Therefore, we are looking forward to future cooperation in Asia.

Taiwan

Asian dust storms occur in the winter and spring, especially from March to May, and mainly originate in the Gobi and Takla Makan deserts of Mongolia and western China. They can move eastward to China, Japan, South Korea, Taiwan, and sometimes to northern Pacific Ocean areas. Particulate matter (PM), especially PM with aerodynamic diameters of <10 μm (PM_{10}), have been recorded at concentrations of >500 μg/m^3, occasionally even exceeding 1,000 μg/m^3, during several ADS in many cities located downwind of these deserts, including Shanghai, Hong Kong, and Taipei. Overall findings of epidemiological studies show that long-range transported Asian dust can increase mortality among residents in downwind areas. Dust derived from mineral soil in deserts and air pollutants from biomass burning are major contributors of long-range transported air pollution across many countries around the world. The negative effects of trans-boundary air pollution on public health are emerging as an important global health issue which needs to be further researched by academics and governments. Global efforts, including alleviating desertification speed in dust-originating areas and reducing industrial emissions along the dust-transporting paths, must be made to tackle the root-causes of trans-boundary air pollution in order to protect the global environment and public health. Collaboration among Asian public health researchers to tackle this trans-boundary pollution problem is recommended as a major step towards protecting public health and environmental quality in this fast growing continent.

Thailand

Thailand is a newly industrialized country and one of the fastest growing economies, ranked 24th on the global market. Thailand is now experiencing environmental issues as a downside of economic growth, which include deforestation and air pollution, among many others. Assessment of health damage due to industrialization and associated remediation approaches are important in Thailand. Climate change, which may affect human health through a range of mechanisms, including the relatively direct risks of floods and storms and the more complex pathways of altered patterns of infectious disease outbreak, is a growing concern of Thailand, and one that might be appropriately addressed by collaborative efforts within Asian countries. Scientific research on common environmental issues, such as trans-boundary air pollution and climate change, is essential given the nature of the problems. It is also important to encourage community empowerment by, for example, organizing practical workshops aimed at distributing research findings to the community or by initiating local-based research activities in accordance with their most critical environmental problems.

Consensus and future directions

We have reached a consensus on the long- and short-term objectives of the forum. As the long-term objective, we should collaborate more closely on Asian environmental health problems at several levels—individual scientist level, academic society level, and governmental level. We believe that collaborations among academic societies can provide the greatest impulse. In pursuing the short-term goals, we agreed it would be important to develop or foster a high-quality scientific journal in environmental health. *Environmental Health and Preventive Medicine* (EHPM) is obviously one of the most potential candidate journals for this role.

To identify priority environmental health issues in the region and to develop a collaborative research network to resolve the challenges, we agreed to organize a steering committee for the Asian Environmental Health Forum, the members of which will be chosen from among the delegates from Asian countries. The committee will support policy-makers in each country by providing knowledge and sharing experiences from other countries. It will also play a pivotal role in identifying those areas warranting the attention of policy-makers and provide channels of communication among policy-makers of countries that share or have shared similar challenges.

To form a steering committee for the forum and to identify environmental health issues in the region that should be given priority in terms of policy, the Korean Society of Environmental Health will take the initiative to organize a series of annual workshops in collaboration with Ministry of Environment of Korea. We hope that the initiative will promote academic communication and identify ideas for synergistic collaborations on environmental health issues during the next 30 years.

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Highlighted Article

Stable isotope ratios and mercury levels in red meat products from baleen whales sold in Japanese markets

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ABSTRACT

We analyzed the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values and Hg concentration in red meat products originating from the predominant types sold in Japan for human consumption: two populations of common minke (J- and O-types), Bryde's and sei whales in the western North Pacific Ocean, and fin and Antarctic minke whales in the Southern Ocean. The order of the trophic positions, evaluated by $\delta^{15}N$ values and Hg concentrations, coincided with their known feeding habits: common minke (J-type)=common minke (O-type) > Bryde's ≥ sei ≥ Antarctic minke ≥ fin. The Hg concentrations in the combined samples from the six samples were significantly correlated with their $\delta^{15}N$ values ($\gamma=0.455$, $n=66$, $p<0.05$), reflecting overall differences in the trophic level. This correlation was not significant for within-species comparison for the common minke (J- and O-types) or the Bryde's whale, probably reflecting the higher $\delta^{15}N$ value and lower Hg concentration in the North Pacific Ocean around Japan. Determination of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ could be used to discriminate between the red meat products originating from the whale species in the North Pacific and Southern Oceans. However, the four whale species or populations in the Pacific Ocean could not be discriminated on basis of these values, nor could the two species in the Southern Ocean. Positive correlations between the $\delta^{13}C$ and $\delta^{15}N$ values and negative correlations between the $\delta^{15}N$ and $\delta^{18}O$ values and the $\delta^{13}C$ and $\delta^{18}O$ values, probably reflecting migration patterns, were found in some whale species in the North Pacific and Southern Oceans.

1. Introduction

Products from whales, dolphins and porpoises (Suborder Cetacea) are sold in Japan for human consumption. Currently, most whale products for human consumption are supplied from the scientific whaling of baleen whales, small-type coastal whaling of toothed whales, and the drive and hand-harpoon fishing of small whales, dolphins and porpoises as well as incidental catch by set nets (Endo et al., 2003). Red meat (muscle) products are the most popular whale products sold in Japan, and most Japanese consumers prefer the red meat originating from mysticetes (baleen whales) to that from odontocetes (toothed whales, dolphins and porpoises). Most red meats from mysticetes sold in Japan originate from the Antarctic minke whale (*Balaenoptera bonaerensis*) and fin whale (*Balaenoptera physalus*) taken in the Southern Ocean and the common minke whale (*Balaenoptera acutorostrata*), Bryde's whale (*Balaenoptera edeni*) and sei whale (*Balaenoptera borealis*) taken in the western North Pacific Ocean. Common minke whales can be categorized into at least two types: the "O type", found primarily in the offshore Pacific Ocean, and the "J type", found primarily in the Sea of Japan and nearshore waters along Japan's Pacific coast (Wade et al., 2010). O-type minke whales are the primary target of Japanese scientific whaling in both coastal and offshore waters of the Pacific, while J-type minke whales are primarily taken as bycatch in coastal set nets around the entire Japanese coastline. Although most baleen whales are assumed to migrate annually between feeding habitat in high latitudes and breeding habitat in low latitudes, the pattern of migration is poorly known for some of the species sampled here.

As odontocetes are long-lived and occupy the top levels of the marine food web, feeding mainly on fish and squid, they biomagnify marine pollutants such as heavy metals and organochlorine compounds (Haraguchi et al., 2000). Among these pollutants,

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contamination with mercury (Hg) is prominent (Endo et al., 2003, 2004, 2005). The contamination levels of pollutants in mysticetes are lower than those in odontocetes, reflecting their preference for plankton and small fish species (i.e., their lower trophic positions). Among the baleen whale species sold in Japan, common minke whales are opportunistic and omnivorous feeders that change their prey temporally and regionally. Compared with common minke whales, Bryde's and sei whales are only moderately omnivorous feeders (Mitani and Bando, 2008; Yasunaga and Fujise, 2009a, b), and Antarctic minke and fin whales are generally zooplankton feeders. In our previous survey of Hg levels (Endo et al., 2003), only one of the 62 red meat products originating from mysticetes showed a Hg concentration exceeding the Japanese permitted level for fish and shellfish (0.4 μg/wet g), whereas all red meat products originating from odontocetes ($n=137$) exceeded the permitted level.

Stable isotope analysis has been used as a tool to obtain information on the feeding ecology of marine species. The $\delta^{15}N$ value shows a stepwise increase in the trophic level of a food chain (Kelly, 2000), and a positive correlation between the $\delta^{15}N$ value and the Hg concentration in biota has been reported (Yoshinaga et al., 1992; Kidd et al., 1995). On the other hand, the $\delta^{13}C$ value is used to indicate the relative contribution to the diet of potential primary sources, and can demonstrate differences between species taking coastal and offshore prey or between those taking pelagic and benthic prey (Kelly, 2000). A significant increase in $\delta^{15}N$ of 3.4 ± 1.1‰ has been shown to occur between consumer and prey (Minagawa and Wada, 1984), whereas only a small enrichment of about 1‰ is found in the $\delta^{13}C$ value (DeNiro and Epstein, 1981). We recently reported that the $\delta^{15}N$ and $\delta^{13}C$ values in odontocetes caught off or stranded on the coast of northern Japan were higher and lower, respectively, than those in whales in the southern area, probably reflecting the variations in marine environment around Japan (Endo et al., 2010). Mitani et al. (2006) analyzed the $\delta^{13}C$ and $\delta^{15}N$ values in the baleen plates of common minke whales caught during scientific research whaling, and tried to elucidate the migration pattern in relation to dietary shift. However, little information is available about the $\delta^{13}C$ and $\delta^{15}N$ values in the muscle of baleen whales, including common minke, Bryde's and sei whales, caught in the western North Pacific Ocean and Antarctic minke and fin whales caught in the Southern Ocean.

Recently, the $\delta^{18}O$ value, in addition to the $\delta^{13}C$ and $\delta^{15}N$ values, has been used to discriminate, verify and identify the habitat of plants and animals, as the $\delta^{18}O$ value reflects the water environment, temperature and humidity of their habitats. For instance, the $\delta^{18}O$ values in beef oil (Heaton et al., 2008), underground water (Mizota and Kusakabe, 1994) and cultured rice (Suzuki et al., 2009) all tend to decrease with latitude (temperature). To our knowledge, however, the $\delta^{18}O$ values in cetacean species have not yet been reported. According to the above latitude-dependent changes, we speculated that the $\delta^{18}O$ value would be lower in cetaceans caught off the northern areas than off the southern areas of Japan, and that $\delta^{13}C$ and $\delta^{15}N$ values would be negatively correlated with the $\delta^{18}O$ value in the whale products sold in Japan.

The purpose of the present study was to analyze the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ and the Hg concentration in red meat products originating from the common minke (J- and O- types), Bryde's and sei whales caught in the western North Pacific Ocean and Antarctic minke and fin whales caught in the Southern Ocean. We discuss the correlations between the trophic level, as evaluated by $\delta^{15}N$ value, and the Hg contamination and among the $\delta^{15}N$, $\delta^{13}C$ and $\delta^{18}O$ values, and the possibility of verifying the species origins of red meat products sold in Japan using these stable isotope ratios.

2. Materials and methods

2.1. Sampling of red meat products and genetic analysis for species origin

Red meat products originating from common minke whale (J- and O-types), Bryde's and sei whales caught in the Northwest Pacific Ocean and Antarctic minke and fin whales caught in the Antarctic Ocean were purchased from retail outlets in Japan between 2000 and 2006, as described previously (Endo et al., 2003, 2005). Samples were stored at −20 °C until analysis.

As reported elsewhere (Baker et al., 1996, 2006), the species origin of cetacean products was identified by mitochondrial DNA sequences (control region and cytochrome *b*) amplified from the products via PCR. The population origin of the common minke whale products (i.e., J- or O-type) was inferred from sequence variation in the mtDNA control region, as described in Baker et al. (2000).

2.2. Chemical analyses

The total mercury (Hg) concentration in the red meat products was determined by a Mercury Analyzer SP-2 (Nippon Instruments Corporation, Tokyo, Japan), as reported previously (Endo et al., 2007). DOLT-2 (National Research Council of Canada) was used as an analytical quality control for Hg. The recovery of Hg was $94 \pm 3\%$ ($n=5$). The Hg concentration in the red meat products was expressed on a wet weight basis.

Dried subsamples of red meat products were analyzed for stable isotopes (^{13}C, ^{15}N and ^{18}O) after the removal of lipids by chloroform/methanol extraction (Logan and Lutcavage, 2008). The $\delta^{13}C$ and $\delta^{15}N$ analyses were performed using a mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany) coupled with an elemental analyzer (EA1108, Fisons, Rodano, Milan, Italy) held in the Center for Ecological Research (CER), Kyoto University (Kyoto, Japan), as reported previously (Endo et al., 2009, 2010). The $\delta^{18}O$ analysis was performed using a mass spectrometer (Delta V PLUS, Thermo Fisher Scientific, Tokyo, Japan) coupled with an elemental analyzer (TC/EA, Thermo Fisher Scientific, Tokyo, Japan) held in the SI Science Co. Ltd. (Saitama, Japan). The natural abundances of ^{13}C, ^{15}N and ^{18}O are expressed as per mil (‰) deviation from the standards as defined by the following equation:

$$\delta^{13}C, \delta^{15}N \text{ or } \delta^{18}O = (R_{sample}/R_{standard} - 1) \times 1000(‰),$$

where $R = {}^{13}C/{}^{12}C$, ${}^{15}N/{}^{14}N$ or ${}^{18}O/{}^{16}O$. CERKU-1, 2 and 5, certified by CER, were used as $\delta^{13}C$ and $\delta^{15}N$ reference materials (Tayasu et al., 2011), and benzoic acid (A and B), certified by Indiana University (IN, USA), was used as the $\delta^{18}O$ reference material.

2.3. Statistical analyses

The data are shown as mean ± S.D., and were analyzed by Turkey–Kramer multiple comparison test and Pearson's correlation coefficient test, using the Statcell program. The level of significance was set at $p < 0.05$.

3. Results and discussion

The stable isotope ratios of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ and the Hg concentration in sixty-six red meat product samples from baleen whale species and populations were analyzed (Table 1), and the analytical results are summarized in Table 2. Fig. 1 shows the relationship between the $\delta^{15}N$ value and the Hg concentration in the combined products from the six samples ($n=66$), and Fig. 2 shows the relationships among the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values. Table 3 shows a summary of relationships among the Hg concentration, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values for each baleen whale species or population.

In agreement with previously published results (Endo et al., 2003), the contamination levels of Hg in the red meat products were in the following order: common minke whales (J-type)= common minke whales (O-type) > Bryde's whale=sei whale=fin whale ≥ Antarctic minke whale (Table 2). A similar order was found in the $\delta^{15}N$ values for these species (Table 2). The Hg concentrations in the combined products of the six samples were significantly correlated with their $\delta^{15}N$ values (Fig. 1, $r=0.455$, $n=66$, $p < 0.05$). As data not shown in Figure, significant correlations were found between the $\delta^{15}N$ values and the Hg concentrations in the combined samples from the North Pacific Ocean ($r=0.418$, $n=46$, $p < 0.05$) and from the Southern Ocean ($r=0.541$, $n=20$, $p < 0.05$). These correlations between the Hg

Table 1
Analytical results of mercury and stable isotope ratios in red meat products originating from baleen whales sold in Japanese markets.

Species origin	Sample code	Hg (μg/wet g)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
Common minke whale, J-type	1	0.050	−18.4	11.5	11.9
	2	0.125	−19.3	11.6	10.6
	3	0.074	−17.2	15.8	9.7
	4	0.070	−19.1	11.4	11.6
	5	0.136	−18.0	15.0	10.5
	6	0.239	−17.5	12.6	11.9
	7	0.180	−18.6	10.3	13.3
	8	0.119	−18.7	11.0	12.8
	9	0.031	−19.1	12.1	12.2
	10	0.027	−18.6	12.7	11.8
	11	0.041	−18.3	11.5	12.9
	12	0.061	−17.5	9.6	13.9
	13	0.029	−19.0	11.2	13.4
Common minke whale, O-type	1	0.053	−19.0	12.1	13.0
	2	0.053	−18.3	12.1	13.9
	3	0.056	−18.5	11.6	13.8
	4	0.044	−19.2	11.5	13.1
	5	0.121	−18.0	11.5	13.6
	6	0.053	−20.3	11.4	12.0
	7	0.160	−17.9	12.0	13.8
	8	0.014	−19.1	9.7	13.7
	9	0.174	−17.6	11.1	12.3
	10	0.176	−17.6	12.0	12.2
	11	0.254	−18.9	10.2	12.9
	12	0.027	−18.9	10.9	12.2
Bryde's whale	1	0.037	−17.2	8.6	15.5
	2	0.090	−17.5	9.3	15.5
	3	0.063	−15.9	11.9	13.7
	4	0.053	−17.6	10.1	16.0
	5	0.070	−16.3	11.6	13.9
	6	0.027	−17.2	9.7	16.7
	7	0.055	−15.9	11.2	14.0
	8	0.056	−16.9	9.5	16.1
	9	0.045	−17.2	9.5	15.0
	10	0.055	−17.1	9.8	14.6
	11	0.067	−16.9	8.4	14.9
Sei whale	1	0.026	−23.1	6.3	15.3
	2	0.082	−18.3	9.6	15.8
	3	0.079	−19.1	8.5	13.7
	4	0.028	−21.7	7.1	16.5
	5	0.045	−19.4	7.6	15.6
	6	0.090	−18.9	8.7	15.4
	7	0.046	−18.7	7.6	14.6
	8	0.054	−18.6	10.3	13.1
	9	0.033	−18.1	8.1	14.9
	10	0.061	−18.7	9.5	15.0
Fin whale	1	0.047	−23.0	5.7	15.2
	2	0.050	−21.2	6.1	15.6
	3	0.026	−23.9	6.0	16.5
	4	0.042	−20.8	6.2	15.4
	5	0.031	−23.0	5.7	15.4
	6	0.052	−22.8	5.9	15.6
	7	0.041	−21.9	5.6	15.3
	8	0.090	−21.9	6.3	13.5
	9	0.026	−23.6	5.4	14.4
	10	0.031	−23.1	4.9	14.4
Antarctic minke whale	1	0.027	−24.3	6.1	14.9
	2	0.051	−24.8	6.0	14.2
	3	0.013	−24.2	5.7	13.8
	4	0.013	−25.1	5.9	14.9
	5	0.077	−24.7	6.0	15.2
	6	0.018	−23.9	6.1	13.2
	7	0.014	−24.7	6.3	14.5
	8	0.027	−25.1	6.4	15.1
	9	0.013	−24.7	6.7	15.5
	10	0.014	−24.7	7.2	14.3

level and the trophic level, as evaluated by $\delta^{15}N$ value, were firstly reported in the food products from Papuan New Guinea (Yoshinaga et al., 1992) and from the freshwater biota in Ontario, Canada (Kidd et al., 1995). Although there was an overall correlation in the combined sample from the six whale species or populations, only the sei whale had a significant within-species correlation between the Hg concentration and the $\delta^{15}N$ value ($\gamma=0.651$, $n=10$, $p<0.05$) (Table 3). This probably reflects the marine environment around Japan (higher $\delta^{15}N$ value and lower Hg concentration in the northern area of Japan; Endo et al., 2010). The fin whale in the Antarctic Ocean had high but non-significant correlation between the Hg concentration and the $\delta^{15}N$ value ($\gamma=0.618$, $n=10$, $p>0.05$), while the Antarctic minke whale had negative correlation. The reason for this negative correlation remains unknown.

According to latest reports (Mitani et al., 2006; Yasunaga and Fujise, 2009a, b), O-type common minke whales may be categorized into coastal and offshore whales. The Hg concentration is lower in the coastal whales (about 0.22 ± 0.07 μg/wet g) than in the offshore whales (about 0.3 μg/wet g) as the coastal whales feed on zooplankton, saury and anchovies (the Hg concentrations in these species were below 0.05 μg/wet g) while the offshore whales feed on these three species as well as on pomfret (0.232 ± 0.027 μg/wet g) (Yasunaga and Fujise, 2009a, b). The present Hg value in the O-type whales (0.099 ± 0.076 μg/wet g) is closer to the Hg value in the coastal whales than to that in the offshore O-type whales. The determination of $\delta^{13}C$ and $\delta^{15}N$ may also allow for the discrimination between the coastal and offshore species (Kelly, 2000). However, there has not yet been any comparison of these values between the coastal and offshore populations of common minke whales. We previously analyzed the Hg levels in cetacean products sold in South Korean markets (Endo et al., 2007), and the Hg concentration in the common minke whale (most of the whales were speculated to be J-type from coastal waters) was 0.22 ± 0.11 μg/wet g (0.03–0.43 μg/wet g, $n=30$), which is higher than the present data for the J-type whale (0.091 ± 0.065 μg/wet g). The difference in Hg concentrations in the common minke whale between the previous and present studies may be due to differences in their diet and habitat.

In the present study (Table 2), no differences were found in the results for Hg concentration, or $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values between the J- and O-types of common minke whales. Compared with the J- and O-types of common minke whales (0.091 ± 0.065 and 0.099 ± 0.076 μg/wet g, respectively), Bryde's and sei whales are only moderately omnivorous feeders and their Hg concentrations were lower (0.056 ± 0.017 and 0.054 ± 0.023 μg/wet g, respectively); Yasunaga and Fujise (2009a) reported similar Hg concentrations in the muscle of Bryde's whales (0.046 ± 0.008 μg/wet g) and sei whales (0.052 ± 0.009 μg/wet g) caught in the western North Pacific Ocean. The Antarctic minke and fin whales in the Southern Ocean are plankton feeders and their Hg levels were slightly lower than those of Bryde's and sei whales in the western North Pacific Ocean (Table 2).

Gendron et al. (2001) analyzed the $\delta^{13}C$ and $\delta^{15}N$ values in skin samples from Bryde's, fin and blue (*Balaenoptera musculus*, a plankton-feeder) whales in the Gulf of California, Mexico. The mean values of $\delta^{13}C$ and $\delta^{15}N$ in the skin samples of the Bryde's, fin and blue whales were −18.1 and 15.8‰ ($n=2$), −16.0 and 15.4‰ ($n=2$), and −18.2 and 12.9‰ ($n=2$), respectively. This order of $\delta^{15}N$ values is consistent with our knowledge of the feeding habits of those whale species, although the $\delta^{15}N$ values in the Bryde's and fin whales are higher than those in the present study (10.0 ± 1.2‰ and 5.8 ± 0.4‰, respectively, Table 2). The variation in $\delta^{15}N$ at the base of the food web is considered to be an important factor in the $\delta^{15}N$ values observed in the upper trophic levels. The $\delta^{15}N$ value in euphausiids (krill) along the west coast of the Gulf of California was 11.0 ± 1.2‰ (Gendron et al., 2001),

Table 2
Summary of analytical results for mercury and stable isotope ratios in red meat products originating from baleen whales sold in Japanese markets.

	Hg (μg/wet g)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
Common minke whale (J-type), $n=13$	0.091 ± 0.065^{a}	-18.4 ± 0.7^{a}	12.0 ± 1.7^{a}	12.0 ± 1.2^{a}
Common minke whale (O-type), $n=12$	0.099 ± 0.076^{a}	-18.6 ± 0.8^{a}	11.4 ± 0.7^{a}	13.0 ± 0.7^{a}
Bryde's whale, $n=11$	0.056 ± 0.017^{ab}	-16.9 ± 0.6^{b}	10.0 ± 1.2^{b}	15.1 ± 1.0^{b}
Sei whale, $n=10$	0.054 ± 0.023^{ab}	-19.5 ± 1.6^{c}	8.3 ± 1.3^{c}	15.0 ± 1.0^{b}
Fin whale, $n=10$	0.044 ± 0.019^{ab}	-22.5 ± 1.0^{d}	5.8 ± 0.4^{d}	15.1 ± 0.8^{b}
Antarctic minke whale, $n=10$	0.027 ± 0.021^{b}	-24.6 ± 0.4^{e}	6.2 ± 0.4^{d}	14.6 ± 0.7^{b}

See Table 1.
Different superscripts indicate significant differences ($p < 0.05$).

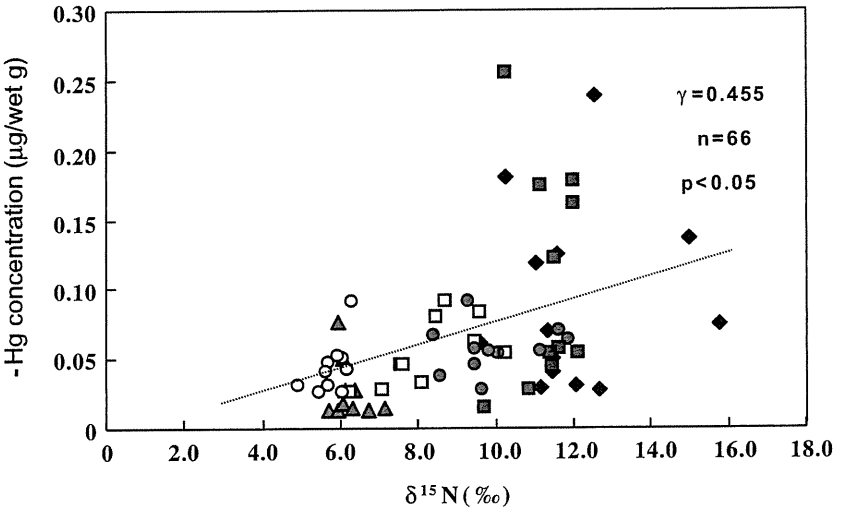

Fig. 1. Relationship between the $\delta^{15}N$ value and the Hg concentration in red meat products originating from baleen whale species or population. See Table 1. J-type common minke whale (◆), O-type common minke whale (■), Bryde's whale (●), sei whale (□), Antractic minke whale (▲), fin whale (○). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

while that in krill found in the stomach of common minke whales caught in the western North Pacific Ocean was 7.2 ± 0.5‰ (Mitani and Bando, 2008). Thus, the trophic positions of Bryde's and fin whales in the western North Pacific Ocean appear to be similar to those in the Gulf of California, respectively.

The $\delta^{13}C$ values in common minke (J- and O-types), Bryde's and sei whales caught in the western North Pacific Ocean were significantly different from those in fin and Antarctic minke whales caught in the Southern Ocean (Table 2), probably reflecting differences in their habitats. Krahn et al. (2008) reported the $\delta^{13}C$ and $\delta^{15}N$ values in the biota of Antarctica: the $\delta^{13}C$ and $\delta^{15}N$ values in the skin of an Antarctic minke whale ($n=1$) were -24.3 and 7.6‰, respectively, and those in the serum of crabeater seals (krill feeders) and in krill were -26.5 ± 1.0 and 8.4 ± 1.6‰ ($n=30$), and -29.8 ± 0.6 and 3.6 ± 0.2‰ ($n=12$), respectively. These $\delta^{13}C$ and $\delta^{15}N$ values in the Antarctic minke whale are in agreement with the present values from the muscle (red meat product) of Antarctic minke and fin whales caught in the Southern Ocean (Table 2). The $\delta^{15}N$ value in krill in the Antarctic Ocean (3.6 ± 0.2‰) was markedly lower than that in the stomach of common minke whales caught in Pacific Ocean (7.2 ± 0.5‰; Mitani and Bando, 2008). Lower $\delta^{15}N$ values in Antarctic minke and fin whales than common minke whale (Table 2) may reflect lower trophic levels of Antarctic minke and fin whales as well as lower $\delta^{15}N$ at the base of Southern food web.

The $\delta^{18}O$ values in common minke whales (J- and O-types) were significantly lower than those in the other whale species (Table 2), whereas the $\delta^{18}O$ values in Bryde's and sei whales caught in the western North Pacific Ocean and those in fin and Antarctic minke whales caught in the Southern Ocean were similar. As far as we know, no information on $\delta^{18}O$ values in cetaceans is available. As the temperature of the Antarctic feeding habitat is lower than that of the temperate North Pacific Ocean habitat, we expected to observe lower $\delta^{18}O$ values in whales in the Antarctic. However, the $\delta^{18}O$ values in the fin and Antarctic minke whales caught in the Antarctic were similar to those of Bryde's and sei whales caught in the North Pacific Ocean. Further study is necessary to explain these unexpected data.

We previously reported the $\delta^{13}C$ and $\delta^{15}N$ values and the Hg concentration in the toothed whale species hunted or stranded along the coast of Japan. The $\delta^{15}N$ values and the Hg concentrations in the toothed whale species (Endo et al., 2005) were markedly higher than those in the baleen whale species in this study (Table 2), reflecting their higher trophic positions. Further determination of $\delta^{18}O$ in the toothed whales from a broad latitudinal range is needed to elucidate whether $\delta^{18}O$ is higher in the toothed whales inhabiting the northern area and whether ^{18}O is bioaccumulated via the food web.

Significant positive correlations ($p < 0.05$) were found between the $\delta^{13}C$ and $\delta^{15}N$ values for Bryde's and sei whales (Table 3), and non-significant positive correlations ($p > 0.05$) were found in the other species caught in the western North Pacific Ocean and the Antarctic Ocean. We previously reported a positive correlation between $\delta^{13}C$ and $\delta^{15}N$ values in wild bluefin tuna taken from different areas around Japan (both values were lower in fish from the northern area), probably reflecting the change in diet due to the wide ranging annual migration from the southern to the northern areas (Hisamichi et al., 2010). Baleen whales, such as the

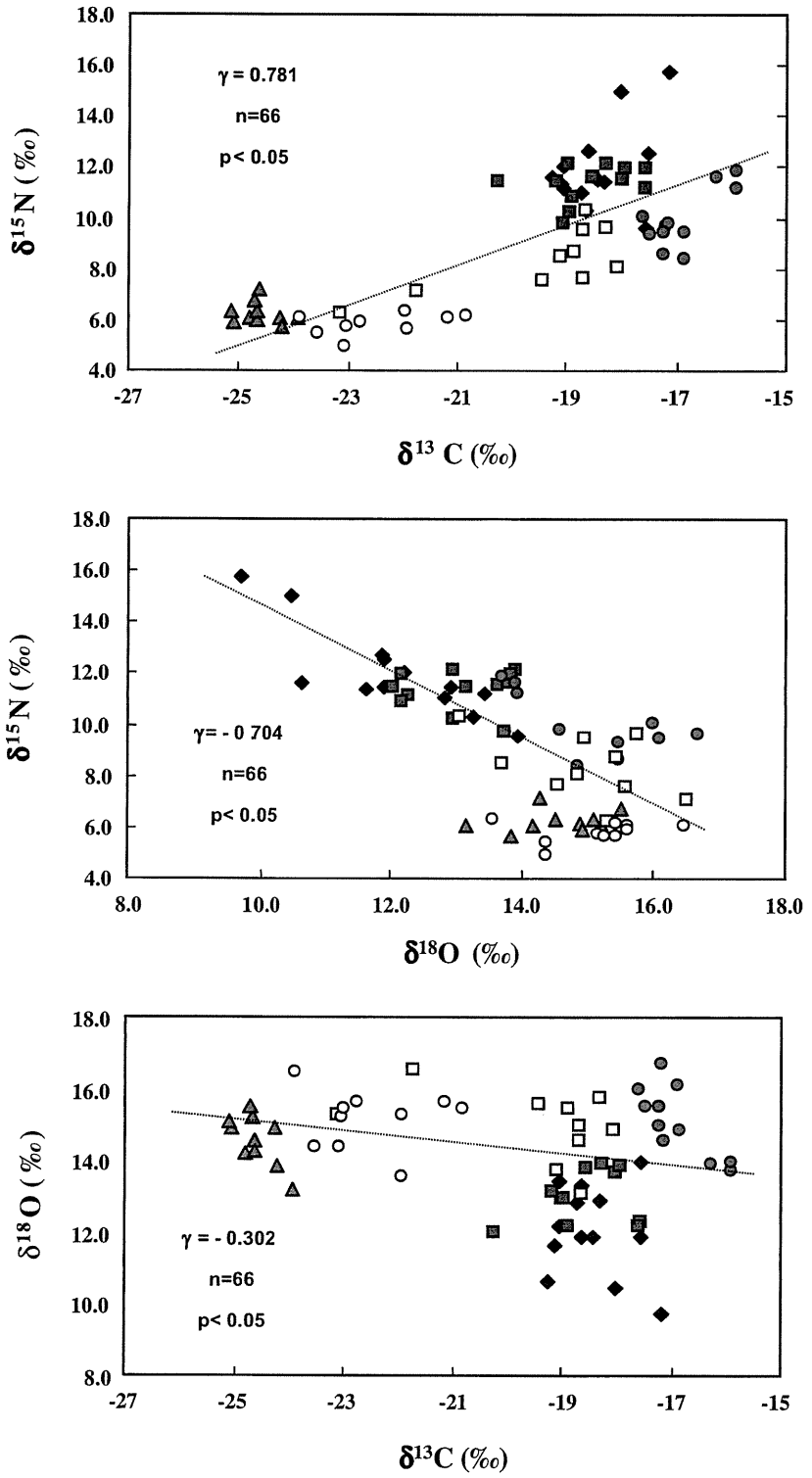

Fig. 2. Relationship among values of $\delta^{13}C$ and the $\delta^{18}O$ in red meat products originating from baleen whale species or population. See Table 1. J-type common minke whale (◆), O-type common minke whale (■), Bryde's whale (●), sei whale (□), Antractic minke whale (▲), fin whale (○). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

common minke (J- and O-types) and Antarctic minke whales, migrate over wide ranges in the North Pacific Ocean and the Southern Ocean, respectively (Kasamatsu et al., 1995; Wade et al., 2010). The positive correlations between the $\delta^{13}C$ and $\delta^{15}N$ values found in the baleen whale species could reflect their migration.

Unfortunately, we do not have any information on whale products with regard to location or date that each whale was killed or the age of the whale. Consequently, it is unclear whether the higher $\delta^{13}C$ and $\delta^{15}N$ values found in the red meat product samples come from whales in the southern or northern areas off

Table 3
Correlation coefficients (γ) of mercury and natural isotopes for within-species samples and overall samples for species or populations of baleen whales.

	Hg vs. $\delta^{15}N$	$\delta^{13}C$ vs. $\delta^{15}N$	$\delta^{15}N$ vs. $\delta^{18}O$	$\delta^{13}C$ vs. $\delta^{18}O$
Common minke whale (J-type), $n=13$	0.074	0.435	−0.852*	−0.185
Common minke whale (O-type), $n=12$	−0.077	0.315	0.199	0.182
Bryde's whale, $n=11$	0.188	0.740*	−0.640*	−0.774*
Sei whale, $n=10$	0.651*	0.751*	−0.484	−0.398
Fin whale, $n=10$	0.618	0.527	0.190	−0.250
Antarctic minke whale, $n=10$	−0.303	0.229	0.229	−0.672*
Overall, $n=66$	0.455*	0.781*	−0.704*	−0.302*

See Table 1.

* $p < 0.05$.

Japan. Based on the negative correlation between the $\delta^{13}C$ and $\delta^{18}O$ values and the $\delta^{15}N$ and $\delta^{18}O$ values (Table 3), it is assumed that the lower $\delta^{18}O$ values in whales in the northern areas result in the higher $\delta^{13}C$ and $\delta^{15}N$ values in whales in the northern area of Japan. However, this hypothesis is not supported by the lower $\delta^{13}C$ and $\delta^{15}N$ values found in wild bluefin tuna in the northern area of Japan (Hisamichi et al., 2010). Further study is necessary to confirm our assumption of spatial variations in $\delta^{18}O$, $\delta^{15}N$ and $\delta^{13}C$ values.

The $\delta^{13}C$–$\delta^{15}N$ plots and the $\delta^{13}C$–$\delta^{18}O$ plots can be discriminated into two groups (Table 2 and Fig. 2): the red meat products originating from the western North Pacific Ocean (J- and O-type common minke whales, Bryde's and sei whales) and the Antarctic Ocean (fin and Antarctic minke whales). We previously analyzed organohalogen compounds such as PCBs and DDTs and reported that the levels were markedly lower in the red meat products originating from the Southern Ocean than in products from the western North Pacific Ocean (Haraguchi et al., 2000). Thus, discrimination between the red meat products originating from the western North Pacific Ocean and the Antarctic Ocean could be achieved by the chemical analysis of stable isotope ratios and the pollutants without the need for genetic analysis. However, Antarctic minke and fin whales, J- and O-type common minke whales and Bryde's and sei whales could not be discriminated on the basis of chemical analysis. On the other hand, the $\delta^{13}C$ and $\delta^{15}N$ values in the red meat products originating from baleen whales sold in Japan were markedly different from those in products originating from toothed whales (Endo et al., 2010). Furthermore, contamination levels of Hg as well as organohalogens found in the baleen whales were markedly lower than those in toothed whales. Thus, the red meat originating from mysticetes and odonotocetes sold in Japan can be discriminated through chemical analysis.

In conclusion, we analyzed the Hg concentration and the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values in red meat products originating from common minke (J- and O-types), Bryde's and sei whales in the western North Pacific Ocean and fin and Antarctic minke whales in the Southern Ocean. The range of Hg concentrations and the $\delta^{15}N$ values in the baleen species and populations were in agreement with the known feeding habits of those. The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values could be used to discriminate between the red meat products originating from the mysticetes in the western North Pacific Ocean and those from the Southern Ocean. However, the four mysticetes in the western North Pacific Ocean and the two mysticetes in the Southern Ocean could not be identified on the basis of these data alone. A positive correlation between the $\delta^{13}C$ and $\delta^{15}N$ values and negative correlations between the $\delta^{13}C$ and $\delta^{18}O$ values and the $\delta^{15}N$ and $\delta^{18}O$ values, probably reflecting migration, were found in some species in the western North Pacific Ocean and the Southern Ocean.

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Levels of Mercury and Organohalogen Compounds in Pacific Bluefin Tuna (*Thunnus orientalis*) Cultured in Different Regions of Japan

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Abstract Contamination levels of total mercury (T-Hg), *p,p′*-DDE, and polychlorinated biphenyls (PCBs) in *akami* (leaner meat) and *toro* (fatty meat) samples from Pacific bluefin tuna cultured in the southern (four locations) and central (three locations) regions of Japan were analyzed. The contamination level of T-Hg in the *akami* and *toro* samples from the southern region tended to decrease with an increase in latitude, whereas those of *p,p′*-DDE and PCBs tended to increase. These spatial trends in contaminants were similar to those reported previously in wild tuna caught off the coast of Japan (Hisamichi et al., in Environ Sci Technol 44:5971–5978, 2010). However, the contamination level of T-Hg in *akami* and *toro* samples from one location in the central region was the highest among all seven locations, whereas the contamination level of *p,p′*-DDE was lower than that from any location studied in the southern region. Thus, contamination levels of T-Hg, *p,p′*-DDE, and PCBs in the cultured tuna may reflect contamination levels not only in the marine environment but also in prey fish used as bait.

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Large predatory fishes, such as tuna, shark, swordfish, and marlin, accumulate high levels of environmental pollutants by way of the food web. Of these species, tuna are particular important as a marine resource, and knowledge regarding the contamination level of mercury (Hg) and related health risks is of great interest to consumers. The permitted levels of total mercury (T-Hg) and methylmercury (M-Hg) in fish and shellfish set by the Japanese Ministry of Health and Welfare are 0.4 and 0.3 μg/wet g, respectively. However, this legislation does not cover the Hg contamination in some of the large predatory fish mentioned previously. Due to concerns over the impact of M-Hg on developing fetuses, the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA 2003) lowered its guideline value for provisional tolerable weekly intake of M-Hg from 3.3 to 1.6 μg/kg body weight (JECFA 2003).

In addition to Hg, tuna accumulate anthropogenic lipophilic compounds, such as polychlorinated biphenyls (PCBs), dichloro-diphenyl-trichloroethane (DDT) and its metabolites (DDTs: *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE), chlordane-related compounds (CHLs: trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor, and oxychlordane), and hexachlorobenzene (HCB) (Ueno et al. 2002, 2003; Hisamichi et al. 2010) as well as naturally produced compounds of tribromoanisol (TBA) (Penta-Abaurrea et al. 2009) and 2,3,3′,4,4′,5,5′-heptachloro-1-2′-bipyrrole (referred to as Q1) (Hisamichi et al. 2010). In contrast to the great emphasis placed on Hg contamination, a little attention has been paid to the potential human health problems associated with the contamination of PCBs and other lipophilic pollutants in tuna. We previously reported that contamination levels of Hg in three tuna species caught off the southern region of Japan were greater than those in tuna caught in the central and northern regions, whereas

contamination levels of PCBs and *p,p*-DDE (a major metabolite of DDTs) caught off the southern region were lower, probably reflecting the contamination levels in the respective marine environment (Hisamichi et al. 2010).

Japan is the world's largest consumer of tuna, and the majority of consumption is in the form of slices of raw fish (sashimi and sushi). Because people living in countries other than Japan have also recently come to eat much more tuna, tuna numbers have begun to dwindle. Recently, the demand for muscle containing a lot of fat (*toro*) has increased as a result of changing preferences among the Japanese population. As a result, the price of *toro* is greater than that of *akami* (lean meat), although there is no fixed standard for distinguishing *akami* from *toro*. To supplement the lack of wild tuna, fatty meat in particular, the business of farming tuna has expanded in Japan.

The technology for farming (culturing) tuna, in which wild tuna are caught and used to stock farms, has been well developed and applied commercially for the Atlantic bluefin tuna (*Thunnus thynnus*) in Mediterranean countries, for the southern bluefin tuna (*T. maccoyii*) in Australia, and for the Pacific bluefin tuna (*T. orientalis*) in Japan and Mexico. The aim of tuna farming in countries other than Japan, which operates through the confinement of captured tuna for short periods of time (usually 2–6 months), is mostly to increase the fat content in flesh (Tudela and Garcia 2004). In contrast, tuna culturing in Japan is usually aimed at cultivating fish captured in the larval stage for long periods (approximately 2.5–3 years) and to up to a fish weight of approximately 30–50 kg. Tuna culturing in Japan has been developed in temperate regions, such as the Okinawa, Kagoshima, Kochi, Nagasaki, Wakayama, and Mie Prefectures (Fig. 1). Recently, due to the development

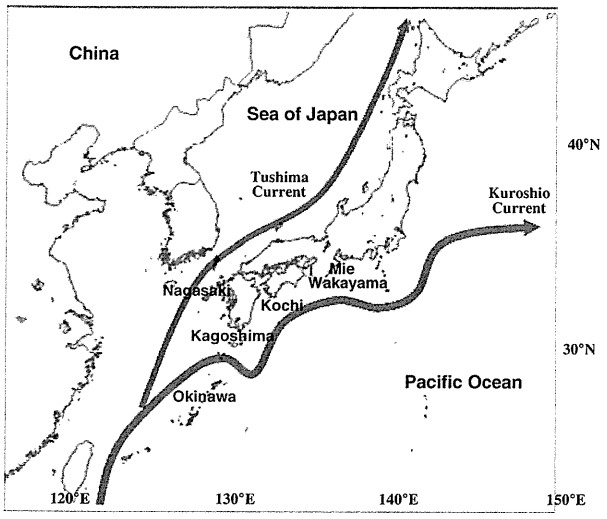

Fig. 1 Map of Japan showing six Prefectures in Japan where bluefin tuna is cultured

of hatchery technology, complete aquacultivation of tuna from gametes (full-cycle cultured Pacific bluefin tuna [FC tuna]), not from wild or larval tuna, has been achieved at Kinki University, Wakayama Prefecture (Nakao et al. 2007; Ando et al. 2008), and the meat of FC tuna cultured for approximately 3 years is now on the market.

Generally in wild tuna, Hg accumulation increases as the size of prey fish increases. However, the size and species of prey fish in farmed tuna can be controlled. Trials for the control of Hg concentration in the muscle (edible portion) have been undertaken in FC tuna (Nakao et al. 2007; Ando et al. 2008) and the farmed southern bluefin tuna (Balshaw et al. 2008a, b), because the increase in lipid content as well as rapid growth of the tuna could result in a decreased Hg concentration in muscle. In contrast, Padula et al. (2008) analyzed the contamination levels of lipophilic pollutants, dioxins, and PCBs in farmed and wild southern bluefin tuna and reported the greater levels of lipophilic contaminants in the farmed tuna than in the wild tuna. However, little is known about the contamination levels of those compounds in the tuna cultured in Japan.

Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ have been used to investigate feeding ecology. The $\delta^{15}N$ value is used to determine the trophic position of the studied species, and the $\delta^{13}C$ value is used to determine the source of carbon by the primary producer in a trophic web, providing information on the foraging habits of the species studied (Kelly 2000). Furthermore, the $\delta^{13}C$ and $\delta^{15}N$ values are known to vary by habitat. For instance, both $\delta^{15}N$ and $\delta^{13}C$ values in wild Pacific bluefin tuna caught off the northern region of Japan were lower than those in tuna caught off the southern region, probably reflecting their wide-ranging migration (Hisamichi et al. 2010). Furthermore, the latitudinal effects on $\delta^{13}C$ and $\delta^{15}N$ values in wild yellowfin and albacore tuna caught off the coast of central and southern Japan have been reported (Hisamichi et al. 2010); $\delta^{13}C$ values in those fish tended to decrease with an increase in latitude, whereas $\delta^{15}N$ values tended to increase. Enrichment of $\delta^{15}N$ by farming was reported in bluefin tuna farmed in the Mediterranean Sea (Vizzini et al. 2010). However, $\delta^{13}C$ and $\delta^{15}N$ values in tuna cultured in Japan have not yet been analyzed and compared with those of wild tuna.

The aim of the present study was to analyze the contamination levels of T-Hg, M-Hg, 13 PCB congeners, *p,p′*-DDE, trans-nonachlor (a major chemical among CHLs), Q1, HCB, and TBA in *akami* and *toro* samples of bluefin tuna cultured at different locations in Japan. Furthermore, we analyzed $\delta^{13}C$ and $\delta^{15}N$ levels in *akami* samples of tuna cultured at different locations. These results were compared among locations and with those for wild bluefin tuna reported previously (Hisamichi et al. 2010).

Materials and Methods

Sampling of Market Products

Fresh meats (*akami* and *toro*) of Pacific bluefin tuna cultured in the Okinawa, Kagoshima, Kochi, Nagasaki, Wakayama, and Mie Prefectures, Japan, were purchased from retail outlets mainly in Sapporo, Hokkaido Prefecture, but also in the Tokyo metropolitan area during April 2003 and December 2007 (Fig. 1). Okinawa Prefecture is the southernmost and Mie Prefecture the northernmost of the six Prefectures in which tuna are cultured. Fresh meat samples from FC tuna (Wakayama Prefecture) were purchased from a retail outlet in Nara Prefecture during July and August 2005 and in June 2008. To distinguish the tuna cultured in Wakayama Prefecture from larval fish and gamete (FC) tuna, we hereafter refer to them as Wakayama-I and -II, respectively.

We discriminated between the cultured bluefin tuna and wild bluefin, yellowfin, and albacore tuna sold in retail outlets not only on the basis of labeling but also by appearance and the results of testing. The tuna samples were stored at −20°C until analysis.

Chemical Analyses

T-Hg concentrations in the tuna samples were determined using a flameless atomic absorption spectrophotometer (HG-1; Hiranuma Sangyo, Ibaraki, Japan) after digestion by a mixture of HNO_3, $HClO_4$, and H_2SO_4 (Endo et al. 2003). M-Hg concentrations in the samples were determined using a gas chromatograph (GC-14A; Shimazu, Kyoto, Japan) with a ^{63}Ni electron capture detector (ECD) (Haraguchi et al. 2000). DOLT-2 (National Research Council of Canada) and CRB463 (BCR [European Commission]) were used as analytical quality-control samples for the determination of T-Hg and M-Hg as reported previously (Endo et al. 2003, 2004, 2008). The mean recoveries of T-Hg and M-Hg from the quality controls were 95% ($n = 5$) and 88% ($n = 4$), respectively. The M-Hg data were corrected by the recoveries.

Concentrations of organohalogen compounds in the tuna samples were determined as reported previously (Hisamichi et al. 2010). Briefly, the lipids in the minced samples were extracted three times by hexane. The combined extracts were concentrated, and the lipid content (hexane-extractable lipid [HEL]) was determined gravimetrically. A portion of the HEL (10–100 mg) was spiked with an internal standard (30 ng CB205), and 13 PCB congeners (CB99, CB101, CB118, CB138, CB146, CB149, CB153, CB170, CB183, CB187, CB194, CB199, and CB208), *p,p′*-DDE, trans-nonachlor, HCB, TBA, and Q1 in the HEL were analyzed using a gas chromatograph (GC-2014; Shimazu, Kyoto, Japan) equipped with ECD. All PCBs congeners, *p,p′*-DDE, trans-nonachlor, and HCB were purchased from Accu Standard (New Haven, CT). Quality assurance for anthropogenic compounds was confirmed by analyzing standard reference materials (cod liver oil 1588b) provided from the National Institute of Standard and Technology (Gaithersburg, MD). Data from our laboratory were in good agreement with the certified values (within 15% difference).

The stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in dried *akami* samples after the removal of lipids using chloroform/methanol extraction were analyzed by mass spectrometry (Delta S; Finnigan, Germany) coupled with an elemental analyzer (EA1108; Fisons, Italy) as reported previously (Endo et al. 2009).

Concentrations of T-Hg and M-Hg in tuna samples were expressed by Hg concentration/wet-weight basis, and organohalogen concentrations were expressed on a wet-weight basis as well as on a lipid-weight basis.

Statistical Analysis

The data were analyzed using Statcell 12 (Scheffe's F or Tukey-Kramer test), and the level of significance was set at $p < 0.05$. All data were expressed as means ± SDs.

Results

Analytical results for $\delta^{13}C$, $\delta^{15}N$, T-Hg, M-Hg, HEL, and organohalogen compounds in *akami* and *toro* samples from Okinawa, Kagoshima, Kochi, and Nagasaki Prefectures (southern region) and Wakayama (-I and -II) and Mie Prefectures (central region) are listed in Table 1.

The average T-Hg and M-Hg levels in *akami* samples from Wakayama-II (0.67 ± 0.14 and 0.43 ± 0.07 μg/wet g ($n = 6$), respectively) were the highest among the seven locations studied, exceeding the Japanese limits for T-Hg (0.4 μg/wet g) and M-Hg (0.3 μg/wet g), respectively (Table 1; Fig. 2). In contrast, average T-Hg and M-Hg in *akami* and *toro* samples from the southern region tended to increase with a decrease in latitude. The average levels of T-Hg and M-Hg found in *toro* samples from all locations were significantly lower than the corresponding levels in *akami* samples ($p < 0.05$), with the percentage of M-Hg to T-Hg found in those samples being in the range of 60–90%.

The average HEL value in *akami* samples tended to increase with an increase in latitude, except for samples from Wakayama-I and -II (Table 1; Fig. 2), and the average HEL in *akami* samples from Wakayama-II was the lowest among all of the locations. The average HEL concentrations in *toro* samples from all locations were significantly greater than the corresponding levels found in

Table 1 Analytical results of bluefin tuna cultured in different regions of Japan[a]

Region	Tissue type	(‰)		(μ/wet g)		(ng/wet g)						(%)
		$\delta^{13}C$	$\delta^{15}N$	T-Hg	M-Hg	PCBs	*p,p'*DDE	Trans-nonachlor	Q1	TBA	HCB	HEL
Okinawa	Akami ($n = 7$)	−17.2 ± 0.7	13.4 ± 1.5[a]	0.33 ± 0.16[a]	0.26 ± 0.11[a]	27.7 ± 15.1[a]	33.5 ± 18.7[a]	5.1 ± 3.9	12.0 ± 3.7	1.29 ± 0.95[a]	1.02 ± 0.51[a]	5.7 ± 3.9
	Toro ($n = 8$)	ND	ND	0.17 ± 0.11[a]	0.15 ± 0.08[a]	368 ± 146	591 ± 431[a]	72.7 ± 44.6[a]	176 ± 104	10.6 ± 8.9[a]	5.98 ± 4.24[a]	33.4 ± 12.6
Kagosima	Akami ($n = 10$)	−17.1 ± 0.8	14.0 ± 0.9[a]	0.31 ± 0.09[a]	0.24 ± 0.07[a]	50.7 ± 26.5	42.4 ± 21.3[a]	8.2 ± 5.1	9.5 ± 5.9	1.31 ± 1.20[a]	0.97 ± 0.82[a]	7.0 ± 2.1
	Toro ($n = 9$)	ND	ND	0.20 ± 0.08[a]	0.16 ± 0.07[a]	528 ± 225	506 ± 257[a]	164 ± 88[b]	191 ± 87	5.90 ± 2.93[a]	5.46 ± 1.80[a]	39.6 ± 11.7
Kochi	Akami ($n = 11$)	−17.1 ± 0.70	14.7 ± 0.3	0.26 ± 0.06[a]	0.21 ± 0.04[a]	45.4 ± 24.2	135 ± 101	7.1 ± 3.7	14.3 ± 5.8	0.75 ± 0.30[a]	0.99 ± 0.56[a]	7.0 ± 3.6
	Toro ($n = 7$)	ND	ND	0.16 ± 0.08[a]	0.13 ± 0.06[a]	519 ± 388	1211 ± 843[a]	76.1 ± 57.6	163 ± 141	5.36 ± 2.22[a]	6.00 ± 1.95[a]	39.5 ± 5.8
Nagasaiki	Akami ($n = 11$)	−17.4 ± 0.9	15.5 ± 1.2[b]	0.21 ± 0.12[a]	0.16 ± 0.08[a]	87.5 ± 69.5[b]	188 ± 193[b]	13.2 ± 9.3	22.7 ± 8.0	3.18 ± 1.90[a]	2.05 ± 1.28	9.2 ± 5.4[a]
	Toro ($n = 7$)	ND	ND	0.11 ± 0.07[a]	0.09 ± 0.04[a]	636 ± 270[b]	2183 ± 1042[b]	122 ± 63	301 ± 134	14.8 ± 4.0[a]	9.16 ± 2.48	38.1 ± 13.0
Wakayama-I	Akami ($n = 4$)	−16.6 ± 0.4	14.4 ± 0.8	0.24 ± 0.05[a]	0.20 ± 0.04[a]	21.5 ± 14.2[a]	16.2 ± 7.7[a]	5.0 ± 4.1	12.9 ± 9.6	1.94 ± 1.87[a]	1.18 ± 1.12	5.1 ± 4.0
	Toro ($n = 10$)	ND	ND	0.14 ± 0.05[a]	0.11 ± 0.03[a]	144 ± 71[a]	127 ± 123[a]	29.9 ± 13.0[a]	81.3 ± 38.9	10.3 ± 5.1[a]	6.11 ± 2.65[a]	29.2 ± 13.2
Wakayama-II (FC)	Akami ($n = 6$)	−16.5 ± 0.2	13.6 ± 0.3[a]	0.67 ± 0.14[b]	0.43 ± 0.07[b]	22.5 ± 25.8[a]	13.4 ± 15.4[a]	8.2 ± 10.0	9.7 ± 9.9	0.53 ± 0.53[a]	0.36 ± 0.35[a]	2.3 ± 1.8[b]
	Toro ($n = 12$)	ND	ND	0.43 ± 0.13[b]	0.29 ± 0.07[b]	224 ± 134[a]	147 ± 94[a]	87.0 ± 55.5[a]	121 ± 83	3.05 ± 2.01[a]	3.06 ± 2.68[a]	25.1 ± 14.3
Mie	Akami ($n = 5$)	−17.5 ± 0.1	13.5 ± 0.3[a]	0.25 ± 0.05[a]	0.19 ± 0.04[a]	37.4 ± 36.0	16.8 ± 7.7[a]	11.5 ± 7.8	15.2 ± 12.3	6.12 ± 2.88[b]	2.77 ± 1.37[b]	10.5 ± 4.6[a]
	Toro ($n = 9$)	ND	ND	0.13 ± 0.05 [a]	0.12 ± 0.04[a]	199 ± 144[a]	91.3 ± 69.7[a]	44.8 ± 23.6[a]	66.0 ± 48.3	33.1 ± 24.7[b]	15.51 ± 13.04[b]	45.7 ± 14.3

Region	(μg/g lipid)					
	PCBs	*p,p'*-DDE	Trans-nonachlor	Q1	TBA	HCB
Okinawa	0.54 ± 0.27	0.60 ± 0.27[a]	0.09 ± 0.05[a]	0.21 ± 0.08	0.022 ± 0.004[a]	0.020 ± 0.007
	1.30 ± 0.52	2.01 ± 0.93[a]	0.25 ± 0.17	0.59 ± 0.21[a]	0.032 ± 0.020[a]	0.017 ± 0.008[a]
Kagosima	0.81 ± 0.51	0.66 ± 0.37[a]	0.13 ± 0.09	0.13 ± 0.05[a]	0.019 ± 0.012[a]	0.014 ± 0.009
	1.32 ± 0.39	1.32 ± 0.40[a]	0.40 ± 0.15	0.47 ± 0.11[a]	0.017 ± 0.013[a]	0.015 ± 0.006[a]
Kochi	0.71 ± 0.37	1.94 ± 1.01	0.11 ± 0.07[a]	0.23 ± 0.10	0.012 ± 0.003[a]	0.014 ± 0.004
	1.29 ± 0.98	2.95 ± 1.88[a]	0.19 ± 0.13[a]	0.40 ± 0.33	0.013 ± 0.005[a]	0.015 ± 0.005[a]
Nagasaiki	0.98 ± 0.41[b]	2.25 ± 1.60[b]	0.15 ± 0.07	0.29 ± 0.13	0.042 ± 0.024[a]	0.026 ± 0.015
	1.63 ± 0.44	5.48 ± 1.82[b]	0.30 ± 0.12	0.76 ± 0.23[a]	0.042 ± 0.018[a]	0.026 ± 0.009
Wakayama-I	0.47 ± 0.13	0.44 ± 0.22[a]	0.09 ± 0.01[a]	0.27 ± 0.04	0.032 ± 0.011	0.021 ± 0.012
	0.47 ± 0.11	0.37 ± 0.22[a]	0.10 ± 0.02[a]	0.27 ± 0.07	0.037 ± 0.013[a]	0.020 ± 0.002
Wakayama-II (FC)	0.84 ± 0.32	0.45 ± 0.21[a]	0.26 ± 0.16[b]	0.36 ± 0.12[b]	0.032 ± 0.011[a]	0.021 ± 0.012
	0.93 ± 0.36	0.59 ± 0.20[a]	0.35 ± 0.13[b]	0.49 ± 0.24	0.012 ± 0.004[a]	0.011 ± 0.004[a]
Mie	0.34 ± 0.15	0.16 ± 0.03[a]	0.10 ± 0.04[a]	0.14 ± 0.07[a]	0.059 ± 0.009[b]	0.026 ± 0.004
	0.43 ± 0.23[a]	0.19 ± 0.09[a]	0.10 ± 0.03[a]	0.14 ± 0.06[b]	0.069 ± 0.033[b]	0.032 ± 0.018[b]

ND not determined

[a] PCB concentration was the sum of 13 PCB congener concentrations. Different superscript letter indicate significant difference between the locations ($p < 0.05$)

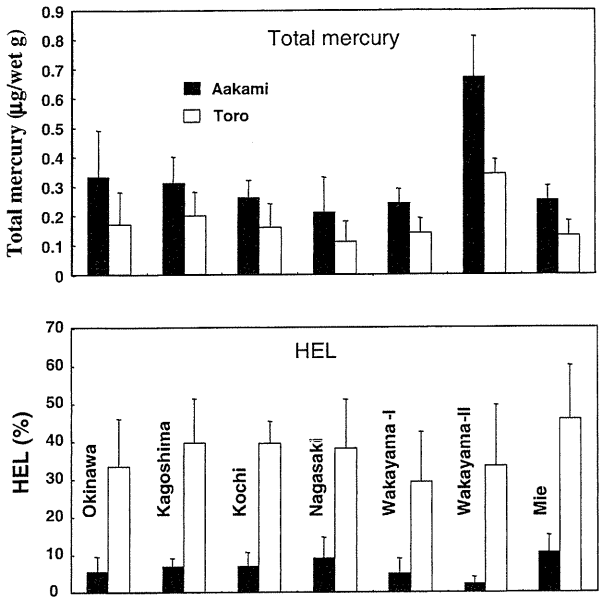

Fig. 2 T-Hg and HEL in *akami* and *toro* samples from tuna cultured in Okinawa, Kagoshima, Kochi, Nagasaki, Wakayama, and Mie Prefectures (see Table 1)

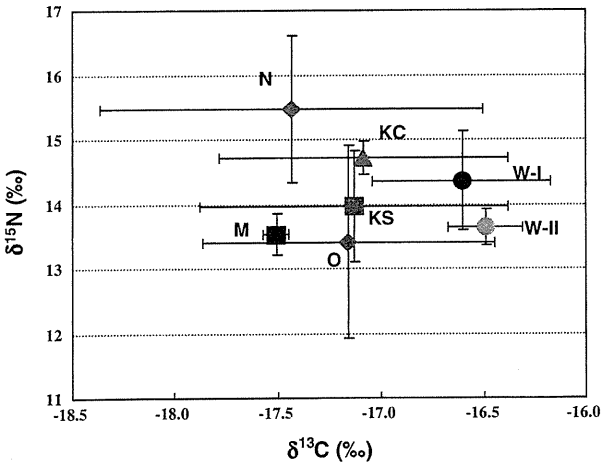

Fig. 3 Stable isotope ratios in *akami* samples from tuna cultured in Okinawa (O), Kagoshima (KS), Kochi (KC), Nagasaki (N), Wakayama (W-I and W-II), and Mie (M) Prefectures (see Table 1)

akami samples, and no clear latitude-dependent increase in HEL concentration was observed in *toro* samples.

The average δ^{15}N value found in *akami* samples from tuna cultured in the southern region was the lowest in Okinawa Prefecture and highest in Nagasaki Prefecture: The δ^{15}N value tended to increase with an increase in latitude (Table 1; Fig. 3). However, δ^{15}N values in Wakayama Prefecture (-I and -II) and Mie Prefecture (central region) were lower than that in Nagasaki Prefecture. In contrast, no spatial trend was observed in the average δ^{13}C values among the seven locations. Furthermore, no correlation was found between δ^{13}C and δ^{15}N values of combined *akami* samples from all seven locations.

PCB (sum of 13 congeners), *p,p'*-DDE, trans-nonachlor, Q1, TBA, and HCB levels, all expressed on a wet-weight basis, were approximately 10 times greater in *toro* samples than in *akami* samples from the seven locations, respectively (Table 1). PCB concentrations found in some *toro* samples exceeded the limit for PCBs set by the Japanese government (500 ng/wet g). Contamination levels of PCBs and *p,p'*-DDE found in *akami* and *toro* samples of Nagasaki Prefectures, expressed on both wet-weight (Figs. 4, S1) and lipid-weight bases (Figs. 5, S2), were the highest among the seven locations, and the levels of PCBs and *p,p'*-DDE tended to increase with increases in latitude for locations in the southern region (Okinawa, Kagoshima, Kochi, and Nagasaki Prefectures). Contamination levels of PCBs and *p,p'*-DDE found in *akami* and *toro* samples from Wakayama-I and -II and Mie Prefecture (central region), expressed on a wet-weight basis, were lower than those from samples obtained from tuna in the southern region, respectively (Figs. 4, S1). No spatial trend was found in contaminations levels of trans-nonachlor or Q1 in *akami* and *toro* samples (Figs. 4, 5, S1, S2). Levels of TBA and HCB in *akami* and *toro* samples from the southern and central regions were one or two orders of magnitude lower than those of *p,p'*-DDE, PCBs, trans-nonachlor, and Q1 (Figs. 4, 5, S1, S2). High levels of TBA and HCB were found in *akami* and *toro* samples from Nagasaki and Mie Prefectures. Contamination levels of *p,p'*-DDE found in *akami* and *toro* samples cultured in the southern region, except for Kagoshima Prefecture, were greater than those of PCBs, respectively, whereas those of PCBs in the central region were lower.

Figure 6 shows the relation between HEL (%) and T-Hg, M-Hg, PCBs, *p,p'*-DDT, trans-nonachlor, Q1, TBA, and HCB concentration (wet-weight basis) in the combined samples of *akami* and *toro*. T-Hg and M-Hg concentrations decreased with an increase in HEL, whereas all organohalogen compounds increased with an increase in HEL, up to approximately 60%, at which point they reached constant levels.

Discussion

Except for Wakayama-II, average concentrations of T-Hg and M-Hg in *akami* and *toro* samples did not exceed the permitted levels in fish and shellfish set at 0.4 and 0.3 μg/wet g, respectively (Fig. 2), although this Japanese legislation for Hg does not cover tuna. In general, the Hg contamination level in tuna correlates to their body length and weight

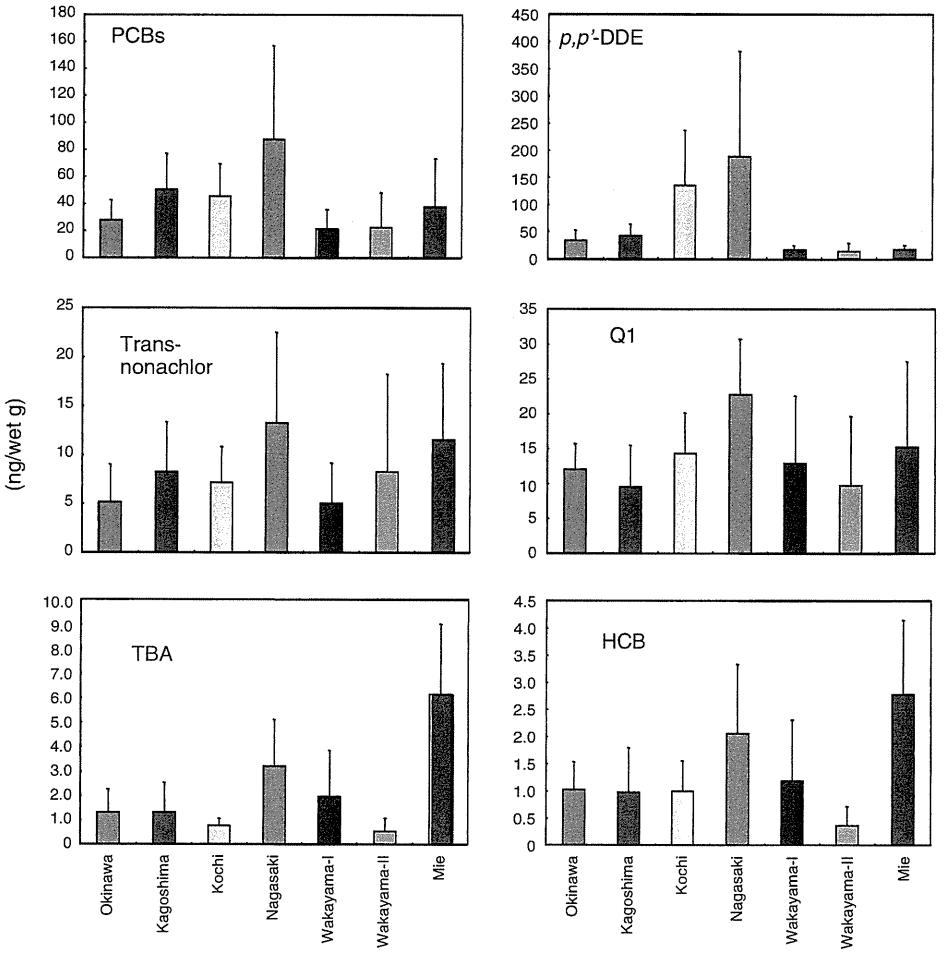

Fig. 4 Levels of organohalogen compounds in *akami* samples from tuna cultured in Okinawa, Kagoshima, Kochi, Wakayama, and Mie Prefectures expressed on a wet weight basis (see Table 1)

(Yamashita et al. 2005; Kojadinovic et al. 2006). We previously reported that the average levels of T-Hg and M-Hg in *akami* samples from wild bluefin tuna caught off the coast of Japan were 1.45 ± 0.73 and 1.25 ± 0.66 μg/wet g, respectively, although the body size of the tuna analyzed was unknown (Hisamichi et al. 2010). Because commercially available tuna cultured in Japan are generally younger and smaller (approximately 2.5–3 years and approximately 30–50 kg), lower contamination levels of T-Hg and M-Hg found in tuna samples cultured in Japan may be due to the younger age and smaller size (Tudela and Garcia 2004). The fattening of tuna has been reported to decrease Hg levels in the muscle of southern bluefin tuna (Balshaw et al. 2008a, b) and probably in that of Atlantic bluefin tuna (Vizzini et al. 2010). However, it is unclear whether the culture techniques used in Japan decrease the Hg concentration in the tuna muscle because no comparative data on Hg concentrations in wild and cultured tuna of similar sizes (and ages) are available.

T-Hg and M-Hg levels in *akami* samples from Wakayama-II (0.67 ± 0.14 and 0.43 ± 0.07 μg/wet g, respectively, $n = 6$) were the highest among all of the locations (Fig. 2). In agreement with the present values for T-Hg, Ando et al. (2008) reported that the average T-Hg concentration in *akami* samples from Wakayama-II (FC tuna, approximately 20–60 kg) was approximately 0.6 μg/wet g. The Wakayama-II farm (Kushimoto, Wakayama Prefecture) was located near the Wakayama-I location. However, contamination levels of T-Hg and HEL (Fig. 2) and some organohalogens (Figs. 4, 5, S1, S2) in the samples from Wakayama-II were markedly different from those in samples from Wakayama-I. The differences in HEL and other contaminants may be ascribed to the differences in HEL and contaminant levels in the prey fish used as bait rather than in the seawater. The contamination level of T-Hg in *akami* samples from Wakayama-II was similar to that in samples from wild bluefin tuna at an average body weight of 50 kg (0.59 ± 0.34 μg/wet g,

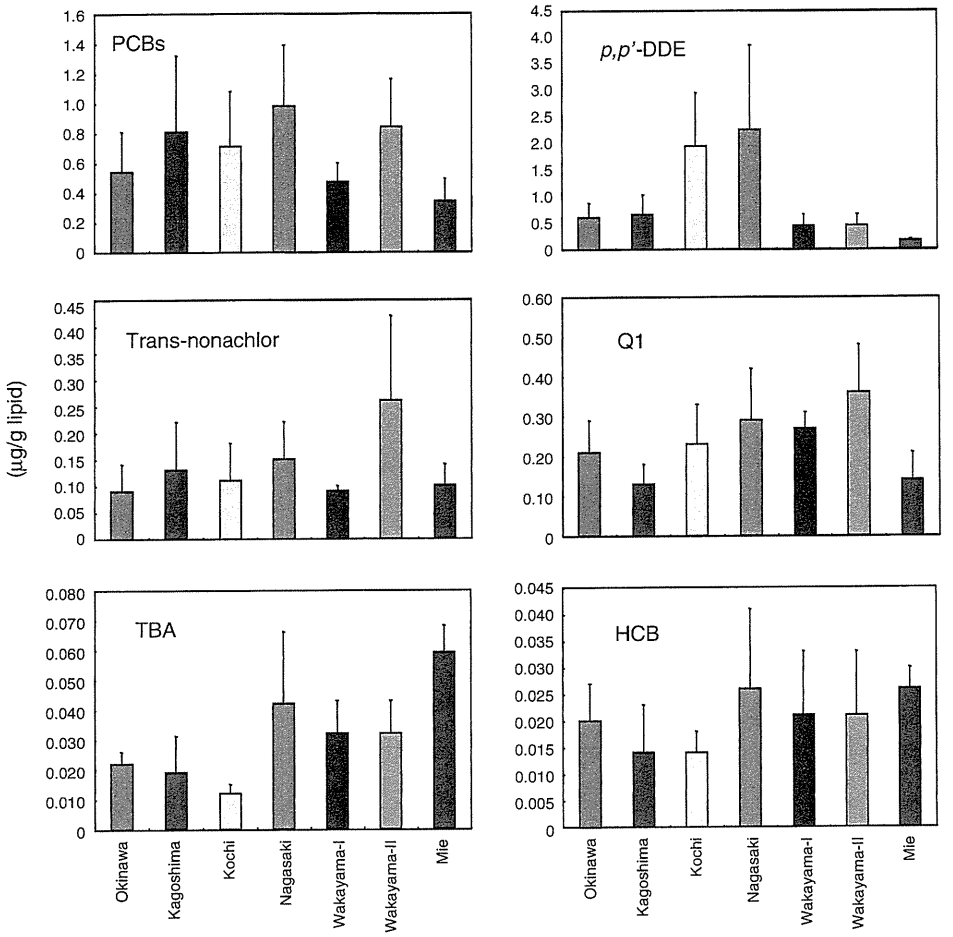

Fig. 5 Levels of organohalogen compounds in *akami* samples from tuna cultured in Okinawa, Kagoshima, Kochi, Wakayama, and Mie Prefectures expressed on a lipid basis (see Table 1)

$n = 15$ [Yamashita et al. 2005]). However, Hg concentration in wild tuna increases with increased body weight, whereas that in Wakayama-II samples was almost constant between 20 and 60 kg (approximately 0.6 μg/wet g [Ando et al. 2008]). Probably the Hg concentration is greater in smaller FC tuna (Wakayama-II) than in wild tuna, whereas it is lower in larger FC tuna. In contrast, HEL in cultured tuna from Wakayama-II (2.3 ± 1.8%) was the lowest among all of the locations and was compatible with that in wild bluefin tuna caught off Japan (1.28 ± 1.00%, $n = 62$ [Hisamichi et al. 2010]). Figure 6 shows the negative correlations between HEL (%) and T-Hg or M-Hg concentration. In agreement with these findings, Balshaw et al. (2008b) reported an inverse relation between lipid content and Hg concentration in muscle of farmed tuna. A lower lipid content, expressed by HEL, may be an additional reason for the greater Hg concentration observed in *akami* samples from Wakayama-II.

T-Hg level in *akami* samples tended to be greater those in tuna farmed in the southern region compared with those from the northern region, except for Wakayama-II (Fig. 2). We previously reported that T-Hg contamination levels in red meat (muscle) of toothed whale species (Endo et al. 2010) and wild bluefin, yellowfin, and albacore tuna (Hisamichi et al. 2010) tended to be greater in the southern region, probably reflecting greater Hg concentrations in the southern marine environment. The same spatial trend in Hg contamination in the tuna cultured in Japan, except for Wakayama (-II) and Mie Prefectures, may reflect Hg concentrations in the marine environment around the farm locations.

Contamination levels of *p,p'*-DDE in tuna cultured in Okinawa, Kagoshima, Kochi, and Nagasaki Prefectures (southern region) tended to increase with an increase in latitude (Figs. 4, 5, S1, S2). A similar but less prominent tendency was also found in the contamination levels of PCBs. Similarly, we previously reported that contamination levels of *p,p'*-DDE and PCBs in wild bluefin, yellowfin, and albacore tuna tended to be greater in the northern and central regions than in the southern region of

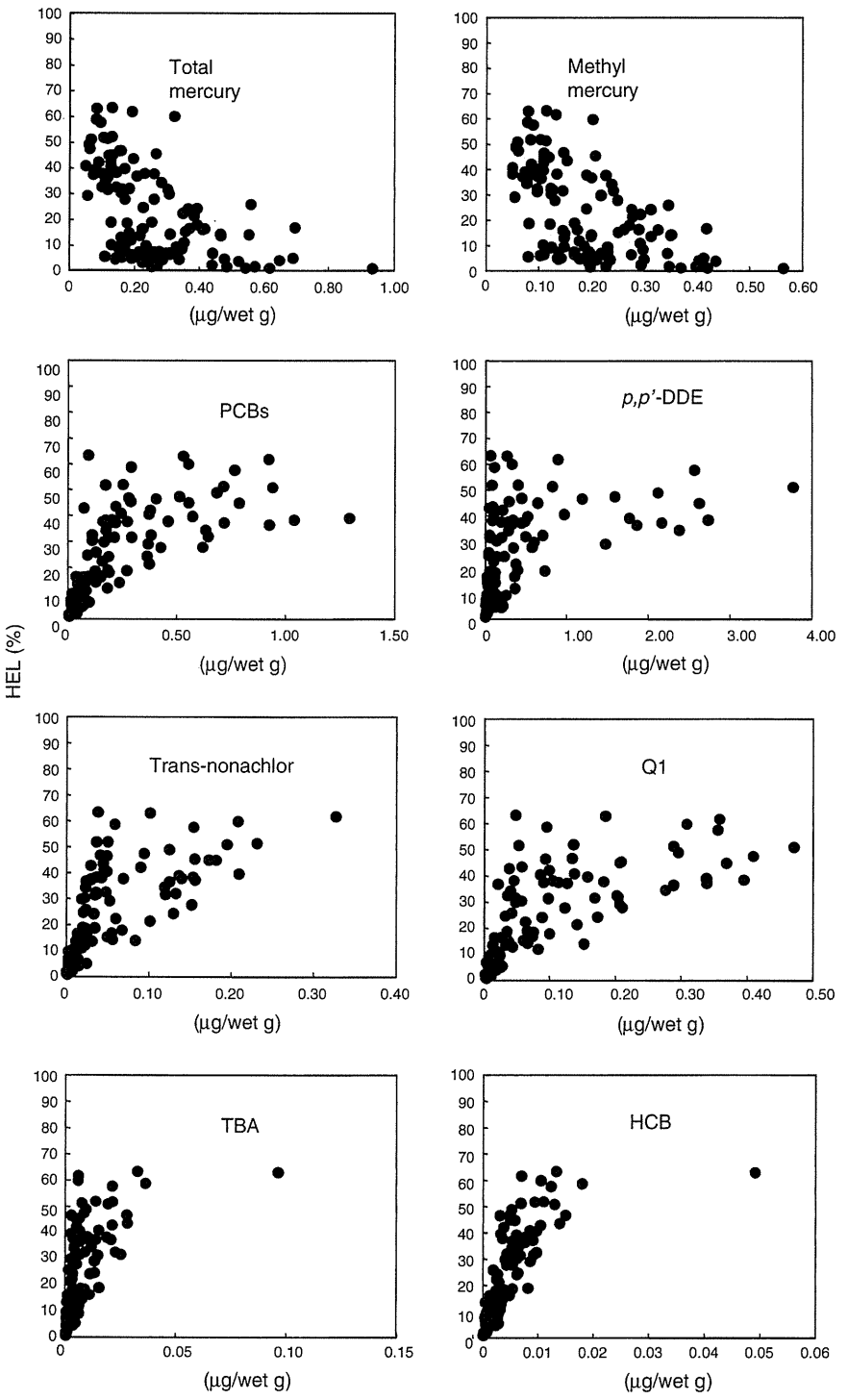

Fig. 6 Correlation between HEL and T-Hg, M-Hg, or organohalogen compounds in combined samples of *akami* and *toro* (see Table 1)

Japan, probably reflecting the spatial contamination pattern of those compounds in the marine environment (Hisamichi et al. 2010). In contrast, contamination levels of *p,p'*-DDE in Wakayama-I and -II and Mie Prefecture (central region) were lower than those in the southern region. The reason for the lower levels of *p,p'*-DDE and PCBs found in the central region remains unclear. A possible reason for these spatial differences in *p,p'*-DDE and PCBs is the existence of differences in the contamination levels of those compounds in the prey fish used as bait.

No clear spatial trends in the contamination levels of trans-nonachlor and Q1 were observed (Figs. 4, 5, S1, S2).

The contamination levels of trans-nonachlor and Q1 were markedly lower than those of *p,p'*-DDE and PCBs. The lower contamination levels of trans-nonachlor have been reported in wild bluefin tuna caught off the coast of Japan, whereas the contamination level of Q1 in the wild tuna was the same as that of *p,p'*-DDE and PCBs (Hisamichi et al. 2010). The reason for the difference in Q1 accumulation remains open.

Contamination levels of *p,p'*-DDE, PCBs, and tans-nonachlor in *akami* samples from cultured tuna (Fig. 5), expressed on a lipid-weight basis, were similar to those in samples from wild tuna caught off the coast of Japan (Hisamichi et al. 2010). Similarly, Vizzini et al. (2010) reported that contamination levels of PCBs, *p,p'*-DDE, and HCB in wild bluefin tuna caught in the Mediterranean Sea, expressed on a lipid basis, were similar to those in farmed bluefin tuna.

Contamination levels of TBA and HCB in *toro* samples, expressed on both wet-weight and lipid-weight bases, were one or two orders of magnitude lower than those of *p,p'*-DDE and PCBs. The highest averages of TBA and HCB (approximately 69 ng/g lipid and 32 ng/g lipid, respectively) were found in the *toro* sample of Mie Prefecture (Table 1), which is contrary to the spatial pattern observed for *p,p'*-DDE and PCB contamination. TBA is reported to be derived from the natural methylation of tribromophenol, which is mostly produced by algae, and found in the muscle of wild bluefin tuna (0.8–6.4 ng/g lipid) and farmed bluefin tuna (0.4–1.0 ng/g lipid) from the Mediterranean Sea (Penta-Abaurrea et al. 2009). The contamination level of HCB in muscle of wild bluefin tuna from the Mediterranean Sea (approximately 36 kg body weight [$n = 7$]) was 5.2 ± 3.5 ng/g lipid (Corsolini et al. 2007), and those in the muscle of skipjack tuna and in the liver of wild bluefin tuna from the Pacific Ocean were 1–10 ng/g lipid (Ueno et al. 2003) and 10–30 ng/g lipid (Ueno et al. 2002), respectively. Contamination levels of TBA and HCB found in bluefin tuna cultured in Japan were greater and similar levels to the reported levels, respectively.

Average δ^{15}N and δ^{13}C values in *akami* samples from tuna cultured in Japan ranged between 13.4 and 15.5 and between -17.5 and -16.5, respectively (Table 1; Fig. 3). In contrast, average δ^{15}N and δ^{13}C values in *akami* samples from wild bluefin tuna caught off the coast of Japan were 13.3 ± 1.1 and -16.9 ± 0.5 ($n = 61$), respectively (Hisamichi et al. 2010). Thus, the average δ^{15}N in cultured tuna in Japan was greater than that in wild tuna. In agreement with these findings, Vizzini et al. (2010) reported a greater δ^{15}N value in farmed bluefin tuna than in wild bluefin tuna from the Mediterranean Sea. Greater δ^{15}N values have also been reported in farmed fish than in wild fish of other species (Serrano et al. 2007).

The average δ^{15}N value in *akami* samples from the southern region (four locations) tended to increase with an increase in latitude, and that in samples from the central region was intermediate compared with those of the southern region. In contrast, there were no clear spatial differences in δ^{13}C values. No correlation was found between δ^{15}N and δ^{13}C values in combined *akami* samples from the southern and central regions. In contrast, we previously reported a positive correlation between δ^{15}N and δ^{13}C values in combined wild bluefin tuna samples from both regions, probably reflecting their wide-ranging migration (Hisamichi et al. 2010). We also reported negative correlations due to latitudinal effect in combined yellowfin tuna samples, combined albacore tuna samples (Hisamichi et al. 2010), and combined toothed-whale samples (Endo et al. 2010). Fish culturing may decrease the strength of these migration- and/or the latitude-related correlations.

Average HEL values in *akami* samples, except for those from Wakayama-I and -II, tended to increase with an increase in latitude (Fig. 2). We previously reported greater HEL values in *akami* samples from wild bluefin tuna caught in the northern region than in those in the southern region of Japan (Hisamichi et al. 2010). Furthermore, we reported that the average HEL value in samples from wild yellowfin and albacore tuna tended to be greater in the central region than in the southern region (Hisamichi et al. 2010). The difference in seawater temperature may be a possible cause for the latitude-dependent tendency observed in HEL values. However, the reason for the lower HEL value in tuna samples from Wakayama-I and -II is unclear. Lower HEL concentrations in prey fish used for tuna culturing is a possible reason.

The average HEL value in *toro* samples from each region was markedly greater than that in the respective *akami* samples. The latitude-dependent increase in HEL observed in *akami* samples was not found in *toro* samples. Fattening may decrease spatial difference in the lipid content of *toro* samples. A greater lipid content in cultured tuna compared with wild tuna was reported in bluefin tuna from the Mediterranean Sea (Vizzini et al. 2010) and in the southern bluefin tuna (Padula et al. 2008).

Nakao et al. (2007) analyzed T-Hg levels in prey fish used in Wakayama-II (FC tuna), but T-Hg levels in the prey fish used in other locations in Japan are unavailable. Furthermore, no data on contamination levels of organohalogen compounds in prey fish used in tuna culturing in Japan are available for business reasons. To elucidate spatial trends in Hg and organohalogen contaminations found in cultured tuna in Japan, analyses of the contamination levels in prey fish are necessary.

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総説

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日中韓における母乳を用いた残留性有機汚染物質のモニタリング

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要　旨

残留性有機汚染物質(Persistent Organic Pollutants; POPs)は環境中のいたるところに遍在し、長期間残留するとともに生物濃縮性を有する環境汚染物質である。POPs汚染に関して母乳をスクリーニングすることは乳児の現在の曝露状況を把握する上で重要である。POPsは脂溶性があるため、血液から母乳に受動輸送される。東アジアの各国で母乳にどのようなPOPsが含まれるかは異なる。中国人の母乳ではDDTやヘキサクロロベンゼン濃度が高いが、日本人ではクロルデン、PCB、ペルフルオロオクタン酸、韓国人ではPBDEsの濃度が高い。こういった特徴の明らかな違いは3国における過去から現在の化学物質曝露の特徴に関連している。POPs濃度は規制されたものは過去に比べて低下してきており、乳児への影響は考えにくいが、新たに検出される物質もある。従って子どもの健康のためにもヒト母乳の継続的なモニタリングが必要である。

緒言

残留性有機汚染物質（Persistent Organic Pollutants; POPs）は環境中のいたるところに遍在し、長期間残留するとともに生物濃縮性を有する環境汚染物質である。2004 年以降、POPs はストックホルム条約により世界的な問題として捉えられている。この種の化合物は鳥類、魚類、哺乳類の組織の化学分析において頻繁に検出されているが、それはヒトの脂肪組織や母乳においても同様である。ヒトが様々な経路を通じてこれら化学物質への曝露を受け得る以上、塩素系 POPs のすべてと、臭素系 POPs のおそらく大部分について、母乳が乳児にとっての主要な曝露経路として作用している。従って、POPs 汚染に関して母乳をスクリーニングすることは乳児の現在の曝露状況を把握する上で重要である。さらに POPs 汚染の時間的傾向を調査することが化学物質規制の効果を理解し、また今後どのような規制が必要になるかを決定する上でも重要となる。その際に特に重点を置くべきなのが、その時代において多量に使用されていた POPs と、その後新たに残留性が明らかになった有害物質のスクリーニングである。即ち、その当時生産量の高かった POPs、POPs 候補物質、他の残留性有毒物質のすべてについてモニタリングすることが重要である。この総説では、比較的新しい POPs について検討する。

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生体異物の母体から母乳への移行

脂肪酸のような低分子量の栄養素は主に二つの経路、受動輸送と能動輸送で血管から母乳へと運ばれる（図 1)。前者は ATP のようなエネルギーは不要であり、濃度勾配によって起こる輸送形態である。この輸送速度は物理化学的性質に依存している。一般的には親油性であり、小さな分子ほど早く輸送される傾向がある。後者はトランスポーターによる特定の分子の輸送であり、血液から乳腺を経て母乳中へ化学物質を輸送することが知られている。

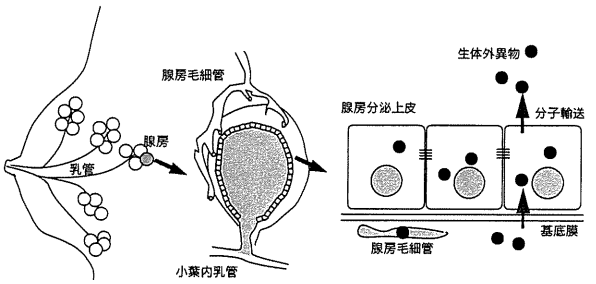

図 1　乳腺での物質輸送の模式図

血液乳腺関門（blood-breast milk barrier）と生体外異物の能動および受動輸送

一般的に免疫学的防御のために血液乳腺関門(blood-breast milk barrier)が存在することが知られている[1)]。 血液乳腺関門は同時に Shennan and Peaker[2)]やAbadin et al.[3)] により化学物質や重金属などの多くの低分子物質の障壁となることが示唆されている。血液乳腺関門がどのような物質を通過させるか、あるいはさせないかについての見地はいまだ限られている。血漿中の物質濃度が平衡に達した時の、血漿中物質濃度に対する母乳中物質濃度が最も信頼性のある母乳/血漿比となることが知られている。受動輸送系の場合は母乳/血漿比は一定になるが、能動輸送系の場合血漿中濃度に依存せず輸送される。これら2つの過程は物質濃度が高い場合に区別できるが、低い場合には本質的には同じ挙動を示す。

血液乳腺関門におけるトランスポーター

乳腺上皮組織にはいくつかのトランスポーターが存在することが知られている（総説[4)]を参照)。これらのデータはトランスポーターの存在を示唆しているが、環境中の物質がトランスポーターによりどの程度輸送されるかについては不明確である。トランスポーターの活性を評価するには技術的に難しい側面がある。遺伝子改変マウス(例：ノックアウトマウス)はトランスポーターの生理的学な役割の検討に使われるが、遺伝子ノックアウトに対する代償的なメカニズム発動はしばし検討の妨げとなる。

血液乳腺関門における受動輸送

物質の物理化学的な特徴は血清から母乳への輸送に大きな影響をもたらす。具体的な例として、多くの類縁物質を持つ代表的な POPs であるポリ臭素化ジフェニルエーテル (Polybrominated diphenyl ethers: PBDEs)とポリ塩素化ビフェニル (polychlorinated biphenyls: PCB) を示す。これらはよく似た炭素骨格を持ち、前者が臭素原子を含み、後者は塩素原子を含む。ハロゲン化数が低いものから高いものにかけて同族体番号が振られている（最大ハロゲン化数は 10 であり、#209 に該当する)。ハロゲン化数の違いはその物質の分子量、官能基の数、脂溶性に違いをもたらす。図 2 では、上記二つの物質について血清と母乳の構成比(全重量に対する割合)を比べている。PBDE 類では分子量の大きい同族体では分子量の小さいものに比べ母乳中%が低くなっている。一方 PCB 類ではこのような傾向は見られない。この明確な対比に対するアプローチとして定量的構造活性相関(Quantitative Structure-Activity Relationship: QSAR)を用いた解析が行われた[5)]。その結果、PBDE 類と PCB 類の母乳/血清の分配について下記の式が導き出された。: