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Natural distribution of $\delta^{13}C$ and $\delta^{15}N$ values of flora and fauna in Tsushima

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1. Introduction

The endangered Tsushima leopard cat (*Felis bengalensis euptilura,* Eliot, 1871) is one of the two species of the wild cats in Japan, the other being the Iriomote leopard cat (*Felis iriomotensis,* Imaizumi, 1967). The population of the Tsushima leopard cat, found only on the Tsushima Islands of Japan (Fig. 1), was estimated to be between only 70 - 90 individuals (Environment Agency & Nagasaki Prefecture, 1997) and has been categorized as IA (Critically Endangered, CR), the highest extinction risk category in the Japanese Red List (Ministry of the Environment, 2002).

Food habit is one of most important ecological aspects to consider the conservation strategy of an endangered species, and that of the cat has been investigated by scat analysis (Asahi 1966 ; Okuhama 1970 ; Inoue 1972 ; Sukigara *et al.* 1988 ; Tatara & Doi 1994). Scat analysis can present a prey list and its seasonal changes, but the data are from unknown individuals and prey items often differ in digestibility. Continued direct observation of feeding and examination of stomachs is hardly applicable because the cats are scarce and difficult to observe or collect. As an alternative study method, carbon and nitrogen stable isotope measurements can estimate what animals assimilate as sources of protein, and have been utilized in many ecological studies (Peterson & Fry 1987 ; Peterson & Howarth 1987 ; Wada *et al.* 1991 ; Hesslein *et al.* 1992).

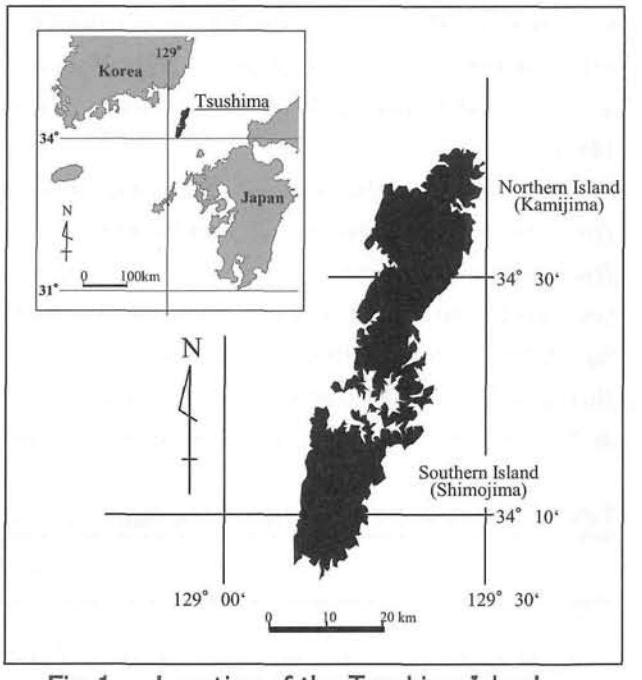

Fig. 1. Location of the Tsushima Islands.

The scat analysis identifies detailed prey items and seasonal changes in food habit, however, by this method it is difficult to make clear quantitatively the ecological niche of the cat within the whole ecosystems. On the other hand, stable isotope analyses cannot identify the prey species, but, they can indicate the primary diets the animal depends on, and the animal's trophic level in the ecosystem.

Because the photosynthetic pathways differ chemically between C_3 and C_4 plants, they produce different degrees of isotopic fractionation : an average $\delta^{13}C$ value for the C_3 plants is about −26.5‰, while that for the C_4 species is about −12.5‰ (Ambrose & DeNiro 1986 ; Smith & Epstein 1971 ; Vogel 1978 ; O'Leary 1981 ; van del Merwe 1982 ; Koike & Chisholm 1996). C_3 plants include most of the temperate zone plants. C_4 plants are represented in about ten plant families and the majority of species are xeric environment grasses, including maize, some species of millets and sorghums, sugar cane and so on. Marine plants and plankton approximate the C_3 cycle but obtain their carbon from dissolved oceanic bicarbonates which have isotope ratios of about 0‰, differing from atmospheric CO_2 of about −7‰. Thus their values average about −19.5‰ (Brown *et al.* 1972 ; Degens *et al.* 1968 ; Deuser *et al.* 1968 ; Sackett *et al.* 1965 ; Chisholm & Koike 1996). Previous studies have used stable carbon $\delta^{13}C$ measurements to trace the relative contribution of marine versus terrestrial foods, or C_3 plants versus C_4 plants in diets of contemporary (Hobson 1986, 1991 ; Hobson & Sealy 1991 ; Hobson *et al.* 1997) and prehistoric consumers (Chisholm *et al.* 1982).

The ratio of stable isotopes can change between diet and consumer due to differential digestion or fractionation during assimilation and metabolic processes. Trophic enrichment of $\delta^{13}C$ is small, mostly about 1‰ (DeNiro & Epstein 1978 ; Fry & Sherr 1984), while that of $\delta^{15}N$ is usually positive about 3 to 4‰ (DeNiro & Epstein 1981 ; Minagawa & Wada 1984), probably because of the preferential excretion of the lighter ^{14}N in urinary waste products (Peterson and Fry 1987). Since the nitrogen isotope ratio is assumed to reflect the trophic position, it has been used as a food web indicator for animals in various ecosystems (Schoeninger & DeNiro 1986 ; Wada *et al.* 1987 ; Fry 1988 ; Hobson & Welch 1992).

The objectives of this study were 1) to determine the natural distribution of $\delta^{13}C$ and $\delta^{15}N$ values of flora at the base of food webs and of fauna as potential prey of the Tsushima leopard cat, 2) to establish representative values of $\delta^{13}C$ and $\delta^{15}N$ of each prey category, and 3) to estimate the trophic level of the cat.

The prey species of the cat have been identified by scat analyses and a few stomach content analyses (Asahi 1966 ; Okuhama 1970 ; Inoue 1972 ; Sukigara *et al.* 1988 ; Tatara & Doi 1994 ; Nakajima 1994 ; Takaesu 1999 ; Hiyama 2004 ; Environment Agency & Nagasaki Prefecture 1997). Prey species of the cat for the isotopic analysis were selected, based on these studies.

2. Materials and methods

A total of 213 samples consisting of 10 species of terrestrial plants, 62 species of potential prey and 3 species of domestic meat providers, and the cat were measured (Table 1). Except for pig and cattle meat, every sample was obtained in Tsushima.

Table 1 $\delta^{13}C$ and $\delta^{15}N$ values of the plants, prey species and the leopard cat.

	Family name	Scientific name	tissue	sample size	Mean±SD $\delta^{13}C$ (‰)	Mean±SD $\delta^{15}N$ (‰)
1. Plants	FAGACEAE	*Quercus acuta*	leave, nut	3	−26.5±1.6	0.6± 1.0
		Quercus glauca	leave, nut	3	−25.8±1.8	−0.2±1.0
		Quercus serrata	leave	2	−24.5±1.7	0.2±0.5
		Quercus variabilis	nut	1	−27.7	1.3
		Castanopsis cuspidata	nut	5	−25.5±1.4	0.4±1.1
		Lithocarpus edulis	nut	2	−27.8±1.4	1.9±1.3
	THEACEAE	*Camellia japonica*	leave, nut	6	−27.5±1.4	0.5±1.2
	POACEAE	*Miscanthus sinensis*	leave	5	−10.9±0.2	−1.2±0.9
		Setaria viridis	leave	4	−9.8±0.3	3.0±1.4
		Setaria italica	seed	1	−10.0	−0.6
2. Gastropod and annelid	(Snail)		muscle	4	−23.2±1.8	1.0±2.6
	(Earthworm)		bulk	4	−25.6±0.6	1.6±0.5

	Family name	Scientific name	tissue	sample size	Mean±SD $\delta^{13}C$ (‰)	Mean±SD $\delta^{15}N$ (‰)
3. Insects	Stenopelmatidae	*Atachycines* sp. *(A)*	bulk	3	−23.7±1.0	3.5±0.7
		Atachycines sp. *(B)*	bulk	1	−25.0	2.5
	Tettigoniidae	*Mecopoda nipponensis*	bulk	3	−28.2±0.7	3.2±0.6
		Paratlanticus sp.	bulk	2	−26.0±0.7	0.8±0.0
		Metrioptera sp.	bulk	1	−25.8	6.8
		Phaneroptera nigroantennata	bulk	1	−26.4	5.4
		Phaneroptera sp.	bulk	1	−24.9	1.4
	Gryllidae	*Teleogryllus emma?*	bulk	7	−22.8±1.2	5.4±0.5
	Acrididae	*Aiolopus* sp.	bulk	2	−9.7±0.5	2.1
		Locusta migratoria	bulk	1	−23.8	7.6
	Catantopidae	*Parapodisma* sp*?*	bulk	2	−27.4±0.9	0.2±0.8
	Cicadidae	*Graptopsaltria nigrofuscata*	bulk, muscle	3	−26.0±2.1	1.9
		Meimuna opalifera	bulk	2	−24.8±0.3	−1.4
		Oncotympana maculaticollis	bulk	1	−25.9	1.5
4. Fish	Monacanthidae	*Stephanolepis cirrhifer*	muscle	3	−15.2±0.3	11.2±1.3
	Zeidae	*Zeus faber*	muscle	2	−15.4±0.1	13.4±0.2
	Sebastidae	*Sebasticus marmoratus*	muscle	1	−13.5	12.8
	Labridae	*Halichoeres poecilopterus*	muscle	1	−12.5	12.8
	Cyprinidae	*Phoxinus oxycephalus jouyi*	muscle	3	−20.5±1.5	5.4±1.0
	Plecoglossidae	*Plecoglossus altivelis altivelis*	muscle	1	−19.2	5.1
5. Amphibians	Ranidae	*Rana dybowskii*	muscle	4	−23.5±1.1	4.3±1.3
		Rana tsushimensis	muscle	16	−22.8±0.8	3.9±1.0
	Hylidae	*Hyla japonica*	muscle	11	−23.4±0.3	5.4±0.7
	Hynobiidae	*Hynobius tsuensis*	muscle,skin	4	−22.3±0.9	3.9±0.7
6. Reptiles	Lacertidae	*Takydromus amurensis*	muscle	2	−24.0±0.7	2.9±0.3
	Gekkonidae	*Gekko japonicus*	muscle	1	−24.5	2.7
	Viperidae	*Gloydius tsushimaensis*	muscle	2	−23.2±0.1	6.3±0.4
	Colubridae	*Elaphe climacophora*	muscle,skin	2	−22.1±0.3	6.8±1.1
		Dinodon rufozonatus rufozonatus	muscle	2	−22.3±0.1	6.3±0.1
7. Birds	Phasianidae	*Phasianus colchicus*	feather	2	−21.8±0.2	4.9±0.1
		Bambusicola thoracica	feather	1	−22.9	6.4
	Muscicapidae	*Turdus pallidus*	feather	2	−21.9±1.7	4.8±0.9
		Turdus naumanni	feather	1	−22.2	7.2
		Zootheta dauma	feather	1	−20.7	3.4
		Turdus obscurus	feather	1	−22.5	7.8
		Cettia diphone cantans	feather	1	−22.6	3.9
		Cyanoptila cyanomelana	feather	1	−23.1	4.0
	Paridae	*Parus major*	feather	1	−23.6	4.1
	Corvidae	*Corvus frugilegus*	feather	1	−22.8	10.2
	Emberizidae	*Emberiza elegans*	feather	2	−24.6±1.3	3.9±0.9
	Zosteropidae	*Zosterops japonica japonica*	feather	1	−21.8	3.7
	Anatidae	*Anas crecca*	feather	1	−30.2	7.4
	Strigidae	*Otus scops japonicus*	feather	1	−22.8	4.9
		Asio flammeus flammeus	feather	1	−23.1	9.6
	Accipitridae	*Accipiter nisus*	feather	3	−20.3±0.5	6.5±1.1
	Ardeidae	*Ardea cinerea*	feather	1	−15.1	14.1
		Nycticorax nycticorax	feather	2	−24.6±4.7	13.0±0.1
		Ixobrychus sinensis	feather	1	−25.5	11.5
	Alcedinae	*Alcedo atthis*	feather	1	−19.4	7.9
	Scolopacidae	*Tringa hypoleucos*	feather	1	−16.1	9.9
	Laridae	*Larus crassirostris*	feather	1	−14.8	14.3
	Alcidae	*Synthliboramphus antiquus*	feather	2	−16.9±0.4	17.5±0.3
	Gavidae	*Gavia pacifica*	feather	2	−17.9±0.8	14.6±0.3

	Family name	Scientific name	tissue	sample size	Mean±SD $\delta^{13}C$ (‰)	Mean±SD $\delta^{15}N$ (‰)
8. Small mammals	Muridae	*Apodemus speciosus*	muscle, hair	15	−24.2±1.0	5.1±1.3
		Apodemus argenteus	muscle, hair	12	−23.0±0.9	3.6±0.8
		Micromys minutus	muscle, hair	8	−22.8±1.8	4.2±1.3
		Micromys minutus	muscle, hair	6	−13.0±2.5	5.1±0.8
		Rattus norvegicus	muscle, hair	3	−22.8±1.3	7.3±0.8
	Talpidae	*Urotrichus talpoidesa*	muscle	4	−22.7±0.8	6.9±1.3
		Mogera robusta	hair	1	−22.4	9.5
9. Carcasses and garbage	Cervidae	*Cervus nippon*	hair	2	−25.2±0.1	2.5±0.1
	Bovidae	*Bos taurus*	muscle	1	−14.6	6.4
	Suidae	*Sus scrofa domesticus*	muscle	1	−15.8	4.9
	Phasianidae	*Gallus gallus*	muscle	3	−16.6±0.3	4.3±0.4
10. Tsuhima leopard cat	Felidae	*Felis bengalensis euptilura*	muscle	25	−20.1±1.2	8.9±1.5

The samples of animals and plants, excluding nuts and feathers, were kept at −20°C until measured. Nuts and feathers were kept in dry conditions at room temperature. After thawing, muscles and leaves were cut into about 1×1 mm fragments using stainless steel scissors. Whole bulk samples of insects were powdered and cotyledons of dried nuts were ground using a mortar and pestle. Hair and feathers were cut into short fragments. Lipids were then removed from the samples with a chloroform-methanol (2 : 1) solution. Small pieces of each sample were placed in a beaker with 5−10 times their volume of the lipid extraction solution, and sonicated for 10 minutes. This lipid removal procedure was repeated at least three times until the solution became clear. The sample was then removed from the beaker, and placed on filter paper, and dried in a draft chamber. After air-drying, the sample was freeze-dried over night in an Eyela freeze drier (Tokyo-Rika, FDU506). The dried sample was ground in a ball mill before analysis. Hair and feather samples were cut into small pieces (<1mm) using stainless steel scissors. $\delta^{13}C$ and $\delta^{15}N$ values were measured twice using a continuous-flow isotope ratio mass spectrometer (ANCA-mass 20-20, Europa Scientific Instruments, UK) with isotope ratio pairs differing by more than 0.1% for $\delta^{13}C$ and 0.3% for $\delta^{15}N$ being analyzed again.

3. $\delta^{13}C$ and $\delta^{15}N$ values of plants

Seven species of C_3 plants (n=22) and three species of C_4 plants (n=10) were collected in Tsushima to establish representative $\delta^{13}C$ and $\delta^{15}N$ values of the primary producers in the terrestrial ecosystem of Tsushima. For C_3 plants, leaves and nuts of oaks *Quercus acuta, Q. glauca, Q. serrata* and *Q. variabilis,* chinquapins *Castanopsis cuspidate* and *Lithocarpus edulis,* and a camellia *Camellia japonica* were sampled. For C_4 plants, leaves or seeds of the grasses *Miscanthus sinensis, Setaria viridis and Setaria italica* were measured.

The $\delta^{13}C$ and $\delta^{15}N$ distributions of C_3 and C_4 plants (Fig. 2-A) showed a clear difference in average $\delta^{13}C$ values between C_3 plants (−26.5±1.3 ‰) and C_4 plants (−10.4±0.6 ‰).. Meanwhile, $\delta^{15}N$ values of C_3 plants overlapped with those of C_4 plants.

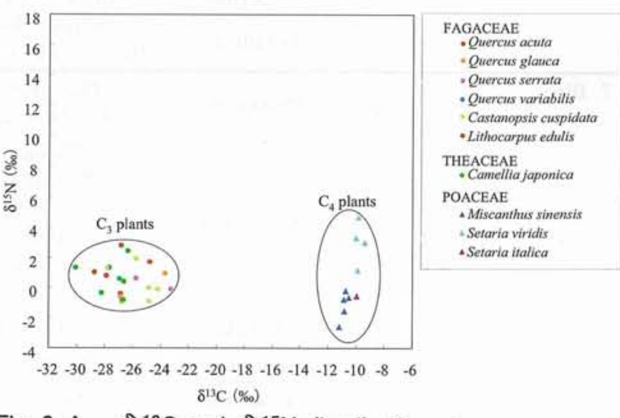

Fig. 2-A. δ 13C and δ 15N distribution of seven species of C3 plants and three species of C4 plants.

The $\delta^{13}C$ values for the C_3 and C_4 plants in this study coincided with other published $\delta^{13}C$ values for C_3 plants, of around −26.5 ‰ (ranging from −34 ‰ to −22 ‰) and for C_4 plants, of around −12.5‰ (ranging from −20 ‰ to −9 ‰) (Ambrose & DeNiro 1986 ; Smith & Epstein 1971 ; Vogel 1978 ; O'Leary 1981 ; van del Merwe 1982 ; Koike & Chisholm 1996). It is also widely accepted that the average values for $\delta^{13}C$ enrichment between the animal and their diet is around 1 ‰ (DeNiro & Epstein 1978 ; Fry & Sheer 1984), so it is possible to

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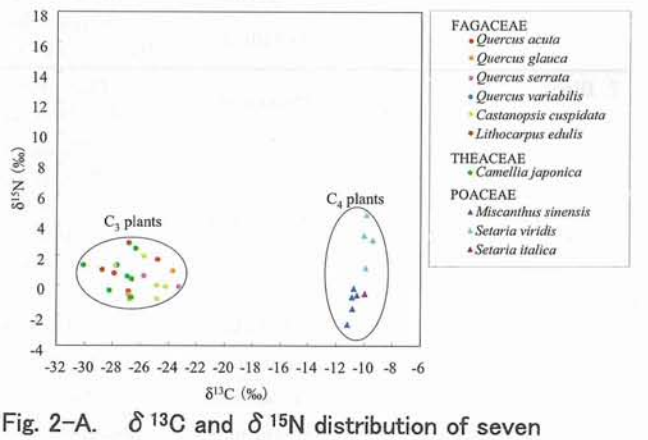

Fig. 2-A. δ 13C and δ 15N distribution of seven species of C3 plants and three species of C4 plants.

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$\delta^{13}C$ value of the *Aiolopus* sp. was different from that of other insects, suggesting that it depends on C_4 plants mainly, because the values overlapped the values for C_4 plants. $\delta^{15}N$ values for omnivorous were higher than those of herbivorous insects. It is considered that this difference in $\delta^{15}N$ values reflects the different trophic levels of their diets. The $\delta^{15}N$ enrichment between herbivorous insects and C_3 or C_4 plants is smaller than the expected value of 3 to 4‰, thus, the plants and insects might not have an active diet-consumer relationship. Similarly the reason why a herbivorous locusts *Locusta migratoria* gave a comparatively high $\delta^{15}N$ value (7.6‰) is unclear, but it may feed on grasses with higher $\delta^{15}N$ values or there may be some other metabolic effects in nitrogen assimilation..

Fish

The possibility of fish as part of the diet of the cat has been reported (Asahi 1966 ; Inoue 1972 ; Yamaguchi & Urata 1976 ; Hiyama 2004), but the cases identified to the species level are rare (Inoue 1972 ; Hiyama 2004). There is no evidence that the cat fed on marine fish in the scat analyses mentioned above. Only a few species of freshwater fish, *Leuciscus (Tribolodon) hakonensis, Morco steindachneri* and other small sized fish were found in the feces (Inoue 1972 ; Hiyama 2004). It is impossible for the cat to capture marine fish in the sea. However, the captive cat consumed marine fish (Yamaguchi & Urata 1976), and residents observed the cats wandering along the shore, especially in winter, and scavenging garbage dumps. This indicates that the cat can use marine fish found on the shore or in garbage as a food source.

Four species of marine fish (n=7) and two species of freshwater fish (n=4), common off and in Tsushima, were captured. These marine fish, filefish *Stephanolepis cirrhifer,* dories *Zeus faber,* scorpion fish *Sebasticus marmoratus* and rainbowfish *Halichoeres poecilopterus* are carnivorous and may be washed ashore. One freshwater fish, minnow *Phoxinus oxycephalus jouyi* is omnivorous while ayu *Plecoglossus altivelis altivelis* is herbivorous.

The $\delta^{13}C$ and $\delta^{15}N$ values of the six species of fish are shown in Fig. 2-D. Average $\delta^{13}C$ values for freshwater fish (−20.2±1.4‰) were more negative than that of marine fish (−15.2±0.3‰). While $\delta^{15}N$ values of marine carnivorous fish (12.5±0.9‰) were obviously higher than those of herbivorous and omnivorous freshwater fish (5.2±0.2‰).

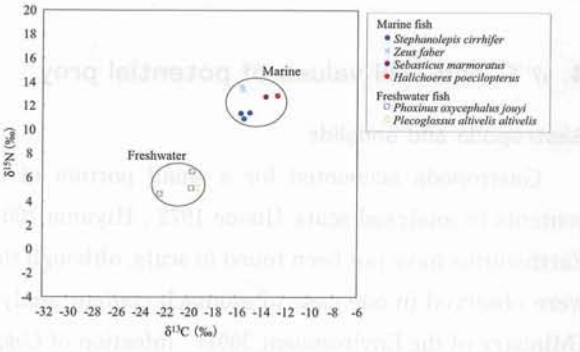

Fig. 2-D. δ 13C and δ 15N distribution of four species of marine fish and two species of freshwater fish.

The $\delta^{13}C$ values for marine fish in this study were within the range of typical marine fish, however, they ranged relatively lower than typical freshwater fish (Koike & Chisholm 1996). One of the causes of the lower $\delta^{13}C$ range for fresh water fish is assumed to be the effects of terrestrial insects in their diets. Meanwhile, $\delta^{15}N$ values for carnivorous marine fish were obviously higher than those for herbivorous and omnivorous freshwater fish, as expected.

Amphibia

Three species of frog, *Rana dybowskii, Rana tsushimensis* and *Hyla japonica,* inhabit Tsushima. All the three species were found in the feces of the cat (Hiyama 2004).

These three species of frog (n=31) and one species of salamander *Hynobius tsuensis* (n=4) were collected. While there is no evidence that the cat preys on the salamander, it is a potential food item.

The average $\delta^{13}C$ values for the three frogs and the

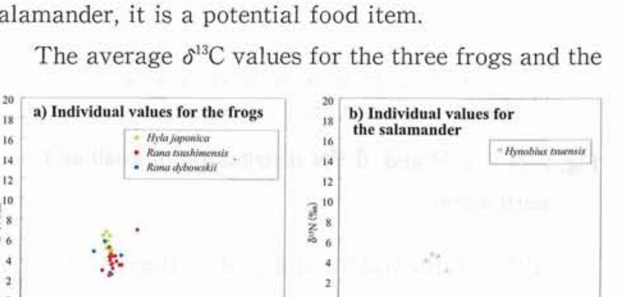

Fig. 2-E. δ 13C and δ 15N distribution of amphibians, three species of frogs and one species of salamander.

salamander were very similar (Fig 2-E). However, the average $\delta^{15}N$ value for *H. japonica* is significantly higher than that of other species (P=0.0002, Mann-Whitney's U-test)

These three species of frogs are carnivorous, feeding mainly on insects such as Pentatomidae (Inoue 1972), and the salamander is also a carnivore, feeding on insects and earthworms. The cause of the higher $\delta^{15}N$ values from *H. japonica* is uncertain, although they may reflect a difference in diet, for example in prey species and size.

Reptiles

Reptiles inhabiting Tsushima include three species of snake, *Gloydius tsushimaensis, elaphe climacophora* and *Dinodon rufozonatus rufozonatus,* two species of lizards, *Takydromus amurensis* and *Scincella reevesii vandenburghi,* and one species of gecko, *Gekko japonicus.* All the three snake species have been found in the cat feces (Inoue 1972 ; Hiyama 2004), however the identified cases of lizards and the gecko at the species level are rare, and only *T. amurensis* has been identified in the feces of the cat (Hiyama 2004), because identifications of the lizard and gecko to species levels are difficult as they rely on minute details.

The $\delta^{13}C$ value of the gecko, *G. japonicus* was −24.5 ‰ and the $\delta^{15}N$ value is 2.7 ‰ (Fig 2-F). $\delta^{13}C$ and $\delta^{15}N$ values for the lizard and the gecko overlapped. While, $\delta^{13}C$ and $\delta^{15}N$ values for snakes were almost 1.4 to 2 ‰ and 3.6 to 3.8 ‰ higher than those of the lizard and gecko, respectively. The lizard and gecko are secondary consumers, feeding mainly on insects, while the snakes are tertiary consumers, consuming mainly frogs, mice, lizards and birds. The $\delta^{13}C$ and $\delta^{15}N$ values of the snakes, the lizard and the gecko coincided with their trophic levels.

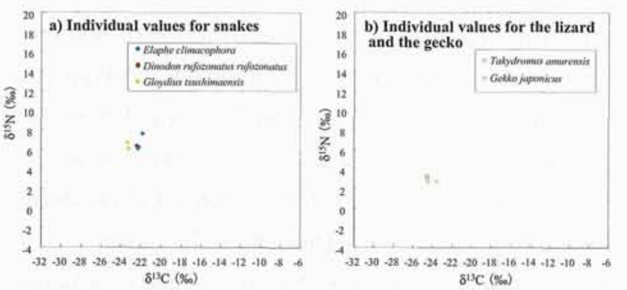

Fig. 2-F. δ 13C and δ 15N distribution of reptiles, three species of snakes, one species of the lizard and one species of gecko.

Birds

The feathers of birds are damaged in the feces of the cat, and rarely identifiable. A few cases of species identifications, of jay *Garrulus glandarius,* quail *Coturnix japonica,* gray heron *Ardea cinerea* and white-eye *Zosterops Japonica,* have been reported (Hiyama 2004). Inoue (1972) estimated the size of prey birds, and indicated that most of them were small (such as thrush *Turdus naumanni* and sparrow *Passer montanus*). Species identified by scat analysis, excluding the gray heron (Hiyama 2004) and most of the small sized birds inhabiting Tsushima are omnivores, suggesting that omnivorous birds are important as a dietary source for the cat.

In this study, representative species of birds inhabiting Tsushima were analyzed. Their feathers were sampled from bird carcasses collected in Tsushima and kept frozen at the Tsushima Wildlife Conservation Center. The species were classified according to Nakamura *et al.* (1995) into omnivorous and carnivorous terrestrial birds, and carnivorous marine / tidal birds. A number of feathers were collected from each of 13 species of terrestrial omnivorous birds (n=16), pheasant *Phasianus colchicus,* partridge *Bambusicola thoracica,* thrushs *Turdus pallidus, T. naumanni, T. obscurus, Zoothera dauma,* tit *Parus major,* warbler *Cettia diphone cantans,* flycatcher *Cyanoptila cyanomelana,* rook *Corvus frugilegus,* bunting *Emberiza elegans,* white-eye *Zosterops japonica,* teal *Anas crecca,* and seven species of terrestrial carnivorous birds (n=10), owl *Otus scops japonicus,* short-eared owl *Asio flammeus flammeus,* sparrow hawk *Accipiter nisus,* grey heron *Ardea cinerea,* night heron *Nycticorax nycticorax,* bittern *Ixobrychus sinensis,* kingfisher *Alcedo atthis,* and four species of marine carnivorous birds (n=6), sandpiper *Tringa hypoleucos,* gull *Larus crassirostris,* murrelet *Synthliboramphus antiquus* and loon *Gavia pacifica.*

The average $\delta^{13}C$ value for the four species of marine carnivorous birds (−16.8±1.2 ‰) was obviously higher than those for terrestrial carnivorous birds (−21.6±3.5 ‰) and terrestrial omnivorous birds (−23.1±2.2 ‰), excluding *A. cinerea.* (−15.1 ‰) which likely depends on marine food sources (Fig 2-G). Among the terrestrial birds analyzed, the average $\delta^{15}N$ value for carnivorous birds (9.6±3.4 ‰) was significantly higher than that for terrestrial omnivorous birds (p=0.0018, Mann-Whitney's U-test).

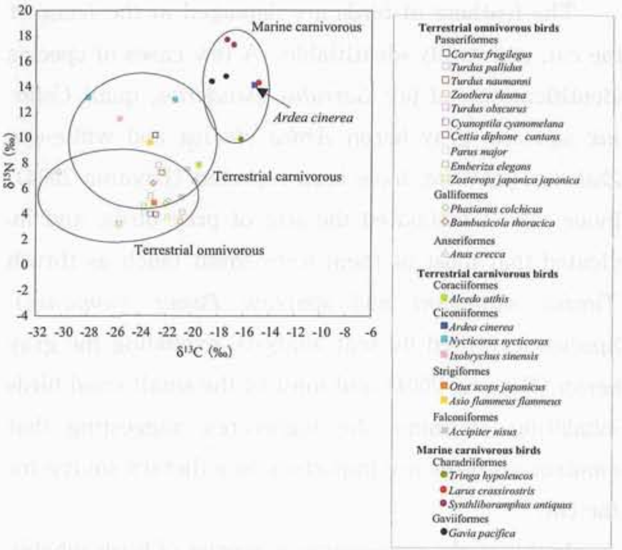

Fig. 2-G δ 13C and δ 15N distribution of 24 species of birds.

δ^{13}C values for seabirds, coastal or at sea, were higher than terrestrial birds. This result seems to reflect the fact that marine and tidal birds consume marine foods. *Ardea cinerea* with higher δ^{13}C values was collected at a coastal village, it likely depends on some marine foods. The general foods of omnivorous birds include seeds, fruit, leaves, insects and earthworms. Carnivorous birds consume small birds, mice, frogs, insects, and fish. On the whole, δ^{15}N values for terrestrial carnivorous birds inland and seabirds on the coasts or at sea were higher than those of terrestrial omnivorous birds. These δ^{15}N results indicate their trophic levels.

Small Mammals

Six species of rodent are found in Tsushima, including the field mice *Apodemus speciosus* and *A. argenteus,* harvest mouse *Micromys minutus,* house mouse *Mus musculus,* rats *Rattus rattus* and *R. norvegicus.* Three species of insectivore, white-toothed shrew *Crocidura suaveolens,* shrew-mole *Urotrichus talpoides* and mole *Mogera robusta,* are also found. All six rodents have been found in the feces of the cat, with two of the species, *A. speciosus* and *A. argenteus* being found in all the scat analysis studies (Asahi 1966 ; Inoue 1972 ; Nakajima 1994 ; Takaesu 1999 ; Hiyama 2000. Samples of all species, except *R. rattus, C. suaveolens,* and *M. musculus* were collected for analysis.

The δ^{13}C and δ^{15}N values for these mammals, including the four species of rodents and the two species of insectivores, are shown in Fig. 2-H. δ^{13}C values for 14 harvest mice *M. minutus* clearly clustered in two separate groups, a higher group (−13.0±2.5 ‰) all collected from the same grassland area, and a lower group (−22.8±1.8 ‰) collected from a wide variety of environments, grasslands, forests and arable land. The higher δ^{13}C values for harvest mice are likely caused by their consumption of C_4 plants, perhaps eulalia, as they were all captured in the same grassland area, dominated by eulalia.

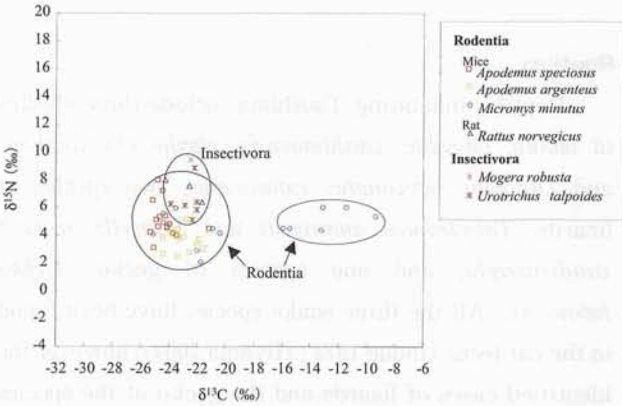

Fig. 2-H. δ 13C and δ 15N distribution of five species of rodents and two species of insectivores.

The average δ^{13}C value of the insectivores was −22.5±0.7 ‰ and their average δ^{15}N value was 6.9±0.8 ‰. The average δ^{15}N value for *R. norvegicus* was significantly higher than those for the three species of mice, *A. speciosus, A. argenteus* and *M. minutus* that also had lower δ^{13}C values ($p=0.0093$, Mann-Whitney's U-test). The average δ^{15}N value for the two species of insectivore fell between those for the rat and mice.

δ^{15}N values of insectivores and *R. norvegicus* were higher than those for mice. *M. robusta* and *U. talpoides* are mostly carnivorous, mainly consuming insects or earthworms. *R. norvegicus* is omnivorous but consumes a comparatively high amount of animal matter (ex ; Abe *et al.* 1994). The three species of mice consume seeds, fruit and insects (ex ; Abe *et al.* 1994), and their δ^{13}C and δ^{15}N distributions overlapped. Among the mice, the δ^{15}N value for *A. argenteus* was lower than that for *A. speciosus. A. argenteus* is omnivorous but consumes a comparatively high amount of plant matter (ex ; Abe *et al.* 1994). Thus the δ^{15}N values for the small mammals seem to reflect their degree of dependence on animal matter and hence their trophic levels.

Carcasses and garbage

It is well known that the cat attacks domestic fowl (Kuroda 1920 ; Yamaguchi & Urata 1976) and fed at garbage dumps in villages in mountainous areas in the past. A NGO has been feeding the cat with chicken since 1993 at Saozaki in the northern island.

Meat (n=5) of chicken, pig, and cattle and hair (n=2) of the deer captured in Tsushima were also measured. The number of deer *Cervus nippon* captured as part of the pest control program increased after 1990 in Tsushima and amounts to more than a thousand since 1994. Many deer carcasses have been left in the field, because the hunters capture the deer not for meat but mainly for subsidies. It has been suggested the cat uses deer meat as an important food source during the winter (Hiyama 2004).

The $\delta^{13}C$ and $\delta^{15}N$ distributions of deer flesh, edible meats and marine fish are shown in Fig. 2-I. $\delta^{13}C$ values for three species of domestic animals, chicken, cattle and pig were -16.6 ± 0.3 ‰, -14.6 ‰ and -15.8 ‰, respectively. Their $\delta^{15}N$ values were 4.3 ± 0.4 ‰, 6.4 ‰ and 4.9 ‰, respectively. Average $\delta^{13}C$ values for the domestic animals were close to those of the C_4 plants, and obviously higher than those of the deer. Average $\delta^{15}N$ values for the edible marine fish, or fish collected from the garbage were the highest among those for individuals of the collected carcasses and from garbage dumps.

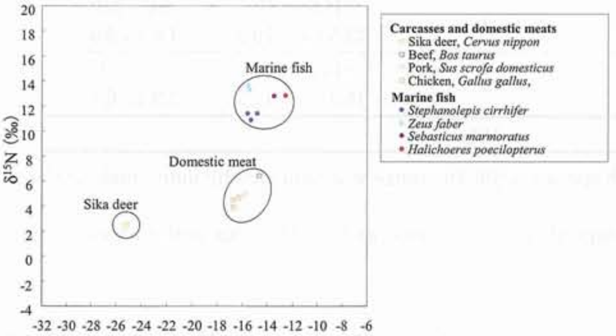

Fig. 2-I. $\delta^{13}C$ and $\delta^{15}N$ distribution of carcass and garbage, sika deer, marine fish and three kinds of domestic meat.

$\delta^{13}C$ values of hair from two deer were -25.3 ‰ and -25.1 ‰ and their $\delta^{15}N$ values were 2.4 ‰ and 2.6 ‰ (2.5 ± 0.1 ‰). The dominant food of the deer in Tsushima, determined from stomach content analysis, was tree leaves, while the portion of graminoids including C_4 plants was lower (Suda 1997). The $\delta^{13}C$ values indicated that the deer was a C_3 plant consumer and supported the previous stomach content analysis. The $\delta^{13}C$ values of each of the farm animals were higher. This was assumed to be the effect of cereals such as corn of C_4 plants being regularly fed to farm animals.

5. $\delta^{13}C$ and $\delta^{15}N$ values of the Tsushima leopard cat

Carcasses of the cat found in the field were immediately collected by the Tsushima Wildlife Conservation Center (TWCC), and a record of the collection date and location, sex, approximate age, external measurements, general body condition and so on, were completed on a prescribed form. The carcasses were then examined pathologically at Kagoshima University. After being examined, the carcasses, without their internal organs, were taken back and kept in a freezer at TWCC. Muscle tissue samples from the wild cats (n=25), that were not fed on chicken, were collected for analysis.

The $\delta^{13}C$ and $\delta^{15}N$ values of the cat, grouped according to four seasons, spring (March to May), summer (June to August), fall (September to November) and winter (December to February), are shown in Fig. 3. The $\delta^{13}C$ values of the cat averaged -20.1 ± 1.2 ‰, ranging from -22.3 to -17.8 ‰, and the $\delta^{15}N$ values averaged 8.9 ± 1.5 ‰, ranging from 7.2 to 12.9 ‰. $\delta^{15}N$ values in summer and fall were restricted to the lower range.

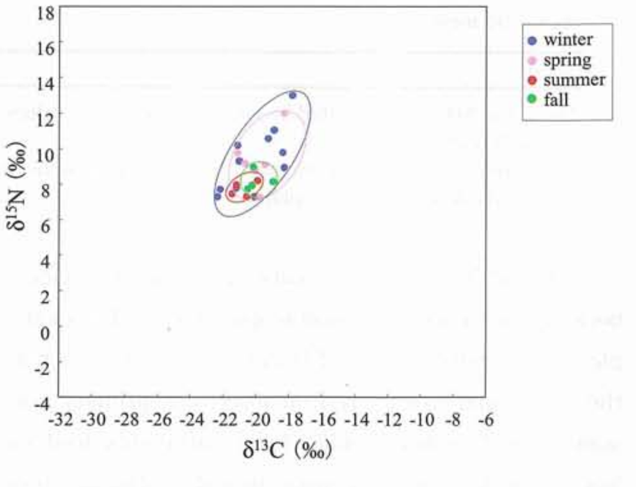

Fig. 3. $\delta^{13}C$ and $\delta^{15}N$ distribution of the Tsushima leopard cat, divided into four seasons.

6. Discussion

Natural distributions of $\delta^{13}C$ and $\delta^{15}N$ of flora, at the base of food webs, and fauna, as potential prey, are shown in Fig. 4. In this figure, the food habit of each taxon was classified as either herbivorous, omnivorous or carnivorous and indicated accordingly. Terrestrial prey with obviously high $\delta^{13}C$ values were assumed to depend completely or mostly on C_4 plants. There was a clear separation in $\delta^{13}C$ values between the terrestrial C_3 ecosystem and the marine ecosystem (Fig. 4). The slope of lines A and B is $\delta^{13}C$: $\delta^{15}N$ = 1 : 3.5 and is representative of the fractionation between the diet and consumer (DeNiro & Epstein 1978 ; Fry & Sherr 1984 ; Minagawa & Wada 1984, Koike 1985). Line A is set up to have more $\delta^{13}C$ than freshwater fish, $Y=3.5X+78$. Line B is set up to have lesser $\delta^{13}C$ than the signature of the marine bird, $Y=3.5X+73$. $\delta^{15}N$ values of herbivorous animals depending on C_3 plants, omnivorous animals and the carnivorous animals ranged from −2.4 to 7.6 ‰ (2.1±0.7 ‰), 0.6 to 10.2 ‰ (4.7±2.3 ‰) and 2.4 to 14.1 ‰ (6.9±3.3 ‰), respectively, and $\delta^{15}N$ values were enriched according to the trophic levels. The representative values for $\delta^{13}C$ and $\delta^{15}N$ for six categories are shown in Table 2.

Table 2 Representative values of $\delta^{13}C$ and $\delta^{15}N$ of plants and prey animals in Tsushima classified by trophic levels

	No. of species	No. of sample	$\delta^{13}C$ mean±SD range	$\delta^{15}N$ mean±SD range
C_3 plants	7	22	−26.5±1.3 −30.1 to −23.3	0.7±0.7 −1.0 to 2.7
C_4 plants	3	10	−10.4±0.6 −11.2 to −9.4	0.4±2.3 −2.6 to 4.6
herbivorous animals depending on C_3 plants	7	17	−24.9±1.1 −28.4 to −21.3	2.1±2.7 −2.4 to 7.6
omnivorous animals depending on C_3 plants	26 *	75	−24.0±2.2 −30.2 to −20.4	4.7±2.3 0.6 to 10.2
carnivorous animals depending on C_3 plants	19	59	−22.3±2.3 −27.9 to −15.1	6.9±3.3 2.4 to 14.1
herbivorous and omnivorous animals depending on C_4 plants	2 *	8	−11.4±2.3 −9.3 to −15.8	3.6±2.1 1.0 to 6.0
carnivorous marine animals	8	13	−15.3±1.7 −18.4 to −12.5	13.3±2.3 9.9 to 17.7
herbivorous and omnivorous animals raised on aquatic ecosystem	2	4	−19.9±0.9 −22.3 to −19.2	5.2±0.2 4.6 to 6.5
domestic meat	3	5	−15.7±1.0 −16.8 to −14.6	5.2±1.1 3.9 to 6.4
total	76	213		

Mean and SD was calculated by mean $\delta^{13}C$ and $\delta^{15}N$ values for each species, while the range was indicated by individual $\delta^{13}C$ and $\delta^{15}N$ values.

* *Micromys minutus* were included both in 'omnivorous animals depending on C_3 plants' and 'herbivorous and omnivorous animals depending on C_4 plants'.

The $\delta^{15}N$ values for carnivores ranged widely because they consume plural trophic levels. For example, carnivorous animals of lower trophic level, such as the gecko and lizard, feed on insects as primary consumers, and higher trophic level carnivores feed on lower trophic level carnivorous animals and omnivorous animals. So it will be possible to compare the trophic levels within carnivorous animals. Meanwhile, the categories with higher $\delta^{13}C$ values include marine matter and food webs depending on C_4 plants. It is not possible to decide whether the diet of an animal is derived from marine or C_4 plants by $\delta^{13}C$ values. The zone between lines A and B in Fig. 4 can possibly include both terrestrial and marine food webs, and food webs depending on C_4 plants. In conclusion, these categories of $\delta^{13}C$ and $\delta^{15}N$ distributions will make it possible to decide and roughly compare the trophic levels and dietary sources of consumers in Tsushima, however, it may be difficult to distinguish marine matter from prey depending on C_4 plants.

As shown in Fig. 4, the averaged $\delta^{15}N$ values of the cat ranged within the 'carnivore' zone. In the previous scat analyses the food habit of the cat was of a carnivore feeding on rodents, birds and insects and so on (Inoue 1972 ; Sukigara *et al.* 1988 ; Tatara & Doi 1994 ; Nakajima 1994 ; Takaesu 1999 ; Maeda 2001 ; Hiyama 2004). The result of $\delta^{13}C$ and $\delta^{15}N$ measurements supported this interpretation. The averaged $\delta^{13}C$ values were near line A. It was supposed that the cat is at a high trophic levels, and fed on prey from various origins, depending on either the marine ecosystem or C_4 plants. The isotopic turnover rate is specific to each tissue, and $\delta^{13}C$ and $\delta^{15}N$ values of each tissue indicate different periods of dietary intake (Tieszen *et al.* 1983 ; Hobson & Clark 1992). $\delta^{13}C$ and $\delta^{15}N$ values of muscle reflect the consumers food habit for several weeks before sampling (Tieszen *et al.* 1983). In this study, seasonal changes in $\delta^{13}C$ and $\delta^{15}N$ values were indicated, and may reflect the seasonal changes in their food habit.

Acknowledgments

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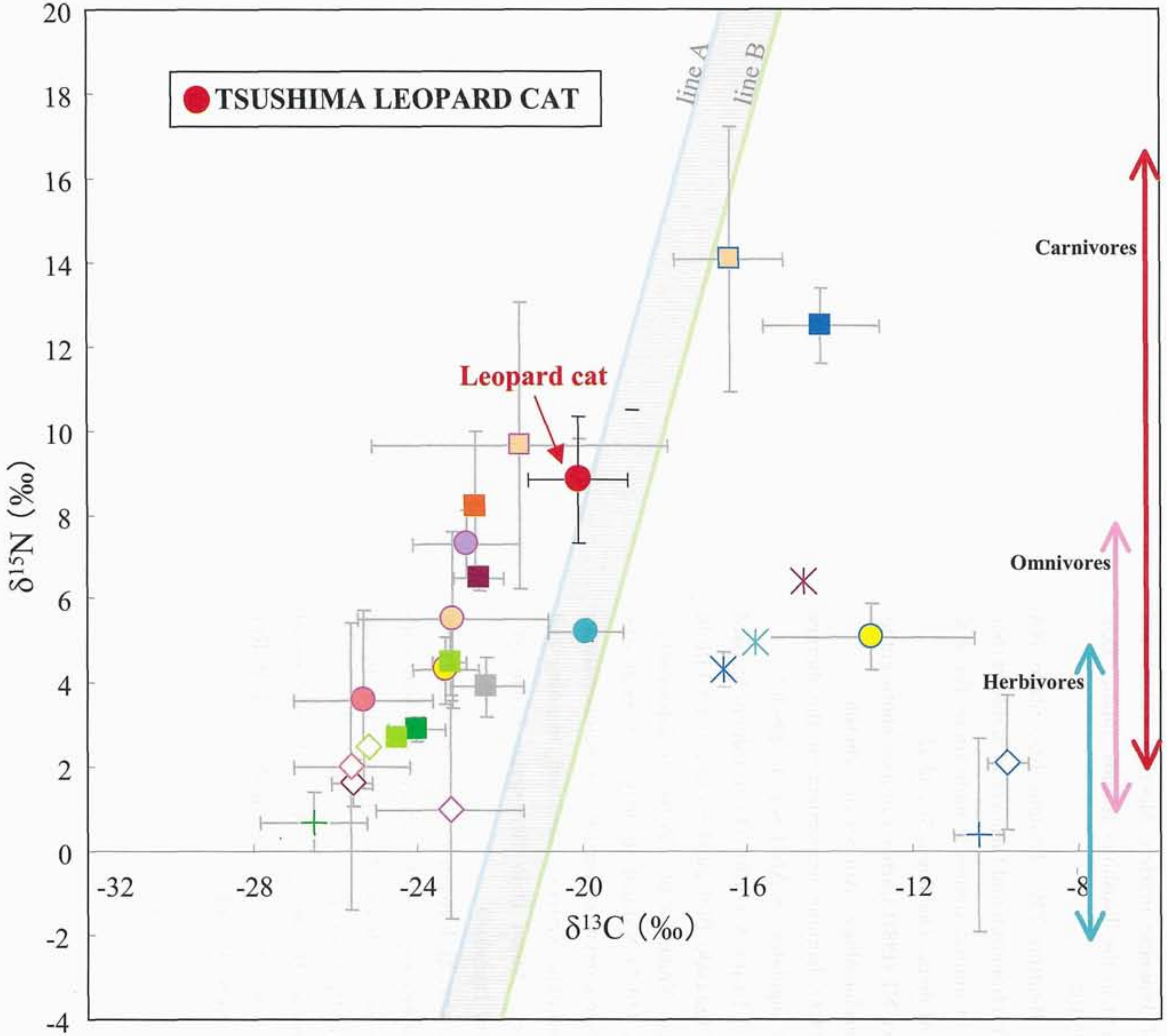

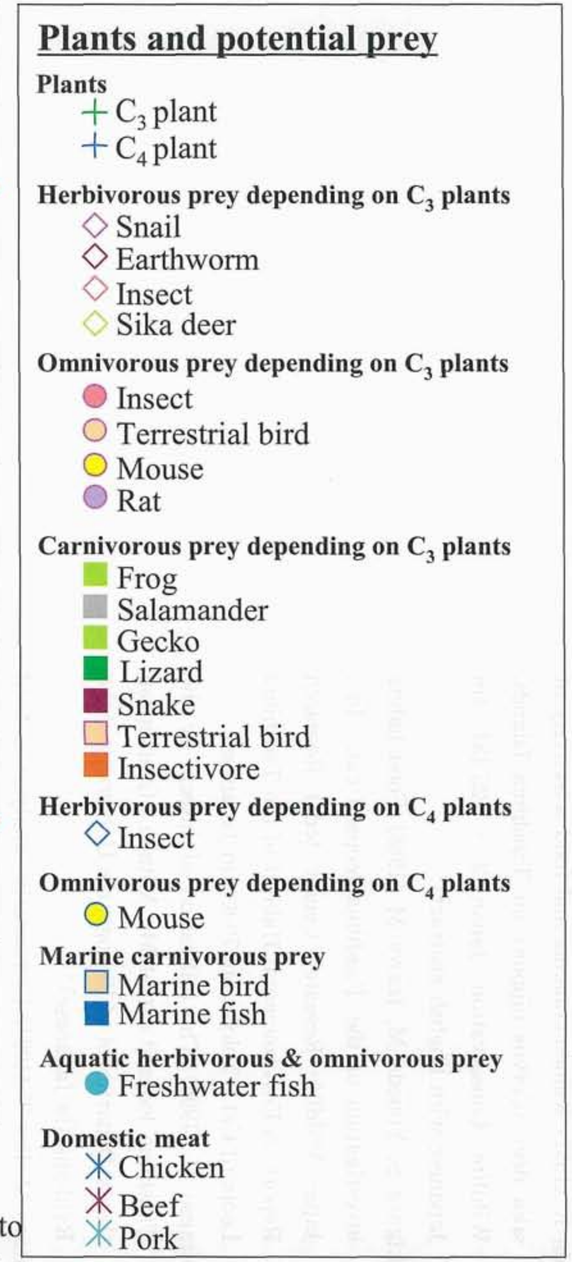

Fig. 4. $\delta^{13}C$ and $\delta^{15}N$ distributions of plants, potential prey and the leopard cat in Tsushima.

Values indicated are means ±SD. The inclinations of line A and B are $\delta^{13}C$: $\delta^{15}N = 1 : 3.5$ according to the average fractionation of trophic shift between the diet and the consumer. Line A is set up to have more $\delta^{13}C$ than freshwater fish, $Y=3.5X+78$. Line B is set up to have lesser $\delta^{13}C$ than the signature of the marine bird, $Y=3.5X+73$.