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Geographical variations in carbon and nitrogen stable isotope ratios in squid

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The magnitude of geographical variations of stable isotope ratios of carbon and nitrogen was examined in ten squid species captured in seven areas of the world's oceans. The average of all 139 squid was −17.4 ±1.4‰ for $\delta^{13}C$ and 11.6 ±1.6‰ for $\delta^{15}N$. The $\delta^{13}C$ of squid showed a clear-cut negative correlation with the latitude of the sampling locations, reflecting the well-known latitudinal characteristics of phytoplankton $\delta^{13}C$, while squid $\delta^{15}N$ did not correlate with the latitude significantly. The $\delta^{15}N$ reflected the regional characteristics of nitrogen metabolism such as denitrification and N_2 fixation, and it was conspicuous in the intraspecific variation in *Sthenoteuthis oualaniensis* ranging from 10.0 ±1.5‰ off Japan to 16.3 ±0.6‰ off Peru in the Pacific Ocean. Significant isotopic variations were also found in the local area among the five species off Japan in the Pacific Ocean and among the three species in the Japan Sea. Attention must be paid to the isotopic variations of primary producers as well as those of squid in examining the trophic positions of squid.

INTRODUCTION

Squid are eaten by many marine animals including toothed whales, dolphins and porpoises, seals, birds such as albatrosses and penguins, and fish such as tunas and sharks (Clarke, 1987), while they are themselves active carnivores feeding mainly upon Crustacea, molluscs and fish at all stages (Nixon, 1987). Squid can thus be regarded as an important species in marine ecosystems everywhere. However, the trophic relationships of cephalopods are extremely complex (Morejohn et al., 1978) and thus it is difficult to examine its trophic position by only conventional methods such as stomach content analyses. As a new powerful method, stable isotope analysis of carbon and nitrogen has been recently used to exhibit the importance of squid as prey of cetaceans (Ostrom et al., 1993) and sea birds (Gould et al., 1997).

The analysis of $\delta^{13}C$ and $\delta^{15}N$ of consumer and prey tissues is a valuable method to examine the trophic relationship and transport of organic matters along a food chain in aquatic and terrestrial ecosystems (e.g. Wada et al., 1987). A significant increase in $\delta^{15}N$ of 3.4 ±1.1‰ has been reported during the single feeding process irrespective of invertebrate and vertebrate (Minagawa & Wada, 1984), while a small enrichment of about 1‰ was found for $\delta^{13}C$ (DeNiro & Epstein, 1978). Consequently, animal $\delta^{15}N$ is a possible indicator of its trophic level and preys, and animal $\delta^{13}C$ can be used to identify a primary producer at the base of a food chain. The $\delta^{13}C$–$\delta^{15}N$ map in an ecosystem can thus show an isotopic food web structure on a corresponding food base.

However, the stable isotope ratios of wildlife often have wide intraspecific and/or interspecific variations in a similar trophic position (Takai & Sakamoto, 1999). Such variations make it difficult to interpret the analysed isotopic values of the animals ecologically. Accordingly, it is necessary to understand the magnitude of geographical variations of stable isotope ratios in squid as a fundamental step before determining the trophic position of squid with this method. In this study, $\delta^{13}C$ and $\delta^{15}N$ of ten squid species captured in seven areas of the world's oceans were measured to evaluate the magnitude of the isotopic variations and to infer determinant factors of the geographical variations.

MATERIALS AND METHODS

Ten squid species were captured in the seven areas from 8 February 1990 to 23 May 1998 (Table 1, Figure 1), *Todarodes pacificus* Steenstrup, 1880 being a major part of the samples. A total of 139 mature squid samples, 47 males and 92 females, were captured by jigging. The reference data of stable isotope ratios of squids are shown in Table 2.

The samples were kept at −20°C. The dorsal mantle length (DML) was measured and then the muscles were excised from the mantle. The muscle tissues were dried, ground to a fine powder, and lipids were removed with a chloroform:methanol (2:1) solution. Stable isotope ratios of carbon and nitrogen were measured by Delta-S mass spectrometry (Finnigan Mat, Germany) after the manual

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Table 1. *The specific names, sampling date and location, DML (mm), and stable isotope ratios (‰) of carbon and nitrogen in the squid.*

Group	N (M:F)	DML (mean ±SD)	δ^{13}C (mean ±SD) (max–min)	δ^{15}N (mean ±SD) (max–min)	Symbol in figures
Pacific Ocean (off Japan)					
OMMASTREPHIDAE					
Sthenoteuthis oualaniensis	5		−16.2 ±0.3	10.0 ±1.5	a
26 Nov.1997 26°30′N, 144°00′E	(0:5)	217 ±61	(−15.8– −16.6)	(12.3–8.3)	
Ommastrephes bartramii	5		−17.5 ±0.2	12.1 ±0.3	b
10 May 1998 39°30′N, 155°00′E	(0:5)	360 ±19	(−17.3– −17.7)	(12.5–11.8)	
Eucleoteuthis luminosa	3		−17.8 ±0.3	11.2 ±11.1	c
23 May 1998 35°00′N, 175°30′E	(1:2)	201 ±20	(−17.5– −18.0)	(12.5–10.5)	
GONATIDAE					
Gonatopsis borealis	5		−17.8 ±0.2	13.1 ±0.2	d
10 May 1998 41°00′N, 155°00′N	(3:2)	224 ±15	(−17.5– −18.1)	(13.4–12.9)	
ONYCHOTEUTHIDAE					
Onychoteuthis borealijaponica	5		−18.0 ±0.4	12.2 ±0.3	e
10 May 1998 41°00′N, 155°00′E	(0:5)	335 ±23	(−17.6– −18.6)	(12.7–11.9)	
Pacific Ocean (off Peru)					
OMMASTREPHIDAE					
Sthenoteuthis oualaniensis	5		−15.6 ±0.3	16.3 ±0.6	f
31 Aug. 1995 14°00′S, 85°00′W	(0:5)	220 ±24	(−15.1– −15.9)	(16.8–15.3)	
The Japan Sea					
OMMASTREPHIDAE					
Todarodes pacificus	70		−18.6 ±0.5	10.5 ±0.4	g
24 Jun.–14 Jul. 1997					
38°10′N–42°00′N 134°00′E–139°00′E	(35:35)	207 ±7	(−17.8– −19.9)	(11.5–9.6)	
LOLIGINIDAE					
Loligo bleekeri	5		−16.6 ±0.5	11.7 ±0.6	h
13 Feb. 1996 34°54′N, 132°04′E	(0:5)	250 ±17	(−15.9– −17.1)	(12.5–11.0)	
SEPIOLIDAE					
Rossia pacifica	5		−16.0 ±0.2	12.0 ±0.5	i
21 Mar. 1997 35°34′N, 135°28′E	(1:4)	65 ±9	(−15.8– −16.2)	(12.7–11.4)	
Indian Ocean (off Australia)					
OMMASTREPHIDAE					
Sthenoteuthis oualaniensis	5		−16.1 ±0.2	11.8 ±0.3	j
3 Jan. 1991 17°59′S, 115°01′E	(5:0)	136 ±5	(−15.9– −16.3)	(12.1–11.4)	
The central Arabian Sea					
OMMASTREPHIDAE					
Sthenoteuthis oualaniensis	14		−15.4 ±0.2	13.8 ±0.7	k
10 Jun. 1996 12°00′N, 64°00′E	(1:13)	171 ±39	(−15.2– −15.8)	(14.6–11.8)	
Atlantic Ocean (off Namibia)					
OMMASTREPHIDAE					
Todarodes angolensis	5		−14.1 ±0.3	13.4 ±0.0	l
8 Feb. 1990 27°48′S, 14°30′E	(0:5)	227 ±14	(−13.8– −14.5)	(13.5–13.4)	
Atlantic Ocean (off Argentine)					
OMMASTREPHIDAE					
Martialia hyadesi	7		−16.5 ±0.2	11.8 ±0.2	m
21 Mar. 1993 47°15′S, 54°25′W	(1:6)	233 ±10	(−16.3– −16.8)	(12.0–11.4)	

N, number of samples; M, male; F, female; SD, standard deviation.

cryopurification of combustion products in a vacuum system (Minagawa et al., 1984), or with a continuous-flow isotope ratio mass spectrometry (CF-IRMS) coupled with element analyser (Carlo Erba, Italy). Isotope ratios, δ^{13}C and δ^{15}N, are expressed as per mil deviations from the standard as defined by the following equation:

$$\delta^{13}\mathrm{C},\ \delta^{15}\mathrm{N} = \left(\frac{R_{(\mathrm{sample})}}{R_{(\mathrm{standard})}} - 1\right) \times 1000 \qquad (1)$$

where $R={}^{13}\mathrm{C}/{}^{12}\mathrm{C}$ or ${}^{15}\mathrm{N}/{}^{14}\mathrm{N}$.

Belemnite (PDB) and atmospheric nitrogen were used as the isotope standards of carbon and nitrogen, respectively. The analytical precision for the isotopic analyses was 0.10‰ for both isotopes by the method of Minagawa et al. (1984), and 0.21‰ for δ^{13}C and 0.25‰ for δ^{15}N by the method using CF-IRMS.

RESULTS

δ^{13}C and δ^{15}N of squid

The average δ^{13}C for all 139 squid was −17.4 ±1.4‰ and that for δ^{15}N was 11.6 ±1.6‰. The δ^{13}C ranged from

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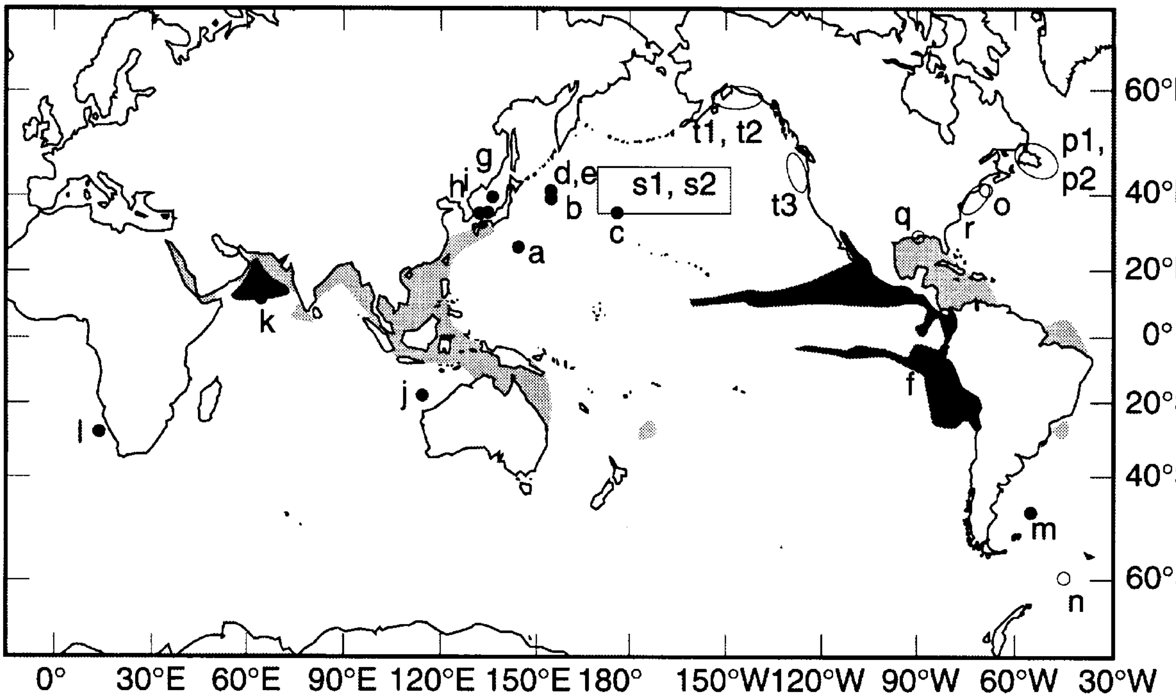

Figure 1. The sampling locations of squid analysed in this study (solid circle; a, f, j, and k, *Sthenoteuthis oualaniensis*; b, *Ommastrephes bartramii*; c, *Eucleoteuthis luminosa*; d, *Gonatopsis borealis*; e, *Onychoteuthis borealijaponica*; g, *Todarodes pacificus*; h, *Loligo bleekeri*; i, *Rossia pacifica*; l, *Todarodes angolensis*; m, *Martialia hyadesi*) and squid analysed in the literature (open circle and square; n, *Kondakovia longimama*; o, the specific name not described; p1 and p2, *Illex illecebrosus*; q, *Lolliguncula brevis*; r, *Loligo pealei*; s1, *O. bartramii*; s2, the miscellaneous squid including *Berryteuthis anonychus*, *Octopoteuthis deletron*, *Histioteuthis dofleini*, and *Taonius pavo*; t1–t3, the specific name not described). The sampling data and the references are listed in Table 1 for a–m and Table 2 for n–t3. The shaded areas show the denitrification areas (dark shade) and the N_2 fixation areas (light shade) after Wada (1988).

−19.9‰ of *Todarodes pacificus* in the Japan Sea to −13.8‰ of *Todarodes angolensis* Adam, 1962 off Namibia in the Atlantic Ocean and the δ^{15}N ranged from 8.3‰ of *Sthenoteuthis oualaniensis* Lesson, 1831 off Japan in the northern Pacific Ocean to 16.8‰ of *S. oualaniensis* off Peru in the southern Pacific Ocean (Table 1). A clear-cut negative correlation was observed between δ^{13}C in squid and the latitude of the sampling location (Spearman's correlation coefficient, $\rho = -0.71$, $P < 0.05$), while no such correlation was observed in δ^{15}N value (Spearman's correlation coefficient, $\rho = -0.22$, ns) (Figure 2).

Neither δ^{13}C nor δ^{15}N showed a marked difference with sex in any species, and there was no significant difference between sexes in *T. pacificus* in the Japan Sea. Accordingly, the sex was not considered in the following analyses.

The intraspecific geographical variation in Sthenoteuthis oualaniensis

Sthenoteuthis oualaniensis was captured in the four areas: off Japan in the northern Pacific Ocean, the Arabian Sea, off Peru in the southern Pacific Ocean, and off Australia in the Indian Ocean (Figure 1). Its geographical variation was conspicuous in δ^{15}N (Figure 3). The average values of δ^{15}N ranged from 10.0 ±1.5‰ (off Japan) to 16.3 ±0.6‰ (off Peru) and those of δ^{13}C from −16.2 ±0.3‰ (off Japan) to −15.4 ±0.2‰ (the Arabian Sea). The difference of the averaged δ^{15}N between two groups in every combination of the four groups was over 1.8‰. Significant difference was shown in both δ^{13}C and δ^{15}N among the four groups (Kruskal–Wallis test; δ^{13}C, P=0.0005; δ^{15}N, $P < 0.0001$).

Variation of squid $\delta^{13}C$ and $\delta^{15}N$ in the local area

Off Japan in the Pacific Ocean, five species, *S. oualaniensis*, *Ommastrephes bartramii* Lesueur, 1821, *Eucleoteuthis luminosa* Sasaki, 1915, *Gonatopsis borealis* Sasaki, 1920, and *Onychoteuthis borealijaponica* Okada, 1927, were captured at four different sampling stations (Table 1, Figure 1). The average δ^{13}C ranged from −18.0 ±0.4‰ in *O. borealijaponica* to −16.2 ±0.3‰ in *S. oualaniensis*, and that of δ^{15}N from 10.0 ±1.5‰ in *S. oualaniensis* to 13.1 ±0.2‰ in *G. borealis*. There was significant difference among the five species in both δ^{13}C and δ^{15}N (Kruskal–Wallis test; δ^{13}C, $P < 0.005$; δ^{15}N, $P < 0.005$).

In the Japan Sea, on the other hand, three species including *T. pacificus*, *Loligo bleekeri* Kerforstein, 1866, and *Rossia pacifica* Berry, 1911 were captured at three different sampling stations (Table 1, Figure 1). The average of δ^{13}C ranged from −18.6 ±0.5‰ in *T. pacificus* to −16.0 ±0.2‰ in *R. pacifica*, and that of δ^{15}N from 10.5 ±0.4‰ in *T. pacificus* to 12.0 ±0.5‰ in *R. pacifica*. There was significant difference among the three species in both δ^{13}C and δ^{15}N (Kruskal–Wallis test; δ^{13}C, $P < 0.0001$; δ^{15}N, $P < 0.0001$).

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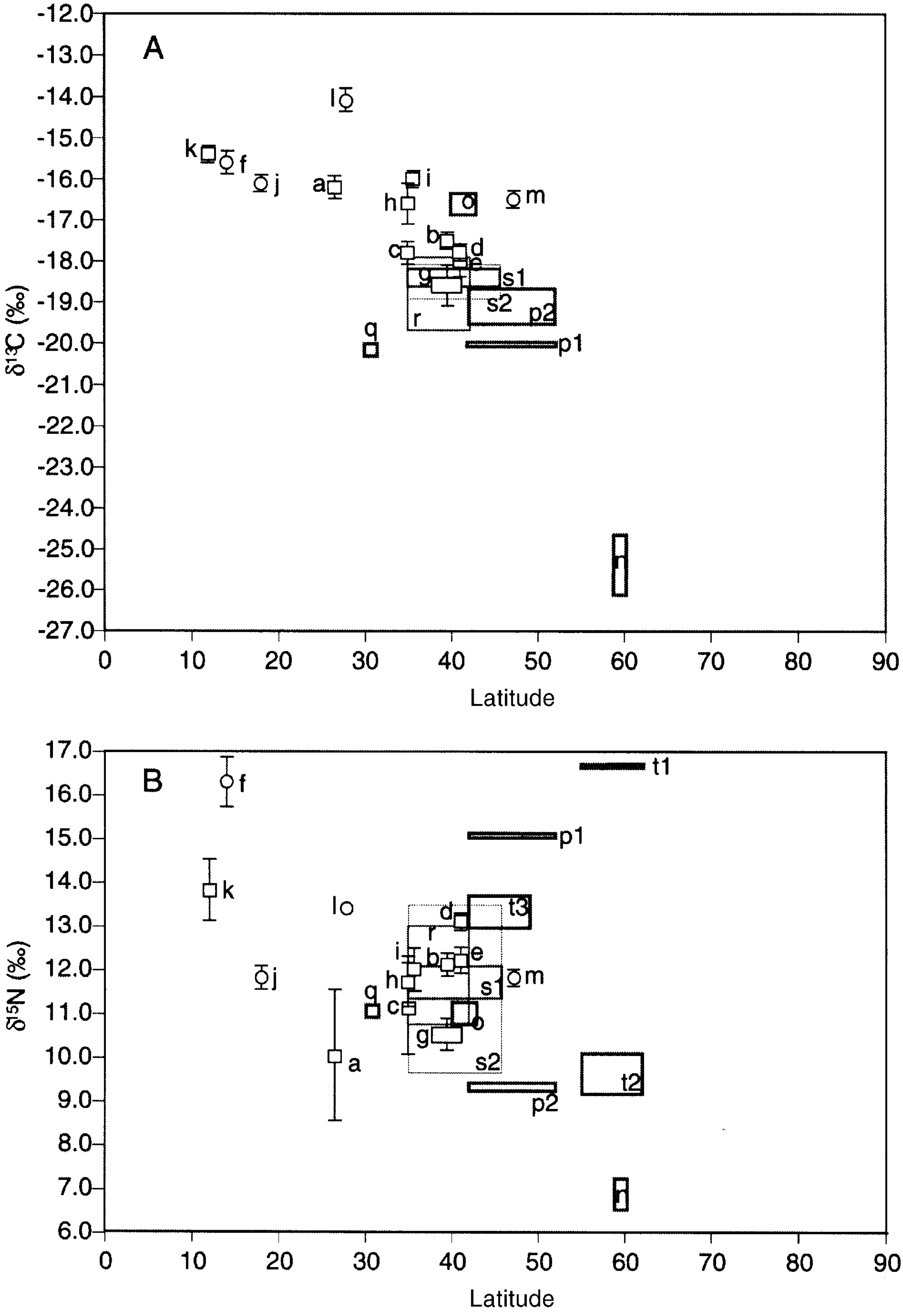

Figure 2. The relationship between the latitude of the sampling locations in the northern (open square) and the southern (open circle) hemispheres and the stable isotope ratios of squid. (A) $\delta^{13}C$, (B) $\delta^{15}N$. The values for the squid analysed in this study (a–m) are shown as mean values ±SD. The reference data (shaded frame squares) are shown as mean values ±SD for n–r, mean ±2SE for s1 and s2, and mean ±SE for t1–t3 according to the descriptions of the original papers. The specific names and the references are listed in Tables 1 & 2.

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Table 2. *The reference data of stable isotope ratios (‰) of carbon and nitrogen in the squids.*

References	N	$\delta^{13}C$	$\delta^{15}N$	Symbol in figures
Wada et al. (1987), the Antarctic (59°34′S–59°57′S, 43°16′W–44°30′W)				
Kondakovia longimama (ONYCHOTEUTHIDAE)	3	−25.4 ±0.8 (m ±SD)	6.9 ±0.4 (m ±SD)	n
Fry (1998), Georges Bank				
squid*1	2	−16.6 ±0.3 (m ±SD)	11.0 ±0.3 (m ±SD)	o
Ostrom et al. (1993), the western North Atlantic in the vicinity of Newfoundland				
Illex illecebrosus (OMMASTREPHIDAE)				
large (24.5 cm), nearshore	2	−20.0	15.1	p1
small (14.5 ±1 cm), offshore, Grand Banks	4	−19.1 ±0.4 (m ±SD)	9.3 ±0.1 (m ±SD)	p2
Sullivan & Moncreiff (1990), Graveline Bay Marsh (30°21′26″N, 88°40′59″E)				
Lolliguncula brevis (LOLIGINIDAE)	2	−20.2	11.1	q
Abend & Smith (1997), Mid-Atlantic Bight to Cape Cod				
Loligo pealei (LOLIGINIDAE)	9	−18.8 ±0.9 (m ±SD)	11.9 ±1.1 (m ±SD)	r
Gould et al. (1997), the North Pacific Ocean (35°N–46°N, 170°E–148°W)				
Ommastrephes bartrami (OMMASTREPHIDAE)	44	−18.4 ±0.2 (m ±2SE)	11.7 ±0.4 (m ±2SE)	s1
miscellaneous squid*2	5	−18.5 ±0.4 (m ±2SE)	11.6 ±1.9 (m ±2SE)	s2
Hobson et al. (1997)				
Gulf of Alaska, large-sized squid*1	1	–	16.7	t1
Gulf of Alaska, small-sized squid*1	4	–	9.6 ±0.5 (m ±SE)	t2
along Washington–Oregon, squid*1	2	–	13.3 ±0.4 (m ±SE)	t3

N, number of samples; m, mean; SD, standard deviation; SE, standard error; *1, the specific name not described; *2, *Berryteuthis anonychus*, *Octopoteuthis*

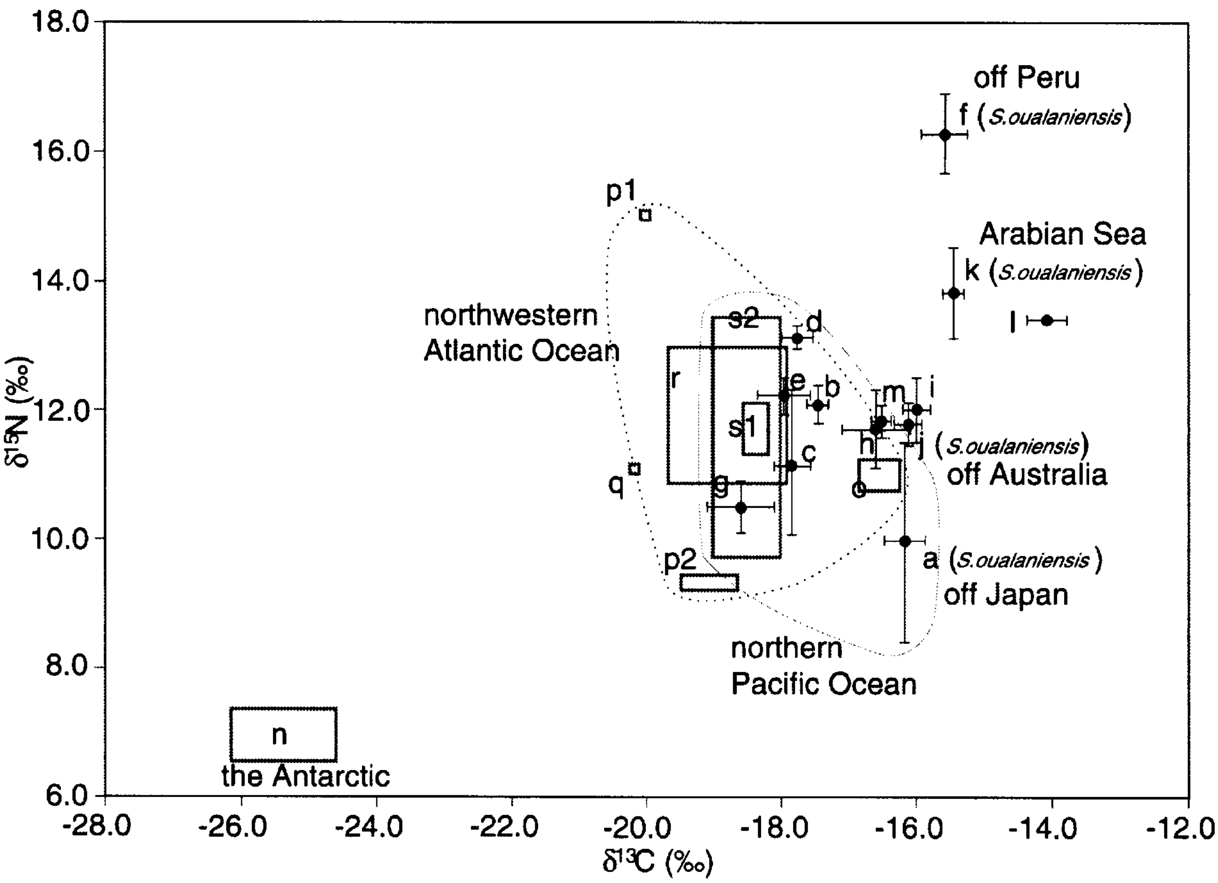

Figure 3. The $\delta^{13}C$–$\delta^{15}N$ map of the squids. The data for the squid analysed in this study (a–m) are shown as mean values ±SD with the solid circles and bars. The reference data (squares) are shown as mean values ±SD for n–r, mean ±SE for s1 and s2, and mean ±SE for t1–t3 according to the descriptions of the original papers. The specific names and the references are listed in Tables 1 & 2.

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DISCUSSION

Variation of squid $\delta^{13}C$ and $\delta^{15}N$ in the world's ocean

The lowest $\delta^{13}C$ (-26.1 to -24.5‰) and $\delta^{15}N$ (6.5 to 7.2‰) of squid were reported in *Kondakovia longimama* Filippova, 1972 captured in the Antarctic (Table 2; Wada et al., 1987), and therefore its maximum difference in the world's oceans was 12.3‰ in $\delta^{13}C$ and 10.3‰ in $\delta^{15}N$. The literary data reinforced the latitude–$\delta^{13}C$ relationship of squid (Figure 2A). Marine phytoplankton $\delta^{13}C$ decreases from equatorial areas toward the pole in the both hemispheres with the decreasing rate of -0.015‰ per 1° in the northern hemisphere and -0.14‰ per 1° in the southern hemisphere (Sackett et al., 1965; Rau et al., 1982). This phenomenon has been explained by mainly water temperature (Fontugne & Duplessy, 1981) and ambient CO_2 concentration in water (Rau et al., 1989), whereas it has recently been shown that the growth rate of phytoplankton affects its carbon isotope discrimination (Laws et al., 1995). It was considered that these multiple factors would cause the latitudinal variation of the phytoplankton $\delta^{13}C$ and consequently be reflected in the squid $\delta^{13}C$.

The latitudinal isotopic variation of phytoplankton has been suggested for also $\delta^{15}N$ as summarized by Wada & Hattori (1991). In higher latitudes and in upwelling regions, nitrate is transported from deeper layers to the euphotic layer by vertical water mixing. The nitrate uptake by natural populations of phytoplankton increases with increasing nitrate concentration in the euphotic layer, but it is saturated at nitrate concentrations higher than 10 μg atoms N l^{-1}. The limiting factor of the primary production may be light intensity in the areas. In the nitrate-rich waters off Kuril Islands (44°N 154°E), significant isotope fractionation of 1.005 was found for nitrate assimilation by marine diatoms. Consequently, low $\delta^{15}N$ values (1–3‰) were observed for phytoplankton collected in the boreal area. On the other hand, the main source of nitrogen for primary production is regenerated ammonium in the tropical and subtropical areas. The primary production is probably limited by nitrogen, and all forms of inorganic nitrogen are taken up and regenerated by organisms within a short period of time. Under such circumstances, inorganic nitrogenous compounds would be used up quickly without occurrence of significant isotope fractionation. The $\delta^{15}N$ value of phytoplankton is therefore close to the average $\delta^{15}N$ value of oceanic nitrate (6–7‰). Reflecting these characteristics, phytoplankton $\delta^{15}N$ often becomes lower at high latitudes than at low latitudes as well as $\delta^{13}C$.

However, there was not a significant correlation between latitude and $\delta^{15}N$ in squid (Figure 2B). The unclear relationship in $\delta^{15}N$ can be explained by the trophic effect. The enrichment per trophic level is striking in $\delta^{15}N$ (3–4‰) relative to that in $\delta^{13}C$ (~1‰). Consequently, the $\delta^{15}N$ of the consumer is strongly affected by the difference of feeding habit of squid and/or lower consumers. In *Illex illecebrosus* Lesueur, 1821 analysed by Ostrom et al. (1993) and the squid analysed by Hobson et al. (1997), in fact, the isotopic difference between the different size groups reached 5.8–7.1‰ (Table 2). This difference is large enough to weaken the negative correlation of latitude–$\delta^{15}N$ of squid, since the difference is comparable to the maximum geographical difference (10.3‰) between *K. longimama* in the Antarctic (Wada et al., 1987) and *Sthenoteuthis oualaniensis* off Peru.

The $\delta^{15}N$ of squid strikingly reflected the locality of nitrogen metabolism such as denitrification and N_2 fixation rather than the latitude-related isotopic variation. In the oxygen-depleted water of the eastern tropical Pacific Ocean, a portion of nitrate upwelled into the euphotic zone from the depth is converted to N_2 by denitrification as shown in Figure 1 (Wada & Hattori, 1991). An extremely high fractionation factor of up to 1.040 was reported for the denitrification in the area (Cline & Kaplan, 1975). In the western part of the Pacific Ocean, on the other hand, bluegreen algae *Trichodesmium* spp. grow actively and supply huge amounts of combined nitrogen to the oligotrophic surface water by nitrogen fixation with low $\delta^{15}N$ values of -2–0‰ relative to atmospheric nitrogen. Little isotope fractionation has been reported for N_2 fixation by leguminous plants (Amarger et al., 1979) and blue–green algae (Minagawa & Wada, 1986) in a variety of marine and terrestrial ecosystems. For this reason, the $\delta^{15}N$ of phytoplankton is high in the eastern tropical Pacific Ocean and low in the western Pacific. The geographical difference of $\delta^{15}N$ of *S. oualaniensis* reflected this regional characteristics of nitrogen metabolism clearly. A high $\delta^{15}N$ value of 13.8 ±0.7‰ found in the Arabian Sea is also explained by the denitrification peculiar to the area as shown in Figure 1 (Altabet et al., 1995).

Variation of squid $\delta^{13}C$ and $\delta^{15}N$ in the local area

Although the feeding habit of *Eucleoteuthis luminosa* is unknown, the other four species, *S. oualaniensis*, *Ommastrephes bartramii*, *Gonatopsis borealis*, and *Onychoteuthis borealijaponica*, captured off Japan in the Pacific Ocean are all supposed to mainly feed on fish, crustacea and squid (Nixon, 1987; Nesis, 1989). It is thus difficult to explain the local variation of the squid in the area only by the interspecific difference of the feeding habit and the subsequent slight isotopic difference. Nakatsuka et al. (1997) showed that the particulate organic matter (POM) in deep water columns exhibits lower $\delta^{15}N$ and higher $\delta^{13}C$ at low latitudes (34°10′N 142°E) than at high latitudes (44°N 155°E) off Japan in the north-western Pacific Ocean, probably reflecting the microbial activity in the surface waters: low isotope fractionation factor of N_2 fixation by blue–green algae and the negative correlation between the latitudes and phytoplankton $\delta^{13}C$. The low $\delta^{15}N$ and the high $\delta^{13}C$ in *S. oualaniensis* were probably influenced by these baseline characteristics of $\delta^{13}C$ and $\delta^{15}N$ in the area, since it was captured at the southernmost station among the five species (Figure 1).

The isotopic values of the three species captured in the Japan Sea are expected to be distinct, reflecting each characteristic habitat use: pelagic *Todarodes pacificus*, coastal *Loligo bleekeri*, and benthic *Rossia pacifica* (Okutani, 1980; Boletzky & Boletzky, 1973). However, the values were peculiar in only *T. pacificus*, and the other two species exhibited similar values (Figure 3). The isotopic difference between the latter two species and the former was striking in $\delta^{13}C$ (2.0–2.6‰) relative to $\delta^{15}N$ (1.2–1.5‰). In order to explain the difference, first of all, it is necessary

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to consider the influence by the water mass structure in the Japan Sea. The sea is divided by the Subarctic Front into two distinct regions, the Subarctic and the Tsushima Warm Current. We captured all samples of *L. bleekeri* and *R. pacifica* in the Tsushima Warm Current, while *T. pacificus* was collected across the Subarctic Front from 38°10′N to 42°00′N (Table 1). A striking change of δ^{13}C of POM across the Subtropical Convergence was observed in the Indian Ocean (Francois et al., 1993) and such baseline variation affected by water mass structure would be reflected in the consumers δ^{13}C (Schell et al., 1998). In fact, the δ^{13}C of *T. pacificus* was significantly more enriched in the Tsushima Warm Current than in the Subarctic (Ikeda et al., 1998). However, the isotopic value of *T. pacificus* was −18.4 ±0.3‰ in the Tsushima Warm Current and 18.8 ±0.5‰ in the Subarctic and thus any area groups of *T. pacificus* were depleted by not less than 1.8‰ in δ^{13}C relative to the other two species. It is therefore difficult to explain this local isotopic variation by the water mass structure.

The isotopic variation among the three species may rather be explained by the inshore–offshore isotopic shift. Algal δ^{13}C is more enriched in benthic algae than in planktonic algae, reaching the greatest difference of 21.5‰ in Lake Baikal (Kiyashko et al., 1998). The average of literary δ^{13}C values for marine phytoplankton is −22‰ relative to −17‰ for marine benthic algae (France, 1995) and the difference gives rise to the difference of animal δ^{13}C between offshore and inshore feeders (Hobson et al., 1994). We suspect that the lower δ^{13}C of *T. pacificus* captured in the pelagic area may be attributable to the lower δ^{13}C of pelagic food web.

CONCLUSION

A number of squid have excellent swimming ability and migrate a long distance (O'Dor & Wells, 1987). Therefore, squid isotopic values would be affected by the baseline variations of the values of primary producers along the migration course. The striking geographical variations shown in both δ^{13}C and δ^{15}N of the squid reflecting the oceanographical factors indicate that we must pay attention to the isotopic variations of primary producers as well as those of squid in examining the trophic positions of squid.

On the other hand, such geographical variations of stable isotope ratios have recently been utilized to study the migration course and season of highly migratory marine animals such as whales and sea birds (Best & Schell, 1996; Minami & Ogi, 1997). These studies are based on the premise that the isotopic values of the marine animals reflect the baseline values of primary producers. The results in this study guarantee that this kind of study can provide useful information for clarifying the migration characteristics of squid as well.

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Appendix 1. The original data of sex, DML (mm), $\delta^{13}C$ (‰), and $\delta^{15}N$ (‰) of the squids.

Group	Sex	DML	$\delta^{13}C$	$\delta^{15}N$
Pacific Ocean (off Japan)				
OMMASTREPHIDAE				
Sthenoteuthis oualaniensis	F	166	−16.0	9.3
	F	300	−16.3	12.3
	F	213	−15.8	9.6
	F	154	−16.3	8.3
	F	254	−16.6	10.5
Ommastrephes bartramii	F	344	−17.4	12.2
	F	346	−17.7	11.9
	F	387	−17.3	11.8
	F	350	−17.6	12.0
	F	375	−17.3	12.5
Eucleoteuthis luminosa	F	194	−18.0	10.5
	F	224	−17.5	12.5
	M	186	−17.8	10.7
GONATIDAE				
Gonatopsis borealis	F	213	−17.5	13.4
	F	230	−17.7	13.3
	M	231	−17.8	12.9
	M	206	−17.7	13.1
	M	242	−18.1	13.0
ONYCHOTEUTHIDAE				
Onychoteuthis borealijaponica	F	301	−18.1	12.1
	F	301	−17.6	11.9
	F	336	−17.9	11.9
	F	354	−18.6	12.5
	F	325	−17.7	12.7
Pacific Ocean (off Peru)				
OMMASTREPHIDAE				
Sthenoteuthis oualaniensis	F	224	−15.7	16.8
	F	240	−15.4	16.3
	F	224	−15.1	16.7
	F	232	−15.8	15.3
	F	179	−15.9	16.3
The Japan Sea				
OMMASTREPHIDAE				
Todarodes pacificus	F	205	−18.9	9.7
	F	204	−17.8	10.3
	F	215	−18.1	10.3
	F	212	−18.1	10.2
	F	201	−17.9	10.5
	F	219	−19.0	10.2
	F	211	−18.5	10.5
	F	215	−18.8	10.2
	F	205	−19.2	10.5
	F	210	−18.5	11.0
	F	214	−18.8	10.4
	F	215	−18.1	10.8
	F	204	−18.4	10.9
	F	213	−18.8	10.2
	F	216	−19.2	9.8
	F	202	−19.4	9.8
	F	190	−19.3	10.0
	F	212	−18.0	11.2
	F	201	−19.5	10.8
	F	212	−19.2	10.7
	F	205	−18.6	10.7
	F	200	−18.6	9.6
	F	211	−19.9	10.7
	F	201	−19.1	10.7
	F	199	−19.1	10.0
	F	205	−17.8	10.0
	F	210	−18.6	10.1
	F	206	−18.4	10.7
	F	207	−18.0	10.6
	F	210	−18.0	10.1
	F	218	−18.1	10.5
	F	215	−18.4	11.5
	F	219	−18.5	10.2
	F	206	−18.3	10.8
	F	212	−18.6	10.1
	M	205	−18.5	10.3
	M	198	−18.2	10.4
	M	200	−18.7	10.5
	M	200	−18.8	10.6
	M	185	−18.4	10.2
	M	210	−18.7	10.9
	M	211	−18.6	10.6
	M	210	−18.9	10.8
	M	212	−18.2	10.6
	M	205	−18.5	10.7
	M	205	−18.6	10.6
	M	203	−19.1	10.0
	M	208	−17.8	11.1
	M	215	−17.9	11.3
	M	205	−18.8	10.4
	M	206	−17.8	11.1
	M	209	−17.9	10.7
	M	210	−18.8	10.5
	M	205	−18.9	10.6
	M	206	−19.0	9.9
	M	211	−18.7	10.0
	M	226	−18.6	10.7
	M	195	−18.5	10.2
	M	206	−19.2	10.1
	M	199	−19.0	10.4
	M	215	−18.5	10.4
	M	207	−18.0	10.7
	M	205	−18.5	10.7
	M	209	−17.9	10.8
	M	214	−18.6	10.9
	M	214	−18.6	10.2
	M	207	−18.4	10.8
	M	205	−19.2	10.3
	M	205	−18.3	10.5
	M	198	−18.6	9.9
LOLIGINIDAE				
Loligo bleekeri	F	225	−16.7	12.0
	F	276	−17.0	11.9
	F	250	−15.9	12.5
	F	240	−17.1	11.0
	F	230	−16.3	11.1
SEPIOLIDAE				
Rossia pacifica	F	60	−16.2	11.4
	F	64	−15.8	12.7
	F	73	−16.0	12.3
	F	74	−16.2	11.7
	M	54	−15.9	12.1
Indian Ocean (off Australia)				
OMMASTREPHIDAE				
Sthenoteuthis oualaniensis	M	135	−16.2	11.4
	M	144	−15.9	11.9
	M	131	−16.3	12.0
	M	138	−16.0	12.1
	M	134	−16.3	11.4
The central Arabian Sea				
OMMASTREPHIDAE				
Sthenoteuthis oualaniensis	F	152	−15.8	14.3
	F	172	−15.4	14.0
	F	151	−15.5	14.2
	F	137	−15.5	14.1
	F	160	−15.4	14.1
	F	120	−15.6	14.6
	F	120	−15.4	14.6
	F	219	−15.4	13.8

continued

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Appendix 1. *(Continued)*.

Group	Sex	DML	$\delta^{13}C$	$\delta^{15}N$
	F	246	−15.5	11.8
	F	226	−15.5	13.4
	F	175	−15.5	13.2
	F	175	−15.3	13.8
	F	193	−15.2	13.8
	M	147	−15.3	13.9
Atlantic Ocean (off Namibia)				
OMMASTREPHIDAE				
Todarodes angolensis	F	229	−13.9	13.5
	F	206	−14.1	13.4
	F	227	−13.8	13.4
	F	228	−14.1	13.4
	F	244	−14.5	13.4
Atlantic Ocean (off Argentine)				
OMMASTREPHIDAE				
Martialia hyadesi	F	222	−16.5	12.0
	F	247	−16.6	11.9
	F	236	−16.3	11.4
	F	233	−16.5	12.0
	F	227	−16.5	12.0
	F	220	−16.8	11.7
	M	243	−16.5	11.9

M, male; F, female.

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