

Habitat Use and Trophic Positions of Kobi Squid *Loliolus sumatrensis* in the Western Seto Inland Sea in Late Spring Inferred from Carbon and Nitrogen Stable Isotope Ratios

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Carbon and nitrogen stable isotope ratios of the Kobi squid *Loliolus sumatrensis* were analyzed to infer its habitat use and trophic positions in the western Seto Inland Sea in late spring. The $\delta^{15}\text{N}$ distribution of this species was strikingly high, compared with the reported $\delta^{15}\text{N}$ values for cephalopods in the oceans of the world. It was considered to clearly reflect ^{15}N -enriched wastewater nitrogen input from industrial areas. The distribution of Kobi squid (60–80 mm in dorsal mantle length) on a $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ map was different among sampling stations and thus the squid were isotopically categorized into three types of groups, I, II, and III. The average isotopic values were $-14.1 \pm 0.3\text{‰}$ in $\delta^{13}\text{C}$ and $17.3 \pm 0.6\text{‰}$ in $\delta^{15}\text{N}$ for group I, $-14.5 \pm 0.6\text{‰}$ in $\delta^{13}\text{C}$ and $16.0 \pm 0.6\text{‰}$ in $\delta^{15}\text{N}$ for group II, and $-16.7 \pm 0.3\text{‰}$ in $\delta^{13}\text{C}$ and $13.7 \pm 0.4\text{‰}$ in $\delta^{15}\text{N}$ for group III. Squid of group I were collected in the northern area of Hiroshima Bay, which has a large river water inflow and thus surface water salinity is relatively low. Squid of group II were widely distributed from central Hiroshima Bay to the northernmost region of Aki Nada, where the freshwater influence is small. Both groups I and II were inferred to be carnivores feeding on decapods and so forth. Squid of group III were collected only in the shallowest edge zone of the northernmost region of Aki Nada. This group was peculiarly depleted in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, being close to the particulate organic matters in the surface layer, and thus was inferred to feed on phytoplankton and/or zooplankton. These results suggest that the life types of this species are diverse in the Seto Inland Sea.

Key words: *Loliolus sumatrensis*, Kobi squid, Seto Inland Sea, habitat use, trophic position, stable isotope ratio, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$

Introduction

Squids of the genus *Loliolus* are abundantly captured in coastal waters around Japan and are utilized as fisheries resources. This genus consists of small species with a longevity of probably one year, and is supposed to inhabit a local area throughout its life history in contrast to pelagic migratory species (Takechi, 1989). Takechi and Kawasaki (1981) reported that in Sendai Bay the Japanese squid *Loliolus japonica* formed three distinct populations with different migration courses and seasons. This predicts that habitat use of *Loliolus* spp. would be diverse within such a local area. In the present study, this diversity of the habitat use in *Loliolus* spp. was examined for the Kobi squid *Loliolus sumatrensis*, which is closely related and morphologically very similar to the Japanese squid, in the western Seto Inland Sea.

The Kobi squid is widely distributed along the western Pacific Ocean from western Japan to the Malay Peninsula (Okutani, 1995) and is also abundant in the Seto Inland

Sea, especially Hiroshima Bay, Aki Nada, and Hiuchi Nada (Ikehara and Ogawa, 1996). In spite of such abundant catch, little is known about its life historical characteristics in the sea. Here we utilized the stable isotope technique in order to infer the habitat use of the Kobi squid in Hiroshima Bay and Aki Nada in late spring.

Carbon and nitrogen stable isotope ratios of an animal are increasingly used to examine time-integrated information on its feeding relationship and/or organic carbon transport through aquatic food webs (Wada *et al.*, 1987; Fry, 1988). The $\delta^{13}\text{C}$ value of an animal shows a slight enrichment of about 1‰ during a single feeding process (DeNiro and Epstein, 1978; Rau *et al.*, 1983). This conservative nature of $\delta^{13}\text{C}$ along a food chain can provide information on the carbon sources of higher consumers in aquatic food webs. On the other hand, $\delta^{15}\text{N}$ of consumers become enriched by 3–4‰ in both vertebrates and invertebrates (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). This stepwise enrichment in ^{15}N can be used to examine the trophic position of consumers. The $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ map in an ecosystem can thus show a schematic food web structure on a corresponding food base.

Recently these isotopic signatures have been utilized to examine the intraspecific variations of habitat use in

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aquatic animals such as fishes, sea birds, and cetaceans (Schell *et al.*, 1989; Hesslein *et al.*, 1991; Best and Schell, 1996; Minami and Ogi, 1997; Takai and Sakamoto, 1999). The isotopic difference in prey species between distant habitat areas gives rise to local differences in isotopic signatures of predators, and thus the isotopic signatures can be used to examine which area the predators have stayed in. Ikeda *et al.* (1998) successfully applied this technique to the migration study of the Japanese common squid *Todarodes pacificus* in the Japan Sea. This typical pelagic migratory species showed significant differences in $\delta^{13}\text{C}$ signatures between the Subarctic water and the Tsushima Current region across the Subarctic Front in summer, supporting the hypothesis that there may be two types of migration groups with different migration patterns, growth rates, and feeding habits in the season (Kidokoro and Hiyama, 1996). If there were any variations in habitat use of the Kobi squid in the western Seto Inland Sea, similar isotopic differences might be expected for the squid among their habitat areas.

In this study, we also estimated the trophic positions of the Kobi squid in Hiroshima Bay and Aki Nada mainly on the basis of the $\delta^{15}\text{N}$ signatures. The stable isotope ratios of fishes and decapods, which were potential food sources of the Kobi squid, collected in Aki Nada on the same sampling date were compared with the values of the Kobi squid in order to clarify their trophic relationship. Finally, a schematic distribution map of isotopically categorized groups, characterized by habitat use and trophic positions, was depicted as a footing for the synthetic study of its life historical characteristics all over the Seto Inland Sea.

Study Area

The Seto Inland Sea is a broad shallow sea located to the

southwest of the mainland of Japan, 450 km long, with an area of 220,000 km² and average depth of 37 m (Fig. 1). The sea consists of several shallower semi-enclosed areas and deeper narrow channels, leading to the open sea at three mouths, the Bungo Channel, the Kii Channel, and the Kanmon Strait. The third mouth leads to the Japan Sea at the westernmost end of the sea and is too narrow to affect the total water exchange of the sea. The former two mouths lead to the Pacific Ocean at the southwestern (the Bungo Channel) and the southeastern (the Kii Channel) end of the sea. The mass of water exchanged through the Bungo Channel is twice that through the Kii Channel (Fujiwara, 1983) and the residence time of the total water in the sea is supposed not to exceed the order of several years (Okubo, 1981; Takeoka, 1984).

Hiroshima Bay and Aki Nada are located to the north of the Bungo Channel (Fig. 1). Hiroshima Bay is dotted with many islands in its central area and thus its topography is very complicated with an area of about 1000 km² and average depth of 24 m. In particular, the Nasami Strait divides the bay into the two semi-enclosed areas, northern and central Hiroshima Bay. The northern bay is one of the most eutrophic areas in the Seto Inland Sea and the Ota River flows into the area from the northern coast. The chlorophyll *a* concentration increases and the dissolved oxygen becomes oversaturated in the surface layer in summer there, in contrast to small seasonal change in the central bay area (Hashimoto *et al.*, 1994). On the other hand, Aki Nada has few islands and bottom undulations in its central area with an area of 600 km² and average depth of 36 m. There is little freshwater inflow in Aki Nada, while the area is exposed to the inflow of the oceanic open water flowing through the Bungo Channel. The water temperature and salinity are ver-

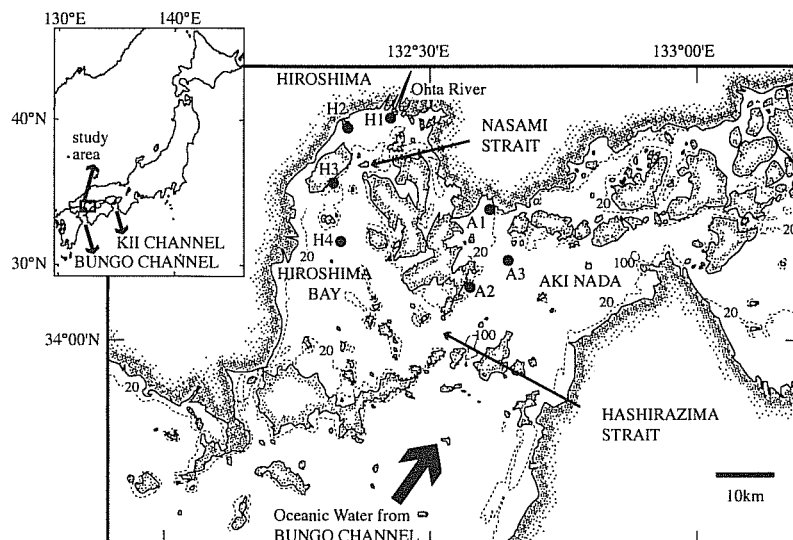


Figure 1. Study area. Kobi squid were collected at Sts. H1-H4 in Hiroshima Bay and at Sts. A1-A3 in Aki Nada.

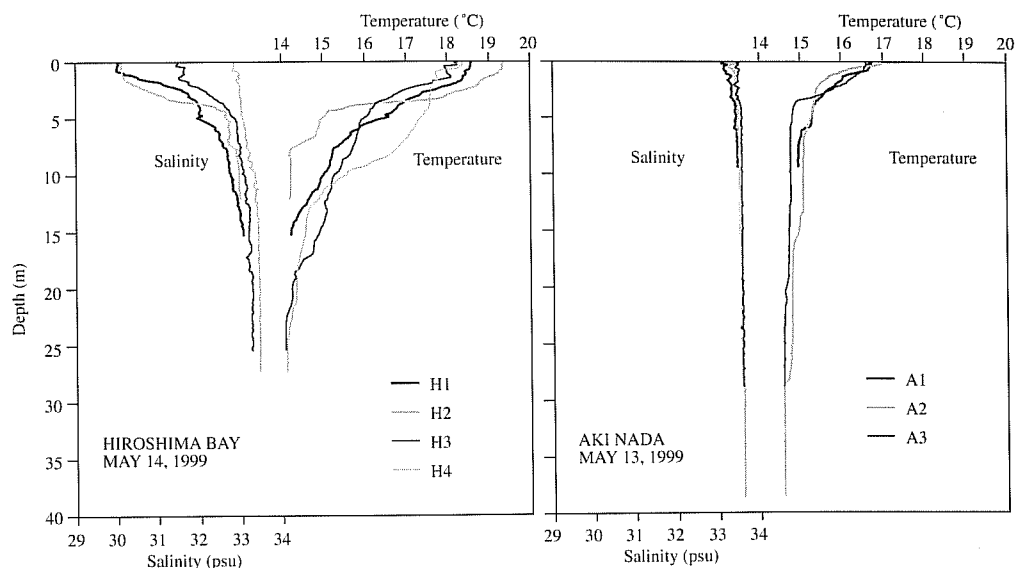


Figure 2. Vertical profiles of salinity and temperature in Hiroshima Bay (Sts. H1-H4) on May 14, 1999 and Aki Nada (Sts. A1-A3) on May 13, 1999.

tically uniform and the pycnocline is not formed there (Takasugi *et al.*, 1983).

Materials and Methods

Sampling

Kobi squid were collected with a bottom trawl net at four stations (H1, H2, H3 and H4) in Hiroshima Bay on May 14, 1999 and at three stations (A1, A2 and A3) in Aki Nada on May 13, 1999 (Fig. 1). The knot to knot size of the net was 25 mm. We compared the isotopic values of the squid of 60–80 mm in dorsal mantle length (DML) among the stations, since the squid of this size class were abundantly captured at all stations. Fishes and decapods were collected with the same net at St. A1. The vertical distributions of water temperature and salinity were also measured by a CTD meter (Alec ACL208).

Stable isotope analysis

The samples were kept at -20°C . The muscle tissues were excised from the mantle of the squid, from the trunk of the fish and the shrimp, and from the ambulatory leg of the crab. These tissues were dried at 60°C , 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 with a MAT 252 mass spectrometer (Finnigan MAT) coupled with an element analyzer (Carlo Erba). Isotope ratios, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, are expressed as per mil deviations from the standard as defined by the following equation:

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

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. Belemnite (PDB) and atmos-

pheric nitrogen were used as the isotope standards of carbon and nitrogen, respectively. The analytical precision for the isotopic analyses was not more than 0.28‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Since marked isotopic differences with sex have not been found for either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of any cephalopods (Takai *et al.*, 2000), no consideration was made for the sexes of the Kobi squid in the following analyses.

Results

Salinity and temperature on the sampling dates

On May 13–14, 1999, the vertical change of salinity was small in the layer deeper than 10 m at every sampling station, while the vertical profile in the surface water above 10 m in depth was different locally (Fig. 2). In northern Hiroshima Bay, the surface water salinity decreased from 32.8 to 30.0 psu at St. H1 and from 33.0 to 30.1 psu at St. H2 vertically, reflecting the freshwater discharge mainly from the Ota River. This freshwater influence gradually weakened to the south of the Nasami Strait with the minimum salinity values of 31.5 psu at St. H3 and 32.8 psu at St. H4. In Aki Nada, freshwater influence was small at every sampling station with a very uniform vertical profile of salinity ranging from 33.1 to 33.6 psu.

The thermoclines in the surface layer were sharp in Hiroshima Bay relative to Aki Nada (Fig. 2). In particular, the water temperature at St. H2 increased sharply 4.4°C at the depth range of 0 to 5 m. By contrast, the vertical change of temperature in Aki Nada did not exceed 2.4°C above 10 m in depth at any station.

Table 1. The carbon and nitrogen stable isotope ratios of Kobi squid (60–80 mm in DML) collected in Hiroshima Bay and Aki Nada on May 13–14, 1999

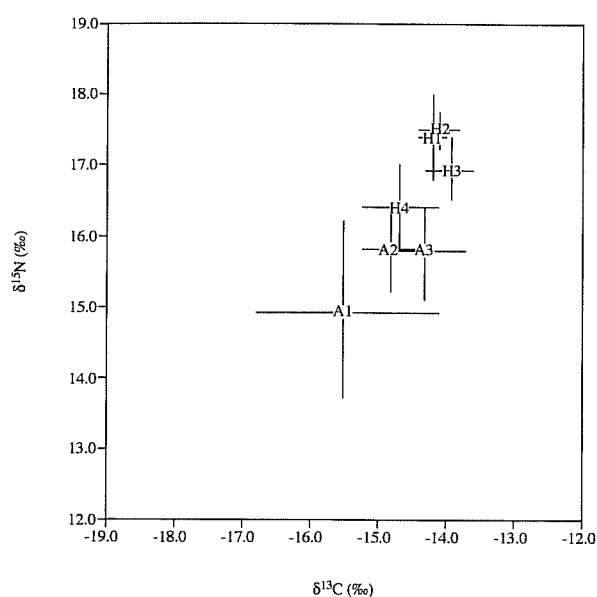
station	depth (m)	n	DML (mm±SD, Max–Min)	$\delta^{13}\text{C}$ (‰±SD, Max–Min)	$\delta^{15}\text{N}$ (‰±SD, Max–Min)
H1	15	28	65±5 (79–60)	-14.2±0.2 (-13.9--14.7)	17.4±0.6 (18.2–15.9)
H2	12	4	66±4 (71–61)	-14.1±0.3 (-13.8--14.5)	17.5±0.3 (17.8–17.2)
H3	25	12	72±7 (80–61)	-13.9±0.3 (-13.5--14.6)	16.9±0.4 (17.6–16.3)
H4	27	14	70±7 (80–60)	-14.7±0.5 (-13.9--15.7)	16.4±0.6 (17.7–15.4)
A1	9	22	68±5 (79–60)	-15.5±1.3 (-13.2--17.2)	14.9±1.3 (16.9–13.2)
A2	43	10	71±5 (77–61)	-14.8±0.4 (-13.8--15.4)	15.8±0.6 (17.1–15.0)
A3	29	9	76±3 (80–70)	-14.3±0.5 (-13.5--15.0)	15.8±0.6 (16.9–14.9)

Stable isotope ratios of Kobi squid

The Kobi squid of 60–80 mm in DML had $\delta^{13}\text{C}$ values ranging from -17.2 to -13.2‰ and $\delta^{15}\text{N}$ values ranging from 13.2 to 18.2‰ (Table 1; Appendix 1). There were significant local differences in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among the squid (60–80 mm in DML) collected at the seven sampling stations ($n=99$; Kruskal-Wallis test; $\delta^{13}\text{C}$, $P<0.0001$; $\delta^{15}\text{N}$, $P<0.0001$). It was obvious that the squid collected in northern Hiroshima Bay were more enriched in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ relative to the squid collected in central Hiroshima Bay and Aki Nada (Table 1; Fig. 3).

The Kobi squid collected in the northernmost region of Aki Nada (St. A1) showed a peculiarly wide isotopic variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Table 1; Fig. 3). Since significant correlations between body size and $\delta^{15}\text{N}$ were reported for some kinds of carnivores (Kasamatsu *et al.*, 1998; Takai and Sakamoto, 1999), we suspected that this wide isotopic variation might be related to the body size of the squid. Thus the squid collected at St. A1 were examined for three size classes, <60 mm, 60–80 mm, and >80 mm in DML. It appeared that the largest five squid of >80 mm in DML showed high isotopic values in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ while the isotopic variations in <60 mm and 60–80 mm in DML were unrelated to the body size (Table 2; Fig. 4).

On the other hand, the correlations between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were remarkable for the squid at St. A1 (Table 2; Fig. 4). There were significant positive correlations between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the squid of <60 mm ($n=33$, $r=0.89$, $P<0.0001$) and 60–80 mm ($n=22$, $r=0.91$, $P<0.0001$) in DML. On the $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ map, most of these squid were separately distributed in two distinct areas (Fig. 4); in one group the $\delta^{13}\text{C}$ value mainly ranged from -17.5 to -16.0‰ and the $\delta^{15}\text{N}$ value from 13.0 to 14.5‰ and in the other group the $\delta^{13}\text{C}$ value mainly ranged from -15.0 to -13.0‰ and the $\delta^{15}\text{N}$ value from 15.0 to 17.0‰. The squid (60–80 mm) with $\delta^{13}\text{C}$ values of at least -16.0‰ at St. A1 had an average $\delta^{13}\text{C}$ value of $-14.4\pm 0.9\%$ and $\delta^{15}\text{N}$ of $16.0\pm 0.7\%$ ($n=12$), being very close to the isotopic values of the squid collected at Sts. A2 and A3 (Table

**Figure 3.** The $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ map for Kobi squid of 60–80 mm in DML. The values are shown as mean values±SD.

1). The squid (60–80 mm) with $\delta^{13}\text{C}$ values lower than -16.0‰ at St. A1 had an average $\delta^{13}\text{C}$ value of $-16.7\pm 0.3\%$ and $\delta^{15}\text{N}$ value of $13.7\pm 0.4\%$ ($n=10$). This isotopic distribution was clearly heterogeneous compared with the other squid collected in this study (Figs. 3, 4).

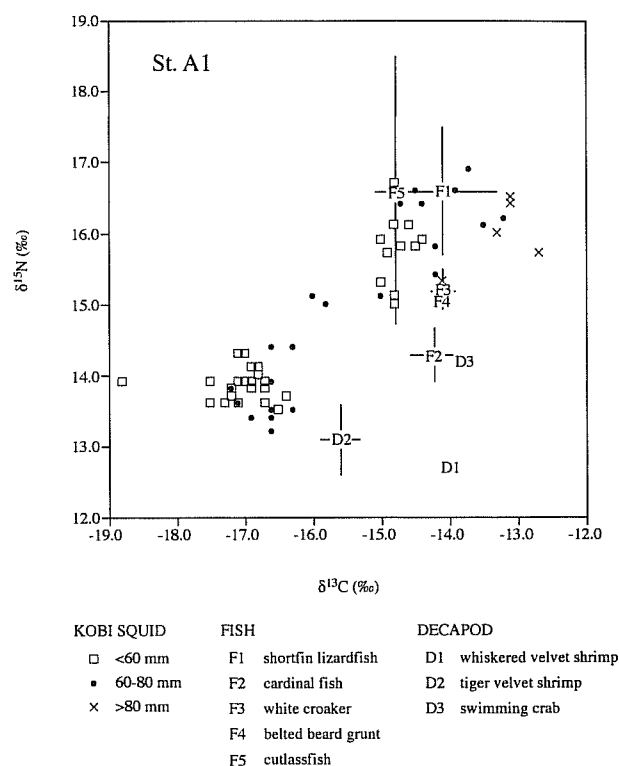
Stable isotope ratios of fish and decapod

According to Takechi (1989), the stomach contents of the Japanese squid in Sendai Bay consisted mainly of fishes and decapods, suggesting that these animals are important food sources of the Kobi squid as well. Therefore, we also analyzed the stable isotope ratios of fishes and decapods captured with the bottom trawl net at St. A1 on May 13, 1999, in order to examine the trophic relationship between the Kobi squid and those animals.

The isotopic values of the fishes ranged from -15.2 to -13.4‰ in $\delta^{13}\text{C}$ and 13.7 to 20.6‰ in $\delta^{15}\text{N}$, closely overlapping with the squid group with high $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ val-

Table 2. the carbon and nitrogen stable isotope ratios of <60 mm, 60–80mm, and >80 mm in DML in Kobi squid collected at St. A1. The correlation coefficients between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are also shown.

size class	n	DML (mm \pm SD, Max–Min)	$\delta^{13}\text{C}$ (‰ \pm SD, Max–Min)	$\delta^{15}\text{N}$ (‰ \pm SD, Max–Min)	correlation coefficient
<60 mm	33	47 \pm 10 (59–25)	-16.2 \pm 1.2 (-14.4–-18.8)	14.6 \pm 1.0 (16.7–13.5)	r=0.89 (P<0.0001)
60–80 mm	22	68 \pm 5 (79–60)	-15.5 \pm 1.3 (-13.2–-17.2)	14.9 \pm 1.3 (16.9–13.2)	r=0.91 (P<0.0001)
>80 mm	5	97 \pm 14 (117–82)	-13.3 \pm 0.5 (-12.7–-14.1)	16.0 \pm 0.5 (16.5–15.3)	r=0.55 (P=0.38)

**Figure 4.** The $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ map for Kobi squid collected at St. A1 on May 13, 1999. The isotopic values are shown not only for 60–80 mm in DML, but also for <60 mm and >80 mm in DML. The isotopic values (mean \pm SD) of fishes and decapods collected at St. A1 on the same sampling date are also shown.

ues collected at St. A1 on the $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ map (Table 3; Appendix 2; Fig. 4). On the other hand, the isotopic values of the decapods ranged from -15.9 to -13.8‰ in $\delta^{13}\text{C}$ and 12.7 to 14.2‰ in $\delta^{15}\text{N}$ (Table 3; Appendix 3). It was notable that whiskered velvet shrimp *Metapenaeopsis barbata* and tiger velvet shrimp *Metapenaeopsis acclivis* were clearly more depleted in $\delta^{15}\text{N}$ than the fishes and the squid with high $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Fig. 4).

Discussion

Stable isotope characteristics of Kobi squid in the western Seto Inland Sea

The Kobi squid in the western Seto Inland Sea showed

strikingly high $\delta^{15}\text{N}$ values, compared with the $\delta^{15}\text{N}$ distribution of cephalopods captured in the oceans of the world (Table 1; Appendix 1). The maximum value of oceanic cephalopods was reported for the purpleback flying squid *Sthenoteuthis oualaniensis* captured in the eastern South Pacific Ocean off Peru, a well known denitrification area (16.3 \pm 0.6‰, 15.3–16.8‰; Takai *et al.* 2000), while a large portion of the Kobi squid collected in this study had $\delta^{15}\text{N}$ exceeding that of the purpleback flying squid, with a maximum value of 18.2‰ (St. H1). Hiroshima Bay is surrounded by industrial areas and thus has a large inflow of wastewater via rivers. In such polluted areas, ^{15}N -enriched nitrogen input from wastewater often increases $\delta^{15}\text{N}$ of the aquatic life (Cabana and Rasmussen, 1996; McClelland and Valiela, 1997). It was considered that the strikingly high $\delta^{15}\text{N}$ values of the Kobi squid would clearly reflect such ^{15}N -enriched nitrogen from wastewater.

In Hiroshima Bay, the Kobi squid were significantly more enriched in $\delta^{15}\text{N}$ in the northern bay than in the central bay (Table 1; Fig. 3). Similar local difference in $\delta^{15}\text{N}$ was observed previously for macroalgae collected in the bay (Takai *et al.*, 2001); the isotopic values of *Gracilaria textorii*, *Ahnfeltiopsis flabelliformis*, *Polysiphonia senticulosa*, *Grateloupia lanceolata* collected in March 2000 and *Ulva pertusa* collected in August 1999 and June 2000 were 0.5–2.9‰ more enriched at the northernmost shore than at the island shore in the central area. In Hiroshima Bay, 63% of freshwater and 82% of total nitrogen load in the bay flows into the northern bay area (Lee and Hoshika, 2000). It was thus considered that the Kobi squid collected in the northern bay area would be particularly under the strong influence of the wastewater inflow.

Isotopic grouping of Kobi squid

The distance between the sampling stations H3 and H4 was only about 10 km (Fig. 1), but the isotopic distributions of the Kobi squid collected at these stations were different on the $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ map (Fig. 3). The squid at St. H3 were close to those at Sts. H1 and H2, while the squid at St. H4 were close to those at Sts. A2 and A3. Accordingly, it was considered that the squid collected at Sts. H1, H2 and H3 and those collected at Sts. H4, A2 and A3 would form isotopically different groups.

The Kobi squid at St. A1 showed a peculiarly wide

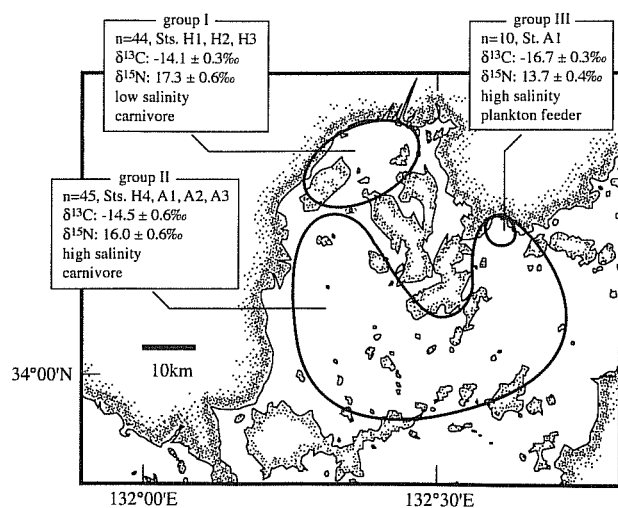


Figure 5. The schematic distribution map of isotopically categorized groups of Kobi squid in late spring. The habitat areas of the groups are encircled by solid lines, respectively.

isotopic variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Table 1; Fig. 3). It was caused by the existence of isotopically different groups, one group with a high $\delta^{13}\text{C}$ value of $-14.4 \pm 0.9\text{‰}$ and a high $\delta^{15}\text{N}$ value of $16.0 \pm 0.7\text{‰}$ ($n=12$) and the other with a low $\delta^{13}\text{C}$ value of $-16.7 \pm 0.3\text{‰}$ and a low $\delta^{15}\text{N}$ value of $13.7 \pm 0.4\text{‰}$ ($n=10$) (60–80 mm in DML; Fig. 4). The former group overlapped closely in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with the squid collected at Sts. H4, A2, and A3 (Figs. 3, 4), and thus they were categorized into the same group. On the other hand, the latter group of the squid at St. A1 was isotopically heterogeneous compared with the other squid collected on the same sampling dates. This group was considered to be distinct from the other groups.

Consequently, the Kobi squid (60–80 mm in DML) collected in late spring were divided into three groups, I, II, and III (Fig. 5). Group I consisted of the squid collected at Sts. H1, H2 and H3, and group II consisted of the squid collected at Sts. H4, A1, A2 and A3. Group III consisted only of the squid with low $\delta^{13}\text{C}$ values and low $\delta^{15}\text{N}$ values at St. A1.

Habitat use of the groups

The squid of group I inhabited the northern semi-enclosed area of Hiroshima Bay (Fig. 5). In Hiroshima Bay, the surface water salinity is relatively low in the northern bay area (Hashimoto *et al.*, 1994) that has a freshwater inflow of 63% (Lee and Hoshika, 2000). This local difference in the salinity vertical profile was also found on the sampling date (Fig. 2), in which the surface water salinity at St. H3 was relatively low as was the salinity at Sts. H1 and H2. It was thus speculated that the squid of group I might prefer a low salinity environment.

The habitat area of group II was broad ranging from

central Hiroshima Bay to the northernmost region of Aki Nada (Fig. 5). Considering that the salinity vertical profiles at Sts. H4, A1, A2 and A3 were similarly uniform (Fig. 2), it was speculated that the squid collected at these stations might prefer a high salinity environment. The local difference in salinity environment might obstruct the frequent mixing of the Kobi squid between Sts. H3 and H4.

Group III was only found at St. A1. This station is located in the shallowest edge zone in Aki Nada and is little influenced by freshwater inflow (Figs. 1, 2). Here it is unclear whether or not this shallow topography would influence the habitat selection of this group.

Trophic positions of the groups

The $\delta^{15}\text{N}$ of POM (0.7–125 μm) collected from the surface water of central Hiroshima Bay averaged $8.3 \pm 1.3\text{‰}$ ($n=10$) from May 14, 1999 to Nov. 10, 2000 (Takai *et al.*, 2001). Also macroalgae collected on the shore near St. H3 showed similar average $\delta^{15}\text{N}$ values from June 15, 1999 to June 30, 2000; $8.5 \pm 1.0\text{‰}$ in Phaeophyceae ($n=30$), $9.1 \pm 0.9\text{‰}$ in Rhodophyceae (except samples with special $\delta^{13}\text{C}$ values of $< -30\text{‰}$; $n=20$), $9.4 \pm 1.4\text{‰}$ in Ulvophyceae ($n=8$), and $8.6 \pm 0.5\text{‰}$ in Chlorophyceae ($n=3$) (Takai *et al.*, 2001). These $\delta^{15}\text{N}$ values indicate that the $\delta^{15}\text{N}$ of the primary producers in central Hiroshima Bay would be mainly distributed from 7 to 10‰. It appears that this $\delta^{15}\text{N}$ distribution of 7–10‰ also applies to the distribution of the primary producers in Aki Nada; the $\delta^{15}\text{N}$ values were 8.8–9.4‰ in the POMs (0.7–125 μm) in the surface layer and 7.0–10.7‰ in 13 species of macroalgae collected in Aki Nada on May 13, 1999 (Takai, unpubl. data).

Compared with this $\delta^{15}\text{N}$ distribution of 7–10‰ in the primary producers, the average $\delta^{15}\text{N}$ of $13.7 \pm 0.4\text{‰}$ in the group III at St. A1 was 3.7–6.7‰ more enriched (Fig. 4). This $\delta^{15}\text{N}$ difference is equivalent to trophic positional difference of 1.1–2.0 trophic level, calculated on the basis of $\delta^{15}\text{N}$ increase of 3.4‰ per trophic level according to Vander Zanden *et al.* (1997). This means that the trophic position of the group III would be 2.1–3.0, being defined primary producers as trophic level “1.” Also considering that the average $\delta^{13}\text{C}$ of $-16.7 \pm 0.3\text{‰}$ (-17.2 to -16.3‰) in the group III was peculiarly close to the $\delta^{13}\text{C}$ values of POMs as an indicator of plankton ($n=10$, $-20.1 \pm 1.7\text{‰}$; Takai *et al.*, 2001), it was inferred that the group III would feed on phytoplankton and/or zooplankton.

Group II collected at St. A1 showed an average $\delta^{15}\text{N}$ of $16.0 \pm 0.7\text{‰}$, being 6.0–9.0‰ higher than the primary producers of 7–10‰ in $\delta^{15}\text{N}$ (Fig. 4). Calculated on the basis of 3.4‰ increase per trophic level in $\delta^{15}\text{N}$, this difference in group II is equivalent to the trophic positional difference of 1.8–2.6. This means that the trophic position of the group II at St. A1 would be 2.8–3.6.

The $\delta^{15}\text{N}$ value of fishes and decapods collected at St.

Table 3. The carbon and nitrogen stable isotope ratios of fishes and decapods collected with a bottom trawl net at St. A1 on May 13, 1999.

species	n	body length (mm±SD, Max–Min)*	$\delta^{13}\text{C}$ (‰±SD, Max–Min)	$\delta^{15}\text{N}$ (‰±SD, Max–Min)
FISH				
shortfin lizardfish <i>Saurida elongata</i>	3	188±17 (203–169)	-14.1±0.8 (-13.6–15.1)	16.6±0.9 (17.6–15.9)
cardinal fish <i>Apogon lineatus</i>	13	57±9 (72–42)	-14.2±0.4 (-13.4–15.0)	14.3±0.4 (14.9–13.7)
white croaker <i>Pennahia argentata</i>	4	123±14 (140–111)	-14.1±0.2 (-13.9–14.4)	15.2±0.3 (15.6–14.9)
belted beard grunt <i>Haplogenyus mucronatus</i>	1	150	-14.1	15.1
cutlassfish <i>Trichiurus japonicus</i>	8	482±215 (820–290)	-14.8±0.3 (-14.2–15.2)	16.6±1.9 (20.6–15.3)
DECAPOD				
whiskered velvet shrimp <i>Metapenaeopsis barbata</i>	4	57±9 (65–44)	-14.0±0.1 (-13.9–14.1)	12.7±0.1 (12.8–12.7)
tiger velvet shrimp <i>Metapenaeopsis acclivis</i>	4	54±4 (59–50)	-15.6±0.3 (-15.2–15.9)	13.1±0.5 (13.8–12.8)
swimming crab <i>Portunus trituberculatus</i>	1	109	-13.8	14.2

* Total length was measured for the cutlassfish and standard length was measured for all the other fishes. Body length of the shrimps was measured from the posterior edge of orbit to the tip of telson. Cephalothorax length was measured for the swimming crab.

A1 on May 13, 1999 ranged from 12.7‰ in whiskered velvet shrimp to 20.6‰ in cutlassfish *Trichiurus japonicus* (Table 3; Fig. 4). The difference in $\delta^{15}\text{N}$ between group II at St. A1 and the two shrimp species was 2.9–3.3‰, being quite similar to the $\delta^{15}\text{N}$ increase of 3.4‰ per trophic level (Vander Zanden et al., 1997), and the average $\delta^{13}\text{C}$ of $-14.4\pm 0.9\text{‰}$ in group II at St. A1 was well consistent with the averages of $-14.0\pm 0.1\text{‰}$ in the whiskered velvet shrimps and $-15.6\pm 0.3\text{‰}$ in the tiger velvet shrimps. These results suggest that the squids in group II at St. A1 are carnivores feeding on decapods and so forth.

The squid of group II collected at Sts. H4, A2 and A3 overlapped closely in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with the group II at St. A1 (Table 1; Figs. 3, 4). This suggests that the former are carnivores feeding on the decapods and so forth like the latter.

The squid of group I collected at Sts. H1, H2 and H3 were clearly more enriched in $\delta^{15}\text{N}$ than those of group II at St. H4 (Table 1; Fig. 3). It appears as if the trophic position of the squid was higher in northern Hiroshima Bay than in the central bay area. However, it was actually considered that the high $\delta^{15}\text{N}$ values would be affected by the inflow of ^{15}N -enriched nitrogen from wastewater into the northern bay area as mentioned above and thus that the trophic positions of groups I and II would probably be almost equal. Also considering that the $\delta^{13}\text{C}$ value of $-14.1\pm 0.3\text{‰}$ in group I was very similar to that of $-14.5\pm 0.6\text{‰}$ in group

II, these squid of group I were inferred to feed on ^{13}C -enriched animals like those of group II.

In this study, the Kobi squid collected in Hiroshima Bay and Aki Nada in late spring were isotopically divided into the three groups, being characterized by habitat use and trophic positions. This suggests that the life types of this species are diverse in the Seto Inland Sea. Hereafter it is necessary to precisely examine the seasonal variation of the habitat use and trophic position all over the sea, in order to clarify their synthetic life historical characteristics in the sea.

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Habitat use and trophic positions of Kobi squid

Appendix 1. The DML (mm), $\delta^{13}\text{C}$ (‰), and $\delta^{15}\text{N}$ (‰) of Kobi squid collected at Sts. H1-H4 on May 14, 1999 and Sts. A1-A3 on May 13, 1999.

St.	DML	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	St.	DML	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
H1 (60–80 mm in DML)				72	-13.9	17.7		
60	-14.4	16.8		73	-14.2	16.4		
60	-13.9	17.3		74	-15.0	16.0		
60	-13.9	17.9		78	-14.2	17.0		
60	-14.4	17.1		78	-14.1	16.5		
62	-14.1	17.8		80	-15.7	15.4		
62	-14.7	17.5	A1(60–80 mm in DML)					
62	-14.3	18.1		60	-17.1	13.6		
62	-14.4	17.8		61	-13.5	16.1		
62	-14.3	16.9		64	-17.2	13.8		
63	-14.1	18.2		64	-14.7	16.4		
63	-14.1	17.1		64	-16.6	13.2		
63	-14.3	17.6		65	-16.6	14.4		
63	-14.5	16.7		65	-16.6	13.5		
63	-14.4	17.6		66	-16.9	13.4		
63	-14.4	17.1		67	-16.3	13.5		
64	-14.0	17.2		67	-16.6	13.4		
64	-14.3	16.9		67	-16.0	15.1		
66	-14.3	17.6		67	-13.7	16.9		
66	-14.1	17.8		67	-14.2	15.8		
67	-14.1	17.9		69	-14.5	16.6		
68	-14.2	17.6		70	-16.6	13.9		
69	-14.4	15.9		70	-14.4	16.4		
70	-14.3	16.2		72	-15.8	15.0		
71	-13.9	18.2		73	-16.3	14.4		
72	-14.2	17.7		76	-15.0	15.1		
74	-14.1	17.9		76	-13.2	16.2		
75	-14.4	17.0		76	-13.9	16.6		
79	-14.1	18.2		79	-14.2	15.4		
H2 (60–80 mm in DML)				A1 (>80 mm in DML)				
67	-14.2	17.2		82	-14.1	15.3		
71	-14.0	17.6		85	-13.1	16.5		
61	-14.5	17.4		96	-13.3	16.0		
66	-13.8	17.8		103	-12.7	15.7		
H3 (60–80 mm in DML)				117	-13.1	16.4		
61	-14.1	16.6	A1 (<60 mm in DML)					
62	-13.7	17.3		25	-14.8	16.7		
64	-14.6	16.3		26	-14.7	15.8		
66	-13.9	16.8		30	-14.6	16.1		
68	-14.3	16.4		31	-15.0	15.3		
75	-13.5	17.6		35	-14.6	16.1		
75	-13.5	17.4		40	-14.9	15.7		
76	-13.7	17.4		40	15.0	15.9		
77	-13.9	16.8		41	-14.8	15.0		
79	-14.0	17.1		42	-14.5	15.8		
80	-14.0	17.0		42	-17.1	13.9		
80	-14.0	16.7		42	-14.8	16.1		
H4 (60–80 mm in DML)				44	-16.7	13.8		
60	-15.0	15.9		45	-16.5	13.5		
61	-14.3	16.7		47	-17.0	14.3		
61	-15.4	16.0		47	-17.1	13.6		
63	-14.6	16.3		47	-17.5	13.6		
67	-14.9	16.0		50	-17.5	13.9		
69	-14.8	16.5		50	-16.9	13.8		
70	-14.8	16.3		51	-16.8	14.0		
71	-14.4	16.7		51	-16.9	13.9		
				52	-14.4	15.9		

Appendix 1. (Continued)

St.	DML	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	St.	DML	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
	52	-17.3	13.6	72	-14.5	16.4	
	54	-17.1	14.3	73	-15.4	15.3	
	55	-16.9	14.1	74	-14.6	16.4	
	55	-17.0	13.9	74	-14.7	15.8	
	56	-16.4	13.7	75	-14.9	17.1	
	56	-16.8	14.1	77	-14.8	15.5	
	56	-16.7	13.6	A3 (60–80 mm in DML)			
	56	-17.2	13.7	70	-15.0	15.2	
	57	-17.2	13.8	75	-14.0	16.9	
	58	-16.7	13.9	75	-14.4	16.6	
	58	-14.8	15.1	76	-13.8	15.6	
	59	-18.8	13.9	76	-13.7	15.9	
A2 (60–80 mm in DML)				76	-14.7	15.5	
	61	-15.3	15.8	76	-14.8	15.8	
	64	-15.1	15.5	76	-14.4	15.6	
	70	-13.8	15.0	80	-13.5	14.9	
	70	-14.6	15.7				

Appendix 2. The standard length (mm), $\delta^{13}\text{C}$ (‰), and $\delta^{15}\text{N}$ (‰) of fishes collected at St. A1 on May 13, 1999.

species	standard length*	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
shortfin lizardfish <i>Saurida elongata</i>			
	169	-13.7	15.9
	191	-15.1	17.6
	203	-13.6	16.2
cardinal fish <i>Apogon lineatus</i>			
	42	-13.8	13.9
	50	-14.6	14.5
	50	-15.0	14.7
	50	-14.1	14.7
	51	-15.0	14.9
	54	-13.9	14.1
	55	-13.4	14.0
	58	-14.3	14.7
	63	-14.0	13.7
	64	-14.4	14.4
	64	-14.3	13.9
	70	-14.0	14.2
	72	-14.3	14.5
white croaker <i>Pennahia argentata</i>			
	111	-14.4	14.9
	112	-14.0	15.0
	127	-14.1	15.2
	140	-13.9	15.6
belted beard grunt <i>Hapalogenys mucronatus</i>			
	150	-14.1	15.1
cutlassfish <i>Trichiurus japonicus</i>			
	290	-14.5	15.5
	325	-15.2	15.6
	335	-15.0	15.4
	345	-15.0	15.3
	355	-14.8	15.5
	688	-14.2	20.6
	700	-14.6	16.5
	820	-15.0	18.1

* Total length was measured for the cutlassfish.

Appendix 3. The body length (mm), $\delta^{13}\text{C}$ (‰), and $\delta^{15}\text{N}$ (‰) of decapods collected at St. A1 on May 13, 1999.

species	body length*	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
whiskered velvet shrimp <i>Metapenaeopsis barbata</i>	62	-13.9	12.7
	65	-14.0	12.7
	56	-14.1	12.8
	44	-14.0	12.7
tiger velvet shrimp <i>Metapenaeopsis acclivis</i>	59	-15.9	12.8
	57	-15.9	13.0
	51	-15.6	12.8
	50	-15.2	13.8
swimming crab <i>Portunus trituberculatus</i>	109	-13.8	14.2

* Body length of the shrimps was measured from the posterior edge of orbit to the tip of telson. Cephalothorax length was measured for the swimming crab.

炭素・窒素安定同位体比から推察した晩春期の瀬戸内海西部海域における ヒメジンドウイカ *Loliolus sumatrensis* の生息場所および栄養段階

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晩春期の瀬戸内海西部海域におけるヒメジンドウイカの生息場所および栄養段階を推察するため、炭素・窒素安定同位体比を分析した。本種の $\delta^{15}\text{N}$ は沖合・外洋域の頭足類に比べて著しく高く、都市排水起源の ^{15}N に富んだ窒素の影響を強く受けていたことが示唆された。外套長 60~80 mm の採集個体の $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ マップ上での分布は、採集地点間で明瞭に異なっていた。この同位体比分布に基づき、採集個体は 3 群 (I, II, III) に分けられた。群 I は河川水流入

量が多く塩分が比較的低い広島湾湾奥部で採集され、群 II は河川水流入の影響が小さい広島湾湾中部と安芸灘で採集された。これらの 2 群は十脚類などを捕食する肉食者であることが示唆された。群 III は、安芸灘北縁部の浅所でのみ採集された。この群は、 $\delta^{13}\text{C}$ 、 $\delta^{15}\text{N}$ ともに特異的に低く、植物もしくは動物プランクトンを摂食するプランクトン食者であることが示唆された。

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