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Biological and geographical variations of carbon and nitrogen stable isotope ratios of squid

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Abstract The $\delta^{15}N$ and $\delta^{13}C$ analysis of consumer and prey tissues is a valuable method for examining trophic relationships and transport of organic matters along the food chain in aquatic and terrestrial ecosystems. Significant increases in $\delta^{15}N$ of 3.4 ± 1.1‰ can occur after a single feeding in both invertebrates and vertebrates, while small increase of about 1‰ can occur for $\delta^{13}C$. $\delta^{15}N$ and $\delta^{13}C$ values can provide information about the trophic levels of squid prey and about the primary producers at the base of the food chain, respectively. In this study, the magnitude of isotopic variations in 10 species of squids captured in 7 areas was examined. Phytoplankton $\delta^{15}N$ and $\delta^{13}C$ are known to be often higher at low latitudes than at high latitudes. $\delta^{15}N$ and $\delta^{13}C$ in the analyzed squid species showed similar patterns. Geographical variations were conspicuous in *Sthenoteuthis oualaniensis* for $\delta^{15}N$ (16.3 ± 0.6‰ off Peru to 10.0 ± 1.5‰ off Japan in Pacific Ocean) and in *Ommastrephes bartramii* for $\delta^{13}C$ (-14.1 ± 0.3‰ off Namibia in Atlantic Ocean to -17.5 ± 0.2‰ off Japan in Pacific Ocean).

Keywords: stable isotope, $\delta^{15}N$, $\delta^{13}C$, squid, biological variation, geographical variation

Introduction

Recent progress in stable isotope ecology has clarified that the carbon and nitrogen stable isotope analysis 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 (DeNiro and Epstein, 1978, 1981; Wada et al., 1987; Hobson and Welch, 1992; Cabana and Rasmussen, 1994). Significant increase in $\delta^{15}N$ of 3.4 ± 1.1‰ was reported during single feeding process irrespective of invertebrate and vertebrate (Minagawa and Wada, 1984), while a small enrichment of about 1‰ was found for $\delta^{13}C$ (DeNiro and Epstein, 1978; Rau et al., 1983; Fry, 1988). Consequently, animal $\delta^{15}N$ is a possible indicator of its trophic level and animal $\delta^{13}C$ can be used to identify a primary producer at the base of a

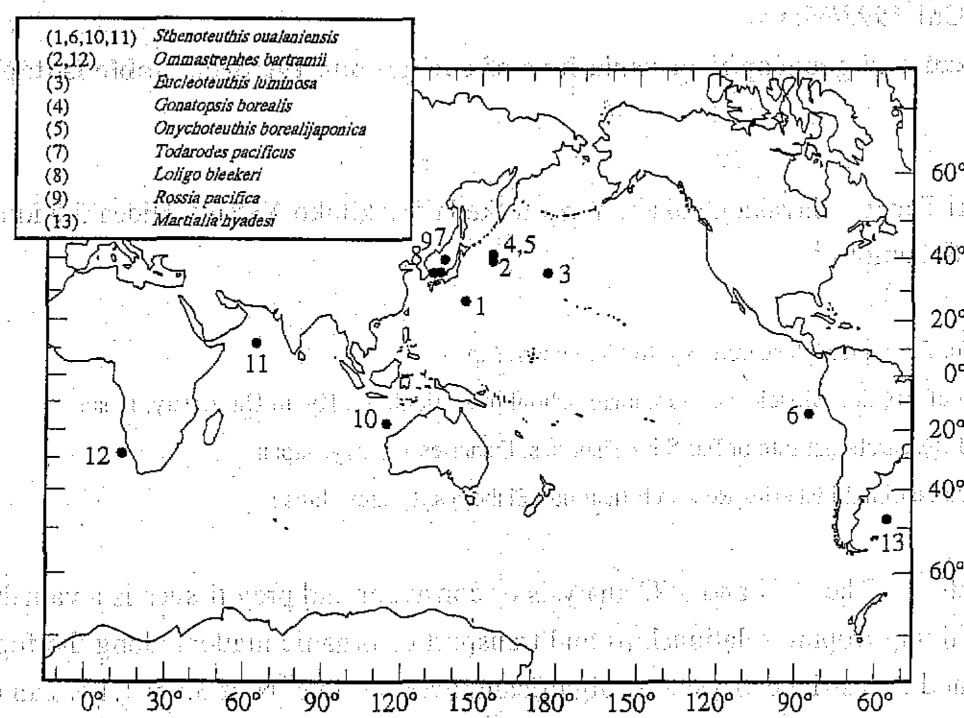

Fig.1. The sampling locations (•) of the analyzed squids. The above 9 species were captured at the numbered circles.

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 variations and/or interspecific variations on a similar niche (Fry and Sherr, 1984; Cabana and Rasmussen, 1996). This characteristics make it difficult to interpret the analyzed isotopic values of the animals ecologically. Accordingly, it is necessary to examine the magnitude of geographical and biological variations of squid stable isotope ratios, in order to evaluate the position and the role of squids in the food web structure with this method. In this study, $\delta^{13}C$ and $\delta^{15}N$ of 9 species of squids captured in 7 areas of the world ocean were measured and the magnitude of the isotopic variations was examined.

Materials and Methods

Nine species of squids, *Sthenoteuthis oualaniensis*, *Ommastrephes bartramii*, *Eucleoteuthis luminosa*, *Gonatopsis borealis*, *Onychoteuthis borealijaponica*, *Todarodes pacificus*, *Loligo bleekeri*, *Rossia pacifica*, and *Martialia hyadesi*, were captured in the 7 areas of the world ocean from 8 February 1990 to 23 May 1998 (Fig.1). The numbers of the analyzed samples and the averaged DML of each species were shown in Table 1. A total of 139 squid samples (male 47, female 92) were analyzed. The squids were captured with jigging.

The samples were kept at -20°C. The DML was measured and then the muscles were

Table 1. The date and the location of the capture, DML (mm), and stable isotope ratios (‰) of the squids.

sampling date	sampling location	n (M : F)*1	DML (mean±S.D.)	$\delta^{13}C$ (mean±S.D.) (max-min)	$\delta^{15}N$ (mean±S.D.) (max-min)
PACIFIC OCEAN (OFF JAPAN)					
OMMASTREPHIDAE					
Sthenoteuthis oualaniensis		5		-16.2±0.3	10.0±1.5
26 Nov.1997	N26°30', E144°00'	(0 : 5)	217±61	(-15.8 — -16.6)	(10.5 — 8.3)
Ommastrephes bartramii		5		-17.5±0.2	12.1±0.3
10 May 1998	N39°30', E155°00'	(0 : 5)	360±19	(-17.3 — -17.7)	(12.5 — 11.8)
Eucleoteuthis luminosa		3		-17.8±0.3	11.1±1.0
23 May 1998	N35°00', E175°30'	(1 : 2)	201±20	(-17.5 — -18.0)	(12.0 — 11.4)
GONATIDAE					
Gonatopsis borealis		5		-17.8±0.2	13.1±0.2
10 May 1998	N41°00', E155°00'	(3 : 2)	224±15	(-17.5 — -18.1)	(13.4 — 12.9)
ONYCHOTEUTHIDAE					
Onychoteuthis borealijaponica		5		-18.0±0.4	12.2±0.4
10 May 1998	N41°00', E155°00'	(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
31 Aug.1995	S14°00', W85°00'	(0 : 5)	220±24	(-15.1 — -15.9)	(16.8 — 15.3)
THE SEA OF JAPAN					
OMMASTREPHIDAE					
Todarodes pacificus		70		-18.6±0.5	10.5±0.4
24 Jun.— 14 Jul.1997	N38°10'—N42°00' E134°00'—E139°00'	(35 : 35)	207±7	(-17.8 — -19.9)	(11.5 — 9.6)
LOLIGINIDAE					
Loligo bleekeri		5		-16.6±0.5	11.7±0.6
13 Feb.1996	N34°54', E132°04'	(0 : 5)	250±17	(-16.3 — -17.1)	(12.5 — 11.0)
SEPIOLIDAE					
Rossia pacifica		5		-16.0±0.2	12.0±0.5
21 Mar.1997	N35°34', E135°28'	(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
3 Jan.1991	S17°59', E115°01'	(5 : 0)	136±5	(-15.9 — -16.3)	(12.1 — 11.4)
ARABIAN SEA (CENTRAL)					
OMMASTREPHIDAE					
Sthenoteuthis oualaniensis		14		-15.4±0.2	13.8±0.7
10 Jun.1996	N12°00', E64°00'	(1 : 13)	171±39	(-15.2 — -15.8)	(14.6 — 11.8)
ATLANTIC OCEAN (OFF NAMIBIA)					
OMMASTREPHIDAE					
Ommastrephes bartramii		5		-14.1±0.3	13.4±0.1
8 Feb.1990	S27°48', E14°30'	(0 : 5)	227±14	(-14.5 — -13.8)	(13.5 — 13.4)
ATLANTIC OCEAN (OFF ARGENTINE)					
OMMASTREPHIDAE					
Martialia hyadesi		7		-16.5±0.2	11.8±0.2
21 Mar.1993	S47°15', W54°25'	(1 : 6)	233±10	(-16.3 — -16.8)	(12.0 — 11.4)

*1 M; male. F; female.

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. Carbon and nitrogen stable isotope ratios were measured with Finnigan Mat Delta-S mass spectrometry after the manual cryo-purification of combustion products in a vacuum system (Minagawa and Wada, 1984), or with continuous-flow isotope ratio mass spectrometry (CF-IRMS) coupled with element analyzer (Carlo Erba, Italy). Isotope ratios, $\delta^{13}C$ and $\delta^{15}N$, are expressed as per mil deviations from the standard as defined by the following equation:

$$\delta^{13}C, \delta^{15}N = \{R_{(sample)} / R_{(standard)} - 1\} \cdot 1000\ (‰)$$

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

Belemnite (PDB) and atmospheric nitrogen were used as the carbon and nitrogen isotope standards, respectively. The analytical precisions for the isotopic analyses were 0.10‰ for both isotopes on the method according to Minagawa and Wada (1984), and 0.21‰ in $\delta^{13}C$ and 0.25‰ in $\delta^{15}N$ on the method with CF-IRMS.

Results and Discussion

$\delta^{13}C$ and $\delta^{15}N$ of the world squids

The $\delta^{15}N$ of the squids ranged from 8.3‰ of *S. oualaniensis* off Japan in northern Pacific Ocean to 16.8‰ of *S. oualaniensis* off Peru in southern Pacific Ocean, while the $\delta^{13}C$ ranged from -19.9‰ of *T. pacificus* in the Sea of Japan to -13.8‰ of *O. bartrami* off Namibia in Atlantic Ocean (Table 1). Phytoplankton $\delta^{13}C$ and $\delta^{15}N$ are reported to be often higher at low latitudes than at high latitudes (Rau et al., 1982; Wada 1997). $\delta^{13}C$ and $\delta^{15}N$ in our analyzed squid species showed similar patterns, in which the negative correlations between stable isotope ratios and the latitude of the sampling location was found (Fig.2). Spearman's correlation coefficient was -0.71 in $\delta^{13}C$ and -0.22 in $\delta^{15}N$. However, the correlation of $\delta^{15}N$ was not significant, while that of $\delta^{13}C$ was significant ($p<0.05$).

Wada et al. (1987) reported that the isotope ratios of *Kondakovia longimama* captured in the Arctic are -26.1 to -24.5‰ in $\delta^{13}C$ and 6.5 to 7.2‰ in $\delta^{15}N$. These values are extremely low compared with our analyzed squids values (Fig.2). Our samples were captured in the lower latitudinal area from N41°00' to S47°15', while *K. longimama* was captured at S59°34' to S59°57'. It suggests that the latitude — stable isotope ratios correlations might become higher near the Pole.

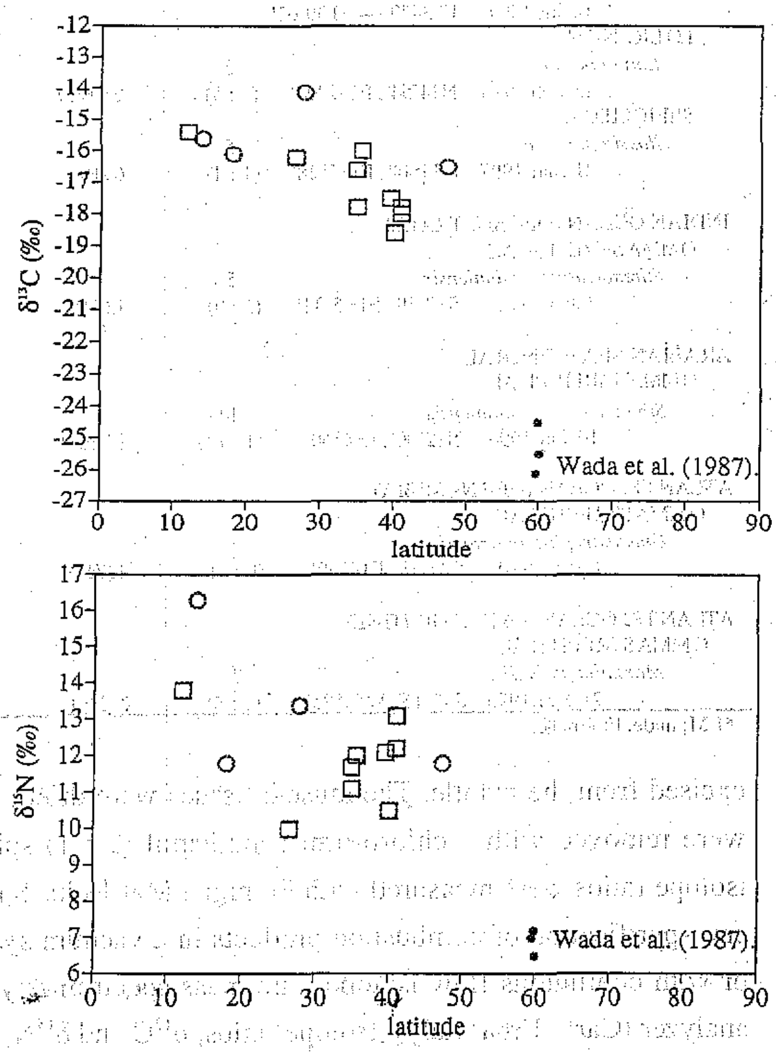

Fig.2. The relationship between the latitude of the sampling locations in the northern (□) and the southern (○) Hemispheres and the stable isotope ratios of squids. The solid circles show the values of *Kondakovia longimama* in the Antarctic (Wada et al., 1987).

The intraspecific geographic variation of $\delta^{13}C$ and $\delta^{15}N$

S. oualaniensis was captured in the 4 areas; off Japan in northern Pacific Ocean, Arabian Sea, off Peru in southern Pacific Ocean, and off Australia

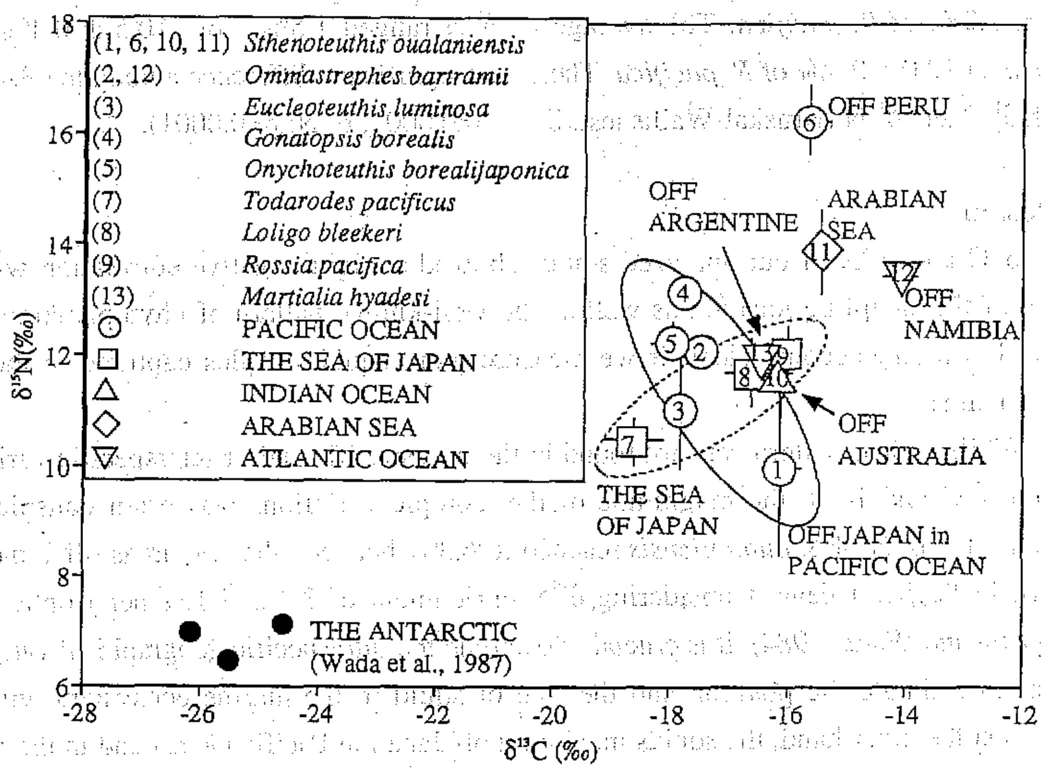

Fig.3. The $\delta^{13}C$-$\delta^{15}N$ map of the world squids. The symbols and numbers show the sampling location and the species, respectively. The bars represent the standard deviation. The solid circles are the values of *Kondakovia longimama* in the Antarctic (Wada et al., 1987).

in Indian Ocean (Fig.1). The geographic variation of *S. oualaniensis* was conspicuous in $\delta^{15}N$; the averages of isotope ratios ranged 0.8‰ from -16.2 ± 0.3‰ (off Japan) to -15.4 ± 0.2‰ (Arabian Sea) in $\delta^{13}C$, and 6.3‰ from 10.0 ± 1.5‰ (off Japan) to 16.3 ± 0.6‰ (off Peru) in $\delta^{15}N$ (Fig.3). The difference of the averaged $\delta^{15}N$ between the area groups was over 1.8‰ in every combination of the area groups. Significant difference was shown in both $\delta^{13}C$ and $\delta^{15}N$ among the 4 area groups (Kruskal-Wallis test, $\delta^{13}C$; $p=0.0005$, $\delta^{15}N$; $p<0.0001$).

O. bartramii was captured off Japan in northern Pacific and off Namibia in Atlantic Ocean (Fig.1). The range of averaged isotope ratios of *O. bartramii* was larger in $\delta^{13}C$ than in $\delta^{15}N$, contrary to *S. oualaniensis*. The difference between the 2 areas was 3.4‰ in $\delta^{13}C$ and 1.3‰ in $\delta^{15}N$. The difference was significant in both $\delta^{13}C$ and $\delta^{15}N$ (Mann-Whitney's U test, $\delta^{13}C$; $p<0.01$, $\delta^{15}N$; $p<0.01$).

The interspecific variation of $\delta^{13}C$ and $\delta^{15}N$ in the same area

Off Japan in Pacific Ocean, 5 species including *S. oualaniensis*, *O. bartramii*, *E. luminosa*, *G. borealis*, and *O. borealijaponica* were analyzed (Fig.1). The average of $\delta^{13}C$ ranged 1.8‰ from -18.0 ± 0.4‰ of *O. borealijaponica* to -16.2 ± 0.3‰ of *S. oualaniensis*. The average of $\delta^{15}N$ ranged 3.1‰ from 10.0 ± 1.5‰ of *S. Oualaniensis* to 13.1 ± 0.2‰ of *G. borealis*. There was significant difference among the 5 species in both $\delta^{13}C$ and $\delta^{15}N$ (Kruskal-Wallis test, $\delta^{13}C$; $p<0.005$, $\delta^{15}N$; $p<0.005$). On the $\delta^{13}C$—$\delta^{15}N$ map of Fig.3, the position of *S. oualaniensis* was distributed away from the positions of the other 4 species.

In the Sea of Japan, 3 species including *T. pacificus*, *L. bleekeri*, and *R. pacifica* were analyzed (Fig.1). The average of $\delta^{13}C$ ranged 2.6‰ from -18.6 ± 0.5‰ of *T. pacificus* to

-16.0 ± 0.2‰ of *R. pacifica*. The average of $\delta^{15}N$ ranged 1.5‰ from 10.5 ± 0.4‰ of *T. pacificus* to 12.0 ± 0.5‰ of *R. pacifica*. There was significant difference among the 3 species in both $\delta^{13}C$ and $\delta^{15}N$ (Kruskal-Wallis test, $\delta^{13}C$; $p<0.0001$, $\delta^{15}N$; $p<0.0001$).

Conclusion

$\delta^{13}C$ and $\delta^{15}N$ of our analyzed squids showed a slight negative correlation with the latitude of the sampling location as well as the well-known pattern of phytoplanktons. The relationship might become clear, if we measure more squid samples captured in the high latitudinal area.

While a clear pattern was not found in the intraspecific and/or interspecific variations of squid $\delta^{13}C$ and $\delta^{15}N$, the magnitude of the isotopic variations was often conspicuous; especially the $\delta^{15}N$ of *S. oualaniensis* reached to 6.3‰ between the two areas off Japan and off Peru in Pacific Ocean. Considering $\delta^{15}N$ enrichment of 3.4 ± 1.1‰ per trophic level (Minagawa and Wada, 1984), it is crucial to examine the intraspecific geographical variations in order to estimate the position and the role of squid in the marine ecosystem with this method. On the other hand, the squids inhabiting off Japan in Pacific Ocean and in the Sea of Japan showed significant interspecific variations. It indicates that the the isotopic variations among species in a same habitat area can not be negligible.

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