Analysis of 13C and 15N isotopes from Eurasian Quaternary fossils: Insights in diet, climate and ecology

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CHAPTER 2

STABLE ISOTOPES: PRINCIPLES AND ANALYTICAL METHODS

2.1 Introduction

This chapter is an introduction to the Chapters 3 to 6, addressing the principles and basics of the application of stable carbon and nitrogen isotopes as a tool for dietary and environmental reconstruction. The popularity of stable isotopes analysis increased almost exponentially over the last few decades (Szpak *et al.*, 2017) and the method is nowadays routinely applied in archaeological investigations. It is, however, important to realize that specific factors have an effect on the stable isotope signals in the different body tissues. The main factors will be discussed in this chapter.

The majority of the stable isotope data discussed in this thesis have been produced at the Centre for Isotope Research (CIO), University of Groningen, the Netherlands. The way the samples are prepared and measured at the CIO is concisely described in this chapter.

2.2 Fundamentals of stable isotope analysis

2.2.1 Isotopes of carbon and nitrogen in nature

In order to understand the behaviour of isotopes, we have to look at atoms first. All atoms consist of a heavy nucleus and a number of electrons moving 'in orbit' around this nucleus. The nucleus consists of protons (charged particles) and neutrons (neutral particles). The number of protons is equal to the atomic number by which a chemical element is identified. However, the mass of a chemical element can differ depending on the number of neutrons in the nucleus. The nucleus of carbon, with 6 protons, can have 6, 7 or 8 neutrons. Hence, there are in nature 3 different carbon isotopes: carbon-12 (^{12}C; 6 protons plus 6 neutrons), carbon-13 (^{13}C; 6 protons plus 7 neutrons), and carbon-14 (^{14}C or radiocarbon; 6 protons and 8 neutrons). Their abundances in nature are respectively 98.9, 1.1 and 10-10%. Nitrogen has two natural isotopes: ^{14}N and ^{15}N, with respective abundances of 99.6 and 0.4%.

The isotopes ^{12}C, ^{13}C, ^{14}N and ^{15}N are stable. ^{14}C is instable or radioactive, and decays through time with a regular pace. Therefore, the amount of ^{14}C in a fossil depends on its age, making this isotope well-known for dating organic samples. The ^{14}C dating method can be applied for organic materials up to around 50,000 years old.

Examples of elements of archaeological interest with more than one stable isotope are carbon (C), nitrogen (N), oxygen (O), hydrogen (H), and sulphur (S), important building blocks of biological molecules. This thesis focusses on carbon and nitrogen.

The different isotopes of a single element have similar chemical properties, and hence, behave chemically more or less the same. However, physically they behave in a different way. The dissimilarity in number of neutrons between isotopes causes different atomic masses. These lead to difference in reaction rates, creating small (predictable) differences in the natural abundances of isotopes in different materials. The lighter

isotopes (that is, the isotopes with the lower mass number) tend to react faster than the heavier, more resistant isotopes which affects the distribution. This process of isotope discrimination is called 'fractionation'. Fractionation leads to an unequal distribution in isotope ratio between the sources (generally more heavier isotopes) and products (generally more lighter isotopes). This is the essence of stable isotope analysis.

A well-known example of a process in which fractionation occurs is photosynthesis. A tree is depleted in ^{13}C (and in ^{14}C) compared to the atmosphere in which it lives, because photosynthesis causes fractionation: photosynthesis is a mass-dependent effect. The heavier isotopes are 'hindered' relative to the lighter ones. This results in a lower $^{13}C/^{12}C$ ratio in the tree than in its surrounding atmosphere.

2.2.2 Stable isotopes of carbon and nitrogen in the laboratory

Stable isotope values can be measured in samples extracted from all kinds of body tissues of both humans and animals, such as hair, bone, and teeth. The main material that is analysed in studies presented in this thesis is (bulk) collagen. Collagen is rich in carbon and nitrogen. It is the main protein found in mammals and facilitates the elasticity of bones. The collagen has to be chemically isolated before its stable isotope values can be investigated.

Stable isotope measurements are performed by mass spectrometry (MS; de Groot, 2004). For isotope ratios, a special form of MS is developed: Isotope Ratio Mass Spectrometry (IRMS). The machinery is based on molecular gases, which means that for this technique, the collagen of the target sample has to be combusted into the gases CO_2 and N_2 before isotope values of respectively carbon and nitrogen can be measured.

After combustion, the gas sample is led via an inlet system into an ion source. This produces a molecular ion beam (CO_2^+ or N_2^+) which is accelerated and steered into the mass analyser (Fig. 2.1). Here, ion beams with different masses are separated by a magnetic field. Heavier isotopes ($^{13}CO_2$ and $^{14}N^{15}N$ as the most relevant examples) follow a straighter flight path than the lighter isotope ($^{12}CO_2$, $^{14}N_2$) beams. The beam intensities of the different ion currents are measured by Faraday-cups. Based on these beam intensities, the isotope ratios are determined.

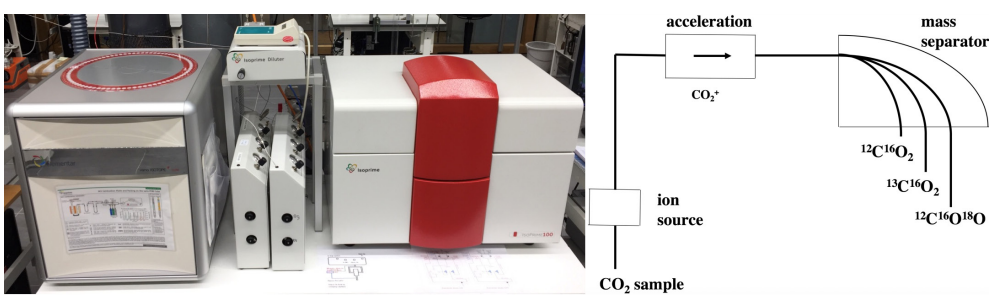

Figure 2.1 Left: Picture of the Elemental Analyser and the IRMS equipment at Groningen, respectively. Right: schematic representation of the way the IRMS operates.

The isotope composition of a sample is given in so-called δ-values. These values are defined as the deviation of the isotope ratio obtained from a sample and that of reference material for normalisation, which is taken along with each measurement. For the carbon and nitrogen isotope ratios these are:

$$^{13}\delta = \frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{reference}} - 1 (\times 1000‰) \quad \text{and} \quad ^{15}\delta = \frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{reference}} - 1 (\times 1000‰)$$

The numerical values for δ are very small. Therefore, they are expressed in per mill (‰, equivalent to 10^{-3}). The notation $\delta^{13}C$ and $\delta^{15}N$ is used in the literature and throughout this thesis instead of $^{13}\delta$ and $^{15}\delta$.

Each time samples are measured by IRMS, the gas of a specific amount of reference material is measured as well. Reference materials consist of 'control standards' and 'normalisation standards', with known isotopic compositions. Control standards are used to monitor the accuracy of the entire pretreatment and measurement procedure and the analytical precision. The measured isotopic value of the normalisation standard has to be 'normalised' and reported relative to the value of internationally recognised reference material. For carbon, this is Vienna PeeDee Belemnite (V-PDB), and for nitrogen, this is ambient inhalable reservoir (AIR). The absolute isotope contents of the reference materials is well known and have been measured very accurately (Mook, 2006 and references therein).

2.3 Principal concepts of the application of stable carbon and nitrogen analysis in archaeological research

Isotope analysis can provide a wide range of information. For instance, $\delta^{13}C$ and $\delta^{15}N$ values can be used as dietary tracers and can serve as indicators for past climatic and environmental conditions. In short, plant $\delta^{13}C$ and $\delta^{15}N$ values are directly controlled by climate and environment (for example, soil processes). Via food consumption, $\delta^{13}C$ and $\delta^{15}N$ values of plants are transferred to their consumers, and finally incorporated into their body tissues.

2.3.1 Stable isotopes of carbon and nitrogen: palaeodiet

Carbon is the universal building block of life. It is a constituent of atmospheric CO_2 and is dissolved in ocean water. Atmospheric carbon forms the key carbon source for terrestrial plants, which they take up by means of photosynthesis, and which is subsequently incorporated by other organisms in the food chain.

Also, nitrogen is a crucial component for all organisms. With a share of about 80% it is the most abundant element in the atmosphere. It is also present (in dissolved form) in ocean water. The N_2 gas is converted by micro-organisms in the soil to ammonium (NH4+; ammonification) or nitrate (NO3-; nitrification) before it is assimilated by

terrestrial plants, or it is made available to plants by nitrogen-fixing microbes living in symbiosis with the plant. In the body of animals and humans, nitrogen is mainly supplied by dietary protein.

$\delta^{13}C$ and $\delta^{15}N$ values, measured in body tissues, can reveal indirect information about the diet of an individual. Food sources have different isotope compositions. Through consumption, these isotopic compositions are incorporated into body tissues, considering some (predictable) change due to fractionation. In turn, stable isotopes in body tissues can provide information on the diet by examining their δ-values.

The entity of analysis - $\delta^{13}C$ and $\delta^{15}N$ values of a single sample - is the individual. It allows to determine differences in diet between individuals. Moreover, isotope composition can also vary between groups. Sample sets representing larger numbers of individuals can provide information at for instance community-scale (for example, about economic or social aspects) or about regional or temporal variability (Schoeninger and Moore, 1992).

For archaeology, the most well-known application of stable isotope research concerns the reconstruction of palaeodiet (Michener and Lajtha, 2007). Based on the $\delta^{13}C$ and $\delta^{15}N$ values of (fossil) animal and human remains it is possible to distinguish herbivores, omnivores, carnivores, C3 and C4 plant consumers, and consumers of aquatic diet - to mention the main categories. This is best illustrated by the famous phrase "you are what you eat (plus a few ‰)" (DeNiro and Epstein, 1976; Kohn, 1999).

It is a general ecological rule that the cohabitation of species leads to niche differentiation that minimises direct competition. Such differentiation is often well reflected in a bivariate diagram that shows the specific $\delta^{13}C$ and $\delta^{15}N$ values. Indeed, a general (and more or less standard) way to present $\delta^{13}C$ and $\delta^{15}N$ data, is to plot the data in a consistent way - $\delta^{13}C$ horizontally, and $\delta^{15}N$ vertically, which enables an easy comparison. Figure 2.2 shows a schematic plot, where the various dietary groups are indicated by their hypothetical, general $\delta^{13}C$ and $\delta^{15}N$ values.

The consumption of C3 or C4 plants, in particular, leads to clearly distinguishable $\delta^{13}C$ signals. Plants using the C3 photosynthetic pathway show a range of $\delta^{13}C$ values around -27‰, whereas C4 plants have much higher $\delta^{13}C$ values of around -13‰ (Marshall *et al.*, 2007). In addition, there is a category known as CAM plants, with $\delta^{13}C$ values in between those of C3 and C4. Most plants are of the C3 type. Prime archaeological examples of C4 plants are corn and millet. Distinctive increases of $\delta^{13}C$ value are also observed in the food chain. There is an enrichment of the $\delta^{13}C$ value by about 1‰ for each trophic level shift (Lanting and van der Plicht, 1998; Fig. 2.2).

More pronounced differences between trophic levels are observed in $\delta^{15}N$ values. The isotopic enrichment of the $\delta^{15}N$ value is about 3-5‰ for each trophic level shift (Bocherens and Drucker, 2003; Hedges and Reynard, 2007). This means for instance, that the bone collagen of a carnivore is enriched in ^{15}N relative to ^{14}N in comparison with the amount of ^{15}N and ^{14}N originally present in their prey species, and herbivores have higher $\delta^{15}N$ values than the plants they consume (Schoeninger and Moore, 1992;

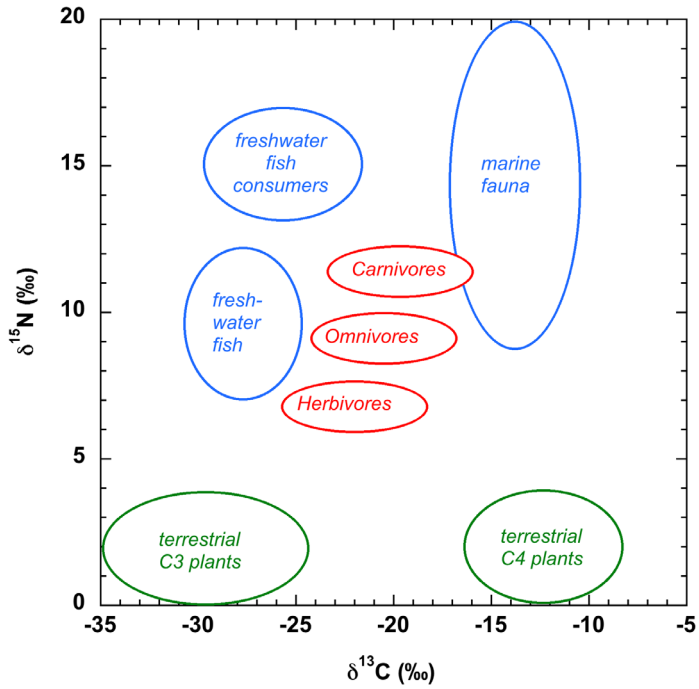

Figure 2.2 Schematic representation of (part of) the food web, indicating the ranges of hypothetical $\delta^{13}C$ and $\delta^{15}N$ values for terrestrial fauna (red), aquatic fauna (blue) and terrestrial plants (green).

Gannes *et al.*, 1998; Sealy, 2001).

Identification of the exploitation of terrestrial and aquatic resources is based on observable differences between organisms living in an aquatic (with in general higher $\delta^{15}N$ values) and terrestrial environment (in general lower $\delta^{15}N$ values) mainly due to differences in the nitrogen and carbon sources within the two systems. For freshwater reservoirs, typical values for animals and humans are around -25‰ and +13‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively (Arneborg *et al.*, 1999; Cook *et al.*, 2001; Philippsen, 2013). For a marine reservoir, these $\delta^{13}C$ and $\delta^{15}N$ values are around -11‰ and +13‰, respectively (for example, van der Plicht *et al.*, 2016).

Diet composition also plays an important role in establishing $\delta^{13}C$ and $\delta^{15}N$ values in tissues of coeval living species from the same trophic level, for instance terrestrial herbivores. In brief, their $\delta^{15}N$ values vary due to nitrogen fixation and type of mycorrhizal associations between consumed plants, and even different $\delta^{15}N$ values can be observed between plant organs of a single plant. In general, grasses and sedges have higher $\delta^{15}N$ values than lichens, mosses and shrubs, and the values in trees are even lower than those in shrubs (Högberg *et al.*, 1996; see summary in Bocherens [2003] and Drucker *et al.* [2010]). Therefore, browsers tend to have lower $\delta^{15}N$ values than grazers (Ambrose, 1991; Drucker *et al.*, 2010).

Specifically, the protein content of consumed plants affects $\delta^{15}N$ values in herbivore tissues. Animals cannot store amino acids to any great extent in their tissues. The higher the protein throughput, the more the body needs to get rid of excess nitrogen by urea

excretion. Due to fractionation, the isotopically lighter ^{14}N is favoured during excretion compared to the heavier ^{15}N, resulting in the retention of heavier ^{15}N in the body. Therefore, protein rich diets lead to higher $\delta^{15}N$ values in body tissues than nutrient poor diets (Ambrose, 1991; Sponheimer *et al.*, 2003a; Fuller *et al.*, 2005).

On the other hand, higher $\delta^{15}N$ values are also found in studies on animals which had very nitrogen-poor diets (Hobson *et al.*, 1993). No consensus has been found explaining the exact mechanism behind the enrichment of ^{15}N in the body of animals that live under extremely nitrogen-poor conditions and/or during water stress. Recycling of internal (body) nitrogen resources for nitrogen concentration purposes is suggested to be contributory, since this would result in the excretion of (small amounts of) concentrated ^{14}N-depleted urea (Sealy *et al.*, 1987; Adams and Sterner, 2000). However, this violates the model of isotopic mass balance: if nitrogen is conserved rather than excreted, ^{15}N enrichment is no option (Ambrose, 1991).

2.3.2 Stable isotopes of carbon and nitrogen: palaeoenvironment

The $\delta^{13}C$ values in plants are primarily determined by the measure of exchange of CO_2 and moisture between the plant leaves and the atmosphere (so-called stomatal conductance; Marshall *et al.*, 2007), which is consecutively affected by various factors. In the terrestrial biosphere, fractionation of $^{13}C/^{12}C$ is mainly governed by the type of the photosynthetic pathway used by plants. In the mammoth steppe, plants used the C3 photosynthetic pathway (Blinnikov *et al.*, 2011). The $\delta^{13}C$ values of C3 plants are mainly determined by (local) environmental factors, such as humidity, atmospheric CO_2 concentrations (Polley *et al.*, 1993), and the occurrence of dense forest (van der Merwe and Medina, 1991). These factors cause geographical gradients in $\delta^{13}C$ values observed on the continent. In addition, there are also altitudinal gradients (van Klinken *et al.*, 1994; Kohn, 2010).

In forests, the 'canopy effect' produces ^{13}C-depleted plants and a gradient of leaf $\delta^{13}C$ values from ground to canopy; the most negative values are near the ground. Explanations for this phenomenon include recycling of ^{13}C-depleted CO_2 in the forest, fractionation due to photosynthesis in low light, and other plant physiological aspects (van der Merwe and Medina, 1991; Noe-Nygaard *et al.*, 2005). Thus, in densely forested regions, the $\delta^{13}C$ values of vegetation tend to be more negative than in open landscapes. In turn, the canopy effect is recognizable in the $\delta^{13}C$ values of bones of animals that feed on plants from a forested habitat (Bocherens and Drucker, 2007; Drucker *et al.*, 2008; Doppler *et al.*, 2017).

Within the terrestrial biosphere, $\delta^{15}N$ values in plants are determined by various biogeochemical processes within the plant and in their surrounding soil, including nitrogen cycling. But also nutrient availability and the type of mycorrhizal fungi living in symbiosis with the plant play a role (Nadelhoffer *et al.*, 1996; Högberg, 1997; Hobbie and Högberg, 2012; Szpak, 2014). Most of these processes are linked to one another and relate to climatic conditions such as humidity and temperature.

Fluctuations in $\delta^{13}C$ and $\delta^{15}N$ values in terrestrial herbivores have been observed that could be linked to changing climatological aspects, such as temperature (Bocherens *et al.*, 2006a,b) and aridity (Kohn, 2010). Besides aspects related to (isotopic) diet composition, climatic and environmental conditions, species-specific physiological aspects can cause differences in $\delta^{15}N$ and $\delta^{13}C$ values between herbivores (Gannes *et al.*, 1998; Schoeller, 1999; Sponheimer *et al.*, 2003b).

2.3.3 Isotope signals in different tissues

Stable isotope analyses can be performed on samples that vary in size from tens or hundreds of milligrams (for example, bone), a few milligrams (for example, hair), to molecular-scale level (for example, individual amino acids). Each sample strategy and sample type have their own possibilities and constraints. Sampling strategy should therefore be carefully considered prior to sample taking, bearing in mind aspects such as research question and properties and conditions of the available material (Phillips *et al.*, 2014).

The signals can represent different time-windows, from days or months up to an average of several years, depending on the turnover time of the sampled tissue and sample strategy. For instance, bone is constantly renewed throughout life and the measured signals in bulk samples of bone reflect dietary records of several years. Short-term dietary signals, such as seasonal change in food supply, can be observed in smaller, serially taken samples from for instance hair.

Among sample types, skeletal elements such as bone, antler and tooth dentine are routinely used for dietary reconstruction. Bone (and antler and bone dentin) is composed of an interconnected mineral and organic structure, which is built from molecules present in food. The mineral component (± 70%) is called bio-apatite. The organic part of bone is mainly made up of collagen (that is, collagen type I). Furthermore, bone consists of other proteins and water (Jans, 2005).

The collagen fraction (including collagen of bone, antler and dentine, henceforward referred to as 'bone collagen' or simply 'collagen') is predominantly used for stable isotope analysis to determine past diet. It can be chemically isolated and since it does not, in principal, exchange carbon with the environment, it is the most inert fraction of bones for ^{14}C dating. The main fraction used throughout this thesis is (bulk) collagen.

2.3.4 Quality aspects of collagen used for isotope analysis

Bone collagen is sensitive to degradation and this explains why the majority of stable isotope analyses using collagen has been carried out on geologically young material with a Holocene or Late Pleistocene age (usually within the ^{14}C time scale, that is, up to 50,000 years ago). The occurrence of good quality collagen in bones that are older than 50,000 years decreases with age. However, if one were excluding bone collagen examination on forehand based on the age, one might miss out on possible valuable

information from the fossils, such as stable isotope or DNA data.

After deposition, bones can be contaminated with exogenous biomolecules and other foreign substances. Several kinds of taphonomical processes, such as hydrolysis, wear and attack by soil bacteria and fungi cause bone degradation or so-called 'diagenesis'. Diagenesis can lead to contamination with exogenous matter from surrounding deposits and/or loss of crystalline or organic components such as collagen. The change in physical and chemical properties of fossils caused by diagenesis may alter the isotope values of proteins, in particular in the case that fossils have a low collagen content. The degree of diagenesis- and in turn preservation state- is related to numerous factors, including local groundwater level, porosity of the skeletal element, temperature, sediment type, and time. Therefore bone (collagen) preservation varies from one burial environment to another (Hedges and Millard, 1995; Bocherens *et al.*, 1997b; Jans, 2005; Harbeck and Grupe, 2009).

It is essential for the validity of the isotope data that the collagen is well enough preserved, or in other words, that the collagen is pure enough and of good quality (Jans, 2005; Koch, 2007; Smith *et al.*, 2007; Harbeck and Grupe, 2009). In order to get rid of potential contaminants, the collagen extraction procedure includes chemical steps with the purpose of removing as many potential contaminants. In general, an organic solvent pretreatment is applied prior to the routine pretreatment in case the sample is covered with glues or preservations. Procedures may vary depending on sample type and properties, and between laboratories.

Collagen integrity can be estimated in different ways. The most common indicators are the carbon and nitrogen extraction yields of the collagen, denoted as respectively %C and %N. These are automatically provided by the mass spectrometer. Also, the atomic C/N ratio ($C:N_{atomic}$ = %C/%N x 14/12) is a widely accepted quality parameter, with values of 2.9-3.6 to be considered acceptable (Szpak *et al.*, 2017). In addition, based on comparison with the chemical composition of collagen extracted from fresh bone using the same purification treatment, the carbon content of genuine collagen should be around 30-40% and its nitrogen content around 11-16% for the results to be reliable (van Klinken, 1999).

The weight proportion of the extracted collagen in relation to the initial sample weight (% yield) is preferably minimally 0.5% (according to van Klinken, 1999) or 1% (according to Ambrose, 1993). For fresh bone, this yield is about 20%. Prior to the collagen extraction, the % yield can be guesstimated by measuring the proportion of the percentage of nitrogen in the initial sample (Bocherens *et al.*, 2005). Such a technique may be efficient to select promising samples in an early stage (with percentages close to the 4% N found in fresh bones [Brock *et al.*, 2010]), and rejecting samples which are examined to yield low quality collagen (Brock *et al.*, 2010, 2012). However, a deficiency is that the high percentage of nitrogen in 'promising' samples might partly be derived from nitrogen-containing contaminants present in the sample (Brock *et al.*, 2012). This can even result in varying nitrogen percentages within one bone. Indeed, ambiguity

exists in the relationship between %N and collagen content (Jacob *et al.*, 2018). Another proxy used to assess the integrity of the collagen is amino acid analysis; for instance, by comparing the amino acid profiles in the extracted collagen with such profiles of a fresh bone (Harbeck and Grupe, 2009).

2.4 Isotope measurements at the Centre for Isotope Research (CIO), Groningen

Chemical preparation, ^{14}C dating, and stable isotope measurements of the samples discussed throughout this thesis were, unless mentioned otherwise, performed at the Centre for Isotope Research (CIO), Groningen University, the Netherlands, following standard procedures (Mook and Streurman, 1983; see for a recent update Dee *et al.*, 2019).

In short, the bone, antler and dentine samples are decalcified over at least a 24-h period using weak acid (HCl, 4% w/vol). When CO_2 release has ceased and the fragments have become soft and pliable they are rinsed thoroughly with distilled water (DW). Following the demineralisation, the samples are soaked into NaOH (1%, > 30 min) to eliminate humic acids, rinsed to neutrality and treated once more with acid (HCl, 4% w/vol, 15 min). The raw collagen fraction is then denatured to gelatine in acidified DW (pH 3) at 90 ºC for 18 h. Before drying, the dissolved gelatine is filtered through a 50 μm mesh to eliminate any remaining foreign particulates.

The collagen product is combusted to obtain CO_2 using an Elemental Analyser (Isocube, Elementar) connected to an IRMS (Isoprime 100, Elementar), providing the isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ (Fig. 2.1). For carbon, the international standards NBS 18, NBS 19 and LSVEC are used to calibrate by two-point normalisation relative to V-PDB. For nitrogen, IAEA-N1 and IAEA-N_2 are the international standards used to calibrate relative to AIR. The combined uncertainty is about 0.3‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. To monitor analytical accuracy and precision, (internal) normalisation standards and control references are used. For $\delta^{13}C$, these are oxalic acid (-17.6‰), caffeine (-38.2‰), CaN (caffeine mixed with $\delta^{15}N$-enriched caffeine, +0.61‰) and collagen (-19.87‰); for $\delta^{15}N$ these are CaN (+19.04‰), caffeine (-6.61‰) and collagen (+10.07‰).

Before 2013, the isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were measured in separate samples by using another Elemental Analyser (NEA). The $\delta^{15}N$ values were measured in duplicate. To monitor analytical accuracy and precision, slightly different (internal) normalisation standards and control references were used. For $\delta^{13}C$, these are oxalic acid (-17.6‰), caffeine (-38.2‰), anthracite (-23.25‰); for $\delta^{15}N$, these are N1 (+0.43‰), N2 (+20.32‰), collagen (+9.8‰) and flower (+1.7‰). The $\delta^{13}C$ and $\delta^{15}N$ values discussed in this thesis and measured at the CIO, have been measured either by using this older Elemental Analyser or by the previous mentioned Isocube.

Throughout the thesis, the $\delta^{13}C$ and $\delta^{15}N$ values are often reported and discussed in conjunction with ^{14}C dates. Most ^{14}C dates in the tables are from Groningen; they can be recognized by the laboratory reference numbers with the following prefixes:

- GrN: conventional (radiometry by proportional gas counters), used until 2011
- GrA: AMS (3 MV Tandetron accelerator, High Voltage Engineering), used from 1994 to 2017
- GrM: AMS (200 kV compact accelerator [MICADAS], IonPlus AG), used since 2017.

^{14}C dates are reported in BP, which by convention means that the ^{14}C radioactivity is measured relative to the Oxalic Acid standard, that the dates are calculated using the Libby half-life of 5568 years, and that isotope fractionation is corrected for using the ^{13}C isotope to $\delta^{13}C = -25‰$ (Mook and van der Plicht, 1999).

2.5 Application of stable isotope analysis from an historical perspective

Today, $\delta^{13}C$ and $\delta^{15}N$ analyses of bones and teeth are almost standardly implemented within archaeological research. However, half a century ago, stable isotope analysis was used only occasionally. The intensification of the use of stable isotopes analysis within the last few decades is partly related to developments within the archaeological discipline itself and to the improved application of chemical, physical, environmental and mathematical research methods. Moreover, the enhanced sophistication and user-friendliness of IRMS, the development of quality controls, the accumulation of available stable isotope datasets and a substantial progress concerning the interpretation of the data have been of crucial value for a useful implementation of isotope studies within an archaeological research context.

Since the invention of the mass spectrometer by Thompson in 1910, several types of IRMS have been developed. The first models required samples to be purified manually before entering the mass spectrometer. In the course of the 1980s-1990s, semi-automated and later fully automated combustion systems (EA, Elemental Analyzer) combined with the mass spectrometer were developed: EA-IRMS.

These technological developments are related to those in the ^{14}C dating realm. ^{14}C fractionates, and hence, ^{14}C dates need to be corrected for this effect. This is done by measuring not only the ^{14}C isotope, but also, in the same sample, the abundance of ^{13}C (Mook and Streurman, 1983). IRMS was introduced in the ^{14}C laboratories in the 1960s. The dating of (archaeological) samples resulted automatically in additional $\delta^{13}C$ data. This became soon supplemented by $\delta^{15}N$ analysis of the same bone (Schoeninger *et al.*, 1983). Initially, this required analysis of a second bone sample - a sample for both C isotopes, and one for ^{15}N. Later, EA-IRMS systems could provide both $\delta^{13}C$ and $\delta^{15}N$

values for one sample. Today, even systems for combined $^{13}C/^{14}C/^{15}N$ analysis (EA-IRMS-AMS) exist (Synal *et al.*, 2013).

Initial applications of $\delta^{13}C$ and $\delta^{15}N$ analysis in archaeology were mainly used to identify the consumption of different resources. In particular, $\delta^{13}C$ values were primarily used to distinguish between consumption of C3 and C4 plants (Vogel and van der Merwe, 1977). This application is based on the observation by the geochemist Craig (1953) who discovered that plants that use different photosynthetic pathways show distinct stable carbon isotope ratios. In the beginning, $\delta^{15}N$ values were mainly used for the determination of trophic levels and discrimination between the exploitation of terrestrial and aquatic resources (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Walker and DeNiro, 1986; Yesner, 1987). The application has expanded since and includes a huge range of research topics including animal husbandry practices (Doppler *et al.*, 2017; Makarewicz, 2017; Cubas *et al.*, 2019) and the estimation of weaning age (Reynard and Tuross, 2015; Guiry *et al.*, 2016). Another significant application of $\delta^{13}C$ and $\delta^{15}N$ of fossil bone is providing information about reservoir effects in ^{14}C dated bone. Aquatic reservoirs are depleted in ^{14}C relative to atmospheric and terrestrial reservoirs. Therefore, archaeological bone of fish consumers date too old on the BP-timescale. The $\delta^{13}C$ and $\delta^{15}N$ values of such bones can be used as proxies in order to estimate the size of the reservoir effect, which can be hundreds of ^{14}C years (Cook *et al.*, 2001; Philippsen, 2013; Wood *et al.*, 2013; van der Plicht *et al.*, 2016).

The sophistication of the IRMS technology over the years has today resulted in lower prices for isotope analysis, faster sample throughput, and smaller sample sizes, allowing to measure on a completely different scale and addressing questions that initially could not be answered.

Wrangel Island, photo: A. Tikhonov