

# Reconstruction of the extinct Ezo wolf's diet

**Short title:** Feeding habits of the extinct Ezo wolf

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14 **Abstract**

15 On Hokkaido, Japan, the Ezo wolf (*Canis lupus hattai*), an apex predator, became extinct at  
16 the end of the 19th century owing to human activities. Top predators often have an important  
17 role in their ecosystems, yet we have no scientific information on the feeding habits of the  
18 Ezo wolf. We performed carbon and nitrogen stable isotope analysis and radiocarbon dating  
19 of specimens of the wolf ( $n = 7$ ) and its prey species and estimated the components of the  
20 wolves' diet using an isotope mixing model. Radiocarbon dating suggested that most of the  
21 wolves examined came from different populations or generations. The mean stable isotope  
22 ratios of the wolves were  $-19.5\text{‰}$  ( $\pm 1.9\text{‰}$  SD) for  $\delta^{13}\text{C}$  and  $8.7\text{‰}$  ( $\pm 2.6\text{‰}$  SD) for  $\delta^{15}\text{N}$ .  
23 The discrimination-corrected isotopic ratios of five of the seven wolves were almost the same  
24 as those of Sika deer at the same sites. In contrast, those of two wolves had clearly higher  
25 isotopic values than those of deer, suggesting that these wolves depended partly on marine  
26 prey such as salmon and marine mammals. Thus, Ezo wolves had similar ecological roles to  
27 Canadian grey wolves, and were a second subspecies shown to have fed on a marine diet, in  
28 addition to the "coastal wolves" of British Columbia.

29

30 **Key words:** dietary reconstruction, extinct mammal, *Canis lupus*, stable isotope, marine prey

31

## 32 **Introduction**

33 Wolf species are distributed widely in the Northern Hemisphere and are typical apex  
34 predators in terrestrial ecosystems. Wolves feed primarily on terrestrial mammals such as  
35 ungulates and rodents (e.g., Ciucci, Tosoni & Boitani 2004, Morehouse & Boyce 2011).  
36 However, recent studies show that grey wolves on the central coast of British Columbia,  
37 Canada, uniquely depend heavily on marine prey. Unlike interior populations, these “coastal  
38 wolves” (Darimont, Reimchen & Paquet 2003) have a distinct genetic structure and distinct  
39 feeding habits (Stronen et al. 2014). The recognition of these coastal wolves is an important  
40 key to understanding drivers of genetic differentiation among neighbouring populations.

41 Hokkaido, at the northern end of Japan, was inhabited by a subspecies of grey wolf, the  
42 Ezo wolf (*Canis lupus hattai*), until the end of the 19th century, when it became extinct owing  
43 to human activities (Inukai 1982). This was the first human-caused extinction of a mammal  
44 species in Japan. Because Ezo wolves disappeared before their ecology could be investigated,  
45 there is almost no information about their past ecological roles. In particular, their feeding  
46 habits are important for understanding their ecological roles. Umeki (2016) found no  
47 scientific information about their feeding habits in the literature. As Hokkaido is similar to  
48 Canada in both environmental conditions and fauna, various marine animals, such as  
49 anadromous salmon species (*Oncorhynchus* spp.) and the carcasses of marine mammals,  
50 could have been prey items for Ezo wolves, as they are in Canada. Therefore, if Ezo wolves  
51 fed on marine animals as well as terrestrial ones, we might detect such a diet by stable isotope  
52 analysis of their bones (e.g. Hilderbrand et al. 1996, Matsubayashi et al. 2015).

53 Here, we aimed at reconstructing the feeding habits of Ezo wolves by using stable isotope  
54 analysis of bone collagen. Ezo wolves and domestic dogs in Hokkaido are distinguishable by  
55 their skeletal size (Ishiguro et al. 2010). Bones excavated from archaeological sites in  
56 Hokkaido have been identified by archaeologists and reported. Few bone fragments of the

57 Ezo wolf have been excavated, but we searched for as many as we could find in the  
58 archaeological reports available at the Hokkaido Archaeological Operations Center, and  
59 obtained samples. We measured radiocarbon values and stable carbon and nitrogen isotope  
60 ratios in the bone collagen of the samples and compared the isotopic values with those of  
61 potential prey species to reconstruct the wolves' feeding habits and differences among  
62 individuals. Investigating their feeding habits, particularly the potential consumption of a  
63 marine diet, is the first step in understanding the ecological role of Ezo wolves in past  
64 ecosystems.

65

## 66 **Materials and methods**

### 67 **Study site**

68 This study was conducted on Hokkaido, the northernmost island of Japan (Fig. 1). The  
69 western half of the island is in the cool temperate zone and the eastern half is subarctic. Sika  
70 deer are distributed across the island, mainly in the central and eastern areas (Yamamura et al.  
71 2008). Chum salmon (*Oncorhynchus keta*) run from October to February in the western and  
72 eastern areas, and pink salmon (*Oncorhynchus gorbuscha*) run from August to October in the  
73 east.

74

### 75 **Sample collection and stable isotope analysis**

76 We collected bone fragments of wolves ( $n = 8$ ) and their potential mammal prey species from  
77 local museums (Fig. 1, Table 1), but we used bones of modern salmon (bought at the market  
78 in eastern Hokkaido in 2012) because salmon bones are rarely excavated from archaeological  
79 sites. All of the wolves used were confirmed as adults, because skeletons of adult wolves,  
80 unlike those of young wolves, are distinguishable from those of domestic dogs.

81 Samples of bone collagen were extracted according to the methods of Schoeninger &  
82 DeNiro (1984), and their carbon and nitrogen stable isotope values were measured by mass  
83 spectrometry. Stable isotope ratios are expressed in  $\delta$  notation in accordance with the  
84 international standard scale, based on the following equation (Bond & Hobson 2012):

$$85 \quad \delta X = (R_{\text{sample}} / R_{\text{standard}}) - 1, \quad (1)$$

86 where  $X$  is  $^{13}\text{C}$  or  $^{15}\text{N}$ ;  $R_{\text{sample}}$  is the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratio of the sample; and  $R_{\text{standard}}$  is that  
87 of Vienna Pee Dee Belemnite for  $^{13}\text{C}/^{12}\text{C}$  or of atmospheric nitrogen for  $^{15}\text{N}/^{14}\text{N}$ . The stable  
88 isotope ratios were measured on a Delta XP mass spectrometer (Thermo Fisher Scientific,  
89 Waltham, MA, USA) connected to a Flash EA 2000 elemental analyser (Thermo Fisher  
90 Scientific) at the Research Institute for Humanity and Nature (Kyoto, Japan). The elemental  
91 concentrations and isotope ratios of carbon and nitrogen were calibrated against alanine ( $\delta^{13}\text{C}$   
92 =  $-19.04$  ‰,  $\delta^{15}\text{N}$  =  $22.71$  ‰) and glycine ( $\delta^{13}\text{C}$  =  $-34.92$  ‰,  $\delta^{15}\text{N}$  =  $2.18$  ‰) laboratory  
93 standards (Tayasu et al. 2011), which are traceable back to the international standards. The  
94 analytical standard deviations (SD) of these standards were  $0.05$  ‰ ( $\delta^{13}\text{C}$ ) and  $0.11$  ‰ ( $\delta^{15}\text{N}$ )  
95 for alanine ( $n = 6$ ) and  $0.02$  ‰ ( $\delta^{13}\text{C}$ ) and  $0.05$  ‰ ( $\delta^{15}\text{N}$ ) for glycine ( $n = 6$ ).

96

### 97 **Correction of isotope values in modern salmon bone collagen samples**

98 We corrected the stable isotope ratios of modern salmon for the expected changes in isotopic  
99 values over time. Carbon stable isotope ratios show a historical decrease after 1860 due to  
100 human consumption of fossil fuels (Suess effect). Ice-core studies (Francey et al. 1999,  
101 Chamberlain et al. 2005) show that atmospheric  $\delta^{13}\text{C}$  decreased by  $0.05$  ‰ per decade  
102 between 1860 and 1960 and by  $0.22$  ‰ per decade since 1960 (Wiley et al. 2013). Therefore,  
103 we increased the  $\delta^{13}\text{C}$  of the modern salmon by  $1.60$  ‰. The  $\delta^{15}\text{N}$  value is also expected to  
104 have changed historically owing to anthropogenic atmospheric nitrogen and biological  
105 nitrogen fixation (the conversion of  $\text{N}_2$  gas to ammonium). For marine prey, the maximum

106 change in  $\delta^{15}\text{N}$  resulting from anthropogenic atmospheric nitrogen is 0.03 ‰, and that  
107 resulting from N fixation is 0.13 ‰ (Wiley et al. 2013). Therefore, we reduced the  $\delta^{15}\text{N}$  of  
108 modern salmon by 0.16 ‰. We used these corrected isotopic values in the subsequent  
109 statistical analyses.

110

### 111 **Mixing model and statistical analyses**

112 Prey items were initially categorized as terrestrial mammals, anadromous salmon, or marine  
113 mammals. We used a  $K$  nearest-neighbour randomization test (Rosing, Ben-David & Barry  
114 1988) to investigate whether the stable isotope ratios of the various food types differed  
115 significantly from each other, and all diet items were isotopically segregated (Bonferroni  
116 adjusted  $P < 0.01$ ). Then we evaluated the proportional contribution of each food resource to  
117 the wolves' diet using Stable Isotope Analysis in R (SIAR), a Bayesian isotopic mixing model  
118 available as an open-source R package (Parnell et al. 2010). The SIAR Markov chain Monte  
119 Carlo algorithm was run for 1 000 000 iterations. The first 100 000 samples were discarded to  
120 avoid the possibility of starting value effects. We also assigned elemental concentration  
121 values of prey items (%C and %N in Table 1) to account for the differences in C and N  
122 concentrations (Phillips & Koch 2002). Because different tissues incorporate isotopes at  
123 different rates, we applied a correction factor for bone collagen to incorporate the isotopic  
124 discrimination (Trophic Discrimination Factor: TDF) between predator and prey before  
125 generating the model. On the basis of several published studies that described wolf–prey  
126 isotopic TDF, we set an isotopic TDF of  $1.1 \text{ ‰} \pm 0.2 \text{ ‰ SD}$  for  $\delta^{13}\text{C}$  (Szepanski et al. 1999,  
127 Bocherens & Drucker 2003, Fox-Dobbs 2007) and  $3.9 \text{ ‰} \pm 1.1 \text{ ‰ SD}$  for  $\delta^{15}\text{N}$  (Schwarcz &  
128 Schoeninger 1991, Szepanski et al. 1999, Bocherens & Drucker 2003, Fox-Dobbs 2007). All  
129 statistical analyses were conducted in R (R Core Team 2013).

130

## 131 **Radiocarbon dating**

132 We used radiocarbon ( $^{14}\text{C}$ ) dating to determine the year of death of the ancient wolves, but  
133 not of the modern wolves (HUB9880 and HUB9890B), for which accurate information is  
134 recorded. We obtained enough collagen from two ancient wolves (TN1-01, WO-01) to  
135 graphitize the carbon in it, but not for the other three (OPA-01, UEN-01 and YNG-01). For  
136 UEN-01 and YNG-01, we used bone collagen of a deer (UEN-03 and YNG-02) which was  
137 excavated with the wolf. The age of OPA-01 was determined from geological information at  
138 the site (BBE 1997).

139 Collagen samples used for carbon and nitrogen stable isotope analysis were also used for  
140 the radiocarbon analysis. Targets were graphitized at the Research Institute for Humanity and  
141 Nature, and  $^{14}\text{C}$  concentrations were measured by accelerator mass spectrometry at the  
142 Institute of Accelerator Analysis Ltd (Kanagawa, Japan).  $^{14}\text{C}$  results are expressed as a  
143 percentage of modern (1950) carbon, which is corrected for isotopic discrimination using the  
144  $\delta^{13}\text{C}$  values. The  $^{14}\text{C}$  dates were calibrated against the IntCal13 calibration curve (Reimer et  
145 al. 2013) in OxCal v. 4.2 software (Ramsey 2009).

146

## 147 **Results**

148 One of the wolf collagen samples (TKR-01; Table 1) showed a higher C/N ratio (8.9) than the  
149 others, well outside the established range of pure bone collagen samples (2.9–3.6; DeNiro  
150 1985), so its isotopic data were not used in further analyses. The mean stable isotope ratios of  
151 the wolves were  $-19.5\text{‰} \pm 1.9\text{‰}$  SD (range,  $-21.0\text{‰}$  to  $-15.2\text{‰}$ ) for  $\delta^{13}\text{C}$  and  $8.7\text{‰} \pm$   
152  $2.6\text{‰}$  SD ( $4.5\text{‰}$ – $13.7\text{‰}$ ) for  $\delta^{15}\text{N}$ . The discrimination-corrected isotopic ratios of five of  
153 the seven wolves were almost the same as those of Sika deer at the same sites (Fig. 2). In  
154 contrast, those of YNG-01 and UEN-01 were higher than those of deer (Fig. 2). The results of

155 the mixing model analysis suggested that these two wolves ate some marine prey; however,  
156 wide range of 95 % credible intervals showed that our model could not successfully dissect  
157 the proportions of salmon and marine mammals (Table 2).

158 <sup>14</sup>C dating showed that the wolves TN1-01, WO-01, and YNG-01 and the deer UEN-03  
159 died between 3961 and 3106 BC (Table 3). Archaeological records showed that OPA-01 died  
160 between AD 1667 (the date of eruption of Mt Tarumae, in the volcanic ash layer of which the  
161 remains were found) and AD 1913 (when the site was found) (BBE 1997).

162

## 163 **Discussion**

164 The results of radiocarbon dating suggested that most of the isotopic data were derived from  
165 different wolf populations or generations and were not biased to particular populations,  
166 although WO-01 and YNG-01 were not temporally isolated. Therefore, the variation in stable  
167 isotope values of the wolves derives from both population-level and individual-level  
168 differences in feeding habits. The ratios were clearly different from those of domestic dogs  
169 (Fig. 2), so these animals were not large domestic dogs.

170 A recent genetic study of the Ezo wolf showed that its mitochondrial DNA was identical  
171 to that of the Canadian grey wolf (Ishiguro et al. 2010). Canadian grey wolves show a variety  
172 of feeding habits at both the population and individual levels. For instance, wolf populations  
173 of coastal British Columbia have three different feeding habits: mainland wolves feed mostly  
174 on terrestrial resources, Outer Islands subpopulations depend strongly on marine resources,  
175 and Inner Islands subpopulations feed on both resources (Darimont et al. 2009). Our stable  
176 isotope results showed that Ezo wolves likely had feeding habits that were similar to those of  
177 the Inner Islands subpopulation in British Columbia, because both populations depend on  
178 both marine and terrestrial prey.

179 Several dietary studies of ancient domestic dogs on Hokkaido showed that all of these



180 dogs fed on marine prey provided by humans (Fig. 2; Naito et al. 2010, Tsutaya et al. 2014).  
181 On the other hand, the  $\delta^{15}\text{N}$  values of the wolves in this study were clearly distinct from that  
182 of domestic dogs, suggesting that the wolves were not fed by humans. However, we cannot  
183 exclude the possibility that UEN-01 and YNG-01 sometimes fed on marine prey provided by  
184 humans. Further analysis to reconstruct the isotope chronology of individual mammals, such  
185 as incremental stable isotope analysis in dentine (Beaumont et al. 2013), should help to  
186 answer the question.

187       Although wolves can feed on carcasses of marine mammals (e.g. Darimont & Paquet  
188 2002, Watts et al. 2010), grey wolves in Canada feed primarily on spawning salmon  
189 (Darimont et al. 2004). Stable isotope analysis suggested that the Ezo wolves also ate more  
190 salmon than marine mammals (Fig. 2). However, YNG-01 is likely to have fed on some  
191 marine mammals (Table 2), which could have been available to some wolf populations on  
192 Hokkaido. Consumption of marine organisms by terrestrial mammals is an important route of  
193 transfer of marine-derived nutrients to terrestrial ecosystems (e.g. Hilderbrand et al. 1999,  
194 Helfield & Naiman 2006). Thus, some wolf populations in coastal areas of Hokkaido could  
195 have had a role in transporting marine-derived nutrients to terrestrial ecosystems, unless  
196 humans were feeding them.

197       To date, the existence of wolves with a high dependence on marine prey has been  
198 reported only on the North American coast (e.g. Szepanski et al. 1999, Darimont & Reimchen  
199 2002, Darimont et al. 2009, Watts et al. 2010). Our data suggest that the Ezo wolf also fed on  
200 a marine diet. The coastal wolves in British Columbia are considered an evolutionarily  
201 significant unit (Ryder 1986), which is valuable for conservation because of its genetic and  
202 ecological uniqueness (Muñoz-Fuentes et al. 2009). This implies that Ezo wolves, with their  
203 partial dependence on marine prey, were important for terrestrial ecosystems on Hokkaido.

204

205 **Competing interests**

206 We have no competing interests.

207

208 **Authors' contributions**

209 J.M. designed the study; O.T. helped acquire the specimens; J.M., T.O. and I.T. performed the  
210 chemical and stable isotope analyses. All authors approved the manuscript for publication.

211

212 **Acknowledgements**

213 We thank F. Takaya of the Hokkaido University Botanical Gardens, K. Ebata of the Kushiro  
214 City Board of Education, K. Morioka of the Historical Museum of the Saru River, T.

215 Arakawa, T. Miyaji, S. Akaishi and M. Takeda of the Tomakomai City Museum, T. Aono of

216 the Date City Institute of Funkawan Culture and T. Kumaki of the Tokyo University for

217 access to specimens. We also thank the anonymous reviewers for their helpful comments on

218 the manuscript drafts. This work was supported by JSPS KAKENHI Grant Numbers

219 16K186270002 and 24\*2469 to J.M. and 16H02524 to I.T.

220

221 **References**

222 Beaumont, J., Gledhill, A., Lee-Thorp, J. & Montgomery, J. (2013). Childhood diet: a closer  
223 examination of the evidence from dental tissues using stable isotope analysis of  
224 incremental human dentine. *Archaeometry*. 55, 277–295.

225 Bocherens, H. & Drucker, D. (2003). Trophic level isotopic enrichment of carbon and  
226 nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems. *Int.*  
227 *J. Osteoarchaeol.* 13, 46–53.

228 Bond, A.L. & Hobson, K.A. (2012). Reporting stable-isotope ratios in ecology: recommended  
229 terminology, guidelines and best practices. *Waterbirds*, **35**, 324–331.

230 BBE (Biratori Board of Education). (1997). *Cultural property investigation report of*  
231 *Opaushinai I site related to a construction of Biratori bypass (National road No. 237).*  
232 Biratori, Hokkaido, Japan (in Japanese).

233 Chamberlain, C.P., Waldbauer, J.R., Fox-Dobbs, K., Newsome, S.D., Koch, P.L., Smith,  
234 D.R., Church, M.E., Chamberlain, S.D., Sorenson, K.J. & Risebrough, R. (2005).  
235 Pleistocene to recent dietary shifts in California condors. *Proc. Natl. Acad. Sci. USA*. **102**,  
236 16707–16711.

237 Ciucci, P., Tosoni, E. & Boitani, L. (2004). Assessment of the point-frame method to quantify  
238 wolf *Canis lupus* diet by scat analysis. *Wildl. Biol.* **10**, 149–153.

239 Darimont, C.T. & Paquet, P.C. (2002). The gray wolves, *Canis lupus*, of British Columbia’s  
240 Central and North Coast: distribution and conservation assessment. *Can. Field Nat.* **116**,  
241 416–422.

242 Darimont, C.T. & Reimchen, T.E. (2002). Intra-hair stable isotope analysis implies seasonal  
243 shift to salmon in gray wolf diet. *Can. J. Zool.* **80**, 1638–1642.

244 Darimont, C.T., Reimchen, T.E. & Paquet, P.C. (2003). Foraging behaviour by gray wolves  
245 on salmon streams in coastal British Columbia. *Can. J. Zool.* **81**, 349–353.

246 Darimont, C.T., Price, M.H.H., Winchester, N.N., Gordon-Walker, J. & Paquet, P.C. (2004).  
247 Predators in natural fragments: foraging ecology of wolves in British Columbia’s central  
248 and north coast archipelago. *J. Biogeogr.* **31**, 1867–1877.

249 Darimont, C.T., Paquet, P.C. & Reimchen, T.E. (2009). Landscape heterogeneity and marine  
250 subsidy generate extensive intrapopulation niche diversity in a large terrestrial vertebrate.  
251 *J. Anim. Ecol.* **78**, 126–133.

252 DeNiro, M.J. (1985). Postmortem preservation and alteration of in vivo bone collagen isotope

253 ratios in relation to palaeodietary reconstruction. *Nature*, **317**, 806–809.

254 Fox-Dobbs, K., Bump, J.K., Peterson, R.O., Fox, D.L. & Koch, P.L. (2007). Carnivore-  
255 specific stable isotope variables and variation in the foraging ecology of modern and  
256 ancient wolf populations: case studies from Isle Royale, Minnesota, and La Brea. *Can. J.*  
257 *Zool.* **85**, 458–471.

258 Francey, R.J., Allison, C.E., Etheridge, D.M., Trudinger, C.M., Enting, I.G., Leuenberger, M.,  
259 Langenfelds, R.L., Michel, E. & Steele, L.P. (1999). A 1000-year high precision record  
260 of  $\delta^{13}\text{C}$  in atmospheric CO<sub>2</sub>. *Tellus B: Chem. Phys. Meteorol.* **51**, 170–193.

261 Helfield, J.M. & Naiman, R.J. (2006). Keystone interactions: salmon and bear in riparian  
262 forests of Alaska. *Ecosystems.* **9**, 167–180.

263 Hilderbrand, G.V., Farley, S.D., Robbins, C.T., Hanley, T.A., Titus, K. & Servheen, C.  
264 (1996). Use of stable isotopes to determine diets of living and extinct bears. *Can. J. Zool.*  
265 **74**, 2080–2088.

266 Hilderbrand, G.V., Thomas, A.H., Charles, T.R. & Charles, C.S. (1999). Role of brown bears  
267 (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. *Oecologia.* **121**,  
268 546–550.

269 Inukai, T. (1982). Eradication of Ezo wolf by humans. *Zenshu Nihon Doubutsushi.* **5**, 11–15  
270 (in Japanese).

271 Ishiguro, N., Inoshima, T., Shigehara, N., Ichikawa, H. & Kato, M. (2010). Osteological and  
272 genetic analysis of the extinct Ezo wolf (*Canis lupus hattai*) from Hokkaido Island,  
273 Japan. *Zool. Sci.* **27**, 320–324.

274 Matsubayashi, J., Morimoto, J.O., Tayasu, I., Mano, T., Nakajima, M., Takahashi, O.,  
275 Kobayashi, K. & Nakamura, F. (2015). Major decline in marine and terrestrial animal  
276 consumption by brown bears (*Ursus arctos*). *Scientific reports*, **5**, 9203.

277 Morehouse, A.T. & Boyce, M.K. (2011). From venison to beef: seasonal changes in wolf diet

278 composition in a livestock grazing landscape. *Front. Ecol. Environ.* **9**, 440–445.

279 Muñoz-Fuentes, V., Darimont, C.T., Wayne, R.K., Paquet, P.C. & Leonard, J. A. (2009)

280 Ecological factors drive differentiation in wolves from British Columbia. *J. Biogeogr.* **36**,

281 1516–1531.

282 Naito, Y.I., Chikaraishi, Y., Ohkouchi, N., Mukai, H., Shibata, Y., Honch, N.V., Dodo, Y.,

283 Ishida, H., Amano, T., Ono, H. & Yoneda, M. (2010). Dietary reconstruction of the

284 Okhotsk culture of Hokkaido, Japan, based on nitrogen composition of amino acids:

285 implications for correction of <sup>14</sup>C marine reservoir effects on human bones, *Radiocarbon*

286 **52**, 671–681.

287 Parnell, A. C., Inger, R., Bearhop, S. & Jackson, A. L. (2010). Source partitioning using

288 stable isotopes: coping with too much variation. *PloS one.* **5**, e9672.

289 Phillips, D.L. & Koch, P.L. (2002). Incorporating concentration dependence in stable isotope

290 mixing models. *Oecologia.* **130**, 114–125.

291 R Core Team. (2013). R: A language and environment for statistical computing. R Foundation

292 for Statistical Computing, Vienna, Austria.

293 Ramsey, C.B. (2009). OxCal Program v4.2. <http://www.rlaha.ox.ac.uk/orau/oxcal.html>

294 (accessed 11 October 2016).

295 Reimer, P.J., Bard, E., Bayliss, A., Beck, J. W., Blackwell, P. G., Ramsey, C. B., Grootes, P.,

296 Guilderson, T., Hafliðason, H., Hajdas, I., et al. (2013). IntCal13 and Marine13

297 radiocarbon age calibration curves 0–50 000 years cal BP. *Radiocarbon.* **55**, 1869–1887.

298 Rosing, M.N., Ben-David, M. & Barry, R.P. (1988). Analysis of stable isotope data: a K

299 nearest-neighbours randomisation test. *J. Wildl. Manage.* **62**, 380–388.

300 Schoeninger, M. J. & DeNiro, M. J. (1984). Nitrogen and carbon isotopic composition of

301 bone collagen from marine and terrestrial animals. *Geochim. Cosmochim. Acta* **48**, 625–

302 639.

303 Schwarcz, H.P. & Schoeninger, M.J. (1991). Stable isotope analyses in human nutritional  
304 ecology. *Am. J. Phys. Anthropol.* **34**, 283–321.

305 Stronen, A. V., Navid, E. L., Quinn, M. S., Paquet, P. C., Bryan, H. M. & Darimont, C. T.  
306 (2014). Population genetic structure of gray wolves (*Canis lupus*) in a marine archipelago  
307 suggests island-mainland differentiation consistent with dietary niche. *BMC ecol.* **14**, 1.

308 Szepanski, M.M., Ben-David, M. & Ballenberghe, V.V. (1999). Assessment of anadromous  
309 salmon resources in the diet of the Alexander Archipelago wolf using stable isotope  
310 analysis. *Oecologia.* **120**, 327–335.

311 Tayasu, I., Hirasawa, R., Ogawa, N.O., Ohkouchi, N. & Yamada, K. (2011). New organic  
312 reference materials for carbon- and nitrogen-stable isotope ratio measurements provided  
313 by Center for Ecological Research, Kyoto University, and Institute of Biogeosciences,  
314 Japan Agency for Marine-Earth Science and Technology. *Limnology.* **12**, 261–266

315 Tsutaya, I., Naito, Y.I., Ishida, H. & Yoneda, M. (2014). Carbon and nitrogen isotope  
316 analyses of human and dog diet in the Okhotsk culture: perspectives from the Moyoro  
317 site, Japan. *Anthropol. Sci.* **122**, 89–99.

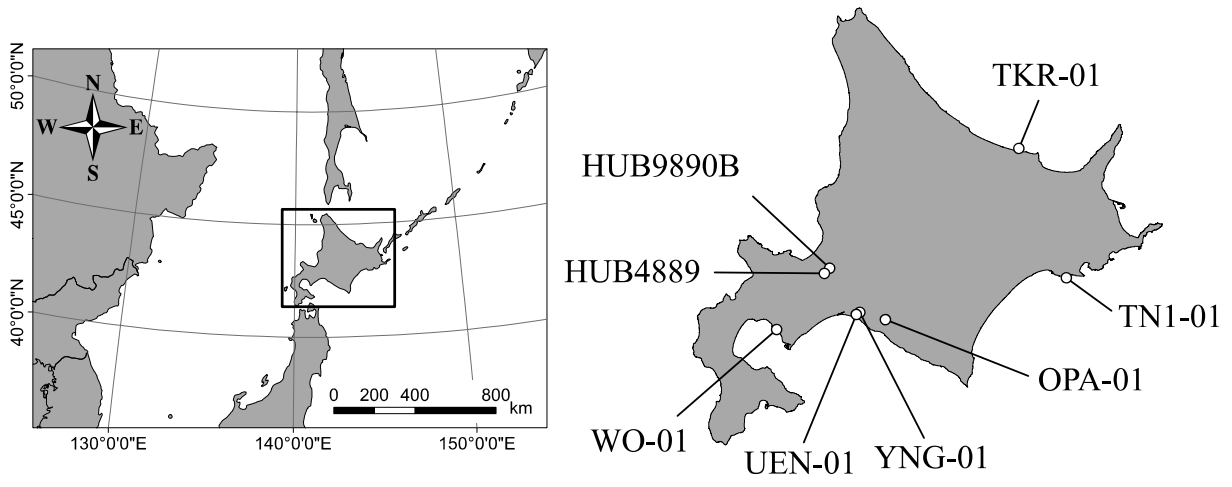
318 Umeki, K. (2016). Research history of extinct wolves in Hokkaido: Overview and issue.  
319 *Research Journal of Graduate Students of Letters.* **15**, 35–67 (in Japanese).

320 Watts, D. E., Butler, L. G., Dale, B. W. & Cox, R. D. (2010). The Ilnik wolf *Canis lupus*  
321 pack: use of marine mammals and offshore sea ice. *Wildl. Biol.* **16**, 144–149.

322 Wiley, A.E., Ostrom, P.H., Welch, A.J., Fleischer, R.C., Gandhi, H., Southon, J.R., Stafford,  
323 T.W., Penniman, J.F., Hu, D., Duvall, F.P. & James, H.F. (2013). Millennial-scale  
324 isotope records from a wide-ranging predator show evidence of recent human impact to  
325 oceanic food webs. *Proc. Natl. Acad. Sci. USA.* **110**, 8972–8977.

326

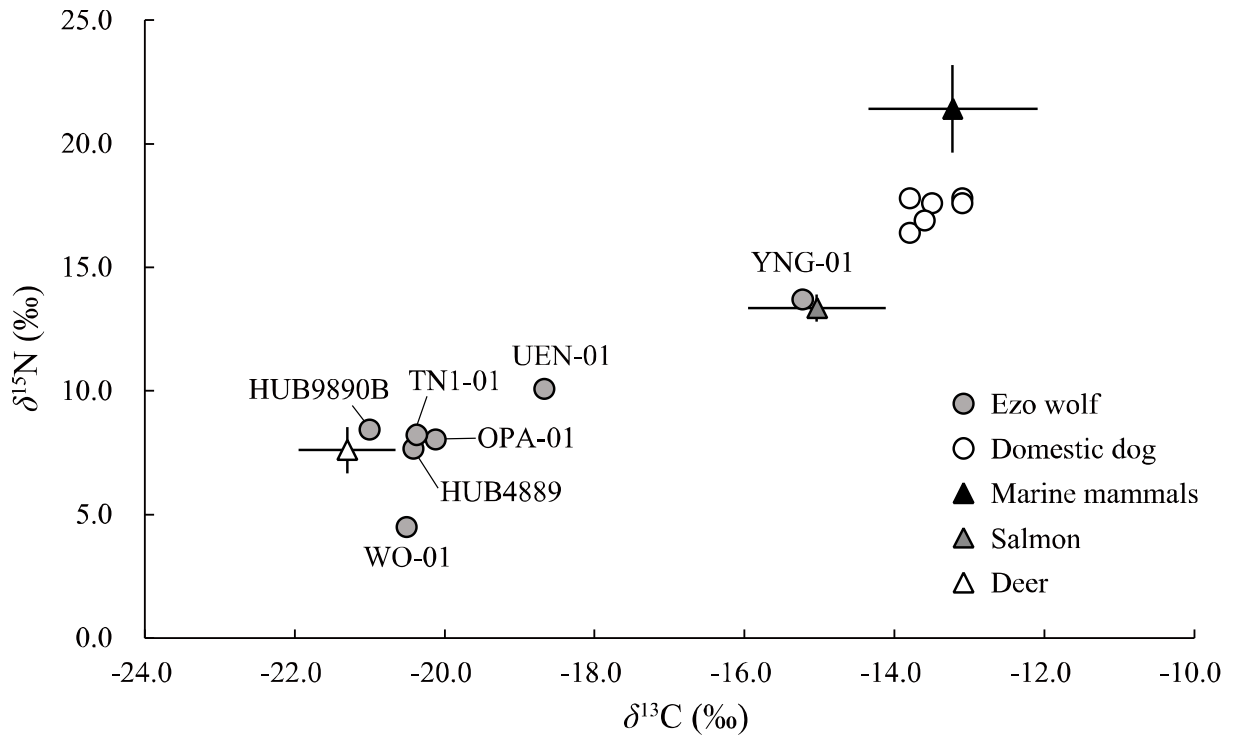
327 **Figure legends**



328

329 Fig. 1. Origins of the specimens on Hokkaido.

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331

332 Fig. 2. Carbon and nitrogen stable isotope ratios in bone collagen of Ezo wolves, domestic  
 333 dogs (Naito et al. 2010, Tsutaya et al. 2014), and potential prey (mean  $\pm$  SD). Ratios of prey  
 334 species have been corrected to account for isotope discrimination factors.

335



336 Table 1. Summary of carbon and nitrogen isotope analyses of faunal bones, and results of the stable isotope mixing models for estimation of the  
 337 diet of individual wolves.

ID	Group	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	%C	%N	C/N
HUB9889	Wolf	-20.4	7.7	46.18	15.99	3.37
HUB9890B	Wolf	-21.0	8.4	44.12	15.70	3.28
OPA-01	Wolf	-20.1	8.0	42.01	14.24	3.44
TN1-01	Wolf	-20.4	8.2	40.91	14.06	3.39
UEN-01	Wolf	-18.7	10.1	39.88	13.77	3.38
WO-01	Wolf	-20.5	4.5	41.20	14.66	3.28
YNG-01	Wolf	-15.2	13.7	45.08	14.68	3.58
TKR-01	Wolf <sup>a</sup>	-22.9	11.9	22.85	3.01	8.86
	Deer <sup>b</sup>	-22.4 ± 0.6	3.7 ± 0.9	39.2 ± 3.3	13.8 ± 1.3	3.3 ± 0.0
	Salmon <sup>b</sup>	-16.2 ± 0.9 <sup>c</sup>	9.5 ± 0.5 <sup>c</sup>	42.1 ± 1.6	15.6 ± 0.7	3.2 ± 0.1
	Marine mammals <sup>b</sup>	-14.4 ± 1.1	17.5 ± 1.8	38.0 ± 3.7	13.5 ± 1.4	3.3 ± 0.0

338 a. This sample showed an unacceptably high C/N ratio.

339 b. Mode ± high and low 95 % credible intervals.

340 c. After correction for temporal isotopic shifts.

341

342 Table 2. Results of stable isotope mixing models for estimation of the diet of individual wolves.

ID	Deer	Salmon	Marine mammals
HUB9889	86.6 (75.1–96.7)	3.8 (0.0–20.0)	1.1 (0.0–13.2)
HUB9890B	88.9 (78.3–97.8)	1.4 (0.0–16.7)	1.1 (0.0–12.5)
OPA-01	82.3 (70.9–94.8)	6.3 (0.0–23.8)	1.4 (0.0–15.0)
TN1-01	84.5 (72.8–95.6)	5.1 (0.0–21.9)	1.2 (0.0–14.6)
UEN-01	61.6 (46.9–76.1)	31.1 (1.8–48.5)	2.0 (0.0–25.8)
WO-01	94.3 (85.0–99.3)	1.1 (0.0–11.9)	0.7 (0.0–7.7)
YNG-01	21.2 (1.6–34.4)	44.7 (17.4–89.5)	33.9 (2.5–52.1)

343 Values are mode  $\pm$  high and low 95% credible intervals.

344

345 Table 3. Radiocarbon dates and estimated age of each Ezo wolf or archaeological site.

ID	Site	Common name	Scientific name	pMC (%)	<sup>14</sup> C age (yrBP)	Calibrated age
HUB9889	Hokkaido Univ.	Wolf	<i>Canis lupus hattai</i>	-	-	1879 AD
HUB9890B	Hokkaido Univ.	Wolf	<i>Canis lupus hattai</i>	-	-	1881 AD
OPA-01	Opaushinai	Wolf	<i>Canis lupus hattai</i>	-	-	1667-1913 AD
TN1-01	Tenneru 1	Wolf	<i>Canis lupus hattai</i>	56.71 ± 0.20	4560 ± 30	3483 - 3106 BC
UEN-03	Uenae	Deer	<i>Cervus nippon</i>	55.11 ± 0.20	4790 ± 30	3643 - 3521 BC
WO-01	Wakkaoi	Wolf	<i>Canis lupus hattai</i>	53.11 ± 0.19	5080 ± 30	3961 - 3798 BC
YNG-02	Yanagidate	Deer	<i>Cervus nippon</i>	53.12 ± 0.19	5080 ± 30	3960 - 3798 BC

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