BRITISH BEEF ORIGIN PROJECT II – Improvement of the British Beef Isotope Landscape Map (Isoscape) for Scotland and Northern Ireland

FS515009: Final report
October 2016




By: Dr James Donarski, Katharina Heinrich (Fera) with contributions from QUB and JHI

Contents

Glossary

ArcGIS = Aeronautical Reconnaissance Coverage Geographic Information System (software package)

BBOP = British Beef Origin Project

BSE = Bovine spongiform encephalopathy

C ratio = $^{13}C/^{12}C$ ratio = $\delta^{13}C$ values expressed in [‰]

CJD = Creutzfeldt-Jakob Disease

CNRC = Conseil National de Recherches Canada (National Research Council Canada)

COOL = Country of Origin labelling/ Labels

CRM = Certified Reference Material

cv = Coefficient of Variation

EA = Elemental Analyser

Fera = The Food and Environment Research Agency, which became Fera Science Ltd from 01/04/2015

FT-IR = Fourier Transform Infra-Red (spectroscopy)

H ratio = $^{2}H/^{1}H$ ratio = $\delta^{2}H$ values expressed in [‰]

H_2O_2 = Hydrogen Peroxide

HCNS ratio = Analysis of hydrogen, carbon, nitrogen and sulfur stable isotope ratios

HNO_3 = Nitric Acid

HT = High Temperature

IAEA =International Atomic Energy Agency

IGFS = Institute for Global Food Security (department of QUB)

IHS = in-house standard

IRMS = Isotope Ratio Mass Spectrometer

JHI = The James Hutton Institute

LIMS = Laboratory Information Management System

MC-ICP-MS = Multi Collector Inductive Coupled Plasma Mass spectrometer

N ratio = $^{15}N/^{14}N$ ratio = $\delta^{15}N$ values expressed in [‰]

NIST = National Institute of Standards and Technology

Pb = Lead

Pb ratios = $^{206}Pb/^{207}Pb$, $^{208}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$

PDO = Protected Designation of Origin

PGI = Protected Geographical Indication

PPM = Parts per Million [mg kg^{-1}]

QUB = Queen's University Belfast

RM = Reference Material

S ratio = $^{34}S/^{32}S$ ratio = $\delta^{34}S$ values expressed in [‰]

SBOP = Scottish Beef Origin Project (FS515009), also referred to as BBOP II by JHI

SD = standard deviation

SE = Standard Error

Sn = Tin

Sr = Strontium

Sr ratio = $^{87}Sr/^{86}Sr$

TIMS = Thermal Ionisation Mass Spectrometer

Executive Summary

1. Scotch beef is a 'protected geographical indication' (PGI) product, and to be labelled Scotch beef, beef must be sourced from specified Scottish farms, where cattle have been raised using defined farming practices and production methods. Scotch Beef is known to attract a market premium and is likely to be a candidate for fraudulent substitution. This project was undertaken to provide an effective method of deterrence, by developing procedures to detect such fraud and reassure consumers about the authenticity of Scotch beef.

2. The project is based on the concept that information on the geographical origin of a sample can be obtained through the analysis of the stable isotopic composition of that sample and linking it to the environment where it was grown/reared. Many chemical elements (e.g. carbon, nitrogen, sulfur) have different stable forms (stable isotopes) due to differences in their atomic structure. The ratio of these isotopes in a food can be influenced by factors in the local environment and can therefore form the basis for determining geographical origin.

3. A stable isotope map of beef cattle from the United Kingdom has been previously reported, which was able to provide some discrimination between Scotch and non-Scotch beef [FA0205 and Defra Seedcorn]. This present project has augmented that initial data with further stable isotope measurements, taken from Scottish and Northern Irish beef (in total 299 beef samples), to improve the coverage of the stable isotope map and to assess seasonal influences on that map. It also used data (n = 200) from FA0152 to include sampling locations from East Anglia, the Midlands, the South and South East of England.

4. The effect of seasonality for carbon and hydrogen isotopes was demonstrated to be minor in relation to the natural variation of the samples.

5. Using this increased dataset, an internet based web-tool has been generated, which is able to take a stable isotope profile from a beef sample and predict, with known confidence, whether the measured isotope profile is consistent with any UK 2-letter postcode region.

6. The performance of the web-tool was tested by sending 15 blind (geographical origin information withheld) beef samples to Fera Science Ltd for analysis and subsequent geographical origin prediction using the web-tool. All 15 samples were assigned 2-letter postcode regions that were consistent with their actual postcode. It was noted though, that the isotopic profiles of several samples were also consistent with significant proportions of the

United Kingdom, i.e. the web-tool showed that the sample could have originated from a large proportion of the United Kingdom. Consequently, the web-tool was updated to provide two separate options for classification, using different confidence settings of ≥95% and 80%.The ≥95% setting provides higher confidence in the results and the 80% setting provides higher discrimination.

7. The web-tool was tested using stable isotope profiles from a limited number of non-UK samples from Ireland, Germany, Brazil, Australia, and New Zealand. Using the current web-tool, an Irish beef sample could not be differentiated from Scotch beef, a German beef sample could be differentiated from parts of Scotland; whereas the web-tool successfully differentiated Brazilian, Australian and New Zealand samples from Scotch beef. Considering that the majority of global production of beef is from the Americas (45% of Global production, 2013, FAOSTAT) the current web-tool can offer significant protection from the mis-labelling of beef as Scotch.

8. Recommended future work is to apply the web-tool for the analysis of Scotch beef to identify potential fraudulent claims. The experimental design of such a survey should be considered carefully to maximise the ability to detect fraudulent samples. It should also include known authentic Scotch beef and known authentic non-Scotch beef. This will ensure that the tool remains fit for purpose and will determine the power of the tool to differentiate Scotch beef from other beef on a global scale. For example, further analysis of samples from the Americas, Asia and from different European locations is recommended.

9. Following the decisions of the United States and Canada to lift the import ban of Scotch beef, it is also recommended that beef, labelled as Scotch and sold in other countries, is tested to ascertain whether fraudulent labelling is occurring. If inferior products are fraudulently labelled as Scotch beef this may undermine aspects of the Export Plan for Scotland's food and drink industry. Currently, the isotope tool is not able to reliably differentiate Scotch beef from specific parts of the UK, this is due to the limited variation associated with the isotopic signatures between these regions of the UK. Further discrimination may be possible, through the use of multiple sampling (analysis of several separate samples from a single specific location), although this aspect requires further research to fully characterise what increase in discrimination this would achieve.

10. The stable isotope ratio analysis data set for Scotch beef has been updated and incorporated into a UK based web-tool, which is able to (i) confirm the origin of Scotch beef and (ii) detect potential food fraud in geographical mislabelling of Scotch beef. The webtool is available for use by Food Standards Scotland, Defra and Fera Science Ltd.

1. Introduction

1.1 General background on geographical origin claims

1.1.1 Social and economic context

The European Union Protected Food Names Schemes came into force in 1992 (Council Regulation (EEC) No 2081/92, 1992) and offers an independent inspection and labelling system for the protection of food names on a geographical basis, comparable to the French system 'Appellation d'Origine Contrôlee´ (AOC)' used for wine. There are three schemes; Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Certificate of Specific Character (CSC) (also known as Traditional Speciality Guaranteed). PDO is the term used to describe foodstuffs, with a strong regional identity, that are produced, processed and prepared in a specific geographical area using prescribed techniques that may be unique to that region, e.g. Orkney beef and Shetland beef. Foods with PGI status must have a geographical link in at least one of the stages of production, processing or preparation, e.g. Scotch beef and Welsh beef. A CSC does not refer to a specific geographical origin, but defines traditional character, either in terms of production techniques or composition.

Manufacturers of protected foods usually charge a premium for their produce due to higher production costs, and consequently economic incentives exist to replace genuine articles with inferior ones for financial gain.

As a result of concerns relating to BSE (Bovine spongiform encephalopathy), Human variant CJD (Creutzfeldt-Jakob Disease) and the impact on the internal market for Beef, the EU introduced far reaching legislation concerning labelling of beef. The first of two stages of legislation was introduced on the 1st September 2000 (Regulation (EC) No 1760/2000 of the European Parliament) and initiated a system to provide correct, complete and transparent information designed to enable consumers to make an informed choice about retail beef in the marketplace and further designed to enable enforcement agencies to trace back retail beef to where it originated.

Required labelling information included:

(a) A traceability reference number or code

(b) The name of the Member State or non-EC country in which the animal or group of animals were born

(c) The name of the Member State or non-EC country in which the animal or group of animals were raised

(d) 'Slaughtered in: [name of Member State or non-EC country]'

(e) Licence number of slaughterhouse

(f) ‘Cutting/cut in: [name of Member State or non-EC country]’

(g) Licence number(s) of cutting plant(s)

The second stage came into force on the 1st January, 2002 and required the inclusion of additional precise information relating to where the source animal of retail beef had been born and reared.

The introduction of compulsory beef labelling legislation means there is a requirement for Enforcement Agencies, such as Customs and Trading Standards, to have access to reliable analytical methods that can be used to verify origin labels on beef whether they are part of the Protected Food Names Scheme, subsidy claims or simply ‘suspect’ imported produce.

1.1.2 Analytical methods of choice for geographical origin claims

The transfer of isotope signals from the bio-elements (H, C, N, O, S) present in local feed and water to animal and plant tissue is understood and forms the basis of the approach.

Similarly, the geochemical and environmental isotope signature of a particular region can be traced through the use of strontium (and sulfur to a lesser extent) and lead isotope analysis.

A fundamental part of this approach to determine the geographical origin of a suspect food sample, in an objective way, is the statistical comparison of a sample’s stable isotopic composition with a database of samples for which the geographical origin is known.

As for all analytical techniques, the method does not always provide unequivocal information about the source of a food sample. In some cases the statistical comparison of a profile to a database of samples with a known origin can provide a very high confidence that a sample has been mislabelled (e.g. >95%); in other cases mislabelling may be indicated, but with lower confidence (e.g. 80%). Hence, users of this approach (and similar approaches) need to make their own judgement about the appropriate response to results, which show (with a particular confidence) that a sample may have been mislabelled.

The methods for beef provenance determination, used in this project, include measurements of light stable isotope ratios from hydrogen ($^{2}H/^{1}H$), carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), sulfur ($^{34}S/^{32}S$) and heavy stable isotope ratios from lead ($^{206}Pb/^{207}PB$, $^{208}Pb/^{207}PB$, $^{208}Pb/^{206}PB$) and strontium ($^{87}Sr/^{86}Sr$).

For further background information, see Appendix 1.

1.1.3 Other methodologies

Defra commissioned in 2013 a ‘Desk study to assess the ability of methods for determining the country of origin of selected foods to support existing and planned legislation’ (FA0129). Methodologies with potential for certain applications are described below, but stable isotope analysis was confirmed as the principal method for determining the country of origin.

DNA speciation

DNA-based methods are particularly suited for determining if meat from a particular species of animal is present in a product. Furthermore, DNA analysis can be used to identify specific animal breeds. An example in cured meats is the use of single nucleotide polymorphisms (SNPs), e.g. 'Vitellone dell' Appennino Centrale' beef should be produced from only three cattle breeds: Chianina, Romagnola and Marchigiana. If a more complete SNP database was generated that included not just the specialty UK breeds but all the breeds farmed in the UK and the EU, then it might be possible to use the method more generally for country of origin determination.

Proteomics

Using mass spectrometry it is possible to unambiguously identify, and quantify, many of the peptides present in a sample, which allows for identification of contamination with other animal species; e.g. chicken in pork meat (Sentandreu M.A. et al (2010)).

Metabolomics

Metabolomics is the systematic study of the unique chemical fingerprints that specific cellular processes leave behind. This has been successfully applied for differentiation of European honey types (Donarski J. et al (2010)).

Metagenomics

Metagenomics is a technique whereby DNA is extracted from environmental samples and sequenced. Bioinformatics techniques are then used to identify the microbial species, the environment, from which the DNA was derived, which might lead to COOL applications.

Spectroscopic techniques

FT-IR spectroscopy has been used to discriminate Italian and non-Italian olive oils, and Ligurian and non-Ligurian olive oils (Caetano S. et al (2007). Similarly, FT-raman spectroscopy has been used to discriminate Corsican and non-Corsican honeys (Pierna J.F. et al (2011). Hence these techniques are more applicable to liquid samples.

1.2 Background to this study

Consumers in the UK are increasingly interested in regional foods. The reasons for this vary from a) patriotism; b) decreased confidence in the quality and safety of food produced outside their local region or country; c) characteristic organoleptic or culinary qualities or d) concerns about 'food miles'. Our objective was to use established techniques to augment an existing database of light stable isotopes (hydrogen, carbon, nitrogen and sulfur) and the heavy stable isotopes (lead and

strontium) measurements to improve a beef origin decision making tool (isoscape). Inclusion of additional samples of authentic beef produced in Scotland and Northern Ireland permits the regional origin of British Beef to be confirmed. This assists in the enforcement of the various beef PDOs/PGIs, detection of mislabelling fraud and protection of the interests of the consumer and honest trader.

An existing isotope landscape or food origin map (Isoscape) was created for British beef in the form of a geographical origin decision making web-tool with previous funding from the FSA (Q01123 renamed Defra FA0205) and Defra seedcorn. This project used the methodology established in Q01123 to improve the robustness of the decision-making tool, which was limited by a lack of authentic beef stable isotope data from Scotland and Northern Ireland. This FSS project addressed the limitations of the existing Isoscape and established a tool that can be used to routinely monitor compliance with Scottish beef origin labelling.

Fera's beef database was boosted by a further 250 Scottish and 49 Northern Irish samples. Furthermore, this proposal also combined the evidence gathered under a separate Defra-Fera R&D framework project (FA0152), which addressed the lack of authentic beef reference data from East Anglia, the Midlands and the South East of England.

This technology supported the needs of the FSA/FSS by providing tools for enforcement of food labelling and standards legislation to determine the accuracy of 'Country of Origin Labels'. This was particularly pertinent with the implementation of the EU Food Information Regulation [(EU) No 1169/2011] significantly extending the information required for consumers on the origin of foods they consume, specifically meat and meat products.

2. Objectives as set out in the contract

This FSS project consisted of 10 objectives, as described in Table 1.

Table 1: Summary of project objectives.

Objective	Description
1	Selection of samples for the study
2	Receipt and cataloguing of authentic beef samples
3	Sample preparation for stable isotope analysis
4	Light element (hydrogen, carbon, nitrogen and sulfur) and heavy element (strontium and lead) stable isotope analyses
5	Mid-term report and progress meeting
6	Bayesian statistical evaluation of data and python code for web-based decision making tool
7	Geo-statistical analysis using arcgis
8	Web-based isoscape decision making tool
9	Complete cross validation and blind testing of web based isoscape decision making tool
10	Final report

For details of tasks and deliverables, see Appendix 2.

3. Extent to which objectives have been met

The project was delivered by Fera Science Ltd (Fera – York, UK) and their 2 subcontractors, The James Hutton Institute (JHI – Aberdeen UK) and Queen's University Belfast (QUB – Belfast, UK).

Objective 1 - Selection of samples for the study

Objective was completed successfully, in April 2014.

Objective 2 - Receipt and cataloguing of authentic beef samples

Objective was completed successfully, in April 2015.

Objective 3 - Sample preparation for stable isotope analysis

Objective was completed successfully, in September 2015.

Objective 4 - Light element (hydrogen, carbon, nitrogen and sulfur) and heavy element (strontium and lead) stable isotope analyses

Objective was completed successfully, in September 2015.

Objective 5 - Mid-term report and progress meeting

Objective was completed successfully. The mid-term report was submitted to the FSS project officer on the 11/12/14, and progress meetings were held on the 29/09/14 at Fera and on the 23/02/15 at JHI.

Objective 6 - Bayesian statistical evaluation of data and python code for web-based decision making tool

Objective was delivered, by using a non-Bayesian Loess Model, which was more appropriate to be implemented for the web tool.

Objective 7 - Geo-statistical analysis using arcgis

Objective was completed successfully. Data was analysed by ArcGIS, but the Loess Model, was implemented for web tool.

Objective 8 - Web-based isoscape decision making tool

Objective was completed successfully, in December 2015.

Objective 9 - Complete cross validation and blind testing of web-based isoscape decision making tool

Objective was completed successfully and results presented at the final project meeting at Fera, 19/11/15.

Objective 10 - Final report

Objective was completed, by submitting the final report for review to the FSS project officer on the 15/12/15. After external (FSS) review, the report has been rewritten and undergone internal review (Fera), prior to re-submission in June 2016.

4. Materials and Methods

4.1 Objective 1 - Selection of samples for the study

A pre-project meeting with FSS officers was held in February 2014 and a sampling plan was devised for all 2-letter postcodes of the counties in Scotland (n = 15) and Northern Ireland (n = 6) involved.

4.1.1 Sampling for the beef data base

The sampling (200 g neck muscle per animal) started at the 7 Scottish plants in May 2014, at the 2 Northern Irish plants in June 2014, and continued into March 2015, to cover changes in feeding regimes. All beef samples were frozen at plant and then shipped chilled to Fera (n = 250, Scottish) and QUB (n = 49, Northern Irish).

The final sampling plan used within the project is provided in Table 2.

Table 2: Sampling plan for Scottish and Northern Irish beef samples.

	May-14	Jun-14	Jul-14	Aug-14	Sep-14	Oct-14	Nov-14	Dec-14	Jan-15	Feb-15	Mar-15	Total
Plant 1101	7	7	5	5	4	5	6	3	6	6	4	58
Plant 1103	5	5	3	3	4	3	4	4	3	5	2	41
Plant 1106	6	6	5	4	5	5	5	4	6	6	5	57
Plant 1541	4	5	4	3	2	3	N/A	N/A	N/A	N/A	N/A	21
Plant 1560	7	6	4	4	6	4	6	6	6	5	5	59
Plant 1118	N/A	N/A	N/A	N/A	N/A	4	3	N/A	N/A	N/A	N/A	7
Plant 1756	N/A	5	2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	7
Plant 9012	N/A	2	3	2	2	2	3	2	3	2	3	24
Plant 9023	N/A	3	2	3	2	3	2	3	2	3	2	25
Total	**29**	**39**	**28**	**24**	**25**	**29**	**29**	**22**	**26**	**27**	**21**	**299**

4.1.2 Sampling of blind samples

In April 2015, 15 raw beef samples were selected by the project officers and sent to Fera for blind testing.

4.2 Objective 2 - Receipt and cataloguing of authentic beef samples

4.2.1 Sample receipt and cataloguing

On reception of raw beef samples, the cattle passports were archived. The beef samples were catalogued with unique sample identifiers using LIMS, aliquoted and stored frozen at -20˚C until preparation for isotopic analyses.

4.2.2 Sample distribution amongst partner institutes

For details, please see Appendix 3.

4.3 Objective 3 - Sample preparation for stable isotope analysis

4.3.1 Sample preparation for light stable isotope analysis

4.3.1.1 Fera

From each beef sample, a portion of the original sample underwent freeze-drying, homogenisation and fat extraction to remove the lipid phase, whilst the remainder was kept frozen for the beef sample archive. The treated sample portion was air dried and stored in a desiccator prior to analysis. For further details see Appendix 4.

4.3.1.2 QUB

On arrival of the chilled beef samples, they were stored at -20°C. Subsamples of ~ 40 g were taken, thinly sliced and freeze-dried for more than 30h. The dried sample was turned into a powder using a ball mill, before performing defatting steps with petrol ether/diethyl-ether (2:1), as described by Camin *et al* (2012). The sample preparation was completed with a second freeze-drying step over night.

4.3.2 Sample preparation for lead ratio analysis

See 4.3.1.1 and 4.3.1.2 for preparation of the freeze-dried and defatted material.

An accurately weighed aliquot of 100 mg of the dried, fat extracted meat powder was digested in concentrated acid and peroxide using a temperature controlled microwave oven, and made up to a known concentration with deionised water.

4.3.3 Sample preparation for strontium ratio analysis

See 4.3.1.1 and 4.3.1.2 for preparation of the freeze-dried and homogenised material.

The freeze dried and milled beef samples, typically between 1.5 g and 2.7 g of sample were weighed out to smooth walled aluminium dishes. A maximum of eight were placed in the muffle oven and ashed overnight at 500°C to remove organic matter. Once cooled they were removed from the oven and 1ml of 2M HNO_3, in two additions, was used to digest and transfer the sample to an acid washed PMP beaker. The sample was dried under hot lamps, converted to a chloride form by dissolving in 2M HCl and dried again.

The sample was again animated in 2M HCl and passed through calibrated ion exchange columns to isolate the Sr fraction. The columns contained Bio Rad Analytical Grade AG 50W X 8 cation resin in 200-400 mesh hydrogen form and the flow rates/volume were controlled by peristatic pump. The Sr fraction was collected, dried down and loaded onto a rhenium filament using a tantalum activator solution.

4.4 Objective 4 - Light element (hydrogen, carbon, nitrogen and sulfur) and heavy element (strontium and lead) stable isotope analyses

4.4.1 Light element (HCNS) stable isotope analyses by EA-IRMS

Samples were analysed, in batches of 12, for CNS and H isotope ratio values. For N, C and S isotope ratio analysis, samples of lipid free homogenised beef protein were weighed into tin capsules, which underwent flash combustion in an elemental analyser. The resulting nitrogen (N_2), carbon dioxide (CO_2) and sulfur dioxide (SO_2) gases were dried and separated using gas traps.

For H isotope ratio analysis, samples of lipid free homogenised beef protein were first weighed into tin capsules and equilibrated with laboratory air for 48 hours prior to the capsules being sealed. Samples underwent thermal degradation (pyrolysis) in an elemental analyser before the resulting hydrogen gas was separated either on a gas chromatography column or with a gas trap.

The gases were quantitatively transferred into a Stable Isotope Ratio Mass Spectrometer (SIRMS) where the ^{15}N, ^{13}C, ^{34}S and ^{2}H isotopes were measured in proportion to their lighter corresponding isotopes of ^{14}N, ^{12}C, ^{32}S and ^{1}H respectively. The isotopic data are reported in per mil [‰] on the relevant IAEA δ-scales.

Specific method details are provided in Appendices 4 (Fera) and 5 (QUB).

Quality control

Several standards, shared by QUB and Fera, were analysed in a pre-defined batch format throughout the project, see Appendices 4 and 6 for further details.

Details about investigation of best practice of sample equilibration for H isotope ratio analysis are provided in Appendix 7.

4.4.2 Heavy element (Pb, Sr) stable isotope analyses

4.4.2.1 Lead stable isotope analysis by ICP-MS

The sample batch size performed for this analysis consisted of 48 samples, 2 blanks (empty tubes, only acid) and 2 CRM (RM8414 beef muscle powder) samples. The resultant extraction was analysed for lead isotopes: ^{206}Pb, ^{207}Pb and ^{208}Pb on a 'Thermo iCAP Q' ICP/MS. Calibration, drift and isotopic correction was performed using a certified Pb isotopic standard with the following run structure: Samples, blanks and CRMs were randomly distributed within each run.

Quality control

The CRM, RM8414, was run twice in each of the three analytical batches.

4.4.2.2 Strontium stable isotope analysis by TIMS

During the TIMS procedure the sample filament was preheated and tuned for approximately an hour, prior to capturing ratio data. The ratios were captured using a three cycle dynamic procedure and the mass fractionation is exponentially corrected for using the naturally invariant $^{86}Sr/^{88}Sr$ ratio of 0.1194. A maximum of 150 ratios were collected.

Quality Control and Reference materials

The acceptance criteria were applied to each measured sample:

i. a minimum of 50 ratios collected
ii. a Standard error (St Err,%) <0.0060
iii. a 85/86 ratio <0.001
iv. a signal intensity on mass 85 (Rb) <0.00005V.

A NIST certified reference material (CRM), SRM987 – strontium carbonate with a certified value of 0.71034 ± 0.00026 (95% CI) and an in-house assigned value of 0.710250 ± 0.00002 (2SD), was measured at least twice with each batch of samples. Several full procedure blanks (muffle to instrument) were carried out throughout the period of analysis, to allow for balance blank exercise.

4.5 Objective 6 - Bayesian statistical evaluation of data and python code for web-based decision making tool - Statistical analysis and modelling

4.5.1 Aim

The goal of the statistical analysis was to produce a procedure for assessing whether the source of a particular piece of beef had been accurately described, by applying isotope ratios. The procedure had to be implementable on a web-based system using basic arithmetic functions. The false "positive rate" associated with the procedure: the probability that a correctly labelled sample is assessed by the procedure to be wrongly labelled needed to be controlled reliably. There was an additional requirement to examine whether seasonal variation might have an effect on isotopic profiles, particularly carbon and hydrogen isotope ratios. The procedure/web-tool was to be tested by using a number of unknown samples. Lastly, we wanted to communicate how the procedure could be expected to perform: its power to detect mislabelled beef, in a way that was transparent and easy to understand.

4.5.2 Tools and data

All analysis was undertaken in the statistical software package R (ver. 3.2.2.).

Data from all three beef projects – FA0152, FA0205 and FS515009 - were combined, where appropriate.

4.5.2 Approach

The statistical data analysis consisted of 5 steps, prior the blind testing (objective 9).

4.5.2.1 Visual inspection of results and consideration of the completeness of each data set and preliminary examination of variation in results

Data from all three beef projects – FA0152, FA0205 and FS515009 - were inspected.

4.5.2.2 Fitting of Loess regression to principal components of isotope profiles to produce a database of expected profiles for UK locations

The aim of this part of the analysis was to produce a database of locations (postcodes) with two parts: an expected HCNS profile for the postcode and a value by which individual samples may vary around the expected profile. Data with complete HCNS profiles were used in this part of the analysis. HCNS profiles were transformed into their principal component scores. This is often done so that a smaller number of variables can be used in an analysis, but in this case principal component scores were used because their lack of correlation simplified subsequent analysis.

A Loess regression model [Cleveland et al 1992] (polynomial degree 2) was used to fit a response surface to latitude and longitude for each of the four principal component scores of the HCNS profiles independently. The following algorithm was applied to each principal component:

- Divide the data into 1000 calibration and test sets (50:50) at random

For each set

- Fit a number of Loess models, each with a different degree of smoothing, to the calibration data.
- Use each model to *predict* the response (principal component score) for the locations in the test set.
- Select the 'best' model: the model with the lowest *prediction* standard error. Record the smoothing parameter (span) and the *prediction* standard error for that principal component score.
- Then the final model and database was produced by fitting a Loess response surface to the whole data using the median value of the recorded 'best' smoothing parameters. The upper limit for the *prediction* standard error was estimated to lie at the observed 95th percentile of recorded 'best' *prediction* standard errors. The expected value of the *prediction* standard error was estimated to lie at the median of the recorded 'best' *prediction* standard errors.

The fitted models were expressed as a database of the expected values of the principal component scores of HCNS profiles for each district level postcode and the prediction standard errors (median and 95th percentile). The database was combined with a simple algorithm for

generating principal component scores for an “unknown” sample and identifying those postcodes with which the unknown profile was consistent and those with which it was not. Two different levels of confidence, “≥95%” and “80%”, were used for making an assessment. “≥95%” meant that we expected a sample to be declared not being from its true source on no more than 5% of occasions, when tested. “80%” meant that we expect a sample to be rejected from its true location on 20% of occasions, when tested. A 95% confidence level is often mandated in legislation (e.g. Regulation (EC) No 333/2007), in order to control the false positive rate, when testing food samples against legal limits for the presence of chemical residues or contaminants. Hence, a 95% confidence level was offered as an option for making an assessment for this procedure, on the basis that “95% confidence” was already applied in legislation, to provide a sufficient level of confidence for the purpose of legal enforcement. An option, which aimed to control the false positive rate with a lower, 80%, confidence was also applied on the basis that it would offer a higher probability of detecting potentially mislabelled beef, which could be followed up by further investigation or conformation. However, the interpretation of the results and decisions about which action should be taken in practice lies with the users of the procedure.

The procedure was applied to the results of a test sample as follows. For each district level postcode a score “y” is produced, which is the sum of the squares of the differences between the principal component scores of the unknown sample and the expected principal component scores for a sample from the district level postcode, scaled by the estimated variation of samples around the expected value. Two values were used for the estimated variation: an upper limit, the 95th percentile of *prediction* standard errors, estimated during the model fitting stage (used for “>95%” assessments) and the median of *prediction* standard errors estimated during the model fitting stage (used for “80% assessments”). The score “y” was compared to a critical value: the 80th or 95th percentile of the Chi-squared distribution with four degrees of freedom. District level postcodes with a score above the critical value were excluded as potential sources for the sample. Two-letter postcodes within which *all* district-level postcodes were excluded, were assessed as *not* being a potential source of the sample. Remaining two-letter postcodes were returned as potential sources for the sample.

4.5.2.3 The specification of a simple method for using an isotope profile to exclude locations from those that may be a source of the sample that produced the profile

A detailed description of the process is given in Appendix 8.

4.5.2.4 Assessment of the expected performance of the procedure

The main assessment was undertaken to provide an estimate of the power of the procedure to discriminate between beef that was from its declared location and beef that may from anywhere else in the UK. In addition, data from some non-UK samples were examined. The main assessment followed this approach:

For each location in the UK

- Look up its expected HCNS profile using the model database (4.5.2.2)
- Use this profile as an input into the assessment procedure (4.5.2.3) to find locations for which the profile is not consistent.
- Record the proportion of locations in the UK, which are excluded as potential sources for a beef sample, with that HCNS profile. This is a measure of the "protection" the procedure provides against beef being incorrectly labelled as being from that location.
- The average of the proportion of excluded locations, for profiles from every other location in the UK, provides a measure of the general power of the procedure.

For this assessment a database of expected profiles, based on a grid of equidistant (5 km) points, was used so that different areas within the UK had equal weighting. In addition a number of different detection confidences for the exclusion of locations were applied ("≥95%", "95%", "90%", "80%"). Every other aspect of the procedure and its assessment was unchanged from the "live" web based model.

A similar assessment was undertaken in which expected profiles from locations in Scotland were compared to expected profiles from all other *non-Scotland* UK locations to provide an estimate of the potential for the procedure to detect beef from other locations that was incorrectly labelled as produced in Scotland.

4.5.2.5 Assessment of seasonal variation in profiles

There is potential for isotope ratios, particularly hydrogen and carbon to vary with season. Hydrogen isotope ratios may change because of changes in rainfall between seasons and carbon isotope ratios may vary because of changes in feed between seasons. The potential effect of seasonal variation was assessed using the Scottish samples analysed between 2014 and 2015 (Table 2, Section 4.1.1). The assessment was undertaken by fitting a seasonal model to the residuals of the observed hydrogen (a) and carbon (b) profiles against the fitted geographic response surface. We assumed that *any* variation in the observed carbon and hydrogen isotope ratios around their geographically expected value was due to a periodic variation within a 12-month

period and then fitted the model to estimate what the amplitude of the assumed periodic variation would be, if it was the cause of the variation.

Details of hydrogen (a) and carbon (b) equation are reported in full in Appendix 8.

There is also potential for longer term trends in isotope ratios to be driven by changes in climate (Brookshire and Weaver, 2015), but that was not investigated within this project.

4.6 Objective 7 - Geo-statistical analysis using ArcGIS

The Loess regression model was used to predict expected isotope profiles for each point on a 5 km^2 vector grid that was created to cover the UK.

Only grid cells that had a centre point within the UK polygon boundary where included in this grid, thereby removing grid cells, which extended out into sea.

For the beef data, isotope distribution information was provided by the Statistics Team at Fera. The centre point coordinates (in latitude and longitude) for the remaining 5 km^2 were calculated and used to calculate the isotope values for each location.

4.7 Objective 8 - Web-based isoscape decision making tool

The implementation of the statistical treatment and the mapping functions were described in Section 4.6.

4.8 Objective 9 - Complete cross validation and blind testing of web based isoscape decision making tool

The capabilities of the procedure were assessed with 15 blind samples. The isotopic data of these samples (all isotopes including Sr and Pb) are listed in Table 3.

Table 3: Stable isotope data of 15 blind samples.

					Pb ratios			
Fera sample ID	2H	13C	15N	34S	206/207	208/207	208/206	87Sr
S15-050194	-99.0	-24.13	6.21	7.45	1.1166	2.3977	2.1932	0.707905
S15-050195	-98.8	-23.08	7.16	6.65	1.0851	2.3478	2.1476	0.708191
S15-050196	-105.8	-25.18	5.57	6.35	1.1053	2.3766	2.1739	0.708801
S15-050197	-105.9	-26.81	5.83	8.05	1.1442	2.4022	2.1973	0.708559
S15-050198	-97.8	-22.90	7.89	7.11	1.1037	2.3642	2.1625	0.708342
S15-050199	-107.9	-25.99	5.34	7.05	1.1093	2.3629	2.1614	0.706198
S15-050200	-104.7	-25.94	7.80	6.26	1.0901	2.3436	2.1437	0.708823
S15-050201	-104.2	-26.48	7.55	8.51	1.0939	2.3507	2.1502	0.709250
S15-050202	-101.8	-23.84	7.65	8.19	1.1313	2.3516	2.1510	0.708067
S15-050203	-99.1	-27.01	9.28	13.26	1.1221	2.3988	2.1942	0.709196
S15-050204	-104.7	-26.54	7.25	6.38	1.1126	2.3893	2.1855	0.708292
S15-050205	-95.6	-21.92	6.74	6.29	1.0948	2.3712	2.1689	0.708190
S15-050206	-106.1	-25.35	6.74	6.31	1.0939	2.3498	2.1494	0.708849
S15-050207	-103.2	-24.86	5.67	8.15	1.1026	2.3439	2.1439	0.709783
S15-050208	-105.0	-25.34	6.61	6.78	1.1020	2.3544	2.1536	0.709074

The statistical tool requires only the input of H, C, N and S isotope values and these were used to produce lists of postcodes which were potential (i.e. not excluded with the specified confidence level) sources of the sample. The assessment was undertaken using two exclusion confidences: "≥95%" and "80%". There were two desiderata for the assessment: that the true source be on the list of potential sources (demonstrating that exclusion is reliable) and that the list of potential sources be short (demonstrating that the procedure is powerful).

5. Results

5.1 Objective 1- Selection of samples for the study

Materials were selected as explained in the Materials and Methods section.

5.2 Objective 2 - Receipt and cataloguing of authentic beef samples

Materials were archived as explained in the Materials and Methods section.

5.3 Objective 3 - Sample preparation for stable isotope analysis

Materials were prepared as explained in the Materials and Methods section.

5.4 Objective 4 - Light element (hydrogen, carbon, nitrogen and sulfur) and heavy element (strontium and lead) stable isotope analyses

Individual data of all 299 analysed beef samples for HCNS and Sr, and of these 150 samples for Pb are listed in Appendix 9.

5.4.1 Light element (HCNS) stable isotope analyses by EA-IRMS

All beef samples were analysed for light elements. CNS ratios were measured at Fera, and H ratios at Fera and QUB. Scottish and Northern Irish mean values, expressed in ‰, are shown in Table 4.

Table 4: HCNS isotopic data.

Isotope	Northern Ireland (n = 49)				Scotland (n = 250)			
	mean	SD	min	max	mean	SD	min	max
Hydrogen	-99.1	3.7	-108.4	-91.8	-102.6	3.5	-110.8	-91.4
Carbon	-23.99	2.68	-27.77	-17.10	-25.78	1.16	-27.98	-21.14
Nitrogen	7.62	0.86	5.84	9.22	7.09	1.03	4.37	9.78
Sulphur	7.38	1.15	4.78	10.05	7.84	2.21	3.46	15.92

Quality control

See Appendix 6 for details.

5.4.2 Heavy element (Pb, Sr) stable isotope analyses

5.4.2.1 Lead stable isotope analysis by ICP-MS, at QUB

Samples

A summary of the lead ratio data is shown in Table 5.

Table 5: Pb ratio data from Scotland and Northern Ireland.

	206/207 Mass bias corrected IR	208/207 Mass bias corrected IR	208/206 Mass bias corrected IR
NI Summary			
Mean	1.1104	2.3905	2.1845
SD	0.0268	0.0289	0.0287
Minimum	1.0739	2.3561	2.1187
Maximum	1.1588	2.4503	2.2413
Range	0.0849	0.0942	0.1226
N (number of samples)	25	25	25
Scottish Summary			
Mean	1.1220	2.4004	2.1945
SD	0.0186	0.0217	0.0221
Minimum	1.0647	2.3354	2.1167
Maximum	1.1514	2.4372	2.2293
Range	0.0868	0.1017	0.1126
N (number of samples)	125	125	125

Quality control

The measured lead concentrations of NIST8414 were tabulated and compared to its certified value (0.38 mg/kg), resulting in a mean recovery of Pb of 71.7% (average 0.272 mg/kg, coefficient of variance cv = 17%, n = 6). The recovery was acceptable, as this certified standard was used for isotopic correction.

The reproducibility of the corrected isotopic values was of more importance and criterion was met with cv < 5%.

For 206/207: The cv was 2.2%

For 208/207: The cv was 1.2%

For 208/206: The cv was 1.2%

Please see Appendix 5 for full QUB final report.

5.4.2.2 Strontium stable isotope analysis by TIMS, at JHI

Samples

A summary of the data is shown in Table 6.

Table 6: Sr ratio data from Scotland and Northern Ireland.

$^{87}Sr/^{86}Sr$	Scotland	Northern Ireland
Mean	0.708666	0.708122
SD	0.000660	0.000488
Minimum	0.706452	0.706770
Maximum	0.710244	0.708894
Range	0.003792	0.002124
N (number of samples)	250	49

Quality Control and Reference materials

Mean value for SRM987 (strontium carbonate) was 0.710242 with a 2 standard deviation of 0.000021 for 72 analyses, see Figure 1 for comparison with other research groups. There is no general beef tissue CRM available but NRC/CNRC RM8414 (bovine muscle powder) was analysed once, as possible reference for future work, producing a ratio of 0.713057 ± 0.000037 (2SE).

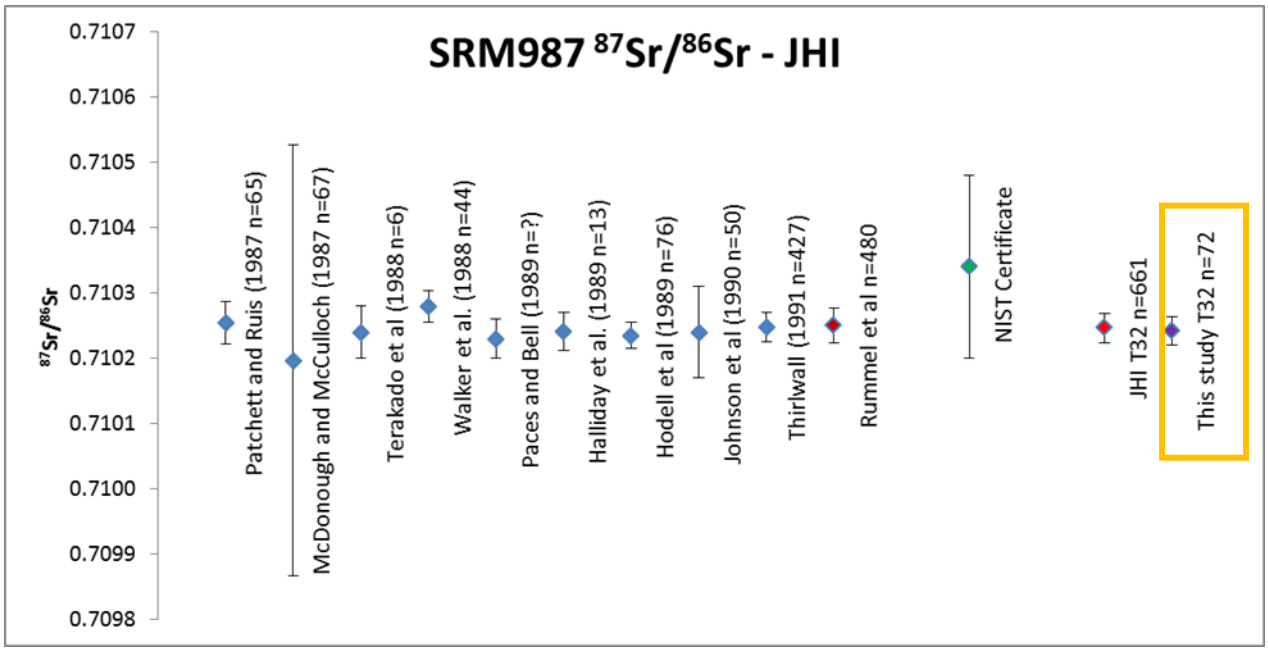

Figure 1: Mean value of SRM987(±2SD), T32, in comparison to other research groups.

Please see Appendix 10 for full JHI final report.

5.5 Objective 6 - Bayesian statistical evaluation of data and python code for web-based decision making tool - Statistical analysis and modelling

5.5.1 Visual inspection of results and consideration of the completeness of each data set and preliminary examination of variation in results

The data set consisted of measurement results produced by the analysis of 791 samples (Table 7).

Table 7: Number of results by region, year and isotope ratio.

Region	Year	Hydrogen	Carbon	Nitrogen	Sulfur	Lead	Strontium
England	2004	1	1	1	1	0	0
England	2005	59	59	59	59	0	44
England	2006	26	26	26	26	0	18
England	2009	7	7	7	6	0	7
England	2014	199	199	199	199	0	0
NI	2014	33	33	33	33	16	33
NI	2015	16	16	16	16	9	16
Scotland	2008	31	31	31	31	0	31
Scotland	2009	68	68	68	68	0	64
Scotland	2014	191	191	191	191	93	191
Scotland	2015	59	59	59	59	32	59
Wales	2005	14	14	14	14	0	14
Wales	2006	5	5	5	5	0	5
Wales	2008	70	70	70	70	0	68
Wales	2009	11	11	11	11	0	11

It was observed that not all isotopic data were available for all samples. Notably, strontium was not measured for the samples from England in 2014. Lead was investigated for the first time in this project, as a possible marker for beef, and therefore measured only in a relatively small number of samples compared to other stable isotopes.

Measurement results are shown in Figures 2 to 9. HCNS was observed to appear stable over the time frame and to show some variation between regions, in particular elevated sulfur ratios for samples from Scotland (Figure 5). The sparse lead isotope data (Figures 7 to 9) did not display any variation between samples from Scotland and Northern Ireland. Measured strontium isotope ratios for samples taken in Scotland were not consistent between the different sampling campaigns. For example, Strontium isotope ratios ($^{87}Sr/^{86}Sr$) were generally lower in samples taken in 2014, compared to samples taken in earlier years, and there was more variation between samples in earlier years (Figure 6).

JHI investigated this variation further in Appendix 10. Because of the sparseness of the lead data and the dissimilarity of the strontium data these two stable isotopes were not included in subsequent analyses.

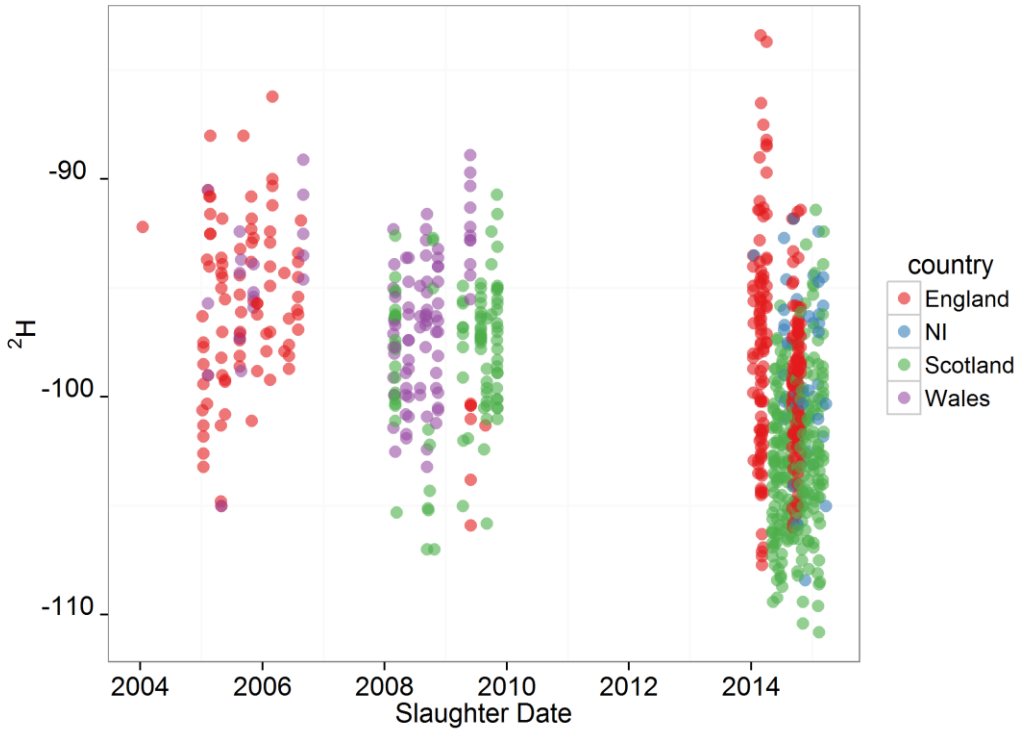

Figure 2: Hydrogen isotope ratios expressed in ‰ vs V-SMOW, against slaughter date.

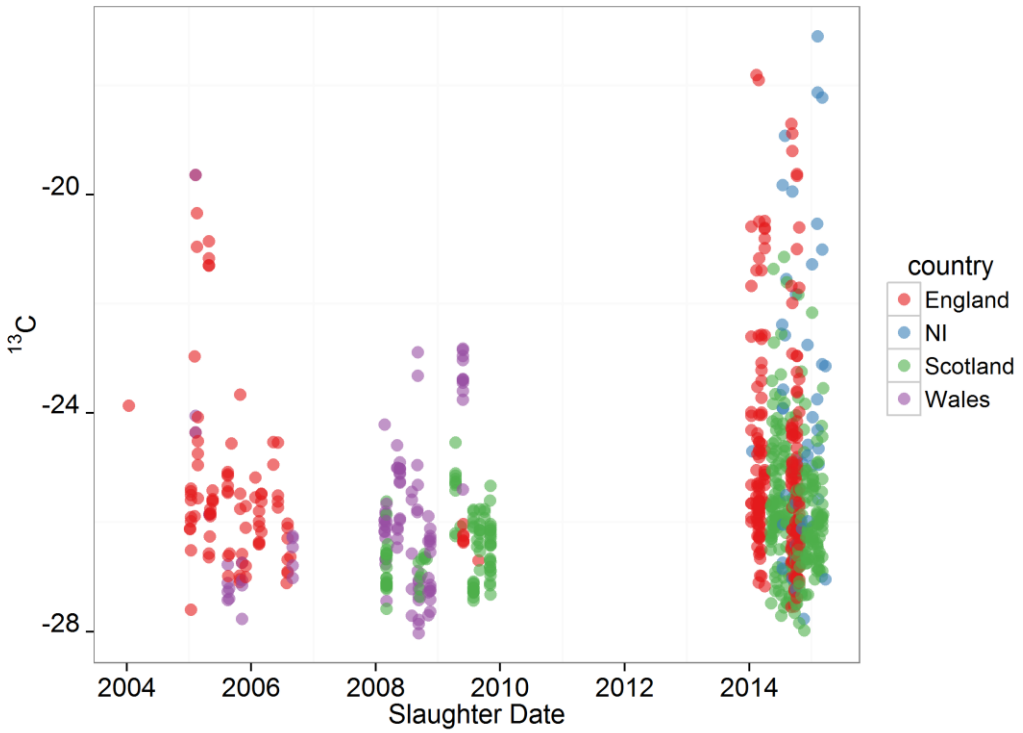

Figure 3: Carbon isotope ratios expressed in ‰ vs V-PDB, against slaughter date.

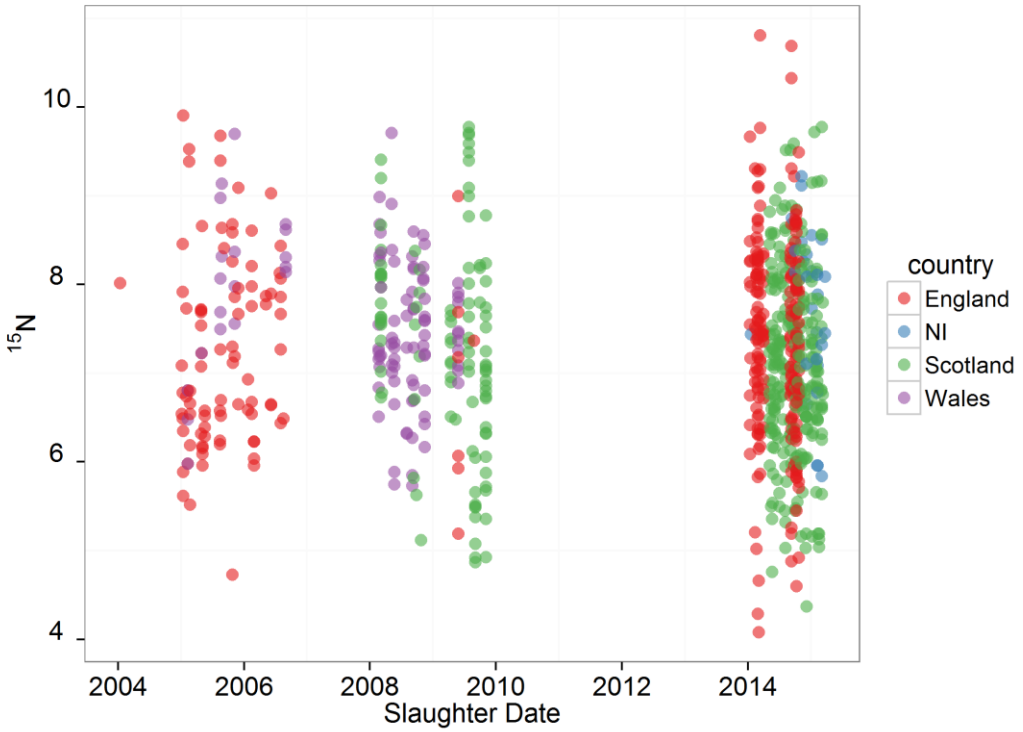

Figure 4: Nitrogen isotope ratios expressed in ‰ vs AIR, against slaughter date.

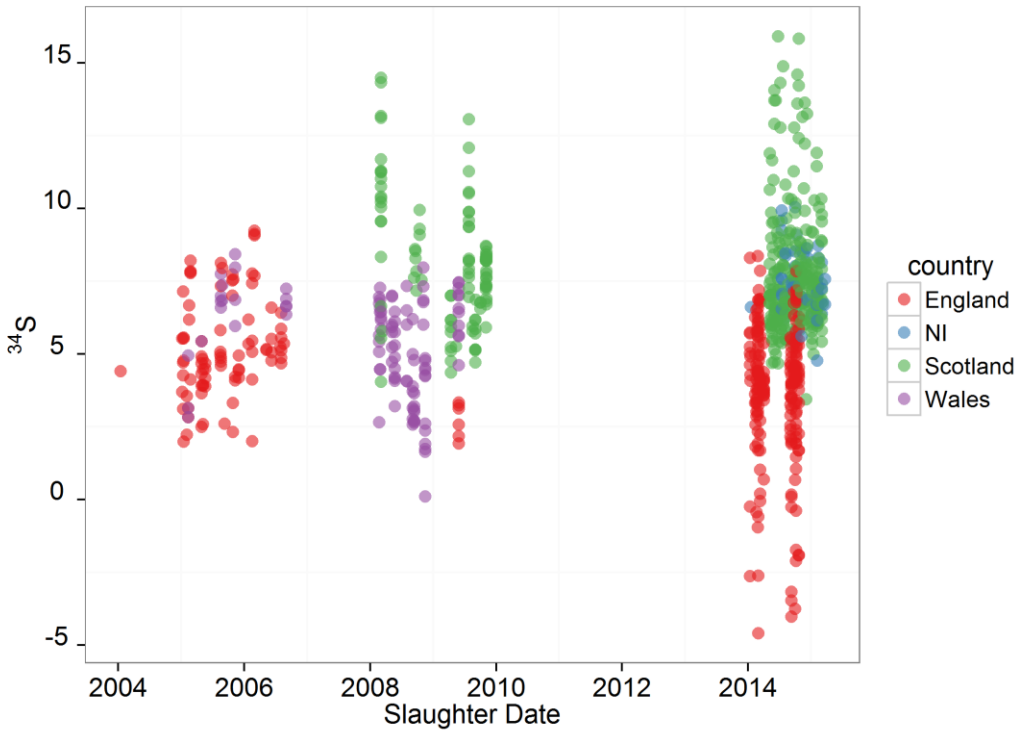

Figure 5: Sulfur isotope ratios expressed in ‰ vs V-CDT, against slaughter date..

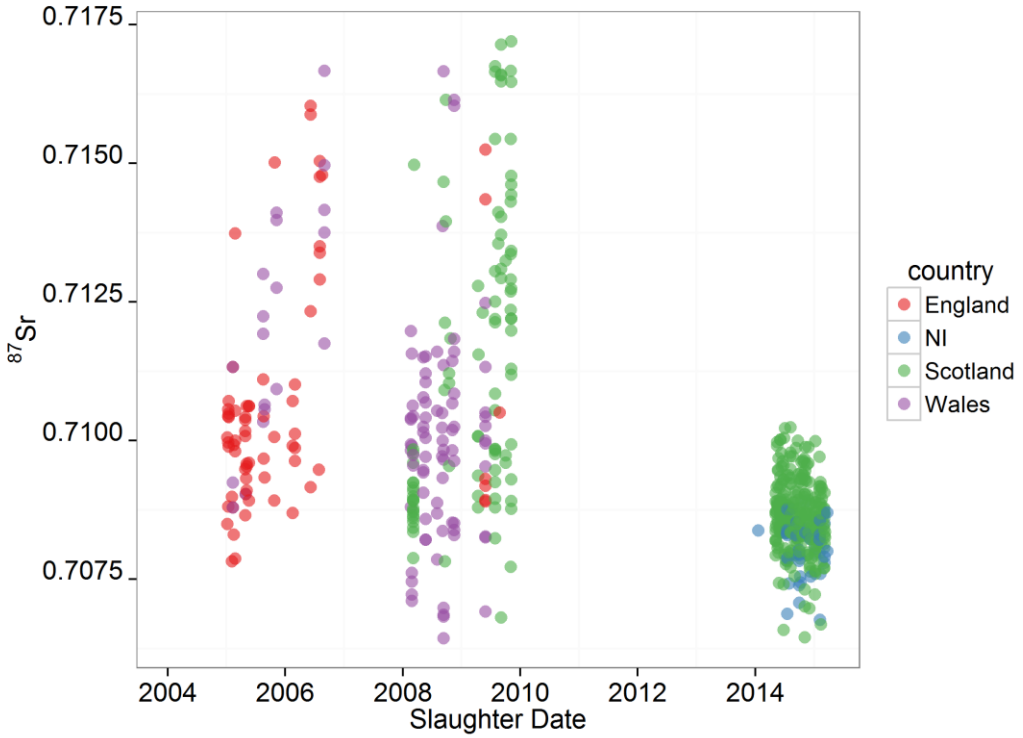

Figure 6: Strontium isotope ratios ($^{87}Sr/^{86}Sr$), against slaughter date.

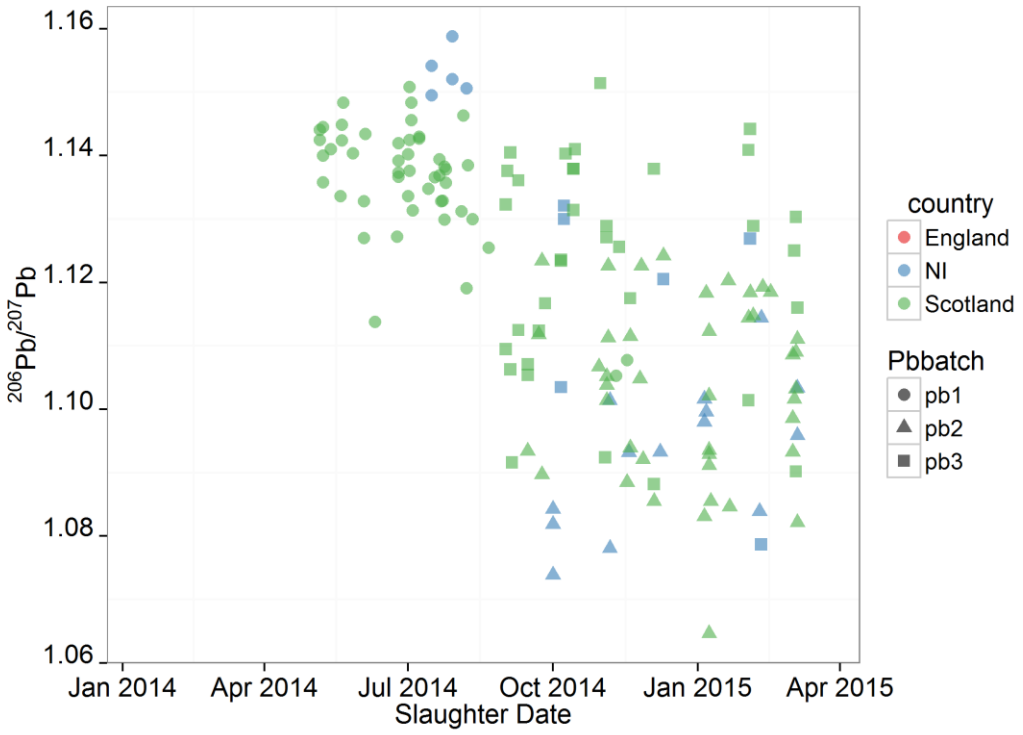

Figure 7: Lead isotope ratios (^{206}Pb /^{207}Pb), against slaughter date.

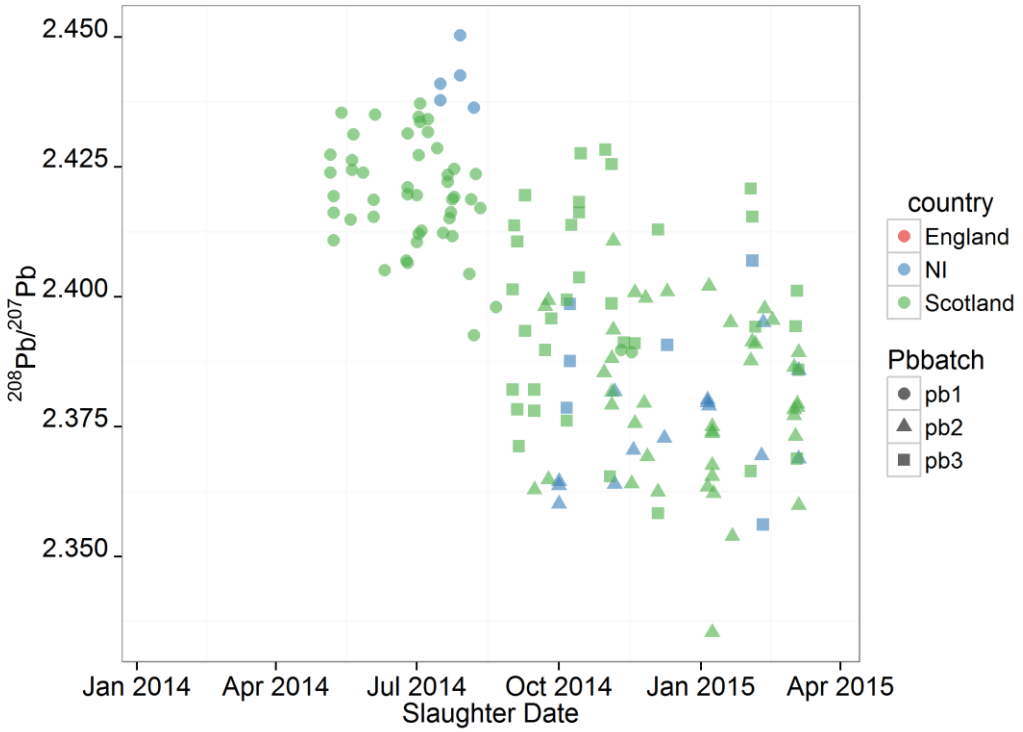

Figure 8: Lead isotope ratios ($^{208}Pb/^{207}Pb$), against slaughter date.

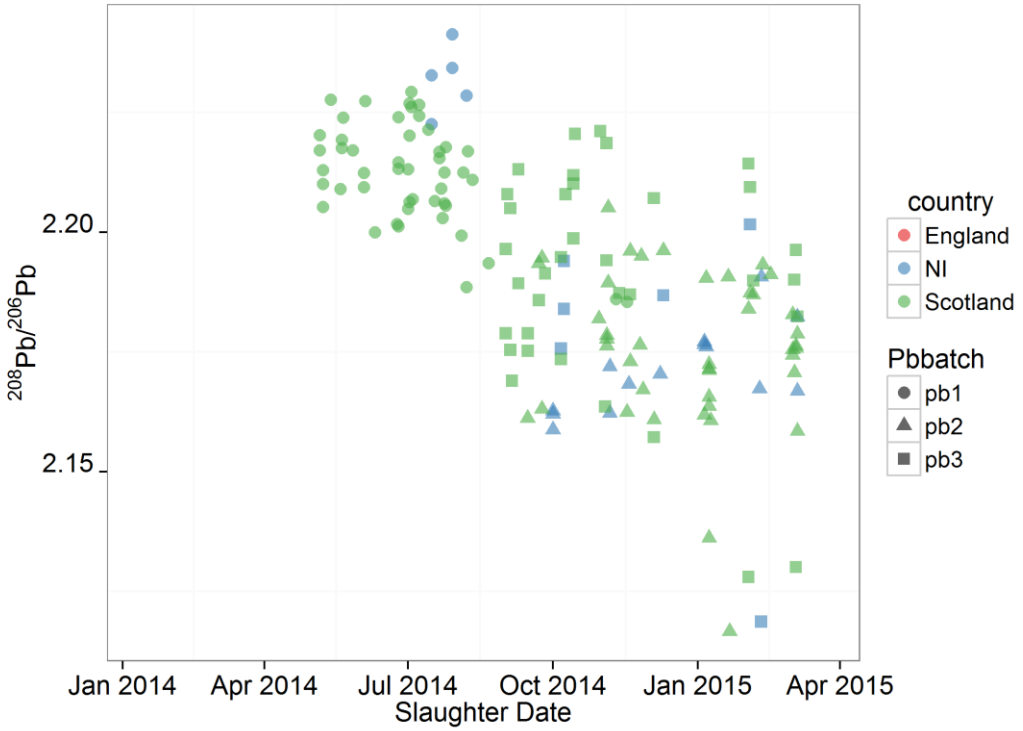

Figure 9: Lead isotope ratio ($^{208}Pb/^{206}Pb$), against slaughter date.

An intercept-only random effect model was fitted to HCNS data, with 'postcode' as a random effect, to examine how the variation in isotope ratios between individual samples from the same postcode compared with variation in the isotope ratios from different locations (Table 8).

Table 8: Variation in isotope ratios.

Isotope ratio	UK Mean	Between location variation (standard deviation)	Within location variation (standard deviation)
Hydrogen	-98.985	2.973	3.363
Carbon	-25.202	0.998	1.425
Nitrogen	7.317	0.470	0.945
Sulfur	5.458	2.336	1.475

Within-location variation was relatively large compared to between-location variation for all isotopes with the exception of sulfur. In the case of sulfur, the between-location variation was larger than the within-location variation.

5.5.2 Fitting of Loess regression to principal components of isotope profiles to produce a database of expected profiles for UK locations

Loess regression was fitted, as explained in the Materials and Methods section.

5.5.3 The specification of a simple method for using an isotope profile to exclude locations from those that may be a source of the sample that produced the profile

Specifications were described in detail, in Appendix 8.

5.5.4 Assessment of the expected performance of the procedure

A summary of the results of the assessment, expressed as the degree of "protection" the procedure gives each UK location is shown in Figure 10. Here, "protection" means the proportion of *other* UK locations which are expected to provide a beef sample, which will be assessed as not being consistent with a specified location. For example, if beef was labelled as being from any one of the locations coloured blue in Figure 10, when it was from some *other UK location*, then we expected that this could be detected on the basis of its HCNS profile for at least 90% of *other UK locations*.

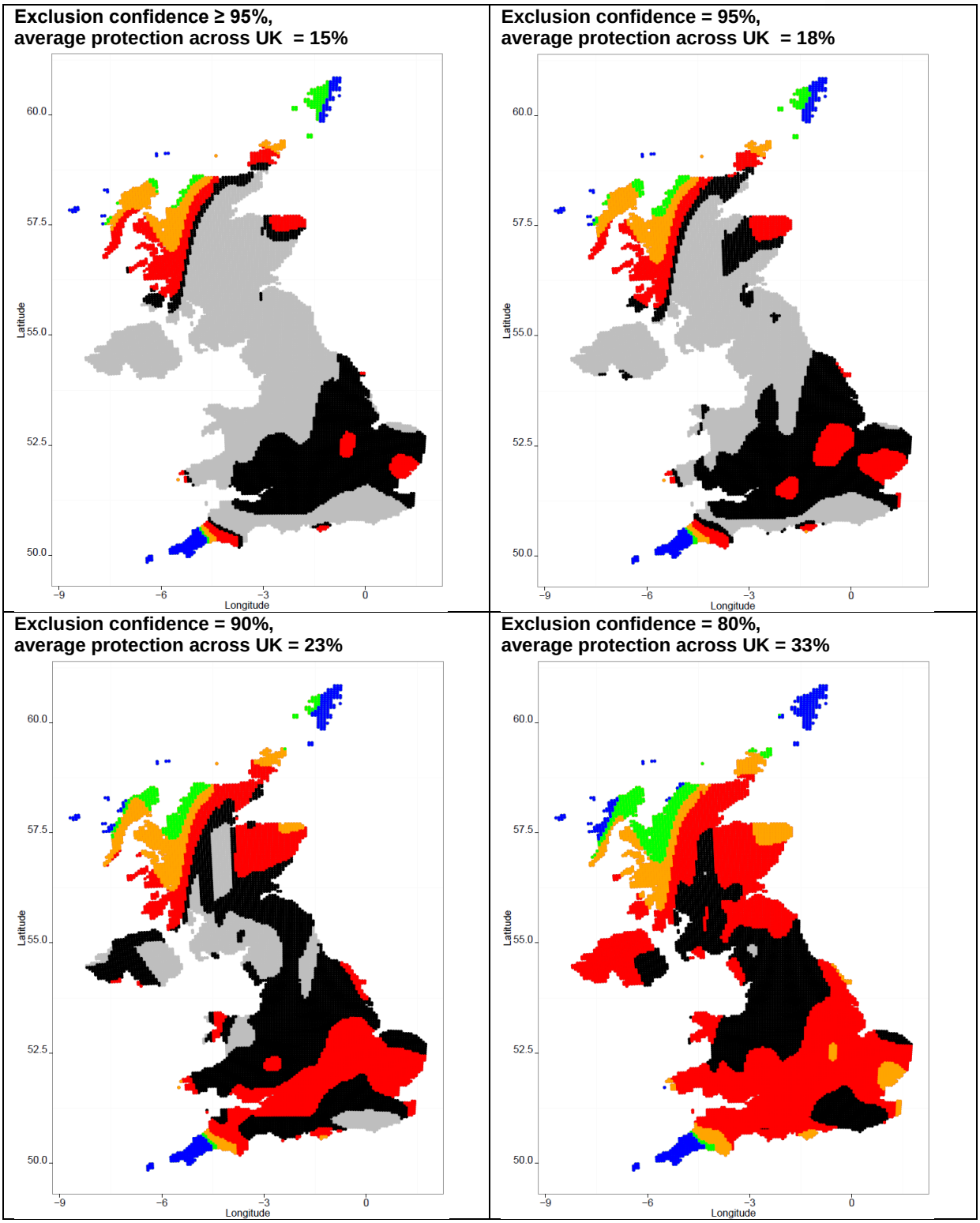

Figure 10: Relation between exclusion confidence and protection.

If the beef was incorrectly labelled as coming from one of the locations coloured black in Figure 10, when it was from some *other UK location*, then we expect that this could be detected on the basis of its HCNS profile for at least 10% of *other UK locations*.

Figure 10 also shows the extent to which there is scope to trade between the confidence associated with the decision to exclude each location as a potential source for a beef sample and the power to detect incorrectly labelled beef. Reducing the confidence, with which locations are excluded, increases the proportion of locations that are excluded on the basis of an observed isotope ratio profile.

The results in Figure 11 suggest that it might be difficult to use the procedure, as it stands, to exclude the possibility that 'non-Scotch, UK produced beef' may have been produced *somewhere* in Scotland. However, there is some power to detect 'non-Scotch, UK produced beef' that has been labelled as coming from a specific location in Scotland.

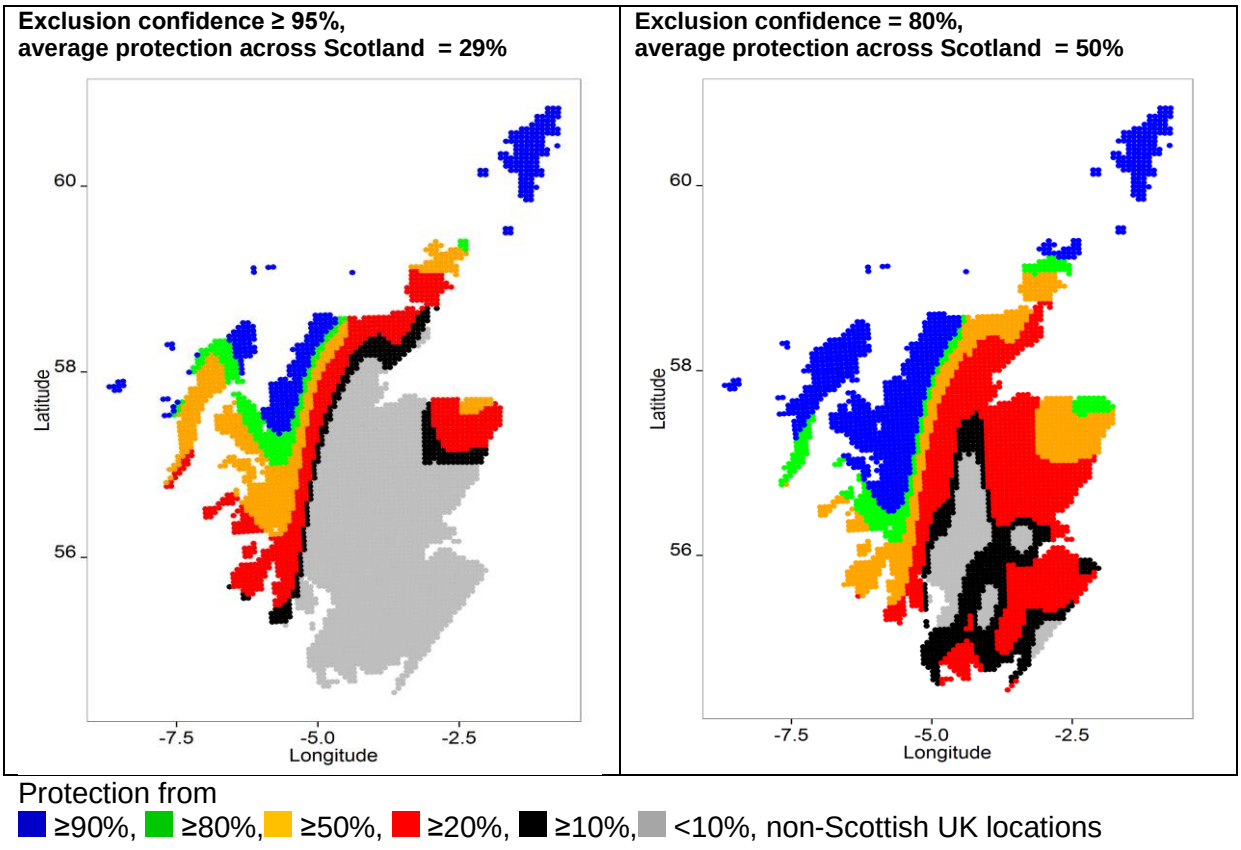

Figure 11: Relation between exclusion confidence and protection of locations in Scotland.

For example, given a measured isotopic profile in a beef sample: H = -96.696, C = -23.537, N = 7.5134 and S = 3.5626, (expected profile for a location in Norfolk) then 14% of UK locations are excluded as potential sources, with a confidence of at least 95%. If locations are excluded with 80% confidence, then 32% of UK locations are excluded as a potential source for that sample (Figure 12) including much of Scotland.

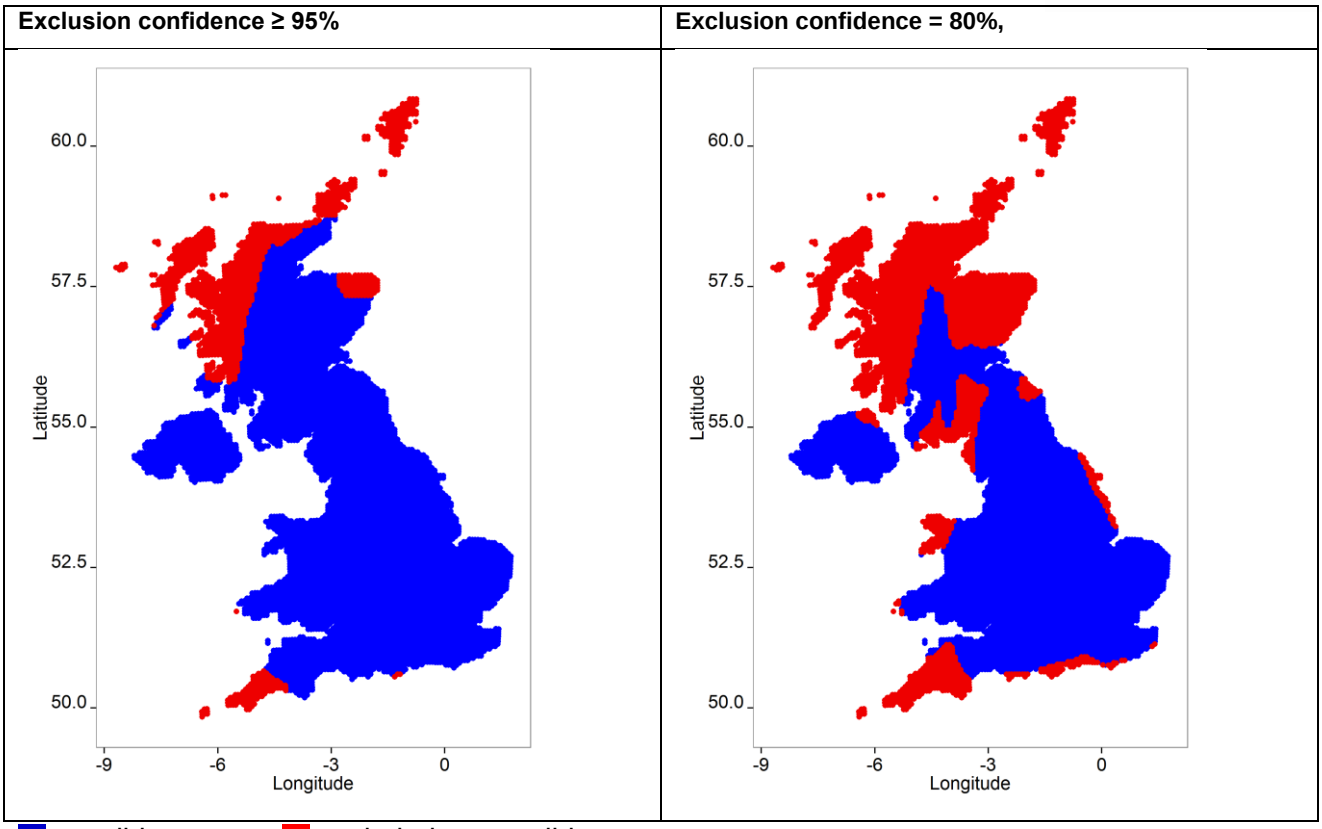

Figure 12: Application of two different exclusion confidence levels (≥ 95% and 80%)

The possibility that the sample with the profile: H = -96.696, C = -23.537 N = 7.5134 and S = 3.5626 comes from *somewhere* in Scotland is not excluded with 80% confidence (Figure 12), but if it is claimed that the sample was produced at a more specific location inside the red area (Figure 12) then this may indicate that the beef is mislabelled.

Results produced by the procedure when applied to some non-UK beef samples were also assessed. Figure 13 shows UK postcodes that were excluded with 95% confidence, or not excluded, based on the HCNS profiles of some previously analysed non-UK beef samples. It can be seen that some non-UK samples had HCNS profiles which are consistent with some particular locations in the UK. However, if there is a more detailed geographic claim made about the samples, such as that they were from a particular part of the UK then it is possible (sample S13-068181), likely (sample S13-068212), or very likely (sample S13-068266) that potential mislabelling could be detected using the procedure developed in this project.

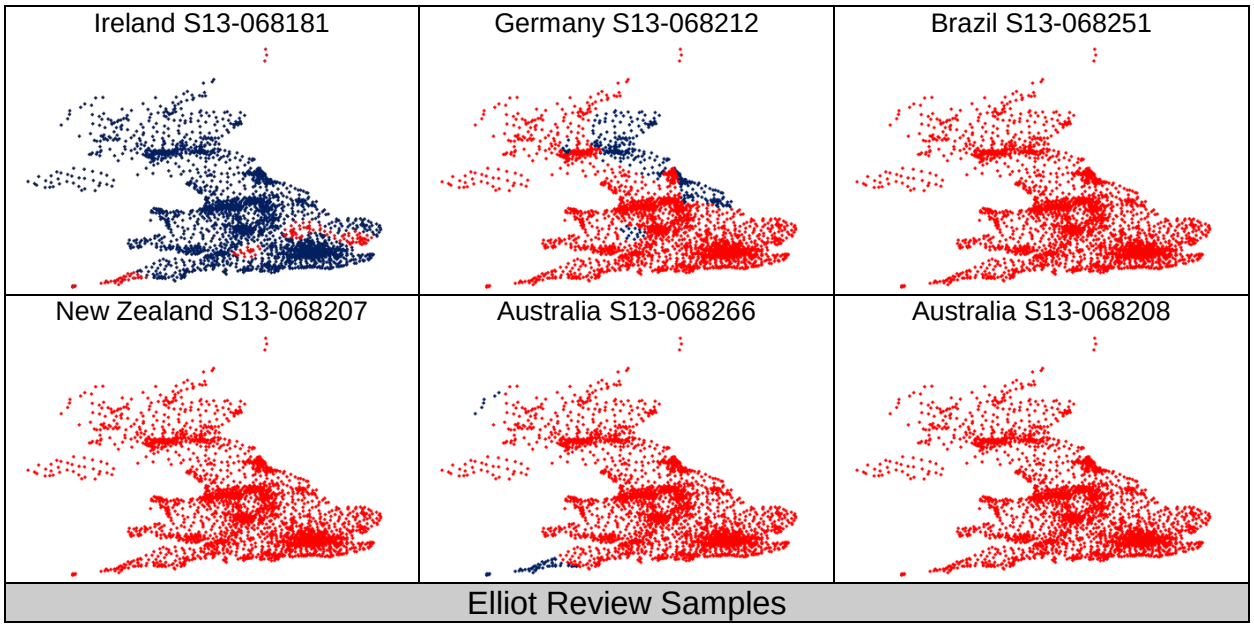

Figure 13: Assessment of some non-UK samples.

5.5.5 Assessment of seasonal variation in profiles

Figures 13 and 14 show the seasonal models fitted to the residuals[1] of the hydrogen and carbon isotope results with their parameter estimates

[1] A "residual" is the difference between an observation and the modelled expected value of the observation. Here it is the difference between an observed isotope ratio for a particular sample from a location and the expected isotope ratio for a sample from that location.

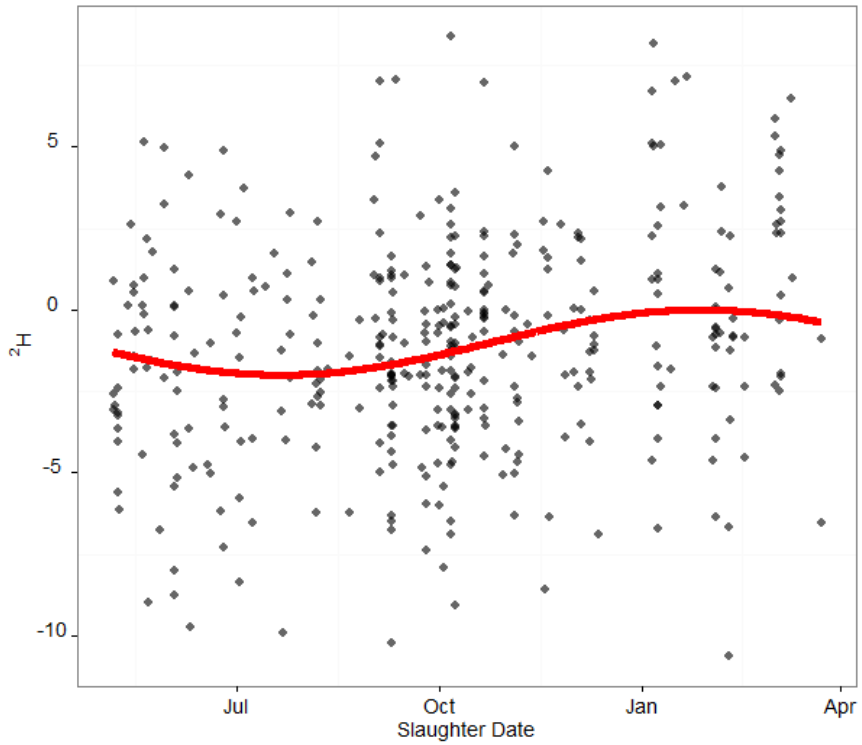

Figure 13: Potential seasonal variation in hydrogen isotope ratios.

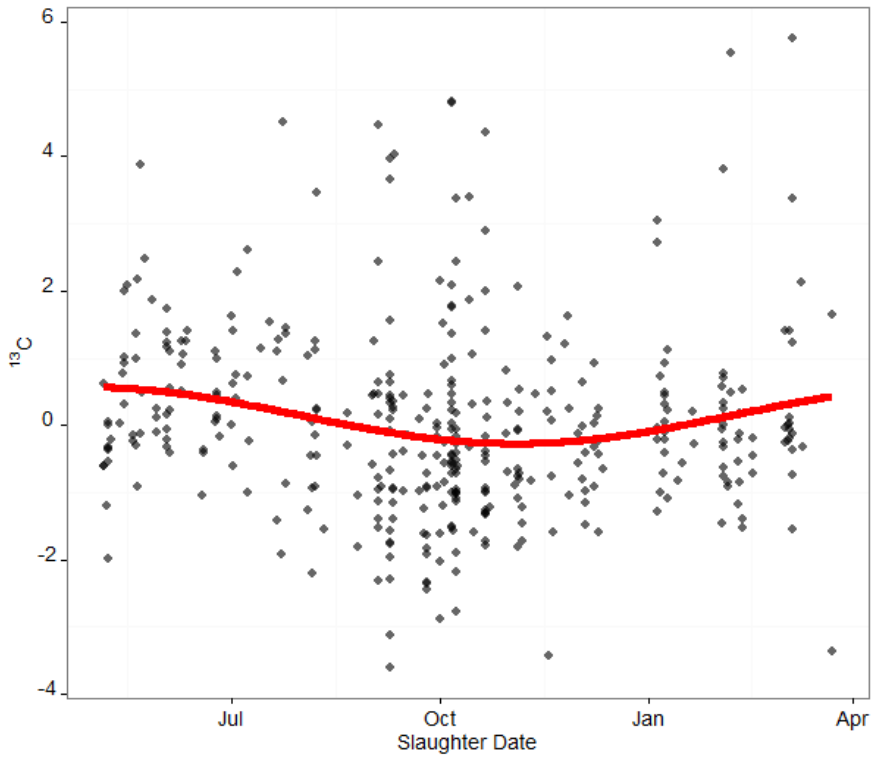

Figure 14: Potential seasonal variation in carbon isotope ratios

While the amplitude parameters “A” of both models are “statistically significant” the potential gain in including seasonal variation in the model appears to be very modest: a reduction in the residual standard error from 3.5 to 3.3 for hydrogen isotope ratios and from 1.41 to 1.39 for carbon isotope ratios. The modest potential decrease in residual error was judged to be not worth the potential bias associated with overfitting a non-existent relationship. Hence, seasonal variation was not included in this version of the procedure.

5.6 Objective 7 - Geo-statistical analysis using ArcGIS

The 5 km^2 grids were provided for the modelling, as described in the Materials and Methods section (4.6).

5.7 Objective 8 - Web-based isoscape decision making tool

A brief tutorial on the usage of the web tool can be viewed at

https://isoscapes-api.fera.co.uk/

Please contact either james.donarski@fera.co.uk or katharina.heinrich@fera.co.uk to enquire about receiving a username and password.

5.8 Objective 9 - Complete cross validation and blind testing of web based isoscape decision making tool

Results of the blind testing are given for expected exclusion confidence at “≥95%” (Table 9) and “80%“(Table 10).

Table 9: Performance of method against blind samples where' expected exclusion confidence' is set to "≥95%"

Sample	True source	Potential locations identified	True source in list	Proportion of Postcodes excluded from list (%)
S15-050194	BT	119	YES	2
S15-050195	BT	119	YES	2
S15-050196	IV	104	YES	14
S15-050197	AB	97	YES	20
S15-050198	BT	119	YES	2
S15-050199	DD	73	YES	40
S15-050200	ML	117	YES	3
S15-050201	DG	117	YES	3
S15-050202	BT	119	YES	2
S15-050203	KW	13	YES	89
S15-050204	PH	116	YES	4
S15-050205	BT	109	YES	10
S15-050206	PH	116	YES	4
S15-050207	AB	109	YES	10
S15-050208	AB	118	YES	2

Table 10: Performance of method against blind samples where expected 'exclusion confidence' is set to "80%"

Sample	True source	Potential locations identified	True source in list	Proportion of Postcodes excluded from list (%)
S15-050194	BT	101	YES	17
S15-050195	BT	109	YES	10
S15-050196	IV	48	YES	60
S15-050197	AB	42	YES	65
S15-050198	BT	86	YES	28
S15-050199	DD	20	YES	83
S15-050200	ML	101	YES	17
S15-050201	DG	76	YES	37
S15-050202	BT	92	YES	23
S15-050203	KW	5	YES	95
S15-050204	PH	100	YES	17
S15-050205	BT	27	YES	78
S15-050206	PH	92	YES	23
S15-050207	AB	55	YES	55
S15-050208	AB	97	YES	19

6. Discussion

During the project, beef samples from 250 Scottish and from 49 Northern Irish cattle were collected. Chemical analysis was conducted on all samples and those isotopes that were shown to have utility in the geographical discrimination of beef (hydrogen, carbon, nitrogen, and sulfur) were used to augment a previously developed isotopic database containing data from approximately 500 UK based cattle.

The isotope data collected for Scotch beef was also assessed for seasonal influences. The isotope ratios that were most likely to be affected by seasonality were carbon (relating to feed source, particularly if grass-based feed is enriched with maize or corn during winter months) and deuterium (relating to climate and changes in temperature). The effect of seasonality was demonstrated to be minor for both isotopes, in relation to the natural variation of the samples.

The performance of the procedure was tested by sending 15 blind (geographical origin information withheld) beef samples to Fera Science Ltd for analysis. The procedure identified those 2-letter postcode regions, which the samples could have originated from. After the geographical origin of these 15 samples was revealed, it was shown that the procedure output correctly confirmed all samples were consistent with their actual postcode. It was noted though, that the isotopic profiles of several samples were also consistent with other regions of the United Kingdom. Consequently, the procedure was updated to provide options for classification, using different confidence settings.

The procedure was also tested with stable isotope profiles from a limited number of non-UK samples from Ireland, Germany, Brazil, Australia, and New Zealand. Using the current procedure, an Irish beef sample could not be differentiated from Scotch beef, a German beef sample could be differentiated from parts of Scotland; whereas the Brazilian, Australian and New Zealand samples were shown to have isotope profiles not consistent with Scotch beef. The latter is directly linked to the carbon isotope composition of the pasture and/or fodder eaten by the cattle. The proportion of C3 versus C4 photosynthetic plant material in the cattle's diet determines whether beef has an isotope signature of around -27 to -24 ‰ or of around -16 to -9 ‰, respectively, if cattle is almost exclusively fed on C4 plants (BBOP-FA0205).

7. Main Implications

The stable isotope ratio analysis data set for Scotch beef has been updated and incorporated into a procedure (UK based web-tool), which is able to (i) confirm the origin of Scotch beef and (ii) detect potential food fraud in geographical mislabelling of Scotch beef, in form of a screening method.

Considering that the majority of global production of beef is from the Americas (45% of Global production, 2013, FAOSTAT) the current procedure can offer significant protection from the mis-labelling of beef as Scotch.

The webtool is available for use by Food Standards Scotland, Defra and Fera Science Ltd and can be configured for the most suitable discrimination.

8. Future work

Recommended future work is to apply the procedure for the analysis of retail samples of Scotch beef to identify potential fraudulent claims of Scotch beef. The experimental design of such a survey should be considered carefully to maximise the ability to detect fraudulent samples. It should also include known authentic Scotch beef and known authentic non-Scotch beef. This will ensure that the tool remains fit for purpose and will determine the power of the procedure to differentiate Scotch beef from other beef on a global scale. For example, further analysis of samples from the Americas, Asia and from different European locations is recommended.

Following the decisions of the United States and Canada to lift the import ban of Scotch beef, it is also recommended that beef, labelled as Scotch and sold in other countries, is tested to ascertain whether fraudulent labelling is occurring. If inferior products are fraudulently labelled as Scotch beef this may undermine aspects of the Export plan for Scotland's food and drink Industry.

Currently, the procedure is not able to consistently differentiate Scotch beef from some parts of the UK, this is due to the natural variation associated with the isotopic signatures. Further discrimination may be possible, through the use of multiple sampling (analysis of several separate samples from a single specific location), although this aspect requires further research to fully characterise what increase in discrimination this would achieve.

9. References

FAOSTAT (livestock primary, UK, 2013): http://faostat3.fao.org/browse/Q/QL/E, accessed 19/05/16.

Sentandreu M.A. et al (2010) A proteomic-based approach for detection of chicken in meat mixes. *Journal of Proteome Research* **9**, 3374-3383.

FA0129: http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=18694, accessed 19/05/16.

Donarski J. et al (2010) Identification of botanical biomarkers found in Corsican honey. *Food Chemistry* **118**, 987-994.

Caetano S. et al (2007) Geographical classification of olive oils by the application of CART and SVM to their FT-IR. *Journal of Chemometrics* **21**, 324-334.

Pierna J.F. et al (2011) Discrimination of Corsican honey by FT Raman spectroscopy and chemometrics. *Biotechnology Agronomy Society Environment* **5**, 75-84.

BBOP – FA0205: http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=18069, accessed 19/05/16.

Camin F. et al (2012) H, C, N and S stable isotopes and mineral profiles to objectively guarantee the authenticity of grated hard cheeses. *Analytica Chimica Acta* **711**, 54-59.

Cleveland W.S., Grosse E, and Shyu W.M. (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.

Brookshire, E.N.J. & Weaver, T. (2015) Long-term decline in grassland productivity driven by increasing dryness. *Nat. Commun.* **6**, 7148.

10. Acknowledgements

The authors like to thank project partners for their contributions to this report.

Fera Science Ltd

Miss Leticia Gaitero

Mrs Celine Pye

Mr Gareth Rees

Mr Malcolm Baxter

Mr Michael Walls

Mr Roy Macarthur

Mr Lee Butler

Mr Andrew Pote

Mr Robert Lake

JHI

Dr Carol-Ann Craig

Dr Barry Thornton

Dr Andrew Midwood

QUB

Mr Manus Carey

Professor Andy Meharg

Disclaimer

Fera hereby excludes all liability for any claim, loss, demands or damages of any kind whatsoever (whether such claims, loss, demands or damages were foreseeable, known or otherwise) arising out of or in connection with the preparation of any technical or scientific report , including without limitation, indirect or consequential loss or damage; loss of actual or anticipated profits (including loss of profits on contracts); loss of revenue; loss of business; loss of opportunity; loss of anticipated savings; loss of goodwill; loss of reputation; loss of damage to or corruption of data; loss of use of money or otherwise, and whether or not advised of the possibility of such claim, loss demand or damages and whether arising in tort (including negligence), contract or otherwise. This statement does not affect your statutory rights.

Nothing in this disclaimer excludes or limits Fera liability for: (a) death or personal injury caused by Fera negligence (or that of its employees, agents or directors); or (b) the tort of deceit; [or (c) any breach of the obligations implied by Sale of Goods Act 1979 or Supply of Goods and Services Act 1982 (including those relating to the title, fitness for purpose and satisfactory quality of goods);] or (d) any liability which may not be limited or excluded by law (e) fraud or fraudulent misrepresentation.

The parties agree that any matters are governed by English law and irrevocably submit to the non-exclusive jurisdiction of the English courts.