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## Animal components in the diet of Japanese black bears *Ursus* thibetanus japonicus in the Kyoto area, Japan

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We measured stable carbon and nitrogen isotope ratios of hair, muscle and potential food items of Japanese black bears Ursus thibetanus japonicus in Kyoto Prefecture and its surrounding area in order to determine the contributions of animal and plant foods. The  $\delta^{13}$ C values of hair samples of bears were  $-22.7 \pm 0.4\%$  in early summer,  $-22.8 \pm 0.3\%$  in late summer and  $-22.7 \pm 0.7\%$ in autumn, while  $\delta^{15}$ N values were  $3.5 \pm 0.4\%$  in early summer,  $3.2 \pm 0.4\%$  in late summer and  $3.6 \pm 0.5\%$  in autumn. The  $\delta^{13}$ C values of muscle samples of bears were -24.6% in early summer, -23.2% in late summer and -23.1  $\pm$  0.3% in autumn, while  $\delta^{15}$ N values of bears captured were 3.7% in early summer, 5.0% in late summer and  $4.5 \pm 0.7\%$  in autumn. We determined the isotopic endpoints of seven food groups from the isotope ratios of food groups and calculated the contribution of each food group using a stochastic method. Our results suggested that animal components were the major constituent of body tissue, contributing > 61% in all samples except for muscle samples collected in early summer. In muscle samples collected in early summer, none of the food items were estimated to be the major source. In cases in which the animal components were estimated to be major food sources, invertebrates were estimated to account for most of the animal components. It was concluded that animal components are an important source of tissue material in Japanese black bears in Kyoto and its surrounding area.

Key words: animal foods, food habits, isotopes, Japan, Japanese black bear

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The Asian black bear *Ursus thibetanus* is a mediumsized bear that is distributed in southern and eastern Asia (Servheen 1990). In Japan, this bear is generally treated as the subspecies *U. t. japonicus* (Japanese black bear; Hashimoto & Yasutake 1999, Horino & Miura 2000). Black bears are regarded as both a natural resource and

a pest in Japan, and they have been hunted for their meat, fur and gallbladder. Because they cause damage to silviculture, agriculture and apiculture and occasionally attack people, local governments permit culling on request. Due to high hunting pressure and environmental change, their distribution has decreased accompanying the emergence of threatened local populations (Ishii 2002).

The black bears in Kyoto Prefecture are considered to be a threatened population (Kyoto Prefecture 2002). Hunting is prohibited in Kyoto Prefecture and scientific surveys on ecology and biology of the bear in this area, which are essential for appropriate management, are now in action. Recently, black bears were shown to be genetically divided into two smaller populations (Saitoh et al. 2001). However, more information about bears in this area, such as food habits and home range, is needed.

Food habits of Japanese black bears is one topic that needs more research. It is important because foraging behaviour is a significant aspect of bear behaviour, and survival or growth is often constrained by the food resources (Rogers 1976, Welch et al. 1997). Understanding bear nutritional needs help us to comprehend their behaviour or to recognise what kind of habitat is necessary for bear conservation and management.

Japanese black bear has been defined as exclusively phytophagous on the basis of results of faeces and stomach content analyses, and the importance of plant materials has often been emphasised (Naganawa & Koyama 1994, Mizoguchi et al. 1996, Hashimoto & Takatsuki 1997, Hazumi et al. 1997, Horiuchi et al. 2000). However, the contribution of plant foods might have been exaggerated in studies in which faeces and stomach contents were examined because the low digestibility of plant materials (Prichard & Robbins 1990) would have biased the results considerably (Hewitt & Robbins 1996). Therefore, we must verify the validity of the current understanding of food habits of the Japanese black bear by using other methods.

We measured carbon and nitrogen stable isotope ratios of Japanese black bears in Kyoto and its surrounding area in order to determine the contributions of animal and plant foods. Compared to traditional methods, methods using stable isotopes give more integrative information about food assimilated (DeNiro & Epstein 1978, 1981, Tieszen et al. 1983, Hilderbrand et al. 1996). For example, if Japanese black bears depend mostly on plant foods, animal foods will not influence the isotope ratios of bears. On the other hand, if certain amounts of animal components are consumed by bears for a certain time span, the effect of consumption of animal foods

must appear in bear tissue as isotopic signatures. Using this feature of stable isotope analysis, it is possible to validate the current understanding of food habits of bears.

#### Material and methods

#### Study area

Our study area extended from the northern part of Kyoto Prefecture to the northwestern part of Shiga Prefecture, Japan (Fig. 1), at elevations from sea level to 972 m a.s.l. Annual precipitation in this region is about 1,786 mm,

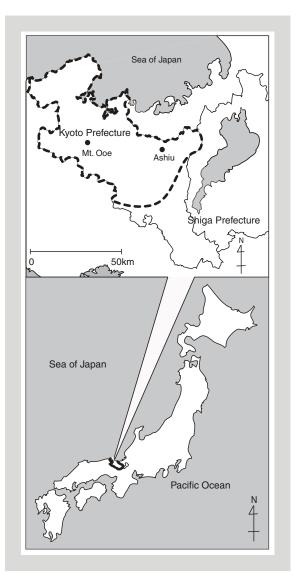


Figure 1. Location (below) and macrograph (above) of the study area in the Kyoto area, Japan. The area enclosed in the broken line indicates where bears were captured. Most of the food samples were collected at Ashiu and Mt. Ooe.

average temperatures are 3.4°C in February and 26.5°C in August, and snowfall occurs between December and March (averages for 1971-2000; Japan Meteorological Agency 2001). Most of the forests in the study area are secondary communities consisting of oaks Quercus crispula and Q. serrata, chestnut Castanea crenata, and red pine Pinus densiflora. The original forests were dominated by beech Fagus crenata, deciduous Q. crispula and evergeeen oaks Q. acuta and Q. salicina, Japanese cedar Cryptomeria japonica, and species of Castanopsis with increasing amounts of the lauraceous Machilus thunbergii at lower elevations. Forest plantations in the study area mainly consist of Japanese cedar and Hinoki cypress Chamaecyparis obtusa. Food materials were collected mainly around Ashiu Forest Research Station of Kyoto University (35°18'N, 135°43'E). Some food materials, including nuts and hymenoptera, were also collected at Mt. Ooe (35°27'N, 135°6'E).

#### Sample collection

We obtained tissue samples from 27 bears during 2001-2002 (Table 1). Some hair samples were collected from

Table 1. Date of capture, weight (in kg) and sex of Japanese black bears captured in Kyoto and its surrounding area during 2001-2002.

					Tissue	
ID	Season	Date	Weight	Sex	Hair	Muscle
01	Early summer	16 June 2001	37.0	ď	0	
02		16 June 2001	45.0	ď	0	
03		23 May 2002	30.0	ď	0	0
04		23 May 2002	48.8	ď	0	
05		19 June 2002	34.0	Q	0	0
06	Late summer	4 July 2001	17.0	ď	0	
07		12 July 2001	50.0	Q	0	0
08		17 July 2001	25.5	ď	0	
09		7 August 2001	31.5	Q	0	
10		11 August 2001	41.5	Q	0	
11		23 July 2002	45.0	Q	0	
12		3 August 2002	50.5	Q	0	
13		6 August 2002	39.0	Q	0	
14		27 August 2002	50.5	Q	0	
15	Autumn	13 September 2001	30.0	?	0	0
16		19 September 2001	30.5	Q	0	0
17		26 September 2001	40.0	ď	0	0
18		10 November 2001	34.0	ď	0	
19		15 November 2001	44.0	Q	0	
20		18 November 2001	64.5	Q	0	
21		6 September 2002	58.0	Q	0	0
22		22 September 2002	45.0	Q	0	
23		13 October 2002	20.0	ď	0	0
24		30 October 2002	81.5	ď	0	
25		28 November 2002	83.0	ď	0	
26		28 November 2002	49.5	ď	0	
27		4 December 2002	71.5	ď	0	

bears captured for another research programme, while others were collected from individuals captured by traps for other animal species. The Kansai Research Center of Forestry and Forest Products Research Institute provided eight muscle and hair samples of bears killed for nuisance control. Body weights ranged within 17-83 kg, and numbers of female and male bears varied with season (see Table 1). Tissue of bears captured in Shiga Prefecture were also included because the sampling sites were very close to Ashiu (see Fig. 1), and their habitats were assumed to be the same as those in eastern Kyoto.

Although approximate carbon and nitrogen isotope ratios of each food material are known (Hilderbrand et al. 1996), the isotope compositions of each food material vary geographically (Tieszen 1989). To estimate representative carbon and nitrogen isotope ratios for major prey groups in our study area, we collected major groups of plant and animal species known to be eaten by bears, referring to Watanabe et al. (1970) and Hashimoto & Takatsuki (1997), from May 2001 to February 2003 (Table 2). Based on local observations, we assumed that bears

Table 2. Composition of food materials of Japanese black bear collected in Kyoto and its surrounding area during 2001-2003.

Food group	Species	Sample size
Foliate diet	Angelica pubescens	1
	Arisaema serratum	2
	Cardiocrinum cordatum	3
	Cynareae spp.	1
	Elastostema umbellatum var. majus	3
	Petasites japonicus	5
	Fagus crenata	4
	Total	19
Berries	Cornus controversa	1
	C. kousa	2
	C. macrophylla	1
	Prunus grayana	1
	Rubs spp.	6
	Sorvus alnifolia	1
	Vitis flexuosa	2
	Total	14
Nuts	Quercus crispula	7
	Q. serrata	1
	Castanea crenata	10
	Fagus crenata	1
	Total	19
Herbivorous	Apis cerana	4
Hymenoptera		
Carnivorous	Vespa simillima xanthoptera	2
Hymenoptera	V. mandarinia japonica	
	Total	3
Omnivorous Hymenoptera	Camponotus obscuripes	5
Crustaceans	Geothelphusa dehaani	2
Herbivorous mammals	Cervus nippon	5

in our study area rarely, if ever, eat maize *Zea mays* (a C4 plant). Similarly, we did not sample any marine food items because Japanese black bears are seldom reported to use marine resources at present. When we identified a nest of hymenoptera, we put the workers from the same nest together as one sample, while workers collected at the same point was put together when identification of the nest was difficult. Nuts and berries from the same tree were also treated as one sample. Muscle of sika deer *Cervus nippon* was collected from dead bodies found in the field and from individuals killed by local hunters.

#### Sample preparation

Samples of muscle, plants and invertebrates were freezedried. Only Du Huo *Angelica pubescens* was oven-dried (60°C). Lipids were extracted from bear muscle using a chloroform and methanol (2:1) solution for  $\delta^{13}$ C analysis (Hilderbrand et al. 1996, Matsubara & Minami 1998). Hair samples for  $\delta^{13}$ C analysis were repeatedly rinsed using a chloroform and methanol (2:1) solution to remove surface lipids and then air-dried (Hilderbrand et al. 1996, Hobson et al. 2000). Dried samples were powdered and enveloped in tin containers for isotopic measurement.

#### Isotope analysis

Carbon and nitrogen isotope ratios were analysed with CF/IRMS (Continuous flow isotope ratio mass spectrometer, Finnigan delta S with an elemental analyzer EA1108; Thermoquest, Germany) at the Center for Ecological Research, Kyoto University. The results are expressed in  $\delta$  notation as follows:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000, (\%e)$$

where X is  $^{13}$ C or  $^{15}$ N and R is the corresponding ratio  $^{13}$ C/ $^{12}$ C or  $^{15}$ N/ $^{14}$ N.  $R_{standard}$  for  $\delta^{13}$ C and  $\delta^{15}$ N corresponds to the ratio of Pee Dee Belemnite (PDB) and AIR, respectively. Based on repeated measurements of a laboratory standard, we estimated our analytical errors to be less than  $\pm 0.21\%$  (SD) for  $\delta^{13}$ C and  $\pm 0.15\%$  (SD) for  $\delta^{15}$ N.

#### Diet estimation

The relative contribution of each diet group was estimated using a stochastic method (Monte Carlo simulation) developed to estimate the contribution of each organic source in a multi-component mixture (Minagawa 1992), which is essentially the same method as that of Phillips & Gregg (2003). We ran the simulation programme until 1,000 possible combinations of contribu-

tion of each food group were obtained. For simulation, we used diet-tissue enrichment of 2‰ for carbon based on the results of experiments using captive bears (Hilderbrand et al. 1996, Felicetti et al. 2003) and on a comprehensive review of mammal species (Kelly 2000). We used the fractionation values of 3‰ for nitrogen when invertebrates were consumed, 4‰ when mammals were consumed, and 5‰ when plant materials were consumed based on the results of experiments using captive bears and other mammals and birds (Hilderbrand et al. 1996, Felicetti et al. 2003, Robbins et al. 2005). In the simulation, a randomly generated combination of source proportions was judged as possible when the following condition was satisfied:

$$\delta X_{\text{bears}} - SD[\delta X_{\text{bears}}] \leq \sum_{k=1}^n f_k \delta X_k \leq \delta X_{\text{bears}} + SD[\delta X_{\text{bears}}] \; ,$$

where X is  ${}^{13}$ C or  ${}^{15}$ N. The variable  $\delta X_{bears}$  corresponds to isotope ratios measured from bear hair or muscle.  $\delta X_k$ represents isotope ratios of the kth food item of n food sources, while fk stands for the relative contribution of that food item.  $SD[\delta X_{bears}]$  corresponds to the standard deviation of measured stable isotope ratios of bear hair or muscle, which were substituted by analytical error in cases where the number of samples was two or less. We used a standard mixing model based on the assumption that C:N ratios in materials assimilated to protein-rich tissue such as hair and muscle do not differ greatly (Krueger & Sullivan 1984, Tieszen & Fagre 1993, Hobson & Stirling 1997, Koch & Phillips 2002, Phillips & Koch 2002, Robbins et al. 2002). With regard to model selection, we believe that we made the best assumption, but we acknowledge the necessity of further experimental studies on fractionation or substrate routing (Gannes et al. 1997, Ben-David & Schell 2001, Koch & Phillips 2002, Phillips & Koch 2002, Robbins et al. 2002, 2005).

The simulation was done for three seasons (early summer = May-June, late summer = July-August, and autumn = September-December) considering isotopic turnover of tissue, the season in which the bears were captured, and seasonal variation in food items. For example, the isotopic signature of hair reflects food habits during hair growth (Nakamura et al. 1982, Hobson 1999), which occurs from early summer to autumn (Jacoby et al. 1999). Besides, black bears in Japan are generally known to eat foliate foods from spring to late summer, whereas berries are consumed through spring to autumn and consumption of invertebrates increases from early summer to late summer, and they eat many nuts in autumn (Watanabe et al. 1970, Yamamoto 1973,

Naganawa & Koyama 1994, Mizoguchi et al. 1996, Hazumi et al. 1997, Horiuchi et al. 2000). Thus, in the simulation using hair collected in early and late summer, we used a model containing food groups except for nuts, whereas a model containing all food groups was used in the simulation using hair samples collected in autumn.

On the other hand, muscle is metabolically active and dietary information obtained will be a temporal integration reflecting its turnover rate. Referring the 26.7 days of half life in gerbil muscle (Tieszen et al. 1983), muscle samples obtained from bears caught in early summer were expected to have gone through the hibernation period and reflect the isotope composition of foods from the previous autumn. Therefore, in the simulation using muscle of early summer, we used a model containing nuts. We acknowledge the uncertainty of this assumption about the turnover rate of muscle and effect of hibernation on isotopic component, and we also acknowledge the necessity of future studies.

#### Results

### Isotopic composition of bears and food materials

The  $\delta^{13}$ C values of hairs were -22.7  $\pm$  0.4% in early summer, -22.8  $\pm$  0.3% in late summer and -22.7  $\pm$  0.7% in autumn, while  $\delta^{15}$ N values were 3.5  $\pm$  0.4% in early summer, 3.2  $\pm$  0.4% in

late summer and  $3.6 \pm 0.5\%$  in autumn (Fig. 2A). The  $\delta^{13}$ C values of two bears captured in early summer was -24.6‰, while that of one captured in late summer was -23.2‰, and that of bears captured in autumn was -23.1  $\pm$  0.3‰ (Fig. 2B). The  $\delta^{15}$ N values of two bears captured in early summer was 3.7‰, while that of one captured in late summer was 5.0‰, and that of bears captured in autumn was  $4.5 \pm 0.7\%$  (see Fig. 2B).

As for the isotope ratios of food items (see Fig. 2), the foliate diet and berries had the lowest  $\delta^{13}$ C value, followed by the herbivores and nuts, and finally invertebrates with the highest ratio. The foliate diet and berries

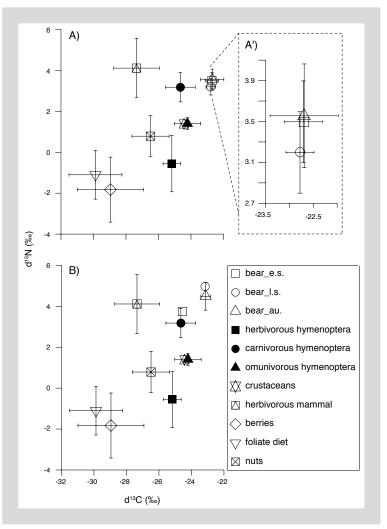


Figure 2. Carbon and nitrogen isotope ratios (in %) of hair (A) and muscle (B) of Japanese black bears and their food materials collected in Kyoto and its surrounding area in 2001-2003. The insert (A') shows a magnification of the area of A) where the isotope ratios of bear hair are plotted. 'bear\_e.s', 'bear\_l.s.' and 'bear\_au' represent bears captured in early summer (May-June), late summer (July-August) and autumn (September-December), respectively. The error bars indicate standard deviation. 'Bear\_e.s.' and 'bear\_l.s.' in B) and crustaceans in both A) and B) have no error bars because of their small sample size (N  $\le$  2). The data on food items in A) and B) are the same.

had the lowest  $\delta^{15}N$  value, followed by nuts, and then the herbivores with the highest ratio. Among the invertebrate groups,  $\delta^{15}N$  values varied from -0.6 ± 1.4% for herbivorous hymenoptera to 3.2 ± 0.7% for carnivorous hymenoptera, while  $\delta^{13}C$  values were similar.

#### Food habit estimation by Monte Carlo simulation

Based on isotope ratios of food items (see Fig. 2), we determined six or seven sources in the models: herbivorous hymenoptera, carnivorous hymenoptera, omnivorous hymenoptera/crustaceans, herbivorous mammals, foliate diet, berries, with nuts added as autumn food.

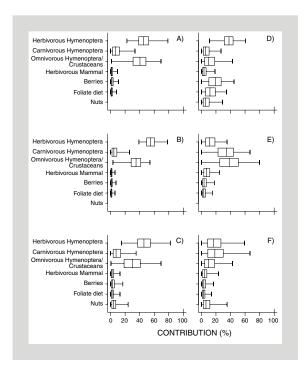


Figure 3. Possible ranges of contributions (in %) of each the seven food groups in the diet of Japanese black bears from the Kyoto area. The results were obtained through Monte Carlo simulation (Minagawa 1992) using hair (A-C) and muscle (D-F) of bears collected in Kyoto and its surrounding area during 2001-2002. The hair and muscle used in A) and D) were collected from bears captured in early summer (MayJune), the hair and muscle used in B) and E) were collected from bears captured in late summer (July-August), and the hair and muscle used in C) and F) were collected from bears captured in autumn (September-December). The horizontal lines indicate the minimum to maximum contribution values calculated for each food group, the boxes show the first to the third quantile, and the vertical bar in the box represents the median for each food group.

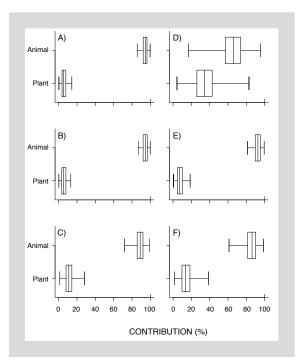


Figure 4. Possible ranges of contributions (in %) of plant and animals to the full diet Japanese black bears from the Kyoto area. The results were obtained through Monte Carlo simulation (Minagawa 1992) using hair (A-C) and muscle (D-F) of bears collected in Kyoto and its surrounding area during 2001-2002. The hair and muscle used in A) and D) were collected from bears captured in early summer (May-June), the hair and muscle used in B) and E) were collected from bears captured in late summer (July-August), and the hair and muscle used in C) and F) were collected from bears captured in autumn (September-December). The horizontal lines indicate the minimum to maximum contribution values calculated for each food group, the boxes show the first to the third quantile, and the vertical bar in the box represents the median for each food group.

Omnivorous hymenoptera and crustaceans were put together because their isotope ratios were very similar with differences of only 0.2 and 0.1‰ for carbon and nitrogen, respectively, and they were therefore regarded as indistinguishable.

The results of simulation using data obtained from analysis of hair samples (Fig. 3) indicated the possibility that the contribution of invertebrates such as insects and crustaceans varies greatly. On the other hand, herbivorous mammals and plant materials, except for nuts in autumn, were estimated to contribute little (see Fig. 3). The possible contribution of nuts in autumn was estimated to have values of 0-35%. When the contributions of different animal materials were summed up and compared to the contribution of plant materials, animal materials as a whole was estimated to have a large contribution with little variation (Fig. 4). Among animal materials

rials, the contribution of invertebrates was relatively large (Fig. 5).

The results of simulation using data obtained from analysis of muscle samples were similar to the results of simulation using hair samples (see Figs. 2B, 3, 4 and 5), though the sample size was small. From analysis of muscle samples obtained from bears captured in late summer and autumn, the contribution of animal components was estimated to be large (see Fig. 4), and the contributions of invertebrates was estimated to be greater than that of herbivorous mammals (see Fig. 5). On the other hand, the  $\delta^{13}$ C and  $\delta^{15}$ N of the muscle of bears captured in early summer were lower than those of bears captured in other seasons (see Fig. 2B), and estimated values of the contribution of both plants and animals varied greatly (see Fig. 4).

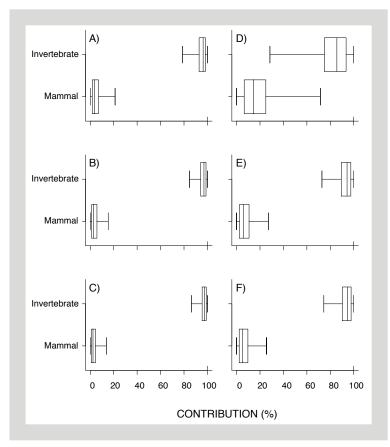


Figure 5. Possible ranges of contributions (in %) of invertebrates and herbivorous mammals to the animal proportion of the diet of Japanese black bears from the Kyoto area. The results were obtained through Monte Carlo simulation (Minagawa 1992) using hair (A-C) and muscle (D-F) of bears collected in Kyoto and its surrounding area during 2001-2002. The hair and muscle used in A) and D) were collected from bears captured in early summer (May-June), the hair and muscle used in B) and E) were collected from bears captured in late summer (July-August), and the hair and muscle used in C) and F) were collected from bears captured in and autumn (September-December). The horizontal lines indicate the minimum to maximum contribution values calculated for each food group, the boxes show the first to the third quantile, and the vertical bar in the box represents the median for each food group.

#### Discussion

Japanese black bears have been considered to be primarily phytophagous because plant materials have been predominantly found in stomachs and faeces (Naganawa & Koyama 1994, Mizoguchi et al. 1996, Hashimoto & Takatsuki 1997, Hazumi et al. 1997, Horiuchi et al. 2000). Although animals such as sika deer and invertebrates have been recognised as items occasionally consumed, their importance has not been ascertained. Our analysis of stable carbon and nitrogen isotope ratios of bear hairs showed that animal components appeared to account for a large proportion of their tissue material (see Fig. 4). Our results suggested a large contribution of invertebrates (see Fig. 5). The wide range of possible

contribution of each invertebrate species indicates that all invertebrate groups may become major contributors (see Fig. 3).

The results of simulation using data acquired from analysis of muscle samples obtained from bears captured in late summer and autumn were similar to the results of simulation using hair samples (see Figs. 3, 4 and 5). In early summer, on the other hand, the values of possible contributions of both animal and plant materials varied widely (see Fig. 4), indicating that both plant and animal materials can be major contributors. Considering the fact that a small amount of invertebrates is consumed in spring (Watanabe et al. 1970, Yamamoto 1973, Takada 1979, Naganawa & Koyama 1994, Hazumi et al. 1997, Horiuchi et al. 2000), it is reasonable to assume that the contribution of plant foods was larger in this than in other seasons, though further investigations are needed to determine whether the isotope ratios change during the denning period. Since our sample size was small, further studies are needed to determine whether the phenomenon observed in muscle is a general trend in the population of bears in our study area.

Determination of the isotope ratios of food sources is one of the factors affecting the accuracy of estimation (Minagawa 1992, Phillips & Gregg 2003). For example, carbon isotopes of plant

materials have been shown to vary with elevation (Körner et al. 1988), and carbon and nitrogen isotope ratios of insects also vary with forest maturity, caste or age (Blüthgen et al. 2003). We therefore agree that further studies on variation in isotope signatures of food sources used by bears are necessary. However, considering the range of the variations and the area of our study site, we expect that the effect of these variations on our conclusion obtained from the simulation is relatively small. Though our sample sizes of food materials were small (partly because of poor crops during the study periods for some plants), we believe that we obtained good representations of isotope ratios of food sources for our study site and study period.

Our results do not deny the importance of plant foods.

If most of the plant foods were consumed mainly as substrates for respiration, it is possible that the contribution of plant foods for body tissue was underestimated (Krueger & Sullivan 1984, Ambrose & Norr 1993, Hobson & Stirling 1997). Therefore, our results should not be interpreted as providing evidence of the contribution of animal foods to the bulk bear diet but rather as providing evidence of the contribution of animal foods to assimilated lean body mass.

Compared to methods available for smaller animals, methods that can be used for studies of food habits of bears are limited. Therefore, a need to combine the advantages of each method exists. As shown in American Ursus americanus and European bears U. arctos, faeces analysis and direct observation are useful for determining the food species consumed (McLellan & Hovey 1995, Mattson 1997, Noyce et al. 1997, White et al. 1998, Swenson et al. 1999). Analysis of stable isotopes is useful for obtaining integrative information about assimilated foods or trophic levels of each bear population (Hilderbrand et al. 1996, Hilderbrand et al. 1999, Jacoby et al. 1999). Using these methods, the means by which bears adapt to each locality, reflecting availability of each food species, have been shown. Japanese black bears may also have different food habits in each locality, which would require different types of management and conservation plans. To establish management and conservation plans that are appropriate for each locality, a comprehensive survey of the ecology of Japanese black bears at the local population level is needed. Stable isotope analysis is a powerful tool for achieving this goal.

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