576

Abelson, P.H. 1955. *Organic constituents of fossils,* Carnegie Institution of Washington Year Book, **54**, 107-9

Bada, J.L., Shou, M.-Y., Man, E.H. and Schroeder, R.A., 1978. Decomposition of hydroxy amino acids in foraminiferal tests: Kinetics, mechanism and geochronological implications, *Earth and Planetary Science Letters* **41**, 67-76

Bowen, D.Q., Hughes, S., Sykes, G.A. and Miller, G.H., 1989. Land-sea correlations in the Pleistocene based on isoleucine epimerization in non-marine mollusks, *Nature* **340**, 49-51

Collins, M.J., Waite, E.R. and van Duin, E.C.T., 1999. Predicting protein decomposition: the case of aspartic-acid racemization kinetics, *Philosophical Transactions of the Royal Society, London*, Series B, **354**(1379), 51-64

Dix, J.K. & Sturt, F. 2011. *The Relic Palaeolandscapes of the Thames* Estuary. ISBN 978-0-907545-67-5. Published by the MALSF. Crown Copyright 2011.

Hare, P.E. and Abelson, P.H., 1967. Racemization of amino acids in fossil shells, *Carnegie Institution of Washington Year Book*, **66**, 526-8

Hill, R.L., 1965. Hydrolysis of proteins, *Advances in Protein Chemistry,* **20**, 37-107

Kaufman, D.S. and Manley, W.F., 1998. A new procedure for determining DL amino acid ratios in fossils using Reverse Phase Liquid Chromatography, *Quaternary Science Reviews* **17** 987-1000

Lisiecki, L. and Raymo, M.E. 2005 A Pliocene-Pleistocene stack of 57 globally distributed benthic $\delta^{18}O$ records, *Paleoceanography*, **20**, 1-17

Penkman, K.E.H., Preece, R.C., Keen, D.H., Maddy, D., Schreve, D.C. and Collins, M.J., 2007. Amino acids from the intra-crystalline fraction of mollusc shells: Applications to geochronology, *Quaternary Science Reviews*, **26**, 2958-2969

Penkman, K.E.H., Kaufman, D.S., Maddy, D. and Collins, M.J., 2008. Closed-system behaviour of the intra-crystalline fraction of amino acids in mollusc shells, *Quaternary Geochronology,* **3**, 2-25

Penkman, K.E.H., Preece, R.C., Keen, D.H. and Collins, M.J., 2010. Amino acid geochronology of the type Cromerian of West Runton, Norfolk, UK, *Quaternary International,* **228**, 25-37

Penkman, K.E.H, Preece, R.C., Keen, D.H., Meijer, T., Parfitt, S.A., White, T.S. and Collins, M.J., 2011. A chronological framework for the British Quaternary based on *Bithynia* opercula. *Nature*, **476**, 446-449

Penkman, K.E.H., Preece, R.C., Keen, D.H., Meijer, T., Parfitt, S.A., White, T.S. and Collins, M.J., 2013. An aminostratigraphy for the British Quaternary based on *Bithynia* opercula. *Quaternary Science Reviews*, **61**, 111-134

Preece, R.C. and Penkman, K.E.H., 2005. Molluscan assemblages and amino acid dating of the Lower Palaeolithic site at East Farm, Barnham, Suffolk, *Proceedings of the Geologists' Association* **116**, 363-377

Sykes, G.A., Collins, M.J. and Walton, D.I., 1995. The significance of a geochemically isolated intracrystalline organic fraction within biominerals, *Organic Geochemistry* **23** (11/12) 1039-1065

Walton D., 1998. Degradation of intracrystalline proteins and amino acids in fossil brachiopods, *Organic Geochemistry,* **28**, 389-410