Stable isotope analysis in raptor and falconry studies

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Abstract: Stable isotopes are forms of an element that are not hazardous and are contained in all natural objects. Abundance of the stable isotopes could be measured in different materials and used in many ecological investigations. The main approach of stable isotope analysis is based on the idea that the isotopic signature of the consumer depends on the isotopic signature of the producer. This basic premise is used in raptor research mainly in the form of foraging and movement ecology studies. According to the history of falconry, the main questions that potentially could be answered by stable isotope analysis are the origin of the falcons found in archeological excavations and the methods of falcon captivity in the Middle Ages. Stable isotope analysis is a very useful tool in the investigation of falcon biology and the history of falconry, but one should understand all the limitations of the method and plan any study using this method very accurately. Stable isotope analysis is a relatively new method, which will develop and improve in the very near future.

Background of the method

What stable isotopes are and how we can measure them

The atom of chemical elements consists of a nucleus (protons and neutrons) and electrons, which are assumed to circle the nucleus. The electrons are charged negatively and balance positively charged protons. Neutrons are not charged and they prevent the nucleus of the element from decaying. The number of protons is equal to the number of electrons and stable for any given element, but the number of neutrons in the atoms of an element may differ. Elements with a different number of neutrons are called *isotopes.* Those isotopes that contain too few or too many neutrons, which makes them unstable, are called *radioactive isotopes,* and those which contain numbers of neutrons that make the atom stable are called *stable isotopes.* The latter usually contain the same number of neutrons as protons or slightly more neutrons. For example, carbon has 6 protons, 6 electrons and different numbers of neutrons (Fig. 1). Those atoms of carbon with 2, 3, 4 or 5 as well as 8, 9, 10 or more neutrons are radioactive isotopes; and those that have 6 and 7 neutrons are stable isotopes. Isotopes with a higher number of neutrons are called heavy isotopes and those with a lower number are called light isotopes. Thus, ^{13}C is a *heavy* stable isotope whereas ^{12}C is a *light* stable isotope (Clayton 2003).

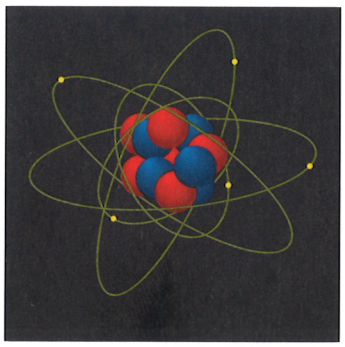

Fig. 1. Carbon atom (^{12}C) with 6 electrons (yellow), 6 protons (red) and 6 neutrons (blue) (drawing L. Foged Thomsen).

Stable isotopes are not hazardous and are contained in all natural objects. It is possible to detect isotopes and measure their abundance using a special technique of analytical chemistry called *mass spectrometry* (Fig. 2). Isotope values are represented by the special notation δ (delta). Delta value refers to the difference between the isotope ratio in the sample and in the standard, and it is often called the *isotopic signature*. This value is calculated in the following way:

$\delta X = (R_{sample}/R_{standard} - 1) * 1000$

δX – is a heavy isotope of the measured element (for example ^{13}C).

R_{sample} and $R_{standard}$ are ratios of heavy isotope to light isotope abundance in the sample and standard respectively. For carbon it will be the ratio $^{13}C/^{12}C$ in the sample and in the PeeDee Belemnite (PDB), which is equal to 0.01118 (Hayes 2002). It is measured in per mille (‰) and usually ranges between -100 and +75 (Fry 2006).

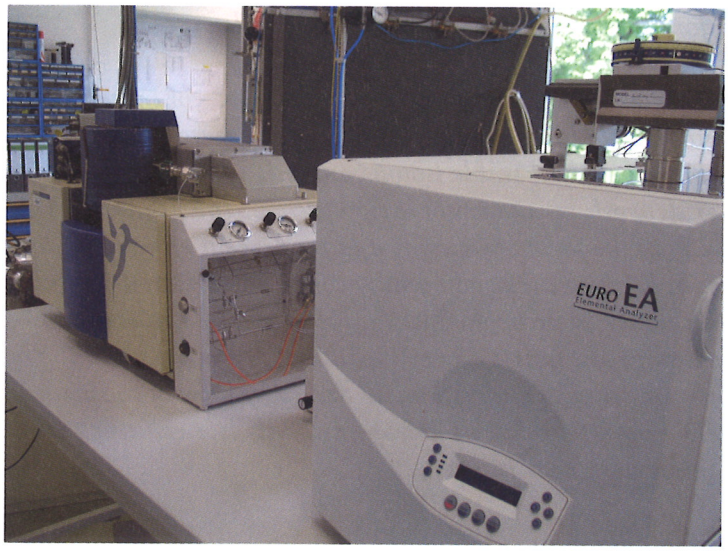

Fig. 2. Mass Spectrometer (MS, background, to the left) connected with an Elemental Analyser (EA, foreground, to the right). Leibniz Laboratory for Radiometric Dating and Stable Isotope Research, Christian-Albrechts-University of Kiel (photo N. Andersen).

The main elements used in stable isotope ecology are carbon, nitrogen, hydrogen, oxygen, and sulfur. Landscape ecology, community ecology, food-webs, and animal migrations are the most popular topics in stable isotope ecology.

Using stable isotopes in animal studies

The fundamental approach to animal studies using stable isotopes is that the isotopic signature of the consumer depends on the isotopic signature of the producer. There are two key issues in using stable isotopes in animal studies: discrimination and turnover.

According to the nature of animal metabolism, the consumer's (e.g. predator's) isotopic signature is always higher than the isotopic signature of the same element in the producer (e.g. prey species). This difference is referred to as the *discrimination factor* (also called the trophic enrichment factor (TEF), trophic fractionation, or just enrichment or fractionation). Thus, the isotopic signature of the consumer is the sum of the producer's isotopic signature and the discrimination factor. That factor is highly variable among species and tissues. It is different for blood, feathers, bones and other tissues within one species, and the discrimination factor of peregrine falcon (*Falco peregrinus*) blood is different from that of the great tit (*Parus major*), warblers (Passeriformes) and other species (Fig. 3). Discrimination factors for certain species and tissues can be determined only by controlled feeding experiments. Researchers have already conducted many studies evaluating the discrimination factor of different tissues and species, but it is still a very small proportion of all species and tissues. Some researchers use discrimination factors of phylogenetically closely related species, but this proxy

estimation of the discrimination factor could lead to certain mistakes (Hobson/Clark 1992a; Bearhop et al. 2002; Post 2002; Pearson et al. 2003).

Fig. 3. Blood sampling of a peregrine falcon at Nenetsky Ridge, Arctic Russia, by the author (photo O. Kulikova).

The second aspect of stable isotope analysis in animal studies is the *turnover rate*. Animal tissues can be metabolically active or inactive. In metabolically active tissues, the isotopic signature is related to the isotopic ratio of the producer (i.e. dietary species) during a particular interval in the past. For example, the blood plasma isotopic signature shows the diet during the past seven to ten days, whereas the muscle tissue isotopic signature shows the diet during the past four to six weeks. Hence, the time period related to the isotopic signature in metabolically active tissues depends on the elemental turnover rate. Isotopic signatures of metabolically inactive tissues (feathers, hairs, fur etc.) indicate the diet during the period of growth of these tissues. For example, the isotopic signature of the feathers being formed during moult shows the bird's diet only during this period. Bones have very slow turnover and their isotopic signature shows the average diet over many years in the past (Hobson/Clark 1992b; Bearhop et al. 2002; Kurle et al. 2013; Ogden et al. 2004).

Stable Isotope Analysis in raptor studies

In raptor studies (and in ornithological studies in general) the most popular topics for stable isotopes are foraging studies and studies of movement ecology (Inger/Bearhop 2008).

Foraging studies

The most widely used methods for the foraging studies of raptors (especially for nestlings) are the analyses of pellets and prey remains left at the nest or at roosting sites. However, these methods lead to biases in quantifying the relative importance of different dietary items (Simmons et al. 1991; Marti et al. 2007; Tornberg/Reif 2007; Pokrovsky et al. 2014). Large prey may be underestimated by pellet analysis because adults dissect large prey and often eat only the meat (or feed it to their nestlings), a tissue they digest completely. On the other hand, large prey may also be overestimated by analysis of prey remains. Birds require more time to consume larger prey items compared to smaller items, which can be swallowed in one piece; thus the remains of large prey have a greater chance to be found at the nest (Redpath et al. 2001; Pokrovsky et al. 2014). In addition, soft-boned juveniles may be totally digested and underestimated. The combination of these methods can also

lead to overestimation of some prey in the diet because of double counting, i.e. fur from one animal could be found in pellets and as prey remains. Furthermore, errors may arise from different ways of calculating prey proportion in the diet. For instance, proportions based on the number of prey may overestimate small prey items (Sonerud 1992). Estimating proportions based on the weight of prey is often difficult due to our inability to evaluate the precise weight of the consumed prey, particularly when prey fragments are absent.

Stable isotope analysis is another powerful method to estimate diet composition (Deniro/Epstein 1977; Hobson/Clark 1992a; Pokrovsky 2012). One of the advantages of this method is that it addresses proportions of assimilated food and thus avoids the biases described above. The proportion of different prey items in the diet can be quantified with stable isotope analysis using *mixing models*. During the past decade these models have been modified from simple (with restrictions of the number of sources and isotopes used) to complex models using the Bayesian approach (Phillips/Gregg 2001; 2003; Moore/Semmens 2008; Parnell et al. 2008; Fernandes et al. 2014). Despite the advances in mixing models, the evaluation of diet composition is a weak point of stable isotope analysis. The isotope signature of each food item varies within a certain range, food items should be specified in advance and the necessity of collecting an adequate sample size of each prey species causes a certain bias in the final evaluation. Therefore, many foraging studies with stable isotope analysis use the mixing models mostly to detect different kinds of spatial and temporal dietary shifts instead of calculating the precise proportions of the food items consumed.

As a case study, the investigation of the dietary shift from main to alternative prey in the rough-legged buzzard population on the Nenetsky Ridge, Arctic Russia, shall be briefly introduced (Pokrovsky et al. 2014). In 2007–2010, the diet of rough-legged buzzards (*Buteo lagopus*) as well as the abundance of their possible prey on the Nenetsky Ridge, Arctic Russia, has been analysed. In that respect, three complementary methods were used to assess the diet of this Arctic predator – pellet dissection, identification of prey remains on nests, and stable isotope analysis – in order to overcome their respective limitations. We used the first two methods to make a list of prey species hunted by buzzards, whereas stable isotope analysis was meant to find out the possible shift from main to alternative prey. It could be documented that the main prey for the buzzards was root voles and water voles, while the alternative (less used) prey was hares and ptarmigans (Fig. 4). In the year when rodent numbers were at their lowest (2010), diet analyses of nestlings showed a shift from rodents to alternative prey. It could therefore be argued that buzzards adopt different feeding strategies along the gradient from generalists to specialists. While the rough-legged buzzard is usually considered a small rodent specialist, our study shows that it can shift to alternative prey where or when rodents are scarce and when alternative prey are sufficiently abundant to provide subsistence for breeding.

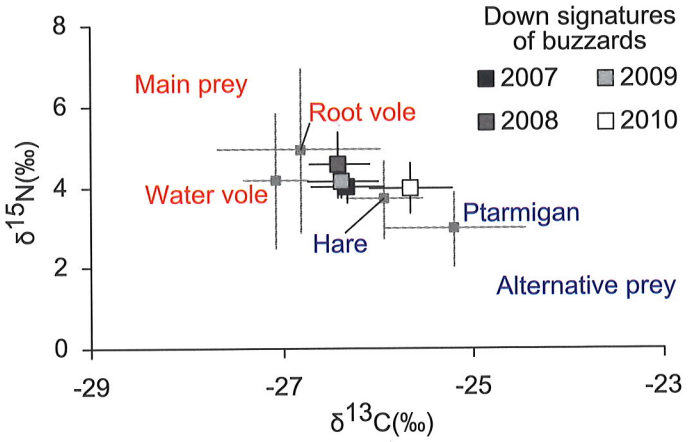

Fig. 4. Results of stable isotope analysis for nestlings of rough-legged buzzards (Pokrovsky et al. 2014).

Studies of movement ecology

The main tool of movement ecology is radio and satellite tracking, which provide very accurate information about bird migration, but are still rather expensive and there is the limitation of battery life. The use of stable isotopes to track movements of raptors is not as precise as GPS tracking, but shows significant promise (Hobson 2007; Hobson/Wassenaar 2008).

There are several stable isotopic patterns in nature that can help to investigate the origin of the individuals. One of the best known patterns is the distribution of the heavy isotope of hydrogen – deuterium – in the world. During the water cycle of evaporation and precipitation different isotopes of hydrogen have different dynamics in different parts of the world. Thus, deuterium is less abundant near the poles than near the equator (Fry 2006). The concentration of deuterium in precipitation is strongly correlated with that in feathers (Bowen et al. 2005), thus it is possible to use the spatial distribution of deuterium to determine the origin of the bird (Meehan et al. 2001; Lott et al. 2003; Lott/Smith 2006). However, there are some potential sources of errors using stable isotopes in movement ecology. We do not know how climate change will affect the deuterium distribution, and it is now clear that deuterium values are different for males and females and for adults and juveniles (Meehan et al. 2001; Smith/Dufty Jr 2005; Hobson 2007). These factors should be taken into account during the planning of stable isotope studies.

The use of deuterium maps to investigate movement ecology is especially popular for raptor studies, because forming feathers during moult of the raptors is well-studied and takes place over a defined period. Other popular applications of stable isotope analysis are the investigations of the role of endogenous reserves (capital) vs. recently acquired nutrients (income) and the role of marine vs. terrestrial nutrients during breeding. There are some studies of raptors using these applications (Hobson 1995). However, these kinds of studies are more popular among other groups of species (Gauthier et al. 2003).

Stable isotope analysis in falconry

Stable isotope analysis could potentially be a good tool in the investigation of the history of falconry. In archeology, stable isotope analyses are used in human diet and paleoclimate studies, and also in investigations focused on livestock and herd management (Balasse et al. 2003; Becker/Grupe 2012). According to the history of falconry, there are several questions that could potentially be answered by stable isotope analysis. The first question is the origin of the falcons found in archeological excavations. Scientists want to know whether falcon remains are related to captive falcons or to wild falcons. The second question relates to the methods of falcon captivity in the Middle Ages or, more precisely, which kind of food falcon keepers provided. In both of these cases we will have the same advantages and limitations described above for studies of modern birds, with additional limitations related to tissue diversity (usually only bones are found in archeological excavations) and the information about the medieval environment. Thus, although we know the deuterium map as it is now, it could have been different in the Middle Ages when the climate was different. Using the current deuterium map for the remains of medieval falcons could lead to significant mistakes. Likewise, we know the isotopic signatures of the potential prey of falcons from present habitats, but this will hardly be useful information in reconstructing the diets of medieval falcons. To correctly use stable isotope analysis in the study of historical falconry we therefore need to undertake a comprehensive investigation of isotopes in medieval material. As for diet reconstruction, falcons and potential prey from the same period would have to be studied, whereas for the investigations of origins, we need to analyse birds from the same period but from different places.

Conclusion

In conclusion, stable isotope analysis is a very useful tool in the investigation of falcon biology and the history of falconry, but one should understand all the limitations of the method and plan any study using this method very accurately. For raptor ecology and especially for the history of falconry, stable isotope analysis is a relatively new method that will develop and improve in the very near future.

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