DIETARY RECONSTRUCTION OF THE OKHOTSK CULTURE OF HOKKAIDO, JAPAN, BASED ON NITROGEN COMPOSITION OF AMINO ACIDS: IMPLICATIONS FOR CORRECTION OF ^{14}C MARINE RESERVOIR EFFECTS ON HUMAN BONES

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ABSTRACT. The relative contribution of marine-derived carbon in the ancient diet is essential for correcting the marine reservoir effect on the radiocarbon age of archaeological human remains. In this study, we evaluated the marine protein consumption of 3 human populations from the Okhotsk culture (about AD 550–1200) in Hokkaido, Japan, based on stable carbon and nitrogen isotopic compositions in bulk bone collagen as well as the nitrogen isotopic composition of glutamic acid and phenylalanine. Despite the similarity of carbon and nitrogen isotopic composition of bulk collagens, nitrogen isotopic composition of their constituent amino acids suggests differences in fur seal contributions among northern Hokkaido (0–24% for Kafukai 1, 0–10% for Hamanaka 2) and eastern Hokkaido (78–80% for Moyoro) populations. It suggests that nitrogen composition of glutamic acid and phenylalanine could provide a detailed picture of ancient human subsistence.

INTRODUCTION

In archaeological studies, the question of what kind of food resource was the staple for ancient humans is an important one. Many researchers have tried to answer this question for a long time based on the composition of stone tools, microwear traces on dental crowns, and organic residue analysis by chemical methods (Richards et al. 2001). Stable isotopic values of bone collagen from ancient human and faunal remains have partly shown great power to resolve the question above because this method provides direct estimation of protein intake by each consumer (Honch et al. 2006). Isotopes of carbon (C) and nitrogen (N) have been often applied for evaluating the impact of marine carbon not only in human remains (Arneborg et al. 1999), but also in charred food residue on pot shards (Imamura 1999) in order to correct partial marine reservoir effects. Although the latter studies were based on the simple isotope mixing model among food resources, the former studies included the following principal assumptions: isotopic values of individual food resources are reflected in those of consumer body tissues; and heavier isotopes are enriched by the physiological processes, which result in higher carbon and nitrogen isotopic values ($\delta^{13}C$, $\delta^{15}N$) for higher trophic level (TL) predators (DeNiro and Epstein 1978, 1981; Minagawa and Wada 1984; Bocherens and Drucker 2003). These isotopic relationships seem to be ubiquitous and widely accepted, if incompletely tested.

Problematic situations often arise, however, when reconstructing omnivorous human diets. Especially, populations that lived on the combination of marine and terrestrial resources share a unique ecological position between 2 or more different ecosystems. Moreover, marine organisms show rel-

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atively large isotopic variations since marine ecosystems usually have more a complicated foodweb structure than terrestrial ones (Minagawa and Wada 1984; Schoeninger and DeNiro 1984). In addition to these isotopic concerns, from the perspective of zooarchaeology, ichthyological remains are usually rarely found compared to other faunal remains. All these uncertainties could lead to contradictory results between isotopic analyses of archaeological samples and zooarchaeological observations. These problems also affected quantitative estimation of marine protein consumption for correcting apparent radiocarbon age offsets in human skeletons (Yoneda et al. 2002, 2004; Naito et al. 2010). Disequilibrium among the carbon reservoirs of the atmosphere, surface water, and deep water makes apparent ^{14}C ages of marine organisms and their consumer older (Stuiver et al. 1986). Indeed, the marine reservoir effect significantly influenced the apparent ^{14}C ages of archaeological marine animals in the western North Pacific (Yoneda et al. 2001).

In this study, we applied a new technique that could clarify marine and terrestrial foodweb structures and give insight into the dietary habits of Okhotsk culture populations, using δ^{15}N analysis of individual amino acids in bone collagen. This technique was originally developed in marine foodweb studies (McClelland and Montoya 2002; Chikaraishi et al. 2007) and applied recently to ancient human remains (Naito et al. 2010; Styring et al. 2010). These studies all paid special attention to the δ^{15}N of glutamic acid and phenylalanine ($\delta^{15}N_{Glu}$, $\delta^{15}N_{Phe}$) from various kinds of biological samples, because these 2 amino acids show quite different ^{15}N enrichment between prey and its consumer (McClelland and Montoya 2002; Chikaraishi et al. 2009). This is ascribed to differences in amino acid metabolism. Glutamic acid, like most amino acids, rapidly undergoes transamination and the C-N bond is cleaved. In contrast, in the case of phenylalanine, the dominant metabolic step does not cleave the C-N bond. Therefore, $\delta^{15}N_{Glu}$ seems to represent the biological enrichment with trophic level (TL) of an organism, while $\delta^{15}N_{Phe}$ represents the value of primary producers in a foodweb.

The Okhotsk Culture

Nitrogen stable isotopic analysis of collagen amino acid was applied to archaeological bone samples unearthed from the Kafukai 1, Hamanaka 2, and Moyoro sites in Hokkaido, Japan (Figure 1). These sites have been assigned to the Okhotsk culture (about AD 550–1200), which spread from Sakhalin into northeastern Hokkaido and the Kuril Islands (Amano 2003). It is generally suggested that Okhotsk culture peoples developed specialized maritime adaptation as evidenced by bone tools such as hooks and harpoons, animal remains including a large number of marine animals, and their site distribution strictly restricted to coastal and island environments (Amano 2003). The isotopic values of human bone collagen previously reported for Okhotsk culture sites also indicate strong reliance on marine mammals (Chisholm et al. 1992; Yoneda 2002). On a general level, it appears that little difference has been observed between the bulk carbon and nitrogen isotopic values of Okhotsk culture groups in northern and eastern Hokkaido (Yoneda 2002), although faunal remains from Okhotsk sites suggested that sea mammals were more important in the east than in the north (Hudson 2004). For example, the Kafukai 1 site on Rebun Island includes a large amount of fish bones, suggesting that the subsistence strategies of the inhabitants primarily relied on fishing rather than hunting marine mammals (Nishimoto 1978). These results have raised intensive discussions about the subsistence pattern of the Okhotsk culture peoples, particularly in relation to the relative importance of fish and larger marine mammals (Nishimoto 1978; Ono 1996; Amano 2003; Hudson 2004).

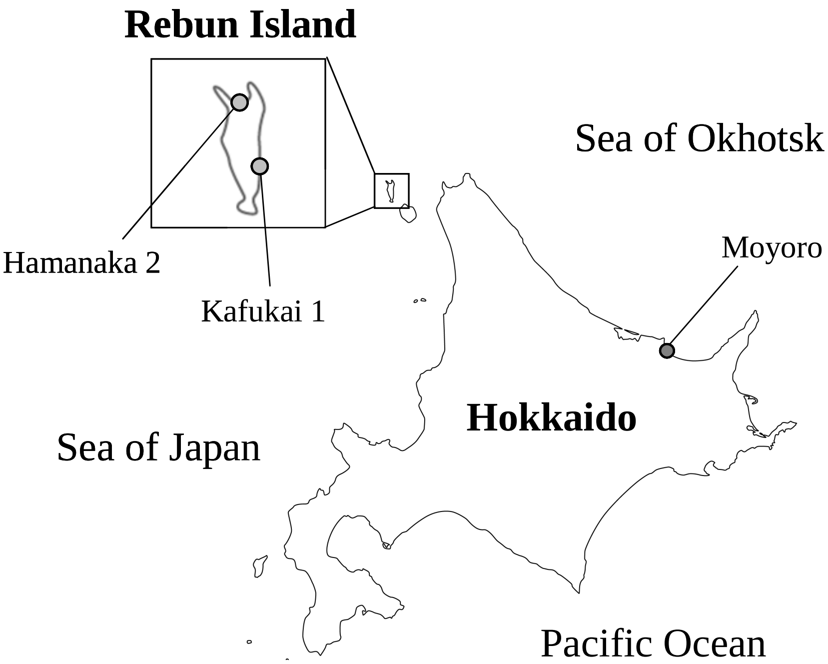

Figure 1 Geographical location of the Kafukai 1, Hamanaka 2, and Moyoro sites

MATERIALS AND METHODS

Materials

The Kafukai 1 and Hamanaka 2 sites are located on the coast of Rebun Island, while the Moyoro site is located on the eastern coast of the main island of Hokkaido. Excavated artifacts from these sites were all assigned to the Okhotsk culture. However, the Kafukai 1 site is characteristic for its occupation layers containing abundant fish bones (Nishimoto 1978; Amano 2003). Human skeletal samples ($n = 7$; Table 1) and faunal bone samples from these sites were analyzed for carbon and nitrogen isotopic composition of bulk bone collagen. In addition, 5 humans and some faunal species ($n = 1$) were selected for nitrogen isotopic analysis of amino acids. The analyzed fauna include: fur seal (*Callorhinus urusinus*), Pacific cod (*Gadus macrocephalus*), Pacific herring (*Clupea pallasii*), greenling, a kind of fish (*Hexagrammidae* family), rockfish (*Scorpaenidae* family), deer (*Cervus nippon*), and boar (*Sus scrofa*), which has less enriched δ^{13}C and δ^{15}N values.

Table 1 Characteristics of individual human remains from the Kafukai 1, Hamanaka 2, and Moyoro sites.

Site	Original ID	Sex	Age	Element
Kafukai 1	No.1	unknown	2.5	frontal
	No.2	unknown	11	parietal bone
	No.3	unknown	6	tibia
Hamanaka 2	1480	male	40–50	skull fragment
	1496	female	30–40	skull fragment
Moyoro	1006	male	30–40	skull
	1011	female	30–35	skull

Carbon and Nitrogen Isotopic Analysis of Bulk Collagen

The collagen extraction procedure was performed according to the improved method of Longin (1971) and Yoneda et al. (2002). Bulk collagen samples (~0.25 mg) were analyzed for stable isotopes by using an EA-IRMS system consisting of a Calro Erba NA1500 elemental analyzer, a Finnigan MAT ConFlo II interface, and a Finnigan MAT 252 isotope ratio mass spectrometer. Based on the standard deviations of replicate analyses of the internal standard material, the uncertainty with each measurement was estimated as 0.2‰ and 0.4‰ for δ^{13}C and δ^{15}N, respectively.

Nitrogen Isotopic Analysis of Amino Acids

Amino acids were prepared by HCl hydrolysis, followed by *N*-pivaloyl/isopropyl (Pv/iPr) addition. Nitrogen compositions of individual amino acids were measured by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) using an Agilent 6890GC coupled to a Thermo-Finnigan Delta^{plus} XP IRMS via combustion and reduction furnaces. For detailed information about these measurements, see Naito et al. (2010).

RESULTS

Measured δ^{13}C, δ^{15}N, and C/N ratios for the bulk bone collagen samples are shown in Table 2. These data suggest that the materials in this study are well preserved, particularly regarding their acceptable C/N ratios (DeNiro 1985). The boar samples showed large isotopic variation, which probably reflects the consumption of marine food by humans (Amano 2003). As reported in the previous studies, the human remains from the 3 Okhotsk culture sites have similarly high δ^{13}C and δ^{15}N values, which indicate a heavy dependence on marine food sources (Chisholm et al. 1992; Yoneda 2002). A similar trend can be also seen in the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values of the samples (Table 3), indicating significantly higher $\delta^{15}N_{Glu}$ values in the humans and marine fauna than those in the terrestrial fauna. On the other hand, there is an unexpectedly large variation in $\delta^{15}N_{Phe}$ values among the marine species. Since phenylalanine shows little ^{15}N enrichment between prey and consumer (McClelland and Montoya 2002; Chikaraishi et al. 2007, 2009), this variation could reflect differences in primary protein sources. In order to examine this, the trophic levels of the marine animals were estimated based on the following equation:

$$TL_{AA} = (\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4) / 7.6 + 1$$

where TL_{AA} indicates the estimated trophic level based on the amino acid δ^{15}N (Chikaraishi et al. 2009). The trophic level (TL) of the fur seal, Pacific cod, greenling, Pacific herring, and rockfish were estimated to be 3.9, 3.6, 3.3, 2.9, 4.0, respectively (see Table 3). These values are compatible with observations from modern ecological and fishery studies (Hokkaido Fisheries Experimental Station 1991; Wada and Ito 1999). These TL values reinforce the hypothesis that those animals belonged to different foodwebs. The human remains from the 3 sites also showed significantly different $\delta^{15}N_{Phe}$ values (ANOVA, $P<0.01$). This could reflect the difference in their dietary habits, as discussed below.

DISCUSSIONS

If one employs a 2-end-point collagen δ^{13}C model consisting of purely terrestrial and purely marine diet consumers as –21‰ and –12.5‰, respectively (Arneborg et al. 1999), the relative contribution of the marine protein in the diets for the 3 Okhotsk culture populations (Kafukai 1, Hamanaka 2, and Moyoro) are calculated as 93%, 94%, and 92% on average, respectively. Considering the measurement errors, there are no significant differences between these estimations. However, the application

Table 2 Stable isotopic ratios, carbon and nitrogen concentrations, and C/N ratios in collagen extracted from human and faunal remains from the Kafukai 1, Hamanaka 2, and Moyoro sites.

Site	Human skeletal sample and faunal species	C%	N%	δ^{13}C	δ^{15}N	C/N
Kafukai 1	No. 1 Human	41.2	14.7	–12.6	23.0	3.3
	No. 2 Human	42.1	15.0	–13.2	20.2	3.3
	No. 3 Human	41.6	14.3	–13.4	19.3	3.4
Hamanaka 2	1480 Human	43.9	15.1	–13.2	19.0	3.4
	1496 Human	43.8	15.7	–12.9	18.6	3.3
Moyoro	1006 Human	43.7	14.7	–13.6	19.3	3.5
	1011 Human	42.3	14.4	–12.7	19.4	3.4
Kafukai 1	Pilot whale (*Globicephala* sp.)	41.8	15.8	–13.3	14.4	3.1
		44.1	16.2	–14.9	15.2	3.2
	Fur seal (*Callorhinus urusinus*)	43.5	16.9	–14.5	17.2	3.0
		44.9	16.8	–14.5	15.8	3.1
		44.0	15.7	–14.0	17.6	3.3
	Steller sea lion (*Eumetopias jubatus*)	41.0	15.4	–15.2	19.4	3.1
		43.0	16.0	–14.5	18.1	3.1
	Spotted seal (*Phoca largha*)	42.5	14.9	–14.0	16.9	3.3
		44.4	15.3	–12.8	13.9	3.4
	Salmon shark (*Lamna ditropis*)	44.1	16.2	–15.1	12.1	3.2
		44.3	16.4	–14.9	12.1	3.2
	Pacific cod (*Gadus macrocephalus*)	44.5	16.9	–14.3	17.0	3.1
		44.9	15.8	–14.3	17.3	3.3
	Rockfish (*Scorpaenidae* family)	43.9	17.1	–13.4	16.4	3.0
		44.9	16.9	–13.1	14.4	3.1
	Large-tooth flounder (*Paralichthyidae* family)	45.1	16.8	–15.0	14.7	3.1
		42.2	16.6	–14.4	14.8	3.0
	Righteye flounder (*Pleuronectidae* family)	44.7	17.0	–13.9	14.0	3.1
		43.6	17.3	–13.5	13.5	2.9
	Pacific herring (*Clupea pallasii*)	43.3	16.9	–13.2	13.7	3.0
		43.6	16.7	–14.7	12.1	3.1
	Greenling (*Hexagrammidae* family)	55.6	20.9	–13.6	14.1	3.1
		41.3	16.0	–15.2	12.7	3.0
	Albatross (*Phoebastria albatrus*)	42.7	15.8	–14.8	18.4	3.1
		44.8	16.6	–14.4	16.3	3.2
	Boar (*Sus scrofa*)	44.4	16.8	–19.9	10.7	3.1
		42.9	16.0	–22.4	3.9	3.1
		46.3	16.8	–17.1	14.3	3.2
		45.4	16.3	–20.2	9.5	3.3
	Domestic dog (*Canis familialis*)	45.3	16.9	–13.5	17.6	3.1
		44.4	16.6	–13.1	17.8	3.1
		44.4	16.5	–13.1	17.6	3.1
		43.3	16.6	–13.8	17.8	3.1
	Brown bear (*Ursus arctos*)	43.0	15.5	–18.2	11.9	3.2
		42.7	16.1	–18.9	8.1	3.1
		43.4	14.4	–19.5	7.3	3.5
Moyoro	Sika deer (*Cervus nippon*)	44.3	14.9	–22.7	7.5	3.5
		45.3	15.6	–23.3	2.1	3.4

Table 3 $\delta^{15}N_{Glu}$, $\delta^{15}N_{Phe}$ and trophic level (TL_{AA}) of the marine species estimated from these 2 values together with $\delta^{13}C$, $\delta^{15}N$ and C/N ratio of bulk bone collagen.

Site	Sample	$\delta^{13}C_{Collagen}$	$\delta^{15}N_{Collagen}$	C/N	$\delta^{15}N_{Glu}$	$\delta^{15}N_{Phe}$	TL_{AA}
Kafukai 1	No. 3 Human	–13.4	19.3	3.4	31.9	6.6	—
Hamanaka 2	1480 Human	–13.2	19.0	3.4	28.9	7.4	—
	1496 Human	–12.9	18.6	3.3	28.6	7.4	—
Moyoro	1006 Human	–13.6	19.3	3.5	26.5	0.5	—
	1011 Human	–12.7	19.4	3.4	28.7	1.1	—
Kafukai 1	Fur seal (*n* = 1)	–14.5	15.8	3.1	24.8	–0.6	3.9
	Pacific cod (*n* = 1)	–14.3	17.0	3.1	28.8	5.8	3.6
	Greenling (*n* = 1)	–13.6	14.1	3.1	30.5	9.4	3.3
	Pacific herring (*n* = 1)	–13.2	13.7	3.0	22.4	4.6	2.9
	Rockfish (*n* = 1)	–13.4	16.4	3.0	27.8	1.3	4.0
	Boar (*n* = 1)	–22.4	3.9	3.1	5.9	5.2	—
Moyoro	Shika deer (*n* = 1)	–22.7	7.5	3.5	7.2	5.1	—

of this method faces major challenges. Firstly, the end-points are defined relative to Greenland Vikings (Arneborg et al. 1999), who inhabited a different environment from the Okhotsk culture humans. Secondly, it may not be safe to assume that there is a linear relationship between the enrichment of bulk collagen $\delta^{13}C$ and the amount of marine protein in diets.

If we select the following as the most likely important 6 food resources for the Okhotsk populations (i.e. fur seal, Pacific cod, greenling, Pacific herring, rockfish, and an aggregate of the boar and shika deer; see Nishimoto 1978), ISOSOURCE software can calculate possible percentages for each (Phillips and Gregg 2003; http://www.epa.gov/wed/pages/models.htm). In addition, we assumed that the variable isotopic enrichment factors from prey to consumer are 0.8–1.3‰ for $\delta^{13}C$ and 3.0–5.0‰ for $\delta^{15}N$ (Bocherens and Drucker 2003), which are the most reliable values given the current difficulties in trophic level studies (see Hedges and Reynard 2007). Subtraction of these values from that of consumers produces 4 extreme carbon and nitrogen isotopic signatures for the prey collagen of each consumer, which can be graphically depicted as a rectangle that includes all the possible isotopic signatures for the collagen of prey (Bocherens et al. 2005) (Figure 2). In this calculation involving ISOSOURCE, a tolerance value and a source increment were set as 0.1% and 2%, respectively, and we treated the Hamanaka 2 and Moyoro humans as a single group on the basis of the similarity of their isotopic values (see Figure 2 caption for detailed information). The percentages of marine protein in diet were estimated to be 94–100% for the Kafukai 1 humans and 90–100% for the Hamanaka 2 and Moyoro humans. These values agree closely with the estimates based on linear modeling of the $\delta^{13}C$ values of bulk collagen. Even if the contribution from terrestrial plants to the human diets is taken into account, the ISOSOURCE program produces similar results (96–100% for Kafukai 1 and Hamanaka 2 humans, 94–98% for Moyoro human; Figure 3). However, it is impossible to discriminate the contributions of Pacific cod and fur seal to the human diets, since they showed quite similar isotopic values.

Amino acid $\delta^{15}N$ analysis could aid the interpretation of the structure of marine foodwebs. It is possible to separate the marine animals into 4 to 5 foodwebs based on $\delta^{15}N_{Phe}$ values (Figure 4). The Pacific cod and Pacific herring might belong to the same foodweb. While rockfish live in association with other kinds of small fish and crustaceans in coastal sea areas (Hokkaido Fisheries Experimental Station 1991), the other species could be migratory and reflect source $\delta^{15}N$ values from different ocean areas. Hence, the Okhotsk culture people seem to have had access to multiple marine foodwebs. Using the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values of the 7 food categories (i.e. fur seal, Pacific cod,

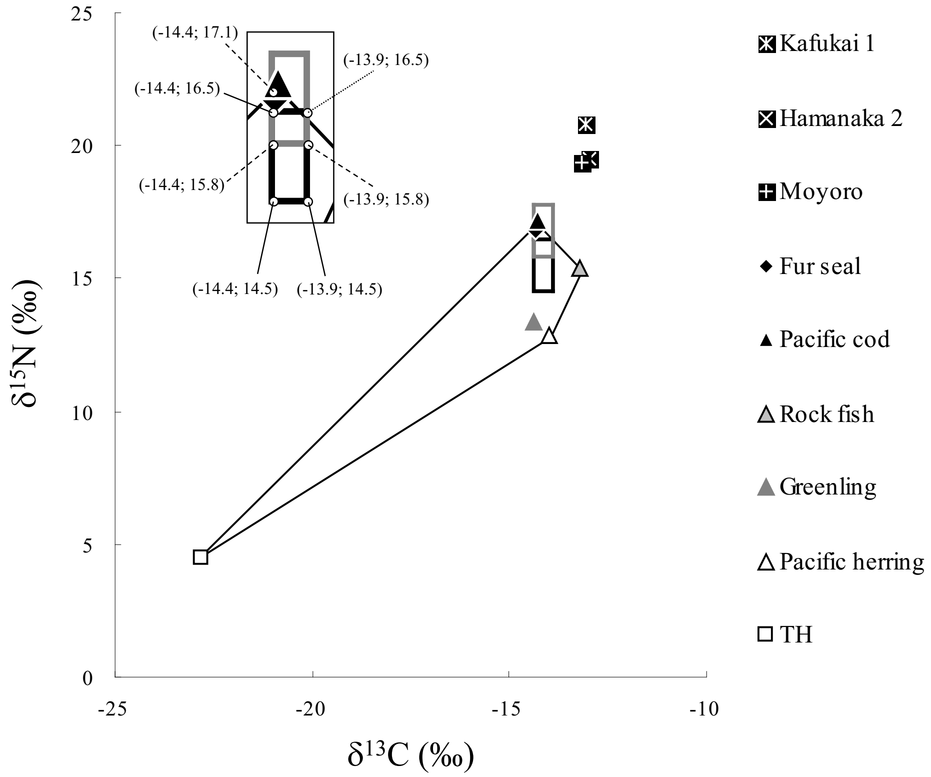

Figure 2 Average $\delta^{13}C$ and $\delta^{15}N$ values of human remains and prey species from the 3 sites. The possible range of prey collagen isotopic signatures for Kafukai 1 (gray rectangle) and the other sites (black rectangle) are also shown. Enrichment factors have been subtracted from the consumer signatures (Bocherens and Drucker 2003). A close-up distribution is shown in the upper left corner. $\delta^{13}C$ and $\delta^{15}N$ values are given for the points of intersection between the surface delimited by the average isotopic signature of potential food source and the gray and black rectangles of the possible food source isotopic signatures, as well as for the extreme values of the rectangles enclosed into this surface (dashed and dotted lines for the gray rectangle, normal and dotted lines for the black rectangle). The percentage calculations were performed for these points (TH: terrestrial herbivores).

greenling, Pacific herring, rockfish, an aggregate of the boar and shika deer and terrestrial plants) and average isotopic values of the diet for the humans that could be estimated by subtracting enrichment factors (8.0‰ for $\delta^{15}N_{Glu}$ and 0.4‰ for $\delta^{15}N_{Phe}$; Chikaraishi et al. 2009) from those of the human bones, ISOSOURCE produces a relative contribution of marine protein in diets of 76–94% for the Kafukai 1 human (No. 3), 60–76% for the Hamanaka 2 humans, and 80% for the Moyoro humans. All these values are low relative to the ones based on the isotopic values of bulk bone collagen. The difference in estimation between the 2 approaches could be attributed to the sample scarcity in amino acid $\delta^{15}N$ analysis, uncertainties associated with the ^{15}N-enrichment factor of the 2 amino acids (especially for the glutamic acid), and the large isotopic variation among the animal remains in bulk $\delta^{13}C$ and $\delta^{15}N$ analysis.

The relative contributions of fur seal in diets, however, show a clear difference among the populations (0–24% for the Kafukai 1 human, 0–10% for the Hamanaka 2 humans, and 78–80% for the Moyoro human). The relative contribution of fur seal to the Kafukai 1 human (0–24%) is compatible with previous reports of their relative caloric contributions of edible parts (6.5%) derived from the

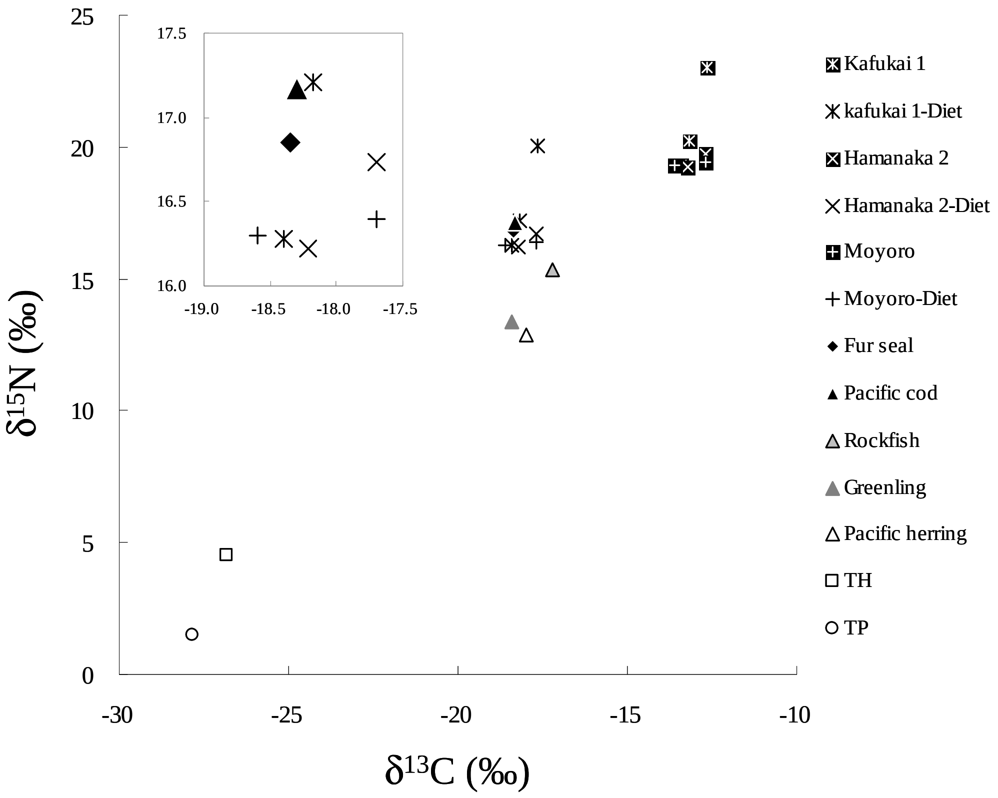

Figure 3 Reconstruction of $\delta^{13}C$ and $\delta^{15}N$ values of protein sources for the Okhotsk human remains. A close-up distribution is shown in upper left corner. The isotopic value of terrestrial plants was estimated by subtracting the enrichment factors (5.0‰ for $\delta^{13}C$ and 3.0‰ for $\delta^{15}N$; Newsome et al. 2004) from those of the terrestrial herbivore collagen. Isotopic values of the human diets were estimated similarly. Bone collagen $\delta^{13}C$ values were assumed to be 4‰ higher than associated muscle tissue carbon values, whereas muscle $\delta^{15}N$ values were assumed to be the same as those of collagen (see Newsome et al. 2004). The percentage calculations were performed only for the value of human diets that are inside the surface delimited by the average isotopic signature of protein sources (TH: terrestrial herbivores, TP: terrestrial plants).

abundance of faunal remains (Nishimoto 1978). These estimations mean the amino acid analysis can provide a more detailed picture of ancient diet even in the case of strong marine-adapted populations. Bearing in mind the data scarcity and the difficulty in applying the isotopic value of marine food resources from the Kafukai 1 site to the other 2 sites, the estimation suggests that there were some differences in dietary habits among the 3 human populations. Although the Okhotsk peoples have been often thought to be fishers rather than hunters, it is possible that subsistence at the Kafukai 1 site did not represent the general subsistence pattern of the culture. The insular environment on Rebun Island might have forced the inhabitants to rely on fish, whereas the Moyoro population settled on the east coast of Hokkaido had difficulty in catching cod and other kinds of fish during the winter because of drift ice (Amano 2003). It therefore seems that the Moyoro people could not help but rely on marine mammals (Ono 1996). Our results are consistent with this suggestion. Nonmetric cranial traits of Okhotsk skeletal remains also suggest the difference between northern and eastern populations (Komesu et al. 2008). However, further isotopic analyses of animal remains from the Hamanaka 2 and Moyoro sites as well as the Kafukai 1 site are required in order to confirm the difference of their dietary habits observed in this study.

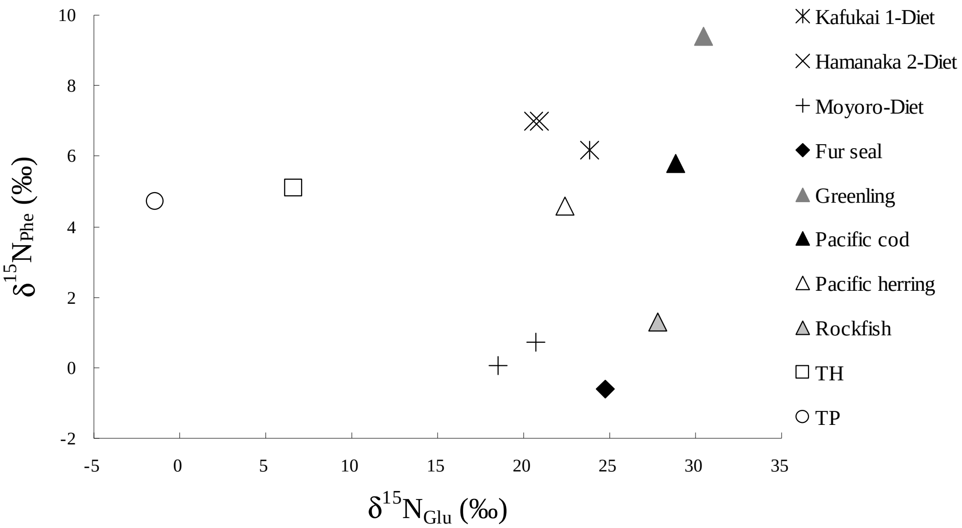

Figure 4 $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values of the selected archaeological samples. Values for the human remains in the graph have been corrected to indicate those of their diets by subtracting nitrogen enrichment factors for glutamic acid (8.0‰) and phenylalanine (0.4‰) from the values of the human bones. The isotopic value of terrestrial plants was estimated by subtracting these enrichment factors from those of the terrestrial herbivores. The percentage calculations were performed on the averaged isotopic values for each site.

Although it is still difficult to estimate the percentages of marine contribution in the ancient diet of Okhotsk peoples in this study, we roughly estimated the calibrated ^{14}C ages under the assumption of 80% and 90% marine contributions for the Kafukai 1 and Moyoro sites (Table 4). The mixed calibration curves of 80% and 90% contributions, Marine04 (Hughen et al. 2004) and IntCal04 (Reimer et al. 2004), respectively, were created using the calibration program OxCal v 4.1 (Bronk Ramsey 1995, 2001, 2009). The local reservoir correction was estimated as 393 ± 32 ^{14}C yr for the Sea of Okhotsk (Yoneda et al. 2007). Because of this large age offset, this small difference of marine contribution is not negligible, suggesting the importance of accurate estimation of marine resources in ancient diets.

Table 4 ^{14}C ages (BP) of Kafukai 1 and Moyoro human remains and their calibrated ages (cal BP) with correction of marine reservoir effects through seafood consumption.

Site	Human sample	^{14}C age (BP)	Calibrated ^{14}C age (80% marine)	Calibrated ^{14}C age (90% marine)	Lab ID
Kafukai 1	No. 1	2014 ± 30	1371–1271	1304–1189	TERRA-080707b10
	No. 2	1976 ± 29	1344–1247	1279–1170	TERRA-080707b13
	No. 3	1687 ± 28	1051–938	980–843	TERRA-080707b14
Moyoro	1006	1693 ± 34	1058–936	996–854	TERRA-06260d04
	1011	1937 ± 35	1305–1187	1260–1126	TERRA-06260d06

In the preceding study, we proposed a quantitative evaluation method for marine protein consumptions, which would be useful for people who exploited food resources from just 2 foodwebs (e.g. marine and terrestrial foodwebs) (Naito et al. 2010). However, this method cannot be applied directly to groups, such as the Kafukai 1 humans, who exploited food resources from more than 3 distinct foodwebs. Nevertheless, it is still worth to use $\delta^{15}N$ values of glutamic acid and phenylalanine because they could provide valuable information about past foodweb structures that could not always be clarified through bulk $\delta^{13}C$ and $\delta^{15}N$ analysis. It is important to understand the human diet with ecological references by analyzing accompanied faunal remains, in order to both accurately estimate marine protein contribution and reconstruct a concrete picture of ancient subsistence.

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