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MEDWAY VALLEY PALAEOLITHIC PROJECT AMINO ACID RACEMIZATION ANALYSIS

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Kirsty Penkman, Simon McGrory and Matthew Collins

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Keywords

Amino-acid Racemization
Geochronology

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Introduction

The Medway Valley Palaeolithic Project (MVPP) is focused on areas of Kent and Essex either side of the Thames Estuary in order to aid the management of the important Palaolithic resource contained within this region. As part of this project a regional Palaeolithic Research Framework was developed, including an integrated chronological and stratigraphic framework for sand/gravel aggregate deposits. This research included amino acid geochronology as part of the chronostratigraphic scheme.

Amino acid analyses were undertaken at the York Laboratory (NEaar) from key sites (Apton Hall Farm, Barling Gravel, Bradwell Hall, East Hyde, East Mersea Restaurant site and Shoeburyness). This involves isolating the intra-crystalline protein fraction of gastropod shells and, in particular, the calcitic opercula from the fluvial gastropod *Bithynia tentaculata*, for which an excellent and growing database of protein degradation data has recently been assembled (Penkman 2005).

This report details attempts to obtain age estimates on the Medway Valley material using amino acid racemization (AAR).

Amino Acid Racemization Geochronology

A new technique of amino acid analysis has been developed for geochronological purposes (Penkman 2005; Penkman *et al* 2007), combining a new Reverse-Phase High Pressure Liquid Chromatography method of analysis (Kaufman and 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. Amino acid data obtained from the intra-crystalline fraction of the calcitic *Bithynia* opercula has been found to be a particularly robust repository for the original protein. This has enabled an increased level of resolution and therefore this material has been focused on in this study.

Theory

Amino acids, the building blocks of proteins, occur as two isomers that are chemically identical, but optically different. These isomers were designated as either D (dextro-rotary) or L (laevo-rotary) 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 from the fact that after death amino acid isomers start to interconvert. This process is 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 age. Other indications of protein decomposition, such as the degradation of unstable amino acids, can also be used to estimate the age of a sample.

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[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.

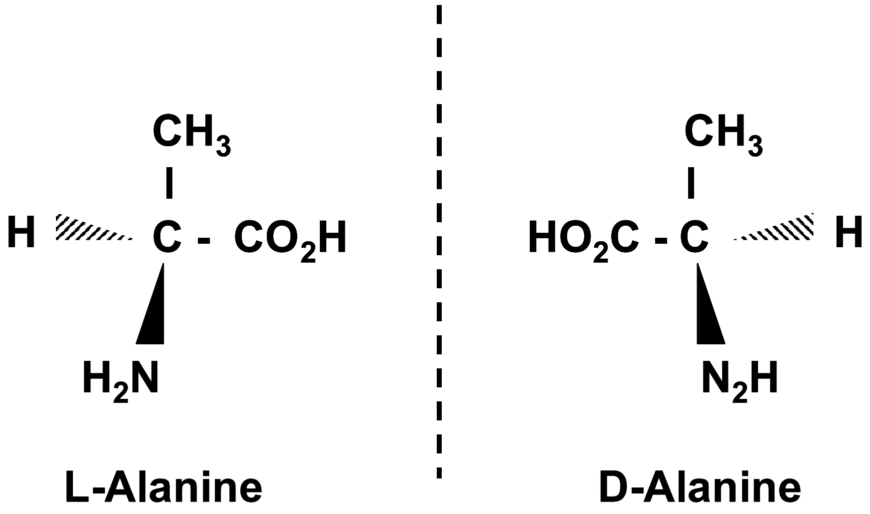


Figure 1: L- and D- amino acid structure

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 way, within a closed environment 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 2005; Penkman *et al* 2007). 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. Thus, as appears to be the case in ostrich eggshell (Miller *et al* 2000), the D/L values of amino acids in the intra-crystalline fraction of shells have been analysed; in the case of ostrich eggshell no bleaching step was used. The molluscan racemisation data reported therefore contrasts with 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 2005; Penkman *et al* 2007) 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.

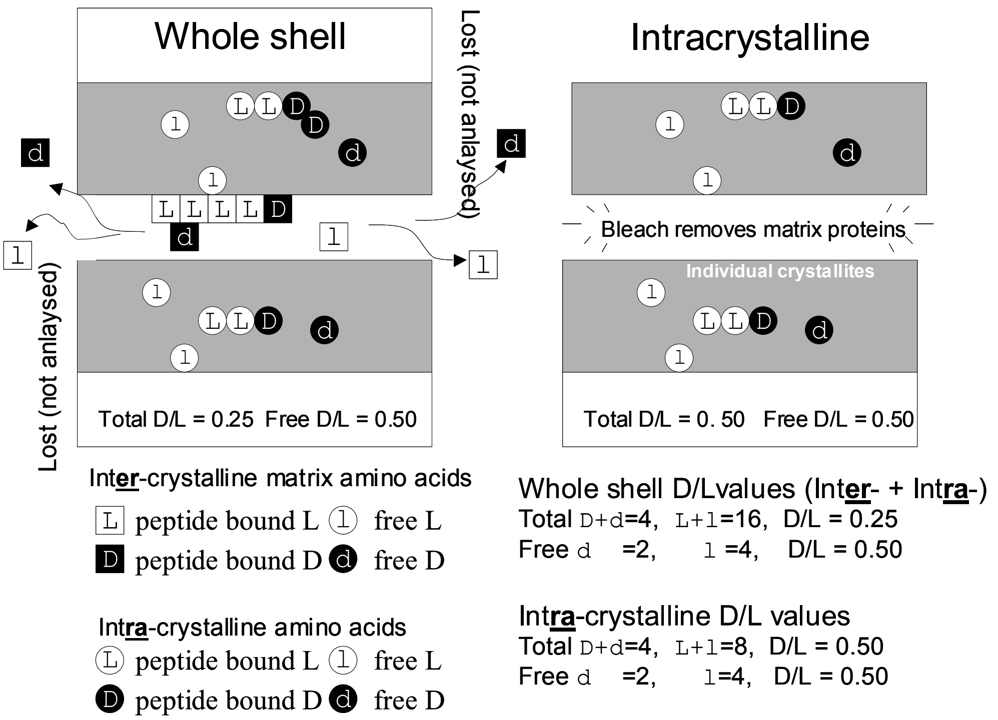


Figure 2: Schematic of intra-crystalline amino acids entrapped within carbonate crystallites. Unlike the proteins of the organic matrix between the crystallites, which leach from the shell with time, in a closed "intra-crystalline" system the amino acids are entrapped. Thus the relationship between the 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

Once a closed system inside mollusc shells has been isolated, then the kinetics of protein decomposition are much simpler to predict. In this laboratory the concept of age estimation using the extent of overall Intra-crystalline Protein Degradation (IcPD) has been devised, 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 information from a single sample to derive an overall measure of the extent of diagenesis of the protein in that fossil. Similar ideas have been used before, although not in such a comprehensive way. Divergence from the normal in a plot of A/I vs Gly/Ala is thought to indicate leaching in molluscs (Murray-Wallace and Kimber 1987). Kaufman (2000) used ratios of Asx to Glx to screen out samples with any unusual values.

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. By linking together different amino acids, and then linking this to a temperature driven model of decay, which includes hydrolysis, racemisation and degradation, the extent of protein degradation can be derived. The pattern of decomposition appears to be different between mollusc genera, requiring separate models for each genus or species studied.

If a closed system is isolated, it should be possible to predict the relationship between geological time and IcPD increase, using not just racemisation but other measures of protein decomposition, such as total and relative concentrations. It follows from the innovations above that, assuming sampling is from an idealised closed system, the pattern of protein decomposition governs the observed racemisation of (a) free amino acids and (b) the total system, (c) the percentage of free amino acids and (d) the total concentration of amino acids.

This model can also be used as a method of assessing the internal reliability of each biomineral used and to determine how closely these substrates approximate to a closed system. 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

Molluscan samples were collected and supplied by David Keen and Richard Preece from 37 samples consisting of eight sites. Amino acid racemization (AAR) analyses were undertaken on 33 individual *Bithynia tentaculata* opercula and 4 individual *Bithynia troschelii* opercula. *Bithynia tentaculata* opercula were analysed from 8 horizons:

5 from Apton Hall Farm borehole, APHF 05 <3A> (NEaar 3737–3739, 3826–3827);
5 from Barling Gravel, 05 <5> (18) (NEaar 3740–3742, 3828–3829);
5 from Bradwell Hall, JCB bulk 13, MIS 11 (NEaar 3731–3733, 3822–3823);
5 from East Mersea Restaurant Site, Sample 3, MIS 5e (NEaar 3728–3730, 3820–3821);
4 from Shoeburyness borehole S1, 13.9 m (NEaar 3746–3748, 3831);
1 from Shoeburyness borehole S1, 14.42-14.44 m (labelled Shoeburyness Channel; NEaar 3132);
3 from East Hyde borehole EH1, 7.55 m (NEaar 3101–3103) and
5 from East Hyde borehole EH1, 9.2 m, MIS 11 (NEaar 3734–3736, 3824–2835).
4 *Bithynia troschelii* opercula (NEaar 3743–3745, 3830) were also analysed individually from Barling Gravel, 05 <5> (18).

Apton Hall Farm borehole, APHF 05 <3A>

TQ 888 931

A borehole was drilled as part of the MVPP project at Apton Hall Farm, designated BH1. This provided a similar sedimentary sequence to that obtained by Roe (1994 ; 1999) at Canewdon, but no pollen assessment was undertaken so the two sequences cannot be correlated in detail. The opercula were obtained from the silty clay, above the sands and gravels, which rested on bedrock. Sample 3a is an amalgamation from samples taken between 5 and 5.5m. Ostracods were most abundant at 4 and 5m depths, all brackish. Opercula were found at *c* 5m depth. An OSL sample was also taken from the base of this sequence, but has not been dated as part of the current round of post-excavation analysis.

Barling Gravel, 05 <5> (18)

TQ 938 906

The samples taken from the Barling Gravel were obtained from a new section (S1) described during the MVPP project, from sample 5, near the base of the sequence. The gravel is believed to overlie the Barling Channel (Bridgland *et al* 2001), although the relationship was not seen in this exposure. The sediment consisted of horizontally bedded very sandy gravel with an organic clay matrix and frequent silty clay drapes, designated as Context 18. The sediments are characteristic of deposition in a cold environment. It is possible that some of the molluscan material found may have been reworked into the cold stage deposits from the interglacial channel deposits at the site. The opercula samples directly underlie OSL sample BLNG05-05, which provided an age estimate of MIS 6. However, the OSL dates in this sequence are inverted.

Bradwell Hall, JCB bulk 13, MIS 11

TL 988 054

Samples from Bradwell Hall were obtained from a new test pit (TP7), context 72. No pollen assessment has been undertaken. The molluscan assemblage included the characteristic "Rhenish fauna". It is thought that this channel can be correlated with the Tillingham Channel at East Hyde (Roe 2001). OSL samples have also been taken from this site, but from a different unit (fine-grained gravel) whose stratigraphic relationship to the channel deposits is unclear (TP5, context 55). Gibbard *et al* (1996) believed the fine-grained gravel to overlie the channel deposits, but evidence of this was not seen in the MVPP investigations.

East Mersea Restaurant Site, Sample 3, MIS 5e

TM 053 136

The second of the two channel deposits at Cudmore Grove has less extensive exposures, and is essentially a fossiliferous gravel, buried by modern beach sand (Bridgland *et al* 1995). The deposits are probably of Ipswichian age, since they contain the remains of *Hippopotamus*.
The samples from this site were archive material obtained from Richard Preece, University of Cambridge, identified as Sample 3 (from Unit 2 [sandy silt with bones and shells]).

Shoeburyness borehole S1, 13.9m and 14.42-14.44 m

TQ 93375-85483

Opercula from Shoeburyness were obtained as archive material from borehole S1 (Roe 1994; 1999) from Richard Preece, University of Cambridge. This borehole material is stored in archives managed by the Department of Geography, University of Cambridge. One operculum was obtained from 14.42–14.44m (labelled 'Shoeburyness Channel') with further samples from a depth of 13.9m. Ostracod analysis suggests that these samples come from a freshwater depositional environment, with brackish influence coming above *c* 10m depth. Detailed pollen analysis has also been undertaken on this borehole (Roe 1999) and the samples come from within a birch-pine phase which is postdated by the development of mixed-oak woodland.

East Hyde borehole EH1, 7.55 and 9.2m MIS 11

TL 9804 0408

The archive borehole EH1 from the Asheldham/Tillingham Channel (Roe 1994; 2001) was sampled at two depths for opercula, 7.55m and 9.2m (this latter is right at the base of the sequence). This borehole material is stored in archives managed by the Department of Geography, University of Cambridge. Ostracod analysis suggests freshwater influence most notable below 8.5m, with more brackish conditions indicated above this. Thus, the 9.2m sample definitely came from the freshwater phase, with the 7.55m sample from the transition zone between freshwater and estuarine conditions. The Rhenish molluscan fauna was reported at a depth of 7.53m (Roe 1994). Pollen assemblages from the East Hyde sequence were assigned by Roe (1994) to the late temperate substage of an interglacial (mixed-oak replaced by coniferous forest) and contained 'Type X'. An *Azolla* plant macrofossil was also reported from the sequence (Roe 2001).

Sample Preparation

Shells were examined under a low powered microscope and any adhering sediment removed. The shell samples were then sonicated and rinsed several times in HPLC-grade water. The shells were then crushed to <100um. Only bleached samples were analysed.

Bleaching

50µl 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°C for 24 hours (H*).

20µl per milligram of sample of 7M Hydrochloric Acid (HCl) was added to each Hydrolysis ("Hyd", H*) sample in sterile 2ml 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 2ml vials were re-tightened to prevent the samples drying out.

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

Demineralisation

Free amino-acid samples ("Free", F) were demineralised in cold 2M 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 with 10µl per mg of Rehydration Fluid: a solution containing 0.01mM HCl, 0.01mM L-homo arginine internal standard, and 0.77mM 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 20µl of rehydrated sample was then placed in a sterile, labelled 2ml 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 the 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 (2µl) were derivitised with 2.2µl *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. 5µm; 250 x 3mm) using a linear gradient of a sodium acetate buffer (23mM sodium acetate, 1.3mM Na_2EDTA; pH6), methanol, and acetonitrile on an integrated HP1100 liquid chromatograph (Hewlett-Packard, 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 = 230nm, Em = 445nm) in each sample and normalised to the internal standard. All samples and blank extracts that had been subjected to identical preparation procedures were run in triplicate. 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, allowing calibration with the analyte samples, were analyzed at the beginning and end of every run, and one standard was analyzed every ten samples. Blanks were randomly interspersed amongst the standards.

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

In total we conducted 148 analyses, all of which were on bleached samples. As previously observed, bleaching reduced the yields of amino acids and also increased reproducibility.

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 Free and Hyd fractions (Appendix 4). 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.

On the basis of the relative D/L values and concentrations (Appendix 4) the amino acid data from the opercula from the East Mersea Restaurant site were consistent with a correlation with MIS 5e. The data from all the other sites analysed indicated an age assignment within MIS 9 or MIS 11. Data from other MIS 9 and MIS 11 sites are also presented for comparison. Discussion of data from opercula from Clacton and Cudmore Grove are also discussed. Channels of different ages are present at Clacton (Warren 1955) but the material from Clacton analysed in the present study comes from samples correlated with the Lower Freshwater Bed at West Cliff, attributed to MIS 11 (Bridgland *et al* 1999). The Cudmore Grove channel deposits are exposed on the foreshore of Mersea Island at Cudmore Grove. The main deposit, containing Middle Pleistocene sediments, was originally associated with the main Thames-Medway system and therefore correlated with the Clacton Channel deposits of the Hoxnian, but are now thought to represent a tributary of the river and likely to be of the interglacial following this (Roe *et al* in prep). These two sites represent intra-crystalline protein degradation values from an early MIS 11 site (Clacton) and a late MIS 9 site (Cudmore Grove), which may aid interpretation of the Medway dataset.

The data obtained from Asx, Glx, serine (Ser), alanine (Ala) and valine (Val) is discussed in detail below. Sample EH9.2Bto1bF (NEaar 3734) from East Hyde borehole, 9.2m, has abnormally low levels of protein degradation in the Hyd fraction compared to that of the Free fraction, indicative of a compromised system. If the amino acids were contained within a closed system, the relationship between the Free and the Hyd fractions should be highly correlated, with non-concordance enabling the recognition of compromised samples (Preece and Penkman 2005). This operculum from East Hyde showed this non-concordance and so was rejected from the dataset.

The pattern of protein degradation with time is slightly different for the opercula of *Bithynia troschelii* compared to that of the *B. tentaculata* opercula. Amino acid racemisation is governed by the original protein sequence and conformation. Whilst developing the research into closed-system protein degradation it became clear that the reaction rates were species-specific, even in the intra-crystalline fraction. This necessitates the comparison of amino acid data only within a single species, meaning that the *Bithynia troschelii* data cannot be directly compared to the *B. tentaculata* data. Whilst analyses at NEaar have shown that the differences in amino acid composition and protein decay patterns between the *B. tentaculata* and *B. troschelii* opercula are negligible (Penkman *et al* submitted 2006), direct comparison of the data between these two species should not yet be undertaken. As *B. troschelii* opercula were analysed only from the Barling Gravel, all the age interpretation made in this study is based on the *B. tentaculata* dataset.

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 values of Asx D/L for the Free and Hyd samples for East Mersea Restaurant Site are the lowest in this set of samples, consistent with a correlation with the Ipswichian.

The D/L values for the other sites fall within the range of that expected from MIS 9 and MIS 11 material (Fig 3).

There is overlap between the Asx D/L data from MIS 9 and MIS 11 sites (Fig 3, first two columns in each graph), due to the plateauing of the increase in D/L at these timescales. This therefore makes discrimination to an interglacial level difficult using this amino acid. However, the data from Apton Hall Farm and the Barling Gravel fall at lower values, with Bradwell Hall tending to yield higher ratios. The spread of data from Barling Gravel is quite high, with one sample (BaGBto4, NEaar 3822) falling at higher values than the rest of the cluster from that site.

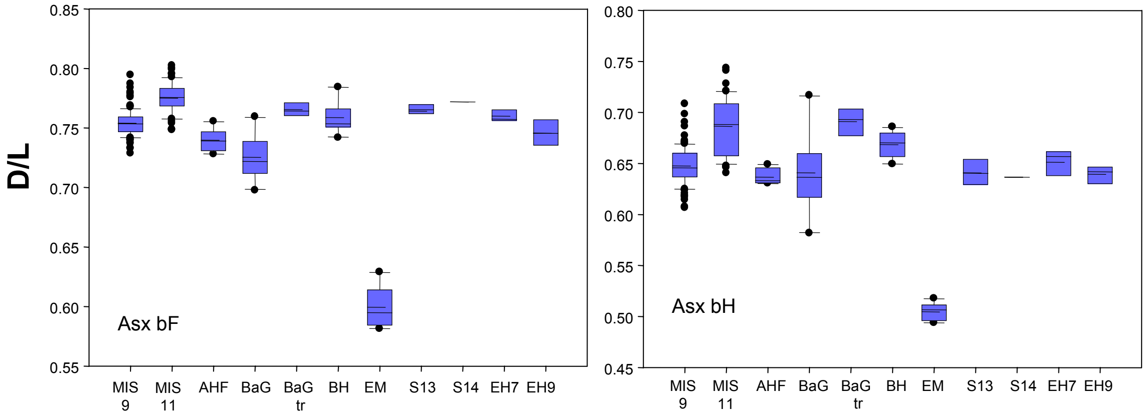


Figure 3: D/L values of Asx for the Free amino acid (FAA; bF; left) and Total Hydrolysable amino acid (THAA; bH*; right) fraction of bleached (intra-crystalline) *Bithynia* opercula from Apton Hall Farm (AHF), Barling Gravel (BaG), Bradwell Hall (BH), East Mersea Restaurant site (EM), Shoeburyness borehole 13.9m (S13), Shoeburyness borehole 14.42m (S14), East Hyde borehole, 7.55m(EH7) and East Hyde borehole, 9.2m (EH9). The species of *Bithynia* analysed from all sites is *B. tentaculata*, but *B. troschelii* is also analysed from Barling Gravel (BaG tr). The data for other MIS 9 sites (including Purfleet, Cudmore Grove and Grays) and MIS 11 sites (including Swanscombe, Ebbsfleet, Woodston, Clacton, Elveden, Beeches Pit and Hoxne) have also been plotted for comparison. For each site, the base of the box indicates the 25th percentile. Within the box, the solid line plots the median and the dashed line shows the mean. The top of the box indicates the 75th percentile. Where more than 9 data points are available, the 10th and 90th percentiles can be calculated (shown by lines below and above the boxes respectively). The results of each duplicate analysis are included in order to provide a statistically significant sample size. Note different scales on the y-axes

The plot of Free to Hyd 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 Medway samples have been plotted in this way (Fig 4), along with data from two relevant sites (enclosed symbols), that of Clacton (MIS 11) and Cudmore Grove (MIS 9). It is clear from this plot that the natural variability within the data does not allow a clear distinction of any of the sites except the East Mersea Restaurant site.

The Free Asx values for EH9.2Bto1 (NEaar 3734) are similar to that from the other samples from this site. However, the values obtained from the Hyd fraction are much lower than expected. When the Free to Hyd graph is plotted (Fig 4), this sample falls significantly off the expected line. This suggests that this operculum's closed system had been compromised.

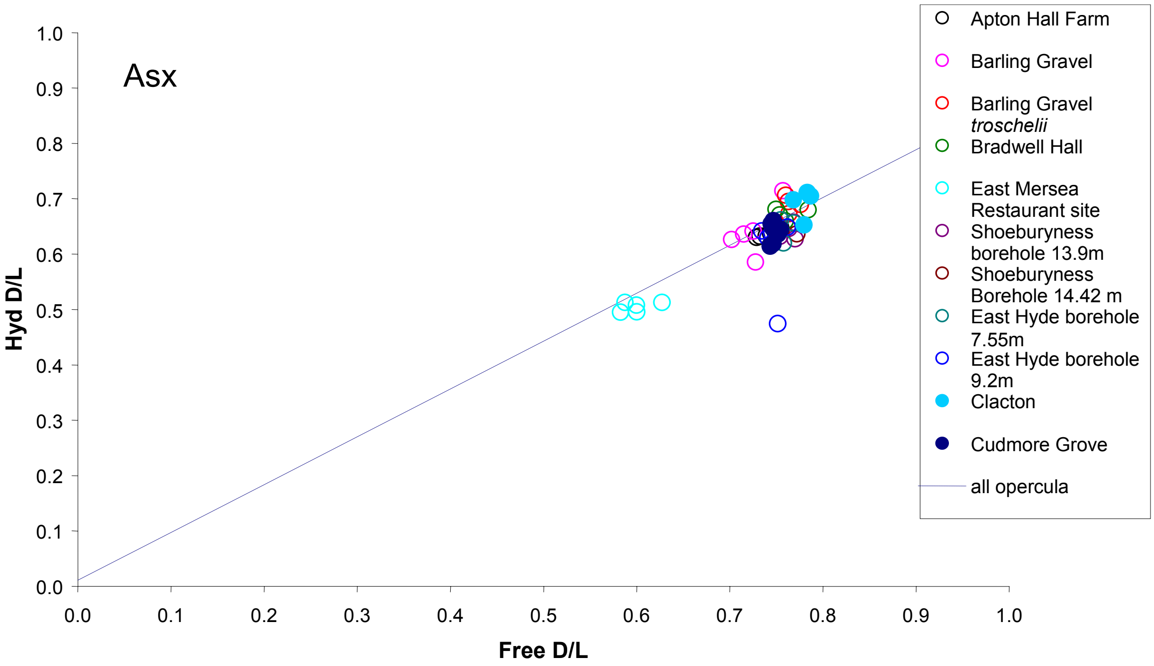


Figure 4: D/L Hyd vs D/L Free for Asx in Medway *Bithynia* opercula, compared to Clacton and Cudmore Grove samples and the trendline observed for fossil samples (in blue). Note the abnormal DL ratios for one of the East Hyde borehole 9.2m samples

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. However, the low levels of racemization do help discriminate between material of MIS 9 and MIS 11 age. 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 values of Glx D/L in the Hyd fraction for East Mersea Restaurant site are significantly lower than those observed for the other samples in this study, and are consistent with a correlation with the Ipswichian.

The 90% confidence limits of the data presented for the MIS 9 and MIS 11 sites in Fig. 5 do not overlap, therefore it should be possible to discriminate between sites of these ages with enough samples. However, for the Hyd fraction much of the Medway dataset falls between these two intervals, indicating that the samples fall either late within MIS 11, or early within MIS 9. The extremes of these interglacials can be demonstrated by comparison with two sites in particular, that of Clacton, which is believed to have been deposited early within MIS 11, and that of Cudmore Grove, which is proposed to represent sediments laid down late within MIS 9 (Fig 6).
Bradwell Hall seems to have the highest values in both the Free and the Hyd fractions, and so is likely to correlate with MIS 11.

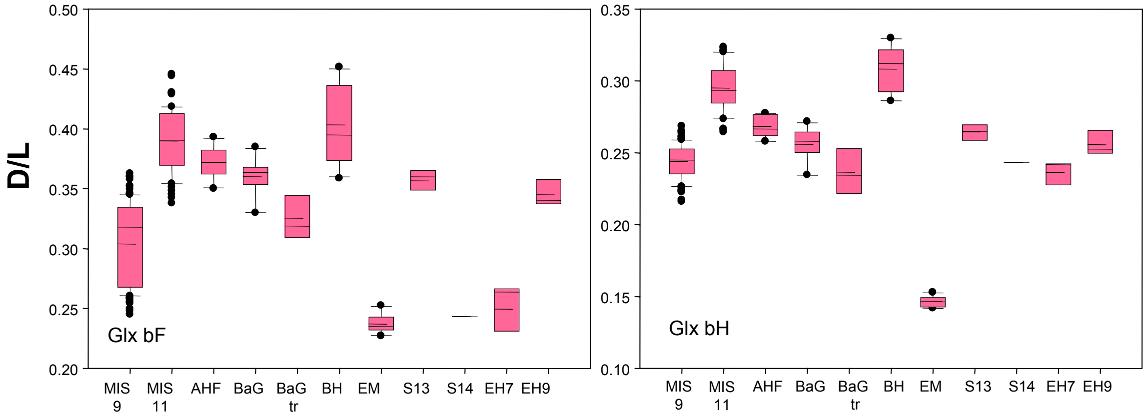


Figure 5: D/L values of Glx for the Free amino acid (FAA; bF; left) and Total Hydrolysable amino acid (THAA; bH*; right) fraction of bleached (intra-crystalline) *Bithynia* opercula from the Medway sites and for the database of MIS 9 and MIS 11 material. For a full description of this figure see the legend for Figure 3

Both the Free and Hyd Glx values for sample EH9.2Bto1 are abnormally low and fall off the expected line, indicative of compromised material (Fig 6).

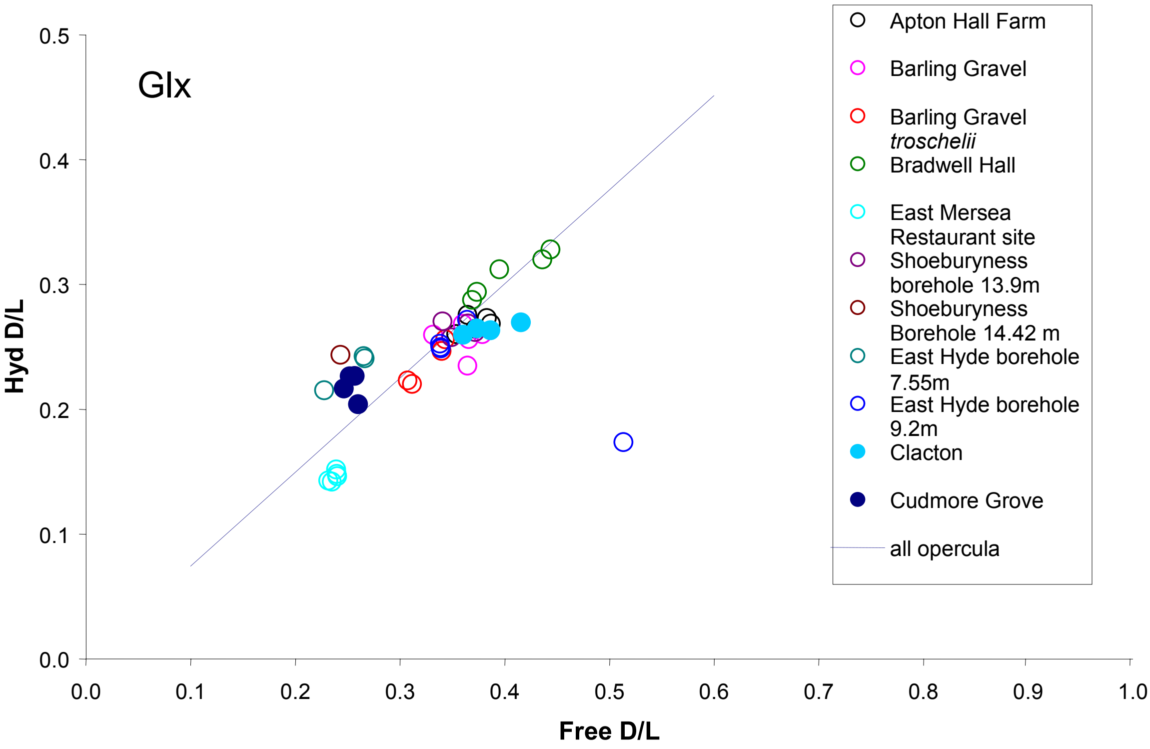


Figure 6: D/L Hyd vs D/L Free for Glx in Medway *Bithynia* opercula, compared to Clacton and Cudmore Grove samples and the trendline observed for fossil samples (in blue). Note the abnormal DL ratios for one of the East Hyde borehole 9.2m samples

Serine (Ser)

Serine is one of the most unstable amino acids, with fast rates of racemisation and decomposition. This results in highly variable data and limits its usefulness in discriminating between sites at the timescales in this study.

However, whilst the degree of racemization observed in the samples from MIS 9 and MIS 11 sites is uninformative in separating them, they do tend to fall at higher values than that of the East Mersea

Restaurant Site (Fig 7). One of the Bradwell Hall samples (BHBto3, NEaar 3733) has very low values but variable replicates, so the Ser data from this sample needs to be treated with caution.

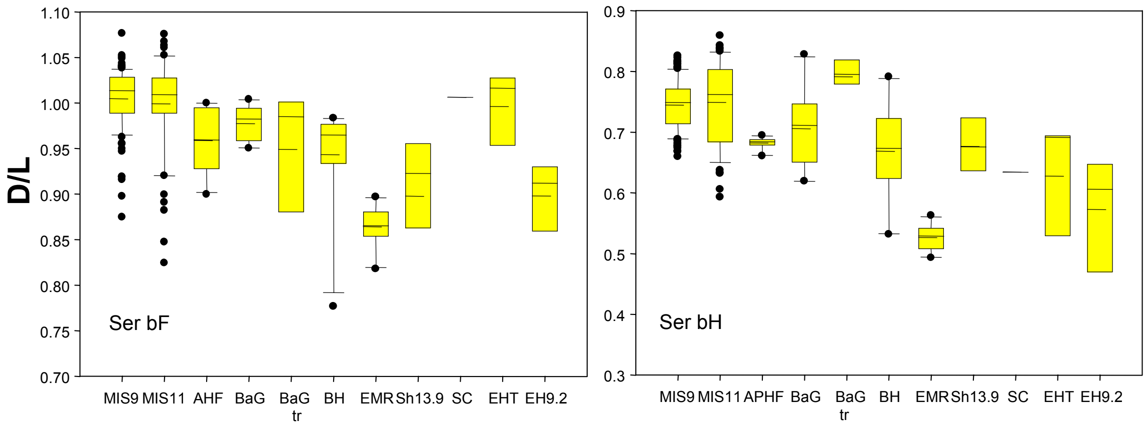


Figure 7: D/L values of Ser for the Free amino acid (FAA; bF; left) and Total Hydrolysable amino acid (THAA; bH*; right) fraction of bleached (intra-crystalline) *Bithynia* opercula from the Medway sites and for the database of MIS 9 and MIS 11 material. For a full description of this figure see the legend for Figure 3

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.

Again, the East Mersea Restaurant Site is easily separable from all the other sites (Fig 8), with ratios consistent with a MIS 5e correlation.

The highest Ala D/Ls are obtained from the Bradwell Hall site, making this site again likely to be of MIS 11 age. The Barling Gravel samples yield some of the younger ratios within this group, which may be interpreted as likely to be of MIS 9 age. Apton Hall Farm also appears to have ratios consistent with MIS 9. The intermediate values of the other sites make it difficult to determine whether they fall late within MIS 11 or early within MIS 9.

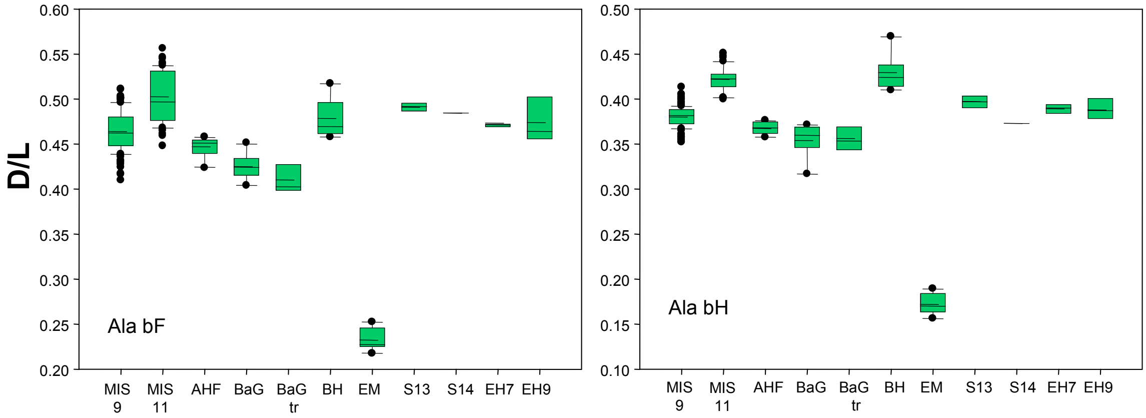


Figure 8: D/L values of Ala for the Free amino acid (FAA; bF; left) and Total Hydrolysable amino acid (THAA; bH*; right) fraction of bleached (intra-crystalline) *Bithynia* opercula from the Medway sites and for the database of MIS 9 and MIS 11 material. For a full description of this figure see the legend for Figure 3

When the Free to Hyd graph is plotted (Fig 9), the EH9.2Bto1 sample falls at lower values than expected. The Bradwell Hall samples have the highest values within the MIS 9/11 group, with the Barling Gravel samples falling lowest.

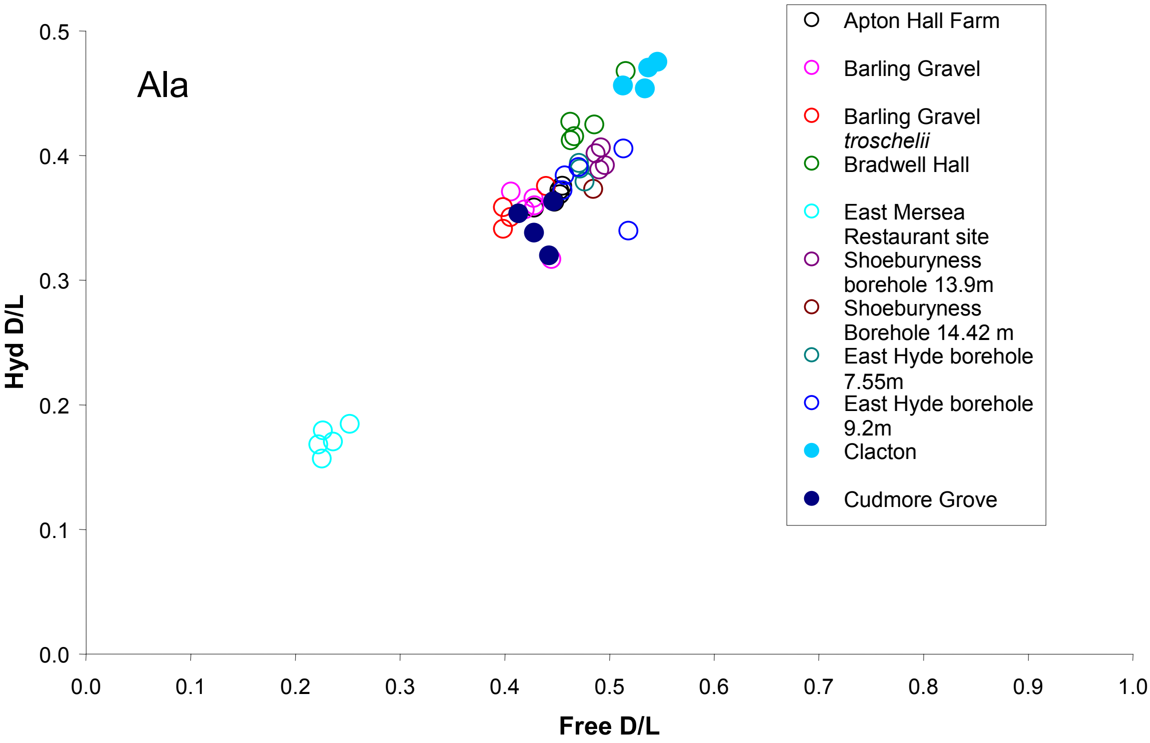


Figure 9: D/L Hyd vs D/L Free for Ala in Medway *Bithynia* opercula, compared to Clacton and Cudmore Grove samples

Valine (Val)

Valine has extremely low rates of racemisation, and so is one of the more useful amino acids for age discrimination within material of this age.

The East Mersea Restaurant site yields the lowest Val D/L values in both the Free and the Hyd fraction (Fig 10), consistent with a correlation with MIS 5e.

The other sites again fall within the range of MIS 9 and MIS 11 sites (Fig 10). The Apton Hall Farm and Barling material have the lowest ratios, indicating a MIS 9 age. Bradwell Hall has the highest ratios, which make it more likely that these represent MIS 11 samples. The Shoeburyness and East Hyde samples again have intermediate ratios, at the lower end of the MIS 11 values and the higher end of the range of MIS 9 values. This therefore indicates that they either fall towards the end of the MIS 11 interglacial or at the beginning of the MIS 9 interglacial.

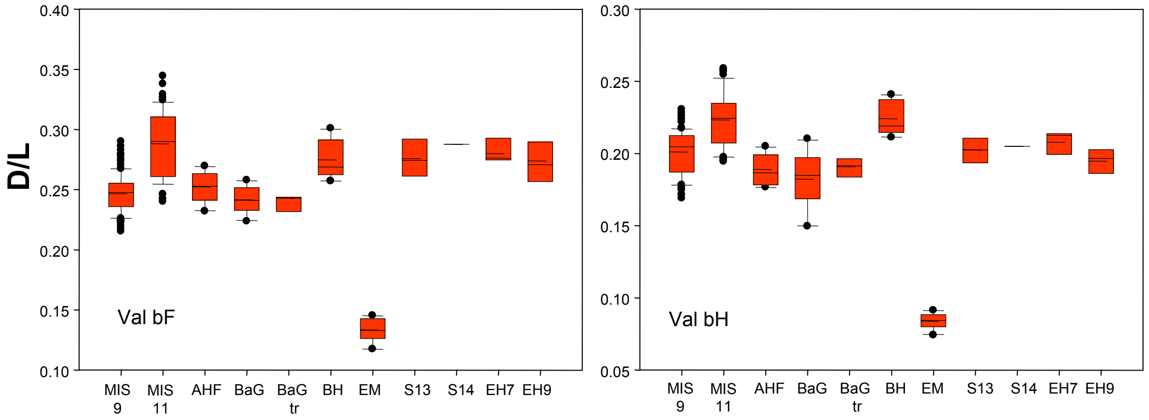


Figure 10: D/L values of Val for the Free amino acid (FAA; bF; left) and Total Hydrolysable amino acid (THAA; bH*; right) fraction of bleached (intra-crystalline) *Bithynia* opercula from the Medway sites and for the database of MIS 9 and MIS 11 material. For a full description of this figure see the legend for Figure 3

The Free to Hyd plot of Val D/L separates out the sites of Clacton and Cudmore Grove distinctly (Fig 11). Whilst the East Mersea restaurant site plots at significantly lower values, consistent with an Ipswichian age, the other Medway sites fall between Cudmore Grove (MIS 9) and Clacton (MIS 11). The Barling and Apton Hall Farm samples tend to fall closer to Cudmore Grove, indicating a MIS 9 age. The Bradwell Hall data has the highest values within the Medway dataset and fall closest to the Clacton data, indicating that this site is likely to be of MIS 11 age.

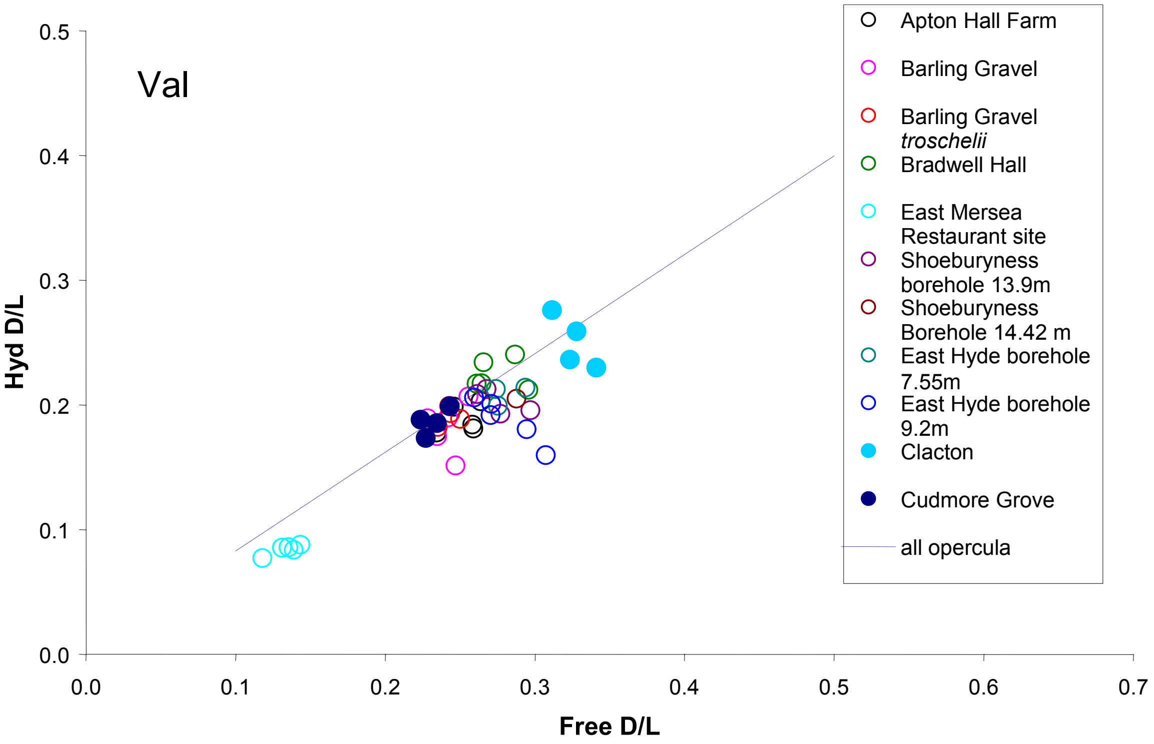


Figure 11: D/L Hyd vs D/L Free for Val in Medway *Bithynia* opercula, compared to Clacton and Cudmore Grove samples and the trendline observed for fossil samples (in blue)

The same problematic sample from East Hyde borehole 9.2m, identified for Asx, Glx and Ala, is also seen to a lesser extent with divergence in the DL ratios of Val, further confirming that this sample is compromised.

[Serine]/[Alanine]

The ratio of the concentrations of Serine and Alanine provides a useful tool for age estimation. Serine is a very unstable amino acid, and it can degrade via dehydration into alanine (Bada *et al.,* 1978). As the protein within a sample breaks down, the concentration of serine will decrease with an increase in the concentration of alanine, thus the [Ser]/[Ala] value will decrease with increasing time. In order to ease the interpretation, the y-axes in Figure 12 are plotted in reverse, so that the direction of increase in protein degradation is the same as for the racemisation graphs.

The samples from the East Mersea Restaurant site fall at higher (and therefore less degraded) values than for the other sites. The second replicate analysis of one of the East Mersea Hyd samples (EMR3Bto5, NEaar 3821) has lower Ser concentrations than the first replicate, and therefore falls at lower values than the other samples; it is likely that this is due to an analytical problem and the first analysis is probably correct.

Interestingly the *B. troschelii* data from Barling falls at lower values, although this is likely to be due to the slight differences in protein composition between the two species analysed here.
On the basis of the [Ser]/[Ala], it is very difficult to separate these sites out on basis of age, other than to say that they are all likely to be of MIS 9 or MIS 11 age.

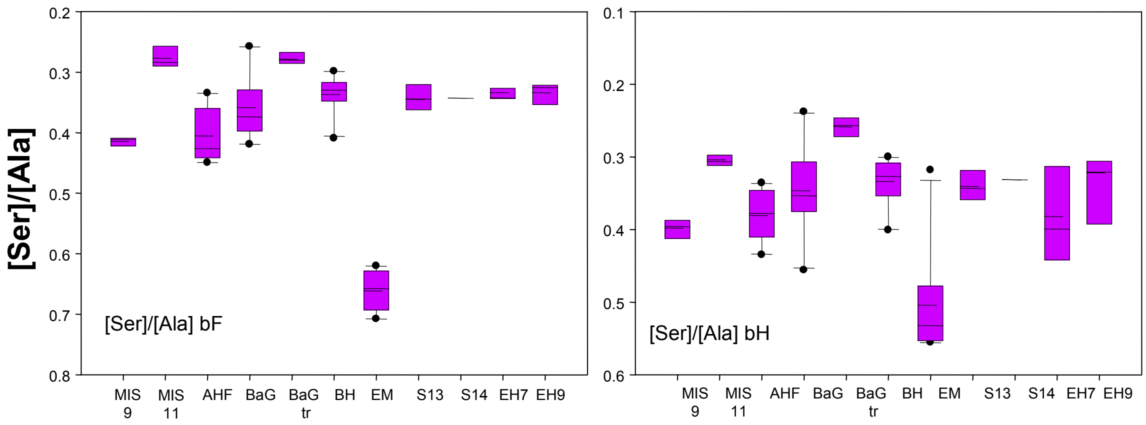


Figure 12: [Ser]/[Ala] for the Free amino acid (FAA; bF; left) and Total Hydrolysable amino acid (THAA; bH*; right) fraction of bleached (intra-crystalline) *Bithynia* opercula from the Medway sites and for the database of MIS 9 (represented by Cudmore Grove) and MIS 11 (represented by Clacton) material. For a full description of this figure see the legend for Figure 3. The y-axes for the [Ser]/[Ala] data are plotted in reverse, so that the direction of increased protein degradation for each of the indicators remains the same

In the Free vs Hyd plot (Fig 13) the large spread of data in the Barling samples is evident – the two outliers being sample BaGBto2 (NEaar 3741) lying at younger values and BaGBto4 (NEaar 3828) lying at older values. The Apton Hall Farm samples plot close to the Cudmore Grove samples, indicating a MIS 9 age. The compromised sample EH9.2Bto1bF plots clearly away from the other samples at much higher [Ser]/[Ala] values than expected.

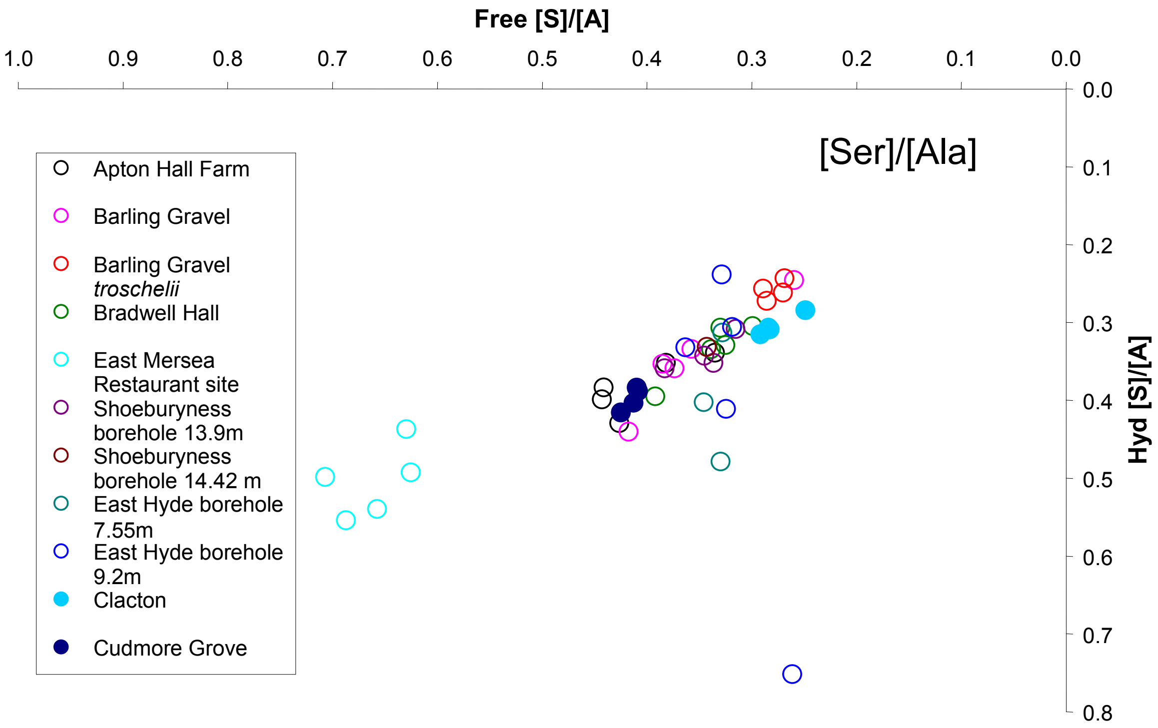


Figure 13: [Ser]/[Ala] Hyd vs D/L Free in Medway *Bithynia* opercula, compared to Clacton and Cudmore Grove samples. As the [Ser]/[Ala] value decreases with increasing protein decomposition, the axes of this plot has been reversed so that the direction of protein decomposition is the same as that for the D/L graphs, with younger samples falling to the bottom left corner and older samples falling to the top right corner of the graph

Discussion

East Hyde borehole compromised sample

Sample EH9.2Bto1bF (NEaar 3734) from East Hyde borehole, 9.2m, has abnormally low levels of protein degradation in the Hyd fraction compared to that of the Free fraction, indicative of a compromised system. The data from this sample has therefore been rejected from the analyses. There are significant indications that the integrity of the closed system of intra-crystalline protein has been compromised in some way. Therefore no age estimation can be made from this sample.

If the sample had been contaminated by microbial action or undergone recrystallisation during its burial history, then the Free to Hyd values of one or more of the amino acids will not match. When the Hyd ratios are compared to the Free ratios from the same sample, they fall clearly well out of the range of any operculum or shell yet analysed (Figs 4, 6, and 13). If the amino acids are contained within a closed system the relationship between the Free and the Hyd would be highly correlated, as evidenced by the tight clustering of Free to Hyd ratios observed for the other opercula (Penkman 2005; Preece and Penkman 2005). That these samples plot well away from these general trends is an indication that post-mortem protein contamination or leaching has occurred.

In the case of the single *Bithynia tentaculata* opercula from East Hyde borehole, 9.2m, the original amino acid composition has clearly been compromised and no age assignment is possible for these samples. The tight correlation of the Free to Hyd ratios allows this alteration to be recognised, and therefore data from the opercula to be rejected. Analysis of just one of these fractions could lead to an erroneous age assignment, without recognition of the composition discrepancies. It is therefore essential that any amino acid analysis is conducted on both the Free and the Hyd fractions from the same sample, and that age correlations using amino acids are not made on the basis of single samples.

The Free amino acid levels from this sample indicate far more protein decomposition than the Hyd amino acids from the SAME shell. One possible explanation for high *Free* and low *Hyd* values is corrosion. If dissolution was the cause then the Free values would be as high or higher than in a reliable sample, whilst the Hyd values would be depressed, as observed in this material.

Comparison with other sites

The analysis of the closed system of protein within shells 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. This measurement, the Intra-crystalline Protein Degradation value (IcPD, formerly DMK) simplifies the presentation of the data to two compound values for each sample, one for the Free and one for the Hydrolysed amino acids. As these should be highly correlated, they can be cross-plotted, giving an aminostratigraphic framework with younger samples lying at low values and older samples with higher values, given a similar temperature history for all the sites. A study has been undertaken of interglacial sites within the UK which has allowed the tentative correlation of the aminostratigraphic framework to the Marine oxygen Isotope Stage (MIS) record (Penkman 2005).

On the basis of the relative D/L values and concentrations (Appendix 4) the amino acid data from the opercula from East Mersea Restaurant site, when compared with unpublished values from Quaternary sites within the UK (Penkman 2005) are consistent with an age assignment within MIS 5e. The amino acid data from the opercula from the other Medway sites are all consistent with an age assignment between MIS 9 and MIS 11. It is likely that the material from Barling and Apton Hall Farm is of MIS 9 age, and that Bradwell Hall is of MIS 11 age. The samples from Shoeburyness and East Hyde fall between the ranges of these two interglacials, and so are likey to correspond to either an early part of MIS 9 or a late part of MIS 11.

Temperature effects on protein degradation

Using modelled protein degradation, it is possible to use estimates of temperature variation in the UK (based here on a combination of long marine and ice core data and terrestrial palaeoclimatic estimates) to produce a curve which illustrates the increase in the IcPD value (i.e. extent of intra-crystalline protein degradation) with time. For example, in Figure 14, a temperature model for a particular site is estimated by interpreting the evidence from two global climate records, at different offset temperatures. Models of protein decomposition, based upon those presented in Collins and Riley (2000), is run with this temperature estimation. A stepwise increase in the predicted IcPD is observed (Fig 14): the rate plateaus in the glacials and rises rapidly in the interglacials, as the activation energies of all the key reactions are in the region of 100–130 kJ mol^{-1}. In the Quaternary it is predicted that during the cold stages little or no racemisation will have occurred in pleniglacial sediments as found in the UK, whereas in warm stages the increase in temperatures will have had a significant impact on the rate of racemisation.

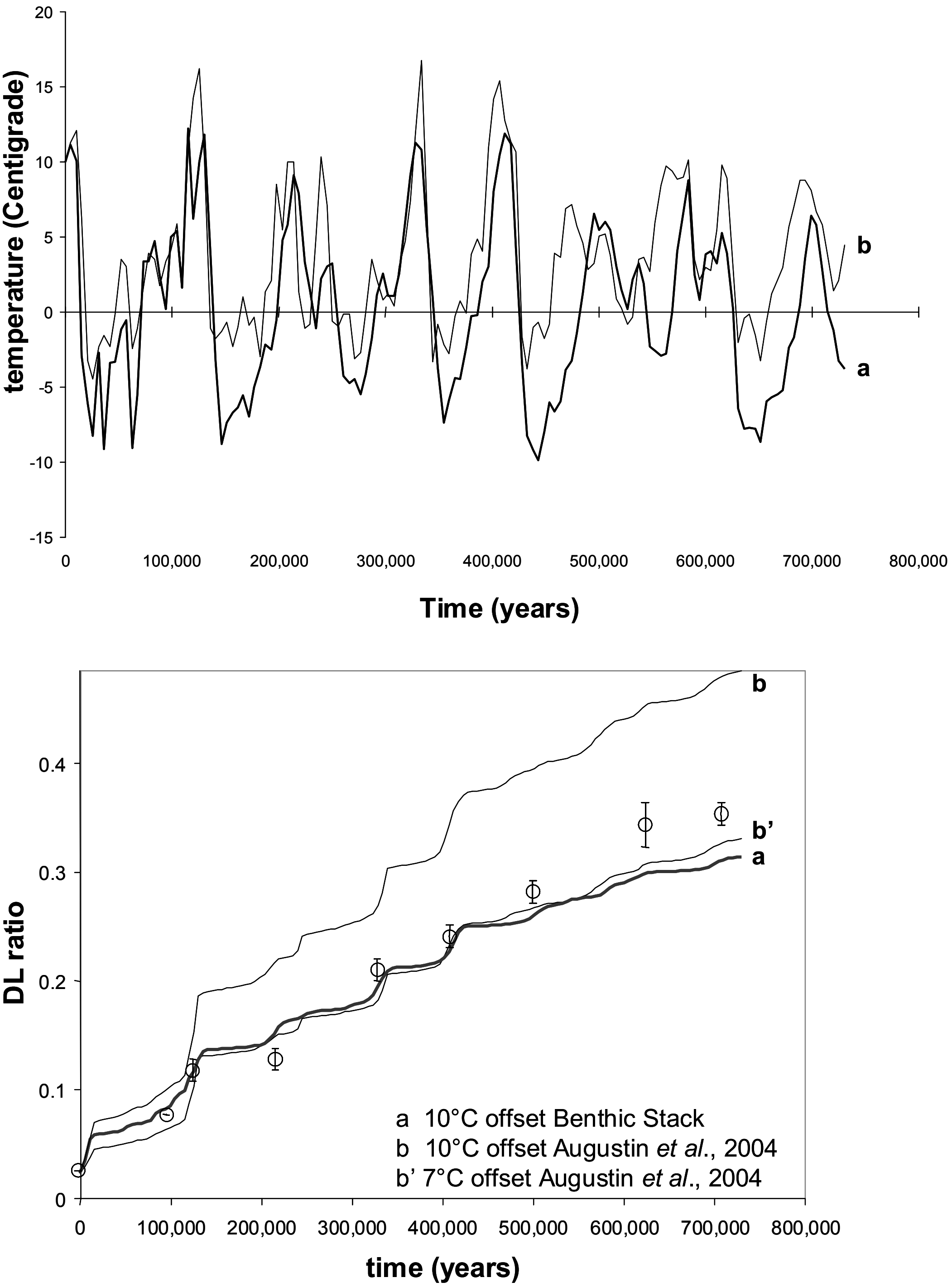


Figure 14: Example of the output from the IcPD model. The upper diagram shows two different temperature records: a benthic stack (a: Karner 2001) and an Antarctic ice core (b: Augustin *et al* 2004). The lower diagram illustrates the effect of the two temperature records on the predicted IcPD. The stepped nature of the increase in racemisation with age is caused by the much faster rates of racemisation in warm than in cold stages. Also observe that the ability to discriminate between isotope stages declines with time, because of the tendency for the reaction to slow (believed to be due to a restriction in available water for hydrolysis). As can be seen, the selection of both the temperature record and the offset temperature can have a significant effect on the extent of the IcPD, and hence any absolute age predictions. It is for this reason that the amino acid results presented are purely in an aminostratigraphic form

Using the thermal models leads to a greater understanding of the sensitivity of the IcPD to subtle changes in temperature and also to the range of values that can plausibly be considered to belong to a single isotope stage. However, the determination of absolute dates is highly dependent on the accuracy of the temperature record, and at this stage is not good enough to be used to determine absolute age estimates from amino acid racemisation data. The models are presented here in order to demonstrate the difficulties of distinguishing samples from the end of one interglacial from the beginning of the following interglacial.

The mechanism of the protein breakdown reactions means that increased degradation occurs during warm stages and there is a slowing in the rates of degradation in cold stages. As so little decomposition occurs in the cold stages, and there is a degree of natural variability in biological samples, it can be difficult to discriminate the end of one warm stage from the beginning of the next. Additional information from these sites would be extremely useful in narrowing down the range of ages possible from the amino acid data alone. In several cases environmental or other data from sites have constrained the time period of a site to early or late within an interglacial, enabling the amino acid data to then pin it to a specific MIS.

Conclusions

Out of the 37 *Bithynia tentaculata* opercula samples analysed in this study, only 1 did not enable relative age estimations to be determined. The one operculum sample analysed from East Hyde borehole 9.2m (EH9.2Bto1, NEaar 3734) showed that the intra-crystalline fraction of protein had been compromised at some point during the burial history of the sample. This is an extremely unusual occurrence with this material, which has been found to be a particularly robust repository for the original protein, with a normal success rate of recovery of ~97%.

Age of the sites

In this study the amino acid data has been used as a relative dating technique to present an aminostratigraphy for the area in question. The conversion of relative sequences into absolute dates and accurate correlation between different areas is currently being undertaken, but preliminary correlations have been made to the MIS record.

In order of youngest to oldest we would place the sites as follows:

East Mersea Restaurant site: has significantly lower protein decomposition that any of the other sites analysed in this study. The amino acid data from this site is similar to that obtained from other Ipswichian (MIS 5e) sites (including Bobbitshole, Trafalgar Square, Coston, Shropham, Tattershall Castle, Cropthorne New Inn, Eckington, and Itteringham).

Data from all the other sites fall within the range of MIS 9 sites (such as Grays, Cudmore Grove, Hackney and Purfleet) and MIS 11 sites (including Hoxne, Swanscombe, Ebbsfleet, Elveden, Woodston, Barnham and Beeches Pit). The levels of natural variability within the samples and the low level of protein breakdown observed in cold stages limits the interpretation of the data. However, tentative conclusions can be drawn to attempt to separate out these sites.

It appears that **Apton Hall Farm** and **Barling** form the youngest samples of this group and are therefore likely to correlate with MIS 9. The pollen correlation of the Apton Hall site indicates that these sediments were deposited in an early phase of an interglacial, which makes a MIS 9 age far more likely than an early MIS 11 age.

It is noted that the Barling dataset had a greater variability than observed in the other sites.

The opercula from **Shoeburyness** and **East Hyde** have intermediate values, and therefore are likely to derive from interglacial sediments that were formed either late within MIS 11 or early within MIS 9. The opercula from Shoeburyness were taken from a pre-oak pollen zone, correlated with the beginning of an interglacial, with birch and pine pollen dominant (Roe 1999). In conjunction with this, the amino acid results would therefore suggest a correlation with early MIS 9 rather than early MIS

11. However, the levels sampled at East Hyde contain pollen correlated with a later temperate zone 3 (Roe 2001). The amino acid data would therefore support an age for this deposit late within MIS 11.

The samples from **Bradwell Hall** seem to have the greatest extent of protein degradation within the sample set. It is likely that these samples correlate with MIS 11, confirming the suggested geological correlation with the Tillingham Channel at East Hyde.

Further information on the sites under study may help resolve the amino acid age determinations further.

Acknowledgements

Thanks to David Keen and Richard Preece for supplying most of the samples for cross-comparison with the Medway dataset reported in this study. Funding from NERC, English Heritage, and the Wellcome 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 3: 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 (eg 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 (Fig 6). In living organisms the amino acids in protein are almost exclusively L and the D/L ratio 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.

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 (Hill, 1965).

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

IcPD: Intra-crystalline Protein Degradation. This is the measure of the overall extent of protein breakdown in the closed system of the intra-crystalline fraction of a shell. Conventional racemization analysis tends to report an allosioleucine / isoleucine (A/I or D/L ratio). 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. However we have avoided trying to give a whole series of D/L values for each amino acid in each sample. Instead we are using a theoretical model of protein degradation. The model outputs are then used to compare observed D/L values of any amino acid against the A/I value at the same stage of protein decomposition. The relative rate of racemization of any amino acid (its DL ratio) is then reported as an A/I equivalent - which as a working title we have named the Intra-crystalline Protein Degradation value (or IcPD) (Collins Penkman and Kaufman in prep).

Instead of getting a single A/I ratio we obtain a series of (IcPD) values, currently IcPD_{Asx}, IcPD_{Glu}, IcPD_{Phe}, IcPD_{Ala}, IcPD_{Val}. Other ratios, notably IcPD_{Ser}, are not currently of implemented in the model – ie we don't have a good degradation model for this amino acid yet.

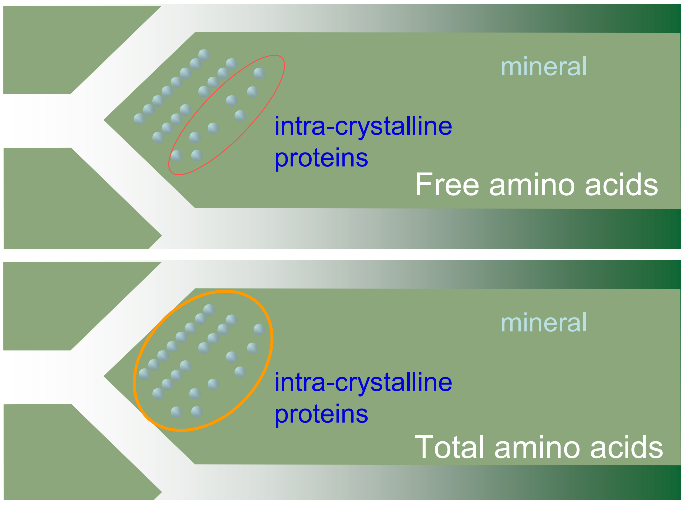
Because each amino acid has its own particular characteristics, only in a well behaved system will $\text{IcPD}_{\text{Asx}} = \text{IcPD}_{\text{Glu}} = \text{IcPD}_{\text{Phe}} = \text{IcPD}_{\text{Ala}} = \text{IcPD}_{\text{Val}}$. If an amino acid has an unusually low ratio (due to modern contamination) or unusually high racemization (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. Indeed we can use elevation of IcPD$_{\mathrm{Asx}}$ = IcPD $_{\mathrm{Glu}}$ and = IcPD $_{\mathrm{Ala}}$ to provide a bacterial contamination index. We have not done so in this case as there was no evidence of contamination.

IcPD values: Intra-crystalline Protein Degradation value, a summary value obtained from multiple amino acid D/L values from a single sample all normalised to a common model of protein degradation and racemization.

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: 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: laevorotary 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 amino acid fraction: 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 zwitterions

What does the date estimated from IcPD mean?

The date is our best estimate based upon the temperature history of the site. If we wanted to constrain this further we would need reliable independent dates. There are considerable differences in racemization rates between different molluscs. This reflects differences in rates of decomposition of proteins within the shell – the so-called species effects (Lajoie *et al* 1980).

Appendix 2

Past Use of Amino Acid Racemization Dating.

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. He suggested the possibility of using the kinetics of the degradation of amino acids as the basis for a dating method (Abelson 1955). In 1967 Hare and Abelson measured the extent of racemization of amino acids extracted from modern and sub-fossil *Mercenaria mercenaria* shells (edible clam). They found that the total amount of amino acids present in shell decreased with the age of the shell. The amino acids in recent shell were all in the L configuration and over time the amount of D configuration amino acid increased (Hare and Abelson 1967). However, even after 35 years this method of dating is still subject to vigorous debate, with the application of AAR to date bone being particularly controversial (Bada 1990; Marshall 1990). Major reviews of AAR include: Johnson and Miller (1997), Hare, von Endt, and Kokis (1997), Rutter and Blackwell (1995), Murray-Wallace (1993), Bada (1991) and Schroeder and Bada (1976). Racemization is a chemical reaction and a number of factors influence its rate (Rutter and Blackwell, 1995). These include: amino acid structure, the sequence of amino acids in peptides, pH, buffering effects, metallic cations, the presence of water and temperature. To establish a dating method the kinetics and mechanisms of the racemization (and epimerization) reaction of free and peptide bound amino acids need to be established. To this end various workers in the late 1960s and the 1970s studied free amino acids in solution and carried out laboratory simulations of post mortem changes in the amino acids in bone (Bada 1972) and shell (Hare and Abelson 1967; Hare and Mitterer 1969). Attempts have also been made to relate the kinetics of free amino acids, with those in short polypeptides and the proteins in various archaeological samples (Bada 1982; Smith and Evans 1980).

The ability of this technique to be used as a geochronological and geothermometry tool has led to its use in many environmental studies, with research into AAR in terrestrial gastropods (Goodfriend 1991; 1992), bivalves (Goodfriend and Stanley 1996), foraminifera (Harada *et al* 1996), ostrich egg shells (Miller *et al* 1992; 1997) and speleothems (Lauritzen 1994). Studies within UK deposits have been based on early methods of chemical separation, using Ion-Exchange liquid chromatography, 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 protein content within marine (Bowen and Sykes 1988) and non-marine mollusc shells (Bowen *et al* 1989) from UK interglacial sites, an amino acid geochronology was developed using the increase in A/I, correlating with the marine oxygen isotope warm stages.

Appendix 3

Abbreviations used in this report

Abbrev	1-letter code	number of chiral centres	
Ala	A	1	Alanine
Arg	R	1	Arginine
Acn			acetonitrile
AA			Amino acid(n)
Asn	N	1	Asparagine
Asp	D	1	Aspartic acid
Asx			Asparagine + Aspartic acid + succinimide
Asu			Succinimide
Cys	C	1	Cysteine
DCM			Dichlormethane
GABA			γ-Aminobutyric acid
Gln	Q	1	Glutamine
Glu	E	1	Glutamic acid
Gly	G	0	Glycine
His	H	1	Histidine
HPLC			High-Performance Liquid Chromatography
Hyp			Hydroxyproline
IBD(L)C			N-Isobutyryl-D(L)-Cysteine
Ile	I	2	Isoleucine
Leu	L	1	Leucine
Lys	K	1	Lysine
MeOH			Methanol
Met	M	1	Methionine
Nle			Norleucine
OPA			ortho-Phthaldialdehyde
Orn			Ornithine
Phe	F	1	Phenylalanine
Pro	P	1	Proline
Ser	S	1	Serine
Thr	T	2	Threonine
Trp	W	1	Tryptophan
Tyr	Y	1	Tyrosine
Val	V	1	Valine

Appendix 4

Data sheets from Medway

Neaar	File	Genus	Species	materials	location	Type	Asx conc pmol/mg	Glx conc pmol/mg	Ser conc pmol/mg	Gly conc pmol/mg	Ala conc pmol/mg
3737bF	G238-0406	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	F	1546	219	422	1247	1261
3737bF	G238-0415	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	F	1563	219	433	1251	1291
3737bH*	G238-2543	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	H*	2691	1768	646	2472	1889
3737bH*	G238-2553	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	H*	2649	1727	636	2632	1891
3738bF	G238-1428	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	F	2305	294	708	1607	1659
3738bF	G238-1464	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	F	2316	295	705	1194	1657
3738bH*	G239-0204	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	H*	3649	2190	918	3054	2110
3738bH*	G240-0203	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	H*	3603	1881	975	2219	2303
3739bF	G238-1529	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	F	2127	272	651	1538	1462
3739bF	G238-1565	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	F	2139	256	656	1057	1489
3739bH*	G239-0305	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	H*	3436	2023	887	3280	2182
3739bH*	G240-0304	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	H*	3461	1677	1089	2652	2782
3826bF	G244-2455	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	F	2222	268	671	1627	1696
3826bF	G244-2463	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	F	2134	247	619	1236	1684
3826bH*	G244-3787	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	H*	2536	1629	595	2361	1670
3826bH*	G244-3795	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	H*	2755	1814	625	2555	1801
3827bF	G244-2556	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	F	2646	288	789	1495	1754
3827bF	G244-2564	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	F	2422	256	718	1158	1657
3827bH*	G244-3888	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	H*	3853	2280	887	3211	2201
3827bH*	G244-3896	*Bithynia*	*tentaculata*	operculum	Apton Hall Farm borehole, APHF 05 <3A>	H*	3846	2289	802	2852	2200
3740bF	G238-0508	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	F	1681	239	534	1301	1363
3740bF	G238-0517	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	F	1677	234	520	1191	1373
3740bH*	G238-2644	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	H*	2950	1836	708	2479	1975
3740bH*	G238-2654	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	H*	2594	1546	626	1964	1800
3741bF	G238-1630	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	F	1965	262	603	1363	1452
3741bF	G238-1666	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	F	1918	247	597	1016	1424

Neaar	File	Genus	Species	materials	location	Type	Asx conc pmol/mg	Glx conc pmol/mg	Ser conc pmol/mg	Gly conc pmol/mg	Ala conc pmol/mg
3741bH*	G239-0406	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	H*	3569	2063	993	3004	2178
3741bH*	G240-0405	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	H*	2764	1244	986	1970	2324
3742bF	G238-1731	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	F	1855	260	555	1533	1479
3742bF	G238-1767	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	F	1821	249	537	1079	1441
3742bH*	G239-0507	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	H*	3011	2001	784	3651	2181
3742bH*	G240-0506	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	H*	2758	1608	803	2920	2239
3743bF	G238-0609	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	F	809	146	340	1429	1154
3743bF	G238-0618	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	F	867	151	340	1464	1198
3743bH*	G238-2745	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	H*	2046	1686	587	4879	2304
3743bH*	G238-2755	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	H*	2045	1632	610	4711	2362
3744bF	G238-1833	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	F	1125	199	395	1786	1381
3744bF	G238-1869	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	F	1115	177	405	1399	1420
3744bH*	G239-0609	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	H*	2289	1891	613	4511	2246
3744bH*	G240-0608	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	H*	2304	1841	623	4147	2290
3745bF	G238-1934	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	F	1778	297	630	2902	2313
3745bF	G238-1970	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	F	1774	287	628	2219	2366
3745bH*	G239-0710	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	H*	2926	2368	763	5080	3112
3745bH*	G240-0709	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	H*	2751	2049	793	4702	3289
3828bF	G244-2657	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	F	1076	195	354	1392	1354
3828bF	G244-2665	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	F	1117	193	349	983	1356
3828bH*	G244-3989	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	H*	2631	2138	721	5367	2847
3828bH*	G244-3997	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	H*	2577	2153	683	4927	2870
3829bF	G244-2859	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	F	1505	171	397	833	1092
3829bF	G244-2867	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	F	1498	170	390	677	1111
3829bH*	G244-4191	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	H*	2743	1569	547	2005	1591
3829bH*	G244-4199	*Bithynia*	*tentaculata*	operculum	Barling Gravel, 05 <5> (18)	H*	2344	1403	475	1639	1465
3830bF	G244-2960	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	F	1260	201	434	2036	1570
3830bF	G244-2968	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	F	1275	198	420	1449	1593
3830bH*	G244-4292	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	H*	3070	2498	868	6637	3160
3830bH*	G244-42A0	*Bithynia*	*troscheli*	operculum	Barling Gravel, 05 <5> (18)	H*	3006	2496	777	5719	3128

Neaar	File	Genus	Species	materials	location	Type	Asx conc pmol/mg	Glx conc pmol/mg	Ser conc pmol/mg	Gly conc pmol/mg	Ala conc pmol/mg
3731bF	G238-0204	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	F	1231	156	393	1089	1195
3731bF	G238-0213	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	F	1236	156	394	1046	1190
3731bH*	G238-2340	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	H*	1394	969	452	1737	1459
3731bH*	G238-2350	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	H*	1108	779	449	1879	1474
3732bF	G238-1023	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	F	1800	242	490	1630	1519
3732bF	G238-1059	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	F	1854	238	489	1184	1493
3732bH*	G238-3276	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	H*	1909	1374	523	2243	1602
3732bH*	G238-3282	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	H*	1987	1393	520	1861	1567
3733bF	G238-1124	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	F	2200	306	712	1862	1740
3733bF	G238-1160	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	F	2244	291	638	1484	1703
3733bH*	G238-3377	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	H*	2402	1641	788	3018	1966
3733bH*	G238-3383	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	H*	2322	1434	782	2506	2011
3822bF	G244-1940	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	F	1582	167	418	1227	1399
3822bF	G244-1949	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	F	1638	163	426	1082	1421
3822bH*	G244-3373	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	H*	1438	752	585	2455	1890
3822bH*	G244-3381	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	H*	1562	783	564	2064	1878
3823bF	G244-2042	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	F	1867	223	466	1399	1379
3823bF	G244-2051	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	F	1891	220	473	1275	1393
3823bH*	G244-3475	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	H*	1529	973	386	1637	1129
3823bH*	G244-3483	*Bithynia*	*tentaculata*	operculum	Bradwell Hall, JCB bulk 13, MIS 11	H*	1424	858	370	1264	1133
3728bF	G238-0103	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	F	937	181	649	1086	993
3728bF	G238-0112	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	F	944	180	656	1007	991
3728bH*	G238-2239	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	H*	2424	1569	920	2597	1664
3728bH*	G238-2249	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	H*	2240	1326	936	2727	1779
3729bF	G238-0821	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	F	865	165	555	999	880
3729bF	G238-0838	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	F	852	158	539	918	869
3729bH*	G238-3074	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	H*	1844	1350	765	2260	1526
3729bH*	G238-3080	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	H*	1605	983	767	1802	1585
3730bF	G238-0922	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	F	996	180	624	924	911
3730bF	G238-0958	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	F	925	163	571	640	829

Neaar	File	Genus	Species	materials	location	Type	Asx conc pmol/mg	Glx conc pmol/mg	Ser conc pmol/mg	Gly conc pmol/mg	Ala conc pmol/mg
3730bH*	G238-3175	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	H*	3350	1998	1067	2891	1931
3730bH*	G238-3181	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	H*	3261	1893	1042	2375	1877
3820bF	G244-1738	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	F	1203	209	860	1342	1217
3820bF	G244-1746	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	F	1212	197	878	1231	1240
3820bH*	G244-3171	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	H*	2527	1731	904	2662	1679
3820bH*	G244-3179	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	H*	2556	1680	754	1980	1644
3821bF	G244-1839	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	F	830	157	565	940	885
3821bF	G244-1847	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	F	818	149	556	870	895
3821bH*	G244-3272	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	H*	2397	1628	883	2521	1590
3821bH*	G244-3280	*Bithynia*	*tentaculata*	operculum	East Mersea Restaurant Site, Sample 3	H*	2491	1641	492	1597	1545
3746bF	G238-0710	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	F	1689	223	493	1548	1421
3746bF	G238-0719	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	F	1756	229	506	1460	1475
3746bH*	G238-2846	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	H*	2418	1532	603	2430	1753
3746bH*	G238-2856	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	H*	2184	1251	615	2033	1800
3747bF	G238-2035	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	F	1856	267	584	1670	1466
3747bF	G238-2071	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	F	1830	243	526	1227	1430
3747bH*	G239-0811	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	H*	3446	2104	773	3396	2150
3747bH*	G240-0810	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	H*	3192	1709	803	2745	2241
3748bF	G238-2136	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	F	2493	336	651	2184	2080
3748bF	G238-2172	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	F	2518	333	666	1618	2095
3748bH*	G239-0912	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	H*	3749	2449	848	4080	2728
3748bH*	G240-0911	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	H*	3529	2055	870	3140	2840
3831bF	G244-3061	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	F	2383	291	690	1749	2107
3831bF	G244-3069	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	F	2342	275	662	1556	1917
3831bH*	G244-4393	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	H*	3966	2435	900	3662	2490
3831bH*	G244-43A1	*Bithynia*	*tentaculata*	operculum	Shoeburyness borehole, 13.9m	H*	3789	2389	845	3099	2466
3132bF	G206-0608	*Bithynia*	*tentaculata*	operculum	Shoeburyness Channel	F	2230	266	668	2140	1946
3132bF	G206-0615	*Bithynia*	*tentaculata*	operculum	Shoeburyness Channel	F	2232	264	671	2114	1958
3132bH*	G207-2118	*Bithynia*	*tentaculata*	operculum	Shoeburyness Channel	H*	3575	2290	901	4442	2648
3132bH*	G207-2135	*Bithynia*	*tentaculata*	operculum	Shoeburyness Channel	H*	3167	1853	897	3872	2783
3101bF	G205-0610	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	F	1729	193	475	1409	1382

Neaar	File	Genus	Species	materials	location	Type	Asx conc pmol/mg	Glx conc pmol/mg	Ser conc pmol/mg	Gly conc pmol/mg	Ala conc pmol/mg
3101bF	G205-0625	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	F	1752	196	479	1409	1376
3101bH*	G207-1411	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	H*	2107	1197	508	2072	1252
3101bH*	G207-1427	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	H*	2131	1176	508	1888	1273
3102bF	G205-2870	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	F	1633	170	462	1572	1349
3102bF	G205-2881	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	F	1680	176	471	1498	1371
3102bF	G205-2887	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	F	1717	172	490	1142	1617
3102bH*	G209-0807	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	H*	1157	764	531	2019	1109
3103bF	G205-2971	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	F	1474	171	383	1193	1174
3103bF	G205-2982	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	F	1480	172	382	1101	1159
3103bH*	G209-1614	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	H*	1755	1229	433	2632	1372
3103bH*	G209-1621	*Bithynia*	*tentaculata*	operculum	East Hyde, 7.55	H*	1783	1212	429	2529	1385
3734bF	G238-0305	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	F	672	109	432	1307	1619
3734bF	G238-0314	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	F	597	86	421	1019	1645
3734bH*	G238-2441	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	H*	5218	4986	3652	11980	4833
3734bH*	G238-2451	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	H*	4933	4162	3676	10375	4917
3735bF	G238-1225	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	F	1938	258	475	1423	1503
3735bF	G238-1261	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	F	2006	254	486	1103	1514
3735bH*	G238-3478	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	H*	2744	1746	573	2177	1876
3735bH*	G238-3484	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	H*	2709	1655	585	2160	1912
3736bF	G238-1327	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	F	1245	166	355	1286	1087
3736bF	G238-1363	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	F	1273	171	353	1032	1097
3736bH*	G239-0103	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	H*	2013	1318	615	2495	1505
3736bH*	G239-0116	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	H*	2151	1322	620	2272	1497
3824bF	G244-2243	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	F	1698	232	482	1707	1486
3824bF	G244-2252	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	F	1738	218	500	1619	1502
3824bH*	G244-3576	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	H*	2182	1523	527	2353	1627
3824bH*	G244-3584	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	H*	2463	1566	238	1296	1558
3825bF	G244-2344	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	F	1782	222	533	1620	1477
3825bF	G244-2353	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	F	1798	220	542	1539	1483
3825bH*	G244-3677	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	H*	2771	2075	1054	4715	3047
3825bH*	G244-3685	*Bithynia*	*tentaculata*	operculum	East Hyde borehole, 9.2m, MIS 11	H*	3123	1885	991	2317	3119

Neaar	File	material	shortened location	Val conc pmol/mg	Phe conc pmol/mg	Leu conc pmol/mg	Ile conc pmol/mg	Asx D/L	Glx D/L	Ser D/L	Ala D/L	Val D/L	[Ser]/ [Ala]
3737bF	G238-0406	B. tent op	Apton Hall Farm	556	184	607	245	0.74	0.38	0.90	0.45	0.27	0.33
3737bF	G238-0415	B. tent op	Apton Hall Farm	559	174	580	231	0.74	0.38	0.95	0.46	0.26	0.34
3737bH*	G238-2543	B. tent op	Apton Hall Farm	1090	425	1187	441	0.65	0.27	0.69	0.38	0.20	0.34
3737bH*	G238-2553	B. tent op	Apton Hall Farm	1091	416	1214	455	0.63	0.28	0.68	0.38	0.20	0.34
3738bF	G238-1428	B. tent op	Apton Hall Farm	663	208	650	211	0.73	0.37	0.93	0.46	0.25	0.43
3738bF	G238-1464	B. tent op	Apton Hall Farm	662	200	669	239	0.73	0.36	0.92	0.45	0.24	0.43
3738bH*	G239-0204	B. tent op	Apton Hall Farm	1078	271	1131	372	0.63	0.27	0.68	0.37	0.20	0.43
3738bH*	G240-0203	B. tent op	Apton Hall Farm	1189	367	1132	406	0.63	0.28	0.68	0.37	0.20	0.42
3739bF	G238-1529	B. tent op	Apton Hall Farm	607	190	607	208	0.73	0.36	0.97	0.43	0.24	0.45
3739bF	G238-1565	B. tent op	Apton Hall Farm	635	170	633	229	0.73	0.35	0.94	0.42	0.23	0.44
3739bH*	G239-0305	B. tent op	Apton Hall Farm	1179	432	1249	434	0.63	0.26	0.69	0.36	0.18	0.41
3739bH*	G240-0304	B. tent op	Apton Hall Farm	1538	457	1516	547	0.63	0.26	0.69	0.36	0.18	0.39
3826bF	G244-2455	B. tent op	Apton Hall Farm	677	248	839	327	0.75	0.38	1.00	0.45	0.25	0.40
3826bF	G244-2463	B. tent op	Apton Hall Farm	660	234	809	337	0.76	0.39	0.98	0.45	0.26	0.37
3826bH*	G244-3787	B. tent op	Apton Hall Farm	926	358	1096	401	0.65	0.26	0.68	0.37	0.18	0.36
3826bH*	G244-3795	B. tent op	Apton Hall Farm	1021	395	1191	432	0.65	0.28	0.66	0.37	0.19	0.35
3827bF	G244-2556	B. tent op	Apton Hall Farm	694	214	753	261	0.74	0.37	1.00	0.44	0.25	0.45
3827bF	G244-2564	B. tent op	Apton Hall Farm	666	198	715	276	0.74	0.37	0.99	0.45	0.27	0.43
3827bH*	G244-3888	B. tent op	Apton Hall Farm	1241	446	1355	484	0.63	0.26	0.69	0.36	0.19	0.40
3827bH*	G244-3896	B. tent op	Apton Hall Farm	1258	454	1344	483	0.63	0.26	0.68	0.36	0.18	0.36
3740bF	G238-0508	B. tent op	Barling Gravel	558	218	631	207	0.73	0.36	0.98	0.44	0.25	0.39
3740bF	G238-0517	B. tent op	Barling Gravel	556	203	620	205	0.73	0.37	0.96	0.45	0.24	0.38
3740bH*	G238-2644	B. tent op	Barling Gravel	1324	536	1530	607	0.59	0.24	0.62	0.32	0.15	0.36
3740bH*	G238-2654	B. tent op	Barling Gravel	1191	461	1372	564	0.58	0.23	0.62	0.32	0.15	0.35
3741bF	G238-1630	B. tent op	Barling Gravel	625	206	591	220	0.72	0.36	1.00	0.43	0.24	0.42
3741bF	G238-1666	B. tent op	Barling Gravel	639	204	637	246	0.71	0.36	0.98	0.43	0.24	0.42

Neaar	File	material	shortened location	Val conc pmol/mg	Phe conc pmol/mg	Leu conc pmol/mg	Ile conc pmol/mg	Asx D/L	Glx D/L	Ser D/L	Ala D/L	Val D/L	[Ser]/ [Ala]
3741bH*	G239-0406	B. tent op	Barling Gravel	1151	416	1148	415	0.63	0.27	0.73	0.36	0.20	0.46
3741bH*	G240-0405	B. tent op	Barling Gravel	1273	350	1170	429	0.64	0.27	0.72	0.36	0.18	0.42
3742bF	G238-1731	B. tent op	Barling Gravel	626	230	638	222	0.73	0.33	1.00	0.42	0.23	0.37
3742bF	G238-1767	B. tent op	Barling Gravel	619	217	646	242	0.72	0.33	0.99	0.42	0.22	0.37
3742bH*	G239-0507	B. tent op	Barling Gravel	1142	469	1191	438	0.64	0.26	0.71	0.36	0.19	0.36
3742bH*	G240-0506	B. tent op	Barling Gravel	1184	442	1185	452	0.64	0.26	0.72	0.36	0.19	0.36
3743bF	G238-0609	B. tros op	Barling Gravel	470	215	377	229	0.76	0.33	0.85	0.40	0.28	0.29
3743bF	G238-0618	B. tros op	Barling Gravel	467	163	384	213	0.76	0.35	0.80	0.40	0.22	0.28
3743bH*	G238-2745	B. tros op	Barling Gravel	1117	491	1071	407	0.71	0.25	0.80	0.36	0.19	0.25
3743bH*	G238-2755	B. tros op	Barling Gravel	1149	475	1129	433	0.71	0.25	0.82	0.36	0.19	0.26
3744bF	G238-1833	B. tros op	Barling Gravel	605	248	651	226	0.77	0.31	0.99	0.40	0.24	0.29
3744bF	G238-1869	B. tros op	Barling Gravel	626	227	652	231	0.76	0.31	0.97	0.40	0.23	0.29
3744bH*	G239-0609	B. tros op	Barling Gravel	1228	559	1404	519	0.67	0.22	0.82	0.34	0.18	0.27
3744bH*	G240-0608	B. tros op	Barling Gravel	1268	535	1427	529	0.67	0.22	0.82	0.34	0.18	0.27
3745bF	G238-1934	B. tros op	Barling Gravel	865	382	895	311	0.78	0.35	1.01	0.44	0.24	0.27
3745bF	G238-1970	B. tros op	Barling Gravel	887	364	913	330	0.77	0.34	0.99	0.44	0.24	0.27
3745bH*	G239-0710	B. tros op	Barling Gravel	1466	725	1524	543	0.69	0.25	0.77	0.37	0.20	0.25
3745bH*	G240-0709	B. tros op	Barling Gravel	1539	675	1583	562	0.69	0.26	0.71	0.38	0.20	0.24
3828bF	G244-2657	B. tent op	Barling Gravel	646	237	754	307	0.75	0.36	0.99	0.40	0.25	0.26
3828bF	G244-2665	B. tent op	Barling Gravel	670	232	702	319	0.76	0.37	0.95	0.41	0.26	0.26
3828bH*	G244-3989	B. tent op	Barling Gravel	1469	635	1414	529	0.72	0.26	0.83	0.37	0.21	0.25
3828bH*	G244-3997	B. tent op	Barling Gravel	1452	633	1431	518	0.71	0.26	0.79	0.37	0.20	0.24
3829bF	G244-2859	B. tent op	Barling Gravel	506	146	560	205	0.71	0.37	0.96	0.43	0.24	0.36
3829bF	G244-2867	B. tent op	Barling Gravel	518	146	592	227	0.70	0.38	0.96	0.42	0.23	0.35
3829bH*	G244-4191	B. tent op	Barling Gravel	1032	321	1074	409	0.63	0.26	0.66	0.36	0.17	0.34
3829bH*	G244-4199	B. tent op	Barling Gravel	957	283	962	380	0.63	0.26	0.66	0.37	0.18	0.32
3830bF	G244-2960	B. tros op	Barling Gravel	695	269	775	327	0.77	0.31	1.00	0.41	0.24	0.28
3830bF	G244-2968	B. tros op	Barling Gravel	725	270	829	363	0.76	0.31	0.98	0.41	0.24	0.26
3830bH*	G244-4292	B. tros op	Barling Gravel	1630	730	1724	604	0.69	0.22	0.79	0.35	0.19	0.27
3830bH*	G244-42A0	B. tros op	Barling Gravel	1621	718	1708	613	0.70	0.22	0.79	0.35	0.19	0.25

Neaar	File	material	shortened location	Val conc pmol/mg	Phe conc pmol/mg	Leu conc pmol/mg	Ile conc pmol/mg	Asx D/L	Glx D/L	Ser D/L	Ala D/L	Val D/L	[Ser]/ [Ala]
3731bF	G238-0204	B. tent op	Bradwell Hall	536	184	583	203	0.75	0.44	0.98	0.46	0.27	0.33
3731bF	G238-0213	B. tent op	Bradwell Hall	542	184	583	208	0.75	0.43	0.98	0.46	0.26	0.33
3731bH*	G238-2340	B. tent op	Bradwell Hall	761	293	834	300	0.69	0.32	0.79	0.43	0.23	0.31
3731bH*	G238-2350	B. tent op	Bradwell Hall	772	292	868	320	0.68	0.32	0.77	0.43	0.24	0.30
3732bF	G238-1023	B. tent op	Bradwell Hall	649	248	679	246	0.75	0.37	0.96	0.47	0.26	0.32
3732bF	G238-1059	B. tent op	Bradwell Hall	673	242	700	275	0.75	0.37	0.97	0.46	0.27	0.33
3732bH*	G238-3276	B. tent op	Bradwell Hall	880	341	932	376	0.66	0.29	0.65	0.42	0.22	0.33
3732bH*	G238-3282	B. tent op	Bradwell Hall	877	343	929	384	0.68	0.29	0.67	0.41	0.22	0.33
3733bF	G238-1124	B. tent op	Bradwell Hall	753	263	760	285	0.74	0.36	0.78	0.46	0.26	0.41
3733bF	G238-1160	B. tent op	Bradwell Hall	754	238	767	283	0.75	0.38	0.93	0.47	0.27	0.37
3733bH*	G238-3377	B. tent op	Bradwell Hall	1062	393	1149	436	0.65	0.29	0.53	0.41	0.22	0.40
3733bH*	G238-3383	B. tent op	Bradwell Hall	1091	382	1116	430	0.65	0.29	0.54	0.41	0.21	0.39
3822bF	G244-1940	B. tent op	Bradwell Hall	588	192	741	252	0.78	0.44	0.98	0.52	0.28	0.30
3822bF	G244-1949	B. tent op	Bradwell Hall	619	197	752	273	0.78	0.45	0.97	0.51	0.29	0.30
3822bH*	G244-3373	B. tent op	Bradwell Hall	928	369	1101	361	0.68	0.33	0.71	0.47	0.24	0.31
3822bH*	G244-3381	B. tent op	Bradwell Hall	964	377	1117	373	0.68	0.33	0.70	0.47	0.24	0.30
3823bF	G244-2042	B. tent op	Bradwell Hall	611	217	743	352	0.76	0.39	0.93	0.48	0.30	0.34
3823bF	G244-2051	B. tent op	Bradwell Hall	593	209	872	408	0.76	0.40	0.96	0.49	0.29	0.34
3823bH*	G244-3475	B. tent op	Bradwell Hall	667	246	741	314	0.66	0.31	0.65	0.43	0.21	0.34
3823bH*	G244-3483	B. tent op	Bradwell Hall	695	242	742	299	0.66	0.31	0.67	0.42	0.21	0.33
3728bF	G238-0103	B. tent op	East Mersea RS	400	161	405	140	0.60	0.23	0.86	0.23	0.13	0.65
3728bF	G238-0112	B. tent op	East Mersea RS	406	155	408	149	0.60	0.23	0.86	0.23	0.13	0.66
3728bH*	G238-2239	B. tent op	East Mersea RS	1024	412	1105	431	0.50	0.14	0.54	0.17	0.08	0.55
3728bH*	G238-2249	B. tent op	East Mersea RS	1052	415	1156	450	0.49	0.14	0.52	0.19	0.09	0.53
3729bF	G238-0821	B. tent op	East Mersea RS	354	139	382	125	0.63	0.24	0.90	0.25	0.14	0.63
3729bF	G238-0838	B. tent op	East Mersea RS	350	130	371	123	0.63	0.24	0.88	0.25	0.13	0.62
3729bH*	G238-3074	B. tent op	East Mersea RS	944	452	1082	443	0.51	0.15	0.51	0.19	0.09	0.50
3729bH*	G238-3080	B. tent op	East Mersea RS	1004	410	1059	461	0.52	0.15	0.50	0.18	0.08	0.48
3730bF	G238-0922	B. tent op	East Mersea RS	391	117	388	125	0.58	0.24	0.86	0.22	0.12	0.69
3730bF	G238-0958	B. tent op	East Mersea RS	365	104	368	130	0.58	0.23	0.87	0.23	0.12	0.69

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3730bH*	G238-3175	B. tent op	East Mersea RS	1311	480	1443	578	0.50	0.14	0.53	0.16	0.07	0.55
3730bH*	G238-3181	B. tent op	East Mersea RS	1296	452	1408	569	0.49	0.14	0.53	0.16	0.08	0.56
3820bF	G244-1738	B. tent op	East Mersea RS	457	202	519	240	0.59	0.23	0.87	0.23	0.14	0.71
3820bF	G244-1746	B. tent op	East Mersea RS	460	195	515	243	0.61	0.25	0.89	0.24	0.14	0.71
3820bH*	G244-3171	B. tent op	East Mersea RS	1159	451	1260	531	0.51	0.15	0.53	0.17	0.09	0.54
3820bH*	G244-3179	B. tent op	East Mersea RS	1162	447	1231	525	0.51	0.15	0.54	0.17	0.08	0.46
3821bF	G244-1839	B. tent op	East Mersea RS	394	140	479	276	0.58	0.23	0.84	0.22	0.13	0.64
3821bF	G244-1847	B. tent op	East Mersea RS	387	134	572	287	0.59	0.25	0.82	0.23	0.15	0.62
3821bH*	G244-3272	B. tent op	East Mersea RS	1087	439	1215	509	0.52	0.15	0.49	0.17	0.09	0.56
3821bH*	G244-3280	B. tent op	East Mersea RS	1166	464	1264	552	0.51	0.15	0.56	0.17	0.08	0.32
3746bF	G238-0710	B. tent op	Shoeburyness borehole	530	211	598	212	0.77	0.36	0.89	0.50	0.28	0.35
3746bF	G238-0719	B. tent op	Shoeburyness borehole	545	215	613	230	0.77	0.38	0.90	0.50	0.27	0.34
3746bH*	G238-2846	B. tent op	Shoeburyness borehole	980	421	1133	403	0.63	0.26	0.63	0.39	0.19	0.34
3746bH*	G238-2856	B. tent op	Shoeburyness borehole	1039	379	1171	419	0.63	0.26	0.61	0.39	0.19	0.34
3747bF	G238-2035	B. tent op	Shoeburyness borehole	574	226	691	210	0.76	0.32	0.72	0.49	0.28	0.40
3747bF	G238-2071	B. tent op	Shoeburyness borehole	575	211	691	229	0.76	0.36	0.85	0.50	0.26	0.37
3747bH*	G239-0811	B. tent op	Shoeburyness borehole	1151	488	1405	446	0.65	0.27	0.69	0.40	0.21	0.36
3747bH*	G240-0810	B. tent op	Shoeburyness borehole	1211	458	1408	471	0.65	0.27	0.69	0.41	0.21	0.36
3748bF	G238-2136	B. tent op	Shoeburyness borehole	792	314	870	291	0.77	0.37	0.96	0.49	0.26	0.31
3748bF	G238-2172	B. tent op	Shoeburyness borehole	810	300	901	327	0.77	0.36	0.94	0.49	0.26	0.32
3748bH*	G239-0912	B. tent op	Shoeburyness borehole	1383	581	1592	537	0.66	0.27	0.74	0.40	0.21	0.31
3748bH*	G240-0911	B. tent op	Shoeburyness borehole	1459	360	1596	561	0.66	0.27	0.73	0.40	0.21	0.31
3831bF	G244-3061	B. tent op	Shoeburyness borehole	699	280	966	432	0.75	0.35	0.95	0.50	0.30	0.33
3831bF	G244-3069	B. tent op	Shoeburyness borehole	705	264	919	429	0.76	0.35	0.96	0.48	0.30	0.35
3831bH*	G244-4393	B. tent op	Shoeburyness borehole	1352	549	1629	538	0.63	0.26	0.66	0.39	0.19	0.36
3831bH*	G244-43A1	B. tent op	Shoeburyness borehole	1369	546	1625	540	0.64	0.26	0.65	0.39	0.20	0.34
3132bF	G206-0608	B. tent op	Shoeburyness Channel	754	296	960	270	0.77	0.24	1.00	0.49	0.29	0.34
3132bF	G206-0615	B. tent op	Shoeburyness Channel	750	293	945	268	0.77	0.24	1.01	0.48	0.29	0.34
3132bH*	G207-2118	B. tent op	Shoeburyness Channel	1524	581	1825	613	0.63	0.24	0.65	0.38	0.20	0.34
3132bH*	G207-2135	B. tent op	Shoeburyness Channel	1647	576	1976	669	0.64	0.24	0.62	0.37	0.21	0.32
3101bF	G205-0610	B. tent op	East Hyde, 7.55	641	207	716	238	0.77	0.27	1.02	0.47	0.28	0.34

Neaar	File	material	shortened location	Val conc pmol/mg	Phe conc pmol/mg	Leu conc pmol/mg	Ile conc pmol/mg	Asx D/L	Glx D/L	Ser D/L	Ala D/L	Val D/L	[Ser]/ [Ala]
3101bF	G205-0625	B. tent op	East Hyde, 7.55	648	209	757	244	0.77	0.27	1.02	0.47	0.27	0.35
3101bH*	G207-1411	B. tent op	East Hyde, 7.55	762	256	863	295	0.66	0.24	0.69	0.39	0.20	0.41
3101bH*	G207-1427	B. tent op	East Hyde, 7.55	777	251	833	310	0.66	0.24	0.68	0.39	0.20	0.40
3102bF	G205-2870	B. tent op	East Hyde, 7.55	546	209	602	201	0.76	0.23	0.95	0.47	0.28	0.34
3102bF	G205-2881	B. tent op	East Hyde, 7.55	559	214	741	207	0.76	0.23	0.98	0.46	0.27	0.34
3102bF	G205-2887	B. tent op	East Hyde, 7.55	601	219	618	233	0.76	0.22	0.94	0.50	0.28	0.30
3102bH*	G209-0807	B. tent op	East Hyde, 7.55	570	242	664	255	0.62	0.22	0.38	0.38	0.21	0.48
3103bF	G205-2971	B. tent op	East Hyde, 7.55	541	159	617	201	0.75	0.26	1.03	0.47	0.29	0.33
3103bF	G205-2982	B. tent op	East Hyde, 7.55	543	160	651	206	0.76	0.27	1.03	0.47	0.29	0.33
3103bH*	G209-1614	B. tent op	East Hyde, 7.55	694	354	931	315	0.66	0.24	0.69	0.40	0.21	0.32
3103bH*	G209-1621	B. tent op	East Hyde, 7.55	692	313	917	298	0.66	0.24	0.69	0.39	0.21	0.31
3734bF	G238-0305	B. tent op	East Hyde BH, 9.2m	690	291	920	350	0.74	0.49	0.90	0.52	0.32	0.27
3734bF	G238-0314	B. tent op	East Hyde BH, 9.2m	724	249	903	340	0.77	0.54	0.90	0.52	0.30	0.26
3734bH*	G238-2441	B. tent op	East Hyde BH, 9.2m	2723	1776	4143	1692	0.47	0.17	0.13	0.34	0.16	0.76
3734bH*	G238-2451	B. tent op	East Hyde BH, 9.2m	2788	1718	4251	1788	0.48	0.17	0.13	0.34	0.16	0.75
3735bF	G238-1225	B. tent op	East Hyde BH, 9.2m	584	213	625	209	0.73	0.35	0.89	0.51	0.27	0.32
3735bF	G238-1261	B. tent op	East Hyde BH, 9.2m	594	206	648	224	0.75	0.33	0.91	0.51	0.27	0.32
3735bH*	G238-3478	B. tent op	East Hyde BH, 9.2m	1155	433	1387	497	0.63	0.25	0.63	0.41	0.20	0.31
3735bH*	G238-3484	B. tent op	East Hyde BH, 9.2m	1163	423	1318	499	0.63	0.25	0.65	0.40	0.20	0.31
3736bF	G238-1327	B. tent op	East Hyde BH, 9.2m	449	174	529	174	0.76	0.37	0.94	0.47	0.25	0.33
3736bF	G238-1363	B. tent op	East Hyde BH, 9.2m	473	187	557	213	0.76	0.36	0.93	0.47	0.27	0.32
3736bH*	G239-0103	B. tent op	East Hyde BH, 9.2m	851	346	987	357	0.65	0.27	0.45	0.39	0.21	0.41
3736bH*	G239-0116	B. tent op	East Hyde BH, 9.2m	862	343	984	379	0.65	0.27	0.45	0.39	0.20	0.41
3824bF	G244-2243	B. tent op	East Hyde BH, 9.2m	679	351	933	752	0.74	0.34	0.85	0.45	0.31	0.32
3824bF	G244-2252	B. tent op	East Hyde BH, 9.2m	689	301	941	725	0.75	0.34	0.83	0.46	0.28	0.33
3824bH*	G244-3576	B. tent op	East Hyde BH, 9.2m	1028	430	1253	504	0.65	0.25	0.59	0.38	0.18	0.32
3824bH*	G244-3584	B. tent op	East Hyde BH, 9.2m	1176	460	1311	544	0.63	0.24	0.54	0.37	0.18	0.15
3825bF	G244-2344	B. tent op	East Hyde BH, 9.2m	629	282	1031	537	0.74	0.34	0.92	0.46	0.29	0.36
3825bF	G244-2353	B. tent op	East Hyde BH, 9.2m	620	249	1043	499	0.73	0.34	0.91	0.46	0.25	0.37
3825bH*	G244-3677	B. tent op	East Hyde BH, 9.2m	1647	795	2193	761	0.64	0.25	0.66	0.39	0.19	0.35
3825bH*	G244-3685	B. tent op	East Hyde BH, 9.2m	2153	905	2501	951	0.64	0.25	0.62	0.38	0.19	0.32

Neaar	File	material	shortened location	Val conc pmol/mg	Phe conc pmol/mg	Leu conc pmol/mg	Ile conc pmol/mg	Asx D/L	Glx D/L	Ser D/L	Ala D/L	Val D/L	[Ser]/ [Ala]	DMKv2
3101bF	G205-0625	B. tent op	East Hyde, 7.55	648	209	757	244	0.77	0.27	1.02	0.47	0.27	0.35	0.40
3101bH*	G207-1411	B. tent op	East Hyde, 7.55	762	256	863	295	0.66	0.24	0.69	0.39	0.20	0.41	0.30
3101bH*	G207-1427	B. tent op	East Hyde, 7.55	777	251	833	310	0.66	0.24	0.68	0.39	0.20	0.40	0.30
3102bF	G205-2870	B. tent op	East Hyde, 7.55	546	209	602	201	0.76	0.23	0.95	0.47	0.28	0.34	0.39
3102bF	G205-2881	B. tent op	East Hyde, 7.55	559	214	741	207	0.76	0.23	0.98	0.46	0.27	0.34	0.38
3102bF	G205-2887	B. tent op	East Hyde, 7.55	601	219	618	233	0.76	0.22	0.94	0.50	0.28	0.30	0.39
3102bH*	G209-0807	B. tent op	East Hyde, 7.55	570	242	664	255	0.62	0.22	0.38	0.38	0.21	0.48	0.28
3103bF	G205-2971	B. tent op	East Hyde, 7.55	541	159	617	201	0.75	0.26	1.03	0.47	0.29	0.33	0.40
3103bF	G205-2982	B. tent op	East Hyde, 7.55	543	160	651	206	0.76	0.27	1.03	0.47	0.29	0.33	0.40
3103bH*	G209-1614	B. tent op	East Hyde, 7.55	694	354	931	315	0.66	0.24	0.69	0.40	0.21	0.32	0.31
3103bH*	G209-1621	B. tent op	East Hyde, 7.55	692	313	917	298	0.66	0.24	0.69	0.39	0.21	0.31	0.31
3734bF	G238-0305	B. tent op	East Hyde BH, 9.2m	690	291	920	350	0.74	0.49	0.90	0.52	0.32	0.27	0.46
3734bF	G238-0314	B. tent op	East Hyde BH, 9.2m	724	249	903	340	0.77	0.54	0.90	0.52	0.30	0.26	0.48
3734bH*	G238-2441	B. tent op	East Hyde BH, 9.2m	2723	1776	4143	1692	0.47	0.17	0.13	0.34	0.16	0.76	0.21
3734bH*	G238-2451	B. tent op	East Hyde BH, 9.2m	2788	1718	4251	1788	0.48	0.17	0.13	0.34	0.16	0.75	0.21
3735bF	G238-1225	B. tent op	East Hyde BH, 9.2m	584	213	625	209	0.73	0.35	0.89	0.51	0.27	0.32	0.41
3735bF	G238-1261	B. tent op	East Hyde BH, 9.2m	594	206	648	224	0.75	0.33	0.91	0.51	0.27	0.32	0.41
3735bH*	G238-3478	B. tent op	East Hyde BH, 9.2m	1155	433	1387	497	0.63	0.25	0.63	0.41	0.20	0.31	0.30
3735bH*	G238-3484	B. tent op	East Hyde BH, 9.2m	1163	423	1318	499	0.63	0.25	0.65	0.40	0.20	0.31	0.30
3736bF	G238-1327	B. tent op	East Hyde BH, 9.2m	449	174	529	174	0.76	0.37	0.94	0.47	0.25	0.33	0.41
3736bF	G238-1363	B. tent op	East Hyde BH, 9.2m	473	187	557	213	0.76	0.36	0.93	0.47	0.27	0.32	0.42
3736bH*	G239-0103	B. tent op	East Hyde BH, 9.2m	851	346	987	357	0.65	0.27	0.45	0.39	0.21	0.41	0.31
3736bH*	G239-0116	B. tent op	East Hyde BH, 9.2m	862	343	984	379	0.65	0.27	0.45	0.39	0.20	0.41	0.31
3824bF	G244-2243	B. tent op	East Hyde BH, 9.2m	679	351	933	752	0.74	0.34	0.85	0.45	0.31	0.32	0.41
3824bF	G244-2252	B. tent op	East Hyde BH, 9.2m	689	301	941	725	0.75	0.34	0.83	0.46	0.28	0.33	0.41
3824bH*	G244-3576	B. tent op	East Hyde BH, 9.2m	1028	430	1253	504	0.65	0.25	0.59	0.38	0.18	0.32	0.29
3824bH*	G244-3584	B. tent op	East Hyde BH, 9.2m	1176	460	1311	544	0.63	0.24	0.54	0.37	0.18	0.15	0.28
3825bF	G244-2344	B. tent op	East Hyde BH, 9.2m	629	282	1031	537	0.74	0.34	0.92	0.46	0.29	0.36	0.41
3825bF	G244-2353	B. tent op	East Hyde BH, 9.2m	620	249	1043	499	0.73	0.34	0.91	0.46	0.25	0.37	0.39
3825bH*	G244-3677	B. tent op	East Hyde BH, 9.2m	1647	795	2193	761	0.64	0.25	0.66	0.39	0.19	0.35	0.29
3825bH*	G244-3685	B. tent op	East Hyde BH, 9.2m	2153	905	2501	951	0.64	0.25	0.62	0.38	0.19	0.32	0.29

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