博士論文

Stable isotopic reconstruction of breastfeeding practices in past human populations

(同位体分析による過去ヒト集団の授乳習慣復元)

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Stable isotopic reconstruction of breastfeeding practices in past human populations

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Contents

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Notes

This dissertation consists from several published and unpublished works. Chapter 1 is derived from Tsutaya and Yoneda (2015) and partly original. Other chapters are mostly based on published or submitted works: Tsutaya and Yoneda (2013) for Chapter 2, Tsutaya et al. (2014a) for Chapter 3, Tsutaya et al. (2015) for Chapter 4, and Tsutaya et al. (2014b, submitted) for Chapter 5. Copyright agreements for these works have been obtained from co-authors and publishers.

Definitions

Weaning is not an event but a process, which starts with an introduction of solid or liquid foods other than breast milk and ends with a cessation of milk intake (Dettwyler and Fishman,1992; WHO, 2008) although a few people typically use the term to refer only to the cessation of breastfeeding. In this dissetation, the start and end of weaning are defined in terms of elemental intake because isotopic studies only measure the contribution of consumed elements into body tissues.

Weaning food is defined here as the generic name for foods and liquids other than mother's milk, which is consumed by subadults during the weaning process.

Adult food means the typical foods consumed by adults in the population.

Fertility is defined as the production of a live birth, that is, a child born alive (Wood, 1994).

Fertility rate is the total number of births a woman would have by the end of the reproductive years while living throughout the reproductive period (Bongaarts, 1978). Because it is difficult to estimate exact number of births in archaeological human populations, I use the word **fertility**, not **fertility rate**, for the indicator of the production of a live birth.

Age categories are basically defined as follows (Lewis, 2007): perinate, around time of birth; infant, less than one year of age; subadult, younger than adults including infant; adult, older than 15–17 years. Cutoff ages for the category of adult varies in each study of this dissertation, because age estimation methods used by collaborator differ among each study. Furthermore, boundary between subadult and adult vary by cultural and biological backgrounds of the target human populations (Halcrow and Tayles, 2008). However, the difference of the cutoff age has little impact on the results and discussion because younger subadults are the main interest of this study.

Abstract

Although fertility is difficult to estimate directly for most archaeological human populations, breastfeeding period, one of the most important determinant of fertility, can sometimes be estimated by using stable isotopes. During the period of breastfeeding, resumption of ovarian activity in the breastfeeding mother is suppressed, owing to hormonal modifications and nutritional costs of lactation. Therefore, a longer period of breastfeeding generally leads to longer inter-birth intervals, and resulting lower fertility in a population. Stable carbon and nitrogen isotope analyses of subadult bone collagen have been used to estimate breastfeeding period in past human populations. The carbon and nitrogen isotope ratios in the tissue of exclusively breastfed infants are 1‰ and 2–3‰ higher than those of their mother's, respectively, because the bioenrichment occur between mothers and breastfed subadults. After the start of the weaning process, the nitrogen isotope ratio of newly formed tissue gradually decreases with decreasing breast milk intake, and finally stabilizes to adult values after the end of the weaning process. In archaeological human skeletal populations, breastfeeding period has been reconstructed by estimating the age at death of subadults and measuring the isotope ratios of the collagen extracted from the skeletons.

However, there are time lags between the actual dietary change and the incorporation of isotopic signals into the tissue because of the slower turnover rate of bone collagen. The equilibrium age of isotopic signals in bone collagen is several months or even years older than the past weaning age. Because of these time lags, weaning ages inferred from the change in isotope ratios of subadult bone collagens have never been directly comparable to those observed in ethnography and reported in historical demography.

To overcome this problem, I estimated temporal changes in human subadult bone collagen turnover rates based on tissue-level bone metabolism, and developed a mathematical model to correct the time lags. Temporal changes in human subadult bone collagen turnover rates were estimated from data on tissue-level bone metabolism reported in previous studies. A model for reconstructing weaning ages was then developed using a framework of approximate Bayesian computation and incorporating the estimated turnover rates. The model is presented as a new open source R package, WARN (Weaning Age Reconstruction with Nitrogen isotope analysis), which computes the age at the start and end of weaning, ^{15}N-enrichment through maternal to infant tissue, and nitrogen isotope ratio of collagen synthesized entirely from weaning foods with

their posterior probabilities.

Then, the developed model was applied to four skeletal populations in Japan to estimate actual weaning ages. Reconstructed weaning ages were used to infer fertility and discuss three demographic events in past Japanese archipelago. In the Hitotsubashi skeletal population, Tokyo (AD 1657–1683: the early Edo period), the age at the end of weaning was reconstructed as 3.1 years (2.1–4.1 years in 95% credible intervals), which agrees with descriptions in various historical documents of the period. The duration of breastfeeding in the Hitotsubashi population was relatively longer than those in modern industrial and traditional societies and four previously reported populations in medieval and in the industrial England. As later weaning closely associates with longer inter-birth interval for mothers, my data suggest a lower natural fertility for the Hitotsubashi population. Assuming that the proportion of married people was also lower in the major cities of the earlier Edo period, my results support the assumption that Edo developed and increased its population by attracting immigrants during urbanization.

In the Yuigahama-minami skeletal population, Kanagawa (AD 12th–14th centuries: the early medieval period), the age at the end of weaning was reconstructed as 3.8 years (2.1–4.1 years in 95% credible intervals). The age at the end of weaning in the Yuigahama-minami population was greater than that in the typical non-industrial populations, a premodern population in the Edo period Japan, and medieval populations in the UK. Kamakura experienced urbanization and population increase in the early medieval period. The younger age-at-death distribution and high nutritional stresses in the Yuigahama-minami population and later weaning, which is closely associated with longer inter-birth interval for mothers, suggests that Kamakura developed and increased its population by immigration during urbanization.

In the Moyoro skeletal population, Hokkaido (AD 6th–10th centuries), the age at the end of weaning was reconstructed as 1.8 years (1.4–2.2 years in 95% credible intervals), which is lower than that in another northern hunter–gatherer–fisher populations and typical modern traditional societies. It is assumed that the Okhotsk people originated from lower Amur River region and expanded rapidly along the northeastern coast of Hokkaido mostly during AD 600–700. Because weaning age is one of the most important determinants of fertility, a shorter breastfeeding period suggests increased fertility. Furthermore, previous studies have shown better nutrition and lower mortality for them, which would further promote the population increase, and thus populations of the Okhotsk culture could expand into new habitats. These findings are consistent with recent emerging evidence of great contributions of the Okhotsk to the formation of later Ainu populations and culture.

The developed model in this study provides a framework for objectively and quantitatively analyzing, interpreting, and comparing subadult bone collagen nitrogen isotope ratios. By using the models to correct the lag time, researchers can compare weaning ages obtained by isotope analysis of past human skeletons with those obtained from participant observations in cultural anthropology and historical literatures as a uniform measure. Applications of this model indi-

cate that process and cause of past human demographic events can be empirically discussed by inferring fertility from isotopically reconstructed age at the end of weaning. However, several proximate and remote factors, as well as breastfeeding period, affect fertility, and these factors are difficult to estimate in most archaeological settings. Furthermore, population dynamics are not only determined by fertility but also by mortality and migration as well. In order to discuss past human population dynamics, one needs to obtain demographic information other than breastfeeding period. Development and application of further methods to estimate demographic parameters would be important to reconstruct past human population dynamics from various aspects.

Chapter 1

Introduction

Reconstruction of mortality and fertility in past human populations is of interest in anthropology and archaeology to discuss population dynamics (Howell, 1986; Chamberlain, 2009). Factual assessments of these demographic parameters are important in studying past demographic events. Although fertility is difficult to estimate directly for most archaeological human populations, breastfeeding period, one of the most important determinant of fertility, can sometimes be reconstructed by using stable isotopes.

1.1 Breastfeeding and fertility

1.1.1 Breastfeeding and weaning in physical anthropology

The reconstruction of breastfeeding and weaning practices of past human populations is one of the most important fields in anthropology, archaeology, evolutionary biology, and history (Stuart-Macadam and Dettwyler, 1995). Infant feeding practices affect the overall health of the population because diet, health status, and growth at young ages have profound effects on an individual's later life (Dettwyler and Fishman, 1992; Katzenberg et al., 1996; WHO, 1998, 2009; Lewis, 2007). Breastfeeding and weaning practices can be viewed from the evolutionary perspectives of life history theory, parental investment, child development, social system, and reproductive strategy (Lee, 1996; Bogin, 1997; Hewlett and Lamb, 2005; Kennedy, 2005; Sellen, 2007; Humphrey, 2010). Furthermore, the length of the breastfeeding period is one of the most important determinants of the fertility of a population (Bongaarts, 1978, 1982; Trussell, 1979; Bongaarts and Potter, 1983; Campbell and Wood, 1988; Wood, 1994). Cultural factors, such as the type of subsistence activities, social constructs, and religious beliefs, also affect human breastfeeding practices (Ford, 1964; Maher, 1992; Fildes, 1995; WHO, 1998; Hewlett and Lamb, 2005). Today, breastfeeding and weaning practices are also of importance in pediatrics, obstetrics, gynecology, epidemiology, and sustainability science (WHO, 1998, 2009; Ip et al., 2007).

Particularly in physical anthropology, breastfeeding and weaning are emphasized from the

standpoint of health, palaeodemography, and the evolution of human life history. The infant feeding practice is one of the most important indicators of health in past human population (Lewis, 2007). Breast milk provides various immunological factors as well as nutrition to infants, and it is important for their survival, especially in the first six months after birth (Cunningham, 1995; Silvia and Clements, 1997; WHO, 1998, 2009; Kramer and Kakuma, 2004). Although the benefits of breast milk consumption continue, complementary feeding is necessary to satisfy an infant's growing need for energy and nutrients after six months of age (Dewey and Brown, 2003). Therefore, sufficient breastfeeding and appropriate food supplementation to subadults is important for the health of populations.

The age at the end of weaning is also a proxy of fertility in past human populations. Shorter breastfeeding periods tend to result in shorter birth intervals and higher fertility because suckling stimuli and the energetic burden of lactogenesis can delay the resumption of a mother's ovulation (Vitzthum, 1994; Wood, 1994; Valeggia and Ellison, 2009; WHO, 2009).

Although there is significant variation in the weaning age, the typical age at the end of weaning in non-industrialized human societies (i.e., 2.4–2.7 years: Ford, 1964; Barry and Paxson, 1971; Sellen, 2001) is younger than that for non-human great apes (i.e., mostly 3.0–6.0 years: reviewed by Harvey and Clutton-Brock, 1985; Hawkes et al., 1998; Kennedy, 2005), despite the fact that the human-life trajectory is characterized by prolonged growth and development (Bogin, 1997; Kennedy, 2005; Emery Thompson, 2013). This relatively early weaning in humans reduced the mother's nutritional and labor costs associated with breastfeeding, facilitating shorter birth spacing (Jones, 2011) and cooperative breeding (Kramer, 2005, 2010; Kachel et al., 2011). To elucidate the evolutionary process of early weaning, it is important to reconstruct breastfeeding periods in past hominins (Kennedy, 2005; Gibbons, 2008; Humphrey, 2010).

1.1.2 Breastfeeding and weaning in reproductive ecology

This study especially focuses on the palaeodemographic aspect of breastfeeding and weaning. During the period of breastfeeding, resumption of ovarian activity in the breastfeeding mother is suppressed, owing to hormonal modifications and nutritional costs of lactation (Ellison, 1995; Valeggia and Ellison, 2009). There are vast amount of researches investigating the biological relationships between breastfeeding and postpartum amenorrhea, which conclude that breastfeeding suppresses resumption of ovarian activity although another factors, such as nutrition and frequency of breastfeeding, could also affect the effect of suppression (reviewed by Wood, 1994; Ellison, 1995). Generally, a longer period of breastfeeding leads to longer inter-birth intervals, and resulting lower fertility in a population. Bongaarts (1982) indicated that fertilities in different populations can be explained almost entirely in terms of four proximate determinants: proportion married, contraception, induced abortion, and duration of postpartum lactational amenorrhea. Among these, lactational amenorrhea is the single most important cause of differences in natural fertility (Bongaarts and Potter, 1983; Campbell and Wood, 1988; Wood, 1994). Trussell (1979)

also indicated that variation in fecundability and length of lactational amenorrhea can produce large differences in the level of fertility, although the effect of the former is marginally greater than that of the latter. Other conditions being similar, the length of the breastfeeding period can be regarded as an estimate of fertility in the population. Actually, demographic studies of modern and historical human populations have frequently found that the breastfeeding period is the strong determinant of fertility in the population (e.g., Hostetler, 1966; Knodel, 1968; Jain et al., 1970; Konner and Worthman, 1980; Lithell, 1981; Wood et al., 1985; Wood, 1994; Hrdy, 1999; Helle et al., 2014).

Although breastfeeding period is one of the most important determinants of fertility, we should be aware that it is not a sole determinant. Fertility is affected directly by biological and behavioral factors, such as proportion married, contraception, induced abortion, lactational infecundability, frequency of intercourse, sterility, spontaneous intrauterine mortality, and duration of the fertile period, when following the classification by Bongaarts (1978); age at marriage, menarche, menopause, onset of pathological sterility, duration of lactational infecundability, duration of fecund waiting time to conception, probability of fetal loss, its associated period, and length of gestation, when following the classification by Wood (1994; Figure 1.1). Breastfeeding only works directly via lactational infecundability among these proximate determinants. Furthermore, these proximate determinants of fertility is affected by socioeconomic, cultural, and environmental factors. All these proximate and remote factors influence fertility. However, most of these factors are difficult to estimate in most of the historical and archaeological settings. Among these, breastfeeding period can be reconstructed by using stable isotopes as just one of the important determinants.

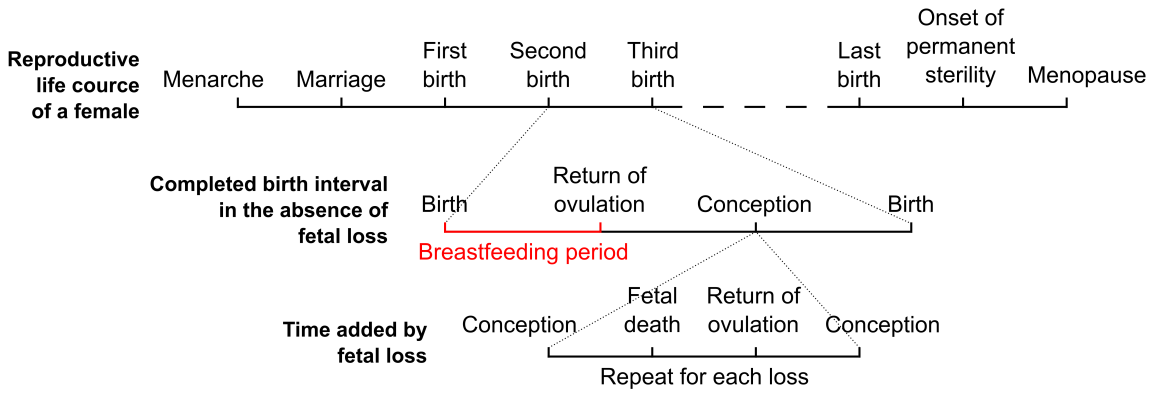

Figure 1.1: Components of the female reproductive life course (adapted from Bongaarts and Potter, 1983; Wood, 1994). Length and timing of these components vary within biologically valid ranges, and combination of them determines fertility rate of a human female.

1.2 Stable isotope analysis

1.2.1 Basics and principles

The body tissues of organisms consist of various elements, which were originally derived from dietary sources, and reflect the elemental signals in the diet. Therefore, organism diets can be reconstructed by analyzing the elemental signals in their tissues. If a certain elemental signal systematically differs among breast milk, weaning foods, and the adult diet, breastfeeding and weaning patterss can also be reconstructed. Research using stable isotopes (van der Merwe and Vogel, 1978; van der Merwe et al., 1981; Tauber, 1981; Chisholm et al., 1982; Ambrose and DeNiro, 1986) have been intensively applied to past and modern human populations since the 1970–1980s (reviewed by Katzenberg, 2008).

Isotopes are variants of a particular element having the same number of protons but a different number of neutrons. For example, ^{12}C, ^{13}C, and ^{14}C are three isotopes of the element carbon. Unlike radioisotopes, stable isotopes do not spontaneously undergo radioactive decay and are stable over a long time period. Because lighter isotopes are preferred in chemical reactions, the relative abundances of isotopes systematically differ through biogeochemical reactions (summarized by Schwarcz and Schoeninger, 1991; Schoeninger and Moore, 1992; Pate, 1994; Fry, 2006; Hoefs, 2010). This relative abundance is referred to as the isotope ratio, and it is generally expressed in terms of δ values that are parts per thousand (‰) differences from a standard:

$$\delta X = (R_{sample}/R_{standard} - 1) \times 10^3,$$

where X is the heavier isotopes such as ^{13}C, and R is the corresponding isotope ratio (e.g., ^{13}C/^{12}C). Isotope ratios are expressed as deviations from the ratios of international standard materials, such as the PeeDee Belemnite for carbon. Therefore, specific isotope ratios are only a relative quantitative measure, and do not have absolute numerical meanings.

1.2.2 Nitrogen isotopes

Stable nitrogen isotope analysis, as well as carbon, has been used in the dietary reconstruction in ecology and bioarchaeology (reviewed by Schwarcz and Schoeninger, 1991; Schoeninger and Moore, 1992; Pate, 1994; Katzenberg and Harrison, 1997; Crawford et al., 2008; Lee-Thorp, 2008; Crowley, 2012; Sandberg et al., 2012). The δ^{15}N value increases with an elevated trophic level (bioenrichment: DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Bocherens and Drucker, 2003), and, in general, is higher in marine organisms than in terrestrial organisms (Schoeninger et al., 1983; Schoeninger and DeNiro, 1984). It is assumed that the trophic level enrichment of ^{15}N occurs because animals preferentially excrete ^{14}N-rich urine which is derived from transamination and deamination processes (Sponheimer et al., 2003b, c).

Nitrogen is the most frequently used element in the isotopic reconstruction of breastfeeding and weaning pattern. The contemporary hair and nail studies of human mothers and fetus (hair segments synthesized *in utero* were sampled after live-birth) have indicated no significant difference (Fuller et al., 2006a) or an approximately 0.9‰ increase (de Luca et al., 2012) in fetal δ^{15}N values compared with those of the mother. Alternatively, fetal and maternal tissues exhibit a parallel decline in δ^{15}N values (from -0.3 to -1.1‰) compared with the preconception period, probably because of a positive nitrogen balance during pregnancy (Fuller et al., 2004). These inconsistent but slight differences between fetuses and pregnant mothers could be a attributed to different nitrogen metabolism statuses among individuals and populations (Duggleby and Jackson, 2002; Kinaston et al., 2009; Burt and Amin, 2014). After delivery, the δ^{15}N value promptly increases to 2–3‰ for exclusively breastfed human infants (Fogel et al., 1989; Millard, 2000; Fuller et al., 2006a; Romeck et al., 2013) and 0.9 ± 0.8‰ for the non-primate terrestrial mammalian neonates (Jenkins et al., 2001) more than that of the milk producer, because the same principle of bioenrichment applies between the mother ("producer") and the breastfed infant ("consumer") through breast milk consumption. Human subadults require energetic and nutritional supplementation after the age of six months (Michaelsen et al., 2000; Dewey and Brown, 2003), and the introduction of weaning food proteins decrease subadult δ^{15}N values during the weaning process. The δ^{15}N values of subadult tissues should decrease to the same range as those of adults after the subadults are completely weaned and consume adult foods. A gradual decrease in subadult δ^{15}N value appears to correspond to a prolonged weaning period and a rapid decrease to a brief period. By analyzing the δ^{15}N values of tissues that were synthesized during infancy and childhood, researchers can estimate the ages when the protein contribution of weaning food began, when that of breast milk ceased, and the tempo of weaning.

The δ^{15}N values of tissue proteins, such as collagen and keratin, reflect those of dietary protein because nitrogen mainly originates from amino acids (Podlesak and McWilliams, 2006; Froehle et al., 2012). Therefore, the relationship between dietary and body tissue δ^{15}N values is relatively more straightforward than that of δ^{13}C values (see below).

1.2.3 Carbon isotopes

The δ^{13}C values of organic matters primarily differentiate with the photosynthetic pathways of plants in the food web; C_3 plants have much lower δ^{13}C values than C_4 plants, such as maize and millet (Smith and Epstein, 1971; O'Leary, 1988; Farquhar et al., 1989). Furthermore, δ^{13}C values is usually greater in marine organisms than in terrestrial organisms depending on the C_3 ecosystem (Tauber, 1981; Chisholm et a., 1982; Schoeninger and DeNiro, 1984; Peterson and Fry, 1987).

Stable carbon isotopes have been used as an indicator of food supplementation rather than that of breast milk consumption (Dupras et al., 2001; Fuller et al., 2006b; Tsutaya et al., 2013), but some studies of marine mammals are exceptions (Polischuk et al., 2001; Newsome et al., 2006;

Ducatez et al., 2008; York et al., 2008; Orr et al., 2012). Contemporary studies of human hair and nail indicated that there seems to be no difference (Fuller et al., 2006a) or an approximately 0.4‰ increase (de Luca et al., 2012) in fetal δ^{13}C values compared with maternal ones. There seems to be no difference in maternal δ^{13}C values between the preconception and gestation periods (Fuller et al., 2006a). The δ^{13}C values of exclusively breastfed infants show an approximately 1‰ increase compared with those of the milk producer for humans (Richards et al., 2002; Fuller et al., 2003, 2006a), no change for the non-primate terrestrial mammals (Jenkins et al., 2001), and a decrease in some marine mammals (Newsome et al., 2006; Ducatez et al., 2008; York et al., 2008; Orr et al., 2012) and polar bears (Polischuk et al., 2001). These species-specific differences probably stem from the differences in breast milk composition (Oftedal and Iverson, 1995): species producing milk with a higher lipid content, such as Pinnipedia, would show less or negative enrichment between mother and infant because ^{13}C is relatively enriched in milk proteins and depleted in milk lipids (Fuller, 2003). The δ^{13}C value of lipids is much lower than that of proteins in the same individual because the lighter carbon isotope is preferentially routed into fatty acids during the fatty acid synthesis (DeNiro and Epstein, 1977). After the onset of the weaning process, subadult δ^{13}C values reflect both breast milk and weaning foods. Thus, weaning foods that are isotopically different from ordinary adult diets in the population can be detected from the change in the δ^{13}C values of subadults during or after the weaning process (Fuller et al., 2006a; Tsutaya et al., 2013).

The protein and apatite fractions of body tissue have been used for isotopic dietary reconstructions. The δ^{13}C values of body tissue proteins, such as bone collagen and hair keratin, primarily reflect those from dietary proteins in breast milk, weaning foods, and adult foods. However, the δ^{13}C values of body tissue protein also record those of dietary carbohydrates and lipids (Krueger and Sullivan, 1984; Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Kellner and Schoeninger, 2007). This is because the δ^{13}C values of essential amino acids in tissue proteins mainly reflect those of dietary protein, but the δ^{13}C values of non-essential amino acids are affected by the δ^{13}C values of dietary carbohydrate and lipid as well as protein (Howland et al., 2003; McCullagh et al., 2005). Conversely, the δ^{13}C values of apatite reflect those of total carbon, including proteins, carbohydrates, and lipids, because apatite forms in equilibrium with blood carbonate, which itself is a product of total dietary metabolism (Krueger and Sullivan, 1984; Schwarcz, 2000).

1.3 Problems and objectives

1.3.1 Cross-sectional reconstruction using subadult bone collagen

By obtaining tissues that record dietary signals in various stages of the weaning process and relating them to biological ages, accurate reconstruction of breastfeeding and weaning patterns is possible. However, soft tissues are not durable in most archeological settings. Therefore, most

studies considering past breastfeeding and weaning concentrate on calcified tissues, that is bones and teeth.

Bone consists of organic (approximately 20% dry weight) and mineral (approximately 70% dry weight) portions, which almost entirely are composed of collagen and calcium phosphates from the apatite family, respectively (Hillson, 1996; Aerssens et al., 1998; Rho et al., 1998; Fratzl et al., 2004). Carbon and nitrogen isotope analyses can be applied to the organic portion. The δ^{13}C and δ^{15}N values of collagen mainly reflect those of dietary protein (Krueger and Sullivan, 1984; Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Kellner and Schoeninger, 2007).

Breastfeeding and weaning practice can be reconstructed in archaeological skeletal populations by estimating the subadult age at death and measuring the isotope ratios of the collagen extracted from skeletons (Humphrey, 2014; Figure 1.2). Subadult ages can be estimated by the physical observations of dental development and eruption (e.g., Smith et al., 1991; Ubelaker, 1999; AlQahtani et al., 2010) and the regression analysis of limb bone length (e.g., Maresh, 1970; Fazekas and Kosa, 1978). These aging methods have been confirmed in anthropology and forensic medicine (Scheuer and Black, 2000). An individual represents only one age point of its death, and many subadult individuals of different ages are needed to reconstruct breastfeeding and weaning practice by the isotope analysis of bone collagen. Thus, breastfeeding and weaning practices reconstructed from such methods represent the cross-sectional typical ones of the skeletal population and not the longitudinal ones of individuals. From the first report by Fogel et al. (1989), over 40 studies have reconstructed cross-sectional breastfeeding and weaning patterns using the carbon and nitrogen isotope ratios of bone collagen (Tsutaya and Yoneda, 2013, 2015; see also Chapter 2).

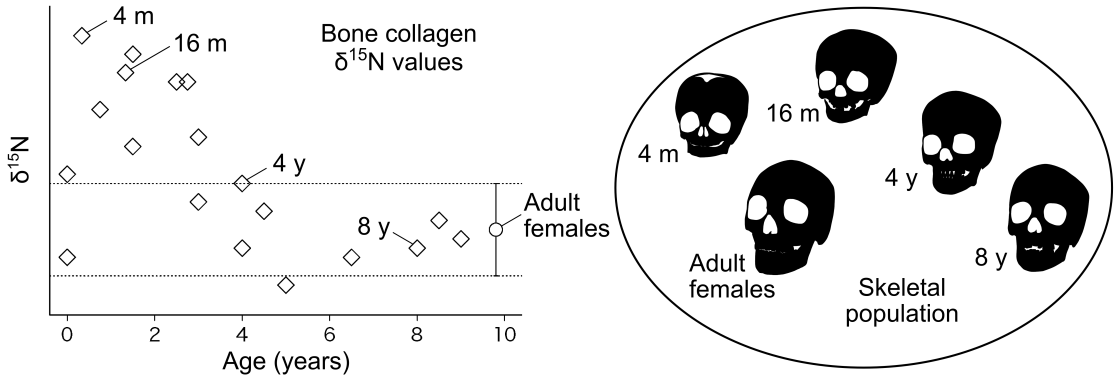

Figure 1.2: Schematic illustrations of the reconstruction of breastfeeding and weaning practices. The practice reconstructed from subadult bone collagen represents the cross-sectional typical ones of the skeletal population and not the longitudinal ones of individuals.

1.3.2 Problems of time lag

Isotopic signals at a certain sampling point should be related to the corresponding biological time period to reconstruct accurate weaning ages. In other words, researchers need to know when the studied tissue was synthesized in the individual's life and how large was the lag time between the actual dietary change and the incorporation of isotopic signals into the tissue. Problems related to the incorporation of elements into body tissues have been intensively discussed in isotopic ecology (reviewed by Crawford et al., 2008; Martínez del Rio et al. 2009; Wolf et al., 2009; Boecklen et al., 2011). The assessment of biological age is relatively straightforward in soft tissues because the potential time lags between actual weaning ages and elemental signals are relatively small. The turnover rates of most soft tissues, such as blood, hair, nail, and muscle, and excreta are relatively high and these reflect diet during relatively short time periods (as little as a few days to months) before sampling for most animal species (e.g., O'Connell and Hedges, 1999; Sponheimer et al., 2003a; McCullagh et al., 2005; Codron et al., 2011; Braun et al., 2013; Bahar et al., 2014).

The turnover rate of bone tissue, however, is relatively low, which requires careful attention to interpret the breastfeeding and weaning signals recorded therein. Typical cortical bone collagen reflects dietary signals over a period of time much longer than 10 years in human adults (Stenhouse and Baxter, 1979; Hedges et al., 2007). Although growing subadult bones indicate higher turnover rates than adult bones, the turnover rates are not high enough to immediately reflect a dietary change in subadults. Thus, the equilibrium age of isotopic signals in bone collagen is several months or even years older than the actual weaning age. Because of these time lags, weaning ages inferred from the change in isotope ratios of subadult bone collagens have never been directly comparable to those observed in ethnography and reported in historical demography. Although weaning ages in past human populations are important estimate of fertility, those inferred from isotope analysis have not usually been discussed from the standpoint of palaeodemography, with the exception of a few studies (Schurr and Powell, 2005; Waters-Rist et al., 2011).

1.3.3 Objectives of this study

To overcome this problem, I estimated temporal changes in human subadult bone collagen turnover rates based on tissue-level bone metabolism, and developed a mathematical model to correct the time lags (Chapter 2). Then, the developed model was applied to three skeletal populations in Japan to estimate actual weaning ages. Reconstructed weaning ages were used to estimate fertility and discuss three demographic events in past Japanese archipelago: urbanization of the premodern city of Edo (Chapter 3), urbanization of the medieval city of Kamakura (Chapter 4), and expansion of the Okhotsk culture in Hokkaido (Chapter 5). Demographic events in Japan were considered in this study because there are a wealth of skeletal samples and

information about socioeconomic, archaeological, and cultural backgrounds.

Chapter 2

Estimation of subadult bone collagen turnover rate and development of a model

Nitrogen isotope analysis of bone collagen has been used to reconstruct the breastfeeding practices of archaeological human populations as explained in Chapter 1. However, weaning ages have been estimated subjectively because of a lack of both information on subadult bone collagen turnover rates and appropriate analytical models. To overcome this problem, I adopt mathematical approaches. Temporal changes in human subadult bone collagen turnover rates are estimated, and a model for reconstructing precise weaning ages is then developed using a framework of approximate Bayesian computation and incorporating the estimated turnover rates. The model is applied to 39 previously reported Holocene skeletal populations from around the world, and the results were compared with weaning ages observed in ethnographic studies.

2.1 Introduction

2.1.1 Problems and objectives

In previous isotopic studies, weaning ages have been subjectively estimated from visual assessments of detectable changes in subadult bone collagen δ^{15}N values. To overcome these difficulties, attempts have been made to simulate changes in δ^{15}N values of subadult bone collagen in two pioneering studies. Schurr (1997) used exponential functions to describe changes in δ^{15}N values and estimate the age at the start of weaning. Millard (2000) suggested that the model proposed by Schurr (1997) suffered from a number of difficulties, and proposed an alternative model that further included a nitrogen mass balance and the age at the end of weaning. However, both models still suffer from the following three problems.

1. The subadult bone collagen turnover rates are not fully considered. The bone collagen turnover rate is high in early infancy (Bryant and Loutit, 1964; Rivera and Harley, 1965), but it decreases over the course of subadult growth (Szulc et al., 2000; Hedges et al., 2007). If not corrected, the lower bone collagen turnover rates at higher ages would generate significant discrepancies between the visible changes in bone δ^{15}N values and actual weaning ages.

2. Some parameters used to describe changes in δ^{15}N values are determined arbitrarily. Two parameters, ^{15}N-enrichment from maternal to infant tissues and the δ^{15}N values in weaning foods, could vary among different individuals and populations; therfore, they should be considered as variables in addition to the weaning ages. First, it has been reported that ^{15}N-enrichment varies to some extent in modern infant-mother pairs (between 1.7‰ and 2.8‰, $n = 7$: Fuller et al., 2006a) and in archaeological populations (between 0.5‰ and 4.4‰, $n = 25$: Waters-Rist and Katzenberg, 2010). Second, it is possible that δ^{15}N values of materials used in weaning foods were different than those used in adult foods (Dupras et al., 2001; Keenleyside et al., 2009).

3. The results are represented as point estimates without either probabilities or confidence intervals. The probabilities of the weaning parameters should be calculated to evaluate the validity of the computation results.

The objective of this chapter is to develop a model for analyzing cross-sectional δ^{15}N data of subadult bone collagen, and to compare weaning ages between modern ethnographic and archaeological skeletal populations. The model is programmed in R language, which is a free software environment for statistical computing and graphics (R Core Team, 2014). The model has the following three important features that are not present in the previous models:

1. The subadult bone collagen turnover rate is estimated anew and incorporated in the equations.

2. The enrichment factor and δ^{15}N values of weaning foods are included as target parameters to be estimated.

3. Using a framework of approximate Bayesian computation (ABC) allows researchers to calculate the probabilities and credible intervals of the weaning parameters.

2.1.2 Bone collagen turnover rate in subadult

Temporal changes in the bone collagen turnover rate must be considered to estimate a precise weaning ages from an observed isotope ratio. Bone collagen is laid down during childhood because of bone modeling, which is a formative process primarily associated with skeletal growth, and is replaced throughout life by bone remodeling, which is a coupled resorptive and formative process

that does not change the quantity of bone (Fratzl et al., 2004; Glimcher, 2006). As indicated in Figure 2.1, turnover refers to the proportion of newly synthesized bone collagen to the total bone collagen during modeling and remodeling over a unit of time. When the turnover rate is high enough (i.e., $\geq$1.0 per unit time), bone collagen at a specific age consist only of newly synthesized collagen, and the isotope ratio will immediately change with dietary changes. When the turnover rate is lower (i.e., $<$1.0), the bone collagen consists not only of newly synthesized but also previously synthesized collagen, the isotope ratio reflects recent and past dietary intakes.

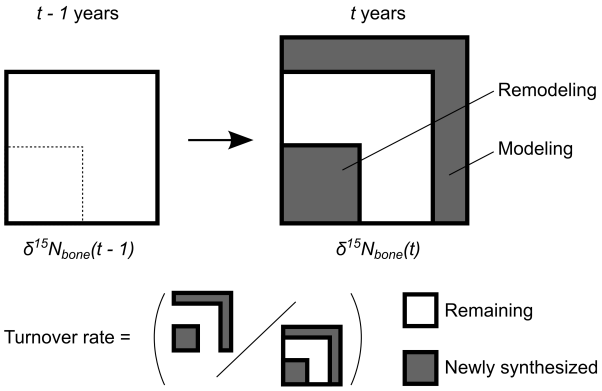

Figure 2.1: Schematic illustration of the bone turnover process. The δ^{15}N value for bone collagen at the unit time age of t years is represented as $\delta^{15}N_{bone}(t)$.

Although temporal changes in turnover rates of subadult bone minerals and collagen have been estimated by analyzing the uptake of ^{90}Sr fallout (Rivera and Harley, 1965; Papworth and Vennart, 1984) and bomb-^{14}C (Hedges et al., 2007), respectively, the estimates produced from these bulk cross-sectional studies were not necessarily precise. Some of the estimates were not based on direct measurements in subadults but on extrapolations from results for adults. In addition, the assumptions made about the dietary intake of tracers in these subadults were simplistic and ignore individual variation. In the present study, I calculated turnover rates from bone metabolism mechanisms at the tissue level so that more precise subadult bone turnover rates could be estimated.

Turnover rates of mineral and organic phases should differ because the mineralization process is much slower than the synthesis of the organic matrix. Bone is a composite material, and is mainly made of a calcified organic matrix (Rho et al., 1998; Glimcher, 2006). The microstructure of bone material consists of assembled collagen fibrils forming the organic phase, and tiny mineral particles reinforcing them (Fratzl et al., 2004). Two coupled processes are responsible for bone remodeling. The resorptive process involves osteoclasts dissolving the mineral phase by creating a low pH environment around the bone surface, and then producing a lysosomal protease to degrade the organic matrix (Teitelbaum, 2000). The next formative process inolves osteoblasts replacing the organic matrix and rapidly mineralizing it up to 70% of full mineralization capacity within

a few days (primary mineralization), the residual 30% of the mineralization occurring gradually over several years (secondary mineralization) (Fratzl et al., 2004; Ruffoni et al., 2007). The mineralization process has been formulated as a mineralization law (Ruffoni et al., 2007). Bone modeling occurs with a similar formative process as bone remodeling but with the resorption of the bone cartilage template instead of mineralized old bone (Scheuer and Black, 2000). Since the growth (Mitchell et al., 1945) and replacement (Leggett et al., 1982) (i.e., turnover, consisting of the modeling and remodeling processes) of bone minerals at the tissue level have been well documented, the turnover of bone collagen can be estimated by correcting the mineralization delay (Ruffoni et al., 2007).

2.1.3 Approximate Bayesian computation

ABC is a modern approach in Bayesian inference that allows posterior distributions to be evaluated when it is difficult to calculate the likelihood function, which describes probabilities under given parameters. Various ABC methods have been applied in diverse fields such as population genetics, evolutionary biology, ecology, and epidemiology (Beaumont, 2010; Bertorelle et al., 2010; Csilléry et al., 2010).

A general ABC algorithm takes a given observation x and repeat the following three steps until J points have been accepted:

1. Draw the candidate parameter θ_j from the prior distribution $\pi(\theta)$.
2. Simulate dataset x_j using θ_j and the model.
3. Accept θ_j if $\rho(x, x_j) \leq \alpha$, and otherwise reject θ_j.

Here $\rho(\cdot)$ is a function measuring the distance between simulated and observed data points, α is a fixed tolerance for the "closeness" of simulated and observed data, and x, x_j, and θ may be vector values. If $\rho(\cdot)$ measures appropriate distances and tolerance is sufficiently small, the accepted parameters reasonably approximate the posterior distributions. This is a rejection sampling algorithm, which is the simplest ABC procedure.

Although ABC has proved to be a flexible and powerful approach for evaluating posterior distributions, its major drawback is its inefficiency. Acceptance rates in the simple rejection sampling described above can be very low, especially when the posterior is a long way from the prior, which wastes computing time. Several algorithms have been proposed to increase the sampling efficiency, by introducing weighting with regression analysis (Beaumont et al., 2002; Leuenberger and Wegmann, 2010), Markov chain Monte Carlo sampling (Marjoram et al., 2003), and sequential Monte Carlo (SMC) sampling (Sisson et al., 2007; Beaumont et al., 2009; Toni et al., 2009). We used SMC sampling with corrected partial rejection control proposed by Sisson et al. (2007) because this method could be implemented more quickly and simply in my model

in the R software environment. SMC sampling is characterized by successively decreasing the tolerance, and weighted resampling from the previous parameter population.

2.2 Model

2.2.1 Estimating bone collagen turnover rates in subadult

In this study, bone collagen turnover rates in subadults were calculated from the modeling (Mitchell et al., 1945) and remodeling (Leggett et al., 1982) rates for cancellous bone minerals, and the mineralization law for the bone organic matrix (Ruffoni et al., 2007). "Turnover" is defined as the aggregated effects of bone modeling (i.e., the addition of bone tissue by skeletal growth) and remodeling (i.e., the replacement of existing bone tissues). Here I present a summary of the procedures, and the detailed mathematical expressions are given in the next two paragraphs. First, following Leggett et al. (1982), the bone mineral turnover rate $T_{min}[t]$ over one unit of time (i.e., one year from $t-1$ to t, Equation 2.4) in childhood was calculated using the functions that describe the temporal change in bone mineral mass $C(t)$ (Mitchell et al., 1945) (Equation 2.1) and the remodeling rate $\gamma(t)$ (Leggett et al., 1982) (Equation 2.2). Next, the bone collagen turnover rates $T_{col}[t]$ over one unit of time from $t-1$ to t years (Equation 2.5) were calculated sequentially with $T_{min}[t]$ and the mineralization law, $\lambda(i)$, which described the bone collagen mineralization process (Ruffoni et al., 2007). The mineralization law was derived from Ruffoni et al. (2007), and represents the rate of mineralization of the collagen portion at the ith year after the collagen matrix was formed (Equation 2.3). Finally, the resulting discrete turnover rates were coerced into a quartic polynomial (QP) formula (Equation 2.6). Turnover rates at ages less than one year were extrapolated from the QP function.

Basic functions to describe the temporal changes of bone mineral were derived from several previous studies. Following Mitchell et al. (1945), the bone mineral mass $C(t)$ at age of t years was represented as:

$$C(t) = 28.0 + 86.828t - 16.5105t^2 + 1.5625t^3 - 0.04114t^4 (0 \geq t \geq 20). \tag{2.1}$$

This equation represents the modeling process of bone turnover. On the other hand, the remodeling rate $\gamma(t)$ at age of t years was represented as follows:

$$\begin{aligned} \gamma(t) &= \frac{104.3}{C(t)}, \text{when t} \leq 1.5, \text{and} \\ \gamma(t) &= 0.975\text{e}^{-0.11t}, \text{when t} > 1.5. \end{aligned} \tag{2.2}$$

This equation was obtained from Leggett et al. (1982) and was derived from direct histological observations of subadult rib bone formation and resorption performed by Frost (1969). Note that a term for the radioactive decay of ^{90}Sr (0.025 per year), included in the original equations, was excluded from my equations. Following Ruffoni et al. (2007), the mineralization law $\lambda(i)$,

which describes the rate of mineralized collagen portion at ith years after the collagen matrix was formed, was set as:

$$\lambda(i) = c_1 \frac{1 + \frac{i}{i_1}}{\frac{i}{i_1}} + c_2 \frac{1 + \frac{i}{i_2}}{\frac{i}{i_2}}. \tag{2.3}$$

In this study, c_1, i_1, c_2 and i_2 were set as 18/23, 1/300, 25/92, and 5, respectively. Equation 2.3 corresponds to over 70% primary mineralization in a few days and protracted secondary mineralization of up to 100% in about 20 years, values that have been given in several previous studies (Akkus et al., 2003; Fratzl et al., 2004; Ruffoni et al., 2007, 2008).

Temporal changes in the bone mineral turnover rate can be represented using these functions. Put simply, the bone mineral turnover rate, $T_{min}[t]$, over one unit of time from $t-1$ to t years, was represented as follows:

$$T_{min}[t] = \frac{C(t) - C(t-1)}{C(t)} + \frac{C(t-1)}{C(t)} \int_{t-1}^{t} \gamma(x)dx. \tag{2.4}$$

The former and latter terms in the function indicate the effects of bone modeling and remodeling, respectively. Using the bone collagen turnover rate, $T_{col}[t]$, over one unit of time from $t-1$ to t years, $T_{min}[t]$ can also be represented as follows:

$$\begin{aligned} T_{min}[t] &= T_{col}[t]\Delta\lambda[1], \text{when t} = 1, \text{and} \\ T_{min}[t] &= T_{col}[t]\Delta\lambda[1] + \sum_{j=1}^{t-1}\left(\frac{C(j)}{C(t)} T_{col}[j]\Delta\lambda[t+1-j]\right), \text{when t} \geq 2. \end{aligned} \tag{2.5}$$

Here, $\Delta\lambda[i]$ is defined as follows:

$$\begin{aligned} \Delta\lambda[i] &= \lambda[i], \text{ when i} = 1, \text{ and} \\ \Delta\lambda[i] &= \lambda[i] - \lambda[i-1], \text{ when i} \geq 2. \end{aligned}$$

The former and latter terms in the second function shown in Equation 2.5 indicate the effects of turnover delay in the bone mineral for the intended unit of time (i.e., $t-1$ to t years) and the aggregated effects of the delay for the former unit times (i.e., 0 to 1 year, 1 to 2 years, .., and $t-2$ to $t-1$ years). The turnover rate over one unit of time (i.e., one year) from $t-1$ to t years can be sequentially calculated using Equation 2.4 and 2.5, as indicated in Table 2.1. The resulting discrete turnover rates were coerced to a quartic polynomial formula using the *nls* function in R, in accordance with the quartic formula for bone mineral mass (i.e., Equation 2.1). The formula is represented as follows:

$$T_{col}[t] = 1.778 - 0.4121t + 0.05029t^2 - 0.002756t^3 + 0.0005325t^4, \tag{2.6}$$

and is shown in Figure 2.2.

Table 2.1: Estimated temporal changes in turnover rates for bone minerals and collagen.

Age		Turnover rate (year^{-1})		
From	To	Mineral	Collagen	Collagen (QP)
0	1	1.217	1.474	1.413
1	2	0.908	1.059	1.134
2	3	0.786	0.892	0.924
3	4	0.700	0.776	0.771
4	5	0.629	0.682	0.664
5	6	0.571	0.611	0.590
6	7	0.527	0.558	0.540
7	8	0.492	0.520	0.507
8	9	0.462	0.489	0.483
9	10	0.434	0.461	0.463
10	11	0.407	0.432	0.441
11	12	0.378	0.402	0.416
12	13	0.349	0.370	0.386
13	14	0.319	0.337	0.349
14	15	0.289	0.302	0.306
15	16	0.258	0.267	0.260
16	17	0.227	0.231	0.213
17	18	0.194	0.193	0.171
18	19	0.158	0.151	0.139
19	20	0.118	0.104	0.124

QP: calculated from the quartic polynomial function.

2.2.2 Changes in δ^{15}N values of diet and bone collagen

Following Millard (2000), the δ^{15}N value of newly synthesized collagen at a given age of t years was defined by four parameters, the ages at the start (t_1) and end (t_2) of weaning, enrichment factor between the infant and mother (E), and δ^{15}N value of collagen synthesized entirely from weaning foods ($\delta^{15}N_{wnfood}$) (Equation 2.7 and 2.8). The δ^{15}N value of newly synthesized collagen equals the sum of the δ^{15}N value of the mothers tissue and enrichment factor before weaning ($t < t_1$), which changes in a parabolic manner during weaning ($t_1 \leq t \leq t_2$), and equals the collagen δ^{15}N value that fully reflects the consumption of weaning food ($t > t_2$).

Then, the incorporation of newly synthesized collagen and replacement of existing collagen in bone are simulated in over each successive unit time using the estimated turnover rate for bones

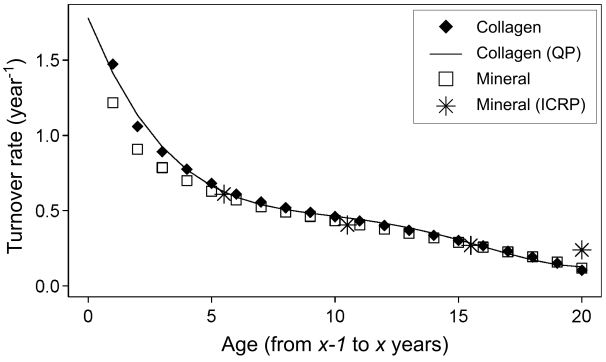

Figure 2.2: Estimated temporal changes in bone mineral and collagen turnover rates. Turnover rates of bone minerals and collagen are represented as discrete values, and that of collagen is fitted to QP plotted against age. Reference turnover rates of bone mineral (midpoint values for those of cortical and trabecular bones) estimated by ICRP are also shown (Valentin, 2002; see below).

(Equation 2.6 and 2.9). As most isotopic studies on weaning have focused on rib bones, because of their assumed fast turnover (Parfitt, 2002) and relatively trivial importance in morphological studies, the rate incorporated into the present model was that of cancellous bones. Although the rib bones that were sampled would have contained cortical parts, the relatively high surface to volume ratio in ribs would have resulted in a high proportion of cancellous parts and only thin cortical parts, making the turnover rate comparable to that of cancellous bones (Parfitt, 2002). Although one unit of time consists of one year, adjustments from the last unit of time enables simulated δ^{15}N values to be calculated for each individual in the dataset (Equation 2.10 and 2.11). Simulated δ^{15}N values, $\delta^{15}N_{bone}$, for each individual can be calculated under the given weaning parameters (t_1, t_2, E and $\delta^{15}N_{wnfood}$) using the model described above. The most appropriate weaning parameters can be estimated by minimizing the mean least square distance between the observed and resultant simulated change in bone collagen δ^{15}N values. The equations used to model the isotopic changes in subadult bone collagen during the weaning process are described in detail in the next four paragraphs.

Following Millard (2000), the δ^{15}N values for newly synthesized collagen $\delta^{15}N_{new}(t)$ at a given age of t years are given by the following equation:

$$\delta^{15}N_{new}(t) = (1 - p(t))(\delta^{15}N_{mother} + E) + p(t)\delta^{15}N_{wnfood}. \tag{2.7}$$

The proportion of non-milk protein in the total dietary protein intake at age of t is represented as $p(t)$. The δ^{15}N value for the mother's milk is described as $\delta^{15}N_{mother} + E$, using the δ^{15}N value for the mothers tissue, $\delta^{15}N_{mother}$ (approximated by the mean δ^{15}N value for adult females), and a ^{15}N enrichment factor for the transfer from the maternal to infant tissue, E. The δ^{15}N value for collagen synthesized from non-milk foods is represented as $\delta^{15}N_{wnfood}$. We considered

$\delta^{15}N_{wnfood}$ to be a variable because children in the past could have eaten weaning foods with different δ^{15}N values from the adult mean δ^{15}N values. This value has been approximated in previous studies as the mean δ^{15}N value for the adults.

The proportion of non-milk protein in the total dietary protein intake is assumed, in my model, to increase exponentially. The relative proportion of non-milk protein at the age of t years, $p(t)$, is described as follows:

$$\begin{aligned} p(t) &= 0, \text{when t} < \text{t}_1 \text{ (breast milk only)}, \\ p(t) &= (\frac{t-t_1}{t_2-t_1})^2, \text{when } \text{t}_1 \leq \text{t} \leq \text{t}_2 \text{ (during the weaning process), and} \\ p(t) &= 1, \text{when t} > \text{t}_2 \text{ (no breast milk)}, \end{aligned} \tag{2.8}$$

where the ages at the start and end of weaning are represented as t_1 and t_2, respectively. Equation 2.8 was derived from a model proposed by Millard (2000) and represents slow initial weaning and rapid final weaning. In the original model, four forms (linear, parabolic, reverse parabolic, and sigmoid) of dietary change were applied to the condition "during the weaning process" in Equation 2.8. Although the form of dietary change during weaning can be selected in the WARN package, I used only the parabolic form because Millard (2000) used a parabolic weaning pattern to model the changes in δ^{15}N in archaeological datasets. This seems to be a reasonable assumption as not only the amount of milk protein consumed decreases during the weaning process but also the proportion of milk protein consumed also decreases because of the increasing total dietary intake in growing subadults.

The δ^{15}N value for bone collagen at the age of t years, $\delta^{15}N_{bone}(t)$, is calculated as follows:

$$\delta^{15}N_{bone}(t) = (1 - T_{col}[t])\delta^{15}N_{bone}(t-1) + T_{col}[t]\int_{t-1}^{t} \delta^{15}N_{new}(x)dx. \tag{2.9}$$

The former and latter parts of the equation represent the remaining and the newly synthesized portion, respectively, of the bone collagen over one unit of time from $t-1$ to t years. Extending equation 2.9, the δ^{15}N value for bone collagen at the age of $t+a$, i.e., a being a part of one year from the unit time point t $(0 < a < 1)$, is represented as:

$$\delta^{15}N_{bone}(t+a) = (1 - T_{col}[t+a])\delta^{15}N_{bone}(t) + T_{col}[t+a]\int_{t}^{t+a} \delta^{15}N_{new}(x)dx. \tag{2.10}$$

In equation 2.10, $T_{col}[t+a]$ is the bone collagen turnover rate over a year from t to $t+a$, given by:

$$T_{col}[t+a] = \frac{\int_t^{t+a} T_{col}[x]dx}{\int_t^{t+1} T_{col}[x]dx} T_{col}[t+1]. \tag{2.11}$$

The bone collagen δ^{15}N values for each unit of time (one year) can be calculated sequentially, as reference values, using Equation 2.9 under the given parameters. The δ^{15}N values that correspond to the observed ages for the samples can then be calculated from the reference values and Equation 2.10. The initial bone collagen values at 0 year of age, $\delta^{15}N_{bone}(0)$, were approximated

using the mean δ^{15}N value of adult females, because the δ^{15}N value for infant tissue is assumed to be the same as to that of the mother (Fuller et al., 2006a). Theoretical δ^{15}N values for the age of each individual in the observed dataset can be calculated using Equation 2.10.

In my model, the differences between the individuals are evaluated by calculating mean square distance, D, between the observed and simulated δ^{15}N values. Put simply, point estimates of the parameters with minimized D can be calculated by solving the optimization problem (the application of the optimization problem to palaeo dietary reconstructions has been described by Little and Little, 1997). These represent point estimates under the framework of maximum likelihood estimates (MLE). Although the point estimates do not provide information on the error ranges, they will be used later in the SMC sampling procedures; therefore, optimized values for weaning parameters under the MLE framework were calculated. We used the *optim* function in R to obtain the optimized parameter value, θ_{opt}, and its resultant minimum mean square distance, D_{opt}.

2.2.3 Incorporation of ABC

To obtain posterior probabilities of the estimated parameters, fitting calculations between the observed and simulated data are performed under the ABC framework with SMC sampling. The details of the SMC procedures proposed by Sisson et al. (2007) that were used in my model are given in the next three paragraphs. Using the ABC framework, a number of weaning parameter sets that give well-fitted δ^{15}N values were sampled and assumed to represent the posterior distributions of the parameters. After applying the ABC procedure, posterior distributions were smoothed using the kernel density estimation (Wand and Jones, 1995), and joint probabilities for weaning ages (t_1 and t_2) and marginal probabilities for E and $\delta^{15}N_{wnfood}$ were calculated. In the density estimation, posterior probabilities were calculated to one decimal places for discrete parameter categories because strictly implementing the density estimation as a continuous distribution requires advanced numerical analysis techniques.

SMC sampling is characterized by a successive reduction in tolerance and a weighted resampling from the previous parameter population, called a "particle". Particles of preliminary simulations are used to calculate the next set of parameter vectors, to generate simulated data within a certain distance D from the observed data. The particles are then repeatedly resampled (according to a weighting scheme that considers the prior distributions), perturbed (using a transition kernel), and judged (on the basis of a successively decreasing tolerance). The particles after this iterative process finally approximate a sample of the posterior distribution of the parameters. In particular, the partial rejection control procedures prune away parameters that have minimal impacts on the final estimation in the parameter weighting step in the earlier stages of the tolerance reduction, and this increases the sampling efficiency (Liu, 2001).

To adopt the ABC framework, I added individual error terms ϵ_i in Equation 2.9 as follows:

$$\delta^{15}N_{bone}(t+a) = (1 - T_{col}[t+a])\delta^{15}N_{bone}(t) + T_{col}[t+a]\int_{t}^{t+a}\delta^{15}N_{new}(x)dx + \epsilon_i. \quad (2.12)$$

These errors were independently sampled from the normal distribution with mean of 0.0 and SD of σ, and individually assigned to simulated δ^{15}N values. By considering this individual error term, parameters that result in D values smaller than D_{opt} can be generated, which represent more plausible estimates for the measured data. In the ABC framework, D values are calculated using randomly generated parameters from the prior distributions, then the parameters that result in D values smaller than D_{opt} become the posterior distributions.

The sequential Monte Carlo algorithm in my model proceeds as follows (see Sisson et al. 2007 for more details):

1. Set prior distributions $\pi(\cdot)$ for the parameters and the number of particles j in one population. Calculate the final tolerance α_K $(= D_{opt})$ under the MLE framework and set decreasing tolerances. Set the population indicator $k = 1$ (initialization).

2. Set the particle indicator $j = 1$ (initialization).

   (a) If $k = 1$, independently sample θ^{**} from the prior distribution $\pi(\theta)$.
   If $k > 1$, sample θ^{*} from the previous population θ_{k-1} with weights W_{k-1}, and perturb the particle to θ^{**} with transition kernel ϕ.
   Simulate the change in the δ^{15}N value, $\delta^{15}N^{**}_{bone}(t)$, with θ^{**} using Equation 2.12. If $D^{**} \geq \alpha_k$, θ^{**} are rejected and then repeat procedure 2(a).

   (b) Set the indicators as follows:

$$\theta_k^{(j)} = \theta^{**},$$
$$W_k^{(j)} = 1 \text{ (if k = 1), and}$$
$$W_k^{(j)} = \frac{\pi(\theta_k^{(j)})}{\sum_{x=1}^{J} W_{k-1}(\theta_{k-1}^{(x)})\phi(\theta_k^{(j)} \mid \theta_{k-1}^{(x)})} \text{ (if k > 1).}$$

   If $j < J$, increment $j = j + 1$ and go to procedure 2(a).

3. Normalize the weights so that:

$$\sum_{j=1}^{J} W_k^{(j)} = 1.$$

   If the requirements for an effective sample size ESS are not met such as:

$$ESS = \frac{1}{\sum_{j=1}^{J} {W_k^{(j)}}^2} < \frac{J}{2},$$

   sample with replacement, the particles $\theta_k^{(j)}$ with weights $W_k^{(j)}$, to obtain a new population $\theta_k^{(j)}$, and set weights $W_k^{(j)} = \frac{1}{J}$.

4. If $k < K$, increment $k = k + 1$ and go to procedure 2.

Prior distributions $\pi(\cdot)$ were set as normal distributions with default means of {0.5, 3.0, 1.9, $\delta^{15}N_{mother}$, and 0.0} and SDs of {3.0, 3.0, 0.9, 3.0, and 1.0} for t_1, t_2, E, $\delta^{15}N_{wnfood}$, and σ, respectively. The mean weaning age was obtained from values recommended by modern pediatricians and the biologically expected ages (Dettwyler, 2004). The mean and standard deviation of the enrichment factor E was obtained from the values reported by Waters-Rist and Katzenberg (2010). The hyper parameter for the individual error term σ was used as an absolute value in the calculation. The default number of particles J was 10,000. Decreasing tolerances α_k were set as D_{opt} + {2, 1, 0.5, 0.25, 0.125, 0.0625, 0} and, therefore, the number of populations $K = 7$. The transition kernel ϕ was set to be a normal distribution with a mean of 0.0 and SD of 0.1.

2.3 Materials and methods

2.3.1 Application to archaeological populations

The developed model was applied to previously reported δ^{15}N datasets of archaeological skeletal populations to validate the model and discuss human breastfeeding and weaning practices in the Holocene. The target populations were chosen according to the following criteria: (1) the numerical values for age and bone collagen δ^{15}N were reported and (2) more than six subadult individuals were included. The mean and SD of adult females and all adults were calculated anew if their individual numerical values were reported, and otherwise the summarized values indicated in article, table, or figure were used. Data from teeth were excluded. The E and $\Delta^{15}N_{adult-wnfood}$ (the difference between the $\delta^{15}N_{wnfood}$ and mean δ^{15}N value for all adults) values were estimated on the basis of the mean δ^{15}N values of adult females and total adults, respectively. If there were no mean δ^{15}N values for adult females, the values for total adults were used instead, and *vice versa*. Information on the time period when the population lived and the type of subsistence practiced (hunting–gathering or not) were extracted from the literature. For the purposes of comparison, weaning ages that had been reported numerically in three independent meta-analyses of the ethnographic literature were used (Ford, 1945; Barry and Paxson, 1971; Sellen, 2001). Although there would been overlap among the target ethnographic populations and problems relating the non-independence of cultures (i.e., the Galton's problem: Mace and Pagel, 1994) for both the archaeological and ethnographic populations, I treated them separately.

The model was applied to 39 archaeological populations that had been reported in 29 different publications (Figure 2.3; Table A.1). Since I targeted diet in early life before adolescence, the age of the individuals used in this study was limited to below 10 years. The prior distributions were set to have normal distributions with means of {0.5, 3.0, 1.9, and $\delta^{15}N_{mother}$} and SDs of {3.0,

3.0, 0.9, and 3.0} for t_1, t_2, E, and $\delta^{15}N_{wnfood}$, respectively, except for one population. For the Isola Sacra population (Prowse et al., 2008), the prior distributions were set to have means of {0.0 and 1.0} and SDs of {1.0 and 1.0} for t_1 and t_2, respectively, because this dataset had the unique features of younger weaning process and higher ^{15}N-enrichment in infants, which complicated the parameter optimization. The mean weaning age was obtained from the ages recommended by modern pediatricians and the biologically expected ages (Dettwyler, 2004). The mean and SD values for E was obtained from values reported by Waters-Rist and Katzenberg (2010). The number of "particles" (i.e., unit sets of weaning parameters resampled) was 10,000. The number of parameter "populations" (i.e., units of successively reducing tolerance) was seven.

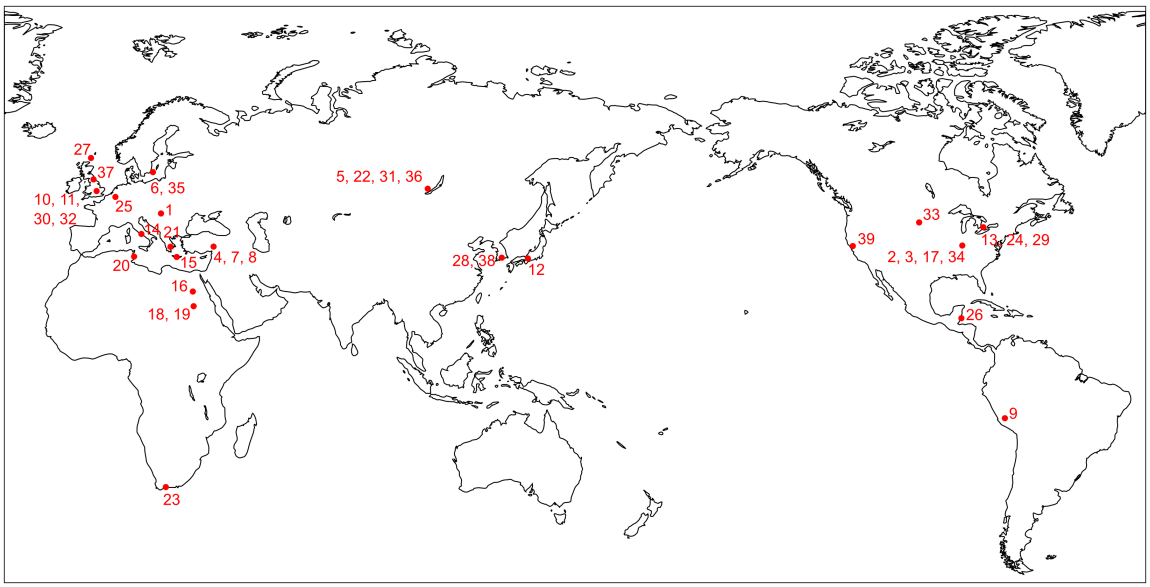

Figure 2.3: Location of the 39 archaeological populations used in this study. See Table A.1 for correspondence between number and name of the populations.

2.3.2 Statistics

All statistical calculations were performed using R software (R Core Team, 2014).

2.4 Results

2.4.1 Subadult bone collagen turnover rate

The calculated turnover rates are shown in Table 2.1 and Figure 2.2. The turnover rate of bone collagen was estimated to be larger than that of bone mineral until an individual reaches their late teens, and to decrease over the course of subadult growth. The integrated bone collagen turnover rate from 0.0 to 1.0 years of age was estimated to be 1.588, and the estimated bone

collagen turnover rate was higher than 1.000 per year by two years of age (see Table 2.1). The integrated turnover rate from 0.0 years of age reached 0.966 at 0.60 years of age, suggesting that it takes 31 weeks for infants to fully reflect post-birth dietary δ^{15}N signals. The integrated turnover rate from the age of 19.0 to 20.0 years of age was estimated to be 0.130.

2.4.2 The implemented model

The model developed in the present study is distributed as the R package WARN (Weaning Age Reconstruction with Nitrogen isotope analysis), which is freely available under the GPL license and can be downloaded from the Comprehensive R Archive Network. Although they are not considered in the present study, credible intervals can be calculated for a given parameter range using the WARN package. Images of the results calculated using the package are shown in Figure 2.4.

2.4.3 Application of the model

The maximum density estimators (MDEs, i.e., weaning parameters that result in maximum probability density), posterior probabilities, and the other information from each archaeological dataset are given in Table A.1. To standardize the results, the estimated $\delta^{15}N_{wnfood}$, which is the δ^{15}N value of collagen synthesized entirely from weaning foods, is represented as a difference from the mean δ^{15}N value for total adults, $\Delta^{15}N_{adult-wnfood}$.

Results for the Bjärby population (Howcroft et al., 2012) were excluded because E for the population was estimated to be negative (i.e., -1.6‰, see Table A.1). There was only one individual less than three years of age in the Bjärby population, so this biologically unexpected temporal change pattern in the δ^{15}N value appears to be an artifact. Relationships between MDEs and the logarithmic probabilities are shown in Figure 2.5. A negative linear correlation was found between the age at the end of weaning, t_2, and the logarithm of the joint probability of the weaning ages (Spearman's rank correlation test: $R = -0.72$, $p < 0.001$) but the other parameters did not indicate evident relationship. Assuming that MDEs with probabilities of less than 0.05 (0.0025 for the joint probability of the weaning ages) were unlikely, they were also excluded from the subsequent analyses. A Kruskal–Wallis test indicated that the MDE sets did not differ by the type of bone analyzed (rib, a combination of a rib and another, and those other than ribs; t_1: $\chi^2 = 0.52$, $p = 0.770$; t_2: $\chi^2 = 0.63$, $p = 0.731$; E: $\chi^2 = 0.42$, $p = 0.812$; $\Delta^{15}N_{adult-wnfood}$: $\chi^2 = 4.43$, $p = 0.109$). Although the parameters have credible intervals (see Figure 2.4 for an example), only MDEs are considered in the subsequent analyses to simplify the discussion.

Histograms of the MDE parameters are shown in Figure 2.6, and summarized in Table 2.2. In MDEs of t_1 and t_2, 18 (52.9%) and 21 populations (68.8%) is in the range between 0.0 and 1.0 years and between 1.0 and 3.0 years of age, respectively, and frequencies decrease with age. MDEs

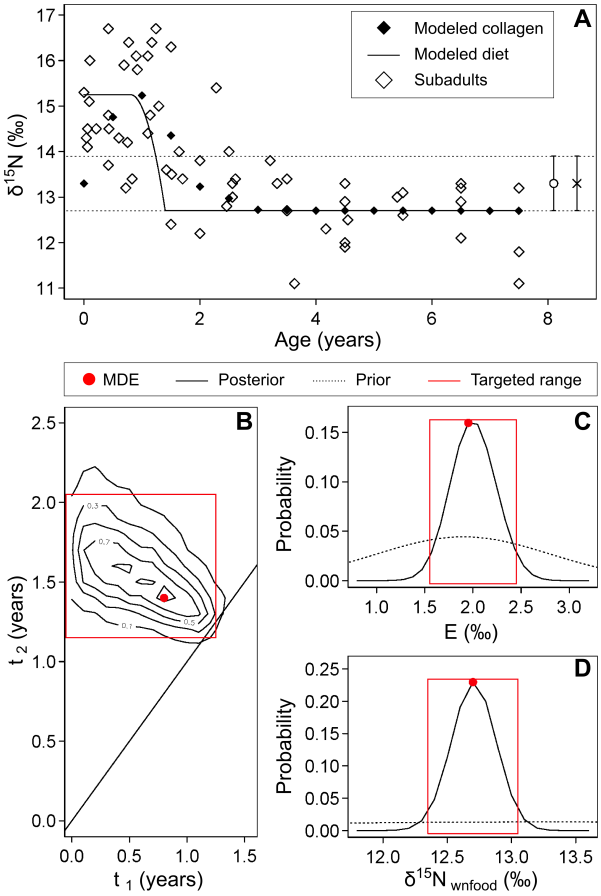

Figure 2.4: An example of the results of applying WARN model using the Spitalfields population (Nitsch et al., 2010, 2011) as a case study. (A) Modeled temporal changes in the δ^{15}N values by subadult age calculated from the reconstructed MDEs. Mean and SD ranges for adult females and all adults are indicated with open circles and crosses, respectively. (B) Contour lines show the posterior probability for the combination of weaning ages. The target ranges for t_1 and t_2 are 0.0–1.2 years and 1.2–2.0 years of age, respectively, and the calculated joint probability for the ranges is 0.942. (C) Distribution of posterior probabilities for the ^{15}N-enrichment from maternal to infant tissues. The target range is 1.6–2.4‰, and the calculated marginal probability for the range is 0.961. (D) Distribution of posterior probabilities for the δ^{15}N values for collagen synthesized entirely from weaning foods. The target range is 12.4–13.0‰, and the calculated marginal probability for the range is 0.960. Subadult ages and bone collagen δ^{15}N values were taken from Nitsch et al. (2010, 2011).

of the other two parameters distribute on the both sides of the mode, and bands ranging between 2.0 and 3.0‰ and between -1.0 and -0.5‰ represented modes for E and $\Delta^{15}N_{adult-wnfood}$, respectively. The means of all of the 38 parameters weighted by their probabilities, including parameters with probabilities below 0.0025 (joint probability of the weaning ages) or 0.05, are

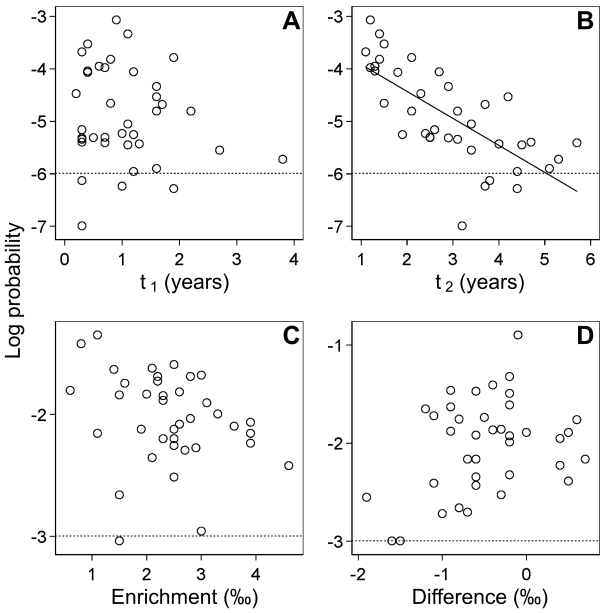

Figure 2.5: Relationships between MDEs and the logarithms of the probabilities of the four weaning parameters for the archaeological populations. Dotted lines indicate the thresholds of probability. (A) and (B) The age at the start and end of weaning, respectively. (C) ^{15}N-enrichment from maternal to infant tissue. (D) The difference between the δ^{15}N value of collagen synthesized entirely from weaning foods and the mean δ^{15}N value for all adults. A regression line is also shown for t_2 (slope = -0.514, intercept = -3.403).

also shown in Table 2.2.

Table 2.2: Summary of MDEs for the archaeological populations calculated using the developed model.

Parameter	Mean	SD	Median	n	Weighted mean
t_1 (year)	1.07	0.78	0.95	34	0.94
t_2	2.80	1.32	2.55	34	2.10
E (‰)	2.44	0.90	2.50	37	2.30
$\Delta^{15}N_{adult-wnfood}$ (‰)	-0.48	0.61	-0.55	38	-0.41

Weighted means are calculated from all 38 MDEs, including those with probabilities less than 0.0025 (joint probability) or 0.05 (marginal probability).

The estimated weaning ages in various archaeological populations were compared in terms of the time period when the population were alive and the type of subsistence. No relationship was found between weaning ages and the midpoint time when the populations were alive (Figure 2.7).

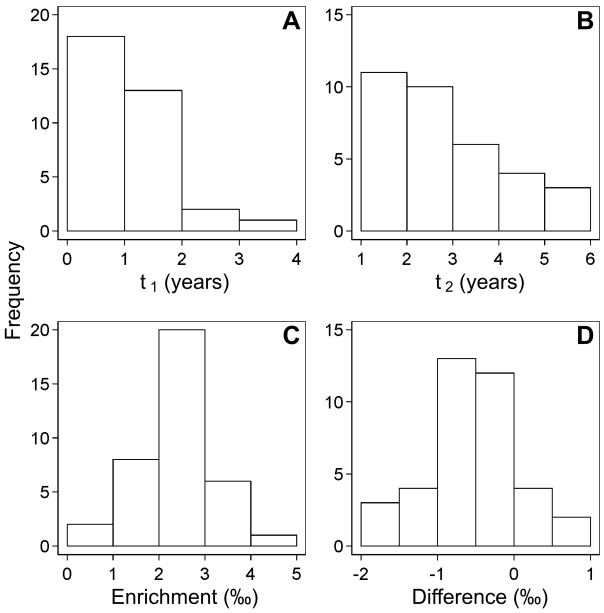

Figure 2.6: Histograms for the MDE distributions of the four weaning parameters for the archaeological populations. See Figure 2.5 for the meanings of the labels.

Mann–Whitney U tests did not indicate significant differences in t_1, t_2, E, and $\Delta^{15}N_{adult-wnfood}$ between hunter–gatherer (HG) and non-hunter–gatherer (NHG) populations (t_1: $U = 97$, $p = 0.932$; t_2: $U = 78.5$, $p = 0.509$; E: $U = 77$, $p = 0.285$; $\Delta^{15}N_{adult-wnfood}$: $U = 66$, $p = 0.055$), but did indicate a large difference for $\Delta^{15}N_{adult-wnfood}$. The mean and SD for $\Delta^{15}N_{adult-wnfood}$ values of the HG and NHG populations were -0.14 ± 0.40‰ and -0.57 ± 0.63‰, respectively. The mean and standard deviations (SDs) of MDEs by type of subsistence are shown in Table 2.3.

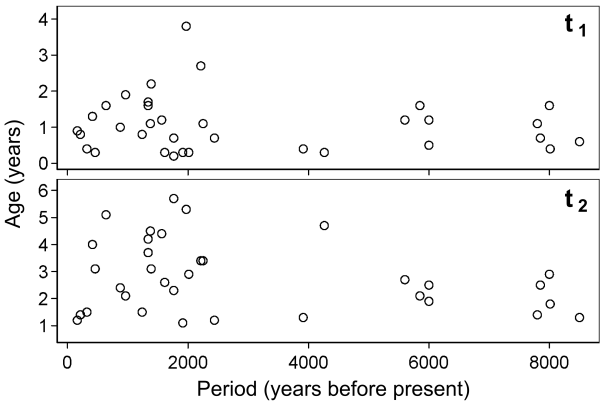

Figure 2.7: Maximum density weaning ages plotted by the midpoint time period for the populations.

Table 2.3: Mean and SDs of MDEs for the archaeological populations by the type of subsistence.

Subsistence	t_1 (year)	t_2 (year)	E (‰)	$\Delta^{15}N_{adult-wnfood}$ (‰)
Hunting–gathering	0.93 ± 0.37	3.01 ± 1.25	2.54 ± 0.68	-0.14 ± 0.40
Non-hunting–gathering	1.11 ± 0.85	2.74 ± 1.35	2.38 ± 0.94	-0.57 ± 0.63

MDEs for the weaning ages of the archaeological populations were compared to those obtained from ethnographic studies (Table 2.4) as illustrated in Figure 2.8. A Mann–Whitney U test indicated that the ethnographic populations were significantly younger ($U = 361.5$, $p < 0.001$) at the start of weaning (t_1). However, the Kruskal–Wallis test showed no significant differences in the age at the end of weaning among archaeological and ethnographic populations ($\chi^2 = 7.15$, $p = 0.067$).

Table 2.4: Summary of ages at the start and end of weaning found in previous studies of ethnographic populations.

Weaning age (year)	Mean	SD	Median	n	Reference
Start	0.48	0.50	0.48	42	Sellen (2001)
End	2.63	0.98	2.50	156	Barry and Paxson (1971)
	2.72	0.72	2.50	44	Ford (1945)
	2.39	0.87	2.50	108	Sellen (2001)

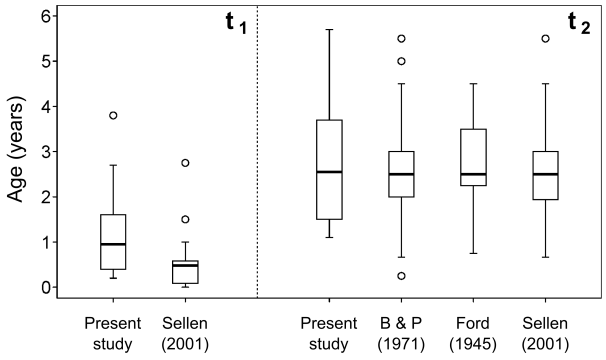

Figure 2.8: Boxplots for the weaning ages estimated in this study and observed in previous ethnographic studies. Data were obtained from Barry and Paxon (1971), Ford (1945), and Sellen (2001).

2.5 Discussion

2.5.1 Bone collagen turnover rate

Tracer intake and biochemical marker studies have shown that the bone mineral and collagen turnover rates are high in the first few years of life (i.e., >1.0 per year: Bryant and Loutit, 1964; Rivera and Harley, 1965; Szulc et al., 2000), which is consistent with my results (see Table 2.1 and Figure 2.2). However, temporal changes in the bone collagen turnover rate after infancy and before adulthood have never been estimated directly and continuously, and the present study allowed them to be estimated. An isotopic study on an archaeological infant of a known age has suggested that infant rib bone collagen can fully reflect post-birth dietary ^{15}N input, in an extreme case, in only five to six weeks (Nitsch et al., 2011), but this is estimated to take 31 weeks from my results. Our study allows typical temporal changes to be estimated, but the bone collagen turnover rate in subadulthood probably varies. Although details of the calculation are unclear, Valentin (2002) indicated typical turnover rates of bone as a reference, which were estimated based on turnover of bone-seeking radionuclides. This reference values correspond and are in a good agreement with the turnover rates of bone mineral estimated in this study (see Figure 2.2).

The integrated bone collagen turnover rate from 19.0 to 20.0 years of age was estimated to be 0.130 per year in my study (see Figure 2.2), which is a little higher than that proposed by previous studies: 10.4 ± 2.7% during adulthood (Stenhouse and Baxter, 1979) and 9.7% and 4.1% for 20-year-old male and female femora, respectively (Hedges et al., 2007). Although the type of bone sampled by Stenhouse and Baxter (1979) is not stated, differences between the turnover rates in different bone types could cause these different results. The turnover rates are higher in bones with greater surface to volume ratios than those in bones with smaller ratios (Parfitt, 2002). Ribs, which were target bones in my study, have relatively high proportions of cancellous and thin cortical parts, whereas femur analyzed by Hedges et al. (2007) has a lower proportion of cancellous and thick cortical parts. although there are slight differences, the overall trend of the temporal changes in bone turnover rates in this study is consistent with previous estimates.

2.5.2 Validity of the model

Although it is desirable to test validity of the model, the absence of proper test data means this is not possible. Archaeological skeletal populations cannot be tested because the true weaning ages are usually unknown, and historical literature, if any, describing breastfeeding practices at the time period when the population lived sometimes differs from actual practices (e.g., Fildes, 1982; Nitsch et al., 2011; see also Dupras et al., 2001; Fuller et al., 2006b; Prowse et al., 2008). Since the model presented here was intended for human subadult bones, conducting an experimental study was difficult, and hair, nail, and other tissues were not suitable for analysis because they

have different turnover rates than bone collagen. Experimental studies of animals other than human would not be appropriate because human growth patterns are unique among mammals (Bogin, 1999; Kennedy, 2005); therefore the nitrogen mass balance in human subadults would probably be different from that in other animals.

Although there is no way of testing the validity of the model under existing conditions, the present model provides advantages in the objective comparison of weaning parameters among different populations. For example, lower and higher ^{15}N-enrichment values of infants than the biologically expected values of 2 to 3‰ (Fuller et al., 2006a) have been reported in Wetwang (Jay et al., 2008) and Isola Sacra (Prowse et al., 2008) populations, and these results are objectively shown to be 0.8‰ and 4.6‰, respectively, by the model presented here (see Table A.1).

2.5.3 Weaning practices in archaeological populations

Assuming that lower probabilities for the weaning parameters suggests greater individual variability, a negative linear relationship between the MDEs of t_2 and the logarithms of their probabilities can be interpreted from two perspectives. First, early end-of-weaning departures from the norm would increase with the norm of older weaning age; therefore, individual variations within the population would increase. Second, the subadult bone collagen turnover rate decreases as their age increases (see Figure 2.2) and the bone takes longer to reflect isotopic changes in dietary input, which would result in individual variations in the weaning age being amplified. Another interpretations could be made on MDEs of t_1 that they are so small that the two factors just mentioned have little effect on the relationship between MDEs of t_1 and its probabilities.

Provided that the developed model produces valid estimates, some aspects of breastfeeding and weaning in the Holocene human populations would emerge. Some bioarchaeological studies have led to the hypothesis that breastfeeding duration become shorter around the time of the subsistence transition from hunting–gathering to agriculture during the Holocene mainly because of the availability of weaning food (Buikstra et al., 1986; Molleson et al., 1993; Larsen, 1995), but no significant differences were observed between HG and NHG population weaning ages. Furthermore, there were no consistent trends of the secular changes in the estimated MDEs of weaning ages (Figure 2.7). These results suggest that the type of subsistence and time period are not determining factors for weaning ages in a population. The hypothesis of shortened breastfeeding periods in NHG populations is not supported by an ethnographic meta analysis (Sellen and Smay, 2001), and the results reconstructed from archaeological populations in the present study support this conclusion. However, $\Delta^{15}N_{adult-wnfood}$ values were greater in NHG populations than those in HG populations although the difference is not significant. Assuming that the cause of this difference is dietary, this could be interpreted as indicating that NHG people fed their subadults more lower trophic level weaning foods, such as plants, than HG people did. Although the type of subsistence did not determine the weaning ages, it could have affected the weaning food used.

MDEs of t_1 were significantly higher than those reported for ethnographic populations (Figure 2.8), and also higher than that required biologically for infant health (i.e., beginning at the age of six months: Kramer and Kakuma, 2004). This difference would have been caused by a discrepancies in the intended "age at start of weaning" among ethnographic, nutritional, and isotopic studies rather than a reflection of actual weaning practices in the past. The δ^{15}N values in bone collagen mainly reflect the ratios of dietary protein (Ambrose and Norr, 1993; Tieszen and Fagre, 1993), but nutritionists and cultural anthropologists see the age when liquid or solid foods are first introduced as the start of weaning, without considering the protein contribution from these foods (Dettwyler and Fishman, 1992; Sellen and Smay, 2001). Subadult nutritional requirements are not necessarily met by protein, and high-protein foods are often avoided as weaning foods, especially in early part of the weaning process (de Kanashiro et al., 1990; WHO, 1998, 2009). It would be valid to assume that the actual age when solid or liquid foods were introduced could be lower, but that the subadult bone collagen δ^{15}N value did not record the event.

There were no significant differences in the age at the end of weaning between archaeological and ethnographic populations, and my results suggest that the age at the end of weaning in human populations without modern artificial baby foods did not change in a consistent manner throughout the Holocene. However, although results from observational and isotopic studies are not necessarily directly comparable, the estimated mean MDE of t_2 (2.80 $\pm$ 1.32 years) is still smaller than the age at the end of weaning in great apes (3.0 years for gorillas, 4.8 years for chimpanzees, and 6.0 years for orangutans: Hawkes et al., 1998). Shortening of the breastfeeding period would have occurred during the process of human evolution in the Pleistocene. Existing nitrogen isotope analysis for reconstructing weaning practices requires many well-preserved subadult bones, new analytical methods, such as stable isotope analysis of serial sections of tooth dentin (Eerkens et al., 2011; Beaumont et al., 2013b), and enamel (Wright, 2013) and the analysis of Sr/Ca ratios in tooth enamel (Humphrey et al., 2008), could allow weaning ages to be reconstructed for more ancient hominins.

Although it is possible that the prior distribution affect the result, set of MDEs of E (mean: 2.44 $\pm$ 0.90‰) is consistent with biologically valid values. Isotope analysis on modern human mother and infant pair have reported that the ^{15}N-enrichment is in the range between 1.7 and 2.8‰ (Fuller et al., 2006a). Reconstructed E vary to some extent (Figure 2.6C), and this would possibly be stem from variations in mother's diet (Prowse et al., 2008; Gardner et al., 2011) and/or physiological factor affecting nitrogen mass balance such as growth (Waters-Rist and Katzenberg, 2010), nutritional adjustment in metabolism (Duggleby and Jackson, 2002), pathology (Katzenberg and Lovell, 1999), and drought (Schwarcz et al., 1999).

Most of the $\Delta^{15}N_{adult-wnfood}$ values were negative, which could be interpreted as having dietary or physiological causes. First, it is probable that the proportion of foods from lower trophic levels with lower δ^{15}N values was universally greater in the subadult than that in the

adult diet. It has been reported in ethnographic studies that lower trophic-level foods such as cereals and legumes are preferred to higher trophic-level foods such as animal and fish foods as weaning foods in various populations (Ford, 1945; Hull and Simpson, 1985; de Kanashiro et al., 1990; Sellen and Smay, 2001), and animal milk would not be universally available as a weaning food in the ancient period. Second, a positive nitrogen balance in developing subadults would probably be one of the reasons for lowered tissue δ^{15}N values (Fuller et al., 2004). Although the effect of growth is not evident in the long bones of juvenile and adolescents from seven to nineteen years of age (Waters-Rist and Katzenberg, 2010), I studied rib bones of younger subadults. The turnover rate in younger subadult bones would be greater than that in juvenile and adolescent bones (see Figure 2.2); therefore, it is possible that the effect of growth on the δ^{15}N values is recorded and evident in the present study.

2.5.4 Limitations of the developed model

Finally, there are two caveats to consider before applying the model presented here. First, the present model is intended for bones with relatively high turnover rates, such as cancellous bones or ribs. Although WARN was applied equally to isotopic data from bones with relatively low surface to volume ratios (e.g., limbs, cranium, and mandible) to maximize the sample size in the present study, attention to this aspect is required for more precise analysis. Second, the WARN approach will always attempt to fit a model, even if the subadult δ^{15}N values do not indicate breastfeeding and weaning signals. If researchers cannot find patterns of isotopic changes by visually inspecting the data, they are urged to examine their data carefully before applying the model, for example, for a biased age distribution or high isotopic variability in subadults. Although the estimated turnover rate and model developed can be further improved, in this study, I propose a framework for objectively and quantitatively analyzing and interpreting subadult bone collagen δ^{15}N values. A precise reconstruction of past breastfeeding and weaning practices over a wide range of time periods and geographic regions could make it possible to understand this unique feature of human life history and cultural diversity in infant feeding practices (Lee, 1996; Bogin, 1997; Hawkes et al., 1998; Kennedy, 2005; Sellen, 2007; Humphrey, 2010).

From the the next chapters, the developed model will be applied to three skeletal populations in Japan to estimate actual weaning ages. Reconstructed weaning ages will be used to estimate fertility and discuss three demographic events in ancient Japanese archipelago.

Chapter 3

Urbanization of the premodern city of Edo (Hitotsubashi)

Urbanization is the physical growth of cities, which affects the lifestyle of people, culture, and population dynamics (Woods, 2003). To investigate population dynamics during urbanization, one needs to evaluate migration and the natural increase of the population (Keyfitz, 1980). Natural increase is established by the mortality and fertility of the population, and the former is generally higher in urban areas (Honda, 1997; Lewis, 2002; Nagaoka and Hirata, 2007). This study aims to reconstruct the weaning ages of the Edo period population by stable isotope analyses and discusses fertility and population dynamics during urbanization in pre-modern Japan.

3.1 Introduction

3.1.1 Population dynamics in the city of Edo

The Edo period in Japan lasted from AD 1603 to 1867 under the governance of the Tokugawa shogunate. This period witnessed improvements in the physical well-being, economic growth, and accomplishments in the lifestyle and diet (Hanley and Yamamura, 1977; Hanley, 1997; Hayami, 1999; Harada, 2009). There were many castle towns with more than a thousand inhabitants, which were connected by an active commercial network. In particular, Edo, Osaka, and Kyoto were developed cities having populations of more than hundreds of thousands although the background and process of urbanization differed among them (Saito, 2002; Hamano, 2007). Edo, former name for Tokyo, was the largest city and capital of feudal Japan with a population that included townspeople, warriors, priests, and loafers of around 1.3 million by early 18th century (Naito, 1966).

Temporal changes in the population of Edo have been reconstructed at the macro level (Sekiyama, 1958; Naito, 1966; Hayami, 1999). Before the end of the 16th century, Edo was a

minor castle town. After the Tokugawa family took over the governance of Japan at the beginning of the 17th century, Edo was established as the capital and experienced rapid development and population increase. The Tokugawa shogunate forced hundreds of feudal lords all over Japan to keep their wives and children in Edo and periodically work there. As a result of these impositions, many warriors immigrated to live in the city as well as craftspeople, merchants, and others. By early 17th century, Edo became the largest city in Japan. The population of Edo was censused every six years by the Tokugawa officials since AD 1721; the number of townspeople reached approximately 0.50 million and stabilized at 0.53–0.54 million until the end of the Edo period (Sekiyama, 1958; Minami, 1978). Historians have assumed that in-migration from neighboring rural areas rapidly increased the population in the early Edo period, and equilibrium between increase (i.e., in-migration and fertility) and decrease (i.e., out-migration and mortality) stabilized the population in the late Edo period (Sekiyama, 1958; Minami, 1978; Saito, 2002). However, approaches at the process of the population increase are needed to empirically evaluate these demographic assumptions.

Historical demography, a study in census documents (*shumon aratame cho*: see Cornell and Hayami, 1986 for detail), is such an approach to investigate population dynamics at the level of fertility and mortality particularly in rural areas in the late Edo period. Historical demographers have revealed the regional and temporal variabilities of population dynamics in the Edo period Japan (Kito, 2000; Hayami, 2001). In rural areas, the population increased fairly rapidly during the early Edo period (17th century), but the rate of population increase began to decrease by the first half of the 18th century. These changes were mainly interpreted from a socioeconomic perspective (e.g., Hanley and Yamamura, 1977; Kito, 2000; Hayami, 2001). In several minor urban areas of the late Edo period, people suffered lower fertility and higher mortality, and urban areas mainteined their population by attracting immigrants from rural areas. This is a so-called "urban graveyard" hypothesis (Smith, 1973; Hayami, 1999, 2001; Kito, 2000; but see Sharlin, 1978; Saito, 2002). However, the historical demographic approach is generally difficult to apply to urban areas because urban areas suffered the effects of fires and turnover of inhabitants more than rural areas; thus, historical documents were more easily lost. Furthermore, a historical demographic study of Edo is hopeless because several aerial attacks in World War II destroyed the historical census documents (Hamano, 2007). Therefore, previous historical demographic studies of Edo are quite limited. Only apprenticeship (Minami, 1978), subsistence composition (Yoshida, 1992), and labor market (Saito, 2002) have been studied in a few areas of Edo at the end of the Edo period. To the best of my knowledge, there is no empirical historical study of the demography or demographic parameters of Edo during the early Edo period.

Therefore, a new approach to study the demographic parameters of Edo in the early Edo period is needed to understand the urbanization of Edo, the world largest city at that time. Bioarchaeological approaches can provide important information concerning the living conditions, mortality, and fertility of inhabitants of Edo, which historical evidence does not furnish.

This study aims to reconstruct infant feeding practices, one of the most important determinants of fertility, in the early Edo population excavated from the Hitotsubashi site, Tokyo, Japan. Mortality patterns of the Hitotsubashi population have already been reconstructed in a palaeodemographic study based on the age structure of the skeletal population (Nagaoka and Hirata, 2007); therefore, population dynamics during urbanization can be empirically discussed in more detail.

3.1.2 Previous isotopic studies of human skeletons from the Edo period

Several isotopic studies have revealed that adult humans in the Edo period Japan generally consumed foods from C_3, freshwater, and marine ecosystems (Table 3.1, but see also Yoneda et al., 2011), which is consistent with historical and archaeological data (Hanley, 1997; Ehara et al., 2009; Harada, 2009). However, infant feeding practice in the Edo period was reconstructed for only one skeletal population from Fushimi, an urban city in Kyoto (Kusaka et al., 2011). Although the number of subadult individuals analyzed was only six and the dates of the samples ranged the entire Edo period, Kusaka et al. (2011) showed older weaning ages in this population (i.e., consumption of weaning foods by the age of four years and end of weaning by the age of six years).

Table 3.1: Comparison of mean isotope ratios of adult human skeletons from different Edo period sites.

Site	Location	δ^{13}C	δ^{15}N	n	Reference
Anrakuji-Higashi	Yamanashi	-18.3 ± 0.2	10.2 ± 0.7	5	Yoneda, 2000
Fushimi	Kyoto	-19.7 ± 0.6	12.1 ± 0.6	27	Kusaka et al., 2011
Kaneiji	Tokyo	-20.2 ± 0.7	12.3 ± 1.3	19	Koike et al., 1990
Kaneiji	Tokyo	-18.3 ± 0.6	12.4 ± 0.9	11	Yoneda, 2012
Kamiizawa-one, Hodokubo	Nagano	-17.7 ± 0.9	12.1 ± 1.5	5	Yoneda et al., 1996
Unseiji	Hyogo	-19.8	13.9	1	Nagaoka et al., 2013a
Uozu	Toyama	-20.1	12.8	1	Kaifu and Yoneda, 1999
Hitotsubashi	Tokyo	-19.4 ± 0.5	11.0 ± 0.8	46	This study

3.1.3 Hitotsubashi site

The Hitotsubashi site (or Hitotsubashi High School site) in Tokyo (Figure 3.1) was the graveyard of the Joan-ji temple before the Great Fire of Meireki in 1657 and that of the Hozen-ji and Gangyo-ji temples after the fire (Kato, 1985). The burials represent the early Edo period between 1657 and 1683. The excavation was conducted in 1975 and was the first major one that specifically

considered Edo as an object of archaeological investigation (Vaporis, 1998). The larger proportion of graves consisted of cheap rounded wooden coffins (*hayaoke*) that were often used for the lower social statuses, suggesting that the people buried represent townspeople. Cinerary urns containing cremated bones, jar burials, and direct burials in pits were also found (Kato, 1985).

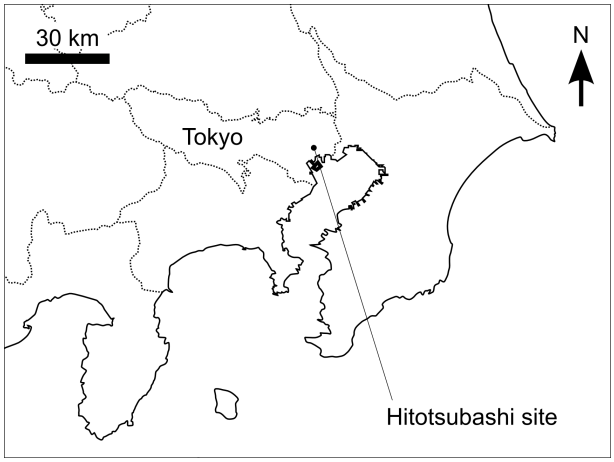

Figure 3.1: Location of the Hitotsubashi site.

The estimated number of individuals recovered from the Hitotsubashi site was reported to approach at least 207, and the percentage of individuals between the ages of 0 and 4 years was as large as 36.3% (Nagaoka and Hirata, 2007). There was no difference in burial types among the different sexes and age groups (Kato, 1985). Studies on palaeodemography and stress markers have suggested that the living environment at the site was highly stressful compared to that of the modern standards (Hirata, 1990), but not as stressful as that estimated for earlier periods in Japan such as the Jomon (Yamamoto, 1989; Nagaoka et al., 2006).

Overall, the reconstructed demographic parameters for the Hitotsubashi site could be directly related to those of townspeople in Edo in the early Edo period because of the social status of the buried people and the large number of subadult individuals which probably represent the actual mortality rate in the Edo period (Nagaoka and Hirata, 2007).

3.2 Materials and methods

3.2.1 Bone samples

Isotope analyses was performed on bone samples from 46 adult (17 females and 29 males) and 84 subadult human skeletons from the skeletal collection housed in the Department of Anatomy, St. Marianna University School of Medicine (Kanagawa, Japan). Individuals aged under and over 15 years are regarded as subadults and adults, respectively. The adult and subadult bone samples are listed in Tables B.1 and B.2, respectively. All samples were obtained from rib bones.

Age and sex of the skeletons were determined by my collaborators (Tomohito Nagaoka and Junmei Sawada of St. Marianna University School of Medicine, Japan). The physical observations of adult skeletons by Nagaoka and Hirata (2007) were basically succeeded by them. The sex of the adults was estimated by the morphology of the pelvis (Phenice, 1969; Houghton, 1974; Ferembach et al., 1980; Bruzek, 2002). The age of death for the adults was re-examined under the Bayesian theorem by a revised method proposed by Buckberry and Chamberlain (2002) instead of that proposed by Lovejoy et al. (1985), which was adopted originally in Nagaoka and Hirata (2007), because the former method is more appropriate for elderly adults (Mulhern and Jones, 2005; Falys et al., 2006; Nagaoka and Hirata, 2008). The morphological observations of the Hitotsubashi adults with the revised method followed the results of Nagaoka and Hirata (2008). By applying Bayesian estimation, modern Japanese skeletons ($n = 177$) described in Nagaoka et al. (2012) were used as the reference population with uniform prior. In this study, the adult ages were classified into the following three: 15–34, 35–54, and $\geq$55 years of age.

A total of 137 subadult individuals were aged by the collaborators, and 84 of them were randomly selected for isotope analysis. Although age estimation methods of dental development and eruption have been confirmed in anthropology and forensic medicine, some variations in the results among different populations exist (Tompkins, 1996). Using a reference chart derived from another population probably results in biased dental ages. Therefore, the age of the subadult skeletons was estimated by them and compared using the two independent reference charts of Ubelaker (1999) and The Japanese Society of Pedodontics (JSP: 1988). The latter was created on the basis of data collected for 46,698 healthy Japanese subadults in 1984. In addition to these criteria, the age categories in Oyamada et al. (2008) were also applied by the collaborators; Phase 1: deciduous dentition not yet complete (about 0.5–2 years of age); Phase 2: deciduous dentition complete (about 3–5 years of age); or Phase 3: mixed dentition (about 6–10 years of age). The age estimated with the Oyamada method is more "accurate" instead of the broader error ranges.

3.2.2 Stable isotope analyses

Collagen extraction followed the modified gelatinization method (Longin, 1971; Yoneda et al., 2004a). The cleaned bone samples weighing 0.1–0.6 g were soaked in 0.2 M NaOH to remove exogenous organic matter, such as humic and fulvic acids. The samples were rinsed with pure water, and then lyophilized and crushed into coarse powders. The crushed samples were sealed in cellulose tubes and treated with 0.5 M HCl at 4°C overnight, and the demineralized remaining portion was recovered by centrifuging. The samples were gelatinized in pure water at 90°C for more than 12 h, filtered using a glass fiber filter (Wattmann GF/F), and lyophilized.

The resulting lyophilized gelatin was analyzed using an EA-IRMS (Thermo Flash 2000 elemental analyzer, Finnigan ConFlo III interface, and Thermo Delta V mass spectrometer) at the laboratory of Isotope Ecology, The University of Tokyo, Japan. The carbon (%C) and nitrogen

(%N) mass concentrations were measured by EA. The analytical standard deviation (SD) was approximately 0.1‰ for δ^{13}C and <0.2‰ for δ^{15}N. The elemental concentrations and isotope ratios of carbon and nitrogen were calibrated against a laboratory alanine standard traceable back to the PDB and AIR international standards.

3.2.3 Statistics

All statistical analyses were performed using the R software (R Core Team, 2014). Weaning parameters were objectively estimated by using the WARN, R software package (Tsutaya and Yoneda, 2013; see Chapter 2) with the default configurations.

3.3 Results

3.3.1 Preservation of bones

The preservation of collagen was evaluated by using the atomic C/N ratios and the yield of extracted gelatin. On the basis of acceptable C/N ratios (2.9–3.6; DeNiro, 1985), six subadult samples were excluded from the dataset. Following the criteria proposed by van Klinken (1999), all samples except the excluded ones were considered to have acceptable yields (greater than 1%).

3.3.2 Isotope analyses of adult bones

The results of the isotope analyses of the adult bones are summarized in Table 3.2 and shown in Figure 3.2 (see Table B.1 for raw data). The Mann–Whitney U test indicates no significant difference between sexes for δ^{13}C ($U = 256.5$, $p = 0.828$) but significantly lower δ^{15}N values for females ($U = 363.0$, $p = 0.008$). Although the correlation coefficient is small, Spearman's rank correlation tests indicate a significant correlation between carbon and nitrogen isotope ratios for adult males ($R = 0.609$, $p < 0.001$) but not for adult females ($R = 0.311$, $p = 0.225$). Consequently, the statistics are performed for both sexes because of the isotopic differences between the sexes.

The adult isotope ratios of the different age categories are shown in Table 3.3. The Kruskal–Wallis test indicates no significant difference among the three adult age categories for both δ^{13}C (females: $\chi^2 = 1.334$, $p = 0.513$; males: $\chi^2 = 2.234$, $p = 0.327$) and δ^{15}N (females: $\chi^2 = 0.769$, $p = 0.681$; males: $\chi^2 = 2.452$, $p = 0.294$). The lower δ^{15}N values of females are evident in all the three age categories (Table 3.3).

The adult isotope ratios from the different burials are shown in Table 3.4. The Mann–Whitney U test indicates no significant difference between adult males and females from the two different types of burials (wooden coffins of rectangular or rounded shape and pit) for both δ^{13}C (females: $U = 3.0$, $p = 0.376$; males: $U = 57.0$, $p = 0.689$) and δ^{15}N (females: $U = 3.5$, $p = 0.476$; males:

Table 3.2: Summary of carbon and nitrogen isotope ratios (‰) of adults and subadults.

		δ^{13}C		δ^{15}N		n
		Mean	SD	Mean	SD	
Adult	Female	-19.3	0.5	10.6	0.8	17
	Male	-19.4	0.5	11.3	0.7	29
	All	-19.4	0.5	11.0	0.8	46
Subadult	<4 years old	-18.8	0.5	13.3	1.4	63
	≥4 years old	-19.0	0.6	11.2	1.2	15

Table 3.3: Summary of isotope ratios (‰) of male and female adults in different age categories.

Age range		δ^{13}C		δ^{15}N		n
(years)		Mean	SD	Mean	SD	
Female	15–34	-19.5	0.4	10.9	0.7	5
	35–54	-19.3	0.7	10.5	0.8	7
	≥55	-19.1	0.0	10.6	1.8	2
Male	15–34	-19.4	0.1	11.7	0.1	2
	35–54	-19.4	0.6	11.2	0.7	23
	≥55	-18.9	0.5	11.8	0.6	3

$U = 2.452$, $p = 0.294$). The isotope ratios of adults from different types of burials are shown in Figure 3.2.

The isotope ratios obtained from past and modern Japanese foods with a fixed offset from dietary protein to collagen of 5.0‰ for δ^{13}C and 4.0‰ for δ^{15}N (Lee-Thorp, 2008) are compared in Figure 3.3 with the results in this study for adults. Although the isotope ratios of these foods are not directly comparable to the results in this study, the data suggested that freshwater fish, marine foods, and C_3-based terrestrial foods are the major protein sources for the adults in the Hitotsubashi site.

The adult isotope ratios of the Hitotsubashi site are compared with those of the Kaneiji site in Edo (Koike et al., 1990; Yoneda, 2012), as shown in Figure 3.3. Historical and archaeological studies have revealed that people buried in the Kaneiji site represents various social statuses from townspeople to *shogun* (Koike et al., 1990; Yoneda, 2012). Previously analyzed skeletons belonged to the Tokugawa shogunate family (Yoneda, 2012) and warriors of relatively higher statuses (Koike et al., 1990). Although the sex differences in the isotope ratios of the Hitotsubashi population and temporal differences in the Kaneiji-shogun population (Yoneda, 2012) are not considered here, the Kruskal–Wallis test indicates significant difference among these three

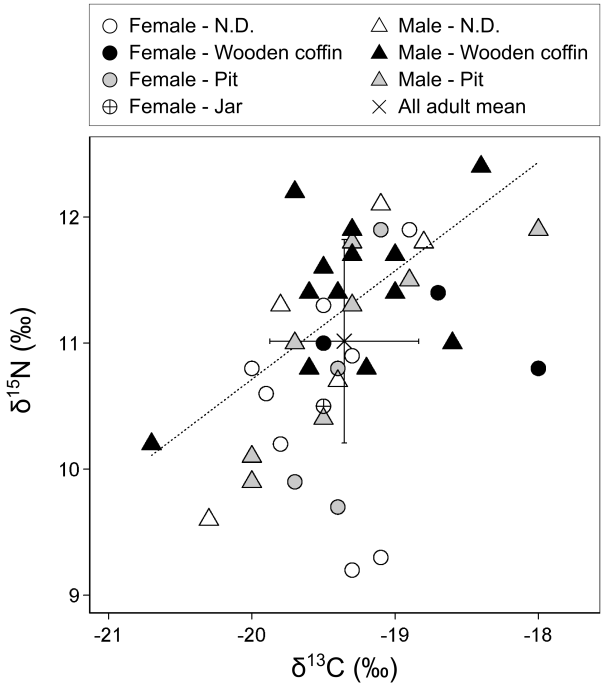

Figure 3.2: Adult δ^{13}C and δ^{15}N results from the Hitotsubashi site. Adult individuals are shown by sex and type of burials. A dotted line represents the regression for males (slope = 0.86, intercept = 27.94).

populations for both δ^{13}C ($\chi^2 = 38.24$, $p < 0.001$) and δ^{15}N ($\chi^2 = 23.70$, $p < 0.001$). A post-hoc Mann–Whitney U test with a corrected p-value accoring to the Bonferroni method indicates an inter-population difference between the Hitotsubashi and Kaneiji-shogun ($U = 40.0$, $p < 0.001$; δ^{15}N: $U = 72.0$, $p < 0.001$) and between the Hitotsubashi and Kaneiji-warrior populations (δ^{13}C: $U = 756.0$, $p < 0.001$; δ^{15}N: $U = 162.5$, $p < 0.001$). In this method, the p-value was adjusted to 0.017 (= 0.05 / 3: Bland and Altman, 1995).

3.3.3 Age estimation of subadult skeletons

The difference between ages estimated by the different reference charts by the collaborators is summarized in Table 3.5. For individuals in the Oyamada age phases 1, 2, and 3 (i.e., less than six years old), 21.9% JSP ages were older than the Ubelaker ages and 8.8% were younger. The mean differences of the JSP and Ubelaker ages were less than 0.10 for individuals in the Oyamada phases of 1–3. The maximum absolute differences between the JSP and Ubelaker ages were 0.25, 1.0, 1.0, and 3.0 years for phases 1, 2, 3, and 4, respectively. The data suggest that the differences in the ages estimated by the different reference charts are small for individuals younger than six years, but large for subadults older than six years.

Therefore, I and the collaborators determined the age by the following two criteria as a total

Table 3.4: Summary of isotope ratios (‰) of male and female adults from different types of burials.

		δ^{13}C		δ^{15}N		n
		Mean	SD	Mean	SD	
Female	Wooden coffin	-18.7	0.8	11.1	0.3	3
	Pit	-19.4	0.2	10.6	1.0	4
Male	Wooden coffin	-19.3	0.5	11.5	0.6	16
	Pit	-19.3	0.7	11.0	0.8	8

Adults excavated from the wooden coffins in rounded or rectangular shape are aggregated.

Table 3.5: Summary of differences in the JSP ages than the Ubelaker ages.

Age phase	n	Proportion (%)	Mean	SD
1 (0–0.5 years)	6	16.7	-0.04	0.10
2 (0.5–2 years)	65	43.1	0.10	0.25
3 (3–6 years)	43	14.0	0.00	0.33
4 (>6 years)	23	52.2	0.52	0.70

Proportion of individuals estimated to be different ages by the two reference charts, and mean and SD of the differences are indicated by the age phases (Oyamada et al., 2008).

estimate for both JSP and Ubelaker: (1) if there is an overlap in JSP and Ubelaker age ranges, then the overlapping range is adopted as the total estimate, and (2) if there is no overlap in the JSP and Ubelaker age ranges, then the Ubelaker age is adopted as the total estimate. I give priority to the Ubelaker estimate because it represents a conservative estimate relative to my alternative hypothesis of later weaning in the Edo period, and it has been the confirmed aging reference in anthropology and forensic medicine (Ferembach et al., 1980). The total age ranges and Oyamada age phases are given in Table B.2 for isotopically analyzed individuals.

3.3.4 Isotope analysis of subadult bones

The δ^{13}C and δ^{15}N values of the subadult samples are shown in Figure 3.4 and summarized in Table 3.2 (see Table B.2 for raw data). The Mann–Whitney U test indicates significantly higher carbon ($U = 861.5$, $p < 0.001$) and nitrogen ($U = 1008.0$, $p < 0.001$) isotope ratios in subadults under the age of four years than adult females. The test also indicates significantly higher isotope ratios in subadults over the age of four years than all adults for carbon ($U = 476.0$, $p = 0.028$) but not for nitrogen ($U = 347.5$, $p = 0.973$). The elevated isotope ratios of subadults under the

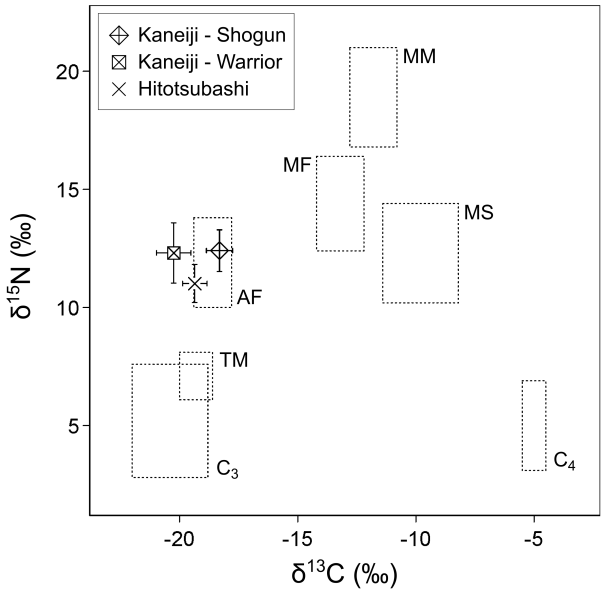

Figure 3.3: Mean and 1SD ranges of the δ^{13}C and δ^{15}N values of adults from the Hitotsubashi and Kaneiji site. Mean and 1SD ranges of isotope ratios of Japanese food groups (Yoneda et al., 2004a) are also shown as dotted squares; C_3: C_3 plants, C_4: C_4 plants, TM: terrestrial mammals, FF: freshwater fishes, MS: marine shellfishes, MF: marine fishes; MM: marine mammals.

age of four years suggests isotopic enrichment via breastfeeding (Fuller et al., 2006a).

The mean ^{13}C-enrichment in subadults aged 0–4 years is 0.6‰ (Table 3.2) and is smaller than the 1.0‰ enrichment expected from breastfeeding (Fuller et al., 2006a), suggesting an introduction of ^{13}C-depleted weaning foods. The elevated δ^{15}N values gradually decrease after the age of one year and approach the adult range around the age of 3–5 years (Figure 3.4B). After correcting for the lag time generated from the bone collagen turnover, the WARN calculations showed that the most probable ages for the start and end of weaning are 0.2 and 3.1 years, respectively (Figure 3.5). The nitrogen isotopic enrichment from mother to subadult and the δ^{15}N value of collagen synthesized entirely from weaning foods was calculated as 3.0–3.8‰ and 10.6–11.9‰, respectively, at 95% credible interval (CI). There is little difference in the calculated weaning parameters among the datasets adopting different aging references (Table 3.6).

3.4 Discussion

3.4.1 Adult diet

Various historical and archaeological studies have suggested that the diet of the Edo townspeople mainly consisted of C_3 plant foods (e.g., rice, vegetables, and soybean products) and fish from freshwater and sea and that terrestrial animal meat was not commonly consumed, particularly

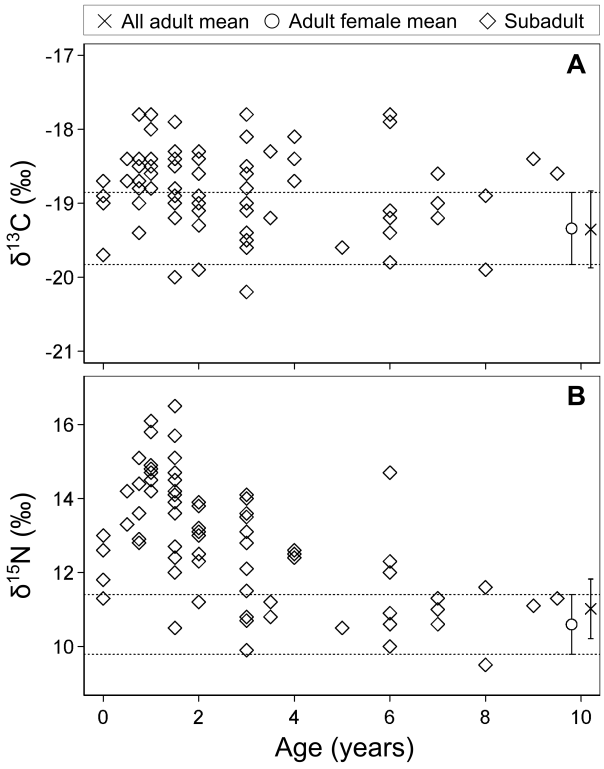

Figure 3.4: Subadult (A) δ^{13}C and (B) δ^{15}N values by estimated age at death. Dotted lines indicate the 1SD ranges for the mean isotope ratio of adult females. Mean and 1SD ranges of all adults are also shown.

during the early period because of Buddhism discipline (Hanley, 1997; Ehara et al., 2009; Harada, 2009). The isotopic results of this study (Table 3.2) well agree with such data considering the isotope ratios of Japanese foods (Yoneda et al., 2004a). However, the results in Figure 3.3 are rough estimates comparing food sources and consumers from different ecosystems. Furthermore, terrestrial animal contribution cannot be excluded by using the isotopic results alone.

Although there is no significant difference in the isotope ratios among the three age categories and burial types, the δ^{15}N values of the Hitotsubashi adult females are significantly lower than those of males. If we assume that the cause of this difference is the diet, then the males probably consumed higher trophic level foods or more marine foods than females. The significant correlation in the isotope ratios of adult males (Figure 3.2) also suggests that their main dietary protein source consisted of two types of foods, and these foods could be derived from C_3 and marine ecosystems. However, this type of isotopic difference by sex is not necessarily found in other populations of the Edo period. The δ^{13}C values of adult females excavated from the Fushimi site is significantly higher than those of the males, and there is no significant sex difference in the δ^{15}N values (Kusaka et al., 2011; see also Table 3.1). In the Kaneiji warrior population, there is no significant sex difference in the δ^{13}C and δ^{15}N values (Koike et al., 1990; see also Table 3.1).

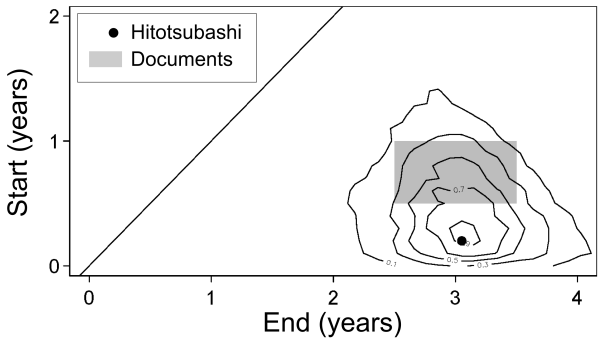

Figure 3.5: Probabilities for specific combinations of ages at the start and end of weaning in the Hitotsubashi skeletal population calculated with the WARN package. A closed point indicates the most probable ages and the contour lines show the posterior probability for the combination of weaning ages relative to that of the most probable one. Shaded range indicates the general weaning ages described in several historical documents of the Edo period (see Table 3.7).

Further isotope analyses on other skeletal populations are needed to clarify the dietary difference between sexes in the Edo period.

It is possible that the sex difference in the δ^{15}N values resulted from the positive nitrogen balance during pregnancy (Fuller et al., 2004; Nitsch et al., 2010) as well as dietary differences between the sexes. It has been hypothesized that the increased use of nitrogen in the diet and urea for tissue synthesis during pregnancy decreased the trophic level effect to maternal tissues by 0.5–1‰ (Fuller et al., 2004). Delivery imposed a remarkable mortality crisis on the pregnant females even in the Edo period (Kito, 2000), and several females could have died soon after successive deliveries and this may be represented in the lower δ^{15}N values.

The isotope ratios of the Hitotsubashi adults were significantly lower for both carbon and nitrogen than those of the Kaneiji-shogun and significantly higher for carbon and lower for nitrogen than those of the Kaneiji-warrior adults (Figure 3.3). Both, these sites were located in Edo. Social status, which can be reconstructed from the type of graves (Tanigawa, 1992), probably caused this difference. The Kaneiji temple was closely connected with the Tokugawa shogunate, and all human skeletons analyzed by Yoneda (2012) were the wives and daughters of the Tokugawa shogun, and some skeletons analyzed by Koike et al. (1990) represented warriors in higher social status (i.e., *hatamoto*). On the other hand, the skeletal population from Hitotsubashi represent townspeople buried in cheap wooden coffins or pit burials (Kato, 1985). Historical studies have indicated some differences in diet according to the economic and social status of people in the Edo period (Ehara et al., 2009; Harada, 2009), although no concrete isotopic assessments could be drawn. Considering the great isotopic variation in human skeletons around Japan during the Edo period (Yoneda et al., 2011), the variations in isotope ratios are relatively small for these three populations in Edo. Therefore, we do not expect large contributions from specific

Table 3.6: Weaning parameters and its probability of the Hitotsubashi population calculated by WARN program.

		MDE	Range		
			Lower	Upper	Probability
Total	t_1	0.2	0.0	1.3	0.95
	t_2	3.1	2.1	4.1	
	E	3.4	3.0	3.8	0.96
	$\delta^{15}N_{wnfood}$	11.3	10.6	11.9	0.96
Ubelaker	t_1	0.4	0.0	1.5	0.95
	t_2	3.1	2.1	4.1	
	E	3.3	2.9	3.8	0.97
	$\delta^{15}N_{wnfood}$	11.3	10.6	11.9	0.95
JSP	t_1	0.3	0.0	1.3	0.95
	t_2	3.2	2.5	4.1	
	E	3.4	3.0	3.9	0.97
	$\delta^{15}N_{wnfood}$	11.3	10.7	11.9	0.96

MDE: maximum density estimator, t_1 and t_2: the age at the start and end of weaning, respectively, E: the ^{15}N-enrichment through maternal to subadult tissue, and $\delta^{15}N_{wnfood}$: δ^{15}N value of collagen synthesized entirely from weaning foods. Joint probabilities are shown for t_1 and t_2, and marginal probabilities for E and $\delta^{15}N_{wnfood}$. Results of application to datasets with the total, Ubelaker, and JSP ages are shown.

food items to complicate the breastfeeding and weaning signals in the subadult isotope ratios.

3.4.2 Age estimation of subadult skeletons

Although the ages estimated by the JSP chart are generally greater than those estimated by Ubelaker (Table 3.5), the fluctuations in the estimated ages do not affect the discussion in this study. The individuals of the Oyamada age phase 2, who suffer a relatively greater shift in their estimated age by the different aging references (i.e., 0.16 ± 0.27 years), show higher δ^{15}N values than adults, and the subadult δ^{15}N values drop within the range of the adults during the Oyamada age phase 3, where the estimated ages represent a smaller effect of the shift (i.e., 0.00 ± 0.33 years). These results suggest that the differences in the ages estimated by using different reference charts have little effect on the isotopically reconstructed weaning ages in this study, as indicated in Table 3.6.

3.4.3 Infant feeding practice

The partially elevated δ^{13}C values of subadults aged 0–4 years would reflect both ^{13}C-enriched breast milk and ^{13}C-depleted weaning food consumption (Fuller et al., 2006a). The age for the start of weaning was calculated to be 0.2 years based on the change in the bone collagen δ^{15}N values (Table 3.6), which is reasonable because infants aged six months and older need weaning foods to sustain their health (Kramer and Kakuma, 2004). As discussed below, various historical documents of the Edo period recommended the supplementation of rice gruel for breastfed subadults older than 6–9 months (Kajitani, 2007, 2008; Sone, 2011). The consumption of such terrestrial C_3 weaning foods would have resulted in the partial carbon isotopic enrichment seen in the Hitotsubashi subadults.

The increased isotope ratios in infants is consistent with breastfeeding signals although the offset of 3.0–3.8‰ in δ^{15}N values calculated by WARN program with respect to the adult female mean is slightly larger than the expected trophic level shift of 2–3‰ between mother and infants (Fuller et al., 2006a). Similar high ^{15}N-enrichment has been observed in a human skeletal population from Roman Italy (4‰, Prowse et al., 2008) and medieval UK (7‰, Burt, 2013). One possible explanation is physiological factors. Amino acid routing into breast milk is not completely understood and the δ^{15}N values of individuals fluctuate because of nitrogen balance (Fuller et al., 2004; Mekota et al., 2006), aridity (Schwarcz et al., 1999), and pathology (Katzenberg and Lovell, 1999). Fuller (2003) investigated carbon and nitrogen isotope ratios in human breast milk and found that bulk breast milk is isotopically heterogenous; furthermore, he considered that differences in the use of milk compounds alter the degree of ^{15}N-enrichment. Recent amino acid compound specific isotope analysis revealed heterogeneities in the δ^{15}N values of individual amino acids in human breast milk (Tea et al., 2013). Different contributions of these isotopically heterogeneous amino acids in breast milk would result in different ^{15}N-enrichment through breastfeeding. Another factor is dietary; if the mothers of infants with high δ^{15}N values actually consumed ^{15}N-enriched foods, then this increases the δ^{15}N value in infants via breastfeeding (Prowse et al., 2008; Burt, 2013). Alternatively, it could be envisaged that the analyzed sample of the adult population is not representative of the mothers of these infants. The early introduction of ^{15}N-enriched weaning foods is also possible although there is no historical document describing such traditions to the best of my knowledge.

Despite the overall pattern of isotopic change, individual variations are also evident in the Hitotsubashi population (Figure 3.4). For example, the δ^{15}N values of some individuals aged less than two years are within the 1SD range of those of adult females (e.g., H-3 and H-85, Table B.2). The most probable reason for such variability is diet as previously mentioned. Furthermore, the "osteological paradox" (Wood et al., 1992) is also one of the possible reasons. Because the excavated subadult skeletons represent dead individuals, it is possible that they represent abnormal weaning practice such as exceptionally early weaning. The other potential

reason is the different treatment based on the sex although it is difficult to infer that from the morphological criteria of young skeletons (Lewis, 2007).

The similarities in the isotope ratios of most subadults over the age of four years with adults suggest that the post-weaning foods consumed by them were isotopically similar to the adult diet. Although there is a significant isotopic difference between subadults and adults for carbon, this difference seems to originate from some individuals with relatively higher isotope ratios (e.g., H-208 and H-265, see Table B.2). There would have been some differences in the constituents of weaning foods, and these individuals consumed weaning foods of a relatively higher trophic level.

The isotopic signal and WARN program show that weaning foods were introduced around the age of 0.2 (0.0–1.3 for 95% CI) years and weaning ended around 3.1 (2.1–4.1 for 95% CI) years in the Hitotsubashi population (see Table 3.6 and Figure 3.5), which agrees well with descriptions in historical documents from the Edo period summarized in Table 3.7. The traditional method of counting the age in the Edo period was that an infant was considered to be one year old at birth and one year older at the subsequent New Year's Day (Cornell and Hayami, 1986). Therefore, in this study, I subtracted 1.5 years from the written age in historical documents and discussed them in the corrected Western form. *Shoni yojoroku*, a medical text published in 1688 by Masayuki Chimura, recommended the supplementation of diluted rice gruel around six to nine months after birth and mainly breastfeeding until 1.5 years of age (Kajitani, 2008). *Okina mondo*, a Confucian text written in 1761 by Toju Nakae, assigned three years for breastfeeding (Yamazumi and Nakae, 1976, pp. 121–128, vol. 1; Sone, 2011). In *Shoni hitsuyo sodate gusa*, the first Japanese book on child care published in 1703, Gozan Kaduki, a famous physician, recommended that subadults should be mainly breastfed until the age of one year with a little weaning foods, mainly fed by weaning foods with little breast milk from 1.5 to 2.5 years, and then weaned by 3.5 years (Yamazumi and Nakae, 1976, pp. 287–366, vol. 1). In *Shoni sodate katagi* a descriptive novel *Ukiyo zoshi* of the daily living of common people at that time and published in 1773, Kiyu Eiseido, a novelist, described a 1.5-year-old male subadult whose whining was stopped by breastfeeding and snacks during theatergoing (Yamazumi and Nakae, 1976, pp. 313–352, vol. 2). In *Ai-iku sadan*, a medical text influenced by Western studies and published in 1853, Ryusai Kuwata, a physician, recommended that subadults should be breastfed at least eight months after birth and gradually rice gruel must be introduced after six to nine months of age (Kajitani, 2007; Sone, 2011). Furthermore, older weaning ages (i.e., start of weaning by the age of four years and end of weaning by six years) have also been suggested in the Fushimi site for the Edo period Kyoto city by interpreting isotopic data on six subadult individuals (Kusaka et al., 2011).

The age of ≈3.1 years at the end of reconstructed weaning in the Hitotsubashi population indicates a relatively longer breastfeeding period and suggests lower natural fertility of townspeople in Edo during the early Edo period. Modern infants in industrialized nations are generally

Table 3.7: Historical literatures giving a mention on ages at the start and end of weaning in the Edo period.

Literature	Year	Start	End	References
Shoni yojoroku	1688	6–9m	>1.5y	Kajitani, 2008
Okina mondo	1761	–	3y	Yamazumi and Nakae, 1976
Shoni hitsuyo sodate-gusa	1703	<1y	3.5y	Yamazumi and Nakae, 1976; Sone, 2011
Shoni sodate katagi	1773	–	>1.5y	Yamazumi and Nakae, 1976
Ai-iku sadan	1853	6–9m	–	Kajitani, 2007; Sone, 2011

breastfed for less than 1–2 years (Dettwyler, 2004; Ministry of Health, Labour and Welfare, 2007). The average age at the end of weaning in traditional human societies is 2.4–2.7 years, and the minimum is less than one year (Ford, 1945; Barry and Paxson, 1971; Sellen, 2001). Compared to these weaning ages, the breastfeeding period in the Hitotsubashi population is relatively longer. Although detailed investigations of the dynamics of the weaning process are important to indisputably connect breastfeeding practice and demographics (Vitzthum, 1994), isotope analysis cannot provide evidence at such fine resolution. Nevertheless, the age at the end of weaning is a good proxy for fertility, especially for populations in preindustrial settings (Bongaarts, 1978, 1982).

If the proportion of married people was also lower in the early Edo period, then the relatively longer breastfeeding period, which usually decreases natural fertility (Bongaarts, 1978, 1982) and relatively higher mortality seen in city populations (Nagaoka and Hirata, 2007), probably did not favor the natural population increase in the Hitotsubashi site. Actually, a lower proportion of married people was assumed in Edo in the early Edo period because of the biased sex ratio (i.e., the number of males was 1.8 times greater than that of females in AD 1721) recorded in the Tokugawa official documents (Minami, 1978). A biased sex ratio of 2.3 times more males is also observed in the Hitotsubashi skeletal population (Nagaoka and Hirata, 2007). If this assumption is correct, then the data suggest that the development and growth of Edo in the early Edo period was based on attracting immigrants. Historical demographic studies have revealed that the population in rural areas increased rather rapidly during the 17th century because of the increase in proportion married resulted from the expansion of farming areas and segmentation of landholding units (*bunke*) (Hanley and Yamamura, 1977; Hayami, 1999; Kito, 2000). Similar mechanisms to the "urban graveyard" of the late Edo period, relatively higher mortality (Smith, 1973; Hayami, 1999, 2001; Kito, 2000) and lower fertility (Sharin, 1978; Saito, 2002) than rural areas, would have already existed. In the future, I anticipate that immigrants to Edo can be identified by using oxygen and strontium isotopes of tooth enamel (Waseda and Nakai, 1983; Kusaka et al., 2012).

The results of this study have further implications for the demography of the Edo period Japan. The reproductive patterns in rural areas of the late Edo period are characterized by lower fertility rates than those reconstructed in various regions of Western Europe (Flinn, 1985; Saito, 1992; Kito, 2000). The prolonged breastfeeding period in some rural areas has been hypothesized to be one of the important causes of the lower fertility in the latter Edo period than that in European populations (Tomobe, 1991, 2002; Kito, 1995). By comparing the stable isotopic results, the breastfeeding period in the Hitotsubashi population was longer than those in four populations of UK in the medieval times and in the industrial era. Stable carbon and nitrogen isotope analyses of bone collagen and tooth dentine have revealed that weaning ended at or before two years of age in the medieval village population of Wharram Percy, Yorkshire (Richards et al., 2002; Fuller et al., 2003) and suburb population of Fishergate House, York (Burt, 2013). The isotopic results suggest that solid foods were introduced before the end of the first year and breastfeeding had entirely ceased by two years of age in the 18th and 19th century skeletal population from Spitalfields, London (Nitsch et al., 2011) and 19th century skeletal population from Lukin Street cemetery, London (Beaumont et al., 2013a). Furthermore, a historical study on the literatures indicated that the age at end of weaning was generally less than two years in UK between1500 and 1800 (Fildes, 1982). The reconstructed breastfeeding period in the Hitotsubashi population (i.e., 3.1 years) is longer than those in these four populations in UK. If the relatively prolonged breastfeeding practice was common during the entire Edo period and in mainland Japan, the results of this study support the hypothesis concerning the cause of the lower fertility in some rural areas of the late Edo period Japan (Tomobe, 1991, 2002; Kito, 1995). However, breastfeeding period possibly varied in different populations and cultures; therefore, it is important to reconstruct the weaning ages in the rural areas of the Edo period Japan.

3.5 Conclusions

Isotope analyses of adult human skeletons from the Hitotsubashi site showed significant depletion in δ^{15}N for females relative to males, suggesting dietary differences and/or the effect of pregnancy in females (Nitsch et al., 2010). There is no significant difference in the carbon and nitrogen isotope ratios among adult skeletons of different age categories and burial types. On the other hand, the isotope ratios of adults in the Hitotsubashi site are significantly different from those in the Kaneiji site (Koike et al., 1990; Yoneda, 2012). Nevertheless, the overall isotopic compositions of the diet in these Edo populations are similar, considering the great isotopic variation in human skeletons around Japan during the Edo period (Yoneda et al., 2011).

The δ^{13}C and δ^{15}N values of subadults under the age of four are higher than those of adult females. These increases well agree with bioenrichment of isotopes between mothers and infants (Fuller et al., 2006a) whereas the ^{15}N-enrichment in the Hitotsubashi population seems relatively large. The high δ^{15}N values gradually decrease after the age of one, and approach the adult range

by the age of four. With the correction for the delay resulted from the bone collagen turnover (Tsutaya and Yoneda, 2013; see also Chapter 2), the age for the start and end of weaning was calculated to be 0.2 (0.0–1.3 at the 95% CI) and 3.1 (2.1–4.1 at the 95% CI) years, respectively. This is consistent with several historical documents describing infant feeding practices in the Edo period and previous isotopic results for subadult skeletons in Kyoto (Kusaka et al., 2011). The isotopic results of subadults from the Hitotsubashi site shows relatively prolonged breastfeeding period compared with that in modern industrialized nations (Dettwyler, 2004; Ministry of Health, Labour and Welfare, 2007), typical traditional societies (Ford, 1945; Barry and Paxson, 1971; Sellen, 2001), and that estimated from four populations from medieval (Richards et al., 2002; Fuller et al., 2003; Beaumont et al., 2013a) and industrial (Nitsch et al., 2011; Burt, 2013) UK. The prolonged breastfeeding period reconstructed in this study, the relatively higher mortality (Nagaoka and Hirata, 2007), and biased sex ratios (Minami, 1978) probably did not favor the natural population increase at Hitotsubashi, which is consistent with historical assumptions that the urbanization of Edo in the early Edo priod was supported by in-migration.

Chapter 4

Urbanization of the medieval city of Kamakura (Yuigahama-minami)

Infant feeding practice is one of the most important proxies of health and fertility in past human populations as explained in Chapter 1. Study of this chapter aims to reconstruct breastfeeding practices in a medieval skeletal population from the Yuigahama-minami site, Kamakura, Japan, using stable isotopes. This reconstruction enabled us to discuss subadult health and population dynamics in the medieval city of Kamakura.

4.1 Introduction

4.1.1 Medieval Kamakura

Medieval Kamakura was established by the Minamoto family in AD 1180 as a historical capital of a military government, the Kamakura Shogunate (Yamamura, 1990). The city of Kamakura is surrounded by mountains along the north, east, and west sides, and its south side is bordered by the Sagami-wan Bay. Kamakura has been inhabited since the late Yayoi period (300 BC – AD 250) and the prototype of the city was already established by the AD 11th century (Gomi and Mabuchi, 2004). After the capital was established by the Minamoto family, the city of Kamakura experienced further development and an increase in population (Amino et al., 1989; Kawano, 1995). Roads, residences of military and townspeople, temples, and shrines were built in the city. Pit buildings expanded into the seashore (Suzuki, 2013). The seashore was used for burial sites as well (Amino et al., 1989). The prosperity of the city of Kamakura continued until AD 1333, the end of the Kamakura period, when the Minamoto family lost the Genko war, governance was taken over by the court, and the capital was moved to Kyoto (Yamamura, 1990).

Archaeological data suggest that people living in Kamakura enjoyed affluent commercial products imported from outside the city, yet suffered from poor living conditions owing to high population density and poor hygiene (Amino et al., 1989; Kawano, 1995). The diet of the

townspeople in Kamakura was common for medieval Japan, which mainly consisted of commercial food products such as rice, wheat, vegetables, fish, shellfish, and meat (Amino et al., 1989; Kawano, 1995; Ehara et al., 2009). The population of medieval Kamakura was assumed to be approximately 64,100–109,000, in which warriors, townspeople, and temple/shrine workers comprised 17,500–29,000, 31,600–56,900, and 15,000 individuals, respectively, by both analyzing historical records and deducing from excavated areas (Amino et al. 1989). The population density in the medieval period was assumed to be higher than that of the present day. This excess population is likely to have had adverse impacts on living conditions. Archaeological remains suggest that the debris and dead bodies of animals were often abandoned in the side ditches and on the streets in Kamakura (Amino et al., 1989; Kawano, 1995) probably because of high population density and the substantial economical gap between the rich and the poor.

Previous bioarchaeological studies suggested severe living conditions in medieval Kamakura. Paleodemographic studies on the townspeople skeletons from the Yuigahama-minami site (162 adults and 98 subadults) and Seika-ichiba location of the Chusei Shudan Bochi site (28 adults and 13 subadults) indicate a younger age-at-death distribution than that of early modern Edo populations, similar to the Mesolithic-Neolithic Jomon population (Nagaoka et al., 2006, 2013b). A higher frequency of several stress markers, such as enamel hypoplasia and cribra orbitalia, was observed for medieval Kamakura skeletons from the Yuigahama-minami site (Hirata et al., 2011) and the Chusei Shudan Bochi site (Nagaoka et al., 2013b).

The reconstruction of breastfeeding practice would provide new insights into subadult health and population dynamics of townspeople in the city of Kamakura. Although the duration of breastfeeding and the adequate complementary feeding are one of the most important aspects of the subadult health (WHO, 1998, 2009), infant feeding practice in medieval Japanese populations is yet to be investigated. Such information would contribute to a better understanding of the higher subadult stress and mortality rates reconstructed from the skeletal populations of townspeople in medieval Kamakura (Nagaoka et al., 2006, 2013b; Hirata et al., 2011). Population dynamics during the urbanization of Kamakura can also be better understood by reconstructing the weaning ages, as the breastfeeding period is an important determinant of fertility in human populations (Bongaarts, 1982; Bongaarts and Potter, 1983; Campbell and Wood, 1988; Wood, 1994). Population dynamics are determined primarily by mortality, fertility, and migration (Keyfitz, 1980). However, less data is available on fertility in medieval Japan because of the methodological limitations in history and archaeology (Farris, 2006). Although historical literature and artistic representations play a major role in reconstructing past ways of human life, the former is confined to the warrior class and the latter is unsuitable for the quantitative reconstruction of child rearing and infant feeding practices in medieval Japan (Yamamura, 1990; Moriyama and Nakae, 2002). Furthermore, limited sources of historical documents are available that describe childcare and infant feeding practices in medieval Japan. Only one literary description of infant feeding practice, *Sekyosho*, a book describing family rules of warriors in the

Muromachi period (AD 1336–1573) of late medieval Japan, recommended that subadults should be wet nursed, female subadults should be supplemented with family foods after the age of 1.5 years, and males should be fed with fish and animal meat to strengthen their body (Yamazumi and Nakae, 1976, p. 59–66, vol. 1); these ages were corrected from the traditional age calculation method in past Japan (Cornell and Hayami, 1986). However, these descriptions cannot be directly applied to townspeople in the Kamakura period. As such, bioarchaeological approaches can provide important information concerning the infant feeding practice of townspeople in medieval Kamakura.

4.1.2 Previous isotopic studies of human skeletons from the medieval Japan

Despite a wealth of isotopic dietary reconstructions for people from the Jomon and Edo periods in mainland Japan (e.g., Minagawa, 2001; Yoneda et al., 2004a, 2011; Kusaka et al., 2008, 2010; Naito et al., 2013), only one study exists that isotopically reconstructs the diet of medieval people in mainland Japan. Minami and Nakamura (2011, same data appeared in Minami et al., 2007) applied stable carbon and nitrogen isotope analysis and radiocarbon dating to excavated adult skeletons of townspeople from the Yuigahama-minami ($n = 16$) and Chusei Shudan Bochi ($n = 5$) sites (Table 4.1). They suggested that diet of both populations comprised of C_3 plants as well as marine and freshwater fishes. They also exhibited relatively higher δ^{15}N values and younger calibrated radiocarbon ages for the Yuigahama-minami skeletons excavated from individual burials relative to those from mass burials. However, there is no previous isotopic data on subadult human skeletons from medieval Japan.

Table 4.1: Summary of previously reported carbon and nitrogen isotope ratios (‰) and radiocarbon ages of medieval human skeletons in Kamakura.

Site	Burial	δ^{13}C		δ^{15}N		n	^{14}C age (calAD)	
		Mean	SD	Mean	SD		Lower	Upper
Yuigahama-minami	Individual	-18.7	0.6	10.3	0.4	10	1160	1391
	Mass	-18.9	0.6	8.6	0.6	6	1174	1380
Chusei Shudan Bochi	Mass	-18.8	1.2	9.8	0.9	5	1040	1258

4.1.3 Yuigahama-minami site

The Yuigahama-minami site is located along the sandy seashore at the southern end of Kamakura City (Figure 4.1), as part of a series of medieval sites, such as Chusei Shudan Bochi, Seiyokan, and Zaimokuza. The Yuigahama-minami site was excavated in 1995–1997, and individual and mass burials were found from layers between the late 13th and late 14th centuries (Saiki, 2002). The

radiocarbon ages of the human skeletons, calibrated with the marine reservoir effect, are within the 95% credible interval (95% CI) range of 1160–1391 calAD (Minami et al., 2007; Minami and Nakamura, 2011; see also Table 4.1). Because the graves of warriors and clergy are located in the hill zones of Kamakura (Kawano, 1995) and burial goods were rarely found (Saiki, 2002), the seashore burials were probably used for the lower segments of society such as townspeople (Muramatsu, 2009). The estimated number of people recovered from the Yuigahama-minami individual and mass burials approaches at least 527 and 3,108, respectively (Hirata et al., 2002; Saiki, 2002).

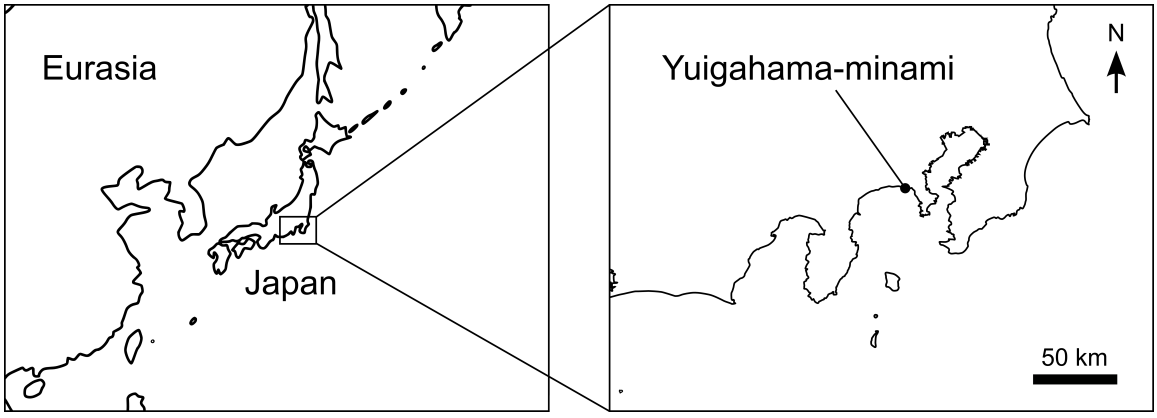

Figure 4.1: Location of the Yuigahama-minami site.

In the original excavation report, physical observations were not provided for all skeletons. A total of 416 skeletons (128 subadults aged ≤12 years, 131 adult females, 131 adult males, and 26 adults of unknown sex) from the individual graves at the Yuigahama-minami site were observed in Hirata et al. (2002). The pathological conditions (e.g., dental caries, cribra cranii, cribra orbitalia, enamel hypoplasia, tuberculosis, and sinusitis) of each individual skeleton were described, although the inclusion of partial skeletons introduces uncertainty in calculating the observed frequency of these pathological conditions (Hirata et al., 2002). In this study, all information about pathological conditions was obtained from Hirata et al. (2002).

The prevalence of enamel hypoplasia among the skeletons from the individual graves at the Yuigahama-minami site has been quantified (Sawada et al., 2007, Hirata et al., 2011). The authors considered all deciduous and permanent teeth on maxilla and mandibles from 250 individuals (78 subadults aged ≤15 years, 90 adult females, 79 adult males, and three adults of unknown sex), except for teeth with severe attrition and calculus (Hirata et al., 2011). Hirata et al. (2011) indicated that enamel hypoplasia occurred in 34.1% of cases for maxillary permanent central incisors ($n = 135$) and 63.9% of cases for mandibular permanent canines ($n = 147$). These figures were comparable to those observed in skeletal populations from the Edo and Jomon periods (Yamamoto, 1988).

Nagaoka et al. (2006) applied paleodemographic analyses on the human skeletons excavated

from individual burials at the Yuigahama-minami site and suggested a highly stressful living environment for townspeople of the medieval Kamakura. Among 260 skeletons with a preserved pelvis, 17.3% of individuals were aged between 0 and 4 years, and the sex ratio was 106 males to 100 females (Nagaoka et al., 2006). They estimated demographic parameters based on juvenility index, in which the numbers of deaths of older subadults are expressed as a ratio of their deaths to the number of adult deaths in the population (Bocquet-Appel and Masset, 1996; Chamberlain, 2009). This was designed to avoid the biasing effects caused by differential mortuary practices and post depositional preservation potential of younger subadult skeletons (Lewis, 2007). Using this juvenility index, the deaths occurring between 0 and 1 year and between 0 and 5 years were calculated as 27.6 ± 1.5% and 44.1 ± 1.5%, respectively (Nagaoka et al., 2006). Comparison of demographic profiles from the Yuigahama-minami series with other skeletal series (Kobayashi, 1967) indicated that while both the survivorship curve and life expectancy of the Yuigahama-minami sample are similar to those of the Jomon population, they are far lower than those of the early modern Edo population (Nagaoka et al., 2006).

4.2 Materials and methods

4.2.1 Bone samples

Isotope analyses were performed on Yuigahama-minami bone samples from three adult (two female and one male) and 45 subadult human skeletons housed at the Department of Anatomy, St. Marianna University School of Medicine (Kanagawa, Japan). These individuals were townspeople (Muramatsu, 2009) and were not included in the previous analyses of Minami et al. (2007) and Minami and Nakamura (2011). I chose skeletons from individual burials. Because these skeletons were found in articulated positions, we could easily identify single individuals without mixing with the remains of other individuals. Individuals aged under and over 16 years are regarded as subadult and adult, respectively. The adult and subadult bone samples are listed in Tables B.3 and B.4, respectively. All samples were obtained from rib bones. I have also included isotope data of adults from the individual burials of the Yuigahama-minami site reported by Minami and Nakamura (2011).

Age and sex of the skeletons were determined by my collaborators (Tomohito Nagaoka and Junmei Sawada of St. Marianna University School of Medicine, Japan). The skeletal analysis of the human remains sampled in this study is taken from Nagaoka et al. (2006) and Hirata et al. (2002, 2011). The sex of adults was estimated by the morphology of the pelvis (Phenice, 1969; Houghton, 1974; Ferembach et al., 1980; Bruzek, 2002). The ages of subadult skeletons were estimated using a reference chart (Ubelaker, 1999). The degree of development and closure of the occipital synchondrosis (Wakebe, 1990), and degree of ossification and epiphyseal union of the pelvis and long bones (Flecker, 1942; Webb and Suchey, 1985) were further used as secondary criteria. Pathological status was already reported for most but not all individuals (Hirata et

al., 2002), and I took these information. Among the 45 subadult skeletons used in this study, some pathological conditions were reported for 23 of these individuals, 14 individuals had no reported pathological conditions, and there is no observation in Hirata et al. (2002) for eight individuals (Table B.4). Dental caries and enamel hypoplasias were found in 47% and 19% of subadult individuals used in this study, respectively (Table B.4).

4.2.2 Stable isotope analyses

Collagen extractions were performed by my collaborator (Akina Shimomi of The University of Tokyo, Japan) at the Laboratory of Isotope Ecology, University of Tokyo, and proceeded according to a modified Longin method (Longin, 1971; Yoneda et al., 2004a). Procedures were generally same as those described in Section 3.2.2, but 1.0 M HCl was used instead of 0.5 M.

Samples were measured by myself in duplicate using an EA-IRMS (Thermo Flash 2000 elemental analyzer, Finnigan ConFlo III interface, and Thermo Delta V mass spectrometer) at the Laboratory of Isotope Ecology, University of Tokyo. The elemental concentrations and isotope ratios of carbon and nitrogen were calibrated against laboratory alanine standards whose values are traceable back to the PDB and AIR international standards, respectively. Reproducibility of replicate analysis of laboratory standards is better than 0.1‰ for standard deviation (SD) of δ^{13}C values and <0.2‰ for δ^{15}N values.

4.2.3 Statistics

All statistical analyses were performed using software R (R Core Team, 2014). Weaning parameters were estimated by the default configurations of the WARN package implemented using the R software environment (Tsutaya and Yoneda, 2013; see also Chapter 2).

4.3 Results

4.3.1 Preservation of bones

The preservation of collagen was evaluated by atomic C/N ratios and the yield of extracted gelatin. Considering acceptable C/N ratios (range of 2.9–3.6; DeNiro, 1985), one subadult sample (No. 1130) was beyond the acceptable range. Following the criteria proposed by van Klinken (1999), all samples, except one (No. 238), had acceptable yields (greater than 1%). These two individuals were excluded from the dataset.

4.3.2 Adults

The results of isotope analyses on adult bones are summarized in Table 4.2 (see Table B.3 for raw data) and shown in Figure 4.2, with previously reported data by Minami and Nakamura

(2011). A Mann–Whitney U test indicated no significant difference between the sexes for δ^{13}C ($U = 8.5$, $p = 0.308$) and δ^{15}N values ($U = 12.5$, $p = 0.926$).

Table 4.2: Summary of carbon and nitrogen isotope ratios (‰) of subadult and adult human bone collagen from the Yuigahama-minami site.

		δ^{13}C		δ^{15}N		n
		Mean	SD	Mean	SD	
Subadult	<4 years old	-18.8	0.3	12.5	1.0	19
	≥4 years old	-18.9	0.6	10.4	0.8	24
	All	-18.9	0.5	11.3	1.4	43
Adult	Female	-18.9	0.6	10.4	0.6	10
	Male	-18.5	0.8	10.4	0.5	3
	All	-18.8	0.6	10.4	0.6	13

The previously reported isotope ratios of the past and modern Japanese foods (Yoneda et al., 2004a) with a fixed difference from dietary protein to collagen of 5.0‰ for carbon and 4.0‰ for nitrogen (Lee-Thorp, 2008) are compared in Figure 4.2 with the Yuigahama-minami adults. The data suggest that marine foods, C_3-based terrestrial foods, and freshwater fish were the primary protein sources for Yuigahama-minami adults although the isotope ratios of these food sources are obviously not directly comparable to the results in this study.

4.3.3 Subadults

The δ^{13}C and δ^{15}N values of subadult samples are shown in Figure 4.3 and summarized in Table 4.2 (see Table B.4 for raw data). A Mann–Whitney U test indicated significantly higher nitrogen isotope ratios in subadults under the age of four years compared to adult females ($U = 164.5$, $p < 0.001$), which was not the case for carbon ($U = 98.5$, $p = 0.890$). No significant difference exists in isotope ratios between all adults and subadults over the age of four for both carbon ($U = 134.5$, $p = 0.503$) and nitrogen ($U = 141.5$, $p = 0.946$). Elevated δ^{15}N values of subadults under the age of four years suggests isotopic enrichment through breastfeeding (Fogel et al., 1989; Millard, 2000; Fuller et al., 2006a). Although statistical analysis is not possible because of the small sample size of each age group, there appears to be no obvious difference in δ^{13}C and δ^{15}N values between subadults with either enamel hypoplasia, cribra cranii, sinusitis, or tuberculosis, and the subadults without these conditions (Figure 4.3). This implies that there is no obvious systematic change in δ^{13}C and δ^{15}N values originating from nutritional stress and pathology.

The mean δ^{13}C values of subadults <4 years of age was only 0.1‰ higher than that of adult females (Table 4.2), which is inconsistent with the 1‰ enrichments expected from breastfeeding (Richards et al., 2002; Fuller et al., 2003; Fuller et al., 2006a) and 0.4‰ enrichment during

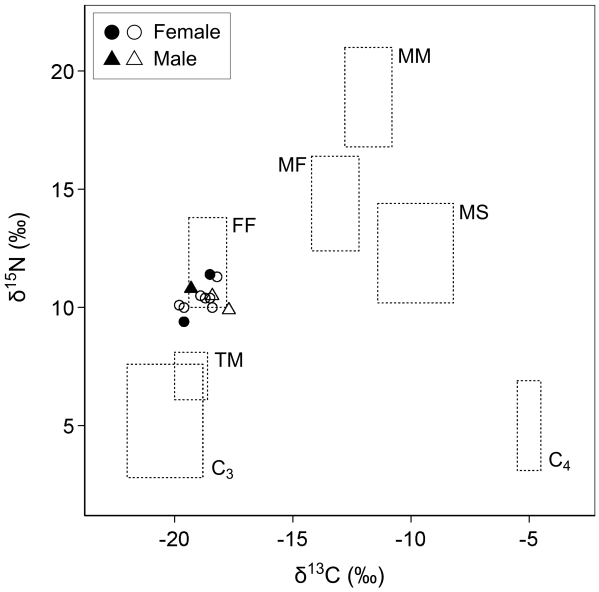

Figure 4.2: Adult δ^{13}C and δ^{15}N results from the Yuigahama-minami site. Individuals represented as empty and solid points were obtained from the Minami and Nakamura (2011) and this study, respectively. Mean and 1SD ranges of isotope ratios of Japanese food groups (Yoneda et al., 2004a) with fixed spacing are also shown as dotted squares; C_3: C_3 plants, C_4: C_4 plants, TM: terrestrial mammals, FF: freshwater fishes, MS: marine shellfishes, MF: marine fishes; MM: marine mammals.

gestation (de Luca et al., 2012). The elevated δ^{15}N values in subadults gradually decreased from 1–2 years of age and approach to the adult range around the age of 3.5–4.5 years (Figure 4.3B). The data suggest that the contribution from weaning foods to dietary protein intake became evident by 1.5 years of age and that weaning proceeded fairly gradually in the Yuigahama-minami population, ending by five years. After correcting for the lag time generated by slower bone collagen turnover, the WARN model showed that the most probable ages for the start and end of weaning were 1.1 and 3.8 years, respectively (Table 4.3). The ^{15}N-enrichment from the mother to the subadult and the δ^{15}N value of collagen synthesized entirely from weaning foods were calculated as 1.8–2.7‰ and 9.7–10.5‰, respectively, with a 95% credible interval (CI).

4.4 Discussion

4.4.1 Adult diet

The reconstructed adult diet in the Yuigahama-minami population is consistent with that shown for medieval townspeople in historical and archaeological records (Ehara et al., 2009). Historical documents described that rice was the staple grain of the people in medieval Japan. Several vegetable and fruit species which can be seen in modern Japan were already consumed. Marine

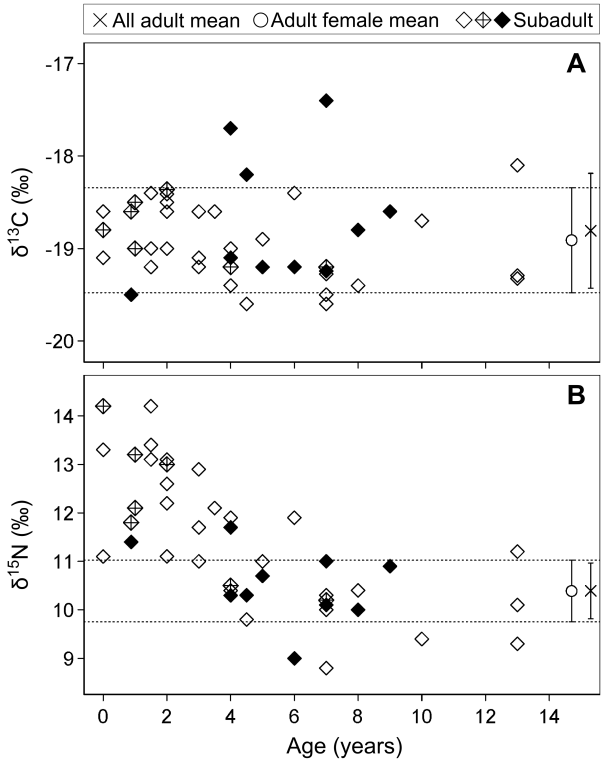

Figure 4.3: Subadult (A) δ^{13}C and (B) δ^{15}N values by estimated age at death in the Yuigahama-minami skeletal population. Dotted lines indicate 1SD ranges for the mean isotope ratio of adult females. Mean and 1SD ranges of all adults are also shown. Solid diamonds indicate individuals with observed pathological conditions, except for caries; empty diamonds indicate individuals with no reported pathology; diamonds with cross mark indicate individuals without observation in Hirata et al. (2002).

and freshwater fish and mollusks as well as terrestrial animals were eaten as protein sources. Analyses of archaeological remains suggest that people in Kamakura consumed various kind of foods, such as C_3 grains, vegetables, terrestrial animals, fish, and mollusks (Amino et al., 1989; Kawano, 1995).

4.4.2 Infant feeding practice

No significant difference in δ^{13}C values between younger subadults and adult females is attributed to ^{13}C-enrichment by breast milk consumption and ^{13}C-depletion by weaning food consumption (e.g., Waters-Rist et al., 2011; Howcroft et al., 2014; Tsutaya et al., 2014a; see also Chapter 3). Subadults aged 6 months and older need weaning foods for good health (Kramer and Kakuma, 2004). Although the age at the start of weaning was calculated as 1.1 years, based on the change in the bone collagen δ^{15}N values (Table 4.3), this calculation only considers proteinous weaning food consumption (Tsutaya and Yoneda, 2013; see also Chapter 2). Increasing needs for dietary

Table 4.3: Weaning parameters and its probability of the Yuigahama-minami population calculated by WARN program.

	MDE	Range		
		Lower	Upper	Probability
t_1	1.1	0.0	2.8	0.96
t_2	3.8	2.9	4.4	
E	2.2	1.8	2.7	0.97
$\delta^{15}N_{wnfood}$	10.1	9.7	10.5	0.96

MDE: maximum density estimator, t_1 and t_2: the age at the start and end of weaning, respectively, E: the ^{15}N-enrichment through maternal to subadult tissue, and $\delta^{15}N_{wnfood}$: δ^{15}N value of collagen synthesized entirely from weaning foods. Joint probabilities are shown for t_1 and t_2, and marginal probabilities for E and $\delta^{15}N_{wnfood}$.

energy (Dewey and Brown, 2003) in younger developing subadults are much higher than protein requirements (Michaelsen et al., 2000). Thus, weaning foods mainly consist of carbohydrates and lipids, which provide energy rather than protein. Several historical sources of the Edo period recommended the supplementation of rice gruel for breastfed subadults older than 6–9 months (Kajitani, 2007, 2008; Sone, 2011). However, these descriptions are not directly comparable to the Kamakura period. Contrary to nitrogen, carbon isotopes from dietary carbohydrates and lipids, as well as those from dietary protein, can be routed into body tissue proteins (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Kellner and Schoeninger, 2007). This is especially the case for individuals living with low protein intake (Schwarcz, 2000). Furthermore, the δ^{13}C values of breastfed subadults decreased to maternal levels more rapidly than δ^{15}N values during the weaning process (Fuller et al., 2006a). Considering this evidence, δ^{15}N values of subadults may reflect predominantly breast milk-derived proteins, and δ^{13}C values are affected by ^{13}C-depleted weaning foods. Subadult δ^{13}C values did not increase, despite enriched δ^{15}N values in several archaeological skeletal populations (e.g., Schurr and Powell, 2005; Choy et al., 2010; Waters-Rist et al., 2011; Beaumont et al., 2013a).

Increased δ^{15}N values in infants are consistent with breastfeeding signals (Fogel et al., 1989; Millard, 2000; Fuller et al., 2006a). Although ^{15}N-enrichment during exclusive breastfeeding was calculated to be 2.2‰ (Table 4.3), individual δ^{15}N differences to the adult female mean varied to some extent in younger subadults (Figure 4.3B). Variation in δ^{15}N values in adult females suggests that maternal baseline values also varied by individual, which was reflected in infant δ^{15}N values through breastfeeding. The effects of isotopic change because of nutritional stress (Fuller et al., 2005; Mekota et al., 2006) and bone pathology (Katzenberg and Lovell, 1999; Olsen et al., 2014) is minimal, if there exists any. This is because of sampling of non-pathological part

of bone, a lack of evidence for systemic stress, and no systematic differences in the isotope ratios of subadult individuals with pathological conditions (Figure 4.3).

The calculated age for the end of weaning (i.e., 3.8 years: Table 4.3) in the Yuigahama-minami population is relatively greater than typical non-industrial populations. Meta-analyses of ethnographic descriptions suggest that the average age at the end of weaning is 2.4–2.7 years although that includes a large variation (Ford, 1945; Barry and Paxson, 1971; Sellen, 2001). Isotopic results revealed an end of weaning age at 3.1 years (95% CI: 2.1–4.1) in the Hitotsubashi population in 17th century Edo city, the capital of feudal Japan (Tsutaya et al., 2014a; see also Chapter 3). An end of weaning age of less than 2 years was isotopically reconstructed in two medieval skeletal populations from the village of Wharram Percy (Richards et al., 2002; Fuller et al., 2003) and Fishergate House, the suburb of York (Burt, 2013) in England, UK. The individuals at these sites were of a low socioeconomic class (Richards et al., 2002; Burt, 2013; Tsutaya et al., 2014a; see also Chapter 3), similar to those from Yuigahama-minami. The diets of these adult populations were similar to those of Yuigahama-minami. C_3 plants and terrestrial animals were consumed in Wharram Percy (Richards et al., 2002; Fuller et al., 2003), and C_3 plants, marine foods, freshwater fish, and terrestrial animals, were consumed in both Hitotsubashi (Tsutaya et al., 2014a; see also Chapter 3) and Fishergate House (Burt, 2013). Comparison of the age at the end of weaning in these skeletal populations suggests a longer breastfeeding period in medieval Kamakura, Japan.

The similarities in $\delta^{13}C$ and $\delta^{15}N$ values of subadults over the age of four years with adults suggest that the foods consumed by subadults after the process of weaning were isotopically similar to the adult diet. However, variations in the isotope ratios of subadult individuals over four years of age were observed (Figure 4.3), suggesting individual variation in post-weaning diet as well as that in adult diet. Different infant feeding practices based on sex, as recommended in *Sekyosho* (Yamazumi and Nakae, 1976) is another likely cause of this variation. However, note that the descriptions in *Sekyosho* should not be directly applied to the Yuigahama-minami results, as the family structure changed, becoming more patriarchical and male-dominated from the Kamakura to Muromachi periods (Suzuki, 1982, 1983), and the historical document refers solely to the warrior classes, not townspeople.

4.4.3 Nutritional status and health

Despite the longer period of breastfeeding, severe stress events and younger age at death distributions were reconstructed from the human remains in medieval Kamakura (Nagaoka et al., 2006, 2013b; Hirata et al. 2011), suggesting overall nutritional stress rather than insufficient breastfeeding. Although breast milk is an important nutritional source and provides various immunological factors, nutritional supplementation is essential for subadults over six months of age (WHO, 1998, 2009). According to Dewey and Brown (2003), energy requirements from weaning foods for growing subadults increases by approximately 1.5 and 2.7 fold at 9–11 and

12–23 months of age, respectively, relative to energy needs at 6–8 months. Specific nutrients such as iron and zinc are candidates for deficiency in subadults and need to be adequately accounted for in weaning foods to ensure subadult health (Dewey and Brown, 2003). Frequent famines and wars occurred in medieval Japan, affecting food provision to commoners (Farris, 2006). Because people in Kamakura were largely dependent on imported food sources, food shortages in rural areas also affected urban Kamakura. Subadults in lower social classes in urbanized cities were especially vulnerable to such food crises (Gracey, 2002; Woods, 2003).

Alternatively, a longer breastfeeding period would be a consequence of weaning food shortage and bad health of subadults. During subadult illness, the appetite for food often decreases and the desire to be breastfed increases, as such breast milk may become the primary source of nutrients (WHO, 2009). However, the isotopic signals for breastfeeding in subadult skeletons are obviously taken from non-survivors and therefore may not be indicative of healthy individuals who survived into adulthood (Katzenberg et al., 1996; Lewis, 2007; see also Wood et al., 1992). In the future, reconstructing infant feeding practices from healthy adults who have survived their subadulthood using isotope analysis of tooth dentin would be more beneficial (e.g., Wright and Schwarcz, 1999; Fuller et al., 2003; Eerkens et al., 2011; Beaumont et al., 2013b; Burt and Garvie-Lok, 2013; Eerkens and Bartelink, 2013; Burt and Amin, 2014; Henderson et al., 2014).

4.4.4 Fertility and population dynamics

The data suggest that the population increases during the urbanization of Kamakura was primarily supported by immigration and not the natural population increases of the townspeople. Kamakura city was only a minor town at the beginning of the Kamakura period, but the region saw rapid development thereafter (Amino et al., 1989; Kawano, 1995; Suzuki, 2013). Population dynamics are determined by mortality, fertility, and migration (Keyfitz, 1980). In medieval Kamakura, living conditions were poor; human remains indicate high mortality and a similarly high frequency of several stress markers (Amino et al., 1989; Kawano, 1995; Nagaoka et al., 2006, 2013b; Hirata et al. 2011). Furthermore, fertility of townspeople in medieval Kamakura would be relatively low because the longer breastfeeding duration would likewise increase the length of postpartum amenorrhea, which is the most important determinant of fertility (Bongaarts, 1982; Bongaarts and Potter, 1983; Campbell and Wood, 1988; Wood, 1994). Insufficient nutrition, as suggested in the townspeople in medieval Kamakura (Nagaoka et al., 2006, 2013b; Hirata et al., 2011), also increased the length of postpartum amenorrhea, thus decreasing fertility rates (Ellison, 1994; Valeggia and Ellison, 2009). The combination of these results strongly suggests that the population increase in the medieval city of Kamakura was a result of immigration from surrounding rural areas and not decreased death rate or an increased birthrate.

Premodern cities in Japan and Europe have comparatively higher mortality (Smith, 1973; Hayami, 1999; Kito, 2000) and lower fertility rates (Sharlin, 1978; Saito, 2002) than rural areas. Premodern cities were "urban graveyards" of the people. Results of this study indicate that this

was already the case in Kamakura, the medieval urban capital of Japan. This is consistent with the suggestion in archaeology, which assumes that Kamakura was a so-called "black hole" for people, materials, and cultures (Kawano, 1995).

4.5 Conclusions

Stable isotope analyses indicated that marine foods, C_3-based terrestrial foods, and freshwater fish were the primarily sources of protein for adults in the Yuigahama-minami site, consistent with the historical (Ehara et al., 2009) and archaeological (Amino et al., 1989; Kawano, 1995) records of medieval Japan. A combination of my results with the isotopic data set of Yuigahama-minami adults reported in Minami and Nakamura (2011) indicated no significant difference between the sexes.

The ages at the start and end of weaning were calculated as 1.1 years (0.0–2.8 in 96% CI) and 3.8 years (2.9–4.4 in 96% CI). The reconstructed breastfeeding duration was relatively longer in the Yuigahama-minami population than in typical non-industrial societies (Ford, 1945; Barry and Paxson, 1971; Sellen, 2001), the Hitotsubashi population in 17th century Edo city (Tsutaya et al., 2014a; see also Chapter 3), and the two medieval skeletal populations from Wharram Percy (Richards et al., 2002; Fuller et al., 2003) and Fishergate House (Burt, 2013) in the UK. Because of the prevalence of stress markers and the younger age at death distributions in skeletons from medieval Kamakura (Nagaoka et al., 2006, 2013b; Hirata et al. 2011), immunological and nutritional benefits of a longer breastfeeding duration did not compensate the adverse effects of severe nutritional stress for the townspeople in medieval Japan. Alternatively, a longer breastfeeding period would be a consequence of weaning food shortage and bad health of subadults. Furthermore, Kamakura experienced development, expansion, and population increase in the early medieval period (Amino et al., 1989; Kawano, 1995) despite a higher mortality (Nagaoka et al., 2006, 2013b) and longer inter-birth interval, a potential consequence of longer breastfeeding duration, not favoring natural population increase. Thus, my results suggest that population increase during urbanization in Kamakura was supported by immigration and not natural increase.

Chapter 5

Expansion of the Okhotsk culture in Hokkaido (Moyoro)

In this chapter, I focused on the fertility of the Okhotsk people, northern hunter–gatherer–fishers in the AD 5th–13th centuries, and performed stable isotope analyses to reconstruct their weaning age, one of the most important determinants of fertility (Bongaarts and Potter, 1983; Campbell and Wood, 1988; Wood, 1994). The Okhotsk culture expanded rapidly from southern Sakhalin to the Okhotsk coast of eastern Hokkaido and the Kuriles along the coastline. Its population dynamics have been inferred by anthropologists and archaeologists, but its demographic parameters, especially of fertility, have not been reconstructed based on bioarchaeological evidences.

5.1 Introduction

5.1.1 Okhotsk culture

The Okhotsk people were sedentary maritime hunter–gatherer–fishers who flourished in Sakhalin, Hokkaido, and the Kurile Islands of northeast Asia during the AD 5th to 13th centuries (Ushiro, 1991; Amano, 2003; Hudson, 2004). They are characterized for substantial maritime adaptation: archaeological sites have been found only in coastal areas, and large amounts of marine food remains and fishing and sealing tools were found at the sites (Okada, 1998; Yamaura, 1998; Amano, 2003; Hudson, 2004).

Stable carbon and nitrogen isotope analyses have also revealed that most of their dietary proteins were derived from marine mammals and fishes (Chisholm et al., 1992; Yoneda, 2002; Naito et al., 2010a). The reported mean δ^{13}C value of adult human bone collagen from two Okhotsk sites in northern Hokkaido (Omisaki and Hamanaka) was -17.5‰ (Chisholm et al., 1992). Yoneda (2002, personal communication) analyzed adult human skeletons excavated from two Okhotsk sites, Hamanaka 2 and Utoro in northern and eastern Hokkaido, respectively. The mean δ^{13}C and δ^{15}N values were -13.1 ± 0.2‰ and 19.2 ± 0.6‰ for Hamanaka 2, and -13.8 ±

0.7‰ and 19.4 ± 0.5‰ for Utoro, respectively. These studies indicate that the Okhotsk people depended on marine mammals as their protein sources more strongly than any other prehistoric population in Japan, such as the Epi-Jomon and Ainu (Chisholm et al., 1992; Yoneda, 2002). Unfortunately, however, they did not conduct isotope analyses of faunal remains from the same sites in the same period, and thus, their conclusions require further scrutiny. Naito et al. (2010a) conducted amino acid-specific nitrogen isotope analyses of adult human and faunal skeletons excavated from three different Okhotsk sites (i.e., Hamanaka 2, Kafukai 1, and Moyoro) and revealed dietary differences between northern and eastern Hokkaido. Although the sample size was small (i.e., $n = 2$ or 3 per one site), they concluded that fur seal contribution was higher in Moyoro of eastern Hokkaido (78–80%) than that in Hamanaka 2 and Kafukai 1 of northern Hokkaido (lower than 24%) populations.

Although the available radiocarbon dates have a much larger range, the Okhotsk culture is divided into several periods (Ohyi, 1975; Amano, 1979; Ushiro, 1991; Hudson, 2004). The origin of the Okhotsk culture is unclear, owing to a lack of archaeological data in Sakhalin. However, strong similarities among Okhotsk people and populations from the lower Amur River region and Neolithic Baikal have been suggested from morphological (Ishida, 1996; Komesu et al., 2008; Kudaka et al., 2013) and ancient DNA (Sato et al., 2007, 2009) analyses. In the earlier period of Okhotsk culture (AD 400–500), they were distributed around the southwestern end of Sakhalin and the northern end of Hokkaido. In the middle period, the Okhotsk people expanded their habitat rapidly from southern Sakhalin and northern Hokkaido to the eastern Hokkaido and Kuriles along the coasts, mostly during AD 600–700 (Yamaura and Ushiro, 1999). The interior region of Hokkaido at these times was occupied by Epi-Jomon and Satsumon peoples, who are descendants of Jomon hunter–gatherers, and the Okhotsk people coexisted with these peoples (Okada, 1989; Yamaura and Ushiro, 1999). During the process of expansion, pit dwellings became larger (Amano, 2003; Hudson, 2004), and the main subsistence changed from fishing to sealing in eastern Hokkaido because drift ice made winter fishing impossible there (Ono, 1996a, b; Naito et al., 2010a). In the final period, the Okhotsk culture receded to the northern and eastern edges of Hokkaido and was absorbed around AD 1000 into the Satsumon culture, which developed into the Ainu culture several hundred years later (Yamaura and Ushiro, 1999). Recent cultural (Masuda et al., 2001), genetic (Sato et al., 2007, 2009; Jinam et al., 2012), morphological (Ishida, 1996; Hanihara, 2010), and linguistic (Lee and Hasegawa, 2013) evidence indicates a large contribution of the Okhotsk to the formation of the Ainu population and culture.

Population increase has been suggested as one of the main causes of the Okhotsk culture's rapid expansion along the coast of Hokkaido (Ohyi, 1978, 1988; Amano, 1979; Ono, 1996b). However, some studies have suggested other factors behind the expansion into eastern Hokkaido, such as the motivation of fur trading with other populations (Amano, 1979) and political and social disturbances along the Amur (Yamaura, 1998). At present, however, actual evidence for population increase of the Hokkaido Okhotsk is ambiguous (Hudson, 2004); only mortality has

been estimated by palaeodemographic methods, and fertility has been little studied. Nagaoka et al. (2012) classified age of death into three categories (15–34, 35–54, and ≥55 years of age), reconstructed distributions of adult age at death from aggregated skeletal series from seven different Okhotsk sites, and concluded that the proportion of elderly individuals (≥55 years of age) was greater in the Okhotsk than in the Jomon series. To characterize the population dynamics of the Okhotsk people, I reconstructed weaning ages in the Moyoro population in eastern Hokkaido by isotope analysis and evaluated fertility in the Okhotsk culture.

5.1.2 Moyoro site

The Moyoro site is a representative shell mound of the Okhotsk culture, located in eastern Hokkaido, Japan and formed on an estuarine sand area of the Abashiri River (Figure 5.1). The site has been excavated by several researchers since the beginning of the 20th century (Yonemura, 1935; Kodama, 1948; Komai, 1964; Ito, 1965; Kiyono, 1969). Pottery, large pit dwellings, faunal remains, shell mounds, and bone and stone tools as well as human remains have been found at the site. The Moyoro site is thought to have been used for some hundred years, and most calibrated radiocarbon ages of excavated adult human skeletons range approximately from 500 to 900 calAD (Komesu et al., 2008; Tsutaya et al., 2014b; see below), which is the Middle Okhotsk period and was characterized by rapid expansion. Radiocarbon ages of carbides and paleomagnetic dating obtained in recent excavations indicate that the site was used as early as the AD 5th to 7th centuries (Abashiri City Board of Education, 2009).

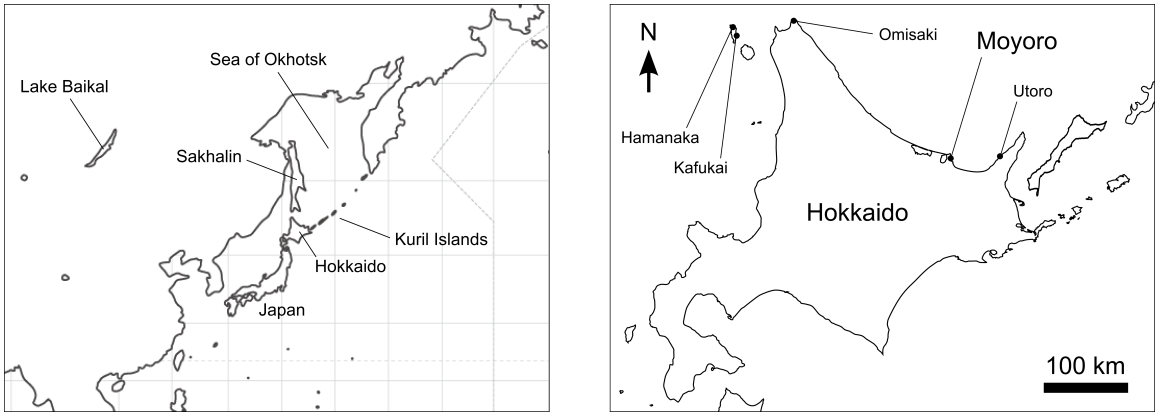

Figure 5.1: Location of the Moyoro and other Okhotsk archaeological sites mentioned in this study.

Previous isotope analyses showed that the Moyoro adult humans obtained approximately 80% of their dietary protein from marine mammals (Naito et al., 2010a). Although most Hokkaido populations in past were highly dependent on marine mammals, this figure is large compared with other populations from the Epi-Jomon culture of southwestern Hokkaido (Minagawa, 2001;

Tsutaya et al., 2013), the Okhotsk culture of northern Hokkaido (Nishimoto, 1978; Naito et al., 2010a), and the Ainu culture (Minagawa, 2001). Isotopic data of subadult individuals of the Okhotsk culture have never been reported.

5.2 Materials and methods

5.2.1 Skeletal samples

I and my collaborators obtained elemental and isotopic data from 18 faunal remains (Table B.5) from the excavated Moyoro faunal collections at the Abashiri City Historical Museum, and 58 adult (22 females, 34 males, and two sex-unknowns, Table B.6) and 58 subadult (Table B.7) human individuals from the skeletal collection at the Hokkaido University Museum. Elemental and isotopic data of two faunal (M14 and M32) and two human adult (1006 and 1011) individuals reported in Naito et al. (2010a) were also included in the dataset.

Faunal species were identified by zooarchaeologist(s) other than myself. Age and sex of the human skeletons were determined by my collaborator (Hajime Ishida of University of the Ryukyus). The sex of the adults was determined by the morphology of the pelvis: the composite arch, the greater sciatic notch, the inferior pelvis, the ischiopubic proportion, and the preauricular sulcus (Bruzek, 2002), the medial aspect of the ischiopubic ramus, the subpubic concavity, and the ventral arc (Phenice, 1969). The ages of subadult skeletons were determined from a reference chart of tooth development and eruption (Ubelaker, 1999).

5.2.2 Stable isotope analyses

Sample collection and collagen extractions were performed by my collaborators (Yuichi I Naito and Minoru Yoneda of The University of Tokyo) for faunal and adult human skeletons, and by myself for subadult human skeletons. All samples were prepared for isotope analysis at the University of Tokyo, Japan, following a modified gelatinization method (Longin, 1971; Yoneda et al., 2004a). Procedures were generally same as those described in Section 3.2.2.

Most of the isotope analyses of faunal and adult skeletons were performed by my collaborators (Y.I.N. and M.Y.) at the National Institute for Environmental Studies, Ibaraki, Japan. Approximately 0.25 mg of the resulting gelatin was measured using an EA-IRMS; Carlo Erba NA1500 elemental analyzer, Finnigan MAT ConFlo II interface, and Finnigan MAT 252 mass spectrometer. The isotope analyses of subadult skeletons were performed by SI Science Co., Ltd. The resulting gelatin was measured with an EA-IRMS consisting of a Flash EA1112 elemental analyzer, ConFlo III interface, and DELTA V Plus mass spectrometer. The analytical standard deviation (SD) was approximately 0.1‰ and 0.2‰ for carbon and nitrogen, respectively. Elemental concentrations and isotope ratios were calibrated against an alanine standard.

To eliminate skeletons from different periods than that of the Okhotsk culture, radiocarbon

ages of the Moyoro adult human skeletons were referred (Komesu et al., 2008). Radiocarbon dating was performed by my collaborator (M.Y.) at NIES-TERRA, the accelerator facility of the National Institute for Environmental Studies, Ibaraki, Japan (Yoneda et al., 2004b; Komesu et al., 2008; see also Tsutaya et al., 2014b). Corrections of +382 ± 16 years for the local reservoir effect (Yoneda et al., 2001) and 90% contribution from marine proteins were applied considering the marine reservoir effect of the ^{14}C age. The ^{14}C ages were calibrated by the OxCal program (Bronk Ramsey, 1995) based on the atmospheric and marine data sets (IntCal13 and Marine13, respectively: Reimer et al., 2013). Skeletons with calibrated ages apparently later than the Okhotsk period were excluded.

5.2.3 Statistics

All statistical analyses were performed with R software (R Core Team, 2014). Dietary contributions of various food sources was estimated by using a Bayesian mixing model SIAR (Parnell et al., 2010, see also Phillips, 2012) with a "very long" run configuration of MCMC (i.e., 1,000,000 iterations and 400,000 burn-in). Food sources were grouped into several categories with distinct isotope ratios. This categorization is meaningful only in isotope ecology and may not necessarily have archaeological significance. The discrimination δ^{13}C and δ^{15}N values from prey to consumer bone collagen were set as 1.5 ± 0.5‰ (5.0 ± 0.5‰ in case of C_3 plants due to the difference discrimination with animal foods) and 4.0 ± 1.0‰, respectively (Lee-Thorp, 2008). Isotope ratios of modern Hokkaido C_3 plants and modern Japanese marine shellfish with correction of the Suess effect (Friedli et al., 1986) were those reported in Tsutaya et al. (2013) and Yoneda et al. (2004a), respectively. Two applications were independently performed: applications into two dogs and all adult humans. Loess curves (Cleveland and Devlin, 1988) were generated using the *lowess* function in R with eight years as the smoothing span and 10 iterations.

Weaning parameters were estimated from the results of subadults younger than 10 years of age using a program adopting approximate Bayesian computation, WARN (Tsutaya and Yoneda, 2013; see Chapter 2) with default configurations. When WARN was applied to previously reported skeletal populations of northern hunter–gatherer–fishers, data of low-δ^{13}C subadults were omitted from the dataset of Tsutaya et al. (2013); the δ^{15}N values of the proximal metaphysis was used for a dataset of Ust'-Ida, and data from Shamanka and Lokomotiv were omitted owing to a small number of younger subadults for Waters-Rist et al. (2011); data obtained from tooth dentin collagen was omitted for Howcroft et al. (2014).

5.3 Results

5.3.1 Preservation of bones

The results of isotopic and elemental analyses of faunal and adult/subadult human bone collagen are shown in Table B.5, B.6, and B.7, respectively. The preservation of collagen was evaluated from the atomic C/N ratios and the yield of collagen. On the basis of acceptable C/N ratios (2.9–3.6; DeNiro, 1985), six adult human samples were excluded from the dataset. Following the criteria proposed by van Klinken (1999), all samples were considered to have acceptable yields (greater than 1%).

5.3.2 Fauna

The δ^{13}C and δ^{15}N values of faunal bone collagen are summarized in Table 5.1 and shown in Figure 5.2. Carbon and nitrogen isotope ratios of terrestrial herbivores range from -23.3‰ to -22.3‰ and 2.1‰ to 7.5‰, respectively. The herbivorous mammals fell within the range of C_3 consumers, which indicates that the mammals consumed little or no C_4 plants. Isotope ratios from wild terrestrial carnivores range from -21.0‰ to -17.6‰ for carbon and from 5.7‰ to 10.2‰ for nitrogen. The δ^{13}C and δ^{15}N values of some terrestrial mammals (e.g., M14, MF18, and MF27) are unexpectedly higher than the natural isotopic abundances in Japanese terrestrial resources (Yoneda et al., 2004a). Such higher isotope ratios could be due to the sea spray effect (Virginia and Delwiche, 1982; Heaton, 1987).

Stable isotope ratios of marine fish range from -15.1‰ to -13.1‰ for carbon and from 11.3‰ to 15.0‰ for nitrogen. The isotope ratios from marine mammals range from -14.2‰ to -13.6‰ for carbon and from 14.0‰ to 16.6‰ for nitrogen. The δ^{13}C and δ^{15}N values of marine mammals are higher than those of fish, possibly because of trophic level enrichment (Minagawa and Wada, 1984; Bocherens and Drucker, 2003). The δ^{13}C and δ^{15}N values of brackish water fish bone collagen were similar and at the lower end of those of marine fishes, respectively (Figure 5.2).

Dogs showed relatively different isotope ratios than the other faunae. Two dog individuals have δ^{13}C and δ^{15}N values similar to marine mammals. Assuming that the dogs and the humans consumed similar foods, the Bayesian mixing model indicates that the 95% credible interval (CI) of dietary protein contributions from brackish water fish, marine fish, and marine mammals are lower than 45%, whereas those from terrestrial foods are lower than 25% (Table 5.2). The data indicate that the dogs were predominantly fed with brackish water fish, marine fish, and marine mammals. The posterior probabilities of the dietary proportions are summarized in Table 5.2 and shown in Figure 5.3A.

Table 5.1: Summary of δ^{13}C and δ^{15}N values for faunal and human bone collagen from the Moyoro site.

		δ^{13}C		δ^{15}N		n
		Mean	SD	Mean	SD	
Fauna	Brackish water fish	-14.8	1.8	11.4	0.2	2
	Dog	-13.7	0.1	16.7	0.4	2
	Marine fish	-14.3	0.8	13.9	1.5	5
	Marine mammal	-14.2	0.3	15.8	0.9	7
	Terrestrial carnivore	-19.2	1.8	8.0	2.1	4
	Terrestrial herbivore	-22.7	0.4	4.1	2.3	4
Adult human	Female	-13.5	0.5	19.3	0.9	21
	Male	-13.7	0.8	19.5	0.8	29
	All	-13.6	0.7	19.4	0.8	52
Subadult human	$\leq$3 years of age	-13.9	0.6	20.6	1.6	18
	3–10 years of age	-13.9	0.5	19.1	0.5	35
	$>$10 years of age	-13.5	0.3	18.6	0.5	5

5.3.3 Adults

The δ^{13}C and δ^{15}N values of adult human bone collagen are summarized in Table 5.1 and shown in Figure 5.4. The stable isotope ratios of adult female range from -14.8‰ to -12.7‰ for carbon and from 16.3‰ to 20.6‰ for nitrogen. The isotope ratios of adult males range from -16.0‰ to -12.7‰ for carbon and from 17.8‰ to 20.8‰ for nitrogen. The Mann–Whitney U test indicated no significant difference between females and males (δ^{13}C values: $U = 300$, $p = 0.937$; δ^{15}N values: $U = 264.5$, $p = 0.437$). However, the variances of the δ^{13}C values of males were significantly greater than those of females (F test: $F = 2.580$, $p = 0.032$), whereas those of the δ^{15}N values between the sexes did not differ significantly (F test: $F = 0.942$, $p = 0.867$).

There is no systematic time change in isotope ratios. Spearman rank correlation tests indicated that the calibrated ^{14}C ages did not correlate significantly with δ^{13}C ($R = -0.113$, $p = 0.481$) and δ^{15}N ($R = -0.032$, $p = 0.845$) in adult human individuals. The 95% CIs of calibrated ^{14}C ages range from 1095 calBP to 334 calBP (Table B.6).

Applying the Bayesian mixing model, the 95% CI of dietary protein contribution from marine mammals is calculated to be approximately 80–90% in humans (Table 5.2). On the other hand, the summed dietary protein contribution from terrestrial foods is lower than 16% (Table 5.2). The data suggest that the Moyoro adult human population heavily depended on marine mammals for dietary proteins. The posterior probabilities of the dietary proportions are summarized in Table 5.2 and shown in Figure 5.3B.

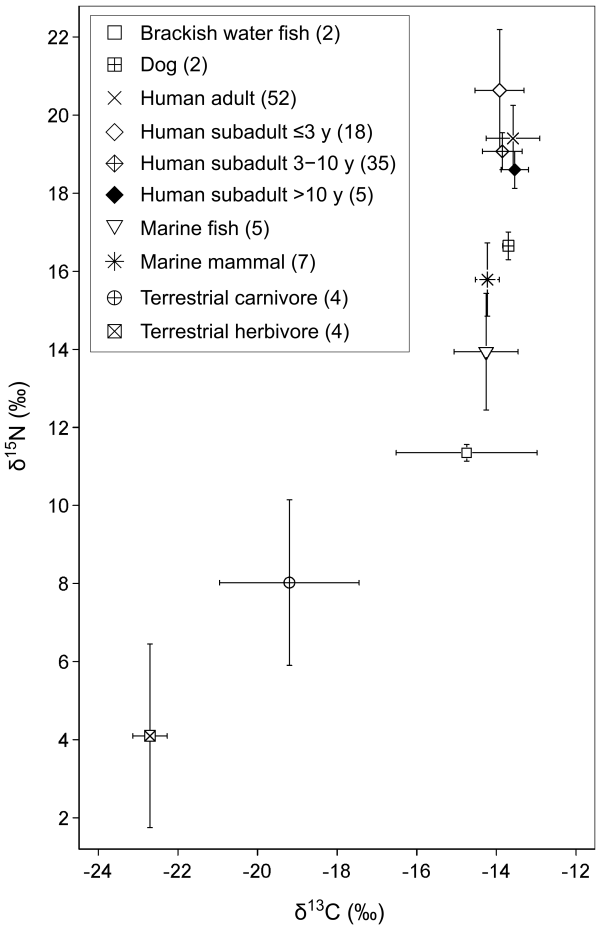

Figure 5.2: δ^{13}C and δ^{15}N values for faunal and human bone collagen from the Moyoro site. The mean and 1SD ranges are indicated. The isotope ratios of modern C_3 plants and marine shellfish are from Tsutaya et al. (2013) and Yoneda et al. (2004a), respectively. Numbers in parentheses indicate number of analyzed individuals. Human subadults are divided into three age categories.

5.3.4 Subadults

The δ^{13}C and δ^{15}N values of subadult human bone collagen are plotted with faunal and adult human isotope ratios in Figure 5.2. Most subadult individuals show isotope ratios similar to those of adults, suggesting that subadults relied on the same dietary ecosystem as adults. The Moyoro population depended highly on marine foods, especially mammals, for its dietary protein intake (Naito et al., 2010a; Table 5.2 and Figure 5.3B). A summary of the isotope ratios for the Moyoro population is shown in Table 5.1.

Some trends are evident in the isotopic changes with age in subadults. Loess curves indicate that δ^{13}C values decreased during 0–3 years, remained lower during 3–10 years, and increased to the adult mean after 10 years of age, and that δ^{15}N values rapidly decreased to the adult mean during 0–3 years, showed a further marginal decrease during 3–10 years, and remained lower

Table 5.2: Mode and lower and higher 95% credible interval ranges of the relative dietary contribution (%) for the Moyoro dog and adult human calculated by SIAR.

		BF	C_3	MF	MM	MS	TC	TH
Dog	Mode	20.5	1.5	19.5	19.5	15.5	1.5	1.5
	Lower	2.0	0.5	3.3	4.7	1.6	0.9	0.5
	Upper	32.7	20.1	39.1	44.8	30.9	25.0	20.8
Human	Mode	0.5	0.5	0.5	86.5	0.5	0.5	3.5
	Lower	0.1	0.1	0.3	80.2	0.0	0.3	0.6
	Upper	4.2	2.4	9.9	90.2	2.0	8.7	6.6

BF: brackish water fish, C_3: C_3 plants (Tsutaya et al., 2013), MF: marine fish, MM: marine mammals, MS: marine shellfish (Yoneda et al., 2004a), TC: terrestrial carnivores, and TM: terrestrial herbivores.

after 10 years of age (Figure 5.5). Mann–Whitney U tests indicate significantly higher and lower δ^{15}N values for subadults aged 0–3 years ($U = 308.5$, $p = 0.001$) and 3–10 years ($U = 626.5$, $p = 0.014$) compared with adult females and all adults, respectively. Elevated δ^{15}N values in infants are a result of breastfeeding (Fuller et al., 2006a). In contrast, the Mann–Whitney U tests indicate significantly lower δ^{13}C values for subadults aged 0–3 years ($U = 101.0$, $p = 0.013$) and 3–10 years ($U = 498.0$, $p < 0.001$) than for adult females and all adults, respectively. There is a significant decrease in δ^{15}N (Mann–Whitney U test, $U = 44.5$, $p = 0.016$) and a nonsignificant change in δ^{13}C (Mann-Whitney U test, $U = 115.5$, $p = 0.692$) values of subadults older than 10 years compared with all adults. Isotopic differences between subadults and adults are summarized in Table 5.3.

Table 5.3: δ^{13}C and δ^{15}N differences and results of U test for subadults from the Moyoro site.

	δ^{13}C			δ^{15}N			v.s.
	Difference	U	p	Difference	U	p	
≤3 years of age	-0.4	101.0	0.013	1.4	308.5	0.001	Adult female
3–10 years of age	-0.3	498.0	0.000	-0.3	626.5	0.014	All adult
>10 years of age	0.0	115.5	0.692	-0.8	44.5	0.016	All adult

Using the WARN program to correct for the lag time resulting from bone collagen turnover, the >95% credible intervals (CIs) of the ages at the start (t_1) and end (t_2) of weaning, ^{15}N-enrichment from mother to infant (E), and δ^{15}N value of collagen synthesized entirely from weaning foods ($\delta^{15}N_{wnfood}$) were calculated as 0.0–1.4 years of age, 1.4–2.2 years of age, 2.0–

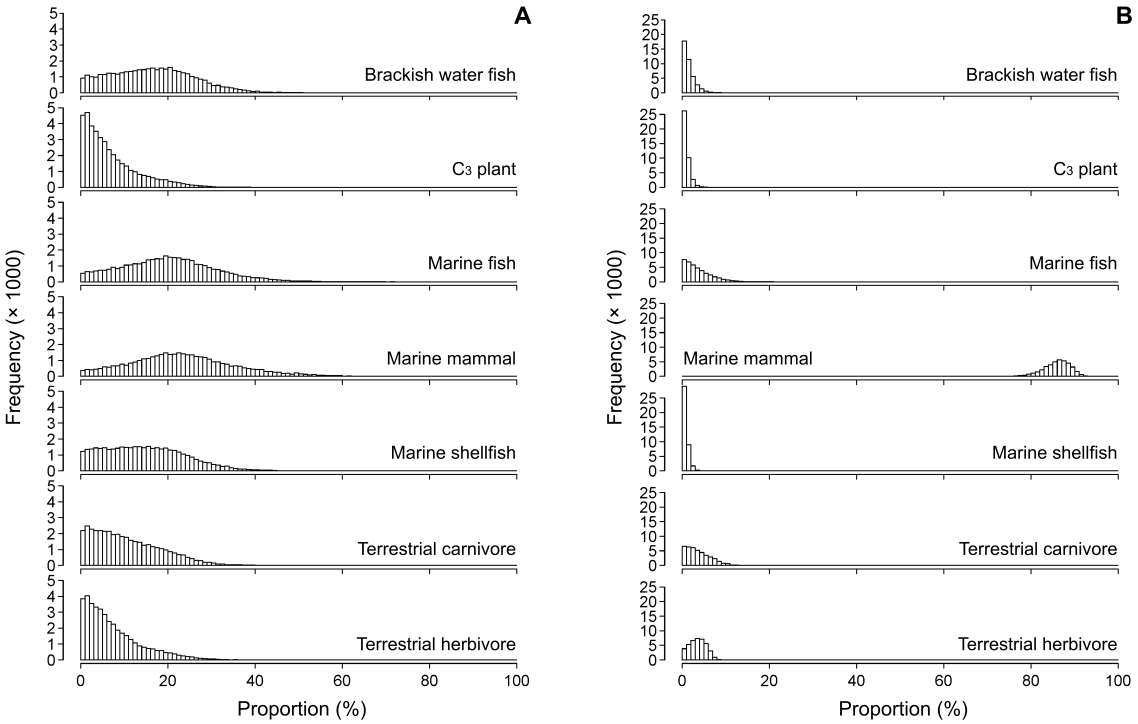

Figure 5.3: Posterior distribution of the dietary proportion for (A) dogs and (B) adult humans calculated by SIAR.

3.4‰, and 19.0–19.3‰ for the Moyoro population (Table 5.4). A summary of the posterior distributions of the age at the end of weaning in previously reported past northern hunter–gatherer–fishers is also shown in Table 5.4.

5.4 Discussion

5.4.1 Dog diet

Unlike other terrestrial carnivores, the isotope ratios of the two dogs suggest marine protein consumption (Figure 5.2). These dogs would have been to some extent fed with marine foods caught by humans. However, the Bayesian mixing model showed that the diet composition of the dogs differed from that of the humans; dietary proteins from marine mammals are 80–90% of the consumed proteins for humans but only 5–45% for the dogs in the 95% CI. The protein contribution of brackish water and marine fish was apparently greater in dogs compared to humans and this contribution from brackish water and marine fishes is possibly comparable with that from marine mammals in dogs (Table 5.2, Figure 5.3A). Humans and dogs have lived sympatrically in the Moyoro site, and their diet were somewhat different (Table 5.2) and thus significant overlap in dietary protein intake would have been avoided.

Several ethnographic studies have reported dog use and dog diet of the indigenous Ainu

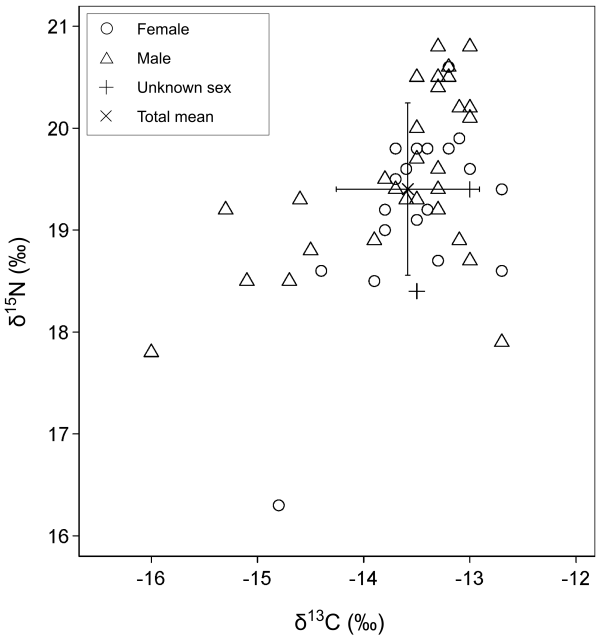

Figure 5.4: δ^{13}C and δ^{15}N values for adult human bone collagen from the Moyoro site.

people in Hokkaido and Sakhalin, and fishers in Kamchatka. Ethnographic accounts of the Ainu population in Hokkaido (Starr, 1904) and Sakhalin (Nishitsuru, 1942; Kasai, 1943) in the beginning of the twentieth century and those in Hokkaido in the 19th century (Batchelor, 1892) reported that dogs were used for hunting of terrestrial mammals and sledging. Dog skins were used to make clothes and shoes (Batchelor, 1892). Several studies also recorded that the Ainu people fed their dogs with low-sodium trout (Nishitsuru, 1942; Kasai, 1943). The isotope ratio of trout in Hokkaido is similar to that of brackish water fish (Tsutaya et al., 2013). Shnirelman (1994) reported that fishers in Kamchatka in the late 19th century fed their domesticated dogs with dried or fermented fish and used them as sledge dogs. Although the cultural traits reported in modern ethnographic studies are not directly comparable with those in the past Okhotsk population, such ethnographic observations agree well with the isotopic results in this study.

Carbon and nitrogen isotopic similarities between dogs and humans have been reported from various archaeological sites around the world (e.g., Cannon et al., 1999; Fischer et al., 2007; Fornander et al., 2008; Choy and Richards, 2009). Furthermore, it has been assumed that the isotope ratios of dogs are a proxy for human diet. On the other hand, there are several differences between the dog and human isotope ratios, and some have assumed that the isotopic differences indicate dietary differences between dogs and humans (Rick et al., 2011; Losey et al., 2013). However, none of these studies has shown what the dogs actually ate because of insufficient faunal isotope ratios. This study has found a lesser contribution of marine mammal protein in the dog diet of the Moyoro site by considering the feeding ecology of the Okhotsk dogs and humans. Thus, it would be better to cover other faunal isotope ratios as well when assessing the

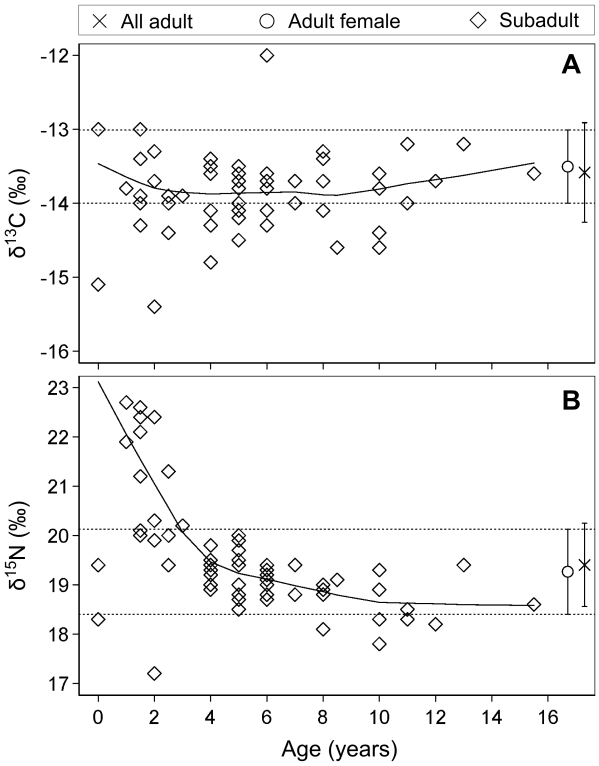

Figure 5.5: Subadult (A) δ^{13}C and (B) δ^{15}N values by estimated age at death in the Moyoro skeletal population. Dotted lines indicate 1SD ranges for the mean isotope ratio of adult females. Mean and 1SD ranges of all adults are also shown. Solid lines indicate Loess curves.

isotopic evidence for domesticated dog diet.

5.4.2 Adult human diet

The mean isotope ratios did not differ by sex; however, the variance of δ^{13}C value was greater for human males (Figure 5.4). This variance is not the result of time change in diet. The larger variance of the male isotope ratios suggests that the male diets were more heterogeneous than female diets in the Moyoro population. Chisholm et al. (1992) reported that the mean δ^{13}C values of human bone collagen for males were more negative than females in several prehistoric Hokkaido sites; furthermore, they inferred that the males would have eaten more terrestrial foods. Considering that the δ^{13}C values of several adult males are more negative than those of most Moyoro adults, there were apparently some adult males who preferentially consumed ^{13}C-depleted foods such as terrestrial foods.

The wide dietary range of the Moyoro males is discussed from several aspects. First, accessible food sources might be greater for males due to cultural or behavioral reasons. While Ainu females mainly handled labor around the household such as making clothing, cooking, and childcare, males were in charge of hunting and fishing (Batchelor, 1927; Nishitsuru, 1942; Kasai, 1943;

Table 5.4: Maximum density estimator (MDE), probability of MDE, the assigned CI, and probability of the CI for weaning parameters calculated by WARN with the Moyoro and other datasets (≤10 years of age).

Population	Parameter	MDE		Range		
		Estimator	Probability	Lower	Upper	Probability
Moyoro	t_1	0.4	0.026	0.0	1.4	0.952
	t_2	1.8		1.4	2.2	
	E	2.7	0.102	2.0	3.4	0.947
	$\delta^{15}N_{wnfood}$	19.2	0.309	19.0	19.3	0.953
Ajvide	t_2	2.3	0.005	1.5	3.5	0.952
Ust'-Ida	t_2	2.7	0.018	2.3	3.1	0.951
Usu-moshiri	t_2	4.9	0.004	3.8	6.3	0.950

t_1 and t_2: ages at start and end of weaning, respectively; E: the ^{15}N-enrichment through maternal to subadult tissue; and $\delta^{15}N_{wnfood}$: δ^{15}N value of collagen synthesized entirely from weaning foods. Joint probabilities are shown for t_1 and t_2, and marginal probabilities for E and $\delta^{15}N_{wnfood}$. Isotopic data of Ajvide, Ust'-Ida, and Usu-moshiri population are derived from Howcroft et al. (2014), Waters-Rist et al. (2011), and Tsutaya et al. (2013), respectively.

Ohnuki-Tierney, 1974). The greater the activity ranges are, the more diverse food sources from different ecosystems can be obtained. Furthermore, some researchers have assumed that the status of females in the eastern Okhotsk culture declined (Yamaura, 1982; Hudson, 2004), which could correlate with the availability of food. The above mentioned considerations should be evaluated from the standpoint of archaeology and ethnography in the future. Second, male-biased immigration could also be a possible reason although patrilocal-like marital systems were described in an ethnographic study of the Sakhalin Ainu (Ohnuki-Tierney, 1974). Adult males who moved in from other villages may have retained the isotope ratios of their ecosystems of origin because it takes more than 10 years for adult bone collagen to fully turn over and record a new diet (Hedges et al., 2007). I anticipate that immigrants to Moyoro can be identified by using oxygen and strontium isotopes of tooth enamel (Waseda and Nakai, 1983; Kusaka et al., 2012).

Although the mixing model provides quantitative estimates for dietary reconstruction, we need to consider two major drawbacks. First, we cannot evaluate the relative contributions from food sources that are not included in the model. Ordinarily, we cannot calculate contribution from food sources that do not remain in the archaeological site. To overcome this problem, the isotope ratios of modern C_3 plants and marine shellfish were included in the SIAR models. A small amount of carbonated boiled barley and specific quantities of several marine shellfish re-

mains were excavated from the Moyoro site (Abashiri City Board of Education, 2009); however, the carbon and nitrogen isotope ratios of their edible part cannot be measured. Although a small amount of carbonated grains of C_4 plants was also excavated, I excluded the C_4 plants from the SIAR analysis, because the C_4 grains were assumed to have been used in rituals considering their excavation conditions (Yamada, 1996; Abashiri City Board of Education, 2009). Although the food sources examined in this study cover the range of excavated food items, it is always possible that there are other unexcavated food sources. Second, we cannot evaluate the relative contributions from food sources that indicate almost the same isotopic signatures. Protein contributions from dogs in human diet cannot be evaluated in this study because the $\delta^{13}C$ and $\delta^{15}N$ values of dogs and marine mammals are quite similar (Figure 5.2). Although 80–90% of dietary proteins are derived from marine mammals, this probably includes the contribution from dogs as well. However, I believe that the contribution from dogs is not that large because the excavated skeletons of dogs are apparently smaller than those of marine mammals in the Moyoro site although the exact quantity has never been estimated (Abashiri City Board of Education, 2009). Furthermore, in the Kafukai site of northern Hokkaido, dogs and pigs together contribute only 2.9% of the estimated caloric intake based on the quantitative evaluation of the excavated remains (Nishimoto, 1978). Although such drawbacks would not affect the overall conclusions, we should be aware of them.

The Bayesian mixing model of carbon and nitrogen isotopes showed that the Moyoro adults obtained approximately 80–90% of dietary proteins from marine mammals at the highest (Table 5.2 and Figure 5.3B), which suggests significant contribution of marine mammals to the dietary protein sources of the Okhotsk people in eastern Hokkaido. Amino acid-specific nitrogen isotope analyses revealed that fur seal protein contribution was 78–80% in the Moyoro humans (Naito et al., 2010a), which is slightly lower than the contribution in this study probably because of the above-mentioned reasons. Furthermore, it is suggested that the relative contributions of fur seals were lower in northern Hokkaido (i.e., 0–24% for the Kafukai 1 and 0–11% for the Hamanaka 2 humans), whereas that of marine proteins was equally high (i.e., 76–94% for the Kafukai 1 and 60–76% for the Hamanaka 2 humans). Nishimoto (1978) calculated the relative caloric contributions of edible parts based on the abundance of faunal remains from the Kafukai 1 site (northern Hokkaido), showing that the contribution was greater than 80% for marine fish and 6.5% for marine mammals. Carbon and nitrogen isotope analysis with a stochastic mixing model for three Jomon, Epi-Jomon, and early modern (Ainu) skeletal populations in Hokkaido suggested that the protein contribution was 28–35% for marine fish and 42–51% for marine mammals (Minagawa, 2001). Carbon and nitrogen isotopic data from the Usu-moshiri skeletal population in Epi-Jomon Hokkaido suggested that the protein contributions from marine fish and marine mammals were 12–42% and 45–76%, respectively (Tsutaya et al., 2013). Comparing these with my results, the dietary protein dependence of the Moyoro adult humans on marine mammals would be greater than that of the other Okhotsk populations in northern Hokkaido

and other prehistoric Hokkaido cultures. This supports the archaeological hypothesis of strong dependence of the Okhotsk people in eastern Hokkaido on marine mammal hunting (Ono, 1996a, b).

Several studies have suggested better nutritional status for the Okhotsk people, which possibly relates with their maritime dietary adaptation. Comparing with the mainland Jomon hunter–gatherers, the Okhotsk populations had better oral health (Oxenham and Matsumura, 2008), lesser frequency of linear enamel hypoplasia (Oxenham and Matsumura, 2008), greater demographic proportion of elderly individuals (Nagaoka et al., 2012), and larger body height (Kudaka et al., 2013). Marine mammals provided abundant fat, which is one of the most important energy sources for northern hunter–gatherer populations (Cordain et al., 2000). Although several studies have suggested the frequent appearance of cribra orbitalia (Oxenham and Matsumura, 2008) and heavy work load (Shimoda et al., 2012) in the Okhotsk culture compared with the skeletal populations of mainland Japan, the maritime dietary adaptation apparently enabled the Okhotsk people to settle in the harsh northern environment along the coast of the Sea of Okhotsk.

5.4.3 Infant feeding practice

Cross-sectional age changes in δ^{13}C values suggest that the Moyoro subadults were partially consumed ^{13}C-depleted foods relative to typical adult foods until around the age of 10 years. Although the difference (-0.3‰) is relatively small, the δ^{13}C values of subadults aged $\leq$3 years old are significantly lower than those of adult females (Table 5.3). An approximately 1‰ δ^{13}C increase in exclusively breastfed infants (Fuller et al., 2006a) would be compensated with lower δ^{13}C values of such weaning foods, resulting in non-elevated δ^{13}C values of infants in the Moyoro population (Figure 5.5A). Subadults older than six months of age would have consumed weaning foods because supplementation by foods other than breast milk is needed for healthy growth of human subadults after the age of six months (Kramer and Kakuma, 2004) and the maximum density estimator of the age at the start of weaning (the introduction of weaning foods) calculated by WARN is 0.4 years (Table 5.4). Such ^{13}C-depleted foods would also have been consumed after the end of the weaning process, because the δ^{13}C values of subadults aged 3–10 years remain significantly 0.3‰ lower than those of all adults (Table 5.3). It is unlikely that the lower δ^{13}C signal before the end of weaning represents subadult bone collagen remaining after the dietary change, because bone collagen turnover rates remain relatively high during subadulthood (e.g., 44% and 26% per year at 10 and 15 years of age, respectively: Tsutaya and Yoneda, 2013). After 10 years of age, a subadult diet is expected to be isotopically similar to an adult diet with respect to carbon.

^{13}C-depleted foods consumed by subadults would have been marine fish, fats, and/or terrestrial foods from a C_3 ecosystem, such as observed in the ethnography of northern populations. Several ethnographic studies of indigenous modern Ainu people in Hokkaido and Sakhalin have

shown that weaning foods from lower trophic levels than marine mammals were consumed by the Ainu people, such as premasticated salmon and ribs of bear, hare, or dog (Ohnuki-Tierney, 1974), a milk substitute consisting of mashed herring roe, cut cow parsnip root, boiled lily root, and seal oil (Kasai, 1943), and soup consisting of boiled cow parsnip, mashed herring roe, seal oil, and edible soil (Chiri, 1976). Hilger (1971) reported that a one-year-old Ainu infant was fed a porridge of finely ground root of a kind of lily (*Cardiocrinum glehnii*) during his trip to Hokkaido in 1965–1966. A dietary survey of traditional infant feeding practices of the Yupik and Inupiat in Alaska revealed that premasticated fish liver and "baby agutuk," mixtures of hydrogenated fats from caribou, moose, and seal, were commonly used as weaning foods (Heller and Scott, 1967). The carbon and nitrogen isotope ratios of these terrestrial, lipid, and fish foods are lower than those of marine mammals (see Figure 5.2; Tsutaya et al., 2013), which provide 80–90% of adult dietary protein (Naito et al., 2010a; Table 5.2).

Interestingly, similar δ^{13}C changes in subadults around and after weaning have been found in several skeletal populations of northern hunter–gatherer–fisher. Two types of infant feeding practice in the Usu-moshiri population of the Epi-Jomon Hokkaido, 2300–1700 years BP, have been suggested (Tsutaya et al., 2013). One group consisted of subadult individuals with δ^{13}C values below the mean of adult female means minus two standard deviations and δ^{15}N values lower than those of subadults in the other groups. In skeletal populations from early (8800–7000/6800 calBP: Shamanka) and late (6000/5800–5200 calBP: Ust'-Ida) Neolithic Cis-Baikal (the southwestern region of Lake Baikal, Siberia, Russian Federation), Waters-Rist et al. (2011) reported that there was no ^{13}C-enrichment in breastfed infants. They discussed that supplementation with ^{13}C-depleted weaning foods would mask a slight breastfeeding carbon isotope effect (Fuller et al. 2006a), although the age changes in δ^{13}C value for most subadults are less than the 1SD ranges of adult female δ^{13}C values. Howcroft et al. (2014) reported no evidence of a trophic level shift between infant and mother δ^{13}C values during the period of breastfeeding in a skeletal population from the Middle Neolithic Pitted Ware Culture in the Baltic island of Gotland (after 3000 BC), with the mean of collagen formed before the age of six months being very similar to that in bone in adult females or that formed in tooth dentin after the age of six months. They also suggested that certain foods with relatively high δ^{13}C value, presumably derived from marine fish, were given to subadults in another skeletal population from the same culture, based on the isotope ratios of tooth dentin collagen (see also Eriksson, 2004). I anticipate that in future, details of such isotopically different subadult foods can be evaluated by amino acid-specific isotope analyses (Naito et al., 2010b, 2013).

Age changes of δ^{15}N values are expected to be a result of breastfeeding, ^{15}N-depleted weaning foods, and positive nitrogen balance during growth. Two subadult individuals (1064 and 1139) aged one year old showed increased δ^{15}N values (+2.6‰ and +3.4‰, respectively: see Table 5.1) than adult female mean, and the WARN calculation yielded a 2.0–3.4‰ increase in exclusively breastfed infants (Table 5.4). These ^{15}N-enrichments are slightly higher than, but consistent with,

those reported for modern exclusively breastfed infants (2–3‰: Fuller et al., 2006a). Because terrestrial foods from C_3 ecosystems and marine fish usually show lower δ^{15}N values, as well as δ^{13}C, than marine mammals (see Figure 5.2; Tsutaya et al., 2013), the abovementioned food supplementation in subadults would result in a δ^{15}N value lower than that of adults. If that were the case, however, isotopic signals of ^{15}N-depleted foods would be compensated by ^{15}N-enrichment of breast milk consumption in infants. The influence of such ^{15}N-depleted foods is visible after the end of weaning. The higher and lower δ^{15}N values of subadults aged $\leq$3 and 3–10 years, respectively, are consistent with this assumption (Figure 5.5B). The significantly lower δ^{15}N values in subadults older than 10 years (Figure 5.5B) may attributed to positive nitrogen balance during growth (Fuller et al., 2004, but see Waters-Rist and Katzenberg, 2010). Because the δ^{13}C values of subadults older than 10 years are similar to those of adults, it is more plausible that their lower δ^{15}N values are not the result of diet.

Although the general trend is evident, there are individual variations in δ^{13}C and δ^{15}N values of the Moyoro subadults (Figure 5.5). These probably reflect intra-population variation in general diet and/or infant feeding practice. Chronological changes in breastfeeding and weaning practices are also possible, although there were no obvious relationships between the stable isotope ratios and calibrated radiocarbon ages in the Moyoro adults. Reconstruction of individual weaning histories by sequential analysis of tooth dentin serial section would address these questions (Eerkens et al., 2011; Beaumont et al., 2013b).

5.4.4 Breastfeeding and fertility

Isotopically reconstructed age at the end of weaning (1.4–2.2 years in 95% CI) in the Moyoro population is lower than those in other northern past hunter–gatherer–fisher populations. In the Usu-moshiri population from the Epi-Jomon Hokkaido, the δ^{15}N values of subadult bone collagen fell within the adult range at around 4–6 years of age (Tsutaya et al., 2013). Stable isotope analysis with an intra-individual sampling methodology of long bones indicated that contributions from breast milk ceased by 3.5–4.0 years and 3.0 years of age in early (Lokomotiv and Shamanka) and late (Ust'-Ida) Neolithic skeletal populations in Cis-Baikal, respectively (Waters-Rist et al., 2011). The δ^{15}N values of bone and tooth dentin collagen fell within the 1SD range of adult females by the age of two years and were equal to the adult female mean around the age of five years in Ajvide, a Middle Neolithic population in Gotland (Howcroft et al., 2014). Although the different sampling methodology prevents an exact comparison, the 95% CIs of age at the end of weaning corrected for the delay of bone collagen turnover were youngest in the Moyoro population (Table 5.4). The abovementioned ^{15}N-depleted subadult foods can contribute to the rapid decrease in subadult δ^{15}N values, but do not affect calculations of WARN. Equations in WARN do not fix a baseline for after-weaning subadult δ^{15}N ($\delta^{15}N_{wnfood}$) values, and thus the age at the end of weaning is not calculated as a relative change from adult δ^{15}N values but from δ^{15}N values of complementary and subadult foods (see Tsutaya and Yoneda, 2013; see also

Chapter 2).

Reconstructed 1.4–2.2 years (95% CI) of age at the end of weaning in the Moyoro population was lower compared with the age at the end of weaning in typical ethnographic populations. Worldwide ethnographic meta-analyses have indicated that the average age at the end of weaning in traditional human societies is 2.4–2.7 years, although their variation is large (Ford, 1945; Barry and Paxson, 1971; Sellen, 2001). Earlier or similar ages at the end of weaning have been reported in several ethnographic studies of the modern Ainu population. Ohnuki-Tierney (1974) reported that the period of breastfeeding in the Ainu population on the northwestern coast of southern Sakhalin during the first half of the 20th century lasted as long as two to three years. Isabella L. Bird, who traveled to Japan in 1878, noted that subadults among the Ainu people in the southwestern Hokkaido were not weaned until they were at least three years old (Bird, 1880).

Considering the age at the end of weaning in past northern hunter–gatherer–fisher and modern Ainu populations, the age of the Moyoro population is clearly earlier. Although chronological periods of subadult skeletons were not determined, the period of rapid expansion overlaps with the duration of habitation of the Moyoro population there (500–900 calAD: Table B.6). Because later weaning leads to lower fertility (Wood, 1994; Ellison, 1995; Valeggia and Ellison, 2009), the results of this study seem to be consistent with the hypothesis of population increase underling the rapid expansion of Okhotsk culture (Ohyi, 1978, 1988; Amano, 1979; Ono, 1996b). The suggested lower adult mortality also supports the population increase in the Moyoro populations, inferred from the greater demographic proportion of elderly individuals than found in the mainland Jomon hunter–gatherers (Nagaoka et al., 2012). Comparative analysis using the R-matrix method (Relethford and Blangero, 1990) of cranial morphology showed low phenotypic variability in eastern Okhotsk populations, suggesting that the Okhotsk people moved into the eastern area with a small population size and then rapidly increased (Komesu et al., 2008). These points of evidences are also consistent with the recent studies indicating large contributions of the Okhotsk people to the formation of the Ainu population and culture (Ishida, 1996; Masuda et al., 2001; Sato et al., 2007, 2009; Hanihara, 2010; Jinam et al., 2012; Lee and Hasegawa, 2013).

An inferred better nutrition in the Okhotsk populations supports the population increase, because improvements in nutritional status marginally increase human fertility and fecundability (Bongaarts, 1980; Wood, 1994). The metabolic load model, which assumes that the major determinant of postpartum ovarian function is maternal energy availability, has been proposed as a complement to the importance of breastfeeding in lactational amenorrhea (Ellison, 1994; Valeggia and Ellison, 2009). For example, Marlowe (2001) analyzed demographic data from 161 forager societies and indicated that higher male provisioning increases female fertility. Potter (1975) showed that the relative increase in birth intervals resulted from breastfeeding is larger in populations subsisted with lower nutrition. Under this view, the Moyoro people tended to a shorter period of lactational amenorrhea and resulting increased fertility because of their good nutritional status, as suggested by studies of physical anthropology. The Okhotsk peoples had

larger body height (Kudaka et al., 2013), a greater demographic proportion of elderly individuals (Nagaoka et al., 2012), better oral health, and lower frequency of linear enamel hypoplasia (Oxenham and Matsumura, 2008), than did the mainland Jomon hunter–gatherers. Marine mammals provided abundant fat, which is one of the most important energy sources for northern hunter–gatherer populations (Cordain et al., 2000), and it is possible that the strong dependence on marine mammals increased the nutritional status of the eastern Okhotsk peoples.

Although the higher fertility and lower mortality suggested by bioarchaeological analyses support population increase during the expansion of the Okhotsk people, they are not direct evidence for the population increase. Changes in population size of the Okhotsk people await evaluation by population genetics (Harpending et al., 1998; Drummond et al., 2005).

5.5 Conclusions

Carbon and nitrogen isotope ratios suggest that the Moyoro humans and dogs heavily depended on marine foods for their dietary protein intake. The Bayesian mixing model suggests that marine mammals may have contributed 80–90% to the human dietary proteins at the highest. Previous archaeological and isotopic studies have suggested that the Okhotsk people in Sakhalin and northern Hokkaido relied on marine fish, whereas the population on the east coast of Hokkaido had difficulty in catching marine fish during winter because of drift ice and thus relied on marine mammals (Ono, 1996a, b; Amano 2003; Naito et al., 2010a). My results are consistent with this suggestion. On the other hand, the Bayesian mixing models suggested that the dietary protein contribution of marine mammals in domesticated dogs was lower than that in humans, suggesting an avoidance of substantial dietary overlap between sympatrically living humans and dogs at the Moyoro site. Although the mean adult human isotope ratios did not differ between sexes, the variance of the carbon isotope ratios was significantly greater in the males.

Although most subadults relied on the same dietary ecosystem as the adults, stable isotope ratios of carbon suggest that Moyoro subadults were fed ^{13}C-depleted foods during and after the weaning process. Contribution from these foods ceased by the age of 10 years, and subadults older than 10 years show δ^{13}C values similar to those of adults. Consumption by subadults of different kinds of foods than the adult diet can be seen in ethnographic descriptions of Ainu populations in Sakhalin and Hokkaido (Kasai, 1943; Chiri, 1976; Hilger, 1971; Ohnuki-Tierney, 1974) and Yupik and Inupiat people in Alaska (Heller and Scott, 1967), and is suggested by isotopic studies of several northern hunter–gatherer–fisher skeletal populations (Waters-Rist et al., 2011; Tsutaya et al., 2013; Howcroft et al., 2014).

Most subadults younger than three years of age showed higher δ^{15}N values than adult females, suggesting bioenrichment of nitrogen isotopes between mothers and infants (Fuller et al., 2006a). The higher δ^{15}N values rapidly decreased and approached the adult range by the age of four years. With correction for the delay resulting from bone collagen turnover, the age for the end

of weaning was calculated as 1.8 (1.4–2.2 at the 95% CI) years. This age is clearly lower than those observed in the Ainu populations (Bird, 1880; Ohnuki-Tierney, 1974) and reconstructed in past northern hunter–gatherer–fishers (Waters-Rist et al., 2011; Tsutaya et al., 2013; Howcroft et al., 2014).

The Okhotsk people rapidly expanded their distributions during the middle period of the Okhotsk culture (Amano, 2003; Hudson, 2004) and their contribution to the formation of the Ainu culture and population was large (Ishida, 1996; Masuda et al., 2001; Sato et al., 2007, 2009; Hanihara, 2010; Jinam et al., 2012; Lee and Hasegawa, 2013). Consistent with these evidences, a shorter period of breastfeeding, which is associated with higher fertility, was reconstructed in the Moyoro population. Although solid evidence is needed for further investigation of the population dynamics of the Okhotsk people, this study indicates a shorter breastfeeding period in an expanded human population in past Hokkaido.

Chapter 6

General discussion

6.1 Development and validation of the model

In Chapter 2, temporal changes in bone collagen turnover rates in human subadult were estimated from data on tissue-level bone metabolism reported in previous studies (Mitchell et al., 1945; Leggett et al., 1982; Ruffoni et al., 2007). A model for reconstructing precise weaning ages was then developed using a framework of approximate Bayesian computation (Beaumont, 2010; Bertorelle et al., 2010; Csilléry et al., 2010) and incorporating the estimated turnover rates. The model is presented as a new open source R package, WARN (Weaning Age Reconstruction with Nitrogen isotope analysis), which computes the age at the start and end of weaning, ^{15}N-enrichment through maternal to infant tissue, and δ^{15}N value of collagen synthesized entirely from weaning foods with their posterior probabilities (see also Millard, 2000). The model was applied to 39 previously reported Holocene skeletal populations from around the world (Table A.1), and the results were compared with weaning ages observed in ethnographic studies (Ford, 1945; Barry and Paxon, 1971; Sellen, 2001). As a result, there were no significant differences in the age at the end of weaning between the archaeological (2.80 ± 1.32 years) and ethnographic populations (Figure 2.8). By comparing archaeological populations, it appears that weaning ages did not differ with the type of subsistence practiced (i.e., hunting–gathering or not). Most of ^{15}N-enrichment (2.44 ± 0.90‰) was consistent with biologically valid values (Fuller et al., 2006a). The nitrogen isotope ratios of subadults after the weaning process were lower than those of adults in most of the archaeological populations (-0.48 ± 0.61‰), and this depletion was greater in non-hunter–gatherer populations.

The most serious problem of this study is a lack of direct validation of the developed model. This is because there is no appropriate dataset consisting of several subadult skeletons in different ages at death with known common weaning age. Data from other tissues, such as hairs and nails, and other species are not suitable because of their different turnover rate with bone collagen of subadult humans. However, there was no inconsistency in indirect validations of the model as shown in Chapter 2; most of the target weaning parameters had biologically valid

distributions (Figure 2.6), and departures from this could be explained from the standpoint of isotope metabolism or model characters.

The developed model in this study rather provides a framework for objectively and quantitatively analyzing, interpreting, and comparing subadult bone collagen δ^{15}N values. By using the models to correct the lag time, researchers can compare weaning ages obtained by isotope analysis of past human skeletons with those obtained from participant observations in cultural anthropology and historical literatures as a uniform measure. These benefits offer a chance to discuss weaning ages obtained by isotope analysis as one of the most important determinants of fertility in past human skeletal populations in the context of palaeodemography.

6.2 Inferring fertility from weaning ages

The developed model was applied to three skeletal populations in Japan to estimate actual weaning ages. Reconstructed weaning ages were used to infer fertility and discuss three demographic events in Japanese archipelago: urbanization of the premodern city of Edo (Chapter 3), urbanization of the medieval city of Kamakura (Chapter 4), and expansion of the Okhotsk culture in Hokkaido (Chapter 5).

The urbanization of the city of Edo, the capital of pre-modern Japan, has been assumed to be not as a result of natural increase but that of in-migration although this assumption has never been verified (Sekiyama, 1958; Minami, 1978; Saito, 2002). To obtain information on natural fertility in Edo, I analyzed stable carbon and nitrogen isotopes in 46 adult and 84 subadult human skeletons excavated from the Hitotsubashi site (1657–1683 AD: the early Edo period), Tokyo, Japan and reconstructed their breastfeeding period in Chapter 3. The changes in the nitrogen isotope ratios of subadults suggest that weaning foods were introduced around the age of 0.2 (0.0–1.3 in 95% CI) years and weaning ended around 3.1 (2.1–4.1 in 95% CI) years (Table 3.6), which agrees with descriptions in various historical documents of the period (Table 3.7). The duration of breastfeeding in the Hitotsubashi population was relatively longer than those in modern industrial and traditional societies (Ford, 1945; Barry and Paxon, 1971; Sellen, 2001) and four previously reported populations in medieval (Richards et al., 2002; Fuller et al., 2003; Beaumont et al., 2013a) and in the industrial (Nitsch et al., 2011; Burt, 2013) UK. As later weaning closely associates with longer inter-birth interval for mothers (Bongaarts and Potter, 1983; Campbell and Wood, 1988; Wood, 1994), my data suggest a lower natural fertility for the Hitotsubashi population. Assuming that the proportion of married people was also lower in the major cities of the earlier Edo period (Minami, 1978), my results support the assumption that Edo developed and increased its population by attracting immigrants during urbanization.

I measured stable carbon and nitrogen isotope ratios in the bone collagen of three adults and 45 subadults from the Yuigahama-minami site (from 12th to 14th century) in Kamakura, the early medieval capital of Japan in Chapter 4. The changes in the nitrogen isotope ratios

of subadults suggest that the relative dietary protein contribution from breast milk started to decrease from 1.1 (0.0–2.8 in 96% CI) years of age and ended at 3.8 (2.9–4.4 in 96% CI) years (Table 4.3). The age at the end of weaning in the Yuigahama-minami population was greater than that in the typical non-industrial populations (Ford, 1945; Barry and Paxon, 1971; Sellen, 2001), a premodern population in the Edo period Japan, and medieval populations in the UK (Richards et al., 2002; Fuller et al., 2003; Burt, 2013). Skeletons of townspeople from medieval Kamakura indicate severe nutritional stress (e.g., enamel hypoplasia and cribra orbitalia: Nagaoka et al., 2006, 2013b; Hirata et al. 2011), yet this longer duration of breastfeeding did not compensate adverse effects for nutritional deficiency. The longer breastfeeding period may have been a consequence of weaning food shortage and bad health of subadults (see WHO, 2009). Kamakura experienced urbanization and population increase in the early medieval period (Amino et al., 1989; Kawano, 1995; Suzuki, 2013). The younger age-at-death distribution and high nutritional stresses in the Yuigahama-minami population and later weaning, which is closely associated with longer inter-birth interval for mothers (Bongaarts, 1982; Bongaarts and Potter, 1983; Campbell and Wood, 1988; Ellison, 1994; Wood, 1994; Valeggia and Ellison, 2009), suggests that Kamakura developed and increased its population by immigration during urbanization.

The Okhotsk people were sedentary hunter–gatherer–fishers who lived and prospered in Sakhalin, Hokkaido, and the Kurile Islands during the AD 5th to 13th centuries (Ushiro, 1991; Amano, 2003; Hudson, 2004). They expanded rapidly along the northeastern coast of Hokkaido. I reconstructed infant feeding practices of the Moyoro population of the Okhotsk culture in eastern Hokkaido, Japan, by measuring stable isotope ratios in 58 subadult human skeletons in Chapter 5. Food supplementation with relatively lower carbon isotope ratio during and after weaning was suggested, as observed in ethnographic descriptions of northern human populations such as the Ainu (Kasai, 1943; Chiri, 1976; Hilger, 1971; Ohnuki-Tierney, 1974) and isotopically suggested in past northern hunter–gatherer–fisher populations (Waters-Rist et al., 2011; Tsutaya et al., 2013; Howcroft et al., 2014). Nitrogen isotope ratios of subadults showed that the age at the end of weaning in the Moyoro population was 1.8 (1.4–2.2 in 95% CI) years (Table 5.4), which is lower than that in another northern hunter–gatherer–fisher populations, such as Usu-moshiri population in the Epi-Jomon culture in Hokkaido (Tsutaya et al., 2013), and typical modern traditional societies (Ford, 1945; Barry and Paxon, 1971; Sellen, 2001). Because weaning age is one of the most important determinants of fertility, a shorter breastfeeding period suggests increased fertility (Bongaarts and Potter, 1983; Campbell and Wood, 1988; Wood, 1994). Furthermore, suggested better nutrition (Kudaka et al., 2013) and lower mortality (Nagaoka et al., 2012) would further promote the population increase, and thus populations of the Okhotsk culture could expand into new habitats (Ohyi, 1978, 1988; Amano, 1979; Ono, 1996b). These findings are consistent with recent emerging evidence of great contributions of the Okhotsk to the formation of later Ainu populations and culture (Ishida, 1996; Masuda et al., 2001; Sato et al., 2007, 2009; Hanihara, 2010; Jinam et al., 2012; Lee and Hasegawa, 2013).

These studies indicate that process and cause of past human demographic events can be empirically discussed by inferring fertility from isotopically reconstructed age at the end of weaning. However, several proximate and remote factors, as well as breastfeeding period, affect fertility (see Chapter 1), and these factors are difficult to estimate in most archaeological settings. Furthermore, population dynamics are not only determined by fertility but also by mortality and migration as well (Keyfitz, 1980). In order to discuss past human population dynamics, one needs to obtain demographic information other than breastfeeding period. Although there was a wealth of previous historical, anthropological, and demographic information in the three demographic events discussed in this dissertation, this is not always the case. Development and application of further methods to estimate demographic parameters would be important to reconstruct past human population dynamics from various aspects.

6.2.1 Future issues: teeth

Unfortunately, reconstruction of weaning ages by using subadult bones is subject to several limitations. First, statistics reconstructed from archaeological skeletal series are not necessarily equal to the actual health status and life history of past populations because there is individual-level heterogeneity in the risk of death and disease, and because the skeletal series represent only dead individuals who do not survive any more ("osteological paradox": Wood et al., 1992; see also Chapter 3). Isotopic signals from subadult skeletons represent breastfeeding and weaning practices in dead individuals rather than those in healthy individuals who survived into adulthood. Because breastfeeding and weaning practices are closely related to subadult health and survival (summarized by Dettwyler and Fishman, 1992; Cunningham, 1995; Katzenberg et al., 1996; WHO, 1998, 2009; Kramer and Kakuma, 2004), it is possible that the dead subadults experienced atypical diet and weaning. Second, in cross-sectional reconstruction of human breastfeeding practices, which requires many subadult individuals of different ages, researchers have assumed that a single, homogenous, culturally determined breastfeeding and weaning practice was observed by all members of the study group (Richards et al., 2002). However, this assumption is not universally true (Vitzthum, 1994; Tsutaya et al., 2013). Finally, subadult skeletons are sometimes subjected to the cultural practice of burial in different places than communal cemeteries, are easily degraded postmortem because of their less-mineralized nature and small size, and are sometimes overlooked during the excavation and curatorial work (Lewis, 2007). These issues make it difficult to apply cross-sectional reconstruction of breastfeeding practice in archaeological populations, which requires a number of subadult bones of different ages.

By using teeth as a target of stable isotope analysis, the above-mentioned limitations can be overcome. Tooth are one of the tissues that retain dietary signals from infancy and childhood after reaching adulthood (Hillson, 1996; Smith, 2008; Nanci, 2013). Generally, unlike bone collagen, teeth tissues are not replaced once they form (Nanci, 2013), and thus retain the isotopic and elemental dietary signals of subadulthood until and after the death of the individual. Over time,

dentin grows sequentially from the crown to the root tip like a series of stacked cones, and the ages at the start and end of dentin formation differ among tooth types (Smith, 1991; Dean et al., 1993; Dean and Scandrett, 1995; Hillson, 1996). A whole tooth represents the average dietary signal of several years between onset and completion, but the resolution of reconstruction has been increased by obtaining subsamples from longitudinal tooth sections. Studies using teeth have increased recently and overcome the above-mentioned limitations (Wright and Schwarcz, 1999; Fuller et al., 2003; Dupras and Tocheri, 2007; Eerkens et al., 2011; Beaumont et al., 2013b; Eerkens and Bartelink, 2013; Henderson et al., 2014). First, the analysis of individuals who survived the weaning process is a good solution to circumvent the osteological paradox in the reconstruction of breastfeeding and weaning practices. Second, Individual variations in breastfeeding and weaning cannot be discussed in most cross-sectional reconstructions using bones, but can be in longitudinal reconstructions using isotopic signals in tooth dentin. Finally, the potential applicability of longitudinal reconstruction of breastfeeding and weaning practices using teeth is wider than cross-sectional studies because the required number of individuals is much smaller in the former. However, because teeth are one of the most informative tissues in morphological studies and possess the greatest potential for the recovery of ancient DNA, it is clear that non- or less-destructive techniques are needed.

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Appendix A

Model application results of the archaeological populations

Table A.1: Summary of model application results and descriptions of the archaeological populations intended in this study.

ID	Site	Location	Period	Subsistence
1	Ajdovska jama cave	Slovenia	6400–5300 calBP	NHG
2	Angel	Ohio, USA	AD 1300–1450	NHG
3	Charlston Annis	Ohio, USA	3500–1000 BC	HG
4	Aşıklı Höyük	Turkey	9000–8000 calBP	NHG
5	Baikal	*Cis*-Baikal, Siberia	9000–3000 BP	HG
6	Bjärby	Sweden	AD 0–200	NHG
7	Çatalhöyük	Turkey	7400–8300 BP	NHG
8	Çayönü Tepesi	Turkey	9000–7000 calBP	NHG
9	Conchopata	Peru	AD 550–1000	NHG
10	Dorset late Iron Age	UK	100 BC–AD 100	NHG
11	Dorset Romano-British	UK	AD 43–	NHG
12	Fushimi	Japan	AD 1600–	NHG
13	Harvie	Ontario, Canada	AD 1825–1892	NHG
14	Isora Sacra	Italy	AD 0–200	NHG
15	Kastella	Greece	AD 1000–1100	NHG
16	Kellis	Egypt	AD 250	NHG
17	Indian Knoll	Ohio, USA	3500–1000 BC	HG
18	Kulubnarti R	Sudan	AD 550–800	NHG
19	Kulubnarti S	Sudan	AD 550–800	NHG
20	Leptiminus	Tunisia	AD 100–400	NHG
21	Lerna	Greece	2100–1700 BC	NHG
22	Lokomotiv	*Cis*-Baikal, Siberia	8800–7000/6800 calBP	HG
23	Matjes River Rcok Shelter	South Africa	12000 BP–recent	HG
24	McPherson	Ontario, Canada	AD 1530–1580	NHG

ID	Site	Location	Period	Subsistence
25	Meuse Basin	Belgium	10000–2000 BC	NHG
26	Marco Gonzalez, San Pedro	Beliz	100 BC–AD 1350	NHG
27	Newark Bay	UK	550–1200 BP	NHG
28	Nukdo	South Korea	550–300 BC	NHG
29	Prospect Hill	Ontario, Canada	AD 1824–1879	NHG
30	Queenford Farm	UK	AD 300–500	NHG
31	Shamanka II	*Cis*-Baikal, Siberia	8800–7000/6800 calBP	HG
32	Spitalfields	UK	AD 1700–1900	NHG
33	Sully	South Dakota, USA	AD 1650–1733	NHG
34	Tinslay Hill	Ohio, USA	AD 1300–1450	NHG
35	Triberga	Sweden	AD 500–1000	NHG
36	Ust'-Ida I	*Cis*-Baikal, Siberia	6000/5800–5200 calBP	HG
37	Wetwang	UK	300–100 BC	NHG
38	Yeanri	South Korea	AD 300–600	NHG
39	Yukisma	California, USA	2200–250 BP	HG

Table A.1 (continued).

ID	MDE				Probability			Element
	t_1	t_2	E	$\Delta^{15}N_{adult-wnfood}$	t_1 and t_2	E	$\delta^{15}N_{wnfood}$	
1	1.6	2.1	2.5	0.0	0.00818	0.111	0.151	–
2	1.0	3.7	2.3	-0.6	0.00196	0.111	0.115	L
3	0.3	4.7	2.5	-0.3	0.00453	0.204	0.080	L
4	0.6	1.3	2.9	0.4	0.01923	0.103	0.108	R
5	0.5	2.5	1.5	-0.2	0.00494	0.048	0.137	–
6	2.1	3.3	-1.6	0.5	0.00566	0.130	0.184	C, L
7	0.7	2.5	1.1	-0.9	0.00497	0.116	0.153	–
8	1.6	2.9	3.9	-0.6	0.01307	0.116	0.096	R
9	0.8	1.5	3.9	-0.4	0.00949	0.127	0.155	C, L, R
10	0.3	2.9	1.5	-1.5	0.00491	0.070	0.050	R
11	3.8	5.3	0.6	-0.8	0.00327	0.165	0.070	R
12	1.3	4.0	1.1	-0.5	0.00438	0.260	0.176	R
13	1.9	4.4	1.4	-1.9	0.00187	0.196	0.078	R
14	0.3	1.1	4.6	0.7	0.02527	0.089	0.115	R
15	1.9	2.1	3.1	-0.9	0.02270	0.149	0.232	R
16	0.2	2.3	3.6	0.5	0.01142	0.123	0.151	L, R
17	1.1	3.4	2.8	-0.4	0.00640	0.185	0.156	L
18	1.7	3.7	1.5	-1.2	0.00929	0.159	0.192	R
19	1.6	4.2	2.6	-0.9	0.01075	0.163	0.196	R
20	0.7	5.7	2.5	-0.7	0.00447	0.105	0.067	L, R

ID	MDE				Probability			Element
	t_1	t_2	E	$\Delta^{15}N_{adult-wnfood}$	t_1 and t_2	E	$\delta^{15}N_{wnfood}$	
21	0.4	1.3	3.0	-0.2	0.01756	0.052	0.225	R
22	1.1	1.4	3.9	-0.1	0.03560	0.107	0.408	L
23	1.2	1.9	2.5	0.5	0.00523	0.081	0.092	C, L, R
24	0.3	3.1	2.5	-1.1	0.00478	0.120	0.090	R
25	0.4	1.8	2.1	-0.2	0.01712	0.095	0.267	L, O
26	2.2	3.1	2.3	-0.8	0.00818	0.152	0.173	R
27	1.0	2.4	2.6	-1.0	0.00534	0.125	0.066	R
28	0.7	1.2	3.0	0.6	0.01872	0.187	0.172	C, L, R
29	0.9	1.2	2.2	-0.3	0.04644	0.178	0.200	R
30	0.3	2.6	1.9	-0.2	0.00574	0.120	0.146	L, R
31	0.3	3.8	1.6	0.4	0.00218	0.175	0.142	L
32	0.8	1.4	2.0	-0.6	0.02195	0.160	0.230	R
33	0.4	1.5	2.2	-1.1	0.02938	0.185	0.179	R
34	1.6	5.1	2.1	-0.6	0.00274	0.198	0.088	L
35	0.3	3.2	3.3	-1.6	0.00092	0.136	0.050	L
36	1.2	2.7	2.7	-0.4	0.01730	0.101	0.245	L
37	2.7	3.4	0.8	-0.6	0.00389	0.242	0.147	C, O, R
38	1.2	4.4	2.3	-0.2	0.00259	0.158	0.098	C, L, R
39	1.1	4.5	2.8	-0.7	0.00430	0.131	0.115	R

Bone elements analyzed were shown: “C” means cranium, “L” means long bones such as limb, “R” means rib, and “O” means other bones.

Table A.1 (continued).

ID	n	Adult female		Total adult		References
		Mean	SD	Mean	SD	
1	12	8.3	0.69	7.5	1.43	Ogrinc and Budja, 2005
2	16	8.0	0.8	8.3	0.7	Schurr and Powell, 2005
3	24	6.7	1.4	7.0	1.3	Schurr and Powell, 2005
4	13	9.6	0.91	–	–	Pearson et al., 2010
5	21	13.3	2.37	12.5	1.98	Weber et al., 2002
6	8	13.2	0.4	13.4	1.4	Howcroft et al., 2012
7	28	10.8	0.92	11.0	0.92	Richards et al., 2003
8	17	6.0	0.53	–	–	Pearson et al., 2010
9	8	10.6	0.99	10.6	1.15	Finucane et al., 2006
10	6	9.4	0.51	9.4	0.61	Redfern et al., 2010, 2012
11	8	9.5	1.16	9.4	0.92	Redfern et al., 2010, 2012
12	6	11.9	0.69	12.0	0.6	Kusaka et al., 2011
13	6	12.1	0.3	12.2	0.4	Katzenberg, 1993; Katzenberg et al., 1993

ID	n	Adult female		Total adult		References
		Mean	SD	Mean	SD	
14	33	10.6	1.1	10.8	1.2	Prowse et al., 2004, 2008
15	7	8.7	0.61	9.1	0.86	Bourbou et al., 2007
16	41	18.0	1.0	17.9	1.09	Dupras, 1999; Dupras et al., 2001
17	30	7.6	0.7	7.9	0.7	Schurr and Powell, 2005
18	33	10.2	0.68	10.3	0.87	Turner et al., 2007
19	41	10.2	0.87	10.4	0.64	Turner et al., 2007
20	31	13.1	1.48	12.9	1.27	Keenleyside et al., 2009
21	11	8.3	0.37	8.4	0.6	Triantaphyllou et al., 2008
22	11	14.1	0.8	–	–	Waters-Rist et al., 2011
23	33	–	–	13.2	1.8	Clayton et al., 2006
24	14	11.7	1.2	13.1	1.1	Katzenberg et al., 1993
25	18	9.7	0.49	9.5	0.65	Bocherens et al., 2007
26	18	9.8	0.81	10.1	0.86	Williams et al., 2005
27	63	11.8	1.75	12.3	1.76	Richards et al., 2006
28	31	10.9	0.42	11.2	0.78	Choy and Richards, 2009
29	33	–	–	12.2	0.64	Katzenberg, 1993; Katzenberg et al., 1993
30	42	9.9	0.86	10.2	0.81	Fuller et al., 2006b
31	21	14.8	0.7	–	–	Waters-Rist et al., 2011
32	61	13.3	0.6	13.3	0.6	Nitsch et al., 2010, 2011
33	28	–	–	11.2	0.49	Tuross and Fogel, 1994
34	19	8.4	0.4	8.7	0.6	Schurr and Powell, 2005
35	16	11.2	1.5	12.9	1.5	Howcroft et al., 2012
36	15	12.1	0.8	–	–	Waters-Rist et al., 2011
37	40	9.6	0.49	9.7	0.6	Jay and Richards, 2006, Jay et al., 2008
38	25	10.1	0.97	10.5	1.1	Choy et al., 2010
39	22	7.7	0.9	–	–	Gardner et al., 2011

Appendix B

Stable isotopic data of individual skeletons

Table B.1: Results of isotope analysis on adult human skeletons from the Hitotsubashi site.

ID	Burial	Sex	Age range	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
H-81	–	F	15–34	-19.3	10.9	43.2	15.9	3.2	11.2
H-116	–	F	15–34	-19.7	9.9	44.3	16.2	3.2	10.6
H-126	Round	F	15–34	-19.5	11.0	46.8	17.3	3.2	8.9
H-238	–	F	15–34	-20.0	10.8	48.3	17.9	3.1	8.4
H-242	–	F	15–34	-18.9	11.9	44.4	16.5	3.1	13.7
H-91	Round	M	15–34	-19.5	11.6	42.1	15.7	3.1	14.5
H-206	Pit	M	15–34	-19.3	11.8	43.0	15.8	3.2	13.5
H-23	Round	F	35–54	-18.7	11.4	42.8	15.6	3.2	14.9
H-24	Rectangle	F	35–54	-18.0	10.8	42.3	15.4	3.2	16.3
H-105	–	F	35–54	-19.8	10.2	44.2	15.6	3.3	12.2
H-130	Pit	F	35–54	-19.7	9.9	44.5	16.1	3.2	10.8
H-185	–	F	35–54	-19.3	9.2	47.7	17.7	3.1	10.4
H-291	–	F	35–54	-19.5	11.3	44.9	16.5	3.2	12.6
H-307	–	F	35–54	-19.9	10.6	43.4	15.7	3.2	12.3
H-1	Pit	M	35–54	-19.5	10.4	41.0	15.2	3.1	14.2
H-12	Round	M	35–54	-20.7	10.2	43.8	16.1	3.2	13.0
H-72	Round	M	35–54	-18.6	11.0	41.9	15.6	3.1	11.6
H-77	Round	M	35–54	-19.7	12.2	43.0	15.9	3.2	14.1
H-88	Round	M	35–54	-19.2	10.8	43.8	16.1	3.2	18.5
H-101	Pit	M	35–54	-19.7	11.0	43.3	15.6	3.2	8.5
H-103	Pit	M	35–54	-20.0	10.1	43.3	15.7	3.2	11.2
H-108	–	M	35–54	-19.1	12.1	44.3	17.2	3.0	12.5
H-114	Round	M	35–54	-19.3	11.9	46.5	17.1	3.2	10.0
H-131	–	M	35–54	-19.4	10.7	43.4	15.6	3.2	12.5
H-139	Round	M	35–54	-19.4	11.4	43.8	16.2	3.2	11.9

ID	Burial	Sex	Age range	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
H-146	Round	M	35–54	-19.3	11.7	43.3	15.8	3.2	16.4
H-154	Pit	M	35–54	-19.3	11.3	41.3	15.1	3.2	14.1
H-160	Pit	M	35–54	-18.0	11.9	42.9	15.7	3.2	18.1
H-163	Round	M	35–54	-19.0	11.4	45.7	17.0	3.1	11.1
H-167	–	M	35–54	-20.3	9.6	44.8	16.2	3.2	16.0
H-183	Round	M	35–54	-19.3	11.9	47.2	17.9	3.1	12.3
H-186	–	M	35–54	-18.8	11.8	46.8	16.9	3.2	9.5
H-212	Rectangle	M	35–54	-19.0	11.7	42.4	15.5	3.2	8.6
H-213	Rectangle	M	35–54	-19.6	10.8	41.3	15.0	3.2	10.3
H-217	Rectangle	M	35–54	-19.4	11.4	42.8	15.8	3.2	11.3
H-220	Round	M	35–54	-19.6	11.4	42.8	15.5	3.2	9.8
H-244	–	M	35–54	-19.8	11.3	44.2	16.2	3.2	15.5
H-162	Pit	F	55+	-19.1	11.9	44.0	16.0	3.2	14.5
H-176	–	F	55+	-19.1	9.3	45.7	16.9	3.2	10.7
H-64	Round	M	55+	-19.4	11.4	42.4	15.5	3.2	11.2
H-127	Round	M	55+	-18.4	12.4	43.3	16.1	3.1	10.0
H-143	Pit	M	55+	-18.9	11.5	42.9	15.7	3.2	13.6
H-98	Pit	F	Adult	-19.4	10.8	43.3	15.9	3.2	12.5
H-189	Pit	F	Adult	-19.4	9.7	43.2	15.1	3.3	9.7
H-190	Jar	F	Adult	-19.5	10.5	43.9	16.2	3.2	10.0
H-2	Pit	M	Adult	-20.0	9.9	42.8	15.6	3.2	15.3

Age (years) and sex were estimated by my collaborators (T.N. and J.S.). Bone sampling, collagen extraction, and isotope analysis were performed by myself.

Table B.2: Results of isotope analysis on subadult human skeletons from the Hitotsubashi site.

ID	Total age	Phase	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
H-8	0	1	-18.7	13.0	43.3	15.9	3.2	16.2
H-107	0	1	-19.7	11.3	42.3	15.6	3.2	8.3
H-119+1	0	1	-19.0	11.8	44.1	15.9	3.2	11.2
H-219	0	1	-18.9	12.6	39.1	14.8	3.1	7.1
H-87	0.5	2	-18.7	14.2	44.9	16.0	3.3	6.7
H-280	0.5	2	-18.4	13.3	47.3	16.8	3.3	12.6
H-7*	0.75	2	-20.9	12.9	32.9	9.3	4.1	1.4
H-9	0.75	2	-18.8	13.6	43.7	16.3	3.1	16.0
H-19	0.75	2	-18.7	14.4	43.5	16.1	3.2	12.7
H-30	0.75<	2	-17.8	15.1	46.1	17.1	3.1	8.9
H-35	0.75	2	-19.4	12.9	42.2	15.8	3.1	10.3
H-93	0.75	2	-18.4	15.1	44.7	16.4	3.2	15.1

ID	Total age	Phase	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
H-102	0.75	2	-19.0	12.8	44.4	15.9	3.3	3.3
H-214	0.75	2	-18.5	14.4	43.1	15.4	3.3	9.5
H-16	1	2	-17.8	16.1	43.2	16.0	3.2	12.0
H-84	1	2	-18.8	14.5	44.5	16.5	3.2	7.3
H-97	1	2	-18.6	14.2	44.6	16.6	3.1	10.9
H-117*	1	2	-20.4	13.4	45.3	12.6	4.2	2.3
H-158	1	2	-18.8	14.7	37.4	13.3	3.3	5.3
H-210	1	2	-18.5	14.9	41.2	14.8	3.3	7.4
H-228	1	2	-18.4	14.8	43.7	16.2	3.2	13.3
H-271	1	2	-18.0	15.8	43.8	15.9	3.2	14.5
H-3	1.5	2	-20.0	10.5	43.3	15.5	3.3	8.7
H-17	1.5	2	-19.0	14.1	45.6	16.1	3.3	15.9
H-18	1.5	2	-18.9	12.4	44.8	16.6	3.2	14.5
H-26	1.5	2	-18.8	13.9	43.6	16.0	3.2	11.8
H-54	1.5	2	-18.5	12.0	44.7	16.6	3.1	17.2
H-60	1.5	2	-17.9	16.5	44.7	16.4	3.2	12.1
H-61	1.5	2	-19.2	12.0	47.3	16.7	3.3	13.1
H-75	1.5	2	-19.0	13.6	45.6	16.1	3.3	8.2
H-86	1.5	2	-18.8	13.6	46.1	16.9	3.2	13.0
H-113	1.5	2	-18.9	14.2	44.8	16.3	3.2	13.7
H-118	1.5	2	-18.5	12.7	47.9	17.1	3.3	12.7
H-155	1.5	2	-18.4	15.7	44.3	16.1	3.2	13.9
H-196*	1.5	2	-23.0	11.5	39.6	7.8	5.9	2.0
H-200	1.5	2	-18.5	14.7	40.6	15.3	3.1	5.9
H-201	1.5	2	-18.9	13.6	43.3	16.1	3.1	17.8
H-227	1.5	2	-18.3	14.5	45.3	16.3	3.2	15.8
H-266	1.5	2	-17.9	15.1	46.0	17.0	3.2	13.6
H-46	2	2	-18.9	12.5	44.0	16.4	3.1	15.2
H-85	2	2	-19.9	11.2	44.7	16.8	3.1	8.3
H-92	2	2	-19.3	12.3	44.0	16.0	3.2	13.9
H-125	2	2	-19.1	13.8	44.3	16.7	3.1	12.4
H-151	2	2	-19.0	13.1	44.7	16.4	3.2	13.8
H-161	2	2	-19.0	13.1	44.4	16.5	3.1	12.4
H-203	2	2	-18.4	13.9	44.9	16.7	3.1	12.2
H-231	2	2	-18.6	13.0	45.2	17.0	3.1	7.0
H-273	2	2	-18.3	13.2	43.9	15.9	3.2	10.3
H-29	3	3	-18.8	13.5	45.2	16.8	3.1	11.3
H-34	3	3	-19.1	12.1	47.2	16.8	3.3	9.2
H-44	3	3	-19.0	12.8	42.3	15.4	3.2	14.3
H-89	3	3	-18.5	11.5	44.8	16.4	3.2	9.3
H-94	3	3	-19.5	10.7	44.9	16.8	3.1	10.1

ID	Total age	Phase	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
H-122	3	3	-19.6	13.1	43.9	14.8	3.5	3.2
H-128	3	3	-18.6	13.5	44.1	16.0	3.2	5.3
H-204	3	3	-19.6	10.8	44.0	16.2	3.2	16.0
H-226	3	3	-18.6	14.1	45.8	16.8	3.2	9.3
H-229	3	3	-17.8	13.6	45.9	17.1	3.1	9.2
H-264	3	3	-19.4	12.8	34.3	12.6	3.2	10.1
H-268	3	3	-18.1	14.0	48.6	17.5	3.2	17.1
H-269	3	3	-20.2	9.9	48.1	17.5	3.2	16.3
H-32	3–4	3	-18.3	11.2	49.4	17.8	3.2	16.1
H-215	3–4	3	-19.2	10.8	48.0	17.2	3.3	16.9
H-15	4	3	-18.4	12.6	44.3	16.2	3.2	13.4
H-69	4	3	-18.7	12.5	47.4	16.4	3.4	7.1
H-120*	4	3	-19.8	11.6	44.4	13.3	3.9	2.2
H-142	4	3	-18.1	12.4	45.7	16.7	3.2	10.3
H-199*	4	3	-21.2	10.9	33.4	9.4	4.2	1.1
H-252	5	3	-19.6	10.5	45.7	16.7	3.2	14.2
H-140	6+	4	-19.2	12.0	46.1	16.7	3.2	10.3
H-144	6	4	-19.4	10.9	47.1	16.8	3.3	12.6
H-178	6	4	-19.1	10.0	46.1	16.5	3.3	11.2
H-208	6	4	-17.8	14.7	43.1	15.5	3.2	4.7
H-263	6	4	-19.8	10.6	43.2	15.9	3.2	17.0
H-265	6	4	-17.9	12.3	44.1	16.6	3.1	16.6
H-53	7	4	-18.6	11.0	47.1	16.7	3.3	14.6
H-111	7	4	-19.2	10.6	43.1	15.8	3.2	12.8
H-234*	7	4	-19.6	13.5	47.0	12.2	4.5	0.7
H-253	7	4	-19.2	11.0	44.5	16.5	3.2	18.0
H-274	7	4	-19.0	11.3	50.3	17.9	3.3	15.3
H-52	8	4	-19.9	9.5	45.6	16.1	3.3	11.0
H-224	8	4	-18.9	11.6	44.6	16.2	3.2	11.1
H-168	9	4	-18.4	11.1	45.0	16.4	3.2	11.9
H-184	9.5	4	-18.6	11.3	45.1	16.8	3.1	13.4

Samples with an asterisk were excluded from the analysis. The total age ranges (years) and Oyamada age phases (Oyamada et al., 2008) estimated by my collaborators (T.N. and J.S.) are also shown. Bone sampling, collagen extraction, and isotope analysis were performed by myself.

Table B.3: Results of isotope analysis of adult skeletons from the Yuigahama-minami site.

ID	Age range	Sex	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
145A	15–19	F	-18.5	11.4	43.0	15.1	3.32	3.8

ID	Age range	Sex	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
12	Adult	M	-19.3	10.8	42.9	15.0	3.34	2.3
245	Adult	F	-19.6	9.4	42.7	15.1	3.30	2.1

Age (years) and sex were estimated by my collaborators (T.N. and J.S.) (Nagaoka et al., 2006). Bone sampling and collagen extraction were performed by my collaborator (A.S.), and isotope analysis was performed by myself.

Table B.4: Results of isotope analysis of subadult skeletons from the Yuigahama-minami site.

ID	Age range	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)	Pathology
235	0	-18.6	13.3	43.1	15.4	3.27	4.7	None
1056	0	-19.1	11.1	43.1	15.0	3.35	3.4	None
1084	0	-18.8	14.2	43.0	15.1	3.32	3.3	–
5121	0.75–1	-19.5	11.4	43.5	15.2	3.34	7.1	EH
1143B	0.75–1	-18.6	11.8	42.6	14.9	3.34	3.6	–
243	0.5–1.5	-18.5	13.2	45.5	16.4	3.24	7.9	–
1168	1	-19.0	12.1	42.7	14.7	3.39	2.3	–
65	1–2	-19.0	13.1	43.2	15.0	3.36	4.3	None
179	1–2	-18.4	14.2	44.8	15.8	3.31	5.3	None
176B	1–2	-19.2	13.4	44.0	15.7	3.27	4.8	None
130	2	-18.5	12.2	42.5	14.9	3.33	5.7	None
172	2	-18.4	13.0	43.4	15.4	3.29	4.2	–
203	2	-19.0	12.6	40.6	14.6	3.24	1.4	None
271	2	-18.6	11.1	44.4	15.2	3.41	1.8	Caries
36B	2	-18.4	13.1	44.9	16.0	3.27	7.4	Caries
54	3	-19.1	11.7	44.3	15.8	3.27	3.1	None
1115	3	-18.6	12.9	42.4	15.0	3.30	3.8	Caries
1047B	3	-19.2	11.0	42.4	15.1	3.28	4.4	Caries
111	3–4	-18.6	12.1	42.4	15.0	3.30	9.0	None
8	4	-19.4	10.4	43.4	15.8	3.20	8.7	Caries
1189B	4	-19.0	11.9	44.0	15.5	3.31	3.1	Caries
138B	4	-19.1	10.3	42.4	15.3	3.23	8.2	Caries, EH
211A	4	-19.2	10.5	42.6	15.0	3.31	8.0	–
232A	4	-17.7	11.7	42.1	15.0	3.27	6.4	Clibra cranii
238*	4–5	-20.4	11.5	34.0	11.7	3.39	0.4	–
292	4–5	-18.2	10.3	42.5	15.0	3.31	1.9	Sinusitis
1076	4–5	-19.6	9.8	42.2	14.1	3.49	4.4	Caries
107	5	-19.2	10.7	43.2	15.0	3.36	3.6	EH
148	5	-18.9	11.0	42.7	15.1	3.30	3.7	None
248	6	-18.4	11.9	42.6	14.9	3.34	3.8	Caries

ID	Age range	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)	Pathology
5C	6	-19.2	9.0	42.0	15.1	3.25	4.2	Caries, F, T?
23	7	-19.5	8.8	44.4	15.6	3.32	3.9	Caries
60	6–8	-19.3	10.0	44.2	15.7	3.28	3.4	None
1108	7	-19.3	10.1	42.6	14.6	3.40	2.1	Caries, EH
118F	7	-19.2	10.2	42.9	15.3	3.27	5.2	–
232B	7	-17.4	11.0	41.9	14.7	3.33	2.7	Caries, EH, P
27B	7	-19.6	10.3	42.5	14.9	3.33	4.9	Caries
254	8	-19.4	10.4	42.9	14.7	3.40	4.9	Caries
206A	8	-18.8	10.0	43.0	15.1	3.32	4.4	Caries, EH
156	9	-18.6	10.9	43.2	15.7	3.21	11.7	EH
22	10	-18.7	9.4	43.1	15.9	3.16	3.1	None
1130*	10	-19.6	10.8	43.3	12.4	4.07	1.4	Caries
72	12–14	-18.1	11.2	43.0	14.5	3.46	2.9	None
110B	12–14	-19.3	10.1	42.5	14.6	3.40	3.3	Caries
200B	12–14	-19.3	9.3	42.9	15.3	3.27	6.8	None

Samples with an asterisk were excluded from the analysis. Age information was shown in years and estimated by my collaborators (T.N. and J.S.) (Nagaoka et al., 2006). Information of pathological conditions were taken from Hirata et al. (2002); "C" means dental caries, "EH" means enamel hypoplasia, "F" means fracture, "T" means tuberculosis, "P" means pyorrhea alveolaris, "None" means no reported pathological conditions, and blank indicates individuals without observation in Hirata et al. (2002). Bone sampling and collagen extraction were performed by my collaborator (A.S.), and isotope analysis was performed by myself.

Table B.5: Results of isotope analysis of faunal skeletons from the Moyoro site.

ID	Scientific name	Category	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
MF22	*Tribolodon hakonensis*	BF	-13.5	11.2	46.7	16.8	3.2	6.3
MF2	*Oncorhynchus keta*	BF	-16.0	11.5	47.6	16.3	3.4	4.9
MF15	*Canis lupus familiaris*	Dog	-13.8	16.4	47.5	17.7	3.1	8.4
MF16	*Canis lupus familiaris*	Dog	-13.6	16.9	47.6	17.7	3.1	6.5
MF1	*Gadinae*	MF	-14.9	15.0	47.7	16.4	3.4	2.9
MF8	*Gadinae*	MF	-14.3	14.5	48.3	17.7	3.2	3.4
MF3	*Paralichthys olivaceus*	MF	-13.9	14.4	47.0	17.1	3.2	4.5
MF13	*Clupea pallasii*	MF	-15.1	11.3	47.3	16.5	3.3	5.6
MF4	*Scorpaenidae*	MF	-13.1	14.5	49.7	17.5	3.3	6.0
MF32	*Delphinidae*	MM	-14.3	15.0	46.9	17.1	3.2	4.9
MF10	*Erignathus*	MM	-14.2	16.1	48.9	17.4	3.3	7.3
MF11	*Erignathus*	MM	-14.5	16.3	43.5	15.8	3.2	6.5
MF12	*Erignathus*	MM	-14.4	14.0	45.7	15.0	3.6	9.0

ID	Scientific name	Category	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
MF31	*Callorhinus ursinus*	MM	-14.2	16.3	51.9	18.4	3.3	4.3
MF9	*Callorhinus ursinus*	MM	-14.4	16.2	47.3	16.9	3.3	6.3
MF33	*Cetacea*	MM	-13.6	16.6	46.6	16.6	3.3	3.9
MF27	*Ursus arctos*	TC	-17.8	9.4	50.2	17.8	3.3	5.8
MF28	*Ursus arctos*	TC	-21.0	5.7	49.5	16.1	3.6	2.7
MF18	*Vulpes*	TC	-17.6	10.2	43.8	14.6	3.5	3.5
MF26	*Martes zibellina*	TC	-20.4	6.8	47.4	16.2	3.4	2.9
MF14	*Cervus nippon*	TH	-22.7	7.5	44.3	14.9	3.5	10.6
MF29	*Cervus nippon*	TH	-22.3	3.3	48.7	16.9	3.4	5.3
MF30	*Cervus nippon*	TH	-22.5	3.5	50.2	16.6	3.5	3.8
MF32	*Cervus nippon*	TH	-23.3	2.1	45.3	15.6	3.4	11.7

Faunal species were identified by zoo archaeologist(s) other than myself. BF: brackish water fish, MF: marine fish, MM: marine mammals, TC: terrestrial carnivores, and TM: terrestrial herbivores. Bone sampling, collagen extraction, and isotope analysis were performed by my collaborators (Y.I.N. and M.Y.). Stable isotopic results of MF14 and MF32 originated from Naito et al. (2010a).

Table B.6: Results of isotope analysis of adult human skeletons from the Moyoro site.

ID	Sex	Element	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	calAD (95%CI)	Yield (%)
1011	F	Cranium	-12.7	19.4	42.3	14.4	3.4	673–844	19.2
1015	F	Cranium	-13.3	18.7	44.4	15.1	3.4	616–761	17.3
1023	F	Cranium	-13.4	19.2	42.5	15.6	3.2	704–883	22.7
1026	F	Cranium	-13.9	18.5	41.4	14.7	3.3	710–895	6.0
1034	F	Cranium	-13.8	19.2	41.6	15.3	3.2	789–984	8.6
1038	F	Cranium	-13.4	19.8	41.9	16.2	3.0	715–896	16.7
1040	F	Cranium	-13.1	19.9	41.8	16.2	3.0	576–709	19.3
1041*	F	Cranium	–	–	41.1	17.0	2.8	666–824	18.2
1044	F	Cranium	-13.0	19.6	42.4	16.3	3.0	770–962	15.8
1056	F	Cranium	-13.2	19.8	41.2	16.1	3.0	711–900	7.3
1081	F	Cranium	-13.7	19.5	41.5	15.4	3.1	674–826	18.8
1082	F	Cranium	-13.5	19.8	42.0	16.1	3.0	553–680	19.5
1086	F	Cranium	-13.2	20.6	41.2	16.5	2.9	658–779	15.5
1089	F	Cranium	-13.5	19.1	42.0	15.4	3.2	573–696	20.5
1095	F	Cranium	-13.5	19.8	42.4	16.7	3.0	685–858	19.5
1109	F	Cranium	-14.4	18.6	42.3	15.4	3.2	418–587	13.9
1114	F	Cranium	-13.7	19.8	43.3	17.2	2.9	718–895	18.9
1121	F	Cranium	-12.7	18.6	41.2	15.3	3.1	–	12.7
1124*	F	Cranium	-13.6	19.6	42.0	15.4	3.2	–	20.8

ID	Sex	Element	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	calAD (95%CI)	Yield (%)
1128	F	Cranium	-14.8	16.3	42.1	15.3	3.2	–	13.8
1132	F	Cranium	-13.4	19.8	42.7	15.9	3.1	–	20.2
1133	F	Cranium	-13.8	19.0	42.1	15.6	3.1	–	12.4
1003	M	Cranium	-13.0	20.2	44.9	14.4	3.6	560–686	7.5
1006	M	Cranium	-13.6	19.3	43.7	14.7	3.5	910–1095	11.3
1010	M	Cranium	-13.3	19.6	42.6	15.0	3.3	677–872	13.2
1012	M	Cranium	-13.7	19.4	44.3	16.0	3.2	690–869	12.6
1017	M	Cranium	-13.2	20.6	44.6	15.3	3.4	561–683	11.4
1024	M	Cranium	–	–	41.7	17.2	2.8	663–804	13.6
1033	M	Cranium	-13.8	19.5	41.8	16.7	2.9	742–960	8.8
1039	M	Cranium	-14.7	18.5	42.2	16.1	3.1	537–675	7.8
1042	M	Cranium	-13.5	19.7	41.6	16.6	2.9	885–1031	11.5
1051	M	Cranium	-13.3	19.2	41.6	15.8	3.1	679–845	20.4
1058	M	Cranium	-15.3	19.2	42.1	15.3	3.2	349–549	4.9
1065	M	–	-13.9	18.9	43.3	16.0	3.2	–	19.5
1067	M	Cranium	-13.0	18.7	42.3	15.4	3.2	643–776	18.8
1068	M	Cranium	-12.7	17.9	41.2	14.5	3.3	558–683	17.3
1069	M	Cranium	-13.1	18.9	40.7	15.4	3.1	654–793	7.4
1070	M	Cranium	-14.5	18.8	39.9	14.8	3.1	403–574	7.8
1079	M	Rib	-13.1	20.2	43.3	15.4	3.3	334–540	18.1
1084*	M	Cranium	–	–	41.2	3.1	15.5	626–769	8.5
1085*	M	Cranium	–	–	41.7	9.7	5.0	550–690	14.6
1087	M	Cranium	-16.0	17.8	41.9	16.2	3.0	701–879	8.1
1088	M	Cranium	-13.5	20.0	41.7	15.9	3.1	633–772	10.8
1107	M	Cranium	-13.3	20.8	41.6	16.2	3.0	725–920	8.1
1112	M	Cranium	-14.6	19.3	43.5	17.2	3.0	550–678	11.3
1113	M	Cranium	-13.2	20.5	43.4	15.8	3.2	520–670	18.1
1119	M	Cranium	-13.3	20.4	42.9	16.9	3.0	653–783	12.8
1120	M	Cranium	-13.0	20.8	42.2	17.3	2.9	519–675	20.3
1123	M	Cranium	-13.5	19.3	41.8	15.3	3.2	–	18.2
1127	M	Cranium	-15.1	18.5	41.6	15.7	3.1	–	23.4
1130	M	Cranium	-13.5	20.5	41.6	15.3	3.2	–	15.0
1131	M	Cranium	-13.3	20.5	42.7	15.2	3.3	–	10.5
1145*	M	Cranium	–	–	34.2	14.3	2.8	–	19.5
1149*	M	Cranium	–	–	33.4	14.1	2.8	–	–
1156	M	Cranium	-13.0	20.1	34.0	12.3	3.2	449–635	20.7
1271	M	–	-13.3	19.4	34.1	13.8	2.9	860–980	9.5
1021	U	Cranium	-13.0	19.4	42.4	15.6	3.2	593–721	22.1
1104	U	Rib	-13.5	18.4	42.1	15.0	3.3	–	19.6

Samples with an asterisk were excluded from the analysis. Sex was estimated by my

collaborator (H.I.). Bone sampling, collagen extraction, and isotope analysis were performed by my collaborators (Y.I.N. and M.Y.). Stable isotopic results of 1006 and 1011 originated from Naito et al. (2010a).

Table B.7: Results of isotope analysis of subadult human skeletons from the Moyoro site.

ID	Element	Age range	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
1004	–	0	-15.1	18.3	43.2	15.2	3.3	16.6
1194	Cranium	0	-13.0	19.4	43.6	15.5	3.3	6.5
1064	Cranium	1	-13.8	21.9	42.9	15.1	3.3	9.8
1139	Cranium	1	-13.8	22.7	43.9	15.3	3.3	12.4
1062	Mandible	1.5	-13.9	22.1	42.6	15.2	3.3	5.3
1063	Cranium	1.5	-13.4	21.2	43.4	15.8	3.2	7.4
1073	Cranium	1.5	-14.0	22.4	40.4	14.6	3.2	3.9
1094	Cranium	1.5	-13.0	22.6	42.7	15.2	3.3	4.6
1181	Rib	1.5	-14.3	20.0	42.7	15.2	3.3	12.3
1205	Cranium	1.5	-14.0	20.1	43.3	15.7	3.2	7.1
1093	Cranium	2	-13.7	19.9	43.6	15.5	3.3	–
1111	Cranium	2	-13.3	22.4	43.4	15.6	3.2	7.3
1137	Mandible	2	-15.4	17.2	41.4	14.0	3.5	5.3
1167	Cranium	2	-13.7	20.3	43.7	15.8	3.2	8.1
1002	–	2.5	-14.4	21.3	43.8	16.1	3.2	11.2
1092	Rib	2.5	-14.0	20.0	43.1	15.6	3.2	20.2
1180	Cranium	2.5	-13.9	19.4	44.0	15.5	3.3	7.3
1055	Cranium	3	-13.9	20.2	43.5	15.5	3.3	5.8
1005	Cranium	4	-13.4	18.9	43.9	15.6	3.3	8.4
1008	–	4	-14.3	19.2	43.7	16.3	3.1	20.6
1052	Cranium	4	-13.6	19.5	44.3	15.9	3.3	7.3
1072	Cranium	4	-14.8	19.0	45.0	15.8	3.3	6.1
1091	Cranium	4	-13.5	19.8	43.3	15.6	3.2	5.4
1148	Rib	4	-14.1	19.3	42.9	15.6	3.2	18.6
1203	Rib	4	-13.6	19.4	42.1	14.9	3.3	12.0
1007	–	5	-13.5	19.7	44.1	16.1	3.2	22.1
1071	Cranium	5	-14.0	19.4	43.2	15.3	3.3	4.2
1102	Cranium	5	-14.5	19.5	43.8	14.7	3.5	5.4
1122	Cranium	5	-13.8	18.5	41.2	14.9	3.2	6.0
1154	Rib	5	-14.1	18.7	43.3	15.2	3.3	7.6
1166	Cranium	5	-13.8	20.0	43.8	15.7	3.3	12.3
1184	Cranium	5	-13.6	18.8	43.9	16.0	3.2	5.5
1186	Cranium	5	-13.7	19.0	42.7	15.5	3.2	5.7
1206	Rib	5	-14.2	19.9	42.9	15.9	3.1	21.0
1029	Cranium	6	-12.0	19.1	44.3	15.7	3.3	6.2

ID	Element	Age range	δ^{13}C (‰)	δ^{15}N (‰)	%C	%N	C/N	Yield (%)
1030	Cranium	6	-13.6	19.0	43.6	15.6	3.3	3.9
1046	Rib	6	-13.8	18.8	43.6	15.8	3.2	19.5
1054	Cranium	6	-13.6	18.7	42.9	15.5	3.2	6.0
1126	Rib	6	-13.7	19.4	42.8	15.4	3.2	16.5
1208	Cranium	6	-14.1	19.3	43.4	15.2	3.3	4.9
1209	Cranium	6	-14.3	19.2	43.8	15.4	3.3	11.0
1188	Cranium	7	-13.7	18.8	43.7	15.8	3.2	4.6
1207	Cranium	7	-14.0	19.4	43.5	15.6	3.3	4.8
1020	Rib	7–9	-14.1	18.1	44.1	15.7	3.3	8.9
1036	Rib	8	-13.7	18.9	42.5	15.5	3.2	14.1
1125	Cranium	8	-13.4	18.8	39.3	14.2	3.2	2.7
1134	Rib	8	-14.1	19.0	43.4	15.6	3.2	18.1
1164	Cranium	8	-13.3	19.0	43.4	15.7	3.2	6.4
1138	Cranium	8–9	-14.6	19.1	42.4	14.4	3.4	6.8
1045	Cranium	10	-14.6	18.9	44.2	15.4	3.3	10.7
1050	Cranium	10	-13.6	17.8	42.5	15.5	3.2	3.2
1106	Rib	10	-14.4	19.3	44.5	15.4	3.4	9.7
1150	Cranium	10	-13.8	18.3	44.1	15.7	3.3	6.6
1022	Cranium	11	-14.0	18.3	42.4	14.9	3.3	4.4
1061	Mandible	11	-13.2	18.5	43.2	15.5	3.3	7.7
1117	Cranium	12	-13.7	18.2	44.1	15.9	3.2	9.6
1016	Cranium	13	-13.2	19.4	42.8	15.5	3.2	6.5
1014	Cranium	15–16	-13.6	18.6	42.9	15.5	3.2	4.4

Age (years) was estimated by my collaborator (H.I.). Bone sampling, collagen extraction, and isotope analysis were performed by myself.