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They became what they ate:

The use of novel isotope techniques for greater insight into Neanderthal dietary choices in Western European Middle Palaeolithic

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Chapter 1: Introduction

The topic of neanderthal and hominin diet has been a regular subject of discussion among archaeologists since the first discoveries of hominin species other than *H. sapiens*, with theories being based on teeth and jaw morphology, to faunal assemblages and tools found in or near hominin camping sites. In the case of *H. neanderthalensis*, it has been traditionally agreed that their diet consisted of high amounts of meat typically hunted from megafauna found throughout their diaspora (Bocherens, 2009, p. 247), with *H. neanderthalensis* gaining the now challenged stereotype and association with the "primitive caveman" trope. Modern research, however, has consistently shown that neanderthals had numerous similarities with modern humans, bridging the gap between a long-extinct species and that of our own. Within this context, the subject of diet becomes increasingly crucial in understanding and reconstructing both social behaviour and structure, survival strategies, and the complex genetic and social link between *H. neanderthalensis* and *H. sapiens*. Further understanding of diet can also drastically increase insight and interpretation of finds within neanderthal sites such as tools, jewellery, use of fire, and potentially art.

As a starting point, there are three elements that must be defined: *H. neanderthalensis*, western Europe, and middle palaeolithic. The first one refers to a now extinct hominin species, vulgarly referred to as neanderthals, who closely shared the planet with our own species: *H. sapiens*. The two species were relatively close both genetically and geographically, even experiencing potential interbreeding, causing modern humans inherit certain genes and traits that would otherwise not be present in our DNA (Sankararam et al., 2012, p. 7). However, there were certain morphological and behavioural differences between the two, although the subject will not be discussed in this thesis. Secondly, the definition of western Europe is geographically debated, however in the context of the literature reviewed, it is referred to primarily within the borders of modern-day Portugal, Spain, and France. Lastly, the middle palaeolithic is defined within marine isotope stages (MIS) 8 through 3 (Lisiecki & Raymo, 2005), or between 300.000 – 30.000 years before the present. While this is an incredibly broad spectrum, the scope of this thesis covers primarily MIS5, or roughly between 130-71kya.

Within this framework, the use of traditional and novel isotope analysis positions itself as a key element in understanding *H. neanderthalensis*' dietary habits, with traditional analysis focusing on stable isotopes and modern research venturing into radioactive and radiogenic nuclides of various elements (refer to Chapter 2 for an in-depth discussion). In the following chapters, isotopic signatures referred as traditional include carbon and nitrogen respectively: $\delta^{13}C$ and $\delta^{15}N$; while novel isotopic signatures correspond to those of calcium and zinc respectively: $\delta^{42/44}Ca$ and $\delta^{66}Zn$. Isotopic analyses can be done on various forms of preserved bioarchaeological tissue such as enamel and bone, as well as collagen found within osseous samples; with tissues typically selected specifically for a particular element or vice versa depending on preservation and amount of finds (Lee-Thorp, 2008, p. 927). Collagen represents part of the organic fraction of bone, with the inorganic being hydroxyapatite (sometimes referred to as bioapatite), and as protein it contains carbon molecules which are extremely useful for isotopic analysis. However, it is highly susceptible to degradation by pH changes, hydrolysis, bacteria, and fungi even though under ideal conditions it can maintain its structure for over 100, 000 years (Brown & Brown, 2010, p. 126). Due to ideal conditions being extremely rare in archaeology, the issue of collagen degradation can be consistent and hence, alternatives should be explored.

While current studies and techniques have greatly expanded and challenged our understanding of neanderthal eating habits, the use of carbon isotopes in collagen has an extremely low preservation rate in deep time, as well as requiring large quantities (10-500mg) of bone material to be analysed, which can severely damage assemblages and delicate remains (Dodat et al., 2021, p. 2). Furthermore, the general scarcity and exclusivity of neanderthal remains does not allow for extensive bioarchaeological samples to be extracted, limiting research potential. Novel techniques could modify and improve our understanding of neanderthal diets while maintaining a low-impact on bioarchaeological remains for preservation and future study. Nevertheless, isotope analysis still requires specialised laboratory equipment and training, even when modern mass spectrometers allow for an easier workflow and data collection, as well as properly excavated samples with appropriate preservation.

The two main sites discussed in this thesis are Regourdou in modern day France (Dodat et al., 2021) and Gabasa (also referred to as “Cueva de los Moros”) in modern day Spain (Jaouen et al., 2022). The following map showcases the geographical location of each under modern satellite imagery for reference, hence icecaps and landmass differences from the middle palaeolithic are excluded:

Figure 1: Western European map showcasing discussed sites. *Google Earth (n.d) generated by author. Retrieved 15/06/2023*

Methodology for this thesis consisted of reviewing and comparing literature regarding isotope studies within the context of neanderthal diet and ecology, with a further discussion on the impact this has on modern research. This framework would then be contrasted with other methods, particularly biomolecular analysis and faunal assemblages found in the sites described in the literature, as well as other European neanderthal sites with relevant information on the subject. Hence, this thesis aims to further understand the potential and limitations of novel isotope research for the benefit of neanderthal diet research, as well as explore how interdisciplinary research in the subject matter opens possibilities that may increase accuracy in dietary conclusions. Because of this, the primary research question is as follows:

Does novel isotope research challenge preconceived notions of dietary habits of H. neanderthalensis, relative to standard research practices?

Dietary studies are a key element in illustrating neanderthal and middle palaeolithic behaviour and ecology, furthering the explanation of trophic relationships and the relationship with *H.* sapiens, as well as the impact of *H.* neanderthalensis extinction. Therefore, the hypothesis for this thesis is presented as follows:

Isotopes of elements extracted from neanderthal remains (such as $\delta^{42/44}Ca$ and $\delta^{66}Zn$) can be of significant use when researching Neanderthal diets without over relying on the bias of collagen-found isotopes; as well as assisting dietary research for studies of biomolecular remains and faunal assemblages.

Furthermore, sub-questions delimiting specific target areas of research apply, such as:

Can isotopes derived from collagen samples be used as proxies for research on novel isotopes such as Zn? Can biomolecular data and faunal assemblages also be used as proxies?

Does desk-based research cover the intricacies and details needed to extract and interpret the required data in studies of this nature?

What is the importance and impact of neanderthal diet in the broad spectrum of palaeolithic and human origins archaeology?

In order to answer and explore the previous questions, the following chapters present a structure meant to present and detail each technique, as well as the archaeological and scientific basis required for this type of research.

Chapter 2 will delve into the nature of isotopes and research, including the chemical/physical properties of these, as well as mass spectrometry with a discussion on its uses and limitations.

Chapter 3 regards the use of $\delta^{42/44}Ca$ in neanderthal diet, with particular examples on neandertal sites in what is now modern France and Spain.

Chapter 4 explores the use of $\delta^{66}Zn$ isotopes for understanding the trophic implications and weaning habits of *H. neanderthalensis.*

Chapter 5 discusses and explores the intricacies of neanderthal diet, with a particular emphasis on faunal assemblages and biomolecular insight; aiming for both a precise and accurate analysis of the combined data.

Chapter 6 is dedicated to the future of isotope research, exploring the previously showcased potential and limitations of it, as well as which elements can be used in the future and their advantages.

Chapter 7 concludes the thesis, reviewing the hypothesis and research, as well as creating a final discussion of the literature reviewed.

Chapter 2: Traditional Isotope Analysis Techniques

Chemical elements have been oftentimes seen as essential building blocks for life to exist and develop, with multitudes of possibilities and adaptations that help create environments, organisms, tools, shelters, among many other essentials for the hominin lineage to flourish. Whilst the periodic table lists and describes 118 elements, these are catalogued based on their atomic weight when neutrally charged, through which the number of protons equals the number electrons orbiting the nucleus of the atom. However, it is important to distinguish that elements, at their atomic level, showcase differences in mass and electrical charge, depending on the amount and behaviour of their subatomic particles[1]. When electrically stable, atoms have an equal ratio of electrons and protons, respectively charged negative and positive. However, the amount of neutrons is oftentimes different than the amount of protons in the nucleus, thus creating an isotope (Brown et al., 2012, p. 876), which can thus increase the amount of neutrons until the atom is no longer stable. Isotopes have multiple properties and states, including being radioactive or radiogenic, and differences in mass can occasionally affect how an element behaves in a determined state. A common example to further understand isotopes is that of hydrogen, with three naturally occurring isotopes:

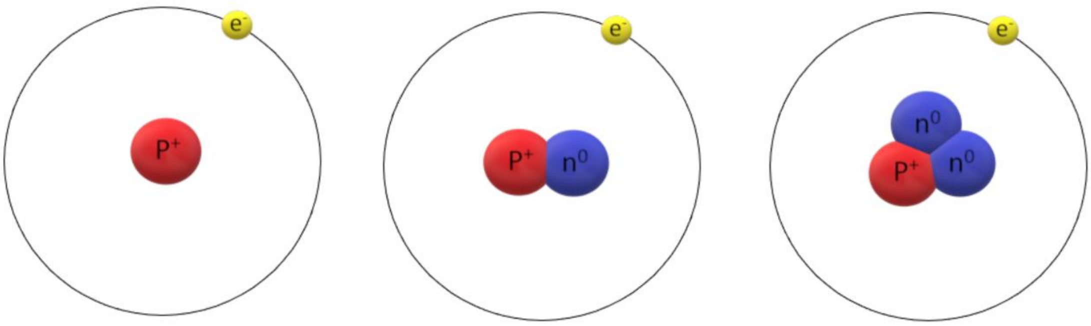


Figure 2: Isotopes of Hydrogen: Protium, Deuterium, and Tritium respectively. Figure by author.

As can be seen, the only variable between the three atoms is the amount of neutrons which can affect its behaviour and radioactivity due to the proportional increase in mass with every change. On elements with larger atomic mass such as carbon, a highly used element in archaeological research, isotopic changes minimally impact chemical or physical behaviour but when their origins and acquisition in a find or bioarchaeological remain are known, it is possible to understand behavioural patterns such as diet or mobility. However, certain isotopes are unstable and later decay into different isotopes of the same element or a different element altogether; which is the main reason why

[1] For the purposes of this thesis, no subatomic particles other than neutrons, protons, and electrons will be discussed.

radiocarbon dating has a limit of about 60kya to date (Higham, 2011, p. 235). Finally, isotopes can go through processes such as fractionation or diagenesis, through which the lighter isotopes are separated from the heavier ones due to chemical or environmental pressures, thus creating isotope ratios that can later be analysed depending on specific research.

For the purposes of archaeology, isotopes are a complex naturally occurring phenomenon that requires observation and interpretation rather than intervention in its study, with minute differences and details being able to provide large amounts of data for an otherwise extremely wide spectrum of research. Said spectrum can determine diet, dating, and mobility for an individual or group within the human past, as well as determine changes in climate and its impact on the biosphere. The combination of these is essential when researching an extinct hominin species like *H. neanderthalensis*, due to the data closing previously existing gaps and scientific stereotypes, as well as providing a baseline for future research.

Isotope research within archaeology can also be applied to the differences in quantities of a specific element noted with the Greek letter δ, to reflect the variation and ratio between isotopes in a determined sample, also known as isotopic signature. Furthermore, this can be applied in bioarchaeological finds as well as other proxies, for example ice cores in palaeoclimate research (Schwarcz et al., 2010, p.337) which can also help indicate changes in ecology and potential sustenance strategies. Below is a short description of some of the most important and commonly used isotopes used in archaeology:

Carbon isotopes are of considerable importance to archaeology, most famously for the capacity of accurately dating organic finds and remains with the radioactive ^{14}C isotope, created in the high atmosphere by cosmic rays, and later introduced into the biosphere via photosynthesis (Bronk Ramsey, 2008, p. 253). Because of this, living organisms acquire ^{14}C through their diet, and due to its radioactive decay, it is possible to calculate how much time has elapsed between the organism dying and the analysis. However, a stable isotope of carbon is also crucial in archaeology: $\delta^{13}C$, which can indicate dietary factors such as freshwater v/s marine protein intake and whether plants consumed by the individual were predominantly C3 or C4 type (Richards & Trinkaus, 2009, p. 16034). Because of this, the $\delta^{13}C$ signature is used as a common proxy for dietary analysis in palaeolithic research, being used for studies in both *H. neanderthalensis* and *H. sapiens* accordingly.

Nitrogen isotopes are extremely useful in diet reconstruction, particularly the $\delta^{15}N$ signature, due to its relationship with trophic levels in various species, generally showcasing a correlation between the $\delta^{15}N$‰ and the trophic position of a determined species or organism (Jaouen et al., 2019). However, certain key factors need to be taken into account with this isotopic signature, particularly the importance of neanderthal mothers breastfeeding and how their children ascend in the trophic chain as a result, combined with the hunting strategies and $\delta^{15}N$ percentages of available herbivore species at the time of the hunt (Wißing et al., 2016, p. 8). Furthermore, $\delta^{15}N$ signature can vary depending on climate factors such as annual precipitation and temperature (Amundson et al., 2003, p. 4), which has to be taken into account especially considering the climactic changes and their consequences towards the end of MIS 5 (Weiss et al., 2022, p. 20). In consequence, when paired with climate and biosphere data, $\delta^{15}N$ presents itself as a valuable element to further the understanding of diet in both *H. neanderthalensis* and *H. sapiens*.

Oxygen isotopes, particularly the $\delta^{18}O$ signature are commonly used for palaeoclimate reconstruction and ice-core analysis. With it, data can be extracted in regards of glaciations, precipitation data, and timeframes of geological periods within the biosphere; as well as ice volumes within glaciers and Earth's poles (Lisiecki & Raymo, 2005, p. 2). Within the context of MIS 5 in Europe, the accuracy of climate and glacial data becomes paramount, and can be further extrapolated to faunal migration and, as a result, *H. neanderthalensis* and *H. sapiens*' migratory and behavioural patterns. Furthermore, $\delta^{18}O$ analysis can be used essentially wherever there is water or ice, though the data can be heavily influenced by fluvial movements throughout time (Koltai et al., 2017, p. 951).

Once a specific element or isotope has been selected and extracted from a desired sample for research, the following step is analysis via mass spectrometry. This technique requires the use of an analytical device called mass spectrometer (likewise, it serves multiple uses in the fields of physics and chemistry) which works by measuring the ratio between the total mass of an atom and its electric charge and later expressing the data in a plot with various resolutions as needed (de Hoffmann & Stroobant, 1999). While the techniques and specifications have varied throughout the years, the physical principles remain constant; though these depend on whether a specific isotope (including radioactive ones) or isotopic signature are required for the determined study. Archaeology has been able to exploit this method to various degrees including dating and mobility, even when isotope studies and ventures into different elements are still considered part of an Avant-garde stage of research. Together with this, technological advances in the field such as the use of Accelerated Mass Spectrometry has allowed research on radioactive isotopes with relatively long half-life, such as ^{14}C, to be conducted (Budzikiewicz & Grigsby, 2006, p. 154). In theory, this could open a plethora of study for radioactive isotopes found in human remains as well as those found in surrounding geographical areas and environments. Furthermore, newer isotope research techniques have permitted the study of zoological remains, key to understanding both diet and the relationship between humans and non-human animals. ZooMS applies the fundamentals of mass spectrometry (hence the MS) with faunal assemblages, in a technique that is minimally invasive when sampling and can thus yield extensive data from various collagen samples while determining different species (Korzow Richter et al., 2022, p. 6). When combining datasets from various isotopes, both from human and non-human bioarchaeological remains, the picture of the past becomes ever more clear, and as a consequence research becomes more accurate and precise.

In conclusion, isotope studies have shown to be of great use in archaeology for reconstruction of multiple elements of past diet, environments, and even hominin behaviour. Because of the scientific nature of type of research, is has to be periodically updated and researched, with new technologies being capable of further improving accuracy and precision in their results, opening possibilities for understanding and interpreting the past from different angles and perspectives. The following two chapters comprise a review of calcium and zinc isotope research to reconstruct neanderthal and hominin diet, with a focus on trophic levels and the challenging notion of carnivory as opposed to newly discovered research on plant consumption.

Chapter 3: $\delta^{42/44}Ca$ Isotopic Signature

As the fifth most abundant element on Earth (Greenwood & Earnshaw, 1998, p. 109) calcium constitutes an important role in hominin evolution and development. With hydroxyapatite being composed with a stoichiometric amount of calcium, noted with formula $Ca_5(PO_4)_3$, the presence of calcium should become apparent when bioarchaeological remains are excavated. However, archaeological research on calcium isotopes and the relationship to paleodiet is still in its infancy, though recent publications have showcased promising results, especially on markers for trophic position and potentially on plant consumption. Furthermore, calcium isotopes can be extensively used in remains with poor preservation, due to the aforementioned heavy presence of the element in mineralized bone tissue as well as being resistant to diagenesis as opposed to carbonates (Tacail et al., 2020, p. 2). This chapter describes the novel use of $\delta^{42/44}Ca$ isotopic signature in diet analysis for *H. neanderthalensis* as well as a comparison of results with faunal assemblages of both herbivores and carnivores in order to objectively understand its presence in diet.

While calcium isotopes have been studied and used experimentally for analysis of dairy consumption and even dinosaur dietary reconstruction (Melin et al., 2014; Heuser et al., 2011), its adoption into archaeology has been slow. Evidence has suggested that "calcium isotope ratios decrease from primary productors to higher trophic levels" (Martin et al., 2017, p. 477), in which organisms with the lowest ratio of the $\delta^{42/44}Ca$ stable isotopic signature should be on the highest trophic position, due to the heavier isotopes being slowly depleted in the food chain. Based on this hypothesis, *H. neanderthalensis*' calcium ratios could help determine carnivory when combined with data from the determined niche's fauna and especially with those of established carnivores such as cave hyaenas (*Crocuta crocuta spelaea*). Originally, calcium analyses were paired with strontium as a ratio of Sr/Ca following the same principle of decrease relative to trophic position (Sponheimer & Dufour, 2009, p. 232); however, the use of $\delta^{42/44}Ca$ can bypass the need for enamel samples and be used directly on cortical or trabecular bone (Dodat et al., 2021, p. 3). More importantly, this allows *H. neanderthalensis*' diet to be analysed and compared under the same parameters as faunal assemblages, ideally found in or around an excavated site, as well as taking advantage of bioarchaeological samples without being limited to collagen preservation or relative osteological remains. Furthermore, the isotopic signature can be used in combination of human and non-human animals to gain deeper insight on trophic relationships, especially if large faunal assemblages such as those found in hyaena dens can be excavated and sampled.

In terms of practical research, few studies have been published regarding *H. neanderthalensis* diet using Ca isotopes as primary method however, the article by Dodat et al. (2021) showcases a significant and detailed advancement on the subject. The study was carried out with specimens excavated from the site of Regourdou in present day France.

Figure 3: 3D satellite image of Regourdou site. Google Earth (n.d) generated by author. Retrieved 15/06/2023

As mentioned beforehand, the $\delta^{42/44}$Ca signature can showcase trophic level of different species both fossil and living, however with signatures being expressed differently through different tissues of the body for the latter, such as muscle, blood or bone signatures (Tacail et al., 2014, p. 533). As such, it influences the calcium absorption in carnivores and omnivores depending on the preferred tissue for consumption, increasing the complexity and precision of the method when reviewing dietary choices. Nevertheless, when combined with other proxies such as δ^{13}C or δ^{15}N signatures, cut and/or burn marks of fauna, $\delta^{42/44}$Ca can be used to significantly improve accuracy on the trophic position of *H. neanderthalensis* in a determined area of research. Figure 2 presents the results on $\delta^{42/44}$Ca analysis for fossils in the site of Regourdou (Dordogne, France) with chronology dating to MIS5.

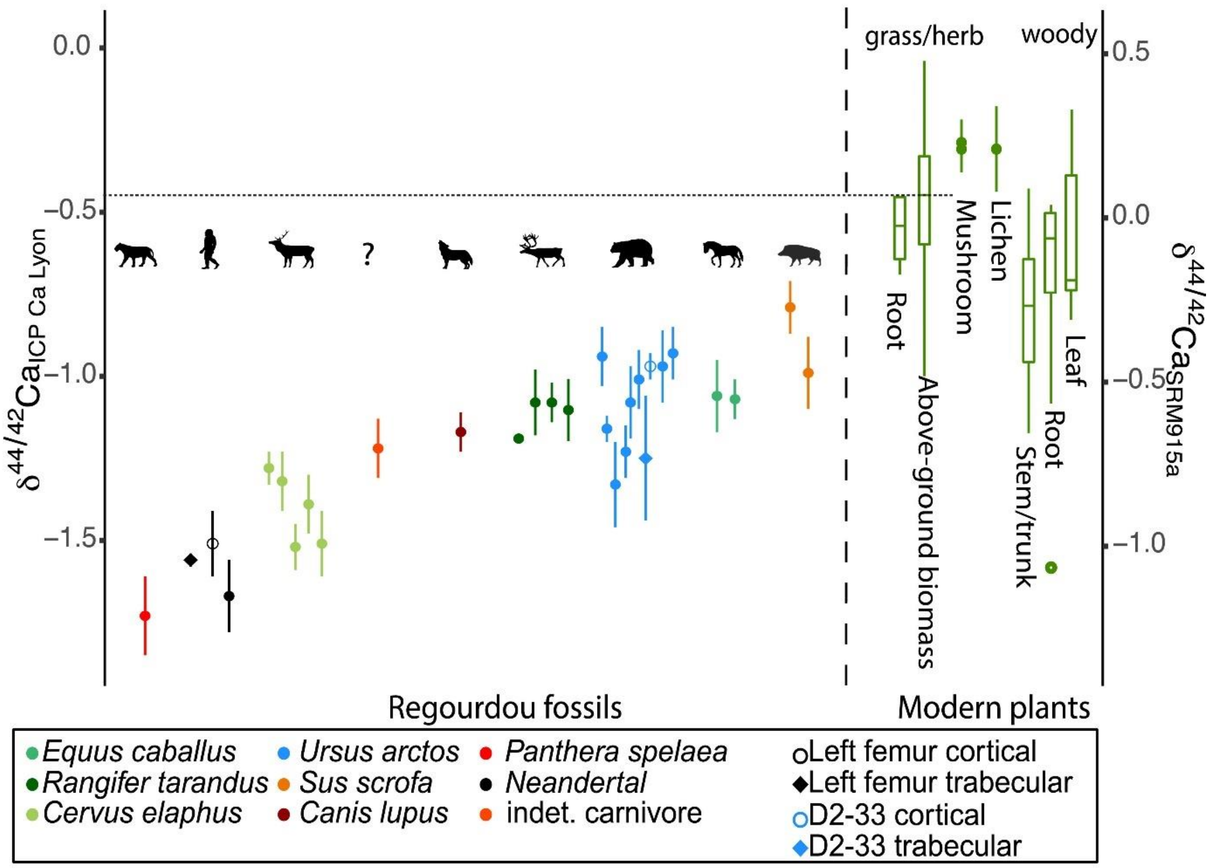


Figure 4: $\delta^{42/44}$Ca chart of Regourdou fossils and modern plants. Extracted from Dodat et al. (2021)

As can be seen, *H. neanderthalensis* signature is considerably lower than that of carnivores such as wolves (*Canis lupus*) or even omnivores such as brown bears (*Ursus arctos*). In turn, these results would maintain the idea that neanderthals were heavy carnivores, though not necessarily exclusive, since the $\delta^{42/44}$Ca results of wolves maintain lower percentages. Interestingly, the only two species that overlap significantly are the cave lion (*Panthera spelaea*) and the red deer (*Cervus elaphus*), which are carnivorous and herbivorous species respectively. Furthermore, it must be noted that cave lions engaged in hunting reindeer heavily and even juvenile cave bears (Bocherens et al., 2011, p. 257), positioning them in an extremely high trophic level as a consequence. However, the position of red deer in this chart challenges the accuracy of the $\delta^{42/44}$Ca signature, due to the species maintaining exclusive herbivory albeit with great variations in plant groups (Gebert & Verheyden-Tixier, 2008, p. 193). This is explained by the authors as a potential correlation with antlerogenesis and Ca fractionation as part of the process but results remain unconclusive (Dodat et al., 2021, p. 4). With this in mind, deer species should be taken under special consideration in $\delta^{42/44}$Ca analysis as to not interfere with results if not previously stated; variations in plant consumption may also play a part in these outcomes, especially when compared to the consistently carnivorous diet of cave lions.

When it comes to *H. neanderthalensis'* diet in this context, comparison with the previously mentioned alternative isotopic and other research techniques is key. In this sense, the trophic position when compared to $\delta^{15}N$ remains relatively constant as high level carnivores, even when the latter could be explained by hunting herbivores that display different $\delta^{15}N$ or the diversification of hunting relative to those of other top level carnivores such as the aforementioned cave lions (Jaouen et al., 2019, p. 4932). Diversification of hunting strategies and prey heavily correlates with modern human behaviour around food and diet, in which various types of food are consumed relative to accessibility and cultural norms. While this does not mean that hunting strategies of various herbivores would necessarily correlate to cultural practices within neanderthal society, it does demonstrate a small insight on availability of food and the adaptation to hunting varied prey. Furthermore, the trophic positioning of *H. neanderthalensis* with the use of Ca isotopes has been shown to consistently correlate with other isotopic ratios, such as Sr/Ca and Ba/Ca, making calcium a reasonable indicator of trophic position (Balter & Simon, 2006, p. 332). Together with this, the Ca turnover has been shown to be relatively stable within bone even when samples are extracted at different locations within a remain (Chu et al., 2006, p. 1661), which once again proves the potential of Ca isotopes when remains are scarce.

Additionally, the $\delta^{42/44}Ca$ signature and the Ba/Ca ratios can be used as a marker for breastfeeding practices in the hominin lineages, giving further insight on maternal dietary choices and biological behaviour within *H. neanderthalensis*. As a result, $\delta^{42/44}Ca$ signature has showcased that the *Homo* genus "marks a strong correlation between $\delta^{42/44}Ca$ and dental ages in the first years of life, suggesting a marked period of breastfeeding" (Tacail et al., 2019, p. 2). Furthermore, neanderthal weaning practices have been shown to be done with younger individuals based on Ba/Ca ratios relative to *H. sapiens* (Austin et al., 2013, p. 218). This creates an interesting marker for both social and maternal behaviour, as well as the element of new-borns and lactating children being on a higher trophic level than their counterparts due to breastfeeding. Likewise, the use of Ba/Ca ratio has shown that neanderthal mothers would consume local foods during the last terms of pregnancy and lactation (Nava et al., 2020, p. 28723). This type of dietary habit is extremely similar to those of *H. sapiens* mothers, closing the gap between the two species and further cementing behavioural similarities even when compared to modern-day individuals.

In conclusion, this chapter has shown that the use of calcium isotopic signatures and ratios can be an effective asset or proxy when studying neanderthal diets and habits. Likewise, it has been shown to function within the ecological niche, providing strong insight on the ecology of *H. neanderthalensis* and other palaeolithic sites. While it requires pairing with other proxies for greater effectiveness,

$\delta^{42/44}Ca$ and its sampling in both bone and enamel can be used as a reliable and accurate alternative to the use of collagen-found isotopes when defining diet, trophic levels, and breastfeeding behaviours. The following chapter explores the use of Zn isotopes and isotopic signatures as a second novel isotope and proxy, with similar comparisons to $\delta^{15}N$ and $\delta^{13}C$ as shown previously.

Chapter 4: $\delta^{66}Zn$ Isotopic Signature

Following the previously described Ca isotopes are zinc (notation Zn) isotopes and its respective signatures are a novel form of research that can indicate trophic levels between organisms including hominins. Similarly to Ca, Zn isotopic studies can be done directly on both bone samples and enamel, offering a wide range of possibilities in relationship to food consumption during early and late stages of life (Jaouen et al., 2016, p. 4a). The isotopic signature used in reconstructing diets is $\delta^{66}Zn$, taking advantage of nuclide stability and overall availability in the biosphere and trophic chains. Neither radioactive nor radiogenic isotopes of zinc have been described for archaeological research.

In terms of ecology, the depletion of $\delta^{66}Zn$ in muscle tissue creates a substantial difference between the levels of $\delta^{66}Zn$ in carnivores and herbivores, with the latter displaying higher results on average (McCormack et al., 2021, p. 2). Paired with data from $\delta^{15}N$ and $\delta^{13}C$, as well as faunal assemblages, the introduction of zinc into archaeological research can prove extremely useful for reconstruction of diet including those of *H. neanderthalensis* and *H. sapiens* across multiple timeframes and ecosystems. However, it must be noted that research on neanderthal diet with the use of zinc isotopes is extremely scarce, with only one article published specifically on the matter (Jaouen et al., 2022), with other research being focused on marine biology and relatively recent archaeology (Jaouen et al., 2016b; Bourgon et al., 2021). This chapter will review the proven potential within previously published research on both $\delta^{66}Zn$ and other isotopic signatures in order to establish the feasibility of incorporating it into neanderthal diet research.

Figure 5: 3D satellite image of the site and surrounding geography. Google Earth (n.d) generated by author. Retrieved 15/06/2023

Even though Zn has been catalogued as a trace element in fauna, it still maintains reliable preservation relative to other elements (Heckel et al., 2014, p. 136), which paired with its aforementioned presence in bone may create an ideal environment for isotopic studies. Zinc is usually acquired into the diet primarily by plant consumption, though later decreasing in overall quantity as the element is passed through the trophic chain (Jaouen et al., 2016, p. 2). Because of this, studies of Zn isotopes in regards to diet should maintain the use of proxy elements such as carbon or nitrogen if available, in order to increase precision of results. Due to the use of $\delta^{66}Zn$ being a novel technique, data remains in its infancy, however it has been shown to be of similar results and quality as previously mentioned isotopes and isotopic signatures alike. Together with this, Jaouen et al. (2022) extracted $\delta^{66}Zn$ from enamel of 14 different species (including *H. neanderthalensis*) in various environments as a benchmark of $\delta^{66}Zn$ absorption and readings. It is key to note that certain plant species process zinc differently, and depending on the environment in which any particular organism consumed determined plants, overall results of $\delta^{66}Zn$ will change (Gupta et al., 2016, p. 90). Below are the box plot results of Jaouen et al. (2022) $\delta^{66}Zn$ study for neanderthal dietary reconstruction:

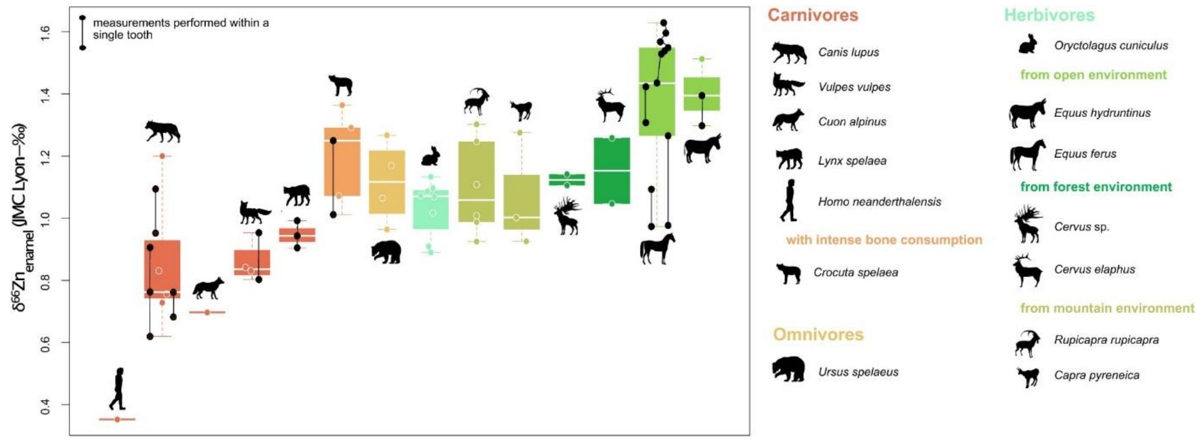


Figure 6: $\delta^{66}Zn$ enamel chart comparing various species and their trophic positions. Extracted from Jaouen et al. (2022)

As can be seen, there is a notorious difference between the animals labelled as carnivores, omnivores, and herbivores, with an overwhelmingly low $\delta^{66}Zn$ concentration in the *H. neanderthalensis* sample, once again suggesting a heavy carnivorous diet. Moreover, samples were taken from species in the same geographical range, thus showcasing that the results have a direct correlation with a carnivorous diet rather than consumption of plants with varied Zn absorption (Jaouen et al., 2022, p. 7). Once again, results correlate with both $\delta^{15}N$ and $\delta^{42/44}Ca$ data, maintaining the notion of *H. neanderthalensis* being a high level carnivore. However, given this precise overlap, consideration has to be taken over the fact that "Zn has better long-term preservation potential than collagen-bound nitrogen" (Bourgon et al., 2021, p. 2). Due to collagen being easily susceptible to degradation by adverse environmental conditions, as well as to the presence of fungi or bacteria (Talamo et al., 2021, p. 62), by implication

Zn isotopes can lead to a change in overall isotope analysis when collagen samples are not available for research or are excavated in poor preservation conditions.

In addition to this, $\delta^{66}Zn$ isotopes can be extrapolated to aquatic ecosystems, which can be useful for identifying potential fish consumption (either saltwater or freshwater), or even marine mammals (Nitzsche et al., 2020; McCormack et al., 2021). While fish consumption has generally not been observed in *H. neanderthalensis*, the potential data compiled from $\delta^{66}Zn$ can give further insight on an otherwise large ecosystem that was, and still is, heavily exploited by *H. sapiens*. Moreover, evidence has pointed out that *H. neanderthalensis* did engage in some form of relationship with aquatic ecosystems, with jewellery made from seashells and even seal hunting in Vanguard Cave, Gibraltar (Stringer et al., 2008, p. 14321). Evidence from sites like these can be paired with coexisting data of $\delta^{15}N$ and $\delta^{66}Zn$ to further understand the trophic relationships of *H. neanderthalensis* and the different environments and ecosystems they formed a part of.

In conclusion, the introduction of $\delta^{66}Zn$ into *H. neanderthalensis* dietary research can provide results extremely similar to those of isotopes of carbon or nitrogen, with the possibility of bypassing the need for collagen-based samples, since these can be extracted from both enamel and bone. Whilst further research is required for palaeolithic and hominin studies, the $\delta^{66}Zn$ signature has presented itself as a reliable and accurate way of understanding trophic levels in the context of palaeolithic archaeology. The following chapter constitutes a discussion on both the calcium and zinc isotopic signatures, combined with previously mentioned carbon and nitrogen studies, and a final comparison with dental calculus and biomolecular studies. The overall chapter would thus comprise results on the feasibility and potential of novel isotope research in the context of neanderthal diet.

Chapter 5: Alternate Dietary Reconstruction Methods

Within the subject of *H. neanderthalensis*' diet, comparisons between both novel isotopes techniques, isotopic ratios in human and animal bioarchaeology, and the use of biomolecular insight on dental calculus and tooth wear for neanderthal sites and remains becomes paramount for an accurate representation of neanderthal ecology. The accurate reconstruction of diet through an interdisciplinary approach, including those of botanical and faunal assemblages within the limits of literature reviewing, would thus require careful evaluation of published data. This chapter constitutes an in-depth discussion and comparison of the previously cited articles of Dodat et al. (2021) and Jaouen et al. (2022), extracting hard data of specific isotope signatures and ratios, in order to graph fauna and *H. neanderthalensis* dietary behaviour.

At length, biomolecular analysis in archaeology comprises the study of organic molecules, particularly proteins, found in remains such as teeth, bone or even food and drink vessels in later periods; with applications ranging from aDNA to organic residue analysis. Because of this, the capabilities of dietary analysis are expanded to the biological signature of a determined species within archaeological remains, whether or not said species is animal, plant, or fungi in nature. As seen previously, research suggests that *H. neanderthalensis* maintained a significantly carnivorous diet, even when directly compared to other carnivores within its niche. While isotope data is in itself accurate, it creates a bias towards understanding dietary choices since it refers to a specific area of caloric intake without necessarily taking into account consumption from sources rich in other macronutrients such as carbohydrates or fat. Furthermore, the existence of a "protein ceiling" (Ben-Dor et al., 2016, p. 369) for consumption in humans directly implies the need of ingesting a larger variety of nutrients from multiple sources. While fat intake also proved essential for meeting nutrient requirements, Hardy (2010) argues that consistent prey size and carcass butchering techniques imply the need for obtaining nutrients from varied sources, thus plants become a plausible cause (p. 667). In consequence, the gathering of plants, berries, roots, fungi, and other edible products was essential for *H. neanderthalensis* subsistence and survival. With this in mind, scientific advances into biomolecular data extracted from dental calculus and botanical remains can breach the gap between our understanding of highly carnivorous dietary choices, or provide a realistic approach into the complete understanding of the hunter-gatherer label.

Firstly, dental calculus constitutes the hardened product of dental plaque accumulation, with biomolecules such as phytoliths and starches having the potential for in situ preservation via the rapid process of mineralization (both pre and post mortem), thus preserving these in its pre depositional context (Hendy et al., 2018, p. 2). As such, it can reveal specific dietary components such as plant species, which can be accurately taxonomically identified, as well as detailing specific uses for instance cooked food and even medicine (Hardy et al., 2012, pp. 623-624). Additionally, phytoliths found within the archaeological context can be of paramount importance for the reconstruction of paleoclimates (Cabanes, 2020, p. 270), furthering the understanding of palaeolithic ecology. Due to their organic nature, recovered phytoliths can also undergo $\delta^{13}C$ isotope analysis for identification of determined carbon fixation methods in specific species (Hodson et al., 2008, p. 336) in order to further complement biomolecular data. Additionally, dental samples can also showcase microwear which can determine abrasives in diet as well as similarities with hunter-gatherer groups as a proxy for palaeolithic diets (El Zaatari et al., 2011, p. 419). Dental calculus then presents itself as a key element in the preservation of extractable archaeological data which, complemented with studies such as those discussed in chapters 3 and 4 of this thesis can aid in improving accuracy of *H. neanderthalensis*' dietary choices and surrounding ecology.

Secondly, the presence of botanical remains in archaeological sites cannot be discarded as an important element in the dietary puzzle, as well as a means of contrast between hunting large herbivores and gathering plants, roots, and berries for sustenance. The middle palaeolithic environment in western Europe showcases a wide and varied array of flora that could have been exploited for food and fuel (Marco et al., 2019, p. 161) by both *H. neanderthalensis* and *H. sapiens* alike. However, the preservation of plant material in the archaeological record is sparse due to rapid decomposing, bacterial and fungal influence, as well as the direct consumption of said resources (Martínez-Varea et al., 2020, p. 1); leading to a research bias on plant consumption before the agricultural revolution. Furthermore, evidence for plant consumption via specific biomarkers such as coprolites can also help identify species and preferred intake of botanical remains (Sistiaga et al., 2018, p. 5). As consequence, when botanical remains are excavated and in reasonably preserved conditions, they can be used for comparative purposes in dietary analysis, both to isotope and biomolecular analysis, however their aforementioned low preservation and overall availability in the archaeological record forces research to focus on dietary from different perspectives.

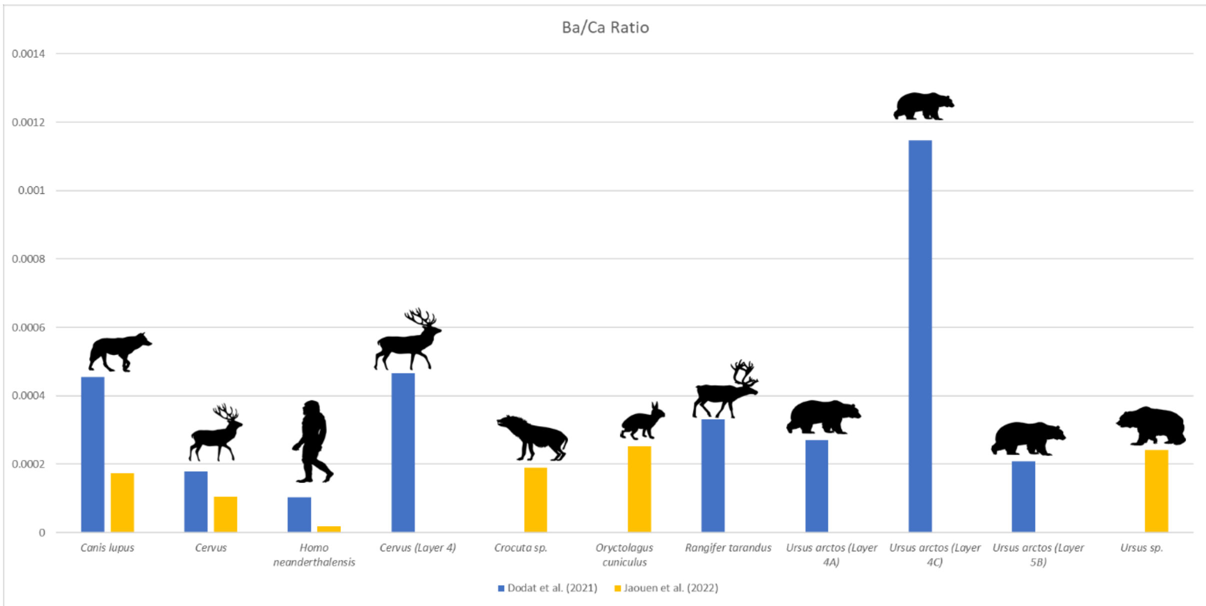
In contrast to biomolecular studies regarding botanical remains, the issue of faunal assemblages within neanderthal sites still establishes a significant case in dietary reconstruction. Due to mineral hydroxyapatite contributing to about 70% of bone mass (Jacobi et al., 2006, p. 557), the post-mortem deposition has astronomically higher preservation rates than those of other biomarkers, as showcased by the bioarchaeological finds in the discussed articles and their general lack of archaeobotanical remains. As a result, the use of faunal assemblages (either anthropogenic or natural) within or in close proximity to *H. neanderthalensis* sites serves both as a key marker and proxy in dietary and ecology studies. Furthermore, assemblages and associated bone complexes showcase dietary choices both through the species selected and the nutritional elements targeted using cutmarks of specific bones and anatomical areas as evidence, as well as potential behavioural conducts (Gaudzinski-Windheuser et al., 2023, p. 6). At its most basic, faunal assemblages can quickly provide a substantial amount of data without the use of complex laboratory equipment due to its extensive research and use, however not without its drawbacks, particularly that of abiotic (e.g., weathering or hydric erosion) as well as biotic (e.g., gnawing or chewing by carnivores) factors which can severely damage preservation of bones and their subsequent cataloguing and interpretation (Pickering, 2002, p. 134). It must be noted that zooarchaeological remains are regularly used for isotope research as contrast or proxies in regards to hominin data excavated in close proximity, whether for dating or dietary purposes. Lastly zooarchaeological analysis can provide details on hunting preferences and overall ecology of hominin sites, largely depending on species and overall volume of excavated remains. In conclusion, faunal assemblages provide a benchmark for dietary reconstruction of *H. neanderthalensis* and other hominins, as well as environmental details of biophysical environments.

Chapter 6: (Re)Defining Neanderthal Diet

Resuming the subject of novel isotopes, direct comparisons between the recurring isotopic signatures of Ca and Zn have to be drawn. This is done by analysis of isotope ratios within faunal and neanderthal remains published on both Dodat et al. (2021) and Jaouen et al. (2022), making use of supplementary data to create the graphs below. The chosen isotopic ratios are: Ba/Ca, Sr/Ca, and Zn/Ca due to their close link to dietary reconstruction and availability on both studies. The species chosen are: *Canis lupus* (wolf), *Cervus sp.* (deer), *Homo neanderthalensis, Crocuta spelaea* (cave hyaena), *Oryctolagus cuniculus* (European rabbit), *Rangifer tarandus* (reindeer), *Ursus arctos* (brown bear), and *Ursus spelaea* (cave bear). The former three are the only ones shown in both studies that match the requirements for this analysis, while the rest have been chosen for their dietary adaptation. The conditions determined for graphing in this thesis are as follows:

1. Species must showcase two or more samples excavated from the same layer or with samples being drawn from the same tissues per study.
2. Samples found in supplementary data must not be labelled as “stained” or “excluded” by the authors.
3. Samples published must have a complete analysis in regards to the requirements published in the articles. In example, a sample that has undergone Ba/Ca analysis but not Sr/Ca will be excluded.

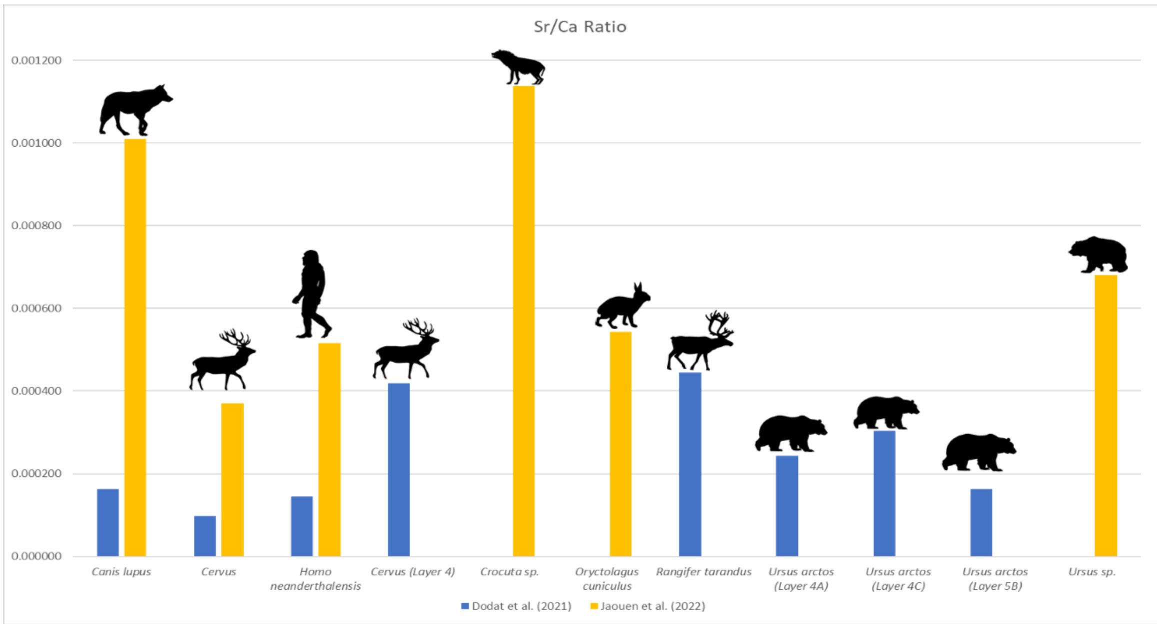
Designed for the scope of this analysis, the data from the species samples has been averaged and displayed as decimal numbers as opposed to scientific notation for increased comprehension when reviewing multiple species, as well as adding silhouettes to improve readability. Refer to Appendix for complete tables used.



Graph 1: Ba/Ca ratio in selected species. Data extracted from Dodat et al. (2021) and Jaouen et al. (2022)

Firstly, Graph 1 displays various differences in ratios between barium and calcium, with some level of consistency between the *Ursus* genus if the notoriously different sample from 4C layer of the Regourdou site is exclude. Due to biological discrimination of Ca in the trophic, the ratio should demonstrate an inverse correlation between Ba/Ca ratio and trophic positioning (Balter et al., 2002, p. 128). Relative to their own excavated sites, this trend is clear between established dietary adaptations for each species, once again showcasing the ample dietary choice of meat for *H. neanderthalensis* found in both Regourdou and Gabasa. Together with this, the notorious difference in the *Ursus* genus for layer 4C can be explained by individual adoption of a mostly herbivorous diet, which is relatively well documented in the overall literature due to brown bears having a high ecological plasticity (García-Vázquez et al., 2017, p. 6); however this explanation is not conclusive due to an overall lack of proof for this individual case.

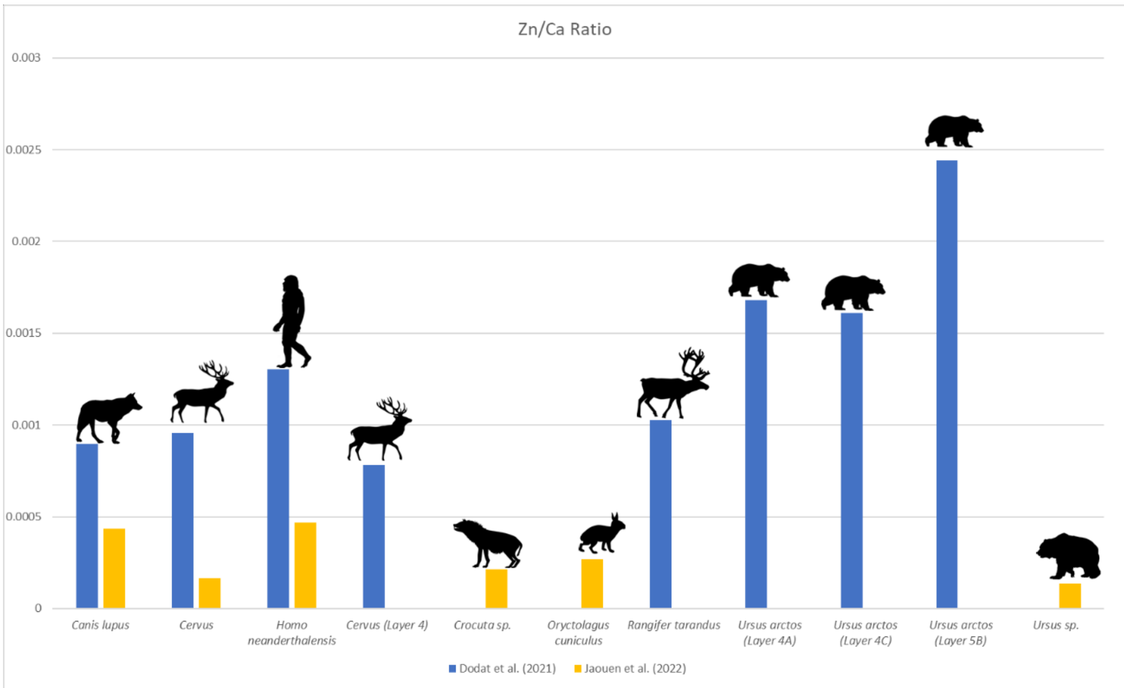
Conversely, the established isotopic ratio Ba/Ca correlates with the conclusion that *H. neanderthalensis* practiced a highly carnivorous diet for remains sampled for both $\delta^{42/44}$Ca and δ^{66}Zn, with little to no isotopic traces of plant or mushrooms consumption indicated in the results. With the use of this ratio as a benchmark or proxy, the use of Ca and Zn isotopes showcase promising results in the overall scope of dietary analysis. Furthermore, the ratio is key in determining overall trophic levels, due to the fact that species of all dietary adaptations are affected similarly regardless of their trophic position, although data requires extra reviewing with each trophic increase (Peek & Clementz, 2012, p. 38), hence drawing comparisons reduces bias and increases the possibilities of niche and environmental reconstruction and ecology. Overall, the Ba/Ca ratio can easily be labelled as an effective control and proxy for dietary isotope analysis.



Graph 2: Sr/Ca ratio in selected species. Data extracted from Dodat et al. (2021) and Jaouen et al. (2022)

In addition, strontium/calcium ratios have also been analysed for dietary comparison as seen on graph 2. While strontium is usually used with the radiogenic isotopes of $^{87}Sr/^{86}Sr$ for understanding mobility and residential change within the archaeological context (Slovak & Paytan, 2011, p. 744), when divided with calcium content in a determined sample it creates a powerful marker for dietary analysis as shown in Graph 2. Traditionally, Sr/Ca ratio has been defined by increasingly lower values inversely correlated with trophic position due to biopurification as the discrimination of against alkali metals in mammals (Burton et al., 1999, p. 609); however, this is not the case for the data presented by Jaouen et al. (2022). Even if the trend were to be reversed, the data is conflictive between ratios of herbivores, carnivores, and omnivores alike, giving no space for interpretation in *H. neanderthalensis*' dietary choices.

Conversely, data shown in Dodat et al. (2021) presents a trend through which carnivores and omnivores generally have lowered values than their herbivore counterparts, however the *Cervus* samples present conflicting results. One explanation for this trend is that consumption of C_3 plants yields a lower Sr/Ca ratio, and since all native European plants are of this carbon fixation (Lee-Thorp & Sponheimer, 2006, p. 136); however, this has to be compared to fauna with access to different niches due to the carnivores presented in the assemblage were excavated in the same sites. Diagenesis could represent the simplest answer, nonetheless the data availability makes it impossible to accurately asses this possibility. If the exception of *Cervus* is assessed, the data published in Dodat et al. (2021) follows the previously established trend of Sr/Ca ratio yielding lower values the higher with increased carnivory, thus positioning *H. neanderthalensis* as a high-level carnivory within the overall assemblage.



Graph 3: Zn/Ca ratio in selected species. Data extracted from Dodat et al. (2021) and Jaouen et al. (2022)

Lastly, zinc/calcium is a isotopic ratio that is generally understudied in archaeological literature due to its complex nature and overall use in maternal diets and enamel formation in infants (Dolphin & Goodman, 2009). Zn presence in enamel is critical and it is where it can be most easily found in archaeological context, for example: Jaouen et al. (2022) presents samples exclusively taken from enamel for δ^{66}Zn research. Due to the high presence of hydroxyapatite in enamel, it is no surprise that Ca also plays a crucial role within this scope, and thus shares a close link to Zn in the process of breastfeeding and later dairy consumption (Chu et al., 2006, p. 1661). Because of this, there are considerable similarities between the two elements for this determined subject, even when on a chemical level they have significant differences.

Therefore, it should be noted that the ratios displayed on Graph 3 are considerably closer to those showcased in the previous two graphs. This could be explained by the similarities between both elements relative to diet; as previously exhibited, the lower values of either element can showcase comparable increase in trophic positioning which would also explain the lower fraction results compared to the aforementioned trace elements and ratios on Graphs 1 and 2. Furthermore, Zn atoms become increasingly incorporated into the crystallized apatite in enamel, thus creating a close link with its Ca counterpart (Weber et al., 2021, p. 16). However, this does not necessarily correlate with an effective biomarker for dietary studies, but rather an explanation for values showcased in dental enamel during teeth formation and lactation (Müller et al., 2019, p. 122). Consequently, Zn/Ca isotopic ratio should not be exclusively used as a control marker within dietary reconstruction, and limit its use to enamel study and formation history.

Taking into consideration the previously mentioned biomolecular techniques and traditional isotopic ratios, neanderthal dietary reconstruction then becomes a dynamic and interdisciplinary subject, through which the application of newer methods such as novel isotopes require thorough testing and comparison. Such is the case for the specific remains of the Regourdou 1 *H. neanderthalensis* specimen previously discussed in Chapter 3 with the $\delta^{42/44}Ca$ isotopic signature, for which additional dietary reconstruction studies have taken place. After revision of teeth wear and palaeoclimate consistent with the temporal space and environment, Fiorenza et al. (2019) concludes that Regourdou 1 neanderthal consumed a wide array of foods including both plant and animal taxa (p. 185).

Following this trend, dental wear constitutes a good indicator of diet due to differences in abrasives within various food groups. At the same time, it can evidence the use of cooked or raw foods when paired with biomolecular data in dental calculus as mentioned previously, and has proven selective plant and cooking behaviour (Henry et al., 2010, p. 489). Additionally, the specimen exhibits uneven wear between the anterior teeth and molars, although such wear in *H. neanderthalensis* teeth can be a consequence of utilizing these as a “third hand” in order to facilitate the use of other tools (Volpato et al., 2012, p. 3). While this showcases an interesting behavioural trait, it does not directly correlate with plant consumption associated with teeth wear even if the tools or artefacts that possibly took advantage of this trait were of plant origin. Lastly, the use of dental wear and microremains analysis can create a distinction in the archaeological record between individuals living in different environments and ecological niches (Estalrrich et al., 2017, p.18). When pairing this data together, the reconstruction for neanderthal environments, diet, and ecology becomes clearer and with increasing accuracy, provided the tools for interdisciplinary study.

As such, the introduction of biomolecular and dental wear studies as a contrasting model does not necessarily challenge the conclusion of heavy meat consumption exhibited in Dodat et al. (2021) for the same specimen, rather both studies complement their research niches for an accurate portrayal of diet that is consistent with reconstructed paleoenvironments and faunal assemblages. In this sense, the use of the $\delta^{42/44}Ca$ isotopic signature presents itself as a suitable marker for dietary analysis in *H. neanderthalensis*, and could even be extrapolated for *H. sapiens* or earlier species in the hominin lineage. Since plant consumption is not directly displayed on $\delta^{42/44}Ca$, but rather trophic levels relative to other species, this possibility should not be excluded. It does show however, that *H. neanderthalensis* consumed meat both often and in large quantities, especially originating from medium and large herbivores; hence plant usage could have had medicinal or complimentary nutritional values on overall diet. As a result, $\delta^{42/44}Ca$ constitutes a novel isotopic signature that could accurately and efficiently portray dietary reconstruction in *H. neanderthalensis*.

To finish, the site of Gabasa 1 reviewed in Jaouen et al. (2022) exhibits a notoriously large faunal assemblage, showcased both in the article itself and in previously published literature. Zooarchaeology plays a pivotal role when determining dietary choices in *H. neanderthalensis*, from the nature of an excavated site, to the aforementioned control samples within isotopic analysis seen in Graphs 1-3. The site of Gabasa 1 has over 23.000 identified specimens of mammals and birds, with the former consisting of the largest fraction and including species of carnivores, omnivores, and herbivores alike (Blasco, 1997, p. 180). This creates ample opportunity for comparisons of species within the same niche and depositional area, which in turn favours understanding both of hunting strategies undertaken by *H. neanderthalensis* and overall ecology of the site as dated; together with opportunities for proxies in isotopic studies, both for traditional and novel techniques. However, the high presence of hyaena activity in the site has shown to distort bone accumulations (Yravedra, 2009, p. 11); which puts pressure on the already highly carnivorous neanderthal presence in Gabasa 1, especially if faunal assemblages are used as a primary marker for diet reconstruction. An alternative view for this issue is the accumulation of herbivore and omnivore bones creates a hunting pattern of hyaenas that can be extrapolated onto other similar sites, and thus keep *H. neanderthalensis* data somewhat on the clear.

In conclusion, the novel isotopic signatures of $\delta^{42/44}Ca$ and $\delta^{66}Zn$ exhibited with an ample amount of comparative evidence, accurate results for dietary analysis in *H. neanderthalensis*. The trophic positioning showcased can be extrapolated into faunal assemblages of the same site, and due to their sample extraction being directly linked to bone and enamel, its use should be of the highest consideration for future researchers. However, it is important to note that results are similar if not identical to previously published data utilising $\delta^{13}C$ and $\delta^{15}N$ in dietary analysis, which in turn gives researchers a reliable choice between these depending on overall preservation of samples, diagenesis, and collagen survivability. Furthermore, the discussion of *H. neanderthalensis* diet still requires ample research, whilst highly carnivorous, neanderthals did consume plant species for a various reasons, and this critical detail should not be left behind on the basis of evidence not showing directly under isotope analysis.

Chapter 7: The Future of Isotope Research

As presented, novel isotopic signatures such as $\delta^{42/44}Ca$ and $\delta^{66}Zn$ can be equally accurate as other methods for dietary reconstruction in *H. neanderthalensis*. While these are notorious advancements in the field of isotope research, it is just beginning to set in motion further types of atomic-related studies within archaeology. Taking into consideration the vastness of chemical elements that take part in human life, and the increasing access to capable scientific equipment, well-trained professionals, and extensive literature sharing and availability, it is no surprise that isotope research beyond the reach of ^{13}C and $\delta^{15}N$ are beginning to appear. As a closing remark, this chapter compiles promising isotopic signatures that can be used to broaden the scope and understanding of the past.

Sulphur isotopes, in particular the $\delta^{34}S$ signature, have showcased potential in detecting and understanding dietary choices regarding consumption of freshwater and terrestrial species (Privat et al., 2007, p. 1202). It can be used in combination and comparison with ^{13}C and $\delta^{15}N$, as well as triple combination as $\delta^{34}S$ - $^{87}Sr/^{86}Sr$ - $\delta^{18}O$ for geographic assignment and seafood intake on individuals (Bataille et all., 2021, p. 16), similar to the use of $\delta^{15}N$ in comparative studies. Yet another application of $\delta^{34}S$ is that of mobility, much alike strontium isotopes, due to differences in sulphur absorption through foodwebs, and its subsequent change depending on geographical position of an individual (Craig et al., 2010, p. 2511). While still relatively underdeveloped, $\delta^{34}S$ analysis poses significant potential within archaeological studies, especially taking into account the mentioned pairings with other isotopic signatures.

A key component in novel isotope research and the future of its application into archaeology is biomedical research, with elements such as calcium, iron, copper, and zinc being researched due to their presence in the human body (Costas-Rodríguez et al., 2016, p. 191). It must be noted that these are mainly focused on blood and soft tissues, although they include bone marrow for study; which in theory makes it virtually impossible to expand into the archaeological record. However, due to the close relationship between bone and blood, the possibility of extrapolating said studies into archaeology is not entirely ruled out. $\delta^{56}Fe$ is a consistently mentioned isotopic signature in the biomedical literature, with values varying between men and women (Albarède et al., 2011, p. 928), nevertheless these values have only been studied in blood samples. Within the same study, calcium and copper played an important role yet further research has to be done in order to determine its feasibility and application outside of medicine and into archaeology.

A different perspective on the issue of future research is the increasing access that the general public has to scientific publications and research without the press acting as a mediator. The rise of the internet and social media has allowed people to read and review in their own words scientific publications that would otherwise be concealed into university libraries or behind expensive subscriptions to magazines. Furthermore, increased access for junior researchers to publish their work and data has given insight into newer ideas and ambitious techniques, although sometimes falling onto so-called “predatory journals” (Fiala & Diamandis, 2019, p. 1). Overall this can also have a negative impact, high availability means a higher amount of data and articles that may not follow strict guidelines or scientific integrity, brewing a dangerous environment for the development of science in archaeology and other fields alike. In contrast, articles that debate and consolidate guidelines for proper archaeological practice in determined fields have also shown increased availability for researchers, including some with Open Access protocols. One such case that refers directly to isotope analysis and its applications in archaeology is Roberts et al. (2017), which attempts to set a benchmark for future archaeologists and researchers in terminology and practice. Examples like these are an essential and positive component for the development of appropriate research and publishing of data.

On a final note, the development and application of $\delta^{42/44}Ca$ and $\delta^{66}Zn$ isotopic signatures constitute the basis for future dietary and trophic analysis ranging from historical archaeology to palaeolithic and beyond. Both articles discussed previously were published in the previous two years of the writing of this thesis, signalling towards increased applications of such techniques in various sites and contexts. With the aid of other dietary proxies which are seeing increased technological advances, the subject of paleodiets gradually becomes more accurate, and the discussion, insight, and interpretation of such topics gives archaeologists a higher quality standard for which to base further research on. This does not mean that these methods are anywhere near perfect or 100% accurate, rather an ideal starting point for current and future archaeologist for which to base ideas, discussions, excavations, and laboratory research.

As can be seen, the future of stable isotope research within archaeology is potentially a bright one, with novel techniques being developed and experimented in a way array of topics that can go further than dating and diets. Increased technological developments and interest within the archaeology community forms the basis of scientific progression in the 21st century. With this in mind, research must continue a trend of progressive and consistent development that explores the many chemical elements that comprise both the natural world and the physical fabric of humanity, both for understanding our past and our present.

Chapter 7: Conclusion

In conclusion, the application of novel isotope research for the context of *H. neanderthalensis* dietary habits and choices, has shown techniques that do not rely on collagen samples, rather utilising enamel and bone; as well as accurate dietary results when paired with different research approaches in the same sites and specimens. Within this framework, the thesis' research questions and hypothesis have been explored thoroughly throughout the thesis, nevertheless a direct discussion of these will serve as part of the concluding chapter. Firstly, the primary research question as follows:

Does novel isotope research challenge preconceived notions of dietary habits of H. neanderthalensis, relative to standard research practices?

The answer to this question is simple: no. Novel isotope research has showcased strikingly similar results to those showcased by traditional isotopes within the subject of *H. neanderthalensis* dietary habits, aiming at a highly carnivorous diet with little to no plant consumption evidenced in isotopic signatures. What novel isotope research can challenge is dietary reconstruction for specimens and samples with little or no surviving collagen, widening the research area and thus creating a more accurate image of neanderthal diets.

As such, the hypothesis followed a similar trend:

Isotopes of elements extracted from neanderthal remains (such as $\delta^{42/44}Ca$ and $\delta^{66}Zn$) can be of significant use when researching Neanderthal diets without over relying on the bias of collagen-found isotopes; as well as assisting dietary research for studies of biomolecular remains and faunal assemblages.

This hypothesis has been proved true for the case of *H. neanderthalensis* diet reconstruction and research, especially in reference to the bypassing of collagen that $\delta^{42/44}$Ca and δ^{66}Zn can offer. Novel isotopes have showcased coherent results when analysing neanderthal diets and should be considered by researchers as a reliable option for investigation. The second statement within the hypothesis is complex, due to the fact that this should not be interpreted as an assist between either approach to the problematic, but rather as a complementary and interdisciplinary solution. Every type of research method showcases limitations, such as plant consumption in isotopic signature and ratios, preservation of samples in biomolecular analysis, and overall preservation and weathering of faunal assemblages. This deepens the notion that these methods should not be seen as competitors of each other, but rather as a combined effort for greater accuracy and precision in diet reconstruction.

Furthermore, the three sub questions for this thesis have been explored, yet a definitive answer to each is displayed below:

Can isotopes derived from collagen samples be used as proxies for research on novel isotopes such as Zn? Can biomolecular data and faunal assemblages also be used as proxies?

Yes, isotopes extracted from collagen samples can be used as proxies, however it is not particularly necessary to this for samples of the same site or individual, since the results of trophic positioning per species would be the same in the wider spectrum. Proxies should be used between datasets that do not express data so similarly as isotopic signatures, whether novel or traditional.

Does desk-based research cover the intricacies and details needed to extract and interpret the required data in studies of this nature?

Taking into account the vast extent that desk-based research can achieve, and depending on the access to various and updated resources, it is possible to cover the details and intricacies of this subject matter; provided that datasets in publications are clear and available to researchers.

What is the importance and impact of neanderthal diet in the broad spectrum of palaeolithic and human origins archaeology?

The importance of neanderthal diets within this subject is evident for various reasons. The first is an overall behavioural dataset that can be accessed via dietary choices, for example the choice of hunting large mammals would require certain social cohesion and communication, as well as furthering and deepening societal structures when it comes to the butchering and processing of food. Secondly, it can serve as a benchmark for environmental reconstruction, such as the presence of C_3 plants or the climactic conditions based on what was hunted or gathered. Lastly, in the subject of human origins, *H. neanderthalensis* was our closest relative, and one we shared more than just genes with, so the reconstruction of diet, an overall mundane matter, becomes of increasing importance if we as a species want to truly understand our nature on a level unachievable with just modern knowledge.

Unfortunately, as much as we can research and try to comprehend and interpret complex data, everything we now know about neanderthals stems exclusively from the archaeological record, even when our understanding has astronomically changed with archaeological practice refinement and scientific as well as technological advances. Everything we could ever learn from them in our modern era has been deposited in time to archaeological sites, layers, and research of such. From the perspective of a distant future looking into a once closely shared past, *Homo neanderthalensis* became to us, what it once ate.

Abstract

The issue of *H. neanderthalensis*' diet has long been a debate between archaeologists, with interpretations ranging from faunal assemblages in neanderthal sites, stable isotope analysis, cooking evidence, and studies of dental calculus. Traditional isotope research has focused on the $\delta^{13}C$ or $\delta^{15}N$ isotopic signature is based on the use of collagen found in bone, however it suffers the problem of easy degradation in deep time. The introduction of novel isotope techniques focused on the signatures of $\delta^{42/44}Ca$ and $\delta^{66}Zn$ can bypass this problem due to samples being extracted directly onto bone and enamel, thus decreasing the chances of lacking evidence and presenting similar and accurate results in dietary research. However, results position species into trophic levels and lack data on plant consumption, thus presenting *H. neanderthalensis* as a nearly exclusive carnivore in the hominin lineage. The objective of this thesis is a comprehensive review of the literature to study the feasibility of adopting such techniques into the research mainstream and the consequences that this would entail. Furthermore, techniques such as biomolecular analysis of dental calculus and faunal assemblages that refer directly to dietary habits and tendencies are both explored and used as proxies for increasing accuracy. While the scope of this thesis has significant limitations to fully asses dietary research techniques used for analysis of middle palaeolithic European neanderthals, the comprehensive review of the latest published literature and a comparative approach between different research methods aims to produce an accurate assessment of both novel isotope techniques and *H. neanderthalensis* diets.

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<u>Figures</u>

Figure 1: Western European map showcasing discussed sites. Google Earth (n.d) generated by author. Retrieved 15/06/2023

Figure 2: Figure by author

Figure 3: 3D satellite image of Regourdou site. Google Earth (n.d) generated by author. Retrieved 15/06/2023

Figure 4: δ42/44Ca chart of Regourdou fossils and modern plants. Extracted from Dodat et al. (2021)

Figure 5: 3D satellite image of the site and surrounding geography. Google Earth (n.d) generated by author. Retrieved 15/06/2023

Figure 6: $\delta^{66}Zn$ enamel chart comparing various species and their trophic positions. Extracted from Jaouen et al. (2022)

Graph 1: Ba/Ca ratio in selected species. Data extracted from Dodat et al. (2021) and Jaouen et al. (2022)

Graph 2: Sr/Ca ratio in selected species. Data extracted from Dodat et al. (2021) and Jaouen et al. (2022)

Graph 3: Zn/Ca ratio in selected species. Data extracted from Dodat et al. (2021) and Jaouen et al. (2022)

Reference for all graphs and Figures 4 and 6:

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Appendix

Species (Dodat et al., 2022)	**Layer**	**Ba/Ca**
Canis lupus	4	0.000455
Cervus	4	0.000438
Cervus	4	0.000494
Cervus	2	0.000156
Cervus	2	0.000172
Cervus	2	0.00021
Homo neanderthalensis	4A	8.72E-05
Homo neanderthalensis	4A	0.000128
Homo neanderthalensis	4A	9.41E-05
Ursus arctos	4A	0.000142
Ursus arctos	4A	0.0004
Ursus arctos	4C	0.000202
Ursus arctos	4C	0.00209
Ursus arctos	5B	0.000187
Ursus arctos	5B	0.000228
Rangifer tarandus	2	0.000367
Rangifer tarandus	2	0.000424
Rangifer tarandus	2	0.000118
Rangifer tarandus	2	0.000417

Species (Jaouen et al., 2022)	Ba/Ca
Canis lupus	0.000206
Canis lupus	0.000113
Canis lupus	0.000201
Canis sp.	0.0000617
Cervus sp.	0.000046
Cervus sp.	0.000165
Homo neanderthalensis	0.0000178
Ursus sp.	0.000131
Ursus sp.	0.000106
Ursus sp.	0.0000808
Oryctolagys cuniculus	0.000285
Oryctolagys cuniculus	0.000288
Oryctolagys cuniculus	0.000236
Oryctolagys cuniculus	0.000198
Crocuta sp.	0.0000805
Crocuta sp.	0.0000831
Crocuta sp.	0.0000635
Crocuta sp.	0.0000588

Species (Dodat et al., 2021)	Layer	Sr/Ca
Canis lupus	4	0.000163
Cervus	4	0.000326
Cervus	4	0.000513
Cervus	2	0.000086
Cervus	2	0.000104
Cervus	2	0.000105
Homo neanderthalensis	4A	0.000145
Homo neanderthalensis	4A	0.000144
Homo neanderthalensis	4A	0.000145
Ursus arctos	4A	0.000187
Ursus arctos	4A	0.000298
Ursus arctos	4C	0.000182
Ursus arctos	4C	0.000425
Ursus arctos	5B	0.000168
Ursus arctos	5B	0.000159
Rangifer tarandus	2	3.27E-04
Rangifer tarandus	2	4.59E-04
Rangifer tarandus	2	6.69E-04
Rangifer tarandus	2	3.21E-04

Species (Jaouen et al., 2022)	Sr/Ca
Canis lupus	0.000982
Canis lupus	0.000554
Canis lupus	0.001490
Cervus sp.	0.000446
Cervus sp.	0.000294
Homo neanderthalensis	0.000516
Ursus sp.	0.000843
Ursus sp.	0.000846
Ursus sp.	0.000354
Crocuta sp.	0.00152
Crocuta sp.	0.000803
Crocuta sp.	0.00053
Crocuta sp.	0.0017
Oryctolagys cuniculus	0.000423
Oryctolagys cuniculus	0.000422
Oryctolagys cuniculus	0.000766
Oryctolagys cuniculus	0.000563

Species (Jaouen et al., 2022)	**Zn/Ca**
Canis lupus	0.000898
Canis lupus	0.000335
Canis lupus	0.000234
Canis lupus	0.000277
Canis sp.	0.000188
Cervus sp.	0.000133
Cervus sp.	0.000171
Homo neanderthalensis	0.000469
Ursus sp.	0.000174
Ursus sp.	0.00013
Ursus sp.	0.000104
Oryctolagys cuniculus	0.000263
Oryctolagys cuniculus	0.000228
Oryctolagys cuniculus	0.000364
Oryctolagys cuniculus	0.000228
Crocuta sp.	0.000218
Crocuta sp.	0.00013
Crocuta sp.	0.000296

Species (Dodat et al., 2021)	Layer	Zn/Ca
Canis lupus	4	0.000898
Cervus	4	0.00075
Cervus	4	0.000812
Cervus	2	0.001
Cervus	2	0.000632
Cervus	2	0.00124
Homo neanderthalensis	4A	0.00196
Homo neanderthalensis	4A	0.0011
Homo neanderthalensis	4A	0.000848
Ursus arctos	4A	0.002
Ursus arctos	4B	0.00191
Ursus arctos	4C	0.00125
Ursus arctos	4C	0.00197
Ursus arctos	4A	0.00136
Ursus arctos	2	0.000946
Ursus arctos	5B	0.00237
Ursus arctos	5B	0.00251
Ursus arctos	7	0.00192
Rangifer tarandus	2	0.000904
Rangifer tarandus	2	0.00126
Rangifer tarandus	2	0.000826
Rangifer tarandus	2	0.00111