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1 **Regional heterothermies recorded in the oxygen isotope**  
2 **composition of harbour seal skeletal elements**

3

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16

17

18 **Abstract**

19 Regional heterothermy is a strategy used by marine mammals to maintain a high and stable  
20 core body temperature, but its identification needs *in situ* measurements difficult to set up in  
21 extant wild organisms and inapplicable to extinct ones. We have analyzed the oxygen isotope  
22 composition of bioapatite phosphate ( $\delta^{18}\text{O}_p$ ) from one permanent tooth and from thirty-six  
23 skeletal elements of one adult male harbour seal (*Phoca vitulina vitulina*) from the Baie de  
24 Somme (Hauts-de-France, France). We propose that the observed intra-skeletal  $\delta^{18}\text{O}_p$   
25 variability reflects tissue temperature heterogeneities typical of the pinniped regional  
26 heterothermy strategy. Our  $\delta^{18}\text{O}_p$  data indicate that bone hydroxylapatite from harbour seal

27 autopod skeletal elements (metacarpals, metatarsals, and phalanxes) mineralises at a lower  
28 temperature than that of the bone from the axial skeleton (e.g. vertebrae, ribs, and girdle  
29 bones). The results suggest that it is possible to locate a history of regional heterothermies in  
30 amphibious marine vertebrates using the  $\delta^{18}\text{O}_p$  values of their mineralised tissues. This  
31 enables direct evaluation of the thermophysiology of both modern and fossil Pinnipedia from  
32 their skeletons opening perspectives on understanding their thermal adaptation to the marine  
33 environment in the fossil record. In addition to thermophysiology, oxygen isotope data from  
34 the permanent teeth of Pinnipedia, which are formed during the *in utero* phase from body  
35 fluid of the mother and at a stable temperature, could be valuable for locating the  
36 geographical areas inhabited by existing Pinnipedia females during their gestation period.

37 **Keywords:** *Phoca vitulina vitulina*; oxygen isotopes; regional heterothermies;  
38 thermophysiology; foraging area.

39

40 **Introduction**

41 Thermoregulatory homeostasis is a major challenge for marine endotherms, i.e. Cetacea  
42 Brisson, 1762 and Pinnipedia Illiger, 1811, inhabiting aquatic environments, mainly due to  
43 the high thermal conductivity of water (Dejours, 1987), a medium that conducts heat  
44 approximately 25 times faster than air (Pendergast and Lundgren, 2009). The marine habitat  
45 offers limited thermal refuges, forcing animals that thermoregulate behaviourally to use the  
46 vertical thermocline or to haul-out when environmental conditions are favourable (Riedman,  
47 1990; Hamilton et al., 2014). As a result, endothermic tetrapods that have made the transition  
48 from terrestrial to aquatic lifestyle have experienced intense evolutionary pressures on  
49 multiple occasions (Reidenberg, 2007; Pyenson et al., 2014; Kelley and Pyenson, 2015).

50 Harbour seals (*Phoca vitulina vitulina* Linnaeus, 1758) have a widespread distribution across  
51 the northern hemisphere, from temperate to polar regions (Burns, 2009). Resting metabolic  
52 heat production allows harbour seals to maintain a core body temperature, the internal  
53 temperature of the body, very close to the main organs, heart, brain, and viscera (Sellier et al.,  
54 2014), of approximately  $37 \pm 1^\circ\text{C}$  (Feltz and Fay, 1966; Miller and Irving, 1975) which  
55 remain stable through time through specific morphological, physiological and behavioural  
56 adaptations (Scholander et al., 1950; Irving and Hart, 1957; Huntley et al., 1984; Folkow and  
57 Blix, 1987; Ryg et al., 1993; Kvadsheim and Aarseth, 2002; Liwanag et al., 2009; Blix et al.,  
58 2010). Because pinnipeds forage at sea but must return to land or sea-ice for breeding and  
59 moulting (Feltz and Fay, 1966; Ling, 1970; Watts, 1992, 1996), they have both fur and a  
60 blubber layer to address these contrasting thermal requirements. The fur plays an important  
61 role in insulating young and adult animals when they are out of water whereas the blubber is  
62 more efficient when they are immersed (Kvadsheim and Aarseth, 2002; Sharwa and Liwanag,  
63 2017). On the other hand, large size reducing surface-area-to-volume ratio (Blix and Steen,  
64 1979), reduced peripheral blood flow through vasoconstriction (Irving and Hart, 1957;  
65 Hansen and Lavigne, 1997) and counter-current heat exchangers located in the nasal cavities

66 (Huntley et al., 1984; Folkow and Blix, 1987) and flippers (Scholander et al., 1950; Blix et al.,  
67 2010) form a whole range of adaptations that enable harbour seals to limit heat loss to the  
68 environment. Harbour seal's appendage extremities lack fur and the adipose layer and due to  
69 their high surface-area-to-volume ratio, constitute a potential thermal window where heat loss  
70 is highly variable and depends on the extent (e.g., Kvadsheim and Folkow, 1997) and the  
71 manner in which the flippers are perfused (Blix et al., 2010). To reduce the rate of heat loss at  
72 sea, harbour seals and some other pinnipeds let the temperature of their appendage decrease  
73 by massive peripheral vasoconstriction and counter-current heat exchange in the limbs (Irving  
74 and Hart, 1957; Miller and Irving, 1975; Blix et al., 2010). Indeed, when exposed to cold or  
75 heat, mammals (i.e. homeothermic endotherms) use regional heterothermy ('core vs. shell'  
76 concept) to adjust heat loss rates in order to achieve a stable core body temperature. This well-  
77 regulated thermoregulatory response to environmental thermal stress is particularly effective  
78 during periods of immersion (Butler and Jones, 1997) and enables marine mammals to limit  
79 excessive heat loss from the body to the environment. It has been argued that the seal mainly  
80 achieves regulated heat loss by means of controlled heat dissipation from the limbs, but  
81 although the rest of the body is often considered to be of minor importance as an area of  
82 regulated heat loss (Irving and Hart 1957), studies have shown that seals may exercise control  
83 over heat loss via the trunk skin, too (Kvadsheim & Folkow, 1997; Øritsland, 1968).

84 One potential method for mapping intra-individual temperature variations and thereby  
85 regional heterothermies in harbour seals involves analysing of the oxygen isotope  
86 composition of phosphate ( $\delta^{18}\text{O}_p$ ) from hydroxylapatite, the mineral which constitutes the  
87 bones, the teeth and the scales of vertebrates (Séon et al., 2022). Oxygen isotope composition  
88 values of phosphate from hydroxylapatite in vertebrates reflects both the oxygen isotope  
89 composition of their body water ( $\delta^{18}\text{O}_{\text{bw}}$ ), mainly derived from food for marine mammals  
90 (Ortiz, 2001; Rash and Lillywhite, 2019; Séon et al., 2023), and their history of tissue

91 temperature due to the thermo-dependency of the oxygen isotope fractionation between  
92 phosphatic tissues and body fluids from which they mineralize (Longinelli and Nuti, 1973a,  
93 1973b; Kolodny et al., 1983; Longinelli, 1984; Luz et al., 1984; Lécuyer et al., 2013). Thus, it  
94 is assumed that harbour seals skeleton record homogeneous  $\delta^{18}\text{O}_p$  values for bones from the  
95 axial skeleton near vital organs and that  $\delta^{18}\text{O}_p$  discrepancies between body regions would  
96 highlight regional heterothermies as shown in the short beaked common dolphin (Séon et al.,  
97 2022). In vertebrates with a high metabolic rate, the bones are remodelled throughout the  
98 animal's life (Castanet, 1980, 2006; Montes et al., 2007; Castanet et al., 2010). To locate  
99 regional heterothermies from the oxygen isotope composition of the phosphate from  
100 hydroxylapatite, it is therefore necessary to consider the turnover rate of skeletal elements,  
101 which varies according to bone function, density, size and age (Hauschka, 1989; Kohn and  
102 Cerling, 2002; Lafage-Proust et al., 2015), and whose stable isotope composition could vary  
103 accordingly (Klepinger, 1984, Lamb et al., 2014; Smith et al., 2020). Earlier studies dedicated  
104 to bone turnover have mainly focused on humans (homeothermic endothermic mammals), and  
105 it has been estimated that the compact cortical bone of adult humans renews slowly (10% per  
106 year), and thus represents a period of up to 10 years (Väänänen, 1993). They mostly concern  
107 species other than pinnipeds and are relatively rare in the literature. Taking all this into  
108 account, it is reasonable to assume that the oxygen isotope composition recorded by the  
109 phosphate group in the hydroxylapatite of skeletal elements from homeothermic endotherms  
110 including the harbour seal, covers a period of several years.

111 Here, we provide new  $\delta^{18}\text{O}_p$  data acquired from tooth, mandible, axial and limb bones of a  
112 single specimen of *Phoca vitulina vitulina* to investigate whether intra-skeletal variability in  
113  $\delta^{18}\text{O}_p$  reflects known regional heterothermies recorded by classical thermometry methods and  
114 we have made an indirect comparison between the measured  $\delta^{18}\text{O}_p$  values to available body  
115 temperature measurements from previous published studies. Subsequently, we discussed

116 whether this proxy could be used as a tool to identify thermoregulatory strategies and  
117 specifically regional heterothermies in both living and fossil pinnipeds. The application of the  
118 isotope method to extinct Pinnipedia would enable a better understanding of the ocean  
119 colonisation and strategies for limiting heat loss within the aquatic environment.

120

## 121 **Material and methods**

### 122 *Sampled specimen*

123 One skeleton of *Phoca vitulina vitulina* (MNHN-ZM-AC-1894-524) curated at the Muséum  
124 national d'Histoire naturelle (MNHN, Paris, France) was sampled. This skeleton belonged to  
125 a wild adult male found stranded on a beach in the Baie de Somme (**Fig. 1**; Hauts-de-France,  
126 France). The third left premolar and thirty-six skeletal elements from mandible, axial and limb  
127 bones were sampled and analysed for their  $\delta^{18}\text{O}_p$  values (**Table 1**). As the aim of this study  
128 was to produce a complete isotope map, a single harbour seal specimen was studied  
129 exhaustively, given the time required to prepare and analyse the samples for the measurement  
130 of the oxygen isotope composition of the phosphate group. In order to detect heterothermies,  
131 the following elements of specimen were sampled in such a way as to form a central-  
132 peripheral representation: the vertebrae, the ribs and the girdle bones making up the Axial  
133 Skeleton set, the humerus, the femur, the radius, the ulna, the tibia and the fibula grouped into  
134 the Stylopod/Zeugopod set, and the metacarpals, the metatarsals and the phalanges forming  
135 the Autopod set. Approximately 30 mg of each skeletal element were sampled as fine powder  
136 by drilling using a Dremel<sup>TM</sup> diamond-studded drill bit. To be consistent across all samples,  
137 cortical bone was preferred.

138

### 139 *Oxygen isotope analysis of bioapatite*

140 Before measuring oxygen isotope ratios, the samples were prepared using the wet chemical  
141 procedure detailed by Crowson et al. (1991) and modified by Lécuyer et al. (1993), which  
142 involves the isolation of phosphate ions ( $\text{PO}_4^{3-}$ ) from the bioapatite which are then precipitated  
143 as silver phosphate crystals ( $\text{Ag}_3\text{PO}_4$ ). For each sample, 20–30 mg of cortical bone or tooth  
144 enamel powder was dissolved in 2 ml of 2M HF. The  $\text{CaF}_2$  residue was separated by  
145 centrifugation and the solution was neutralized by adding 2.2 ml of 2M KOH. Amberlite™  
146 IRN78 anion-exchange resin beads were added to the solution to isolate the  $\text{PO}_4^{3-}$  ions. After  
147 24 h, the solution was removed, and the resin was rinsed with deionized water and eluted with  
148 27.5 ml of 0.5M  $\text{NH}_4\text{NO}_3$ . After 4 h, 0.5 ml of  $\text{NH}_4\text{OH}$  and 15 ml of an ammoniacal solution  
149 of  $\text{AgNO}_3$  were added and the solutions were placed in a thermostated bath at 70°C for 7 h,  
150 allowing the slow and quantitative precipitation of  $\text{Ag}_3\text{PO}_4$  crystals. The  $\text{Ag}_3\text{PO}_4$  crystals were  
151 filtered, dried and cleaned. For each sample, five aliquots of  $300 \pm 20 \mu\text{g}$  of  $\text{Ag}_3\text{PO}_4$  were  
152 mixed with  $400 \pm 50 \mu\text{g}$  of graphite in silver foil capsules. Oxygen isotope ratios were  
153 measured using a high temperature vario PYRO cube™ elemental analyser (EA), equipped  
154 with the “purge and trap” technology (Fourel et al., 2011) and interfaced in continuous flow  
155 mode to an IsoPrime™ isotope ratio mass spectrometer (Elementar UK Ltd Cheadle, UK). To  
156 calibrate the measurements, the NBS127 (barium sulfate precipitated from seawater from  
157 Monterey Bay, California, USA, Sigma-Aldrich Co.), and a silver phosphate precipitated  
158 from the international standards NIST SRM 120c (natural Miocene phosphorite from Florida,  
159 Sigma-Aldrich Co.) were used. Their respective  $\delta^{18}\text{O}_p$  value was set at the certified value of  
160 9.3 ‰ V-SMOW (Hut, 1987; Halas and Szaran, 2001) and fixed at 21.7 ‰ V-SMOW  
161 (Vienna Standard Mean Ocean Water) according to Lécuyer et al. (1993), Chenery et al.  
162 (2010) and Halas et al. (2011), for correction of instrumental mass fractionation during CO  
163 isotope analysis. Along with the silver phosphate samples derived from bones and the third  
164 premolar hydroxylapatite, silver phosphates precipitated from standard NIST SRM 120c were



165 repeatedly analysed ( $\delta^{18}\text{O}_p = 21.9 \pm 0.2 \text{‰}$ ,  $n = 12$ ) to ensure that no isotope fractionation  
166 occurred during the wet chemistry. Although the SI unit for isotope ratio is the Urey (Ur), for  
167 better understanding we are reporting data with delta permil values expressed with respect to  
168 V-SMOW with  $1 \text{‰} = 1 \text{ mUr}$ .

169

### 170 *Statistical analyses*

171 Considering the number of samples for each set (between 6 and 20 samples), we used the non-  
172 parametric Mann-Whitney-Wilcoxon test to compare the median values between two sets of  
173 measurements. Statistical analyses were performed using a level of significance at  $p$ -value  $<$   
174 0.05. The  $\delta^{18}\text{O}_p$  values from the third left premolar and the mandible being represented by  
175 single measurements, they were not statistically compared with those of the other sets. As seal  
176 locomotion is achieved by lateral undulation of the pelvic region (Bebej, 2009; Kuhn and  
177 Frey, 2012; Berta, 2018), we decided to subdivide the Stylopod/Zeugopod set into 'anterior'  
178 and 'posterior' to identify any differences in isotope values.

179

## 180 **Results**

181 The oxygen isotope compositions of hydroxylapatite for the specimen of *P. vitulina vitulina*  
182 (MNHN-ZM-AC-1894-524) have been reported in **Table 1** and range from 15.7 ‰ to 18.3 ‰  
183 V-SMOW. The mean value is equal to 17.2‰, the median value is equal to 17.3 ‰ and the  
184 mid-range value ( $[\text{maximum value} - \text{minimum value}] / 2$ ) is equal to 17.0 ‰. Intra-skeletal  
185  $\delta^{18}\text{O}_p$  variability has been illustrated in **Fig. 2**. Intra-bone variability was assessed by paired  
186 samples taken from vertebrae (neural spine vs. centra). The highest intra-bone  $\delta^{18}\text{O}_p$   
187 variability measured was 1.6 ‰, and is smaller than the global inter-bone  $\delta^{18}\text{O}_p$  variability  
188 (2.5 ‰; **Table 1**). Mean oxygen isotope composition from each set was reported in **Table 2**,

189 oxygen isotope compositions of bones ranged from 16.0 ‰ to 17.6 ‰ for the Axial skeleton  
190 set, from 17.2 ‰ to 17.5 ‰ and from 16.5 ‰ to 17.1 ‰ for the anterior and posterior  
191 Stylopod/Zeugopod set respectively, and from 17.9 ‰ to 18.3 ‰ for the Autopod set. The  
192 Autopod set  $\delta^{18}\text{O}_p$  values are 0.4 to 2.3 ‰ higher than those of the rest of the body ( $p$ -value <  
193 0.05, Mann-Whitney-Wilcoxon statistical test; **Fig. 3**). A significant difference of  $\delta^{18}\text{O}_p$   
194 values of approximately 0.5 ‰ was observed between the anterior Stylopod/zeugopod set and  
195 the posterior Stylopod/zeugopod set ( $p$ -value < 0.05, Mann-Whitney-Wilcoxon statistical test;  
196 **Table 2** and **Fig. 3**). However, no significant  $\delta^{18}\text{O}_p$  difference was observed between bones  
197 belonging to the Axial skeleton set and those from anterior and posterior Stylopod/zeugopod.  
198 The third left upper premolar has the lowest  $\delta^{18}\text{O}_p$  value of the sample set with a value of 15.7  
199 ‰V-SMOW.

200

## 201 **Discussion**

### 202 *Intra-skeletal $\delta^{18}\text{O}_p$ values, a clue about regional heterothermies*

203

204 As expected, especially due to the thermodependence of oxygen isotope fractionation between  
205 phosphatic tissues and body fluids and the isotope composition of the latter, we observed  
206 intra-skeletal variability in the  $\delta^{18}\text{O}_p$  value between the skeletal elements of the axial skeleton  
207 and those of the limbs. Although no direct relationship could be determined between  $\delta^{18}\text{O}_p$   
208 values and body temperature, because of the absence of direct body temperature  
209 measurements for the studied specimen, the covariation of the values of these two parameters  
210 suggests that the intra-skeletal variability of  $\delta^{18}\text{O}_p$  values would reflect the known regional  
211 heterothermies in harbour seals. Harbour seal intra-skeletal  $\delta^{18}\text{O}_p$  variability can be explained  
212 by either the variability of  $\delta^{18}\text{O}_{\text{bw}}$  or variations in mineralisation temperatures assuming that

213  $\delta^{18}\text{O}_{\text{bw}}$  varies little over time. In Pinnipedia, the body water from which bioapatite mineralises  
214 mainly comes from the water contained in prey and to a lesser extent from the water produced  
215 by metabolism containing oxygen derived from inhaled dioxygen (Depocas et al., 1971; Feng  
216 et al., 2022). The oxygen isotope composition of body water is therefore strongly influenced  
217 by variations in the oxygen isotope composition of prey, i.e. fish and cephalopods (Tollit and  
218 Thompson, 1996; Hall et al., 1998; Kuhn et al., 2006; Sharples et al., 2009) whose  $\delta^{18}\text{O}$  value  
219 is equal to that of environmental water ( $\delta^{18}\text{O}_{\text{w}}$ ) and varies seasonally, because of the high  
220 turnover rate in fishes whose main source of water is seawater (Longinelli and Nuti, 1979;  
221 Kolodny et al., 1983). There are currently no data available on the  $\delta^{18}\text{O}_{\text{bw}}$  values of seals and  
222 their variability over time, but as semi aquatic mammals it could be reasonable to assume that  
223 these values slightly higher than those of the environment (about +2‰), as measured on  
224 extant hippopotamuses (Cerling et al. 2008). However, seasonal variations in  $\delta^{18}\text{O}_{\text{w}}$  cannot  
225 explain by themselves the intra-skeletal variability of  $\delta^{18}\text{O}_{\text{p}}$  values in bioapatite since bones  
226 record a period of few years (Rosenthal, 1963; Riccialdelli et al., 2010; Browning et al., 2014;  
227 Teixeira et al., 2022). Furthermore, if the intra-skeletal variability of  $\delta^{18}\text{O}_{\text{p}}$  was linked to  
228 variations in  $\delta^{18}\text{O}_{\text{bw}}$ , the variations would be random and not dependent on the bones sets  
229 considered as illustrated in **Fig.2**.

230 The second possible explanation for this variability is the difference in mineralisation  
231 temperature. The extremities of the limbs, or autopods, which include the metatarsals,  
232 metacarpals, and phalanges, are not covered by fur or by the thick layer of adipose tissue  
233 present on the Phocidae trunk (Scholander et al., 1950; Irving and Hart, 1957). Flippers  
234 extremities in seals, represent important effector structures for thermoregulation because of  
235 their poor insulation, but also due to a vasculature that allows rapid shifts between heat  
236 conservation and heat dissipation (e.g., Kvadsheim and Folkow, 1997, Blix et al., 2010).  
237 During heat conservation, flippers may have temperatures more than 20 degrees lower than

238 those of the core body temperature (Irving and Hart, 1957, Folkow and Blix, 1987; Favilla et  
239 al., 2022). Body temperature differences could therefore explain the differences in  $\delta^{18}\text{O}_p$   
240 values observed between the Axial skeleton and Autopod sets, and between the elements of  
241 the Stylopod/Zeugopod and Autopod sets (**Fig. 3**). According to the relationship established  
242 by Lécuyer et al. (2013) and assuming that the oxygen isotope value of the body water is  
243 constant or weakly variable, the  $\delta^{18}\text{O}_p$  difference of  $\sim 1$  ‰ (**Table 2**) between the Axial  
244 skeleton and the Autopod sets represents a mineralization temperature difference of 4 to 5°C  
245 (**Fig. 2**). This estimated difference in mineralisation temperature is lower than that recorded  
246 by conventional methods (Miller and Irving, 1975; Andrews, 1999). This discrepancy could  
247 be explained by the time period of multiple years recorded within the bones (Hedges et al.,  
248 2004). Consequently, the temperature estimates inferred from bone  $\delta^{18}\text{O}_p$  may differ from a  
249 punctual body temperature measurement (Favilla et al., 2022). Nevertheless, the use of  
250 skeletal elements to address thermophysiological issues offers the possibility of examining  
251 Pinniped specimens in museum collections and observing any differences between species  
252 based on their ecology or geographical distribution, without manipulating wild living  
253 organisms. By focusing on key species, it is also possible to identify the thermoregulatory  
254 strategies adopted by Pinnipeds through geological time and climate.

255 Another major difference observed in terms of  $\delta^{18}\text{O}_p$  variations at the individual level is  
256 between the anterior Stylopod/Zeugopod set and the posterior Stylopod/Zeugopod set (**Table**  
257 **2; Fig. 3**), which have respective mean values of  $17.4 \pm 0.1$  ‰ V-SMOW ( $n = 6$ ) and  $16.9 \pm$   
258  $0.2$  ‰ V-SMOW ( $n = 6$ ). Such  $\delta^{18}\text{O}_p$  values difference could be explained by the heat  
259 production generated by the muscle activity of the pelvic region during locomotion (Bebej,  
260 2009; Kuhn and Frey, 2012; Berta, 2018; **Fig. 3**). This hypothesis could be confirmed or  
261 rejected by comparing the intra-skeletal variability of the  $\delta^{18}\text{O}_p$  of harbour seals with those  
262 obtained for Otariinae Gray, 1825 (data currently unavailable). Indeed, unlike the Phocidae,

263 Otariinae do not swim using the lateral undulation of the pelvic region but propulsion by the  
264 forelimbs (Bebej, 2009; Kuhn and Frey, 2012; Berta, 2018), which should hence record lower  
265  $\delta^{18}\text{O}_p$  values (i.e., higher temperatures) compared to the pelvic region.

266

267 ***Teeth  $\delta^{18}\text{O}_p$ : an open window on the in-utero period***

268 The sampled permanent third left premolar has a very low  $\delta^{18}\text{O}_p$  value that likely reflects  
269 peculiar ontogenetic and regional conditions. In Pinnipedia, the mineralisation of permanent  
270 teeth takes place during the *in utero* phase of the animal development (Beloborodov, 1975;  
271 Stewart and Stewart, 1987; Meyer and Matzke, 2004; Kahle et al., 2018), as crown enamel  
272 hydroxylapatite is not subsequently remodeled. The recording of the oxygen isotope  
273 composition of the tooth, and more specifically of the enamel, which is asynchronous with the  
274 isotope recording of the bone, regularly modified during bone remodeling in endothermic  
275 animals, represents a distinct time interval in terms of mineralisation. The permanent dentition  
276 becomes entirely functional by the end of the brief suckling period, when the offspring are 4-6  
277 weeks old (Reijnders, 1992; Meyer and Matzke, 2004). Unlike most of the phocid species  
278 (Bowen et al., 1992; Boness and Bowen, 1996; Bowen et al., 2009), female harbour seals feed  
279 during a large part of the lactation period (Bowen et al., 1994; Thompson et al., 1994) in order  
280 to meet the energy requirements of lactation and of their metabolism (Bowen et al., 1992,  
281 2001). The energy requirements of pinnipeds, and therefore potentially their foraging  
282 behaviour, are different between males and females depending on their activity and life stage  
283 (pregnant or not, pupping, moulting, reproduction; Beck et al., 2003).

284 The studied male harbour seal specimen (MNHN-ZM-AC-1894-524) comes from the Baie de  
285 Somme, France. Despite being capable of travelling long distances of several hundred  
286 kilometers (Bonner and Witthames, 1974; Wiig and Oeien, 1988), most harbour seal  
287 individuals, a species considered to be philopatric, return to their natal area (Thompson, 1993;

288 Vincent et al., 2021). In this region, it has been observed that female harbour seals,  
289 particularly when they are pregnant, have a fairly restricted distribution and foraging range  
290 compared with males from the same population (Vincent et al., 2021) and are confined to the  
291 estuary of the nearby Somme river. This latter has an oxygen isotope composition comprised  
292 between -7.5 ‰ and -5.8 ‰ V-SMOW (Négre and Petelet-Giraud, 2005; Clauzel, 2022) and  
293 mixes with water from the English Channel, having  $\delta^{18}\text{O}_w$  values between 0 ‰ and 1 ‰ V-  
294 SMOW (LeGrande and Schmidt, 2006). Thus, it is expected that the  $\delta^{18}\text{O}$  value of the body  
295 water of pregnant females living in the estuary will be substantially lower than that of males  
296 and non-pregnant females living offshore or at the margin of the river mouth. Since the  
297 permanent teeth of juveniles are mineralized *in utero* from female body water, these temporal  
298 aspects could very likely explain the extreme  $^{18}\text{O}$ -depletion of the  $\delta^{18}\text{O}_{bw}$  and thus the low  
299  $\delta^{18}\text{O}_p$  value of the third premolar of the male specimen relative to the rest of its skeleton,  
300 which records the last years of the life of the animal and not the early stages of the  
301 development because of bone remodeling (Castanet, 1980; 2006; Montes et al., 2007;  
302 Castanet et al., 2010). This seems to be the most parsimonious explanation since the elements  
303 of the axial skeleton (vertebrae, girdle bones and ribs) are close to the vital organs, having a  
304 relatively stable temperature of  $37 \pm 1^\circ\text{C}$  (core body temperature; e.g. Favilla et al. 2022), and  
305 therefore mineralise at approximately this temperature. The mean value of the "axial skeleton"  
306 set is equal to 17.0 ‰ and ranged from 16.0 ‰ to 17.6 ‰, the variability being the  
307 consequence of a variation in the  $\delta^{18}\text{O}_{bw}$  value of the specimen during its life and whose  
308 origin is a variation in the  $\delta^{18}\text{O}$  value of the ingested environmental water. For the third  
309 definitive premolar tooth, mineralisation temperature is close to  $37^\circ\text{C}$  (core body temperature  
310 of the pregnant female) and using the relationship of Lécuyer et al. (2013), we have estimated  
311 the  $\delta^{18}\text{O}$  value of the water from which the tooth mineralised corresponding to the female  
312 body water in the uterus, ultimately reflecting the value of ingested environmental water.

313 From the measured  $\delta^{18}\text{O}_p$  value of the third left definitive premolar equal to 15.7 ‰ V-  
314 SMOW, the  $\delta^{18}\text{O}$  value of the water from which the tooth mineralised is estimated to be equal  
315 to -2.2 ‰ V-SMOW. After subtracting 2‰ from this value, which correspond to the isotope  
316 enrichment between body water and ingested water of semi-aquatic mammals (Cerling et al.,  
317 2008), this estimated ingested water value of about -4.2 ‰ V-SMOW lies between the  $\delta^{18}\text{O}_w$   
318 values measured for the English Channel (between 0 ‰ and 1 ‰ V-SMOW, LeGrande and  
319 Schmidt, 2006) and that of the Somme River (-7.5 ‰ to -5.8 ‰ V-SMOW, Négrel and  
320 Petelet-Giraud, 2005). This value would therefore correspond to a mixing between pure  
321 marine and fresh waters expected in the estuary of the Baie de Somme where the pregnant  
322 female likely lived during the formation of the third left premolar of the juvenile. This  
323 hypothesis is congruent with independent telemetric studies performed on harbour seals  
324 documenting this behaviour (Vincent et al., 2021).

325

## 326 **Conclusion**

327 In this study, we highlighted the intra-skeletal  $\delta^{18}\text{O}_p$  variability in a harbour seal specimen. A  
328 comparable trend was indirectly observed in  $\delta^{18}\text{O}_p$  values and temperature heterogeneities  
329 known by classical methods of thermometry from the literature. Our  $\delta^{18}\text{O}_p$  data reveal that  
330 bone hydroxylapatite of the autopod of harbour seal mineralise at a lower temperature than  
331 that of the axial skeleton (e.g. vertebrae, ribs, and girdles bones). Such observation and  
332 method open a promising avenue for determining the thermophysiology of fossil Pinnipedia  
333 as the oxygen isotope composition from the phosphate group of the hydroxylapatite may be  
334 preserved in the fossil record. This opens considerable prospects, particularly in the field of  
335 paleontology. The study of Pinniped specimens that lived during key periods in the Earth's  
336 history, notably during global temperature increases, promises to provide important insights

337 into the evolutionary mechanisms and survival strategies adopted by semi-aquatic organisms  
338 when confronted with global warming. In addition, our results indicate that the  $\delta^{18}\text{O}_p$  values  
339 of harbour seal teeth, and probably Pinnipedia in general, are indicators of the feeding areas of  
340 their mother during the gestation period (estuarine/marine or neritic/pelagic). In future studies,  
341 the use of  $\delta^{18}\text{O}_p$  from permanent teeth could provide a better understanding of the sex-  
342 segregation of fishing areas and explore the ecological and behavioural adaptations of  
343 pregnant females. Finally, high-resolution  $\delta^{18}\text{O}_p$  transects along growth layer groups (GLGs),  
344 annual deposits of dentine, could be used to constrain the geographical origin and the  
345 resources used by Pinnipedia during their lifetime. These data could then be used within  
346 habitat models to define the impact of climate and environmental parameters, such as rising  
347 ocean temperatures, on the occupation of marine areas by these organisms through geological  
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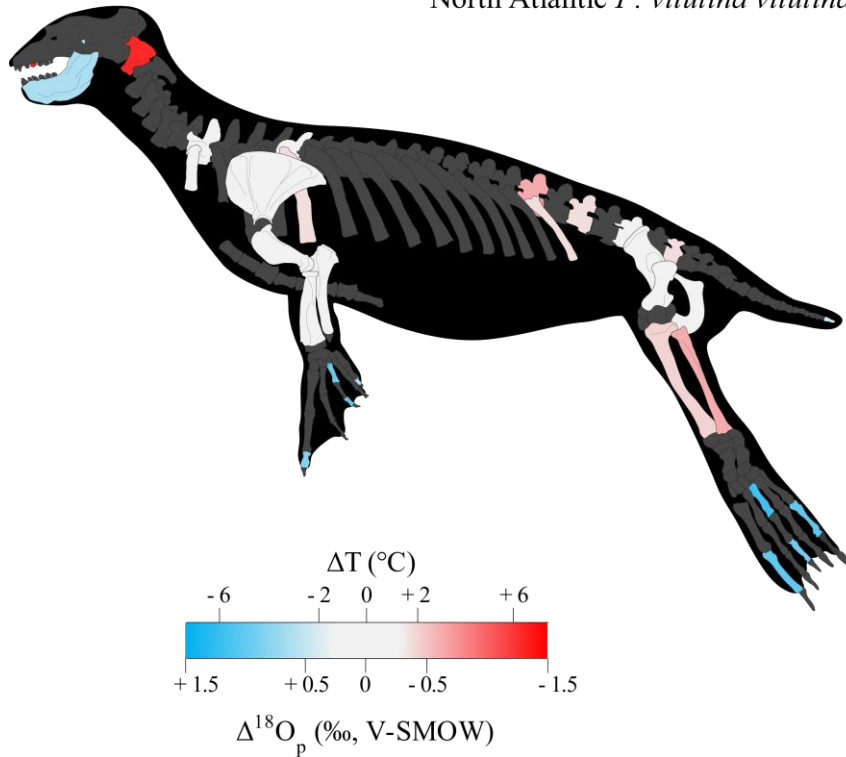
606 **Figure caption**



607

608 **Figure 1:** Geographical location of the Baie de Somme where the harbour seal stranded corps  
609 has been collected.

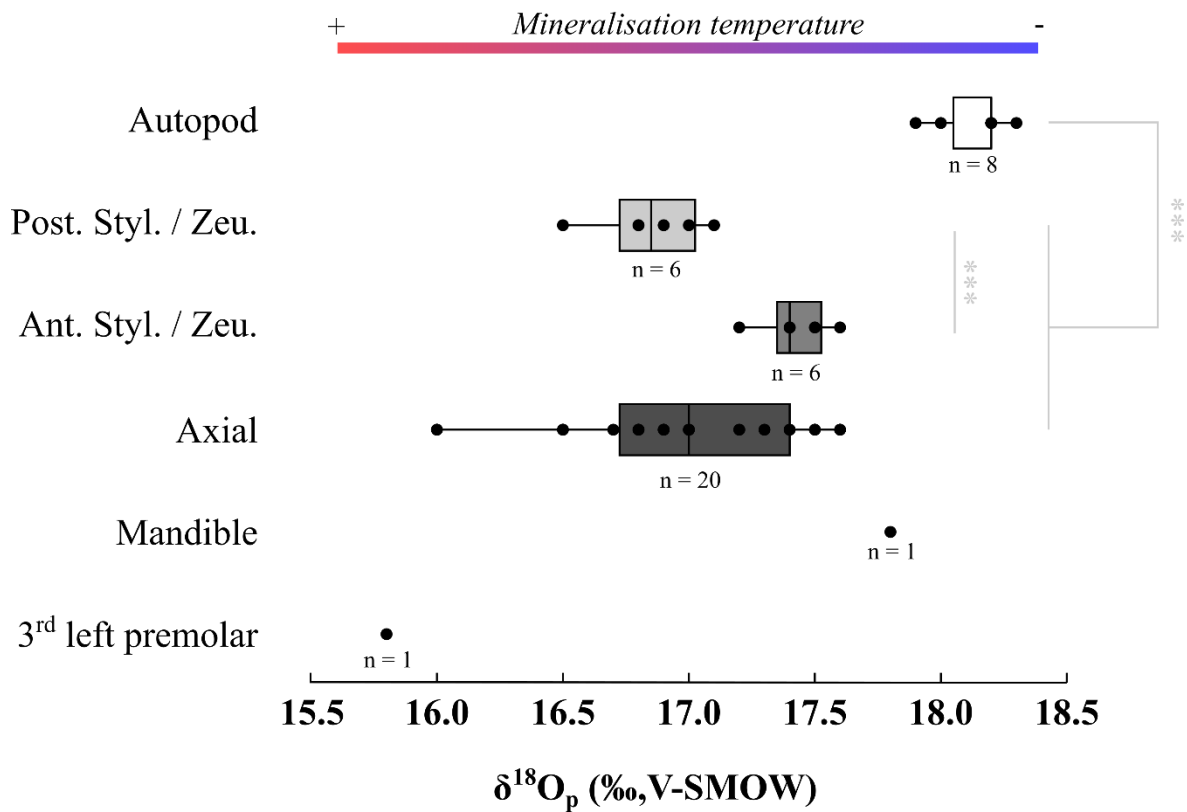
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611

612 **Figure 2:** Oxygen isotope variability within the skeleton from the specimen (MNHN-ZC-AC-  
613 1894-524) of *Phoca vitulina vitulina*. Color in tooth and bones correspond to the  $\delta^{18}\text{O}$   
614 difference between bone or tooth and the mid-range value ( $(\delta^{18}\text{O}_{\text{max}} - \delta^{18}\text{O}_{\text{min}}) / 2$ ) of the  
615 skeleton. For paired skeletal elements as well as vertebrae centra and neural spines, the mean  
616 value is illustrated.

617



618

619 **Figure 3:** Boxplots showing the  $\delta^{18}\text{O}_p$  values distribution of bones sets for the specimen  
 620 (MNHN-ZC-AC-1894-524) of *Phoca vitulina vitulina*. Asterisks (\*\*\*) indicate  $p$ -value <  
 621 0.001. The vertical bars in the boxes correspond to the medians and the whiskers to the  
 622 minimum and maximum values. The average analytical error for each bone analysed is of the  
 623 order of 0.2%.

624



625 **Table caption**

626

627 **Table 1:** Oxygen isotope composition of the phosphate group ( $\delta^{18}\text{O}_p$ ) of the bioapatite of the  
 628 skeletal elements of the specimen of *Phoca vitulina vitulina* (MNHN-ZC-AC-1894-524).

629 **Abbreviations:** Aut. = autopod bone set, Axial = axial skeleton bone set, Styl./Zeu. =  
 630 stylopod and zeugopod bone set with Ant. for the anterior one and Post. for the posterior one.

# Sample	Skeletal element	Sample location	Bone set	$\delta^{18}\text{O}_p$ (‰, V-SMOW)		
				Mean	SD	N
PV1	1 <sup>st</sup> cervical vertebra	centrum	Axial	16.0	0.2	4
PV2	2 <sup>nd</sup> lumbar vertebra	centrum	Axial	16.5	0.1	5
PV3	2 <sup>nd</sup> lumbar vertebra	neural spine	Axial	17.3	0.1	5
		$\Delta$ centrum - neural spine		-0.8		
PV4	5 <sup>th</sup> lumbar vertebra	centrum	Axial	16.8	0.2	5
PV5	5 <sup>th</sup> lumbar vertebra	neural spine	Axial	17.5	0.1	5
		$\Delta$ centrum - neural spine		-0.8		
PV6	1 <sup>st</sup> caudal vertebra	centrum	Axial	16.9	0.2	4
PV7	Last caudal vertebra	bulk	Axial	17.6	0.1	5
PV8	1 <sup>st</sup> thoracic vertebra	centrum	Axial	16.8	0.1	5
PV9	1 <sup>st</sup> thoracic vertebra	neural spine	Axial	17.5	0.1	5
		$\Delta$ centrum - neural spine		-0.7		
PV10	5 <sup>th</sup> thoracic vertebra	centrum	Axial	16.5	0.2	4
PV11	5 <sup>th</sup> thoracic vertebra	neural spine	Axial	17.4	0.1	5
		$\Delta$ centrum - neural spine		-0.9		
PV12	Last thoracic vertebra	centrum	Axial	16.0	0.1	5
PV13	Last thoracic vertebra	neural spine	Axial	17.6	0.1	4
		$\Delta$ centrum - neural spine		-1.6		
PV20	Left scapula	anterior side	Axial	17.4	0.1	4
PV21	Right scapula	anterior side	Axial	17.0	0.3	5
PV24	Left pubis	medial face	Axial	17.2	0.1	5
PV29	1 <sup>st</sup> left rib	medial part	Axial	17.0	0.2	5
PV30	5 <sup>th</sup> left rib	medial part	Axial	17.0	0.1	5
PV31	5 <sup>th</sup> right rib	medial part	Axial	16.7	0.3	5
PV32	15 <sup>th</sup> left rib	distal part	Axial	16.9	0.2	5
PV14	Right radius	diaphysis	Ant. Styl. / Zeu.	17.4	0.1	5

PV15	Left radius	diaphysis	Ant. Styl. / Zeu.	17.5	0.1	4
PV16	Right ulna	diaphysis	Ant. Styl. / Zeu.	17.5	0.1	5
PV17	Left ulna	diaphysis	Ant. Styl. / Zeu.	17.3	0.1	5
PV18	Left humerus	diaphysis	Ant. Styl. / Zeu.	17.2	0.1	5
PV19	Right humerus	diaphysis	Ant. Styl. / Zeu.	17.4	0.1	3
PV22	Left femur	diaphysis	Post. Styl. / Zeu.	17.1	0.2	5
PV23	Right femur	diaphysis	Post. Styl. / Zeu.	17.0	0.2	5
PV25	Left tibia	diaphysis	Post. Styl. / Zeu.	16.8	0.2	5
PV26	Right tibia	diaphysis	Post. Styl. / Zeu.	16.9	0.2	5
PV27	Left fibula	diaphysis	Post. Styl. / Zeu.	16.5	0.3	5
PV28	Right fibula	diaphysis	Post. Styl. / Zeu.	16.8	0.2	5
PV33	Left forelimb phalanx I.2	diaphysis	Aut.	18.0	0.1	5
PV34	Left forelimb metacarpian III	diaphysis	Aut.	18.2	0.2	5
PV35	Left forelimb phalanx III.2	diaphysis	Aut.	18.2	0.2	5
PV36	Left forelimb phalanx V.1	diaphysis	Aut.	17.9	0.1	5
PV37	Left hindlimb phalanx I.2	diaphysis	Aut.	18.2	0.1	5
PV38	Left hindlimb metatarsian III	diaphysis	Aut.	18.3	0.2	5
PV39	Left hindlimb phalanx III.2	diaphysis	Aut.	18.2	0.1	3
PV40	Left hindlimb phalanx V.1	diaphysis	Aut.	18.2	0.1	5
PV41	Left mandible	medial face	Mandible	17.8	0.2	5
PV42	Third left upper premolar (P3)	crown enamel	Tooth	15.7	0.2	5
		Min. $\delta^{18}\text{O}_p$		15.7		
		Max. $\delta^{18}\text{O}_p$		18.3		
		Mean $\delta^{18}\text{O}_p$		17.2	SEM	0.6
		$\Delta\delta^{18}\text{O}_p$		2.6		
		Mid-range		17.2		

631

632

633 **Table 2:** Summary of the mean oxygen isotope composition (‰, V-SMOW) of harbour seal  
634 skeletal regions.

$\delta^{18}\text{O}_p$ (‰, V-SMOW)		
<b>Bone set</b>	<b>Mean <math>\pm</math> SEM</b>	<b>n</b>
Third left premolar	15.7	1
Mandible	17.8	1
Axial skeleton bone set	17.0 $\pm$ 0.5	20
Anterior Stylopod/zeugopod bone set	17.4 $\pm$ 0.1	6
Posterior Stylopod/zeugopod bone set	16.9 $\pm$ 0.2	6
Autopod bone set	18.1 $\pm$ 0.1	8

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