Res. Org. Geochem. **25,** 15−27 (2009)

Article

Traces of sea mammals on pottery from the Hamanaka 2 archaeological site, Rebun Island, Japan: Implications from sterol analysis, stable isotopes, and radiocarbon dating

Yoshiki Miyata[*, a], Akiko Horiuchi[**], Paleo Labo AMS Dating Group[***] and Toyohiro Nishimoto[*]

(Received February 17, 2009; Accepted November 16, 2009)

Abstract

To reconstruct prehistoric human diets, we studied pottery excavated from the Hamanaka 2 archaeological site, Rebun Island, Hokkaido, Japan, where marine mammals were cooked (boiled) in pottery vessels to obtain animal oils and fats. We analyzed lipids adhering to the pottery and demonstrated that cholesterol made up more than 80% of the detected sterols, suggesting that they were of animal origin. The stable isotopes of carbonized material adhering to the inner surfaces of potsherds indicated that the charred materials likely reflected a diet of marine mammals or finfishes. Radiocarbon dates on the same charred materials showed a large marine reservoir effect, which supported the interpretation that they were derived from marine products cooked in the pottery vessels. The apparent radiocarbon age differences between the charred materials on the inner surfaces of the potsherds and charred wood from the same layer that the potsherds came from indicated a correction value for the marine reservoir effect (ΔR) in the northwestern Pacific of 444 ± 55 ^{14}C years at 3010 BP. The results of these three analyses, namely, sterol analysis, stable isotope analysis, and radiocarbon dating, are consistent with the archaeological hypothesis that during the latter half of the Late Jomon period (1300–1200 cal BC), sea mammals were cooked in pottery vessels to obtain animal oils and fats at the Hamanaka 2 archaeological site.

1. Introduction

When prehistoric peoples cooked foods in pottery, chemical components from the foods, such as lipids, sometimes adhered to or were absorbed by the pottery, and charred food materials may be found on the inner surfaces of potsherds from archaeological sites (Fig. 1; Evershed et al., 1992 & 2002; Copley et al., 2004). By identifying these lipids, we can learn about the food materials that were cooked in the pottery vessels. Sometimes, the food material can be identified by examining the charred materials under a microscope. For example, the oldest charred grains of common millet (*Panicum miliaceum*) in western Japan were identified from charred materials on the inner surfaces of pottery excavated at Ryugasaki A site, Siga Prefecture, Japan, by Matsutani (2006). The conventional radiocarbon age was found to be 2550 ± 25 BP (PLD-5304; 800–555

* National Museum of Japanese History, 117 Jonai-cho, Sakura, Chiba 285-8502, Japan
** College of Liberal Arts, International Christian University, 10-2 Osawa, 3-chome, Mitaka, Tokyo 181-8585, Japan
*** Paleo Labo Co. Ltd., AMS Dating Facility, 1900-65, Shimo-tazawa, kurohone-cho, kiryu, Gunma 376-0144, Japan
a Corresponding author: e-mail: miyata@nendai.nagoya-u.ac.jp
Present address: Center for Chronological Research, Nagoya University, Furou-cho, Chikusa, Nagoya 464-8602, Japan

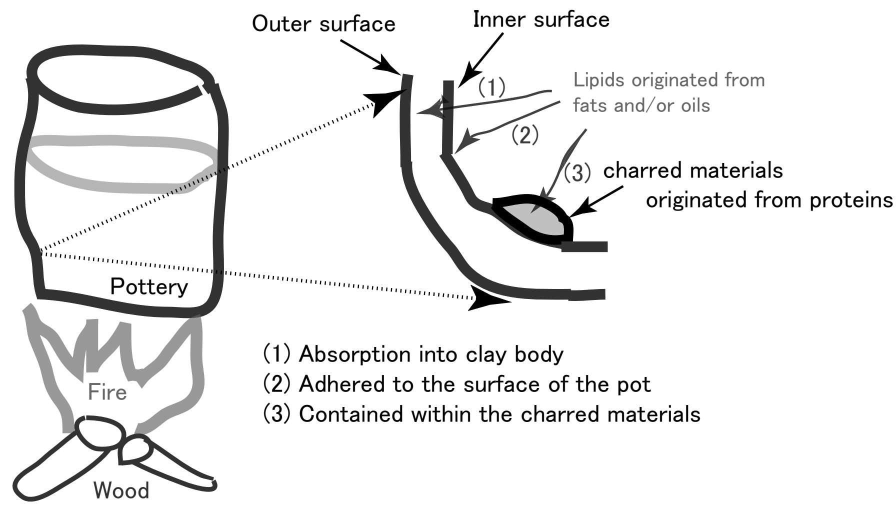

Fig. 1. Diagram showing the possible origins of the analyzed materials. Carbonized matter adhering to pottery may originate from proteins and lipids that adhered to the inner pot surface or infiltrated the pottery during cooking.

cal BC; Miyata et al., 2007a). This result was the first scientific evidence in Japan that common millet was cooked in pottery vessels around Lake Biwa area between 800 and 555 years BC, corresponding to the period from the Final Jomon to the beginning of the Yayoi period. However, direct identification of carbonized matter on the inner surfaces of potsherds is not always possible. More often, chemical analysis of the organic compounds in the charred material is required. Stable isotope analyses such as of carbon and nitrogen isotopes are powerful tools for identifying the origin of the food materials. For example, the carbon and nitrogen isotope composition of the carbonized common millet grains from the Ryugasaki A site (Fig. 2, SGMB 8) are consistent with the isotope composition of C_4 plants, whereas the compositions of other carbonized materials (Fig. 2, squares) adhering to pottery from the same site are clearly different (Miyata et al., 2007a). However, the original chemical compositions of the charred materials are not always preserved during the cooking process, the charring, or the burial in the soil, and possible secondary contamination (diagenesis) by other types of organic matters, such as humic acids from soil or groundwater, may be substantial (Miyata et al., 2006 & 2008). However, materials of sea mammal origin adhered to the pottery can be identified by the combination of a lipid analysis with the determination of the marine reservoir effect on radiocarbon dating. From sterol analysis results, the origin of the lipids can be deduced as mainly animal or plant, and the marine reservoir effect can show whether the substance is a marine product.

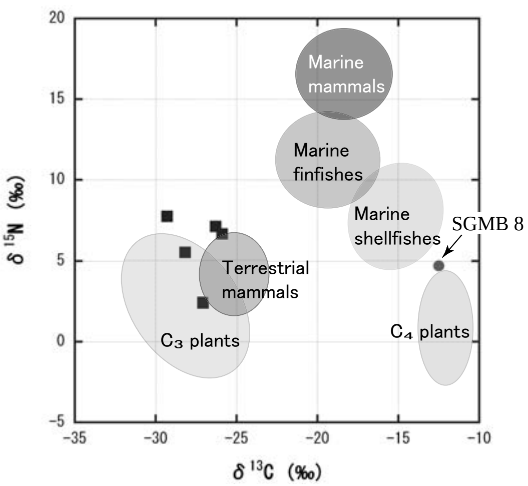

Fig. 2. Stable isotopic data of charred material adhering to the inner surfaces of potsherds from the Ryugasaki A site in relation to typical values for various types of organisms (Yoneda, 2004). Circle, charred grains (SGMB 8) of common millet; squares, other charred materials (Miyata et al., 2007a).

At certain archaeological sites, archaeological evidence indicates that one or more animals were killed

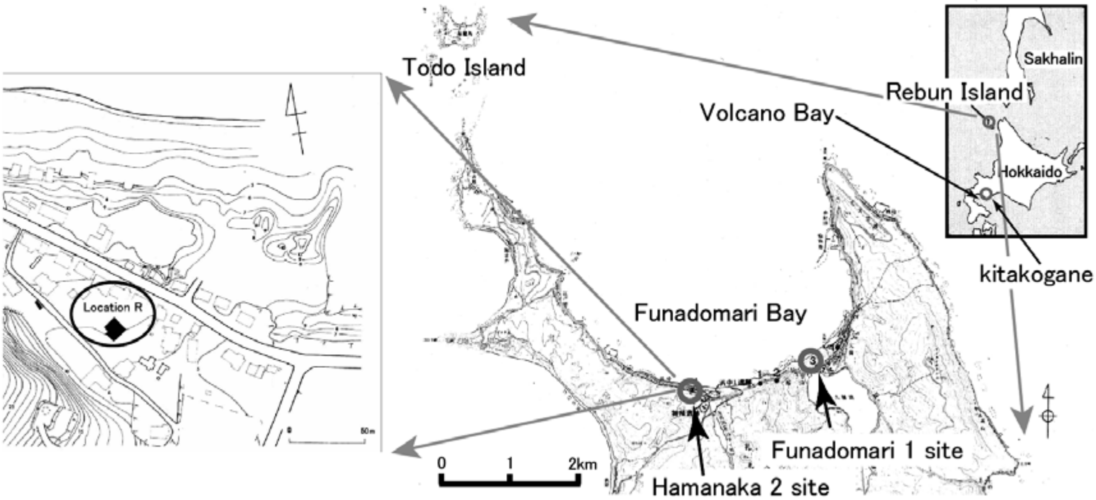

Fig. 3. Hamanaka 2 archaeological site (Location), Rebun Island, Hokkaido, Japan. This figure was modified after Maeda and Yamaura (1992) and Nishimoto (2000).

and butchered by hunters, and the meat and fat were processed by cooking in pottery vessels. At such sites, animal bones, weapons, pottery, and other items used for hunting and animal processing are typically found during excavation. These sites are known as "kill sites" and "processing sites" (Rackham, 1994; Nishimoto, 2000). Hamanaka 2 site is known as one of these kill sites of sea mammals.

The aim of this research was to scientifically verify and characterize the traces of sea mammals processed at the Hamanaka 2 site by using lipid analysis, stable isotope analysis, and radiocarbon dating, because heretofore the inference that a large quantity of Japanese sea lion meat was cooked in pots at the Hamanaka 2 site during the latter half of the Late Jomon period has been based only on archaeological context and has not been supported by any independent scientific analyses. The objects of the analysis were materials adhering to or absorbed in pottery excavated from layer V at Hamanaka 2, where the archaeological context suggested that the pots were used only to boil marine mammals for their oils and fats, thus eliminating, as far as possible, the complication of the chemical composition reflecting two or more food materials.

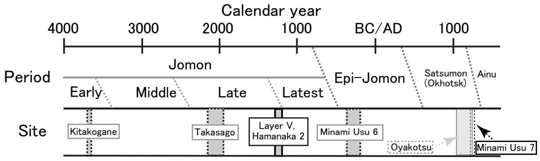

Fig. 4. The positions of the Hamanaka 2 site (this work) and other sites situated around Funka Bay (Yoneda et al., 2001) on an archaeological timeline for Hokkaido, Japan.

2. Hamanaka 2 archaeological remains

Hamanaka 2 is an archaeological midden site (Nishimoto, 2000) on Rebun Island, which is at the northernmost tip of the Japanese archipelago between Hokkaido, Japan, and Sakhalin Island (Fig. 3). This site, which was excavated by the National Museum of Japanese History (NMJH) from 1994 to 1997, was used intermittently from the latter half of the Late Jomon to the Epi-Jomon and Okhotsk periods (see Figure 4). Layer V (location R) at the Hamanaka 2 site (Fig. 5), corresponds to the latter half of the Late Jomon period (Nishimoto, 2000). Although the area excavated is limited (only 11 m×11 m), a large quantity of Japanese sea lion bones were found, along with about 3000 potsherds. The potsherds excavated from layer V belong to the Dobayashi pottery type. This pottery is associated with the latter half of the Late Jomon period, which lasted for the comparatively short time of several decades. The excavated

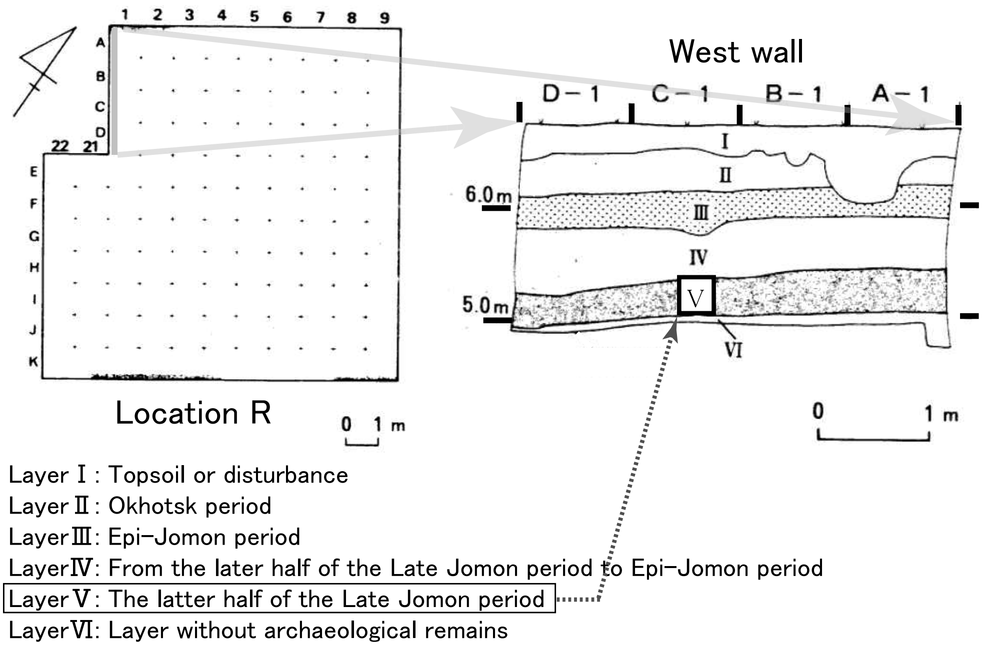

Fig. 5. Plan view of location R, Hamanaka 2, Rebun Island, Japan (left), and archaeological stratigraphy (right) showing the position of layer V. This figure was modified after Nishimoto (2000).

pinniped bones are mainly from Japanese sea lions; only a few are from other mammals or birds. Fish bones and mollusk shells are also rare. The archaeological features of this site include more than 34 traces of fireplaces but no pit dwellings, in contrast to the Funadomari 1 archaeological site, a settlement site only 2 km from Hamanaka 2 (Nishimoto, 2000; see Figure 3). Therefore, people probably did not live year-round at Hamanaka 2, but used it only during the summer as a temporary campsite for hunting Japanese sea lions (*Zalophus californianus japonicus*) (i.e., a kill site; Rackham, 1994; Nishimoto, 2000). Japanese sea lions were cooked (boiled) and processed for animal oils and fats at this site as well, so it is also a processing site. Thus, it is likely that lipids derived from Japanese sea lions adhered to and were absorbed by the pots used for cooking and processing the meat and fat, and that proteins adhered to the inner surfaces of the pots and became charred (Nishimoto, 2000; Kami, 2001). These data together constitute the archaeological context on the basis of which it has been inferred (hypothesized) that a large quantity of Japanese sea lion meat was cooked in pots at the Hamanaka 2 site during the latter half of the Late Jomon period, as described in section 1 (Nishimoto, 2000; Kami, 2001).

3. Materials and methods

Two potsherds excavated from Hamanaka 2 were used for the sterol analysis. One potsherd was from layer V (the latter half of the Late Jomon period), and the other was from layer III, which dates to the Epi-Jomon (see Fig. 4). The lipids were analyzed in this work according to the following procedure (Fig. 6). The two potsherds were supersonicated in a solution of chloroform and ethanol (2:1) to extract the lipids adhering to their surfaces. Then, the potsherds were crushed to powder in a ball mill, and the lipids absorbed into the clay were extracted similarly using a 2:1 chloroform:ethanol solution. In this way, the lipids adhering to the surface of the potsherd and those infiltrated the pottery and absorbed into the clay were determined separately. α-cholestane was added to the extracted lipids as an internal standard (IS), and the lipids were hydrolyzed with 0.4 M MeONa/MeOH to convert the sterol esters, the major component of the sterols in the living body, to free sterols to increase

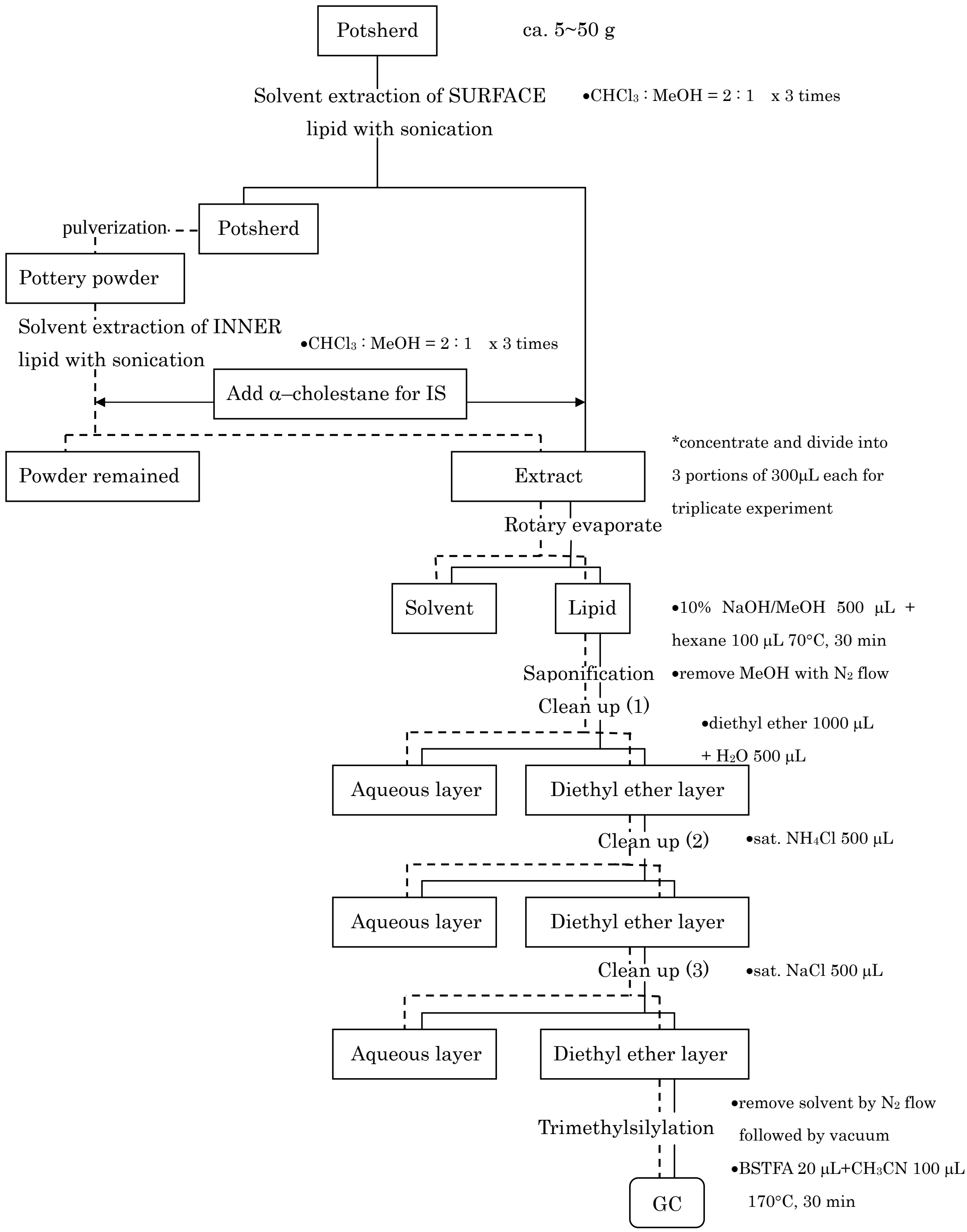

Fig. 6. The sterol analysis procedure. IS, internal standard; sat., saturated; BSTFA, N,O-bis(trimethylsilyl)trifluoroacetamide; GC, gas chromatography. A Shimadzu GC-14B with flame ionization detector was used with a 60 m×0.25 mm GL Sciences Inert Cap-17 column at a constant column temperature of 280 °C.

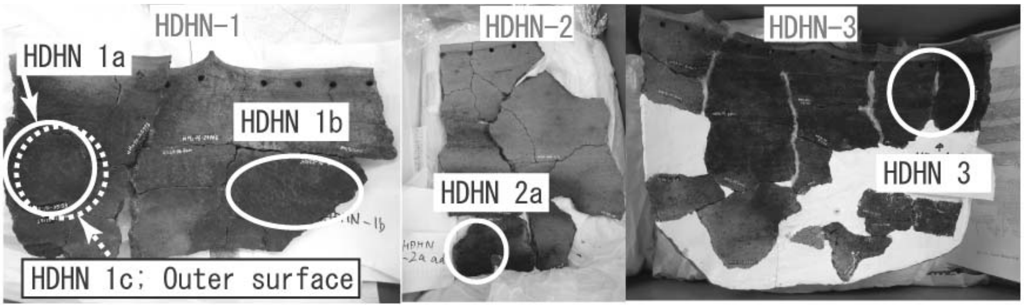

Fig. 7. Parts of three Dobayashi type pots excavated from the Hamanaka 2. This pottery type was used during the latter half of the Late Jomon period. HDHN 1c was the only potsherd with charred material on its outer surface.

detectable sterols. This process also hydrolyzes glycerols, the major components of lipids, and facilitates their removal by the extraction. After trimethylsilylation by treatment with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 170 °C, the sterols were identified quantitatively by gas chromatography, as described by Horiuchi et al. (2007). A Shimadzu GC-14B gas chromatograph with a flame ionization detector was used with a 60 m×0.25 mm GL Sciences Inert Cap-17 column at a constant column temperature of 280 °C. The following sterols were detected: cholesterol (an animal sterol); campesterol, β-sitosterol, and stigmasterol, which are the major phytosterols (plant sterols); and fucosterol, which is the main sterol in brown algae (Sugano and Imaizumi, 1987). All of the sterol analyses were done in the Department of Chemistry, College of Liberals Arts, International Christian University (ICU), Tokyo, Japan.

Five samples of charred materials on potsherds from three "Dobayashi" pots (Fig. 7) and charred wood from a fireplace were used for radiocarbon dating. All were excavated from layer V, location R (see Figures 3 & 5). The procedures for preparation and graphitization of the charred materials for radiocarbon dating, conducted at the Radiocarbon Dating Materials Laboratory, National Museum of Japanese History (NMJH), have been described by Miyata et al. (2005). In brief, the charred materials were treated by a conventional acid-alkali-acid treatment (AAA treatment, wherein the sample is reacted 2–3 times with 1 N HCl, 1–2 times with 0.1 N NaOH, 4–5 times with 1 N NaOH, and 2 times with 1 N HCl for 1 h at 80 °C). The purified sample and CuO(II) combusted together with Sulfix (an adsorbent for sulfur containing gas) to CO_2 at 850°C for 3 h in a sealed quartz glass tube. The CO_2 gas was then purified in a high vacuum line.

The purified CO_2 gas was reduced to graphite by iron powder catalysis. The graphite was measured with a NEC Compact AMS at Paleo Labo Inc., Gunma, Japan (Kobayashi et al., 2007). Calibrated ages of identifiable wood charcoal samples were calculated using RHcal 3.2 (Imamura, 2007) based on the Intcal04 data set (Reimer et al., 2004).

A local marine reservoir correction value ($\varDelta$R) is defined as the difference between the observed ^{14}C age of marine samples and the model marine ^{14}C age in the same calendar year (Southon et al., 1995; Stuiver and Braziunas, 1993). However, the calendar age of the charred materials on the inner surface of potsherds was not always available, because the analyzed charred materials, could be affected by the marine reservoir effect; this case is different from the case of a museum collection of pre-bomb molluscan shells. In this work, charred wood (terrestrial samples) and charred materials on the inner surface of potsherds (marine samples) were collected from the same horizon (Layer V) at Hamanaka 2 to ensure as far as possible that the samples were contemporaneous. Here, we consider the ^{14}C age of the terrestrial charred wood samples to correspond to the model atmospheric ^{14}C age. We first determined the calendar age of the charred wood samples by calibrating the measured ^{14}C age using IntCal04 (Reimer et al., 2004), and then we used the calibrated age to estimate the model marine ^{14}C age of the charred materials from potsherds using Marine04 (Hughen et al., 2004). Finally, the model marine ^{14}C age was subtracted from ^{14}C age of the charred materials (marine samples) from potsherds to evaluate the ΔR. The uncertainty of the ΔR was estimated as the standard error of the mean (Southon et al., 1995; Yoneda et al., 2001; Nakamura et al., 2007).

The carbon and nitrogen isotope compositions of the charred materials on pottery were also measured after AAA treatment by SHOKO Co. Ltd., Saitama, Japan. The analysis was performed with an elemental analyzer (CE Instruments EA1110) and a mass spectrometer (Thermo Electron DELTAplus Advantage) using a continuous-flow system. Carbon and nitrogen isotope results are reported in per mil notation, relative to V-PDB for $\delta^{13}C$ and air for $\delta^{15}N$. Table 1 shows the changes of carbon and nitrogen isotope compositions that occurred as a result of AAA treatment of charred materials from the inner and outer surfaces of the potsherds. The samples of the SGMB series consisted of seven potsherds excavated from a wetland archaeological site near Lake Biwa, Shiga Prefecture. These potsherds were from pottery of the Kitashirakawa-kasou IIc type, which was made during the Early Jomon period. Comparison of the results before and after the AAA treatment shows that the values of $\delta^{13}C$ and $\delta^{15}N$ were changed by at most one per mil deviation by the AAA treatment. Accordingly, if samples of charred materials on potsherds are of good quality, as in the case of samples excavated from a wetland archaeological site, AAA treatment should have little effect on the estimation of the $\delta^{13}C$ and $\delta^{15}N$ values of the original materials cooked in the pots from the charred materials adhering to the pottery surface.

Table 1. Changes in carbon and nitrogen isotope compositions of charred materials from the inner and outer surfaces on potsherds caused by AAA treatment.

	Before AAA		After AAA	
Sample No.*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Charred materials from the inner surfaces of potsherds: 'food residue'				
SGMB-4232a	-26.1	7.6	-26.9	7.5
SGMB-4233a	-26.7	7.1	-27.5	7.3
SGMB-4236a	-26.8	5.4	-27.1	5.9
SGMB-4238a	-26.7	7.0	-27.2	7.0
SGMB-4239a	-27.0	6.8	-26.8	5.8
average	-26.6	6.8	-27.1	6.7
Charred materials from the outer surfaces of potsherds: 'soot'				
SGMB-4234b	-25.5	13.2	-25.6	13.6
SGMB-4236b	-25.7	11.5	-25.4	12.2
SGMB-4237b	-25.7	10.7	-25.5	11.7
SGMB-4238b	-25.6	11.7	-25.0	11.7
SGMB-4239b	-25.3	11.0	-25.9	11.8
average	-25.6	11.6	-25.5	12.2

*The samples of the SGMB series consist of seven potsherds excavated from a wetland archaeological site near Lake Biwa, Shiga Prefecture. The potsherds are from Kitashirakawa-kasou IIc type pottery, which was used during the Early Jomon.

4. Results and discussion

4.1. Sterol analysis

Sterols are one of the major constituents of cell membranes in eukaryotes. Vertebrates synthesize cholesterol, whereas invertebrates rely on an external sterol supply. In many animal tissues, over 95% of the sterol fraction is cholesterol. On the other hand, in plants and algae, major sterols are sitosterol (~70%), stigmasterol (~20%), capmesterol (~5%), and cholesterol (~5%) (Gurr and Harwood, 1991). However, it should be noted that, as with most situations in biology, there are many exceptions.

The analysis of sterols as biomarkers is a strong analytical tool available to archaeologists for identifying the environment in which the inhabitants of archaeological sites lived. For example, Kimpe et al. (2004) analyzed sterols, along with fatty acids and glycerides, to classify different ceramic pots. Lin and Connor (2001) examined the steroids in a coprolite from a Greenland Eskimo mummy and found that 99% of the total sterols (phytosterols/cholesterol = 0.004). They concluded that the phytosterol intake of that person must have been very low because his diet lacked foods of plant origin (Lin and Connor, 2001). In the research presented here, the sterols from the materials adhering to the surfaces of the two potsherds were analyzed to determine the probable origin of the lipids cooked at Hamanaka 2.

Figure 8 shows the sterol compositions of the materials recovered from the surfaces of the potsherds. One potsherd was from the bottom of a pot excavated from

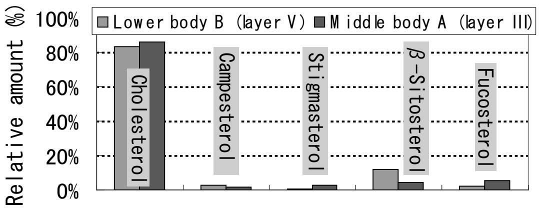

Fig. 8. Sterol compositions of material adhering to two potsherds excavated from the Hamanaka 2 archaeological site.

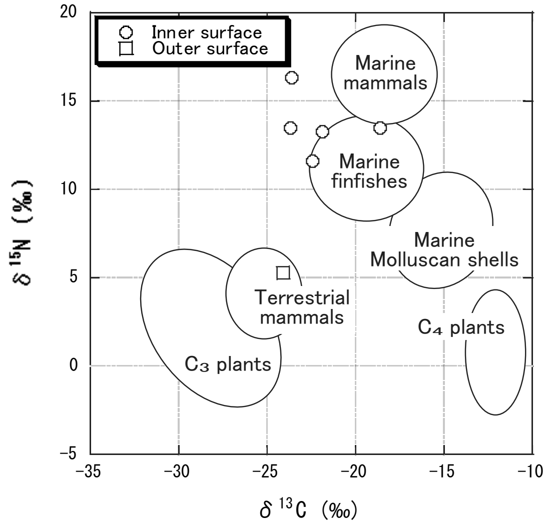

Fig. 9. Likely sources of the charred materials on potsherds at Hamanaka 2 according to their δ^{15}N and δ^{13}C values. The areas of end members are modified after Yoneda (2004).

layer V (latter half of the Late Jomon period), and the other, used as a reference sample, was from the body of an Epi-Jomon pot from layer III. Cholesterol made up more than 80% of the sterols detected on both potsherds, a result consistent with the results of a sterol analysis of materials adhering to a potsherd from the Mawaki archeological site, Ishikawa, Japan (from end of the Early Jomon period to Early Middle Jomon period), where many dolphin bones were found along with pottery (Nakano, 1986).

In contrast, lipids recovered from potsherds from various settlement sites of the Jomon and Yayoi periods, studied at International Christian University (Horiuchi et al., 2007), had total phytosterol (campesterol, β-sitosterol, and stigmasterol)/cholesterol ratios of ~2.6 (Jomon pottery) and ~1.4 (Yayoi pottery); that is, cholesterol made up only ~28% and ~42%, respectively, of total detected sterols (Horiuchi, 2004; Horiuchi and Yamashita, 2006). Therefore, the exceptionally high abundance of cholesterol at Hamanaka 2 strongly suggests that materials mainly of animal origin were cooked in the pots. This result supports the inference that Japanese sea lion meat was cooked in pots to get fat or oil at the site, as described in section 2 (Nishimoto, 2000; Kami, 2001).

However, the evidence is not conclusive. Sterols were scarcely detected in the lipids extracted from within the potsherd (the absorbed lipids) (Miyata et al., 2007b). The reasons for this may be twofold. It is possible that the lipids derived from sea mammals could not be absorbed into the clay body, perhaps because the inner pottery surface became coated by charred materials early in the cooking process, blocking the absorption of lipids. Accordingly, the lipids absorbed into the clay body might not have been sufficiently abundant to be detected.

Another possibility is that after the potsherds were buried, the surfaces of the potsherds were affected by lipids from the bones of sea mammals in the soil. We did not analyze the sterols in the soil; therefore, the possibility of contamination by lipids in the soil cannot be evaluated. A few studies have attempted to distinguish lipids derived from cooking from those originating from secondary contamination. For example, the Evershed research group analyzed lipids from within potsherds after first removing the pottery surface to prevent the possibility of secondary contamination (Charters et al., 1995; Copley et al., 2001). Bones of marine mammals were buried at Hamanaka 2 along with the potsherds, so lipids from the bones, as secondary contamination from the soil, might have affected the analytical results of this study. However, although the lipids may have been from either bones left by the site's occupants beside the pots or meat boiled in the pots, or both; both bones and meat were likely from the same type of marine mammal. Therefore, although the lipids associated with the pots may not date to the exact time that the marine mammals

Table 2. Radiocarbon and stable isotope dates of carbonized materials adhering to pottery and of charred wood samples excavated from the Hamanaka 2 archaeological site.

Sample No.	Lab. No.	Material	Material source	^{14}C age BP (± 1σ)	δ^{13}C (‰)	δ^{15}N (‰)
HDHN 1a	PLD-6305	Charred material on potsherd	Inner upper body	3805±20	-21.9	13.3
HDHN 1b	PLD-6306	Charred material on potsherd	Inner upper body	3815±20	-23.6	16.3
HDHN 1c	PLD-6453	Charred material on potsherd	Outer neck	3730±35	-24.1	5.25
HDHN 2a	PLD-6454	Charred material on potsherd	Inner lower body	3715±35	-18.6	13.5
HDHN 3	PLD-6455	Charred material on potsherd	Inner rim	3840±35	-22.4	11.6
HDHN C2	PLD-6456	Wood	Abies	2980±35	-	-
HDHN C4	PLD-6457	Wood	broad-leaved tree	3035±35	-	-

were cooked, the detected sterols were likely derived from the same sea mammals as those that were cooked.

The sterol analysis results therefore are consistent with the archeological hypothesis that Japanese sea lion was cooked in pots at the Hamanaka 2 site, as described in section 2 (Nishimoto, 2000; Kami, 2001).

4.2. Stable isotope analysis of carbonized materials adhering to pottery

Carbon and nitrogen isotope data of the carbonized materials adhering to the surfaces of potsherds from Hamanaka 2 are shown in Table 2. The isotope compositions of the charred materials from the inner surfaces strongly suggest that the materials were derived from marine mammals or finfishes (Fig. 9), whereas the composition of the charred material from the outer surface of a potsherd (HDHN 1c) suggests a different origin, perhaps from the fuel (wood) used, as shown in Fig. 1.

The stable isotope analysis results of the charred materials on potsherds are also consistent with the archeological hypothesis that a large quantity of Japanese sea lion meat was cooked in pots at Hamanaka 2, as described in section 2 (Nishimoto, 2000; Kami, 2001).

4.3. Radiocarbon analysis of carbonized material adhering to pottery

When marine products are dated by the radiocarbon method, the apparent radiocarbon age is often several hundred years older than the real age. This difference is the marine reservoir effect. In the North Pacific region, the marine reservoir effect is known to be larger than in other oceanic areas because upwelling deep waters in the North Pacific are associated with particularly old radiocarbon ages. Therefore, sea mammals from North Pacific region are expected to show a large reservoir effect.

The AMS radiocarbon dating results for five samples of charred matter from the inner or outer surfaces of potsherds from three different pots and for two charred wood samples are also summarized in Table 2. The charred wood, excavated from a fireplace, was dated to 3008 ± 35 BP (N = 2), which is consistent with the date of the archaeological horizon in which the samples were found (latter half of the Late Jomon period) (Nishimoto, 2000). The calibrated ages of the charred wood (Fig. 10) correspond to a calendar age between 1200 and 1300 cal BC for layer V, location R, at Hamanaka 2. Since the dated charred wood presumably was from wood used as fuel for the cooking fires, we assumed that the age of the charred wood corresponded to the actual age of the archaeological site. As mentioned in section 2, pottery in layer V is of the Dobayashi type, which was used during the latter half of the Late Jomon, and only for the relatively short time of several decades. Accordingly, layer V accumulated over at most a few decades. Therefore, the difference in time between the generation of the charred materials on the potsherds and the generation of the charred wood was also at most a few decades, which is a very short span of time relative to the radiocarbon measurement error.

The charred materials from the inner surfaces of the potsherds were dated to 3715–3840 BP (average, 3794

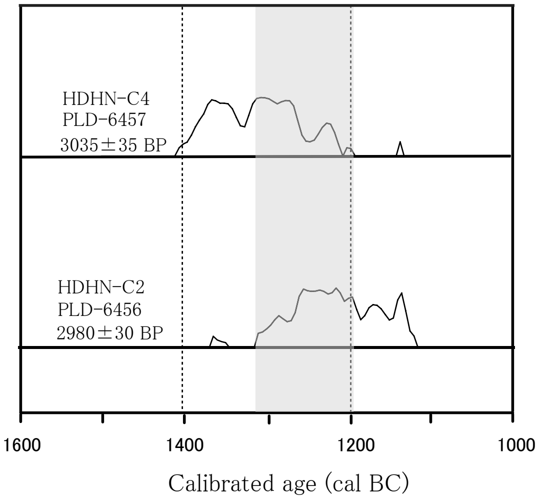

Fig. 10. Calibrated ages of charred wood samples from Hamanaka 2.

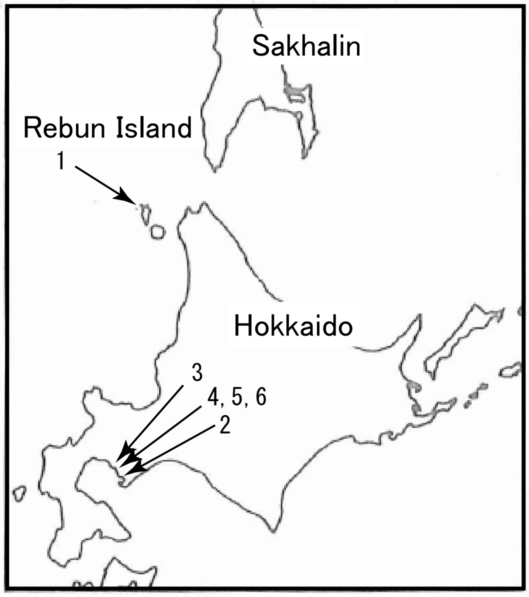

Fig. 11. Sampling sites at which ΔR has been calculated, indicated by numbers on the map (1, Hamanaka 2; 2, Kitakogane; 3, Takasago; 4, Minami Usu 6; 5, Minami Usu 7; 6, Oyakotsu). This figure is based on Yoneda et al. (2001).

± 55 BP; N = 4). Thus, the apparent radiocarbon age of these charred materials was 710–830 ^{14}C years older than that of the charred wood. Moreover, the δ^{13}C values of the charred materials from the inner surfaces of the potsherds were –18‰ to –23‰, which are in the range of oceanic values (see Figure 2). Therefore, it is reasonable to infer that these charred materials were derived from marine products cooked in these pots and that they show the marine reservoir effect.

These radiocarbon dating results support the archaeological hypothesis that Japanese sea lion meat was cooked in pots at Hamanaka 2 during the latter half of the Late Jomon period, as described in section 2 (Nishimoto, 2000; Kami, 2001).

Marine molluscan shells are usually used to evaluate the marine reservoir effect. Because marine molluscan shells take up dissolved inorganic carbon from seawater to form their calcium carbonate shells, the radiocarbon ages of fossil marine shells are representative of the ^{14}C activity of the contemporary seawater. Yoneda et al. (2001) measured the radiocarbon ages of animal bones excavated from the Kitakogane site, Hokkaido, Japan, to estimate the marine reservoir effect in the northwest Pacific (Fig. 3). They compared fur seal and deer bones, instead of shell and charcoal, to evaluate the marine reservoir effect, because they reasoned that fur seals, which mainly eat marine fish, would probably show the marine reservoir age, similarly to mollusk shells, and that deer bones would likely reflect the ^{14}C activity of the air when they were alive, since deer are herbivores. In fact, sea mammals, which are at the highest trophic level in the food chain, have higher nitrogen isotope ratios than marine molluscan shells (Fig. 9). The apparent radiocarbon age differences between fur seals and deer suggest a regional correction value for the marine reservoir effect (ΔR) in the northwestern Pacific for the 5000-year period from the Early Jomon to the Ainu period of 382 ± 16 ^{14}C years (N = 4; 392 ± 12 ^{14}C years at the Kitakogane site; 456 ± 66 ^{14}C years at the Minami Usu 6 site; 357 ± 26 ^{14}C years at the Minami Usu 7 site; 370 ± 80 ^{14}C years at the Oyakotsu site; the Takasago site is an outlier at 297 ± 16 ^{14}C years. These four sites are located close together on Funka Bay but they were occupied at different times; Yoneda et al., 2001; see Figures 4 and 11). Yoneda et al. (2002) recalculated the ΔR value at Kitakogane using the radiocarbon ages of all 30 deer bone samples from that site and obtained a value of 483 ± 61 ^{14}C yr (5600–5490 cal BP) for the Early Jomon period (see Figure 4).

In this study, the apparent age difference between the charred materials on potsherds, which we assumed to be composed of 100% marine products, and the charred wood, which we assumed to indicate the real age of the archaeological site, indicated a *Δ*R value for the northwestern Pacific of 444 ± 55 ^{14}C yr at 3010 BP (N = 4: 365 ± 75, 455 ± 60, 465 ± 60, and 490 ± 75 ^{14}C years). This value is a little larger than the value of 390 ^{14}C years calculated for the Northwestern Pacific by Stuiver and Braziunas (1993), but it is consistent with the value reported by Yoneda et al. (2002), within the margin of error. Even if these charred materials on potsherds all originated from Japanese sea lions, the meaning of this *Δ*R value cannot be discussed further without evaluating possible diagenesis of the charred materials from potsherds during burial in the soil. This problem needs to be examined in the future.

5. Conclusion

The results of the sterol and stable isotope analyses and the radiocarbon dating are consistent with the archaeological hypothesis that many sea mammals were cooked (boiled) in pots to obtain animal oils or fats at the Hamanaka 2 archaeological site during the latter half of the Late Jomon period.

The estimated regional correction value for the marine reservoir effect (*Δ*R) in the northwestern Pacific as calculated from our data is 444 ± 55 ^{14}C yr at 3010 BP ($N = 4$).

However, the origin of the detected sterols remains uncertain. They may (1) have adhered to or been absorbed by the pots during cooking, (2) have been secondary contaminants from the soil, or (3) have been a mixture of both. In the future, we should consider methods for evaluating or eliminating the effect of secondary contamination on information extracted from lipids adhering to or absorbed by pots during the cooking process. Key to solving these problems may be isotope analysis of specific compounds or radiocarbon dating of the lipids themselves.

To determine the food materials cooked in the pots more specifically, we should consider how charred materials are formed on pots and how lipids infiltrate pottery, as well as the possible diagenesis of charred materials on pottery buried in soil.

Acknowledgments

The carbon and nitrogen isotope analyses were done by SHOKO Co. Ltd., Japan. We acknowledge two anonymous reviewers for their detailed and constructive comments on our manuscript, and Dr. Y. Sampei, Chief Editor of Researches in Organic Geochemistry, for his kind encouragement. We thank Ms. N. Kami, Mr. S. Onbe and Drs. Y. Kudo and D. Kunikita for helpful discussions, Drs. M. Yoneda, K. Yoshida, and M. Kobayashi for their critical comments, Ms. S. Kobayashi for her support with sampling, Mr. T. Shinmen, and Ms. I. Nanbu for their help with the radiocarbon dating, and Ms. Y. Ueta for her identification of wood. Dr. M. Imamura gave useful comments on our draft manuscript. This work was financially supported by Grants-in-Aid for Young Scientists (B) Nos. 18700679 and 20700663 (Y.M.), and Creative Scientific Research Grant No. 16GS0118 (T.N.) and Scientific Research Grant (C) No. 19500870 (A.H.) from the Japan Society for the Promotion of Science.

References

Charters S., Evershed R. P., Blinkhorn P. W., and Denham V. (1995) Evidence for the mixing of fats and waxes in archaeological ceramics. *Archaeometry* **37**, 113-127.

Copley M. S., Pamela R. J., Clapham A., Edwards D. N., Horton M. C., and Evershed R. P. (2001) Detection of palm fruit lipids in archaeological pottery from Qasr Ibrim, Egyptian Nubia. *Proc. R. Soc.* **268**, 593-597.

Copley M. S., Hansel F. A., Sadr K., and Evershed R. P. (2004) Organic residue evidence for the processing of marine animal products in pottery vessels from the pre-colonial archaeological site of Kasteelberg D East, South Africa. *South African J. Sci.* **100**, 279-283.

Evershed R. P., Heron C., Charters S., and Goad L. J. (1992) The survival of food residues: New methods of analysis, interpretation and application.

Proceedings of British Academy **77**, 187-208.

Evershed R. P., DuDD S. N., Copley M. S., Berstan R., Stott A. W., Mottram H., Buckley S. A., and Crossman Z. (2002) Chemistry of archaeological animal fats. *Acc. Chem. Res.* **35**, 660-668.

Gurr M. I. and Harwood J. L. (1991) *Lipid Biochemistry, An Introduction,* London, New York, Tokyo, Melbourne, Madrae: Chapman and Hall. pp. 263-265.

Horiuchi A. (2004) Is it possible to study ancient diet from the analysis of organic remains from ancient pottery? *J. Lipid Nutrition* **13**, 126 (in Japanese).

Horiuchi A. and Yamashita N. (2006) A study of organic residues from ancient pottery. Abstracts of the Papers presented at the 23rd Annual Meeting of Japan Cultural Properties and Sci. Association. pp. 286-287 (abstr.) (in Japanese).

Horiuchi A., Takagi A., Yamane T., Takeishi N., and Oshikawa T. (2007) A study on the limitations of fatty acid analysis in archaeological materials using model systems and the perspectives of organic analysis, *Archaeology and Natural Science* **56**, 13-26 (in Japanese with English Abstr.)

Hughen K. A., Baillie M. G. L., Bard E., Beck J. W., Bertrand C. J. H., Blackwell P. G., Buck C. E., Burr G. S., Cutler K. B., Damon P. E., Edwards R. L., Fairbanks R. G., Friedrich M., Guilderson T. P., Kromer B., McCormac G., Manning S., Ramsey C. B., Reimer P. J., Reimer R. W., Remmele S., Southon J. R., Stuiver M., Talamo S., Taylor F. W., van der Plicht J. and Weyhenmeyer C. E. (2004) Marine04 marine radiocarbon age calibration, 0–26 Cal Kyr BP. *Radiocarbon* **46**, 1059-1086.

Imamura M. (2007) On the Radiocarbon calibration program files GHC3.2. *Bulletin National Museum Japanese History* **137**, 79-88 (in Japanese with English Abstr.).

Kami N. (2001) A study of marine mammal hunting in the Late Jomon period. *Hakubou* **2**, 90-98. (in Japanese).

Kimpe K., Drybooms C., Schrevens E., Jacobs P. A., Degeest R. and Waelkens M. (2004) Assessing the relationship between form and use of different kings of pottery from the archaeological site Sagalassos (southwest Turkey) with lipid analysis *J. Archaeol. Sci.* **31**, 1503-1510.

Kobayashi K., Niu E., Itoh S., Yamagata H., Lomtatidze Z., Jorjoliani I., Nakamura K., and Fujine H. (2007) The compact ^{14}C AMS facility of Paleo Labo Co., Ltd., Japan. *Proceedings 10th Int'l Conference Accel. Mass Spectr., Nuc. Instr. Meth. Phys. Res. B* **259**, 31-35.

Lin D. S. and Connor W. E. (2001) Fecal steroids of the coprolite of a Greenland Eskimo mummy, AD 1475: a clue to dietary sterol intake. *Am. J. Clin. Nutr.* **74**, 44-49.

Maeda U. and Yamaura K. (1992) "*Archaeological investigation in Hamanaka II, Island of Rebun, Hokkaido.*" In: Maeda U. and Yamaura K. editors. Hokkaido: "Rebun Kyouikuiinkai." (in Japanese).

Matsutani A. (2006) Determination of the charred particle adhering to pottery excavated from Ryugasaki A site using SEM analysis. In: Kojima, T. editor. *"Ryuugasaki A iseki"*. Shiga: "Shigakenkyouikuiinkai". pp. 173-178 (in Japanese).

Miyata Y., Sakamoto M., Ozaki H., Shinmen T., Onbe S., Nishimoto T., and Imamura M. (2005) Preparation of AMS-14C samples for the archaeological research at National Museum of Japanese History. *10th Int'l Conference Accel. Mass Spectro.*, pp. 78 (abstr.).

Miyata Y., Onbe S., Sakamoto M., and Imamura M. (2006) AMS carbon-14 dating of carbonized material adhering to pottery: The difference of carbon-14 age between inside and outside pottery in swale archaeological site. *Program and abstracts on Japan Association for Quarter. Res.*, pp. 96-97 (abstr.) (in Japanese).

Miyata Y., Kojima T., Matsutani A., Onbe S., and Nishimoto T. (2007a) Oldest common millet from western Japan: Carbonized material adhering to pottery from the Ryugasaki A site, Shiga prefecture. In: Imamura M. and Kobayashi, K. editors. *Bulletin National Museum Japanese History* **137**. Chiba: National Museum Japanese History. pp. 255-265 (in Japanese with English Abstract).

Miyata Y., Horiuchi A., Paleo Labo AMS dating group, and Nishimoto T. (2007b) Sterol analysis and chronological study of pottery excavated from the site of Hamanaka 2, Rebun Island, *Geological Survey Japan Interim Report* **42**, p. 102.

Miyata Y., Horiuchi A., Kondo M., Onbe S., Paleo Labo AMS dating group, and Nishimoto T. (2008) Dietary analysis of archaeological remains using radiocarbon dates and sterol analysis in pottery. *11th Int'l Conference Accel. Mass Spectro.*, p. 34 (abstr.).

Nakamura T., Nishida I., Takada H., Okuno M., Minami M., and Oda H. (2007) Marine reservoir effect deduced from 14C dates on marine shells and terrestrial remains at archeological sites in Japan. *Proceedings 10th Int'l Conference Acceler. Mass Spectro.*, *Nucl. Instr. Meth. in Phys. B* **259**, 453-459.

Nakano M. (1986) "Mawakiisekisyutudodokinizannzonnsurudoubutuyushi." In: Takahori K. and Yamada Y. editors. *"Mawakiisekihakkutsuchousasho."* Ishikawa: Notochoukyouikuiinkai. pp.401-406 (in Japanese).

Nishimoto T. (2000) Report on the research excavation at the site of Hamanaka 2. In: Nishimoto T. editor. *Bull. National Museum Japanese History* **85**. Chiba: National Museum Japanese History (in Japanese with English Summary).

Rackham J. (1994) Early Human Exploitation. *Animal Bones.* Berkeley: Univ. of California Press, pp. 32-43.

Reimer P. J., Baillie M. G. L., Bard E., Bayliss A., Beck J. W., Bertrand C. J. H., Blackwell P. G., Buck C. E., Burr G. S., Cutler K. B., Damon P. E., Edwards R. L., Fairbanks R. G., Friedrich M., Guilderson T. P., Hogg A. G., Hughen K. A., Kromer B., McCormac G., Manning S., Ramsey C. B., Reimer R. W., Remmele S., Southon J. R., Stuiver M., Talamo S., Taylor F. W., van der Plicht J., and Weyhenmeyer C. E. (2004) IntCal04 terrestrial radiocarbon age calibration, 0–26 Cal Kyr BP. *Radiocarbon* **46**, 1029-1058.

Southon J. R., Rodman A. O., and True D. (1995) A comparison of marine and terrestrial radiocarbon ages from northern Chile. *Proceedings 15th Int'l ^{14}C conference, Radiocarbon* **37**, 389-393.

Stuiver M. and Braziunas T. F. (1993) Modeling atmospheric ^{14}C and ^{14}C ages of marine samples to 10,000 BC. *Radiocarbon* **35(1)**, 137-189.

Sugano M. and Imaizumi K. (1987) *Cholesterol*. Sankyo Publishing Company, p.47 (in Japanese).

Yoneda M. (2004) "Tansothissodouitainiyorukosyokuseifukugen" In: Yasuda K., editor. *"Kankyoukoukogakuhandobukku"* Tokyo: Asakurashoten. pp. 411-418 (in Japanese).

Yoneda M., Hirota M., Uchida M., Uzawa K., Tanaka A., Shibata Y., and Morita M. (2001) Marine radiocarbon reservoir effect in the western north pacific observed in archaeological fauna. *Proceedings 17th Int'l ^{14}C conference, Radiocarbon* **43**, 465-471.

Yoneda M., Tanaka A., Shibata Y., Morita M., Uzawa K., Hirota M., and Uchida M. (2002) Radiocarbon marine reservoir effect in human remains from the Kitakogane site, Hokkaido, Japan. *J. Archaeological Sci.* **29**, 529-536.