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1 **A Neandertal dietary conundrum: new insights provided by tooth enamel Zn isotopes from**
2 **Gabasa, Spain**

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Significance statement

Neandertals' diets are a topic of continued debate, especially since their disappearance has been frequently attributed to their subsistence strategy. There is no clear consensus on how variable their diets were in time and space. Isotope studies have helped quantify meat consumption in Neandertals, but usually rely on nitrogen isotope analyses of collagen, a protein rarely preserved in samples older than 50 ka. Moreover, collagen-extraction for isotope analyses is rarely successful in Iberian skeletal material. Here, we employ zinc isotope analysis of dental enamel of a Neandertal and associated fauna (Gabasa, Spain), which can be applied to contexts >50 ka. This proxy confirms a high level of carnivory in an Iberian Neandertal.

Abstract

The characterization of Neandertals' diets has mostly relied on nitrogen isotope analyses of bone and tooth collagen. However, few nitrogen isotope data have been recovered from bones or teeth from Iberia due to poor collagen preservation at Paleolithic sites in the region. Zinc isotopes have been shown to be a **reliable** method for reconstructing trophic levels in the absence of organic matter preservation. Here we present the results of zinc (Zn), strontium (Sr), carbon (C), and oxygen (O) isotope and trace element ratios analysis measured in dental enamel on a Pleistocene food web in Gabasa, **Spain**, to characterize the diet and ecology of a Middle Paleolithic Neandertal individual. **Based** on the extremely low $\delta^{66}\text{Zn}$ value observed in the Neandertal's tooth enamel, **our results** support the interpretation of Neandertals as carnivores as already suggested by $\delta^{15}\text{N}$ isotope values of specimens from other regions. Further work could help identify if such isotopic peculiarities (lowest $\delta^{66}\text{Zn}$ and highest $\delta^{15}\text{N}$ of the food web) are due to a metabolic and/or dietary specificity of the Neandertals.

83 **Introduction:**

84

85 Over the last thirty years, analyses of nitrogen isotopes in collagen ($\delta^{15}\text{N}_{\text{collagen}}$) have provided direct
86 evidence for Neandertal diets across Europe and Asia. These studies all indicate a carnivorous (1–12),
87 or at least a meat-heavy diet for European Neandertals. However, one peculiarity of Neandertal
88 $\delta^{15}\text{N}_{\text{collagen}}$ remains the subject of an ongoing debate. From the one Siberian and eight Western
89 European sites, where both Neandertal and associated fauna have been analyzed, nitrogen isotope
90 ratios in Neandertal collagen are systematically higher than that of other carnivores (3, 6–8, 10, 11, 13,
91 14). An explanation for such elevated values could be the consumption of herbivores, such as
92 mammoths, which themselves exhibit elevated $\delta^{15}\text{N}$ values due to the consumption of plants growing
93 on arid soils (1, 2, 7). While mammoth remains are usually scarce at Neandertal fossil localities, they
94 were nonetheless occasionally consumed, as suggested by remains with cut marks and other human
95 butchery signatures (15). The absence of mammoth remains at Middle Paleolithic sites could be a
96 result of a) Neandertals chose to leave large bone elements at the kill-site, and transport other edible
97 carcass products, mainly meat, back to the habitation site (15) or b) mammoths were not frequently
98 consumed, and the $\delta^{15}\text{N}$ peculiarity consequently reflects the consumption of other resources enriched
99 in ^{15}N .

100 Alongside this $\delta^{15}\text{N}$ peculiarity, one major obstacle to our knowledge of Neandertals'
101 subsistence patterns is that the preservation of organic matter limits the application of collagen-bound
102 nitrogen isotope analysis to fossil specimens. Collagen degrades over time at a varying speed
103 depending on climatic and environmental conditions (16). The oldest hominin specimen in which bone
104 protein is preserved is that of Scladina (Belgium), which dates to 90,000 cal BP (17), but most studied
105 specimens are younger than 50,000 cal BP (1–3, 6–8, 10–13, 18). Furthermore, these specimens are
106 only from sites in northwestern and central Europe and Siberia, where climatic conditions favored
107 collagen preservation. As a result, the variability of Neandertals' diet over time and between regions
108 may not accurately reflected by the currently available isotope data. In Iberia, where the latest
109 surviving Neandertals have been discovered (19, 20), collagen was successfully extracted for only one
110 site (21). Therefore, our knowledge of Iberian Neandertal diets mostly relies on zooarcheological and

111 dental calculus data, which show some inconsistencies (21–25). For instance, similar to other Western
112 European sites, zooarcheological studies emphasize the consumption of terrestrial mammals and birds
113 (21). In contrast, analysis of dental calculus for micro-remains and aDNA metagenomic analysis (26–
114 28) highlight the frequent consumption of plants and mushrooms. Indeed, Weyrich et al. (26) even
115 suggest that Neandertals at El Sidrón (Figure 1) rarely consumed meat, but often ate mushrooms,
116 which would also result in elevated $\delta^{15}\text{N}$ values (29). The consumption of marine foods is also attested
117 for coastal Neandertals, but its frequency cannot be truly assessed in the absence of isotope studies
118 (21, 23–25, 30). Finally, cannibalism has been documented at two Iberian sites (El Sidrón and
119 Zafarraya) (22, 31) (Figure 1), though such practices appear limited and most likely occurred only
120 during periods of nutritional stress (32). Therefore, it is challenging to confirm the homogeneity of
121 Neandertals' diets across time and space, calling into question a direct link between their subsistence
122 strategy and disappearance.

123 This study aims to investigate if the novel Zn isotope proxy could help elucidate the dietary
124 behaviors of Neandertals and the source of their $\delta^{15}\text{N}$ peculiarity, specifically by studying a Late
125 Pleistocene Iberian food web where the presence of mammoth has not been documented (33). The
126 development of Zn isotope analysis ($^{66}\text{Zn}/^{64}\text{Zn}$, expressed as $\delta^{66}\text{Zn}$) has proven that trophic level
127 information can be retrieved from mammalian tooth enamel ($\delta^{66}\text{Zn}_{\text{enamel}}$) (34, 35), including fossil
128 samples from Pleistocene food webs where organic matter is typically not preserved (36, 37). Previous
129 studies have demonstrated that $\delta^{66}\text{Zn}_{\text{enamel}}$ decreases by ca. 0.30 to 0.60 ‰ with each step in
130 archeological and modern food webs (34–38) and that $\delta^{66}\text{Zn}$ values associated with breastfeeding are
131 higher than post-weaning associated values (39). While the main source of variation of $\delta^{66}\text{Zn}_{\text{enamel}}$
132 values is diet, local geology can also likely influence the isotope ratio of a given animal (36, 39). To
133 date, three modern assemblages from Koobi Fora (Kenya), Kruger Park, and the Western Cape (South
134 Africa)(40), a few animals from a historical site (Rennes, France) (41), and three Late Pleistocene sites
135 (Tam Hay Marklot, Nam Lot and Tam Pà Ling, Laos) (36, 37) are the only terrestrial food webs for
136 which Zn isotope data in teeth and/or bones have been published (Figure S14). In the modern Koobi
137 Fora savannah food web, $\delta^{66}\text{Zn}_{\text{enamel}}$ differences have been observed between browsers and grazers

138 (35), but this pattern was not seen in any of the three Pleistocene Asian forest food webs (36, 37).
139 Among modern and historical human populations, historically-documented diets relying on plants are
140 associated with higher $\delta^{66}\text{Zn}$ values than those that include a substantial quantity of animal products
141 (41–44). Zinc isotopes of ancient hominins have been analyzed only in one Pleistocene *Homo sapiens*
142 individual (37) from Southeast Asia, but not yet in any Neandertal specimen.

143 This current study contributes significantly to our understanding of Iberian Neandertal diets by
144 providing information on their trophic ecology for a region where traditional nitrogen isotope analyses
145 are unfeasible due to the poor preservation of organic matter. We use the novel Zn isotopic tool as
146 well as other mobility, ecological and dietary proxies applied on tooth enamel from hominin and
147 animal remains from the cave site Cueva de los Moros 1 (Gabasa – Pyrenees – Spain, Figure 1). The
148 site, excavated in the 1980's, is very well documented (for stratigraphic context, see Montes and
149 Utrilla (45) and Supplementary Information 1). All remains come from the layers e, f, and g of a single
150 stratigraphic layer directly above layer h dated to 143 ± 43 ka. Numerous carnivore remains were
151 recovered along with Neandertal remains (layers e and f), allowing for comparison of the different
152 meat-eating taxa. Co-existing herbivores from three different types of environmental contexts are
153 homogeneously represented in layers e, f, and g: mountains (Iberian ibex [*Capra pyrenaica*], chamois
154 [*Rupicapra rupicapra*]), forest (cervids including red deer [*Cervus elaphus*]), and open environments
155 (horses [*Equus caballus*], European wild asses [*Equus hydruntinus*]).

156

157 **Sample collection**

158

159 ***Species, sample, and proxy selection***

160

161 We analyzed the tooth enamel of 65 samples belonging to 43 different teeth of 12 taxa for
162 $\delta^{66}\text{Zn}_{\text{enamel}}$ (Figure 3, Table S15), 42 samples belonging to 39 different teeth for $\delta^{13}\text{C}_{\text{enamel}}$, $\delta^{18}\text{O}_{\text{enamel}}$
163 (Table S19), 23 samples belonging to 18 different teeth for $^{87}\text{Sr}/^{86}\text{Sr}$ (Table S7) and 40 samples for
164 trace element ratios (Table S11). We sampled 12 to 50 mg of tooth enamel (1 to 20 mg for Zn
165 isotopes) of five carnivore taxa (lynx [*Lynx spelaea*], wolf [*Canis lupus*], fox [*Vulpes vulpes*], dhole
166 [*Cuon alpinus*], hyena [*Crocuta spelaea*]), one omnivore (bear [*Ursus spelaeus*]) and at least five
167 herbivores (Iberian ibex [*Capra pyrenaica*], chamois [*Rupicapra rupicapra*], red deer [*Cervus*

168 *elaphus*], rabbit [*Oryctolagus cuniculus*], horse [*Equus caballus*], European wild ass [*Equus*
169 *hydruntinus*]), as well as one of the four Neandertal dental remains recovered from the site. Hyenas
170 (*Crocota spelaea*) are treated as a separate dietary group (bone-eating carnivores) based on the results
171 of the Koobi Fora and Nam Lot food webs (35, 37).

172 ***Sampling strategy related to breastfeeding***

173 The single sampled Neandertal tooth is a first molar (M1; Ga1.Rev.303)(46). Previous studies based
174 on tooth wear and trace elements suggest that Neandertals were likely weaned between the age of 1 to
175 2.5 years old (47–50), thus possibly overlapping with the crown’s formation period for this tooth. To
176 minimize destructive analyses, we sampled an enamel fragment from the lower part of the crown, next
177 to the cervical margin. This part of the tooth probably formed after weaning or at least when maternal
178 milk consumption could have been strongly reduced (51). The specimen was heavily worn, making it
179 difficult to evaluate the enamel formation period of the part that we sampled. Still, we were able to
180 evaluate trophic spacing between species while taking into account the influence of maternal milk
181 consumption, which triggers higher $\delta^{66}\text{Zn}$ values in human M1 (39). To do so, we analyzed $\delta^{66}\text{Z}_{\text{enamel}}$
182 on different types of non-hominin teeth (dp4, C, P2, P3, P4, M1, M2, and M3, See SI Table S4)
183 formed at different times during ontogeny and obtained values associated with breastfeeding and/or
184 solid food consumption for all species analyzed from Gabasa. In addition, we analyzed Sr/Ca and
185 Ba/Ca ratios that also show variation between tooth enamel mineralized in utero and during pre-or
186 post-weaning periods (52).

187 ***Sampling strategy related to Neandertal mobility and ecology***

188 Other factors such as diagenesis, geographical origin, environmental context, or metabolism can be a
189 source of Zn isotope variability (35, 36, 39, 53). In recent studies, linear mixing models highlighted a
190 possible link between Zn and Sr isotope ratios in teeth, which has been interpreted as resulting from
191 the influence of the local geology on the Zn isotope ratios of its associated food webs (36, 37, 39). To
192 control for this potential bias, we analyzed strontium isotope ratios of Gabasa tooth enamel
193 ($^{87}\text{Sr}/^{86}\text{Sr}_{\text{enamel}}$) for samples with sufficient material (Supplementary Information 5). We also collected
194 Sr isotope data from rocks, soils, plants, and teeth to assess the regional bedrock isotopic data
195 ($^{87}\text{Sr}/^{86}\text{Sr}_{\text{bedrock}}$) and identify whether some animals could have been derived from areas proximal to

196 Gabasa with different **geology** (Supplementary Table S18). We hypothesize that the Neandertal
197 consumed local food sources, whereby its $^{87}\text{Sr}/^{86}\text{Sr}$ should fall within the ranges observed both for the
198 local bedrock and assumed sympatric animals.

199 We estimated carbon and oxygen isotope compositions ($\delta^{13}\text{C}_{\text{enamel}}$ and $\delta^{18}\text{O}_{\text{enamel}}$, respectively) of the
200 Neandertal and associated fauna. Notably, $\delta^{13}\text{C}_{\text{enamel}}$ and $\delta^{18}\text{O}_{\text{enamel}}$ values can offer insights into
201 ecological proxies, such as the environment from which the Neandertal individual obtained its **prey**
202 (i.e. open arid vs. closed mesic habitats), and possibly reveal information about which types of prey
203 were consumed (54–56). Moreover, carbon and oxygen isotope data allow us to compare the food web
204 of Gabasa to other food webs where Neandertals have been discovered. For one horse tooth, five
205 samples were taken serially along the growth axis of the teeth to evaluate potential seasonality and
206 intra-tooth variability over time on Sr and Zn isotope ratios.

207 *Sampling strategy to assess the degree of chemical tooth alteration*

208 Although it has been shown that dental enamel preserves biogenic Zn isotope signatures very well in
209 Pleistocene or even older samples (36, 57), we **independently confirmed** that Zn isotope variability is
210 not related to diagenetic processes. For this purpose, we analyzed trace elements that are abundant in
211 soils but not in tooth enamel (Fe, Al, Mn) and hence are indicative of post-mortem trace element
212 uptake (Supplementary Information 3). Additionally, we examined the carbonate content of dental
213 enamel **to eliminate samples with obvious diagenetic alteration (>10 wt% CO_3)** (Supplementary
214 Information 3). **To capture the influence of diagenesis on isotope ratios, we** also performed
215 measurements on dentin samples from two wolves for Sr, C, and O isotopes and trace elements and
216 one wild ass for Zn isotopes. Finally, four roots of teeth whose enamel was sampled (one chamois,
217 two foxes, and one lynx) were used to attempt collagen extraction (Table S9, S10), using a **high**
218 **extraction-yield** protocol adapted for radiocarbon dating (58, 59) in the hope of getting additional
219 trophic level information and possibly **permitting high-precision** radiocarbon dating.

220

221 **Results**

222 Datasets, details on the method, and additional discussions are given in detail in the supplementary
223 information (SI).

224

225 ***Zinc isotope data***

226 Zinc isotope **values** of Gabasa mammalian tooth enamel range from 0.35 to 1.63 ‰ ($n = 65$) and are
227 mainly influenced by the dietary group of the animals (Figures 2 and 3) (Kruskal-Wallis $\chi^2 = 19.496$,
228 p -value = 0.0002159 and $\chi^2 = 18.298$, p -value = 0.0003818; samples with elevated Al, Mn, and Fe
229 content are excluded), which are assigned based on those used in other $\delta^{66}\text{Zn}_{\text{enamel}}$ isotope studies and
230 zooarcheological data (dietary groups: carnivores, bone-eating carnivores, omnivores, and herbivores)
231 (35, 60, 61). Animal values from different archeological layers fall in the same range (Supplementary
232 Information 4). As previously observed for modern, historical, and Late Pleistocene tooth enamel,
233 there is no correlation between Zn isotope values and Zn concentration data (35, 36, 39, 41, 42). This
234 is an excellent indicator to assess the preservation **of** biogenic Zn isotope signatures (62). On average,
235 large herbivores have higher Zn isotope **ratios** ($\delta^{66}\text{Zn}_{\text{enamel}} = 1.24 \pm 0.17$ ‰ 1σ , $n = 16$) than carnivores
236 ($\delta^{66}\text{Zn}_{\text{enamel}} = 0.92 \pm 0.18$ ‰ 1σ ; $n = 16$, 0.85 ± 0.14 ‰ if hyenas and **the Neandertal** are excluded). The
237 Neandertal tooth exhibits the lowest $\delta^{66}\text{Zn}_{\text{enamel}}$ value (0.35 ± 0.00 ‰), substantially lower
238 ($\Delta^{66}\text{Zn}_{\text{Neandertal-carnivores}} = 0.57$ ‰) than that of the lowest carnivore's value. Mirroring patterns in the
239 modern Koobi Fora (East Africa) food web, the difference between herbivore and carnivore values is
240 even more pronounced when hyenas are excluded, as bone-consumption is thought to induce higher
241 $\delta^{66}\text{Zn}$ values (35). The omnivorous cave bears ($\delta^{66}\text{Zn}_{\text{enamel}} = 1.15 \pm 0.13$ ‰ 1σ ; $n = 3$) exhibit Zn
242 isotope ratios closer to those of large herbivores and rabbits rather than the carnivores', similar to a
243 pattern often observed in nitrogen isotopes (63–66). The herbivores show the highest $\delta^{66}\text{Zn}$ values
244 (0.96 to 1.60 ‰ 1σ ; $n_{\text{measurements}} = 40$ from $n_{\text{tooth}} = 27$ teeth) with grazers (European wild ass and horses,
245 $\delta^{66}\text{Zn} = 1.35 \pm 0.18$ ‰ 1σ ; $n_{\text{tooth}} = 6$) exhibiting the highest values, another similarity to observations
246 in modern food webs (35, 40).

247 ***Strontium isotope data***

248 The $^{87}\text{Sr}/^{86}\text{Sr}_{\text{enamel}}$ range from 0.70834 to 0.70916 ($n = 24$) Strontium and Zn isotope ratios show no
249 correlation, and the Neandertal individual shows an $^{87}\text{Sr}/^{86}\text{Sr}_{\text{enamel}}$ overlapping with those of deer and
250 rabbits (Figure 4A). Strontium isotope ratios from dental enamel and dentin are compatible with the

251 values expected for the region of Gabasa (Supplementary Information 1 and 5). As dentin is likely to
252 be affected by diagenetic alteration and Sr exchange with the soil, these values might reflect soil
253 values and can thus be an indicator of the local bioavailable Sr isotope composition at Gabasa.
254 (Supplementary Information 3).

255

256 ***Carbon and oxygen isotope data***

257 $\delta^{13}\text{C}_{\text{enamel}}$ data range from -14.13 to -8.35 ‰ ($n = 44$), and $\delta^{18}\text{O}_{\text{enamel}}$ data range from -7.46 to 1.64 ‰ (n
258 = 43). Mountain herbivores (chamois and Iberian ibex) have distinct higher $\delta^{13}\text{C}_{\text{enamel}}$ and $\delta^{18}\text{O}_{\text{enamel}}$
259 compared to other herbivores, which might be due to water evaporation on mountain slopes and
260 consumption of plants from more arid contexts (Supplementary Information 6, Figure 2). The
261 Neandertal's $\delta^{13}\text{C}_{\text{enamel}}$ and $\delta^{18}\text{O}_{\text{enamel}}$ values overlap with those of cave animals (Figure 4A and B,
262 Figure S12. Five carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) data were possibly impacted by diagenetic
263 carbonate, and those values were excluded for ecological interpretation (mostly rabbits, see
264 Supplementary Information 3 and 6).

265

266 ***Trace element ratios (Ba/Ca and Sr/Ca)***

267 Trace element ratios are mostly discussed in Supplementary Information 3 and 7. The $-\log(\text{Ba}/\text{Ca})$
268 ranges from 3.25 to 4.75 ($n = 40$) and $-\log(\text{Sr}/\text{Ca})$ from 2.77 to 3.62 ($n = 40$). The two ratios measured
269 in dental enamel do not correlate. As expected (67), mixed feeders exhibit lower Sr/Ca than grazers,
270 but carnivores (especially hyenas) show Sr/Ca ratios overlapping with those of grazers (Figure S13).
271 We report the highest Sr/Ca and Ba/Ca ratios for mountain and forest animals and the lowest Ba/Ca
272 for the Neandertal individual. Here, these ratios do not correlate with tooth developmental stages (i.e.,
273 ontogenetic time of crown mineralization).

274

275 ***Correlations and comparisons between different dietary proxies***

276 Zn isotopes do not correlate with Sr/Ca or Ba/Ca ratios (Figure 4, Supplementary Information 3 and
277 4). Equids have higher $\delta^{66}\text{Zn}$, $\delta^{13}\text{C}$, and $^{87}\text{Sr}/^{86}\text{Sr}$, and lower Ba/Ca than the rest of the herbivores

278 (Figure 4). When performing a PCA based on five proxies ($\delta^{66}\text{Zn}$, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, Sr/Ca, and Ba/Ca), the
279 PC1 places the Neandertal among cave animals (hyenas and bears). This first component is mostly
280 based on $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and Ba/Ca values. Strangely, it is precisely when cave animals are removed that a
281 positive correlation appears between $\delta^{13}\text{C}$ and $\delta^{66}\text{Zn}$ ($R^2 = 0.41$, $p = 0.00009$). This correlation could
282 be due to a combination of a trophic level effect and higher $\delta^{13}\text{C}$ and $\delta^{66}\text{Zn}$ values among grazers. No
283 clear trend has been observed between $\delta^{66}\text{Zn}$ values and the type of tooth sampled (Supplementary
284 Information 4), but it should be noted that this could result from the low sample size per tooth group
285 and different timing of weaning and dental development between species.

286

287 *Collagen extraction*

288 The collagen extraction performed on the dentin from four teeth was unsuccessful, and not enough
289 collagen was extracted for $\delta^{15}\text{N}_{\text{collagen}}$ and/or radiocarbon analyses. This failure is not surprising for
290 samples dating back to about 150 ka (62) (Supplementary Information 2).

291

292

293 **Discussion**

294

295 *New insights into the diet and ecology of Iberian Pleistocene mammals from Zn isotopes in tooth* 296 *enamel*

297 Our study shows similar trends to those previously reported in modern and fossil food webs:
298 Herbivores exhibit higher $\delta^{66}\text{Zn}$ than carnivores ($\Delta^{66}\text{Zn}_{\text{herbivores-carnivores}} = +0.31\text{‰}$ and $+0.38\text{‰}$
299 excluding hyenas). The trophic level depletion is similar to that of the modern food web of Koobi Fora
300 (dental enamel values: $+0.40\text{‰}$, hyenas included and $+0.44\text{‰}$ excluding hyenas), Kenya, or the
301 marine mammal food webs of Arctic Canada ($+0.32\text{‰}$ on average in bones) (34, 38) but lower than
302 that of the Laotian fossil sites ($+0.63\text{‰}$, hyenas excluded). Grazers (horses and European wild ass)
303 tend to show higher $\delta^{66}\text{Zn}$ than browsers, as observed in the savannah of Koobi Fora (35), but not in
304 the tropical rain forests of Tam Hay Marklot and Nam Lot (36, 37). Hyenas have more

305 elevated $\delta^{66}\text{Zn}_{\text{enamel}}$ values than other carnivores (Figure 3), as systematically observed in other sites
306 (35, 37, 40). These higher values are likely due to bone consumption (35), although hyenas have
307 extended breastfeeding time than other carnivores (68), and milk would also similarly drive $\delta^{66}\text{Zn}_{\text{enamel}}$
308 up. However, high Sr/Ca and low Ba/Ca ratios observed in the different teeth of all hyenas support the
309 interpretation of the signature of solid food consumption and consequently of bones as a likely driving
310 factor behind their elevated $\delta^{66}\text{Zn}_{\text{enamel}}$ compared to other sympatric carnivores (Figure S13, Table S4).
311 Cave bears exhibit $\delta^{66}\text{Zn}_{\text{enamel}}$ overlapping with rabbits, deer, and chamois (Figure 3), which confirms
312 the frequent observations based on low $\delta^{15}\text{N}_{\text{collagen}}$, indicating they were mostly herbivorous (12, 63,
313 66).

314 The $\delta^{66}\text{Zn}_{\text{enamel}}$ values of the entire food web of Gabasa are the most elevated values ever
315 measured in dental enamel (Mean values about 0.2 ‰ higher than in the modern food web of Koobi
316 Fora and 0.7 ‰ higher than in the Late Pleistocene food webs of Marklot, Nam Lot and Tam Pa Ling
317 (35, 36, 41, 42), Supplementary Information 4, Figure S14). This is probably not due to the geology
318 because limestones, known to show the highest $\delta^{66}\text{Zn}$ (69), are present at both Gabasa and the Laotian
319 sites. Gabasa food web's uniqueness comes from its environmental context as it is the coldest
320 terrestrial biotope ever studied for faunal $\delta^{66}\text{Zn}$. Pollen data suggest an arid and cold climate (70) with
321 periods characterized by steppe vegetation and some forest patches mainly composed of conifers (45,
322 70, 71). Carbon and oxygen isotopes exhibit patterns similar to those observed for mammals from
323 various Middle Paleolithic Western European sites (54, 55, 72) and are compatible with **mid-mountain**
324 **mosaic landscape** (Supplementary Information 1 and 6). Tree leaves usually exhibit relatively low
325 $\delta^{66}\text{Zn}$ and they were probably scarce in Gabasa, as opposed to the forest of Tam Hay Marklot and the
326 fynbos of Western Cape(40), where the lowest $\delta^{66}\text{Zn}$ in herbivore teeth were measured.

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328

329 *The diet of a Neandertal at Gabasa*

330 Our results demonstrate that the Neandertal individual from Gabasa shows a Zn isotope
331 signature of a top-level carnivore, similarly to that observed for nitrogen isotopes for other sites with

332 Neandertal occupation. Carbon and oxygen isotope and trace element data suggest that the individual
333 inhabited the local area around the cave (Supplementary Information 6 and 7). Of all the animal taxa
334 analyzed in Gabasa, the Neandertal specimen easily exhibits the lowest Zn isotope ratio. As the Sr
335 isotope ratio of the Neandertal tooth enamel is similar to those of other animals (Figure 4), the low
336 $\delta^{66}\text{Zn}_{\text{enamel}}$ value is unlikely the result of a different geographic origin (and consequently different
337 bedrock and isotope baseline) of this individual, but reflect diet instead. Previous work on animal food
338 webs documented a trophic spacing for $\delta^{66}\text{Zn}_{\text{enamel}}$ of 0.3 to 0.6 ‰ (34–36, 38). In Gabasa, the average
339 difference between carnivores and herbivores is 0.31 ‰, but the Neandertal shows a $\delta^{66}\text{Zn}$ value 0.57
340 ‰ lower than that of the carnivores – 0.50 ‰ if hyenas are excluded– and 0.85 ‰ lower than the
341 herbivores, which would be about three trophic levels higher than that of the herbivores if we consider
342 a trophic spacing of 0.31 ‰. This mirrors dietary reconstructions based on nitrogen isotopes from
343 individuals from other European sites such as Goyet (18) (Belgium), Jonzac (France) (13), or Les
344 Cottés (7) (France), where Neandertals exhibit higher $\delta^{15}\text{N}_{\text{collagen}}$ values than sympatric carnivores.

345 Dietary peculiarities other than high-trophic level carnivory either do not explain the low
346 $\delta^{66}\text{Zn}_{\text{enamel}}$ values or conflict with other observations. Here we consider: 1) a signature of
347 breastfeeding; 2) Specific diets (the consumption of either light Zn-bearing foods or the absence of
348 consumption of heavy Zn-bearing foods); 3) a metabolic origin.

349 The breastfeeding signature hypothesis can be ruled out because it leads to high $\delta^{66}\text{Zn}_{\text{enamel}}$. In
350 a previous study, teeth formed during the breastfeeding period of two human populations from very
351 different geographical and archeological contexts exhibit average $\delta^{66}\text{Zn}_{\text{enamel}}$ values that are 0.3 ‰
352 higher than the teeth whose dental enamel forms after weaning (39). Furthermore, the low Ba/Ca of
353 the Neandertal dental enamel suggests that it was formed after the breastfeeding period (49, 52, 73)
354 (Table S20). Moreover, the region of the tooth sampled was very close to the tooth's cervical margin
355 because of tooth wear, which usually corresponds to formation ages of 2-3 years old (74). The
356 turnover of Zn in the body occurs over the course of just a few months, and weaning age (or at least an
357 age with substantial consumption of solid food) among Neandertals is assumed to be around 1 year old
358 (49). Even if milk consumption extended to the period from which enamel was sampled from the

359 tooth, it is unlikely that it was a major source of Zn in the diet at this stage. Therefore, the value
360 observed in the Neandertal tooth enamel is likely a reflection of an adult diet. An early weaning age
361 also prevails in recent humans and has also been documented with Zn isotopes in a Late Pleistocene
362 *Homo sapiens* (37), strongly contrasting with the conditions observed among apes, *Australopithecus*
363 (75), and earlier representatives of the genus *Homo* (76). This adaptation results from the necessity for
364 the breastfeeding mother to share the energetic burden of her child's large brain with other adults able
365 to contribute to solid food. It is central to the human pattern of "cooperative breeding" and has critical
366 social and behavioral implications.

367 Some dietary explanations for the low $\delta^{66}\text{Zn}_{\text{enamel}}$ of the Neandertal individual involving light Zn
368 bearing foods are also excluded:

369 a. **Adult vs juvenile prey:** In principle, if (as in humans (39)) milk consumption leads to higher
370 $\delta^{66}\text{Zn}$, preferential consumption of post-weaning juveniles or adults might lead to lower
371 $\delta^{66}\text{Zn}$. However, this explanation seems unlikely for two reasons. First, such an isotopic
372 disparity has not been observed in herbivore teeth in relationship to weaning in this study or
373 elsewhere (36), so the age of the prey does not appear to matter. Secondly, Neandertals
374 targeted younger deer and horses (61) (Supplementary Information 1) and while other
375 carnivores targeted adult ibexes. As such, if prey age mattered, Neandertal Zn isotope
376 compositions should be biased towards higher $\delta^{66}\text{Zn}$, not lower. Although we cannot rule out
377 the possibility that the formation period of the Neandertal tooth is asynchronous with the site's
378 period of occupancy, there is currently no evidence to support this..

379 b. **Cannibalism or hypercarnivorism:** Cannibalism or carnivore meat consumption could be
380 associated with low Zn isotope ratios, but the absence of cut marks on hominin and carnivore
381 bones rules out **this hypothesis**.

382 c. **Food with unusually low $\delta^{66}\text{Zn}$ values:** Archaeological evidence for some isotopically light
383 foods that Neandertals might have specifically targeted may be missing (e.g., animal livers
384 (53, 77, 78), birds, mushrooms, fruits, leaves (79–81), insects (82, 83) or aquatic resources
385 (43)). However, $\delta^{66}\text{Zn}_{\text{enamel}}$ of the consumers of those foods have been measured and are not

386 associated with lower values than that of sympatric carnivores, both in Gabasa (e.g., lynx are
387 known to consume liver, deer consume leaves, while foxes, bears, and rabbits have been
388 known to consume mushrooms) and other food webs (apes and tapirs for insects and fruits
389 (36)).

390 d. **Aquatic foods:** The consumption of aquatic resources has been demonstrated for some Iberian
391 Neandertals, though not at Gabasa (23, 25, 30). Still, the consumption of common trout
392 (*Salmo trutta*) has been suggested for a Mousterian site at the Grotte du Noisetier (84), located
393 in the French Pyrenees (Figure 1). The trophic level of this species could potentially explain
394 low $\delta^{66}\text{Zn}_{\text{enamel}}$ values, but would imply that the main source of Zn in the diet comes from
395 freshwater fish. Trout Zn content is about ten times less than that of liver or muscle from
396 mammals (85), requiring Neandertals to have consumed trout almost exclusively to shift their
397 $\delta^{66}\text{Zn}_{\text{enamel}}$ to the observed low value. As no fishbone or other evidence for aquatic resource
398 use were found in the Gabasa region, we can disregard this hypothesis as unlikely.

399 e. **Food processing:** Elevated $\delta^{15}\text{N}$ values among Neandertals have been suggested to result
400 from food processing such as fermentation or cooking. However, Zn **fractionates only** at a
401 temperature above $\sim 900^\circ\text{C}$ (86), which is not reached in open fires, and even if **fractionation**
402 **did occur**, it would enrich the Neandertal food in heavy Zn isotopes (86). Biotic fractionation
403 **during meat processing** is not documented, but could happen **within muscle tissues if the**
404 **reactions were incomplete** during fermentation (87) **or other curing processes. However, this**
405 **would only influence the final $\delta^{66}\text{Zn}$ of the consumed food if both reagents and products of**
406 **the curing process would not be contained in the final food product.**

407

408 A combination of dietary practices might explain the low $\delta^{66}\text{Zn}_{\text{enamel}}$ value of the Neandertal. Zinc
409 isotopes are highly fractionated in mammal tissues (77, 78, 88, 89), and different mammal species
410 have different isotope compositions. Thus, a low $\delta^{66}\text{Zn}$ value might reflect consumption of
411 muscles and liver (low $\delta^{66}\text{Zn}$) from deer and rabbits (lower $\delta^{66}\text{Zn}$ than other herbivores, Figure 3,

412 also supported by $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ for which Neandertals exhibit values close to these animals
413 (Figure 2, 4, S10)), while excluding bones and blood (high $\delta^{66}\text{Zn}$). Although cut marks suggest
414 Neandertals hunted deer and horses (61, 90), rabbits are the most abundant taxon of the site
415 (Figure S2), and some rabbit bones show cut marks (Figure S3). Disarticulation of rabbits requires
416 minimal use of tools (61), so they could have been heavily consumed without physical evidence.
417 Temporal asynchrony between cave deposits and tooth growth might explain a discrepancy
418 between zooarchaeological and isotopic dietary conclusions if the deposits represent a period
419 when Neandertals preferentially ate horses and deer, whereas the portion of the tooth corresponds
420 to a period when rabbits were preferentially eaten. Higher $\delta^{66}\text{Zn}$ among other sympatric carnivores
421 hunting the same species as Neandertal (Table S3) (61, 91) might partially reflect bone
422 consumption: hyenas tend to have elevated $\delta^{66}\text{Zn}_{\text{enamel}}$ values, likely due to bone consumption
423 (35, 37). Red foxes and wolves gnaw on bones (although they ingest much less bone than hyenas
424 (92)), while lynxes partially digest the bones of rabbits, which make up 80-100% of their typical
425 diets (91). In addition, all these carnivores may consume substantial blood, possibly helping to
426 explain higher $\delta^{66}\text{Zn}$ values than the Neandertal.

427 Metabolic specificity (different diet-tissue isotopic offsets) for Neandertals compared to other
428 animals might also explain the unusually low $\delta^{66}\text{Zn}$ value and possibly the higher $\delta^{15}\text{N}_{\text{collagen}}$ values
429 that have been measured elsewhere. It is indeed striking how similar the patterns between $\delta^{66}\text{Zn}_{\text{enamel}}$
430 and $\delta^{15}\text{N}_{\text{collagen}}$ mirror each other: in both proxies, Neandertals typically exhibit isotope ratios that
431 would appear to imply a higher trophic level than sympatric carnivores (1, 3–5, 7, 11, 63). Although
432 such distinct compositions have been interpreted to reflect aquatic food resources for some Paleolithic
433 humans (11), compound-specific isotope analysis of amino acids shows that anatomically modern
434 humans from Buran Kaya (Crimea) relied on terrestrial resources, even though they exhibit the highest
435 $\delta^{15}\text{N}_{\text{collagen}}$ known for the Paleolithic (93). These authors suggest preferential consumption of
436 mammoths (absent from Gabasa), but a metabolic origin might yield similarly high $\delta^{15}\text{N}_{\text{collagen}}$ values.
437 We acknowledge that some studies show minimal offsets in $\delta^{15}\text{N}_{\text{collagen}}$ between humans and sympatric

438 herbivores (94). However, the trophic spacing between the Paleolithic and Neolithic periods did not
439 decrease to the extent expected for a transition from hunter-gatherer to a more cereal-based diet (95).
440 Furthermore, for Zn isotopes, a medieval population with expected high meat and marine fish
441 consumption shows strikingly lower $\delta^{66}\text{Zn}_{\text{enamel}}$ values than for sympatric herbivores (0.6 ‰ lower)
442 and for a dog, cat, and pig (0.3 ‰ lower), while $\delta^{15}\text{N}_{\text{collagen}}$ values were strikingly higher than
443 herbivores (4 ‰) and dog, cat, and pig (1 ‰) (41). These observations closely resemble patterns for
444 Neandertals at Gabasa (for Zn isotopes) and elsewhere (for N isotopes), and suggest a metabolic
445 effect. Controlled feeding experiments on animals with an omnivorous diet are necessary to assess this
446 further. The reason for this metabolic difference remains unknown, but it should be noted that the
447 Neandertal is the most carnivorous species among primates, and the only meat-eater not from the order
448 *Carnivora* within the Gabasa faunal assemblage. Therefore, Neandertals may have had some
449 metabolic peculiarities compared to other carnivores and primates regarding adaptations of their
450 gastrointestinal tract and their dietary preferences (96).

451

452 **Concluding remarks.**

453

454 Zinc isotope analysis of tooth enamel can successfully characterize past animal and human diets in the
455 absence of collagen preservation for $\delta^{15}\text{N}$ analysis. Whichever method is used to characterize
456 Neandertals' diets ($\delta^{66}\text{Zn}_{\text{enamel}}$ and $\delta^{15}\text{N}_{\text{collagen}}$), isotope values represent an extreme in carnivore trophic
457 levels, and Iberian Neandertals appear to be no exception. Meat consumption is supported by both the
458 zooarcheological data at Gabasa and $\delta^{66}\text{Zn}_{\text{enamel}}$ analysis. Furthermore, the low Zn isotope ratio
459 observed in the single measured Gabasa Neandertal specimen suggests that this individual might have
460 had a distinct diet compared to other carnivores (possibly avoiding the consumption of bones and
461 blood) and/or may not have fractionated Zn isotopes like other sympatric carnivores. Our study
462 represents the first successful analysis of Zn isotopes from a Neandertal tooth, and further work should
463 focus on understanding whether such unusual compositions result from specific diets or metabolic
464 fractionation in these extinct hominins.

465

466 **Material and Methods**

467 *Material*

468 Details on the context of Gabasa cave deposits, geology, dating, zooarcheology, paleoenvironment and
469 sampling strategy are available in the Supplementary Information. The material used in this study is
470 associated with the permit “Resolution” by the Gobierno de Aragon on the February 10th 2016, and
471 August 31st 2018.

472

473 *Methods*

474 Tooth enamel fragments and powders were sampled at the Department of Human Evolution of the
475 Max Planck Institute for Evolutionary Anthropology (MPI-EVA, Leipzig, Germany) using a drill with
476 a diamond saw and at the Géosciences Environnement Toulouse of **the Observatoire Midi Pyrénées**
477 **(GET/OMP, Toulouse, France)** using a MicroMill. For Zn isotope analyses, 1 to 20 mg enamel
478 fragments were dissolved in 1mL **1N HCl**. Samples were cleaned with double-distilled H₂O in an
479 ultrasonic bath and dried at 50°C. **Zinc** was then extracted by column chromatography using the
480 modified protocol of Moynier et al. (2006) described by Jaouen et al (2016b) (34, 97). The $\delta^{66}\text{Zn}_{\text{enamel}}$
481 analyses were conducted on three different MC ICP MS instruments: a Nu 500 (ENS Lyon, France), a
482 Neptune (MPI-EVA, Leipzig, Germany) and a Neptune Plus (GET/OMP, Toulouse, France) using a
483 Cu doping technique (see Supplementary Information 4). The $\delta^{66}\text{Zn}_{\text{enamel}}$ values were corrected to take
484 into account the offset (+0.27 ‰) existing between our in-house standard, bracketing standard (AA-
485 MPI) and the JMC-Lyon solution. **Zinc isotope data are thus reported toward the JMC-Lyon values,**
486 **and this correction does not impact the relative offsets or our interpretations. Strontium isotope**
487 **analyses were conducted on 5 to 20 mg of enamel powder, after performing a purification step based**
488 **on a modified protocol of Deniel and Pin (2001) (98) described by Richards et al (2008) (13), using a**
489 Triton plus TIMS and a Neptune Plus at GET/OMP Toulouse. O and C isotope data were obtained
490 from untreated tooth enamel powder (<100 µg) using the “cold trap” method with a Thermo Scientific
491 Delta V IRMS in the Vonhof laboratory at the Max Planck Institute for Chemistry (MPIC, Mainz,
492 Germany) after Vonhof et al. (2020) (99). The reference materials **NIST SRM 1400** (for Zn), **NIST**
493 **SRM 1486** (for Sr), IAEA-603, NBS18, **NIST SRM 120c** and three internal house standards - a

494 carbonate standard (VICS) and a tooth enamel structural carbonate (AG-Lox) (for C and O) and a
495 dentin sample (AZE) for Zn were analyzed in the same runs as the samples and gave consistent results
496 for each lab and compared to previous studies (Supplementary Tables S3 to S5). Trace element
497 analyses were conducted at the GET using an ICap (ThermoFischer) Triple Quadrupole (TQ-ICP-
498 MS). Finally, collagen was extracted from four tooth roots for $\delta^{15}\text{N}$ analyses using the protocol of
499 Talamo and Richards (2011) (58). All trace elements and isotope results are available in the
500 supplementary data.

501

502

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804 **Figure 1. A. Location of the Gabasa site as well as other Neandertal sites mentioned in the text.**

805 **B. Detailed map of the Gabasa region. San Estaban de Litera and Benabarre are nearby modern**
806 **cities.**

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808 **Figure 2. Average dental enamel isotope and trace element ratios for the different dietary**

809 **categories at Gabasa, Spain. A: $\delta^{66}\text{Zn}_{\text{enamel}}$, B: $^{87}\text{Sr}/^{86}\text{Sr}$ C: $\delta^{18}\text{O}_{\text{enamel}}$, D: $\delta^{13}\text{C}_{\text{enamel}}$, E: Ba/Ca.**

810 **Omnivores correspond to one single taxon (cave bears) as well as carnivores with significant**
811 **bone consumption (cave hyenas).**

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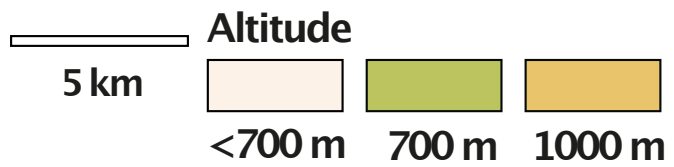
813 **Figure 3. Enamel $\delta^{66}\text{Zn}$ values per mammal taxon for different dietary groups from Gabasa,**

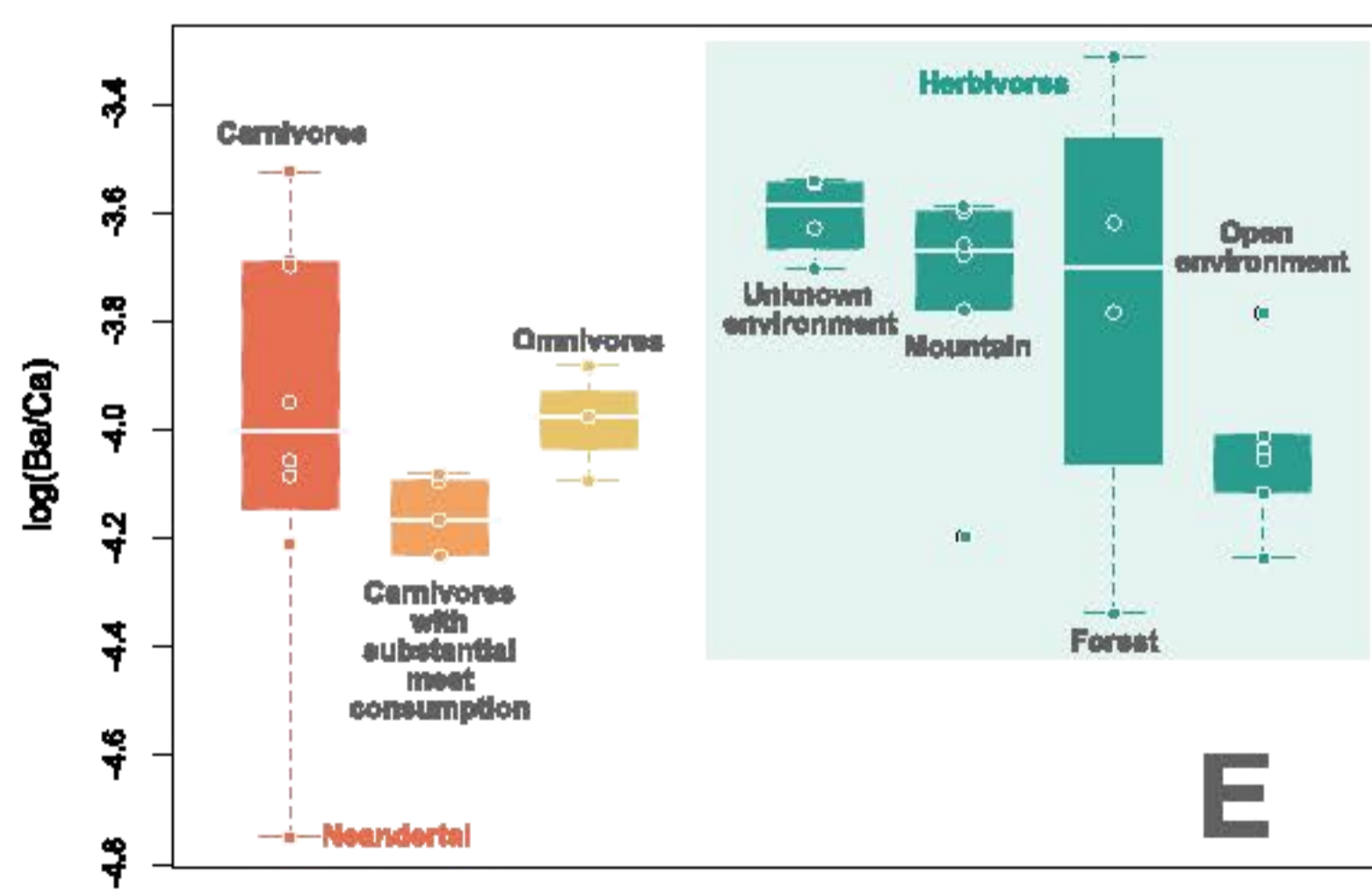
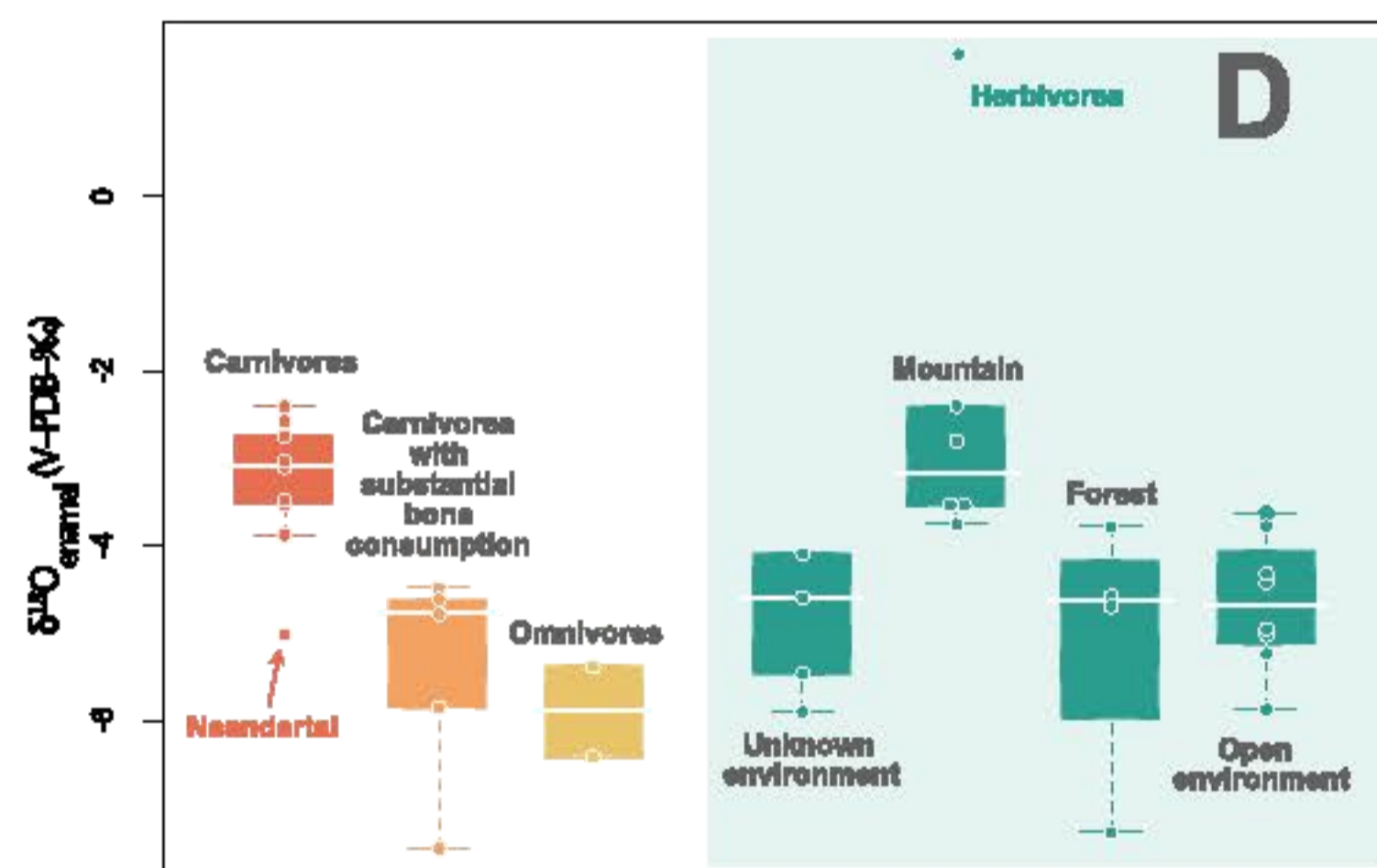
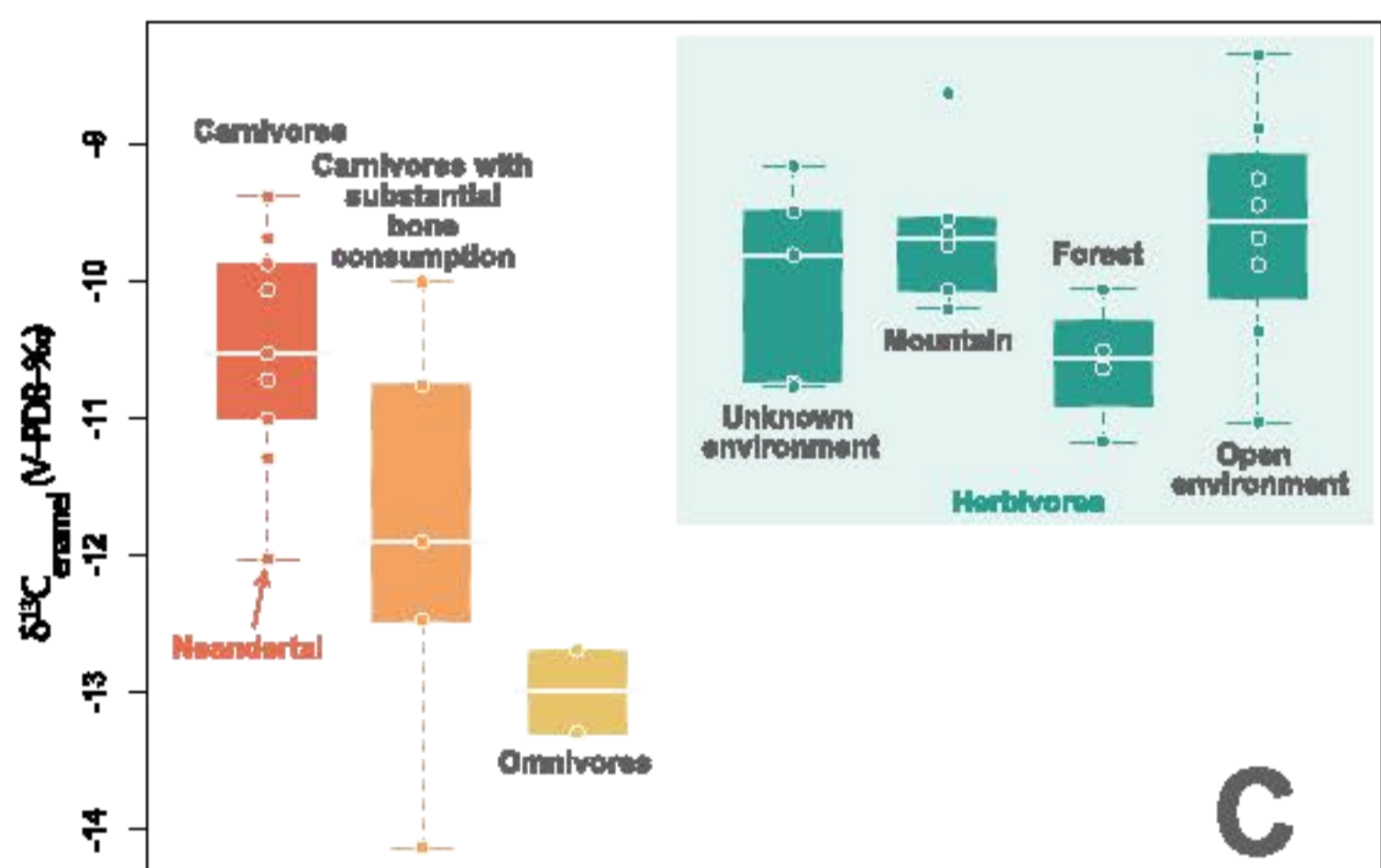
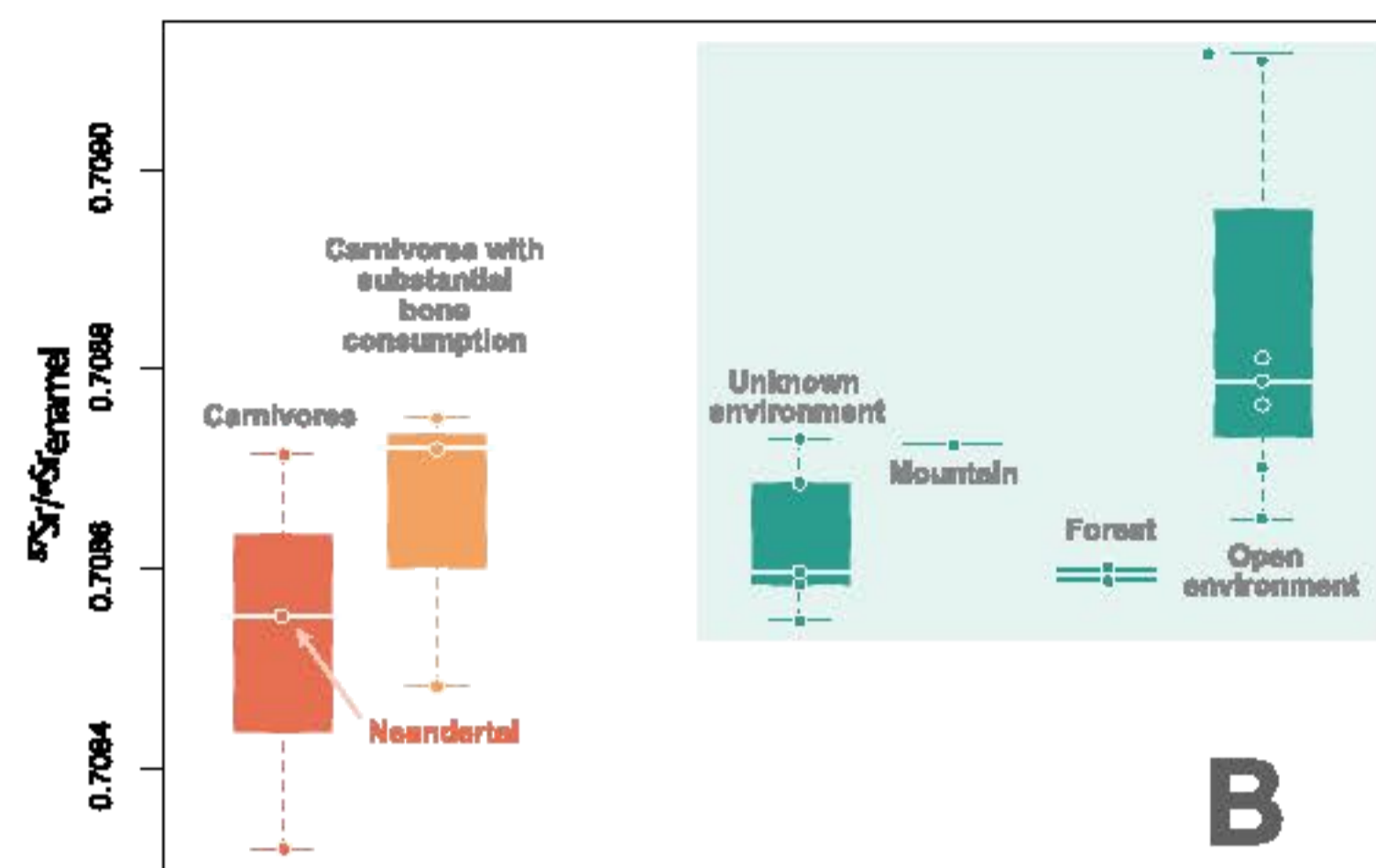
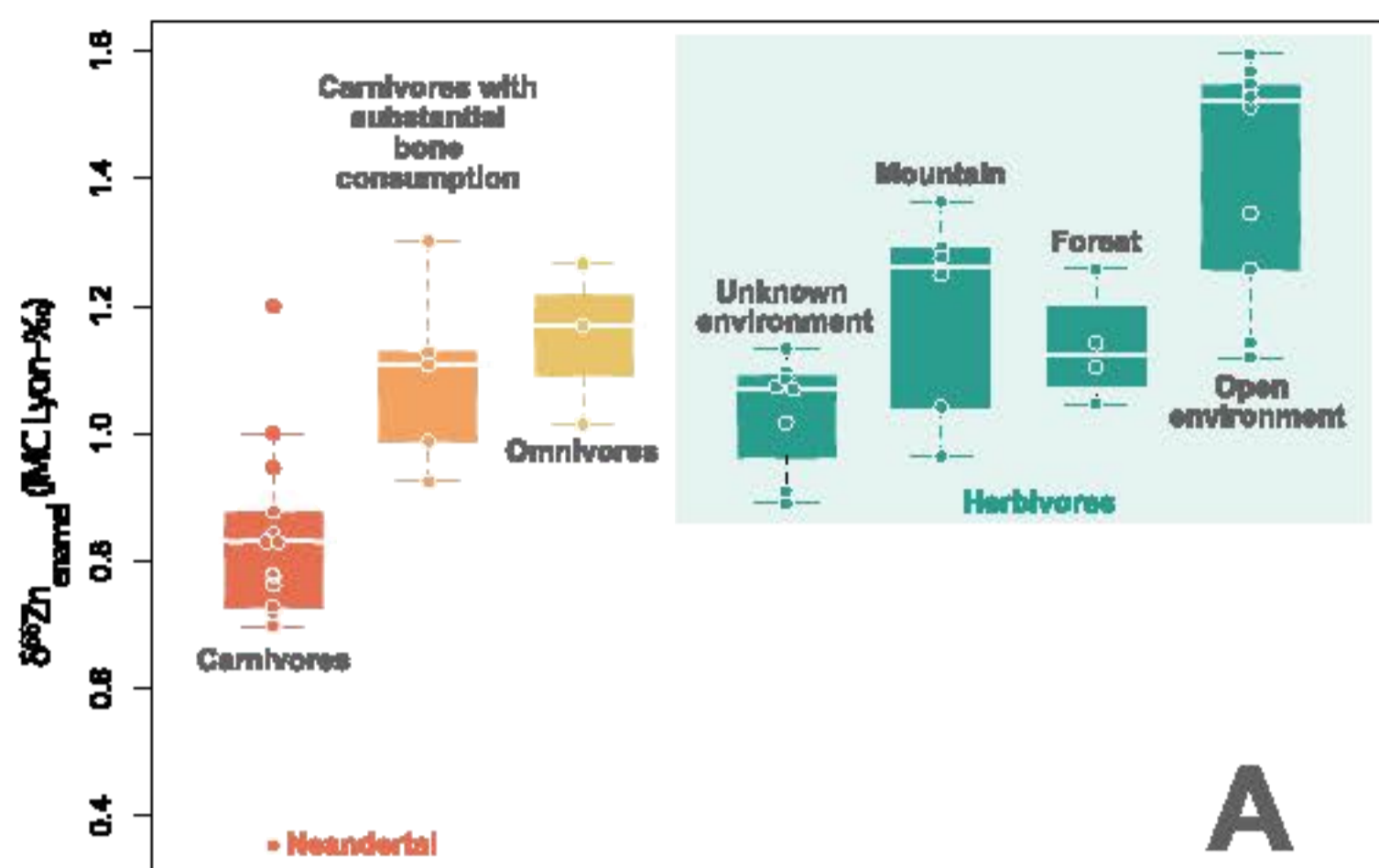
814 **Spain given as box plots. Note that sub-samples taken from one single tooth reflect differences in**

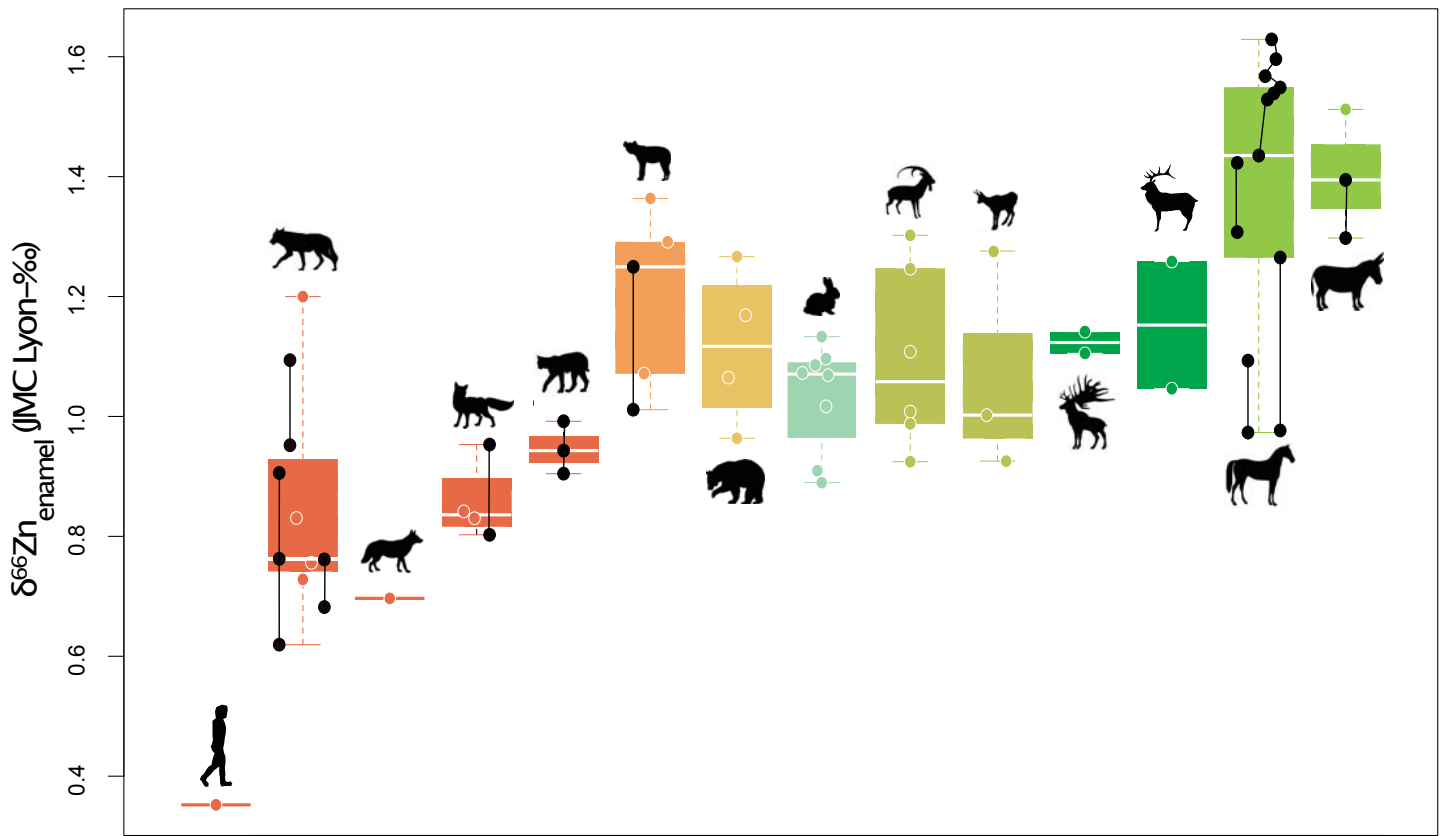
815 **dietary ^{66}Zn values. See text for detailed discussion about the low Neandertal $\delta^{66}\text{Zn}$ value.**

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Figure 4. Zinc isotope values ($\delta^{66}\text{Zn}$, average dental enamel values per tooth) cross plotted versus A) carbon ($\delta^{13}\text{C}$), B) oxygen($\delta^{18}\text{O}$) and C) strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) isotope composition as well as D) Ba/Ca ratios measured in faunal and Neandertal tooth enamel at Gabasa, Spain. Each point corresponds to a single tooth, except for the horse tooth for which five samples were taken (samples are connected with a line). Error bars correspond to the SD between analytical duplicates for C and O isotopes (0.02 to 0.36 ‰; one sample with higher SD has been removed) and for Zn (analytical duplicates: 0.00 to 0.04 ‰; SD for separate samples taken from a single tooth: 0.04 to 0.21 ‰). SE for strontium isotope ratios are 0.000003 to 0.000010 and are too small to display.










Carnivores

-  *Canis lupus*
-  *Vulpes vulpes*
-  *Cuon alpinus*
-  *Lynx spelaea*
-  *Homo neanderthalensis*
- with intense bone consumption**
-  *Crocuta spelaea*



Omnivores

-  *Ursus spelaeus*



Herbivores


-  *Oryctolagus cuniculus*
- from open environment**
-  *Equus hydruntinus*
-  *Equus caballus*

from forest environment

-  *Cervus sp.*
-  *Cervus elaphus*

from mountain environment

-  *Rupicapra rupicapra*
-  *Capra pyreneica*

 measurements performed within a single tooth

