

Stable Isotope Ratios of Carbon, Nitrogen, Oxygen, and Mercury Concentrations in North Pacific Baleen Whales and the Comparison of Their Calves with Toothed Whale Calves

Keywords: Humpback whale (*Megaptera novaeangliae*); Fin whale (*Balaenoptera physalus*); North Pacific right whale (*Eubalaena japonica*); Common minke whale (*Balaenoptera acutorostrata*); Killer whale (*Orcinus orca*); Dall's porpoise (*Phocoenoides dalli*); Lactation

Abstract

We quantified ^{13}C , ^{15}N , ^{18}O , and Hg concentrations in the muscle of calf and immature humpback whales stranded along the coast of the North Pacific Ocean around Hokkaido, Japan, and investigated those changes owing to the lactation. Next, we compared these concentrations in stranded humpback whale calves with those in stranded fin whale and North Pacific right whale calves, and stranded calves from other species reported previously [1,2]. We further compared those concentrations in stranded fin whales with those in fin whales hunted from the North Atlantic and Antarctic Oceans. The $\delta^{13}\text{C}$ value in humpback whale calves increased with body length (7.0-8.7 m), whereas the $\delta^{18}\text{O}$ values tended to decrease. In contrast, a small $\delta^{15}\text{N}$ -enriched peak was found in middle-sized calves. Humpback whale calves had trace Hg concentrations ($\leq 0.05 \mu\text{g/wet g}$), whereas these concentrations exceeded $0.10 \mu\text{g/wet g}$ in immature humpback whales. These changes in the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values and Hg concentrations in humpback whales could reflect a feeding shift from milk to solid foods. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ levels of calves, humpback and fin whales, and common minke whales reported previously [1] were similar, slightly higher than those of North Pacific right whales and significantly lower than those of killer whales [2]. These findings suggest that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the milks and weaning solid foods of humpback, fin, and common minke whales are similar (opportunistic fish eaters), slightly different from North Pacific right whales (zooplankton eater), and markedly different from killer whales (highest predator). Fin whales stranded in the North Pacific Ocean could be distinguished from fin whales hunted from the North Atlantic and Antarctic Oceans using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values. The $\delta^{18}\text{O}$ values, combined with the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values could be an excellent proxy to discriminate fin whales from the three oceans.

Introduction

Stable isotope analysis is a useful tool for obtaining information on the feeding ecology, migration, and physiology of marine mammals [3,4]. The stable isotope ratio of nitrogen ($\delta^{15}\text{N}$) increases as the trophic level in a food chain increases, whereas the stable isotope ratio of carbon ($\delta^{13}\text{C}$) is used to estimate the origin of base of food chain. The stable isotope ratio of oxygen ($\delta^{18}\text{O}$), usually combined with ^{13}C and ^{15}N , has been increasingly used to discriminate, verify, and identify the habitat of marine mammals, as it reflects the geographic and climatic conditions of habitats. The $\delta^{18}\text{O}$ level in surface seawater tends to be depleted at high latitude and low salinity [4-6]. Thus, the $\delta^{18}\text{O}$ values in the bones [7-9] and teeth [10-12] of marine mammals reflect the latitude and salinity of their habitat.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are particularly useful in evaluating



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mother-to-offspring nutrient transfer; nursing offspring of mammals generally have higher $\delta^{15}\text{N}$ levels, with similar or slight higher $\delta^{13}\text{C}$ levels to those of their mothers depending on the milk composition and nursing period [3,4,6]. Many studies of marine mammals have reported the elevated levels of $\delta^{15}\text{N}$ in calf tissues such as bone, tooth, muscle, blood, and skin; these seem to be associated with milk consumption [1,4,8,9,13-17].

Elevated levels of $\delta^{18}\text{O}$ in bones and teeth owing to breastfeeding have been reported in prehistoric human new born and infants [18-20]. Ancient infants accumulated more ^{18}O from ingesting milk than drinking water, and the elevated $\delta^{18}\text{O}$ values in teeth were found in greater in teeth developed at a younger age of infants than those developed at an older age [18]. However, to the best of our knowledge, no studies have focused on changes in $\delta^{18}\text{O}$ values of marine mammals due to lactation.

Most baleen whales (mysticetes) have relatively brief lactation periods (nursing and weaning: 5-7 months), whereas the lactation periods in humpback whales (*Megaptera novaeangliae*) and North Pacific right whales (*Eubalaena japonica*) may be slightly longer, which is in contrast with the lactation periods in toothed whales, dolphins, and porpoises (odontocetes), they have more extensive lactation periods (1-3 years) [21]. Mysticetes feed lower biota in the food chain (i.e., zooplankton and small fish), and typically have less significant exposure to pollutants such as mercury (Hg) and organochlorines, whereas as apex predators, most odontocetes are exposed to high levels of pollutants by ingesting contaminated large fish [22-24]. Positive correlations are generally observed between the $\delta^{15}\text{N}$ value and the Hg burden in marine animals as Hg is a typical contaminant that accumulates through the marine food web [25]. A sharp increase in the Hg burden is reported to be a weaning proxy for piscivore [26].

Hokkaido is the northern most large island in Japan, surrounded by the North Pacific Ocean, Sea of Japan, and Sea of Okhotsk (Figure 1), with more than 50 cetaceans stranded annually along its coast (SNH; <http://www.kujira110.com/>). Most stranded cetaceans are odontocetes of dalli-type Dall's porpoise (*Phocoenoides dalli*), harbor porpoise (*Phocoena phocoena*), and Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), and a mysticete of common minke

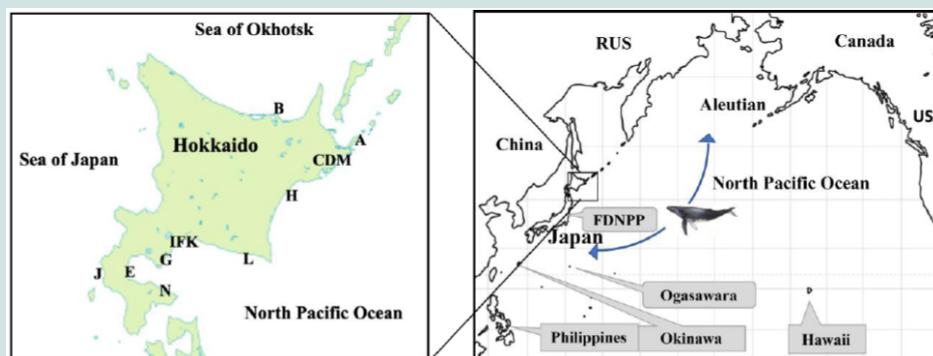


Figure 1: Map of Hokkaido, Japan, and stranding locations of baleen whales. Alphabets and arrow indicate the stranding locations of baleen whales in Hokkaido, Japan, and a putative migratory route of humpback whales. A-I: humpback whales, J-L: fin whales, M and N: North Pacific right whales, FDNPP: Fukushima Dai-ichi Nuclear Power Plant.

whale (*Balaenoptera acutorostrata*); other odontocete of killer whale (*Orcinus orca*), and mysticetes of humpback whale, fin whale (*Balaenoptera physalus*), and North Pacific right whale, are rarely stranded [27,28]. Many calves of mysticetes strand in Hokkaido, although the cause of strandings is unknown.

Among the baleen whales stranded along the coast of Hokkaido, common minke whale is an opportunistic fish predator that temporally and regionally adapts to prey type [29,30]. Humpback and fin whales stranded in Hokkaido are also opportunistic fish feeders, whereas the North Pacific right whale generally only feeds on zooplankton [31,32]. Dietary overlap and resource partitioning among sympatric baleen whales, humpback, fin and common minke whales, in the North Atlantic Ocean have been investigated [33-35]. However, little information is available on the feeding ecology and dietary overlap of humpback, fin, common minke, and North Pacific right whales inhabiting in the North Pacific Ocean around Hokkaido.

Endo et al. reported a small $\delta^{15}\text{N}$ -enriched peak in the muscle of common minke whale calves stranded in Hokkaido during the lactation period [1], which fitted to a quadratic function; the increase in $\delta^{15}\text{N}$ value before the peak may represent nursing, whereas the following decrease may represent weaning. However, the increase of $\delta^{15}\text{N}$ value due to the lactation has not yet reported in other mysticetes, humpback, fin and North Pacific right whales inhabiting in waters around Hokkaido. According to literature, a brief weaning period results in a rapid decrease in $\delta^{15}\text{N}$ values [36], whereas a prolonged weaning period results in gradual decrease in $\delta^{15}\text{N}$ values [4,20]. Endo et al. [1] also reported a trace burden of Hg in nursing common minke whales and the increase in the Hg burden with growth related to the shift from nursing to weaning (consuming solid foods). The degree of Hg burden in mysticetes is generally low, but the Hg burden in opportunistic feeders of mysticetes could be high in proportion to the amount of fish consumed [30].

Studies on lactation and mother-to-offspring nutrient transfer in marine mammals have been increasingly conducted using pinnipeds because they give birth and nurse pups on land or ice in accessible areas; it is easy to observe them and collect paired sampling from lactating mother and suckling pup, in comparison with large whales [21,37,38]. For studies on pinnipeds, if samples from the mother are

not available, foraging habitats and trophic position of mothers are indirectly estimated from the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of their suckling pups [38], using the $\Delta^{15}\text{N}_{\text{pup-mother}}$ and $\Delta^{13}\text{C}_{\text{pup-mother}}$ values. In contrast, the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data of cetacean calves are scarce and the $\Delta^{15}\text{N}_{\text{pup-mother}}$ and $\Delta^{13}\text{C}_{\text{pup-mother}}$ data are even scarce, due to difficulties of sample collection. Available data for $\Delta^{15}\text{N}_{\text{calf-mother}}$ and $\Delta^{13}\text{C}_{\text{calf-mother}}$ values calculated from killer whales [39] in addition to $\Delta^{15}\text{N}_{\text{calves-matures}}$ and $\Delta^{13}\text{C}_{\text{calves-matures}}$ values calculated from common minke whales [1] and Dall's porpoises [36] are all small less than 2‰ for $\Delta^{15}\text{N}$ values and less than 1 for $\Delta^{13}\text{C}$ values, respectively. Thus, the means of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in lactating mothers and mature animals seem to be similar and slightly lower than those of their calves, respectively, as in the case of pinniped [16,38,40].

In contrast to the fin whale inhabiting in the North Pacific and North Atlantic Oceans (opportunistic fish eater), this species in the Antarctic Ocean only feeds on zooplankton [41,42]. To the best of our knowledge, a comparative study of the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values, and Hg concentrations in fin whales from the North Pacific, North Atlantic, and Antarctic Oceans has not yet been conducted. As far as I know discrimination of fin whales inhabiting the three oceans is not possible by genetic analysis.

In the present study, we quantified the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values, and Hg concentrations in muscle samples from humpback whale calves and weaned immature animals stranded along the coast of the North Pacific Ocean around Hokkaido. (1) We investigated changes in the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values and Hg concentration in humpback whales owing to the lactation, and compared these changes with those reported previously in common minke whale calves [1]. (2) Next, we compared the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values in calf muscle samples from several cetaceans, humpback, fin and North Pacific right whales stranded in Hokkaido (this study) and common minke whales [1], killer whales [39] and Dall's porpoises [36] stranded in Hokkaido, and investigate whether the trophic position of mothers (mature animals) could be indirectly estimated from the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of their calves. (3) Lastly, we compared quantified values in the muscle samples of fin whale calves stranded in Hokkaido (North Pacific Ocean) with those values in the red meat products of fin whales hunted by whaling operations from the North Atlantic and Antarctic Oceans, and investigated whether fin whales from the three

oceans could be discriminated using the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values, and Hg concentration.

Methods

Sampling of humpback, fin, and North Pacific right whales

We collected muscle samples from humpback whales (n = 9), fin whales (n = 3), and North Pacific right whales (n = 2) stranded along the coast of Hokkaido, Japan, in 2012 and 2018 (Figure 1 and Table 1). In addition, we collected liver samples from six humpback whale individuals. Most samples were obtained from whales stranded along the coast of the North Pacific Ocean around Hokkaido, with samples obtained from one humpback whale from the Sea of Okhotsk and one fin whale from the Sea of Japan, provided by the Stranding Network of Hokkaido (SNH). Unfortunately, the sex of some stranded individuals could not be determined owing to their advanced decomposition (Table 1).

The body length (BL) of humpback whale newborns, of calves at the cessation of weaning, and at sexual maturity are 4.5-5.0, 8-9, and

Table 1: Stable isotope ratios of carbon, nitrogen and oxygen and mercury concentration in muscle of humpback whales, Fin whales and North Pacific right whales stranded along the coast of Hokkaido.

	Location	Area	Date	Sex	BL (m)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{18}\text{O}$ (‰)	Hg ($\mu\text{g}/\text{wet g}$)
Humpback whale	A	P	Sep 2016	UK	7.0	-19.6	10.5	13.0	0.04 (ND)
	B	O	Oct 2015	F	7.4	-19.6	10.7	13.6	0.03 (0.03)
	C	P	Jul 2011	M	7.6	-18.9	11.3	12.8	0.05 (0.04)
	D	P	Oct 2013	M	7.9	-19.4	12.3	13.4	0.04 (ND)
	E	P	Dec 2017	F	8.2	-18.8	11.5	14.2	0.05 (0.03)
	F	P	Oct 2013	M	8.3	-18.8	12.1	12.5	0.03 (0.03)
	G	P	Sep 2012	M	8.7	-18.0	11.0	11.7	0.02 (0.02)
	H	P	Aug 2018	UK	10.0	-19.3	11.0	11.8	0.13 (ND)
	I	P	Jun 2016	F	10.0	-19.9	10.5	11.5	0.11 (0.05)
				Mean	8.3	-19.1	11.2	12.7	0.06 (0.04)
Fin whale	J	J	Oct 2016	F	5.0	-20.5	10.6	12.6	0.01
	K	P	Jul 2014	UK	8.4	-19.2	11.1	12.3	0.09
	L	P	Jul 2009	UN	10.0	-19.0	10.9	12.1	0.08
				Mean	7.8	-19.6	10.9	12.3	0.06
				SD	2.6	0.8	0.3	0.3	0.04
North Pacific right whale	M	P	Jun 2014	UK	4.6	-21.8	10.3	14.5	0.03
	N	P	Oct 2016		9.5	-23.0	9.4	13.2	0.03

Alphabets indicate the sample ID of stranded whales (see Fig. 1). Parentheses are Hg concentrations in liver samples.
*Stranded along the coast of the Pacific Ocean (P) or the Sea of Okhotsk (O), UK: sex unknown. ND. Not determination.

11-12m, respectively [43]. Considering our data for $\delta^{13}\text{C}$ values and Hg concentrations (Figure 2), we categorized the humpback whale of 10.0 m BL (sample ID, H, and G, Table 1) as weaned but not yet mature animals. Thus, our humpback whale samples included seven calves and two weaned immature animals (Table 1).

The BL of fin whale newborns, of calves at cessation of weaning, and at sexual maturity are 6.0-6.5, ~11, and ~18 m, respectively [44], and the largest fin whale fetus ever reported had a BL of 5.0 m [45]. Thus, we categorized all fin whale samples (5.0, 8.4, and 10.0 m BL; Table 1) as calves, and the smallest calf of 5.0 m BL might be a premature animal.

The BL of North Pacific right whale newborns and weaned calves are ~4.2 and ~10.3 m, respectively [46]. Thus, we considered the two samples (4.6 and 9.5 m BL; Table 1) were from a newborn and a weaning calf.

We purchased the red meat products of fin whales caught off Japan Scientific Research Whaling from the Antarctic Ocean in 2000 and 2006 at retail outlets in Japan (Table 2). We also purchased the red meat products of fin whales from retail outlets in Japan in 2012 and 2013, which were caught off Iceland from the North Atlantic Ocean for human consumption (Table 2).

All stranded whale and red meat product samples were stored at -20°C until chemical analysis.

Chemical analyses

Before the ^{13}C , ^{15}N , and ^{18}O analyses, the lipids in the muscle samples and red meat products were removed by chloroform/methanol extraction [47]. Lipid extraction was repeated three or more times until the color of the extraction solvent became clear.

The analyses of ^{13}C and ^{15}N in the muscle and red meat product samples were performed using an IRMS (Delta S, Finnigan MAT, Bremen, Germany and EA1108, Fisons, Roano, Milan, Italy), as

Table 2: Stable isotope ratios of carbon, nitrogen and oxygen and mercury concentration in red meat products of fin whales caught off North Atlantic Ocean and Antarctic Ocean sold in Japan.

		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{18}\text{O}$ (‰)	Hg ($\mu\text{g}/\text{wet g}$)
North Atlantic	1	-18.9	7.3	16.4	0.22
	2	-19.1	8.4	17.1	0.34
	3	-19.1	8.8	15.8	0.28
	4	-19.1	8.6	16.7	0.31
	5	-19.2	9.0	16.4	0.31
	Mean \pm SD	-19.1 \pm 0.1 ^a	8.5 \pm 0.7 ^{ab}	16.5 \pm 0.5 ^{ab}	0.29 \pm 0.05 ^{ab}
Antarctic Ocean	1	-23.0	5.7	15.2	0.047
	2	-21.2	6.1	15.6	0.050
	3	-23.9	6.0	16.5	0.026
	4	-20.8	6.2	15.4	0.042
	5	-23.0	5.7	15.4	0.031
	6	-22.5	5.9	15.6	0.052
	7	-21.9	5.6	15.3	0.041
	8	-21.9	6.3	13.5	0.090
	9	-23.6	5.4	14.4	0.026
	10	-23.1	4.9	14.4	0.031
	Mean \pm SD	-22.5 \pm 1.0 ^{ab}	5.8 \pm 0.4 ^{ab}	15.1 \pm 0.8 ^b	0.044 \pm 0.019 [*]

Significantly different from the fin whales from the Antarctic Ocean^a and the North Pacific Ocean^b. *quoted from Endo et al. [2].

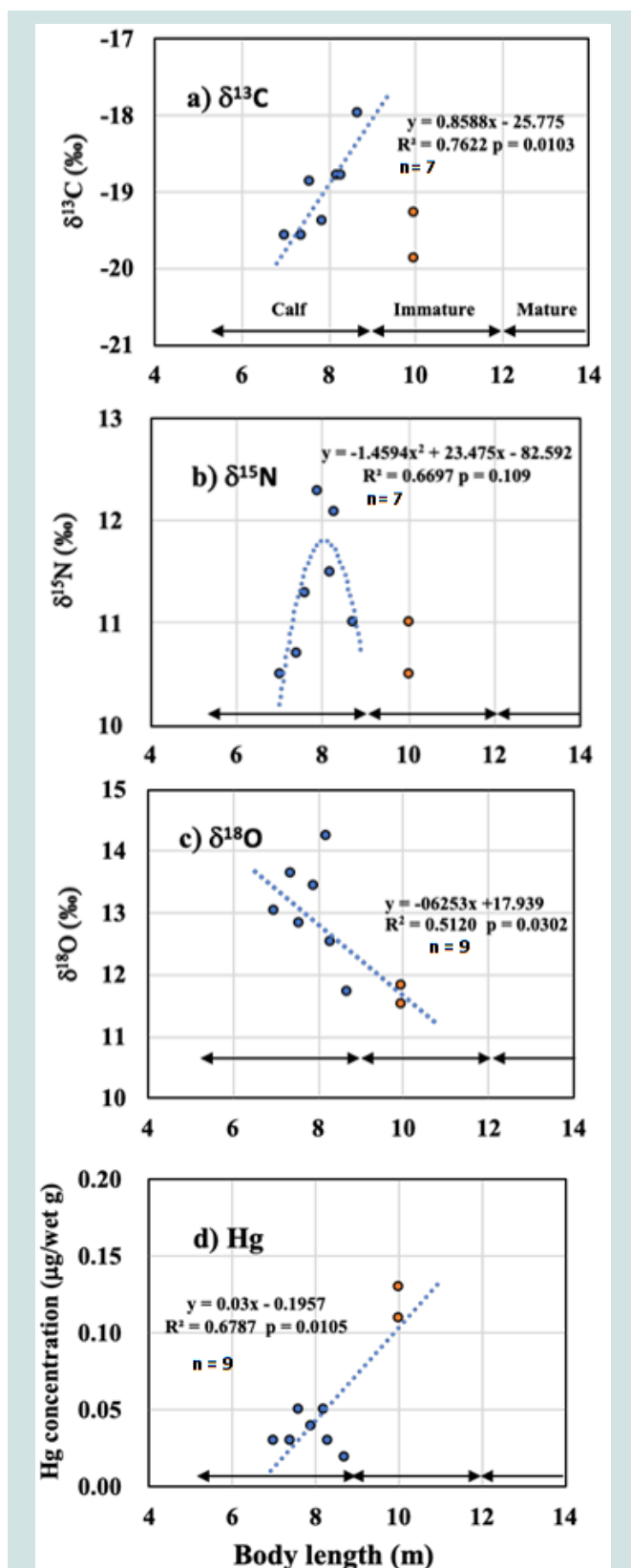


Figure 2: Relationship between body length and $\delta^{13}\text{C}$ value, $\delta^{15}\text{N}$ value, $\delta^{18}\text{O}$ value or Hg concentration in the muscle of calf and immature humpback whales.

described previously [1,48]. The analyses of ^{18}O in the muscle and red meat product samples were also performed using an IRMS (Delta V PLUS, Thermo Fisher Scientific, Tokyo, Japan), as described previously [2,39]. CERKU-1, -2, and -5, certified by the Kyoto University and Institute of Biogeosciences, Japan [49], were used as the working standards for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. NBS127, and benzoic acids (A and B) certified by Indiana University (IN, USA), were used as the working standards for $\delta^{18}\text{O}$. The replicate errors of the working standards for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ were within 0.2%, 0.3%, and 0.4%, respectively, and the R^2 values of their calibration curves were greater than 0.99.

Isotope ratios are in the standard delta (δ) notation relative to the internal standard of Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$), atmospheric nitrogen ($\delta^{15}\text{N}$), and the standard mean ocean water ($\delta^{18}\text{O}$) using the following equation:

$$\delta (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

The total Hg concentrations in the muscle, liver and red meat product samples were quantified using a flameless atomic absorption spectrophotometer (HG-310; Hiranuma Sangyo Co. Ltd., Ibaraki, Japan). As reported previously [1], approximately 0.5 g of sample was digested in a mixture of HNO_3 , H_2SO_4 , and HClO_4 . DOLT-2 (National Research Council of Canada) was used as the analytical quality control for Hg, and the recovery of Hg was $94 \pm 3\%$ ($n = 5$). The Hg concentrations in the muscle, liver and red meat product samples were expressed on a wet weight basis, and the determination limit of Hg was approximately $0.01 \mu\text{g/wet g}$.

Statistical analyses

The values of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and Hg concentration presented for each sample are the means of at least two measurements. Data are shown as mean \pm S.D, and the level of significance chosen was $p < 0.05$.

We investigated whether the relationship between BL and isotopedata ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values) or Hg concentration could be fitted to a linear, Quadratic, or exponential function using JMP (SAS Institute Japan Ltd., version 14.3, Tokyo, Japan). The 95% confidence ellipses were also calculated using JMP. Significant difference among multi groups was tested using Tukey-Kramer's method.

Results

$\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values and mercury concentration in humpback whale

Table 1 shows the ^{13}C , $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values in muscle samples and the Hg concentrations in muscle and liver samples of humpback whales. The average $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values for all humpback whales (sample ID; A-I, $n = 9$) were $-19.1 \pm 0.6\text{‰}$, $11.2 \pm 0.7\text{‰}$, and $12.7 \pm 1.0\text{‰}$, respectively, and those for only humpback calves (C-I, $n = 7$) were $-19.0 \pm 0.6\text{‰}$, $11.4 \pm 0.7\text{‰}$, and $13.0 \pm 0.8\text{‰}$, respectively. The average Hg concentrations in muscle and liver samples were $0.06 \pm 0.04 \mu\text{g/wet g}$ ($n = 9$) and $0.03 \pm 0.01 \mu\text{g/wet g}$ ($n = 6$), respectively.

Figures 2a, b, c, and d show the relationships between the BL and the isotopic or Hg data of humpback whales. The $\delta^{13}\text{C}$ values for calves ($n = 7$) increased linearly with increases in BL ($F_5 = 16.03$, $R^2 = 0.7622$, $p = 0.0103$, Figures 2a). The $\delta^{13}\text{C}$ values for the two immature

whales of 10.0m BL were -19.3‰ and -19.9‰, respectively, lower than that of the largest calf of 8.7 m BL (-18.0‰).

The $\delta^{15}\text{N}$ values for calves peaked in animals with ~8 m BL, as the highest and the next highest $\delta^{15}\text{N}$ values were found in the calves with BL of 7.9 m (12.3‰) and 8.3 m (12.1‰). The $\delta^{15}\text{N}$ values for humpback whale calves were fitted to a quadratic equation, although this was not significant ($F_5 = 4.055$, $R^2 = 0.6697$, $p = 0.109$, Figure 2b); the peak $\delta^{15}\text{N}$ value calculated from this equation was 11.8‰ at 8.0 m BL. The lowest $\delta^{15}\text{N}$ value (10.5‰) was found in the smallest calf (7.0 m BL) and the weaned immature animal (10.0 m BL). Among all humpback whales, the difference of $\delta^{15}\text{N}$ value between the maximum (12.3‰) and minimum (10.5‰) was 1.8‰, and the difference of $\delta^{13}\text{C}$ value between maximum and minimum was 1.9‰ (Table 1).

For all humpback whales, $\delta^{18}\text{O}$ values decreased linearly with increases in BL ($F_7 = 7.344$, $R^2 = 0.5120$, $p = 0.0302$, Figure 2c): The $\delta^{18}\text{O}$ values in the seven calves did not fit to a quadratic function ($F_5 = 1.909$, $R^2 = 0.4880$, $p = 0.262$) although the two highest $\delta^{18}\text{O}$ values were found in the middle-sized calves (14.2‰ at 8.2 m BL and 13.6‰ at 7.4 m BL). There was no correlation between $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of calves ($n = 7$, $p = 0.865$) and all humpback ($n = 9$, $p = 0.386$). Among all humpback whales, the difference of $\delta^{18}\text{O}$ values between the maximum (14.2‰) and minimum (11.5‰) of all humpback whales was 2.7‰ (Table 1).

Trace amounts of Hg were found in the muscle samples of humpback calves (0.02–0.05 $\mu\text{g}/\text{wet g}$), whereas the Hg concentrations of two immature humpback whales were slightly greater than 0.10 $\mu\text{g}/\text{wet g}$ (Table 1) (Figure 2). The Hg concentrations increased linearly as BL increased in all humpback whales ($F_7 = 13.46$, $R^2 = 0.6787$, $p = 0.0105$, Figure 2d). The Hg concentrations in humpback whale liver samples ($0.03 \pm 0.01 \mu\text{g}/\text{wet g}$, $n = 6$) were similar to or slightly lower than those in humpback whale muscle samples ($0.06 \pm 0.04 \mu\text{g}/\text{wet g}$, $n = 9$) (Table 1).

$\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values and Hg concentration in fin and North Pacific right whale muscles

As BL increased, the $\delta^{13}\text{C}$ values of fin whale calves (Table 1, $n=3$) tended to increase (-20.5, -19.2, and -19.0‰), whereas the $\delta^{18}\text{O}$ values tended to decrease (12.6, 12.3, and 12.1‰). In contrast, the $\delta^{15}\text{N}$ values of calves did not change with BL (10.6, 11.1, and 10.9‰). Trace Hg was found in the smallest calf (0.01 $\mu\text{g}/\text{wet g}$), but the Hg concentrations in the larger calves were 0.08 and 0.09 $\mu\text{g}/\text{wet g}$.

We only obtained two samples of North Pacific right whale calves (Table 1). The $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values of the large calf (weaning calf) were lower than those of the small calf (newborn), respectively (Table 1). The $\delta^{13}\text{C}$ values of right whale calves (-21.8 and -23.0‰) were markedly lower than those of humpback and fin whale calves. Similarly, the $\delta^{15}\text{N}$ values of right whale calves (10.3 and 9.4‰) were lower than those of humpback and fin whale calves, whereas the $\delta^{18}\text{O}$ values of right whale calves (14.5 and 13.2‰) were higher than those of fin whale calves. Trace level of Hg was found in the two right whale calves (0.03 $\mu\text{g}/\text{wet g}$).

Isotopic discrimination of calves from sex cetacean species stranded in Hokkaido using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

We investigated whether calves from humpback, fin, and North

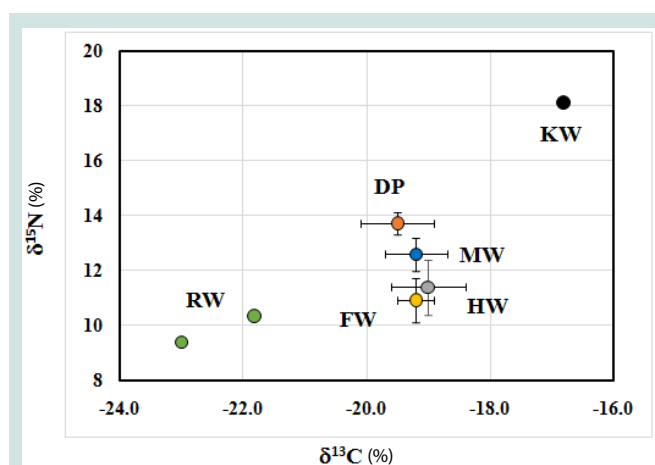


Figure 3: Isotopic discrimination of calves of mysticetes and odontocetes stranded in Hokkaido using stable isotope ratios of carbon and nitrogen. HW: humpback whales ($n=9$), FW: fin whales ($n=3$), RW: North Pacific right whale ($n=2$), MW: common minke whales ($n=12$)[1], KW: killer whales ($n=3$)[39], DP: Dall's porpoises ($n=7$, BL ≥ 130 cm)[36].

Pacific right whale (mysticetes, Table 1), and previously reported calves from common minke whale (mysticete, [1]), killer whale (odontocete, [39]) and Dall's porpoise (odontocete, [36]) stranded in Hokkaido, could be discriminated using their $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ values. The respective $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ values previously reported were $-19.2 \pm 0.5\text{‰}$ and $12.6 \pm 0.8\text{‰}$ ($n = 12$) in common minke whale calves [1], $-16.8 \pm 0.10\text{‰}$ and $18.1 \pm 0.06 \text{‰}$ ($n = 3$) in killer whale calves [39], and $-19.5 \pm 0.62\text{‰}$ and $14.6 \pm 0.21\text{‰}$ ($n=7$) in Dall's porpoise calves [36]. The $\delta^{15}\text{N}$ values of calves were in the following order ($F_{5,30} = 74.51$, $p < 0.01$): killer whale > Dall's porpoise > common minke whale = humpback whale = fin whale \geq North Pacific right whale. On the other hand, the order of $\delta^{13}\text{C}$ values was in the following order ($F_{5,30} = 23.65$, $p < 0.01$): killer whale > Dall's porpoise = common minke whale = humpback whale = fin whale \geq North Pacific right whale (Figure 3).

Figure 3 depicts the dual-isotope plot of calves from six species. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of killer whale calves were the highest among six species ($p < 0.05$), whereas those values of North Pacific right whale calves were the lowest. The dual-isotope plot could apparently discriminate killer whale calves and North Pacific right whale calves from other whale calves. The $\delta^{15}\text{N}$ value of Dall's porpoise calves was the next highest, and slightly but significantly higher than that of common minke, humpback, and fin whale calves ($p < 0.05$) and markedly higher than North Pacific right whale calves ($n = 2$), whereas the $\delta^{13}\text{C}$ value was intermediate between killer and North Pacific right whale calves, and similar to that of common minke, humpback and fin whale calves.

Comparison of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values and Hg concentration in fin whale from the North Pacific, North Atlantic, and Antarctic Oceans

We compared the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values and the Hg concentration in the muscle samples off in whale calves stranded in Hokkaido (North Pacific Ocean, Table 1) with those in the red meat products of fin whales hunted from the North Atlantic and Antarctic Oceans (Table 2). Figures 4a, b, c, and d show biplots of $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ vs. $\delta^{18}\text{O}$, $\delta^{18}\text{O}$ vs. $\delta^{15}\text{N}$, $\delta^{15}\text{N}$ vs. Hg, respectively. As the red meat

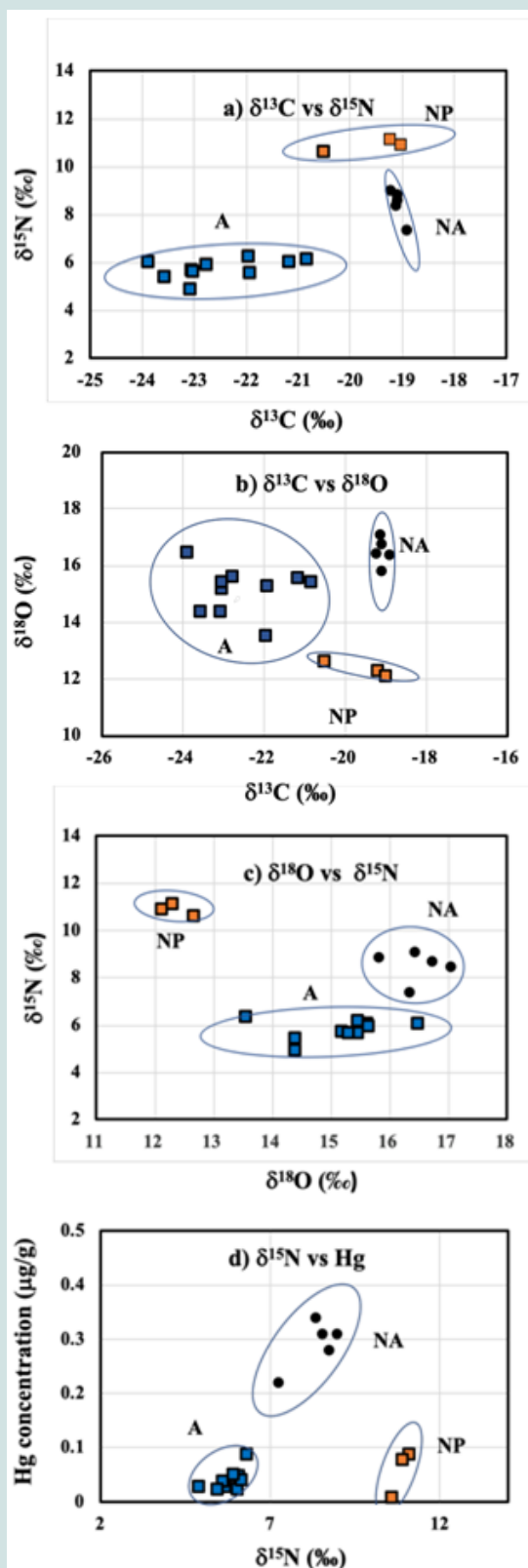


Figure 4: Discrimination of fin whales taken from three oceans using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values and Hg concentration. The ellipses show the 95% confidence areas. NP: North Pacific Ocean, NA: North Atlantic Ocean, A: Antarctic Ocean.

products of fin whale hunted from the North Atlantic and Antarctic Oceans were purchased from retail outlets, their BL were unknown. In contrast, all fin whales stranded in the North Pacific Ocean were calves.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and Hg concentration in fin whales from the North Atlantic Ocean were higher than those from the Antarctic Ocean, respectively, whereas the $\delta^{18}\text{O}$ value was lower (Table 2). All four biplots (Figures 4a, b, c, and d) clearly discriminated fin whales from the North Pacific, North Atlantic, and Antarctic Oceans. The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of fin whales from the Antarctic Ocean varied more than those from the North Pacific and North Atlantic Oceans (Figure 4b), whereas the variation in $\delta^{13}\text{C}$ value was small in fin whales from the North Atlantic Ocean (Figure 4a).

Discussion

Changes in $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ values and Hg concentration in calves of humpback whale by lactation

We found a small $\delta^{15}\text{N}$ -enriched peak in the muscle sample of humpback whale calf at ~8 m BL, which may be due to the nursing and weaning of $\delta^{15}\text{N}$ -enriched milk (Figure 2b). Endo et al. previously reported a similar $\delta^{15}\text{N}$ -enriched peak in the muscle samples of common minke whale calves [1], which fitted to a quadratic function ($p < 0.05$); the increase in $\delta^{15}\text{N}$ value before the peak may represent nursing, whereas the decrease in this value after the peak likely represents weaning. However, in this study, the curve fitting of $\delta^{15}\text{N}$ peak in humpback whale calves (Figure 2b) was not statistically significant ($p = 0.109$) because of the small sample size ($n = 7$) and biased sample (no newborns, few nursing animals). It is also expected that the weaning period of humpback whale may be short compared with the nursing period. Further investigation with a larger sample size is needed to confirm the $\delta^{15}\text{N}$ -enriched peak of humpback whale calves.

In all humpback whales (seven calves and two weaned immature animals), $\delta^{18}\text{O}$ values decreased with growth ($p = 0.0301$; Figure 2c), and the possibility of a $\delta^{18}\text{O}$ -enriched peak, due to nursing and weaning of calves, was not statistically supported ($n = 7$, $p = 0.262$). In addition, there was no correlation between $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of humpback whale calves ($n = 7$, $p = 0.865$). The $\delta^{18}\text{O}$ values likely decreased by weaning because the ^{18}O concentration in milk is higher than that in solid foods [18,19,50]. To the best of our knowledge, this is the first study showing a decrease in $\delta^{18}\text{O}$ values in the muscle of weaning and immature whales. In this study, the difference between the maximum and minimum values of $\delta^{15}\text{N}$ and that of $\delta^{18}\text{O}$ were 1.8‰ and 2.7‰, respectively (Table 1). These differences are consistent with the small increases in $\delta^{15}\text{N}$ value [3,4,17] and $\delta^{18}\text{O}$ value [20] due to lactation.

In contrast, $\delta^{13}\text{C}$ values of humpback whale calves increased with increases in BL; however, the $\delta^{13}\text{C}$ values of weaned immature humpback whale (-19.3 and -19.9‰) were markedly lower than that of the largest calf (8.7 m BL; -18.0‰; Figure 2a). Thus, the $\delta^{13}\text{C}$ -enriched peak could be assumed between the largest calf of 8.7 m BL and the weaned immature animals of 10 m BL, and this putative peak may be associated with the feeding shift from milk to solid foods at least in part. Endo et al. [1] previously reported no particular change in the $\delta^{13}\text{C}$ values in muscle samples of common minke whale because

of the variability in $\delta^{13}\text{C}$ values in calves and the decreasing trend of $\delta^{13}\text{C}$ values in mature animals. Thus, the change in $\delta^{13}\text{C}$ values in the small number of humpback whales (Figure 2a) does not contradict with that in common minke whales previously reported [1]. Although $\delta^{13}\text{C}$ values may be highly variable, $\delta^{13}\text{C}$ -enriched peaks are visible in the ontogenetic changes of vibrissae and skull bones in South American fur seals (*Arctocephalus australis*) [15], and a decreasing trend of $\delta^{13}\text{C}$ values is observed in the bone from weaning Northern fur seals (*Callorhinus ursinus*) [14]. The fat (lipids) concentration in humpback whale milk may increase during mid-lactation [21], but change in $\delta^{13}\text{C}$ level in cetacean milk during lactation has not yet been reported. Changes in $\delta^{13}\text{C}$ level as well as $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ levels and the fat concentration in humpback whale milk during lactation are needed to investigate.

The different patterns of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values found in humpback whale calves (Figures 2a,b, and c) could be ascribed to their different origins: the $\delta^{15}\text{N}$ -enriched peak could be derived from the nursing and weaning of $\delta^{15}\text{N}$ -enriched proteins in milk [4,17], whereas the high $\delta^{18}\text{O}$ values in calves could be derived from $\delta^{18}\text{O}$ -enriched water, proteins, and other milk components [18, 20]. Furthermore, the increase in the $\delta^{13}\text{C}$ values of calves and its putative peak may be derived from the feeding shift from milk to solid foods at least in part. In addition to their different origins, the different turnover rates of components such as protein, water, and lipids may explain the different patterns of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\delta^{13}\text{C}$ values in calf muscle samples (Figures 2a, b, and c). This study quantified the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values in the decomposed muscle samples obtained from stranded whales. Payo-Paya et al. reported that the decomposition of marine mammal muscles does not affect $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values quantified [51]; however, this effect has not yet been investigated for $\delta^{18}\text{O}$ value and requires consideration.

Most baleen whales migrate seasonally, staying in low-latitude breeding grounds in winter and moving to high-latitude feeding grounds where food is more abundant in summer. In the western North Pacific Ocean, female humpback whale gives birth mainly in November around Okinawa (Ryukyu) and Ogasawara regions, and believed to migrate with her calf to the Aleutian region (see Figure 1) [52-54]. However, details of where humpback whale calves stranded in Hokkaido during June and December were born and where they were migrating to are unknown. The available migratory information is the detection of considerable contamination of ^{134}Cs and ^{137}Cs in humpback whale calf stranded in Hokkaido, July 2011 (sample ID, C; Table 1), shortly after the disruption of Fukushima Dai-ichi Nuclear Power Plant (FDNPP) in May 2011 (Figure 1) [27]. The contaminations of ^{134}Cs and ^{137}Cs are direct evidences that this humpback whale calf migrated through the rapidly and temporarily contaminated sea area off FDNPP shortly after the disruption. No information is available about $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values in muscle of humpback whales stranded along the coast of the western North Pacific Ocean around Hokkaido. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the skin of humpback whales inhabiting in waters around Okinawa, Ogasawara and Philippines (see Figure 1) were reported to be $-18.3 \pm 0.06\text{‰}$ and $12.1 \pm 0.13\text{‰}$, respectively [54].

The Hg concentrations in cetacean milk are trace; for instance, $0.003 \pm 0.002 \mu\text{g/wet g}$ for striped dolphins (*Stenella coeruleoalba*, [55]) and 0.22 ng/mL for franciscana dolphins (*Pontoporia blainvillei*,

[56]). In agreement, the Hg concentrations in the muscle of humpback whale calves were trace ($0.02\text{-}0.05 \mu\text{g/wet g}$, $n = 7$), and those in two immature humpback whales slightly above $0.10 \mu\text{g/wet g}$, reflecting the feeding shift from milk to solid foods. The Hg concentrations in the muscle of calves and immature animals of humpback whales (Table 1) and are compatible levels of those of calves and mature animals of common minke whales (0.031 ± 0.024 and $0.133 \pm 0.035 \mu\text{g/wet g}$, respectively [1], as humpback and common minke whales are opportunistic fish-eaters [29,31]. No previous studies have assessed Hg concentrations in humpback whale tissues. To the best of our knowledge, we are the first to report Hg concentrations in the muscle and liver of humpback whale calves (Table 1).

As fin and North Pacific right whales stranding along the Hokkaido coast are rare, the number of samples of fin whales ($n = 3$) and right whales ($n = 2$) collected in 2012 and 2018 were limited. However, the $\delta^{13}\text{C}$ values off in whale calves tended to increase in BL, whereas the $\delta^{18}\text{O}$ values tended to decrease, and the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values in small right whale calf were higher than those in large right whale calf, respectively (Table 1). These changes in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values found in fin and North Pacific right whale calves are not inconsistent with those found in humpback whale calves (Figures 2a, b, and c).

Isotopic discrimination of calves from sex cetacean species stranded in Hokkaido using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

To our best knowledge, this is the first study to compare the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of calves from several mystecetes and odontocetes. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of killer whale calves were the highest, which reflect the fact that mature killer whales occupy the highest position in the marine food chain [57]. The $\delta^{15}\text{N}$ value of Dall's porpoise calves (odontocete) was the next highest and higher than that of mystecetes of common minke, humpback, fin and North Pacific right whale calves, whereas the $\delta^{13}\text{C}$ value was similar to those whale calves. The distribution of calves from six species is similar to the distribution inferred from trophic positions of their mature animals (Figure 3). Thus, we could indirectly estimate the values of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of lactating mothers and mature animals from those values of their calves. To confirm the universality of small values of $\Delta^{15}\text{N}_{\text{calves-mothers}}$ and $\Delta^{15}\text{N}_{\text{calves-matures}}$ (less than 2‰) and $\Delta^{13}\text{C}_{\text{calves-mothers}}$ and $\Delta^{13}\text{C}_{\text{calves-matures}}$ (less than 1‰) calculated from killer whales [39], common minke whale [1], and Dall's porpoises [36], further analyses of these values of humpback, fin, North Pacific right whales, etc. are needed.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of North Pacific right whale calves were the lowest although the sample number was limited ($n = 2$), which may be associated with the fact that mature right whales are zooplankton feeders, and their trophic level seems to be lower than that of opportunistic fish feeders of mysticetes and odontocetes. As the $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ values of milk and plankton may vary by season and region, further samples of right whale calf, in addition to fin and humpback whale calves, are necessary to enable the statistical discrimination of those species of calves. There are no other reports on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values in the muscle sample of North Pacific right whales. Comparable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values have been reported in the bone of southern right whales (*Eubalaena australis*) ($-20.4 \pm 3.1\text{‰}$ and $9.3 \pm 2.3\text{‰}$, respectively [8].

The $\delta^{13}\text{C}$ values of humpback, fin, and common minke whale calves stranded in Hokkaido were of similar ranges ($-19.0 \pm 0.6\text{‰}$, $-19.6 \pm 0.8\text{‰}$, and $-19.2 \pm 0.5\text{‰}$, respectively), with the $\delta^{15}\text{N}$ values of common minke whale calves ($12.6 \pm 0.6\text{‰}$) being slightly higher than those of humpback whale calves ($11.4 \pm 0.7\text{‰}$) and fin whale calves ($10.9 \pm 0.3\text{‰}$). Similar $\delta^{13}\text{C}$ levels and little difference of $\delta^{15}\text{N}$ values in humpback, fin, and minke whale calves simply similar $\delta^{13}\text{C}$ levels in their milks and weaning solid foods. Gavrilchuk et al. [35] reported similar $\delta^{13}\text{C}$ levels in the skin of mature baleen whales from the Northwest Atlantic Ocean (fin whales, $-18.6 \pm 0.4\text{‰}$; common minke whales, $-18.6 \pm 0.4\text{‰}$; humpback whales, $-18.7 \pm 0.4\text{‰}$), with humpback whales having the highest $\delta^{15}\text{N}$ values ($14.3 \pm 0.6\text{‰}$), followed by common minke whales ($13.0 \pm 1.4\text{‰}$) and fin whales ($12.2 \pm 1.3\text{‰}$). They suggest the dietary overlap of prey species among those mysticete species and the consumption of different portions of shared prey.

Discrimination of fin whale from three oceans using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values and Hg concentration

Fin whales from three oceans were discriminated by the biplots using the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values, and Hg concentration (Figures 4a, b, c, and d). As fin whale samples from three oceans are not strictly comparable because the fin whale samples from North Pacific Ocean were calves and only three ($n = 3$) and the BL of fin whales from the North Atlantic and Antarctic Oceans was unknown. However, the target of commercial whaling is mature whales prohibition of hunting for immature and lactating females, and we believe that the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of fin whale calves are only slightly higher and similar to those values of mature whales as in the case of $\Delta^{15}\text{N}_{\text{calves-matures}}$ and $\Delta^{13}\text{C}_{\text{calves-matures}}$ found common minke whales and other marine mammals. Thus, the discrimination of fin whales from three oceans, at least between the North Atlantic and Antarctic Oceans, may be possible because of: 1) the low trophic position of fin whales from the Antarctic Ocean (low $\delta^{15}\text{N}$ and Hg concentration), 2) lower ^{13}C concentration in Antarctic Ocean seawater [58], and 3) high $\delta^{18}\text{O}$ concentration in Antarctic Ocean seawater owing to geographical conditions [5,12]. We previously reported the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values and Hg concentration in the red meat products of common minke whales from the North Pacific Ocean and those of Antarctic minke whales (*Balaenoptera bonaerensis*) from the Antarctic Ocean (zooplankton feeder) [1]. In agreement with the present results, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and the Hg concentration in minke whales from the North Pacific Ocean are apparently higher than those of minke whales from the Antarctic Ocean, respectively ($-18.4 \pm 0.7\text{‰}$ vs. $-24.6 \pm 0.4\text{‰}$, $12.0 \pm 1.7\text{‰}$ vs. $6.2 \pm 0.4\text{‰}$, and $0.091 \pm 0.065 \mu\text{g/wet g}$ vs. $0.027 \pm 0.021 \mu\text{g/wet g}$), whereas the $\delta^{18}\text{O}$ value is lower ($12.0 \pm 1.2\text{‰}$ vs. $14.6 \pm 0.7\text{‰}$). The variability in the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values found in fin whales from the Antarctic Ocean were larger than those from the North Pacific and North Atlantic Oceans (Figures 4a, b, and c), which may suggest a wide migration range for fin whales in the Antarctic Ocean [59].

The Hg concentration in the smallest fin whale calf stranded in Hokkaido was trace ($0.01 \mu\text{g/wet g}$), whereas the Hg concentration in the weaning calves were 0.08 and $0.09 \mu\text{g/wet g}$ (Table 1), which may reflect the consumption of solid foods. However, so far we know, the Hg concentrations in the mature fin whale in the North Pacific

Ocean (opportunistic fish eater) have not yet been reported. The Hg concentration in the red meat products of fin whales from the Atlantic Ocean (opportunistic fish eater) has been reported to be 0.150 (0.08 - 0.350) $\mu\text{g/wet g}$ [60], which is higher than those in the muscle of fin whale calves in the present study (Table 1). In contrast, the Hg concentration in fin whale from the Antarctic Ocean (zooplankton feeder) was only $0.044 \pm 0.019 \mu\text{g/wet g}$ (Table 2), reflecting the low trophic level. The Hg concentrations in newborn and weaning North Pacific right whales were only $0.03 \mu\text{g/wet g}$ (Table 2). A similar Hg concentration was reported in the muscle and kidney samples of southern right whales (zooplankton feeder; $0.04 \mu\text{g/wet g}$ [61]).

Comparison of $\delta^{18}\text{O}$ values in the muscle, bone, and tooth samples of marine mammals

Mammalian calcified tissue, such as enamel, dentine, and bone, are all mineral/organic compounds; the mineral component in these tissues is hydroxylapatite ($\text{Ca}_{10}(\text{PO}_4)_6\text{OH}_2$), often referred to as bioapatite, whereas the organic component is mostly collagen [62]. Studies of feeding ecology and migration using $\delta^{18}\text{O}$ values have preferentially investigated mammal bone and tooth samples and rarely investigated muscle samples. The $\delta^{18}\text{O}$ levels in bones and teeth are markedly higher than those in muscle. More specifically, the ^{18}O values in the teeth of cetaceans were 22.8 - 32.6‰ [10], and those in the bones of right whale and fin whale were $29.5 \pm 1.2\text{‰}$ and $29.8 \pm 0.4\text{‰}$ [8,9], respectively, whereas in this study, the $\delta^{18}\text{O}$ levels in the muscle samples were 11.5 - 14.5‰ and those in the red meat samples were 13.5 - 17.1‰ (Tables 1 and 2). Furthermore, the $\delta^{18}\text{O}$ levels in the red meat products of six baleen whale species were 9.7 - 16.7‰ [1]. The difference in $\delta^{18}\text{O}$ levels between muscle sample and tooth and bone samples may be ascribed to different origins (bioapatite vs. protein). In contrast, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ levels in bone samples [8,9], which are mostly collagen, are compatible with those in the present muscle samples and those from previous studies [2,39] (Table 1, and 2).

Muscle is metabolically more active than bone and tooth. As such, we believe that muscle samples may be suitable for investigating the relatively rapid changes in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ values (e.g. brief lactation period of mysticetes), whereas bone and tooth samples maybe suitable for obtaining information on slow changes over a long period. Williams et al. found small enrichments of the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ levels in prehistoric human infants' bones, and estimated that breastfeeding had ceased between 3 and 4 years [19].

Conclusion

A small $\delta^{15}\text{N}$ -enriched peak, likely related to nursing and weaning, was found in the muscle of middle-sized humpback whale calf. In contrast, the patterns of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ changes were different from that of $\delta^{15}\text{N}$ change and no correlation was found among $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values. The different patterns of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ changes may be because of different origins of nutrients and turnover rates.

The Hg concentrations in the muscle of immature humpback whales were higher than those of calves. These changes in Hg concentrations as well as $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values likely reflect the feeding shift from milk to solid foods.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of calf muscles from six species were

as follows: killer whale > Dall's porpoise > common minke whale = humpback whale = fin whale ≥ North Pacific right whale. This order of $\delta^{15}\text{N}$ values in calves is similar to the expected order of their mature animals: The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of lactating mothers and mature animals seem to be estimated from those of calves.

The $\delta^{18}\text{O}$ value, combined with the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and Hg concentration, discriminated fin whales from the North Pacific, North Atlantic, and Antarctic Oceans. The $\delta^{18}\text{O}$ value could be an excellent proxy to discriminate fin whales inhabiting these three oceans.

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