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Diet shift of upland buzzards (*Buteo hemilasius*) : evidence from stable carbon isotope ratios *

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Abstract We measured the stable carbon isotope ratios for muscle of the upland buzzards (*Buteo hemilasius*), plateau pika (*Ochotona curzoniae*), root vole (*Microtus oeconomus*), plateau zokor (*Myospalax fontanierii*) and passerine bird species at the Haibei Alpine Meadow Ecosystem Research Station (HAMERS), and provided diet information of upland buzzards with the measurement of stable carbon isotopes in tissues of these consumers. The results showed that $\delta^{13}C$ values of small mammals and passerine bird species ranged from - 25.57 ‰ to - 25.78 ‰ (n = 12), and from - 24.81 ‰ to - 22.51 ‰ (n = 43), respectively. $\delta^{13}C$ values of the upland buzzards ranged from - 22.60 ‰ to - 23.10 ‰ when food was not available. The difference in $\delta^{13}C$ values (2.88 ‰ ± 0.31 ‰) between upland buzzards and small mammals was much larger than the differences reported previously, 1 ‰ - 2 ‰, and showed significant difference, while 1.31 ‰ ± 0.34 ‰ between upland buzzard and passerine bird species did not differ from the previously reported trophic fractionation difference of 1 ‰ - 2 ‰. Estimation of trophic position indicated that upland buzzards stand at trophic position 4.23, far from that of small mammals, i.e., upland buzzards scarcely captured small mammals as food at the duration of food shortage. According to isotope mass balance model, small mammals contributed 7.89 % to 35.04 % of carbon to the food source of the upland buzzards, while passerine bird species contributed 64.96 % to 92.11 %. Upland buzzards turned to passerine bird species as food during times of shortage of small mammals. $\delta^{13}C$ value, a useful indicator of diet, indicates that the upland buzzards feed mainly on passerine bird species rather than small mammals due to "you are what you eat" when small mammal preys are becoming scarce [*Acta Zoologica Sinica* **49** (6) : 764 - 768, 2003].

Key words Upland buzzard (*Buteo hemilasius*), Passerine birds, Small mammals, Alpine meadow, Stable carbon isotope, Diet

大𫛭的食性改变：来自稳定性碳同位素的证据 *

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摘 要 通过对大规模灭鼠后的海北高寒草甸生态系统大𫛭、小型哺乳类以及雀形目鸟类肌肉稳定性碳同位素比值的测定发现，大𫛭的肌肉稳定性碳同位素比值介于 - 22.60 ‰和 - 23.10 ‰之间；小型哺乳类和主要雀形目鸟类肌肉稳定性碳同位素比值分别介于 - 25.57 ‰和 - 25.78 ‰以及 - 24.81 ‰和 - 22.51 ‰之间，且它们之间差异性显著。基于碳同位素的分馏模式 (即动物和它食物之间稳定性碳同位素分馏在 1 ‰～2 ‰之间)，我们推断经大规模灭鼠后，大𫛭的食性发生了较大变化，其食物主要来源于高寒草甸的雀形目鸟类，而非原来的小型哺乳类。通过稳定性同位素营养级模型的运算发现，大𫛭处在 4.23 左右的营养级；雀形目鸟类处在 2.4 到 3.39 左右的营养级，而小型哺乳类则处在 1.45 到 1.82 左右的营养级。进而采用稳定性同位素质量平衡模型计算得出，大𫛭的食物由 35.04 %的小型哺乳类和 64.96 %的雀形目鸟类所组成，进一步说明小型哺乳类在大量灭鼠后仅占大𫛭食物的很小一部分。由此可见，采取大规模的化学灭鼠，不仅降低了小型啮齿类天敌——大𫛭的数量，而且使得其食谱发生了巨大改变而转向草甸主要雀形目鸟类 [动物学报 **49** (6) : 764～768, 2003]。

关键词 大𫛭 雀形目鸟类 小型哺乳类 高寒草甸 稳定性碳同位素 食性

Food chains can reveal trophic relationships between organisms. An organism's position within a

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particular food web is defined by its function whether it is a predator, herbivore or decomposer. Natural stable isotope techniques have been successfully applied to define the functional role of organisms (Ehleringer *et al*., 1986; Riera, 1998). In trophic interactions, as one organism feeds on another organism, the consumer tends to be isotopically heavier than its food source. Various studies (Minagawa *et al*., 1984; Tayasu, 1997) have attempted to quantify the difference in stable nitrogen isotope concentration ($\delta^{15}N$) between trophic levels and to reveal trophic relationships within communities. Early papers demonstrated the potential of carbon isotopes to differentiate among sources of organic matter and to clarify carbon flow pathways.

In the analysis of animal's diets, the most commonly used methods are analysis of stomach contents, faecal pellets or food remains. This gives a picture of the last meal at a specific time, but might not be typical of the overall diet. To obtain a more general picture of the diet one can collect faecal pellets or food remains over longer periods, but it is then often difficult or impossible to obtain information about individual variation in diet, especially for birds. In contrast, the analysis of stable isotopes will provide information about the average diet of individuals over a longer period (DeNiro *et al*., 1978), even a lifetime in a relatively short-lived animal. The stable isotope approach is based on the fact that naturally occurring stable isotope ratios in consumer tissues can be related to those in consumers' diets (DeNiro *et al*., 1978, 1981). Changes in, or fractionation of, stable carbon isotope ratios ($^{13}C/^{12}C$) occur with trophic level and are of the order of 1‰ - 2‰ (Hobson *et al*., 1992a; Hilderbrand *et al*., 1996). Thus, isotope measurement of consumers' tissue can reveal information about their ingested foods in ecosystems that are relatively simple and do not involve multiple isotopic inputs. Elevations in an animal's $^{13}C/^{12}C$ relative to those of the community food base have thus been used to infer a consumer's food base (Schoeninger *et al*., 1984). The analysis of the natural variations in stable isotope ratios in animal tissue has proven to be a powerful tool for analyzing the diet of predators (Hobson *et al*., 1992a). In contrast to more direct methods such as gut content analysis, stable isotope analysis looks at the diet over a longer period of time and therefore reflects the long-term feeding behavior of animals (Peterson *et al*., 1987).

In the alpine meadow ecosystem, the upland buzzard is one of the most important raptors controlling small mammals. However, local governments and shepherds tended to reduce small mammal populations using poisonous chemicals in recent years. In fact, the number of small mammals has apparently decreased so much that upland buzzards may face extinction. In the present study, the naturally occurring stable isotopes of carbon ($\delta^{13}C$) is used to test the hypothesis that the upland buzzards tend to feed mainly on local passerine bird species when small mammals become rare.

1 Materials and methods

1.1 Study area

The HAMERS of the Chinese Academy of Sciences was established in 1976 in order to understand the structure and function of the alpine meadow ecosystem, form and development of biodiversity, the adaptive and evolutionary strategies of species, and the impact of global changes on the grassland ecosystem. The HAMERS is located in the region of the Qinghai-Tibet Plateau, in a large valley oriented NW-SE and surrounded on all sides by the Qilian Mountains with N latitude 37°29′- 37°45′ and E longitude 101°12′- 101°33′. The average altitude of mountain area is 4 000 meters above sea level and 3 200 meters for the valley area. The climate of the HAMERS is dominated by the Southeast monsoon and the higher-pressure system of Siberia. It has a continental monsoon type climate, with severe and long winters and short cool summers. The average air temperature is -1.7 ℃ with extreme maximum of 27.6 ℃ and minimum -37.1 ℃. During winter months, the average temperature drops to -15 ℃ to -20 ℃ in highland area; during summer, the temperature in the warmest month (July) averages 14 - 22 ℃ in valleys and 4 - 10 ℃ in mountains. Average annual precipitation ranges from 426 to 860 mm, 80% of which falls in the short summer growing season from May to September. The annual average sunlight is 2 462.7 hours with 60.1% of total available sunshine. Vegetation was characterized by alpine shrub, alpine meadow, and swamp meadow. The research site was roughly confined in alpine meadows, with *Kobresia humilis* as dominant species and *Polygonum viviparum*, *Carex atrofusca*, *Saussurea superba*, *Elymus nutans*, and *Gentiana straminea* as subdominant species.

1.2 Analysis of stable carbon and nitrogen isotopes

Samples were collected from individual animal species for isotope analysis within the HAMERS. Sample size was two for upland buzzards (captured by local herdsman) due to their protection status by the state. For passerine birds the sample size was no less than three except three migrant bird species. Sample sizes of small mammals were more than three. Because the turnover cycle of stable carbon isotope in muscle is no more than two months and muscles truly reflect the variation in carbon isotopes of animals, all samples were collected during April to July 2002 one year after large-scale application of chemicals to control mammals. Muscles of animals (for the upland buzzard and all passerine bird species, the pectoral muscles were collected; for small mammals the muscles of back legs) were air dried indoors to constant mass in an oven at 70 ℃ for 48 hours and ground finely. Samples contained in tin boat were dispatched to isotope ratio mass online spectrometer under EAMS (element-analysis meter and spectrometer) conditions. Interface between element-analysis meter and spectrometer is ConF Ⅲ (continuous flow Ⅲ). Operation conditions: oxidizing furnace temperature is 900 ℃, reducing furnace is 680 ℃, pillar temperature is 40 ℃. The resulting CO_2 was purified in a vacuum line and injected in a Finnigan MAT DELTA^{PLUS}XL mass spectrometer (Finnegan Mat, Bermen, Germany) fitted with double inlet and collector systems respectively. Standards consisted of the Peedee Belemnite (PDB) formation from South Carolina, USA. Five state-classed standards (black carbon) were intervened between every ten samples to make the precision < 0.2 ‰ and meet international standards. The results are expressed in $\delta^{13}C$ relative to the PDB in the conventional δ per mil notation as follows:

$$\delta^{13}C\ (‰) = [\ (^{13}C/^{12}C)_s / \ (^{13}C/^{12}C)_{sta} - 1] \times 1\,000$$

where $^{13}C/^{12}C$ are the isotopic ratios of sample (s) and standard (sta). The overall (sample preparation plus analysis) analytical precision is ±0.2 ‰

1.3 Estimation of trophic positions

Trophic positions of animals are calculated according to the following equations (modified from Hobson *et al*., 1992b, because our results indicated that $\delta^{13}C$ would better reveal trophic positions in the alpine meadow ecosystem than would $\delta^{15}N$): $TL = 1 + (D_C - D_{PPC}) / \Delta T_C$

Where TL is the trophic position of a given animal in an ecosystem; D_C is values of $\delta^{13}C$ of muscle (or whole body) of a given animal respectively; D_{PPC} is the average of $\delta^{13}C$ of primary producers, which is near - 26.51 ‰ and calculated through 101 plant species living at the HAMERS (data not shown here); ΔT_C is isotopic enrichment between $\delta^{13}C$ of animals and their diets which is near 1.15 ‰ ± 0.50 ‰ and is calculated through four animal species (three small mammals and one insect) living at HAMERS and their known isotopic diets or stomach contents (data not shown here).

1.3 Data analysis

All the data were analyzed using SPSS (Statistical Package for Social Scientists) for windows 11.0. Data were expressed as mean ± *SD*. Stable carbon isotope ratios were determined whether there were significant differences between animal species by independent-samples *t* test or one-sample *t* test. All tests were two-tailed.

2 Results

The results of this study showed that and $\delta^{13}C$ values of small mammals and passerine bird species ranged from - 25.57 ‰ to - 25.78 ‰, and from - 24.81 ‰ to - 22.51 ‰, respectively (Table 1). While $\delta^{13}C$ values of the upland buzzards ranged from - 22.60 ‰ to - 23.10 ‰ when food was not available. $\delta^{13}C$ values of the upland buzzards had significant differences from those of small mammals (*Ochotona curzoniae*, *Microtus oeconomus*, and *Myospalax fontanierii*) and passerine bird species, respectively ($t = -16.972$, $df = 3$, $P < 0.001$; $t = -3.423$, $df = 18$, $P = 0.003$, respectively) (Table 1). Significant differences also existed between $\delta^{13}C$ values of small mammals (*Ochotona curzoniae*, *Microtus oeconomus*, and *Myospalax fontanierii*) and passerine bird species ($t = 5.111$, $df = 18$, $P < 0.001$). The differences in $\delta^{13}C$ values were 2.88 ‰ ± 0.31 ‰ between the upland buzzards and small mammals, while nearly equal to 1 ‰ (1.31 ‰ ± 0.34 ‰) between the upland buzzards and passerine bird species (Table 1). The $\delta^{13}C$ value of 2.88 ‰ ± 0.31 ‰ was significantly different from the reported value 1 ‰ - 2 ‰ (one-sample *t* test, $t = -21.695$, $df = 2$, $P = 0.002$), while 1.31 ‰ ± 0.34 ‰ showed no difference ($t = 1.482$, $df = 16$, $P = 0.158$) (where the reported value of $\delta^{13}C$ was defined as mean of the range, 1.5 ‰). Moreover, the results show no significant seasonal variations in isotope values as a group (data not shown).

3 Discussion

The stable isotope approach is based on the fact that naturally occurring stable isotope ratios in consumer tissues can be related to those in consumers' diets (DeNiro *et al*., 1978, 1981). Changes in, or fractionation of, stable carbon isotope ratios ($^{13}C/^{12}C$) occur with trophic level and are of the order of 1 ‰ - 2 ‰ (Hobson *et al*., 1992a; Hilderbrand *et al*., 1996). Thus, isotope measurement of consumers' tissue can reveal information about their ingested foods and about trophic position in systems that are relatively simple and do not involve multiple isotopic inputs. Increases in an animal's $^{13}C/^{12}C$ relative to the community food base have thus been used to infer a consumer's trophic distance from the food source (Schoeninger *et al*., 1984). The recent study showed that the difference in $\delta^{13}C$ values of 2.88 ‰ ± 0.31 ‰ between the upland buzzards and small mammals was much more than the difference reported,

Table 1 $\delta^{13}C$ values of animal species at the HAMERS

Common name	Scientific name	Sampling date (y. m. d)	$\delta^{13}C$ (‰)	*SD*
Upland buzzard (2) [a]	*Buteo hemilasius*	2002.04 - 05	- 22.80	0.20
Passerine birds				
Black redstart, adult male (1)	*Phoenicurus ochruros*	2002.05.11	- 23.86	- [b]
Brandt's rosy finch, adult (4)	*Leucosticte brandti*	2002.04.12 - 13	- 24.30	0.45
Horned lark, adult (4)	*Eremophila alpestris*	2002.04.11 - 14	- 24.28	0.32
Hume's ground jay, adult (3)	*Pseudopodoces humilis*	2002.04.12	- 24.72	0.16
Hume's ground jay, adult (4)	*Pseudopodoces humilis*	2002.06.06	- 24.01	0.08
Long-billed calandra lark, adult (4)	*Melanocorypha maxima*	2002.04.11 - 13	- 24.54	0.32
Long-billed calandra lark, adult (3)	*Melanocorypha maxima*	2002.07.06	- 24.40	-
Robin accentor, adult (3)	*Prunellidae rubeculoides*	2002.04.14	- 24.81	0.12
Small skylark, adult (1)	*Alauda gulgula*	2002.05.09	- 24.08	-
Small skylark, adult (dead) (1)	*Alauda gulgula*	2002.05.09	- 23.44	-
Tree sparrow, adult (2)	*Passer montanus*	2002.04.12 - 27	- 24.10	0.05
Tree sparrow, adult (3)	*Passer montanus*	2002.05.01 - 09	- 24.22	0.03
Tree sparrow, adult (1)	*Passer montanus*	2002.07.08	- 24.27	-
Twite, adult (4)	*Acanthis flavirostris*	2002.05.09	- 24.28	0.20
Twite, adult (3)	*Acanthis flavirostris*	2002.04.09	- 24.17	-
White wagtail, adult (1)	*Motacilla alba*	2002.04.11	- 22.51	-
Yellow-browed warbler, adult (1)	*Phylloscopusinornatus*	2002.05.15	- 23.91	-
Small mammals				
Plateau pika (6)	*Ochotona curzoniae*	2002.04 - 07	- 25.57	0.24
Root vole (4)	*Microtus oeconomus*	2002.04 - 07	- 25.74	0.16
Plateau zokor (2)	*Myospalax fontanierii*	2002.05.14	- 25.78	0.21

a: Sample size b: One datum

1 ‰ - 2 ‰ (Hobson *et al*., 1992a; Hilderbrand *et al*., 1996; DeNiro *et al*., 1978, 1981) and showed significant difference (One-Sample *t* test, $t = -21.695$, $df = 2$, $P = 0.002$), while 1.31 ‰ ± 0.34 ‰ between upland buzzards and passerine bird species showed no significant difference ($t = 1.482$, $df = 16$, $P = 0.158$) (Table 1). These data revealed that small mammals (*Ochotona curzoniae*, *Microtus oeconomus*, and *Myospalax fontanierii*) are not likely to be the dominant food sources of the upland buzzards, and passerine bird species would make a greater contribution to the upland buzzard's diet. Based on the model for estimating trophic position of animals, the upland buzzards would have a trophic position of 4.23, and small mammals and passerine bird species stand at 1.45 to 1.82 and 2.4 to 3.39, respectively. Estimation of trophic position indicated that the upland buzzards stand at trophic position 4.23, far from that of small mammals, i.e., upland buzzards rarely consumed small mammals during food shortage. However, Hobson *et al*. (1992b) used stable nitrogen isotope to infer trophic levels. In our study to trace carbon flow to the upland buzzards, $\delta^{13}C$ acts a more accurate role in evaluating trophic levels compared with $\delta^{15}N$. We tentatively used $\delta^{13}C$ as an indicator of trophic levels here and obtained satisfactory results. According to mass balance model, $\delta^{13}C_{upland\ buzzards} = \delta^{13}C_{small\ mammals} = \delta^{13}C_{small\ mammals} \times P + \delta^{13}C_{passerine\ species} \times (1 - P)$, where P refers to percentage of small mammals to diets of the upland buzzards and $\delta^{13}C_{upland\ buzzards}$ was decreased by 1.5 ‰ - 2 ‰ according to trophic enrichment of stable carbon isotope (Forsberg *et al*., 1993; Hobson *et al*., 1999), small mammals contributed 7.89 % to 35.04 % of carbon to food source of upland buzzards, while passerine bird species contributed 64.96 % to 92.11 %. Small mammals contributed few carbon sources to the food of the upland buzzards during the time of food shortage of small mammals. Furthermore, lipids were not removed form all muscle sam-

ples prior to stable isotope analyses, which would account for some of the isotopic differences. The results would further biased towards of passerine birds since lipids are isotopically light (lipid content would vary slightly among species as all samples of birds were pectoral muscles and get rid of obvious lipid tissues). In this study, $\delta^{13}C$ value, a useful indicator of diet, indicated that the upland buzzards foraged mainly on passerine bird species rather than small mammals because of "you are what you eat" (Eggers *et al.*, 2000).

Since 1990s, governments and scientists all over the world have paid more attention to biodiversity and sustainable development, especially focusing on the effects on the structure and function of ecosystem. The Qinghai-Tibet Plateau, a unique geographic unit as the highest plateau in the world, has a great impact on the Eurasia atmospheric circulation and distribution of various ecosystems, especially on structure, function, adaptation, and evolutionary patterns. Due to the high altitude climate, the alpine meadow ecosystem is fragile and sensitive to species loss. Results of this study indicated that application of poisonous chemicals at the meadow diminished the amount of small mammal biomass, would decrease the number of rodent-controlling upland buzzards, and would eventually change their foraging habits and diets. It is suggested that local governments and shepherds stop utilizing poisonous chemicals and attempt to rely more on biocontrol means, such as rearing raptors for rodent control.

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