Past, present and future perspectives in stable isotope analysis: capabilities and constraints

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The author gives an introduction to stable isotope analysis in general and an overview of the most commonly used stable isotopic systems (hydrogen, carbon, nitrogen, oxygen, sulphur, strontium) in bioarchaeology, prehistoric and historical research, and their application to different materials. The focus of the article is on the most important possible materials for examination, such as bone and tooth enamel. For each isotopic system, basic principles, application examples and possible constraints are given.
Keywords: stable isotopes, bioarchaeology, bone, enamel

Questo articolo è una introduzione alle analisi degli isotopi stabili in generale, con una panoramica dei principali isotopi stabili (idrogeno, carbonio, azoto, ossigeno, zolfo e stronzio) e delle loro applicazioni in bioarcheologia, ricerca storica e preistorica.Il contributo mette a fuoco inoltre i possibili materiali di analisi, come osso e smalto dentario. Per ogni sistema isotopico sono forniti principi basilari, esempi applicativi e limiti.
Parole chiave*: isotopi stabili, bioarcheologia, osso, smalto*

1. Introduction

Information on individual and collective life histories is stored in the substance of skeletons, which need to be deciphered.

Fossil vertebrates have been studied since the late 1970s for their stable isotope composition. These isotopic data are sometimes used as proxies for environmental factors, such as precipitation or temperature. Research on biological issues like diet, thermoregulatory physiology, habitat preference and migration, was pushed by paleoanthropologists studying ancient humans and their ancestors (van der Merwe 1982; DeNiro 1987; Ambrose, Krigbaum 2003).

Information on the development of human populations in time and space especially in early times without written records needs careful research on ecological and natural conditions in certain niches. According to existing climatic parameters such as forest cover, average temperature or precipitation, humans adapt their subsistence strategies to the available ecosystem. This ecosystem will be subdued to anthropogenic influence that might change fauna and flora. Thus, archaeological skeletal findings, human or animal, are the material of choice to reconstruct ancient ecological, economic and social systems and their development in time and space.

Modern application fields apart from archaeology are found in ecology, where these analyses are established for a long time. Additionally, stable isotopes are used in forensic science and food protection. For ecologists the definition of migration and habitat preferences of animals matter (DeNiro 1987; Koch *et alii* 1995; Chamberlain *et alii* 1997; Hobson 1999; Cerling *et alii* 2006). With advancing globalisation it is more and more important to ascertain the origin and authenticity of food (Rossmann *et alii* 2000; Rummel *et alii* 2010; Voerkelius *et alii* 2010). In forensics most of the work is about the identification of the geological origin of unknown dead individuals (Vogel, Eglington, Auret 1990; Cerling *et alii* 2006; Rauch *et alii* 2007). In addition, technological advances have made isotopic analysis more routine.

Vertebrates consist of tissues with different macromolecular and elemental composition, different styles of growth and turnover. All these tissues have different potentials for post-mortem preservation. Soft tissues (skin, muscle, hair and feathers) contain protein and lipids, and usually do not persist over longer periods in usual settings (except permafrost, dry environments). For isotopic analyses mineralized tissues are best suited, such as bone, tooth enamel and dentin, eggshell, and otoliths. Bone, tooth dentin and enamel are composites of mineral, protein, and lipids. The mineral portion consists mainly of a highly substituted hydroxylapatite ($Ca_{10}[PO_4]_6[OH]_2$), best described as bioapatite (Grupe *et alii* 2012). In bioapatite hydroxyl and phosphate groups are substituted by carbonate in a few weight percent. Diverse cations can be found substituting for calcium, such as Sr or Pb. The bioapatite crystal lattice is interwoven with organic matrix, mainly consisting of collagen. The weight percent for collagen is about 30 % of the dry weight. Enamel is denser than bone and consists of more than 95 % mineral. Tooth dentin resembles bone in its composition, but is more solid than bone (Lowenstam, Weiner 1989).

The comparison of isotopic signals from different skeletal elements of a single individual can show mobility during life time, e.g. a change of res-

idence from the one geological or ecological region to a different one. The isotopic signal of tooth enamel only shows the isotopic mixture of the first living years during which to tooth was built (according to crown formation time). The bone reflects the isotopic history of an individual in its last living years (depending on the bone and its specific turnover rate). Are both isotopic signals of enamel and bone different e.g. from the expected local isotopic values, the individual moved just relatively short before its death (Price *et alii* 2003; Schweissing, Grupe 2003).

All molecules that are used in archaeometric analyses have to be tested on integrity (Kohn, Schoeningen, Barker 1999; Sharp *et alii* 2003). More or less all archaeological or soil embedded findings are in differing states of decomposition. Therefore, it is indispensable to keep the possibility of degradation in mind, as always considerable contamination can be present, for example due to microbial biomass or other substances from the surrounding substrate (Grupe *et alii* 2012). The different causes of degradation and the suitable methods for quality proof are discussed in literature (Turner-Walker 2008; Harbeck, Grupe 2009).

2. What are stable isotopes?

Several elements on Earth occur in more than one stable form. These forms are called "isotopes". Stable isotopes are energetically stable and do not decay and therefore are not radioactive. Isotopes are atoms that differ in atomic masses because of different numbers of neutrons in the nucleus (Hoefs 2004). All stable isotopes of the same element are chemically equivalent because they have the identical number of protons and electrons. However, their chemical behaviour (reaction rate and bond strength) varies because chemical and physical properties are related to the atomic mass. These differences in chemical and physical behaviour result in a variation in the ratios of heavy to light isotopes in organic compounds which are called "isotope effect" (Sulzman 2007).

Elements, that are especially susceptible for fractionation events are hydrogen, carbon, nitrogen, oxygen and sulphur. There are the most abundant elements on earth and closely associated with biosphere, hydrosphere and lithosphere. Therefore, a lot of possibilities can be found to gain information about geological or ecological processes and their connections in different habitats, environments and milieus (Faure 1986).

Table 1 lists the most common elements used in isotopic research and their abundances. The lighter isotopes dominate research because they dominate biological compounds as well as the relative increase in mass

Element	Isotopes	Abundance (%)	International Standard
Hydrogen	^{1}H	99,985	Vienna Standard Mean Ocean Water (VSMOW)
	^{2}H (also D)	0,0155	
Carbon	^{12}C	98,892	Vienna Pee Dee Belemnite (VPDB)
	^{13}C	1,108	
Nitrogen	^{14}N	99,635	Atmospheric nitrogen (AIR)
	^{15}N	0,365	
Oxygen	^{16}O	99,759	VSMOW in water, generally VPDB in CO_2 or carbonate
	^{17}O	0,037	
	^{18}O	0,204	
Sulphur	^{32}S	95,00	Vienna Cañon Diablo meteorite troilite (VCDT)
	^{33}S	0,75	
	^{34}S	4,21	
	^{36}S	0,014	
Strontium	^{84}Sr	0,56	US Geological Survey *Tridacna*
	^{86}Sr	9,87	
	^{87}Sr	7,04	
	^{88}Sr	82,53	

Tab. 1. Relative abundances of common elements used in isotopic research in order of increasing mass (modified after Hoefs 2004; Sulzman 2007).

caused by the addition of a single neutron is greatest in these. Iron, lead and strontium are among the heaviest isotopes used and their application is not very wide spread (except for strontium).

As the isotopic differences are remarkably small, the isotopic composition is reported relatively to an internationally accepted standard and expressed in terms of 'delta' (δ) values (for light elements).

$$\delta^H X = [(R_{SAMPLE}/R_{STANDARD}-1)] \times 1000$$

The δ notation is specified here for a particular element (X = H, C, N, O or S), the superscript H gives the heavy isotope mass of the element

(^{2}H, ^{13}C, ^{15}N, ^{18}O, or ^{34}S), and R is the ratio of the heavy isotope to the light isotope, ^{2}H/^{1}H, ^{13}C/^{12}C, ^{15}N/^{14}N, ^{18}O/^{16}O, or ^{34}S/^{32}S (Fry 2006).

Most δ values range between -100 and +50 ‰ for natural samples, while many δ values are negative (Fry 2006). These indicate relatively less heavy isotope in the sample than in the standard. Samples with higher δ values are relatively enriched in the heavy isotope.

3. Reactivity and fractionation

The chemical reactions of an atom are controlled by its electrons in the shell and the chemical behaviour of two isotopes is qualitatively similar. The atomic mass determines the vibrational energy of the nucleus. Thus, differences in mass lead to differences in both reaction rate and bond strength.

Two isotopes are therefore quantitatively different in their physical behaviour, with the greatest differences between the light isotopes that show the greatest relative difference in mass. A deuterium atom has for example is double the mass of a hydrogen atom. In contrast, ^{87}Sr is only 1,2 % heavier as ^{86}Sr.

Mass differences cause differences in physical behaviour because the kinetic energy is constant for a given element in fixed environments. Molecules of greater mass will travel at slower velocity as a smaller molecule in the same physical environment, e.g. at the same temperature (Sulzman 2007). The energy of a molecule with a heavy isotope is lower than with a lighter one and therefore it forms more stable, stronger bonds and is less easily removed from such a bond.

These differences in velocity and bond strength lead to fractionation effects which cause differences between a source and the product compound of a chemical transformation.

Two isotopic fractionation mechanisms are very common: 1) equilibrium and 2) kinetic reactions. Equilibrium fractionation reactions happen when the distribution of isotopes differs in chemical substances or phases (vapour and liquid), when back reactions can take place and a chemical equilibrium can be realized. In kinetic reactions usually the lighter isotope reacts faster and the play a role when for some reason the reverse reaction can not happen such as evaporation in an open system (Fry 2006).

Without fractionation only a uniform distribution of isotopes would be detectable. The degree of isotopic fractionation can be used to examine the magnitude or rate of a process (Koch 2007).

4. Isotope circulation in the biosphere

4.1. Hydrogen isotopes

The main reservoir of hydrogen in the biosphere is the ocean water. The water cycle shows various processes that lead to large-scale geographic patterns with characteristics that help in defining ecological habitats, niches and provenance. Hydrogen in animal tissues derives mainly from three sources: diet (60 ‰), drinking water (30 ‰) and exchangeable hydrogen (10 ‰) (Fry 2006).

Numerous conversions between liquid and water vapour during evaporation and condensation involve kinetic and equilibrium reactions with isotope effects. Evaporating water from the ocean has δ^2H (or δD; for Deuterium) values of -10 to -20 ‰. δ^2H decreases further as water vapour moves inland and up mountains it continuously loses water. Large rivers that have continental origins, and that are fed by melting snow can have much lower δ^2H values than coastal marine waters. Studies showed that strong fractionations occur during photosynthesis, cellulose formation and transpiration, but in the end they balance each other so the resulting δ^2H values are strongly related to those of local water (Fry 2006). With care, these indicators for source water isotopic composition can be a parameter for reconstructions of past climates and ecologies.

Water isotopes (hydrogen and oxygen) can be used as markers for water sources. Global-scale maps and animations of hydrogen and oxygen isotope variation are available on the Web (see e.g. www.waterisotopes.org). West *et alii* (2010) pooled research on hydrological isotopes from all over the world and collected the papers in their book on "isoscapes" (West *et alii* 2010). Water vapour in normal lab air can exchange with hydrogen in organic materials about 10 to 20 ‰, but the effect is well studied and can be corrected (Fry 2006).

Hydrogen and oxygen isotopic ratios can be powerful tools for studies of migration (Bowen, Wassenaar, Hobson 2005; Brettell *et alii* 2012a; Hobson *et alii* 2012), palaeoclimate (Hafner *et alii* 2011; Hobson *et alii* 2012) and ecological networks (Van Wilgenburg, Hobson 2010; Peters *et alii* 2012).

4.2. Carbon isotopes

Carbon is an essential element in our biosphere as it occurs in any living creature and is (after oxygen) the most important element according to mass. Therefore it is found in many varying compounds on Earth from

highly reduced organic compounds in the biosphere to highly oxidized inorganic compounds like CO_2 and carbonates (Hoefs 2004).

The carbon cycle includes active exchanges of CO_2 among the atmosphere, terrestrial ecosystems and the surface water (mainly ocean). In response to input of ^{13}C depleted CO_2 from fossil fuel burning and decomposition the $\delta^{13}C$ value of atmospheric CO_2 is decreasing by almost 1 ‰ during the past 100 years, from about -7 to -8 ‰ (Fry 2006).

The main source for uptake of carbon in mineral and organic substrates is food. Each tissue, bones, teeth, etc., varies in $\delta^{13}C$ value from diet in a characteristic amount. Between food and bioapatite or calcium carbonate an enrichment of ^{13}C of 9 to 10 ‰ can be expected because of fractionation reactions among dissolved carbon dioxide, body fluid bicarbonate, and carbon-bearing minerals at mammal and bird body temperatures (Koch 2007).

During diet-to-mineral fractionation can occur a number of differences in different species, but studies have shown that a relatively large enrichment in ^{13}C can be found where microbial fermentation is involved (Hedges 2003; Passey *et alii* 2005). During fermentation methane is produced which is depleted in ^{13}C and than leaves the body. While ^{13}C enriched carbondioxide can diffuse from the gut to the blood and enter body fluid bicarbonate and mineral carbonate compound (Koch 2007). In marine and in terrestrial mammals the diet-to-bioapatite fractionations are similar (Clementz, Koch 2001). Carbon in bioapatite of ectothermic acquatic animals stems from respiration and surrounding water (Vennemann *et alii* 2001), which means, that any change in the environment (e.g. temperature) has an influence on the isotopic composition of body fluids.

Proteins are composed of amino acids and essential amino acids must originate from dietary protein (Koch 2007). The bulk diet-to-collagen fractionation is 3 to 5 ‰ in mammals and birds. For herbivores and carnivores the picture is quite clear. The effects of carbon routing is more complicated in the case of omnivores, because different sources of food can add different $\delta^{13}C$ values to the bulk diet value (Fogel, Tuross 2003). There is still not enough research present on fish, but it seems to be that fish exhibit more variability in the chemical composition of their bone than mammals (Szpak 2011).

The isotopic composition of terrestrial ecosystems is controlled by the $\delta^{13}C$ values of plants respectively by the photosynthetic pathway, which means it is controlled by the primary producers at the base of the food web (Koch 2007). The most common is the C3 photosynthetic pathway, performed by all trees, most shrubs and herbs, and grasses with a cool growing season.

Carbon uptake by C3 plants, which are dominant on land, involves a net fractionation of about 20 ‰ between the atmospheric CO_2 and plant biomass (-27 ‰; range -22 to -35 ‰). In contrast, C4 plants show a less pronounced fractionation during carbon uptake of about 5 ‰, which results in average in a δ ^{13}C value of -13 ‰ (range -19 to -9 ‰). C4 photosynthesis can be found in some sedges and dicotyledons, and in grasses in arid regions of warm growing seasons (Koch 2007). A rare pathway is that of succulent plants, called the crassulacean acid metabolism (CAM). Their δ ^{13}C values range between those of C3 and C4 plants.

The exchange reaction between the atmospheric and the surface of the ocean involves an equilibrium fractionation between atmospheric CO_2 (-8 ‰) and the total CO_2 ($\sum CO_2$, mostly bicarbonate) in surface water (about 1 ‰). Algal values are about -19 to -24 ‰ due to larger kinetic fractionations during uptake of dissolved inorganic carbon in plankton photosynthesis. The δ ^{13}C values in components of the carbon cycle in freshwater vary depending on the source of dissolved CO_2 in the waters. Possible sources in freshwater are carbonate rock weathering, atmospheric CO_2, mineral springs and organic matter respiration. Strong input from the latter can result in δ ^{13}C values for dissolved CO_2 up to -20 ‰, whereas algal fractionation during carbon uptake can decrease δ ^{13}C C values to -45 ‰ (Fry 2006).

There are some effects known that can affect δ ^{13}C values. C3 plants for example can exhibit large differences depending on their habitat. In closed canopy forests the δ ^{13}C value of forest floor leaves can be ^{13}C depleted by up to 8 ‰ relative to leaves from the top of the canopy. This is due to the recycling of ^{13}C depleted carbon dioxide (Koch 2007). δ^{13}Cvalues vary in vertebrates with diet (photosynthetic pathway; marine vs. freshwater vs. terrestrial food), location and ecological parameters related to plant type and carbon cycling.

Analysis of carbon isotopes (together with δ^{15}N in collagen) it has been shown that for example weaning ages in populations can be detected (Richards, Mays, Fuller 2002). Communities living along the coast were found to be relying on mainly terrestrial food supplies (Polet, Katzenberg 2003).

4.3. Nitrogen isotopes

The largest portion of nitrogen in the biosphere is found as N_2 gas in the atmosphere where the reservoir is well mixed and the δ^{15}N is essentially constant at 0 ‰. Basically, δ^{15}N values vary naturally between -10 to +10 ‰. Nitrogen often is the limiting factor in plant growth or bacterial mineralization, so all of it is consumed without any regard to isotopes. The large world ocean appears to be less variable in isotope com-

position than lakes. Here, large isotope contrasts can be expected where primary production is limited by N.

Nitrogen in animal protein nearly completely derived from dietary protein and $\delta^{15}N$ has been used in numerous research works to assess trophic levels in naturals systems. The heavy isotope ^{15}N is accumulating in the course of a food chain (Ambrose 2000; Fry 2006). In many studies it has been shown that the diet-to-tissue fractionation is about 3 ‰ (Ambrose 1993; Koch 2007) and that the amount of fractionation increases with rising protein content in the food (Sponheimer *et alii* 2003). The successive increase in ^{15}N in trophic levels, from plants to herbivores to carnivores, and the enrichment of ^{15}N in marine compared to terrestrial environments, has been used in several studies to find an approximation to meat vs. plant and/or marine vs. terrestrial food source consumption (Deniro, Epstein 1981; Schoeninger 1985; Ambrose 2000). For example, the analysis of animal bones can show in settlements if there was a nutritional change in supply with animals (Millard *et alii* 2011) in times of uprising and decline. Another application can be the detection of subsistence strategies changes in hunter/gatherer communities to agricultural methods (Grupe, Peters 2011).

4.4. Oxygen isotopes

Oxygen dynamics are controlled mainly by the water cycle. Evaporation and condensation are well known processes that can be monitored easily on regional and global degrees to model the global distribution of isotope composition (West *et alii* 2010).

Organic matter is able to exchange in parts oxygen with the environmental water, so this organic oxygen has to be considered before focusing on the bound non-exchangeable oxygen. $\delta^{18}O$ values in cellulose for example are enriched in ^{18}O by about 27 ‰ versus source water, which seems to mirror equilibrium fractionation reactions between CO_2 and water. Studies with animals are mainly based on questions about migration, e.g. migratory birds (Hobson 1999) and determining which local sources of water are used. The degree to which food influences $\delta^{18}O$ variation in mammals is not fully understood yet, but some research work is trying to figure it out (Daux *et alii* 2008; Pellegrini, Lee-Thorp, Donahue 2011).

$\delta^{18}O$ values in bone and enamel reflect climatic conditions during the lifetime of an individual Air temperature changes with altitude and geographic latitude and so do the $\delta^{18}O$ values in water precipitation. This pattern can be used to reconstruct mobility and migration in humans and animals over lateral and vertical distances between climatic different re-

gions (Sharp, Cerling 1998; Evans, Stoodley, Chenery 2006; Chenery *et alii* 2010; Brettell *et alii* 2012a). The ratio of oxygen isotopes in ongoing precipitation decreases which results in declining $\delta^{18}O$ values by following effects (also in combination!): 1) increasing distance from the coast (continental effect), 2) increasing altitude (altitude effect), 3) decreasing temperature in precipitation (temperature effect), 4) increasing latitude (latitude effect) (Ayliffe, Chivas 1990; Hoefs 2004; Fry 2006).

Reconstruction of ecological parameters for finescaled archeological and ecological purposes needs a very precise quantification of the proportion of the isotopic signal deriving from food and drinking water, which mixes up in the analysed tissue. All different kind of factors potentially affecting metabolism, and therefore tissue isotope values, have to be described exactly. This is necessary to guarantee as much as possible precision of these reconstructions (Kirsanow, Tuross 2011).

The isotopic composition ($\delta^{18}O$, but also true for δD) of mammalian calcified tissue reflects a mixture of several sources of uptake. These can be ingested food and water (δD and $\delta^{18}O$) and inspired air ($\delta^{18}O$) (Luz, Kolodny 1985; Kohn 1996). The relative contribution and consistency of each source are not well understood in mammals yet (Kirsanow, Tuross 2011). Body water is controlled by the uptake of atmospheric O_2, drinking water, water in food, along with an unknown contribution from metabolic effects. The body water is then incorporated into tissue during synthesis (Longinelli 1984; Luz, Kolodny, Horowitz 1984; Ayliffe, Chivas 1990; Bryant, Froelich 1995).

The isotopic values of tissues based on proteins are assumed to be strongly influenced by the isotopic composition of body water (Hobson *et alii* 1999; Sharp *et alii* 2003; Podlesak *et alii* 2008). The different proportions of the sources were calculated by a model (Podlesak *et alii* 2008): drinking water contributes 56 % of the oxygen in body water, while food contributes 15 % of the oxygen, while the rest of 30 % of the oxygen in body water is derived from atmospheric O_2.

Also non-proteinaceous, calcified tissues are influenced by environmental variables. Tooth enamel is widely used to extract apatite. Carbonate and phosphate extracted from enamel are the preferred substrates for these analyses as they are likely to be better preserved in historic or prehistoric terms than bone or hair (Sharp *et alii* 2000; Kohn, Cerling 2002). Analysis of $\delta^{18}O$ in tooth apatite are a common method to study palaeodiet, palaeoecology, palaeoclimate and seasonality (transhumance) (Bocherens *et alii* 1995; Blaise, Balasse 2011; Horbe 2011; Brettell *et alii* 2012a; Frémondeau *et alii* 2012). Carbonate is formed from bicarbonate in blood (Krueger, Sullivan 1984; Ambrose, Norr 1993) and the $\delta^{18}O$ of carbonate is controlled by body water $\delta^{18}O$ (Bryant, Froelich 1995; Bocherens *et alii*

1996; Bryant *et alii* 1996). Phosphate in mammalian bone and teeth precipitates at body temperature and is in isotopic equilibrium with body water. As body water $\delta^{18}O$ reflects the local meteoric water, $\delta^{18}O$ derived from apatite phosphate is linearly correlated with local drinking water (Longinelli 1984; Luz *et alii* 1984; Luz, Kolodny 1985; Daux *et alii* 2005).

4.5. Sulphur isotopes

Phytoplankton fixates sulphate with a small fractionation of 1 to 2 ‰, in contrast to a large isotope effect in dissimilatory sulphate reduction in marine sediments which can vary between 30 to 70 ‰. Continental vegetation averages in $\delta^{34}S$ values about 2 to 6 ‰ over large areas and can be easily distinguished from the approximately 17 to 21 ‰ values in marine plankton and seaweeds. Sulphate in precipitation over open ocean shows lower $\delta^{34}S$ values than sea-spray sulphate (13 vs. 21 ‰) due to slower oxidation of reduced sulphur gases. Much lower $\delta^{34}S$ values (0 to 10 ‰) can be found in terrestrial sulphates (Fry 2006).

Analyses of stable sulphur isotopes on archaeological bone material have been increasingly applied in late research studies (Craig *et alii* 2006; Privat, O'Connell, Hedges 2007; Nehlich, Richards 2009). This has been possible due to technical advances in mass spectrometry, which allowed the use of relatively small amounts of collagen.

Sulphur isotope ratios ($\delta^{34}S$) can be used to distinguish between freshwater and terrestrial ecosystems. It has been shown, that it is possible to define the distance to seawater in animal tissue (Zazzo *et alii* 2011). An additional help and accumulative evidence is given, when sulphur is measured together with carbon and nitrogen isotope ratios. It is possible to discriminate between the consumption of foods from different geographical regions (Hesslein *et alii* 1991; Craig *et alii* 2006; Nehlich, Richards 2009). The problem with sulphur isotopic analysis is that a considerably higher amount of collagen is needed than for nitrogen and carbon isotopes, as the amount of sulphur in collagen is clearly lower (0,22±0,02 %) (Craig *et alii* 2006). Instead of 0,5 mg, 6 mg (fish) to 10 mg (mammalian, avian) of bone collagen is needed (Nehlich, Richards 2009).

4.6. Strontium isotopes

The strontium isotopic system has proven to be most effective to reconstruct migratory events in studies on archaeological material. There are studies on human and animal skeletal material in historic and prehistoric context (Bentley 2006; Evans *et alii* 2006; Chenery *et alii* 2010; Brettell *et alii* 2012a). The Sr concentration is generally very low in non-

skeletal tissue (Bentley 2006). The method can also be used on other organic material like archaeological artefacts, plants or wood (Gillmaier *et alii* 2009; Hollauer 2010). Ericson (1985) presented the strontium isotope method to archaeology (Dupras, Schwarcz 2001; Price *et alii* 1994; Sealy *et alii* 1991) before it became a common tool for archaeological questions (Burton *et alii* 1999; Price *et alii* 2003; Schweissing, Grupe 2003; Bentley 2006).

Strontium isotopes are used as markers to identify the geological origin of an individual and to reconstruct mobility during lifetime. The method is based on the principle that strontium is washed out from minerals in eroding rock by ground water and enters the food chain (Faure 1986).

^{87}Sr is radiogenic which means that it decays over time from ^{87}Rb (β decay, $t\frac{1}{2} = 4,88 \times 10^{10}$ years). This means, for historic or prehistoric questions the concentration of ^{87}Sr can be considered stable. The measurement of $^{87}Sr/^{86}Sr$ ratios is derived from geochronology and is dependent on the age of the rock as well on the original concentration of ^{87}Rb in the rock (Hoefs 2004).

It should be considered that it is hard to distinguish between a natural strontium isotopic signal and a diagenetically altered one. Especially bone is supposed to be susceptible for ionic exchange with the surrounding soil. Only if the measured $^{87}Sr/^{86}Sr$ signal is different from the expected local signature it can be tagged as non-local. Is it the same it could be a false negative result which leads to an underestimation of migration rate (Schweissing, Grupe 2003). On the other hand, it can not be assumed that bone and dentine are altered in any case and could be used as indicators for a local isotopic signature (Krueger, Sullivan 1984). In general, enamel seems to be more reliable in terms of stability of the biogenic signal and resistance to contamination (Sharp *et alii* 2000; Zazzo *et alii* 2010).

Very old rocks (>100 mya) with high original Rb/Sr-values show $^{87}Sr/^{86}Sr$ ratios of more than 0,710, whereas younger rocks (<1 to 10 mya) with low original Rb/Sr values exhibit less than 0,704 (Stanley, Faure 1979). These differences seem to be very little, but are relatively high compared to the measuring error in modern mass spectrometers (± 0,00001 or better) (Bentely 2006; Rummel *et alii* 2010).

Research on environmental ^{90}Sr has shown, that the Sr/Ca ratio decreases up the food chain. This process, called biopurification (Elias, Hiras, Patterson 1982; Ezzo 1994), happens because only 10 to 40 % of Sr ingested by mammals is physiologically absorbed (Burton, Price, Middleton 1999). The decreasing in Sr concentration leads to a shift per each trophic level of about 20 % of the consumed good (Bentley 2006). This should be kept in mind if analysing strontium concentration in different tissues.

Recent discussion within the scientific community and international literature indicates that it is important to determine the local bioavailable isotopic signatures in archaeological horizons for each site under study. This is preferably achieved by analyzing bones from residential animals with a small home range, archaeological or modern finds, and also collected wood or plants. Bentley (2006) and Price *et alii* (2003) suggest that the average value of the bioavailable strontium of local feeders could help to predict the local strontium signature for mammals.

5. Summary

Before applying any isotopic system the hypothesis should be carefully chosen, to avoid unnecessary analyses. The researcher should be clear about what he or she wants to know and what should be shown. The next step is to choose the material, bone or tooth, what time frame should be looked at, what conclusion should be drawn from the results.

For this purpose table 2 gives an (not complete) overview of the materials on hand, which isotopes can be extracted from which material and what possible evidence one might find.

skeletal element	material	fraction	isotopes	evidence	
tooth enamel (formation time)	apatite	carbonate	O	water source	climate
			C	provenance	nutrition (whole diet)
		phosphate	O	water source	climate
		Sr		provenance	
bone (last living years)	apatite	carbonate	O	water source	climate
			C	provenance	nutrition (whole diet)
		phosphate	O	water source	climate
		Sr		provenance	
	collagen	protein	C	nutrition (protein)	
			N	nutrition (protein)	
			S	nutrition (protein)	provenance
			H	nutrition	water source

Tab. 2. Overview of the different isotopic systems, the possible material and the evidence that can be found.

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