Journal of
*Marine Science and Engineering*

MDPI

*Article*

# Changes in Carbon, Nitrogen, and Oxygen Stable Isotope Ratios and Mercury Concentrations in Killer Whales (*Orcinus orca*) during and after Lactation

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**Abstract:** The changes in the stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$), and mercury (Hg) concentrations in muscle and liver tissues during and after lactation were studied in killer whales stranded along the coast of Hokkaido, in the northern area of Japan ($n$ = 16). Calf muscles displayed $\delta^{13}C$- and $\delta^{15}N$-enriched peaks and a $\delta^{18}O$-depleted peak during lactation. The $\delta^{13}C$- and $\delta^{15}N$-enriched peaks appear to reflect the extensive nursing of $^{13}C$- and $^{15}N$-enriched milk and the onset of weaning, whereas the $\delta^{18}O$-depleted peak may be attributable to the extensive nursing of $^{18}O$-depleted milk and the onset of weaning. The $\delta^{13}C$ and $\delta^{15}N$ values tended to gradually increase after the weaning, whereas the $\delta^{18}O$ values tended to decrease. The $\delta^{13}C$ and $\delta^{15}N$ levels in calves were similar between liver and muscle samples, whereas those in mature animals were higher in liver than in muscle samples. The isotopic turnover rates of C and N may be similar between the liver and muscle tissues in calves, which are rapidly growing animals. The Hg concentrations in muscle tissues were slightly higher in small calves than in large calves, probably due to the Hg transfer across placenta. The Hg concentrations in liver and muscle samples increased with increasing body length, and those in two liver samples from mature animals exceeded the high-risk threshold for marine mammal health effects (82 μg/wet g).

**Keywords:** lactation; feeding ecology; lactation; stable isotope of carbon; stable isotope of nitrogen; stable isotope of oxygen; mercury; *Orcinus orca*; *Balaenoptera acutorostrata*



**Citation:** Endo, T.; Kimura, O.; Terasaki, M.; Kato, Y.; Fujii, Y.; Haraguchi, K. Changes in Carbon, Nitrogen, and Oxygen Stable Isotope Ratios and Mercury Concentrations in Killer Whales (*Orcinus orca*) during and after Lactation. *J. Mar. Sci. Eng.* **2024**, *12*, 623. https://doi.org/10.3390/jmse12040623

Academic Editor: Francesco Tiralongo

Received: 1 March 2024
Revised: 24 March 2024
Accepted: 29 March 2024
Published: 6 April 2024



## 1. Introduction

Cetaceans, especially large whales, give birth and suckle in inaccessible oceans, making it difficult to observe their reproduction [1]. Currently, stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are exclusively used to study the reproduction of cetaceans. The $\delta^{15}N$ levels in calves are higher than in their lactating mothers owing to the suckling of $^{15}N$-enriched milk [2–5]. The $\delta^{15}N$-enriched peaks were found in calf muscles during the lactation period in common minke whales (*Balaenoptera acutorostrata* [6]), humpback whales (*Megaptera novaeangliae* [7]), bowhead whales (*Balaena mysticetus* [1]), and Dall's porpoises (*Phocoenoides dalli* [1]) owing to the extensive nursing and the onset of weaning. The $\delta^{13}C$-enriched small peaks due to lactation are also found in bowhead whale and Dall's porpoise muscles [1], whereas the $\delta^{13}C$ peak attributable to lactation was not found in the muscles of common minke whales [6] and humpback whales [7]. Cetacean milk generally contains high concentrations of $^{13}C$-depleted lipids and moderate concentrations of $^{13}C$-enriched proteins [8,9], and the lipid concentrations vary during the lactation period [8,10].

The stable isotope ratio of oxygen ($\delta^{18}O$) in teeth and bones has been increasingly used to discriminate, verify, and identify the habitat of marine animals because this value

strongly reflects the geographic and climatic conditions of habitats [11–17]. Drinking water is the main source of oxygen in the tissues of terrestrial animals [18]. However, cetaceans do not drink water, and they obtain water from ingested food, in which the $\delta^{18}O$ levels are related to environmental water [19]. Small increases in $\delta^{18}O$ values, probably due to breastfeeding, have been reported in the tooth enamel of ancient humans, in addition to the $\delta^{15}N$ enrichment [9,20]. However, the changes in $\delta^{18}O$ values attributable to the lactation have not been reported in cetacean muscles.

Mercury is a global pollutant emitted to the atmosphere from both natural and anthropogenic sources. The mercury accumulation in marine animals generally increases with increasing trophic levels (i.e., increasing $\delta^{15}N$ values) [21–25] and in an age- and/or body length (BL)-dependent manner, with particularly high levels in the liver of odontocetes [26–28]. Methylmercury passes through the placenta and causes fetal Hg poisoning [29].

Killer whales (*Orcinus orca*) are large apex predators; the ages when 90% of the life span has been realized are 34 ± 0.3 for males and 52 ± 0.5 for females [30]. They are the most widespread cetaceans in the ocean, from polar water to tropical seas, although they appear to prefer high latitudes and coastal waters rather than tropical offshore and deep-sea waters [17,31]. There are at least three ecotypes of killer whales in the North Pacific [32], which are genetically distinct and have different dietary preferences: the 'transient' ecotype, which feeds on marine mammals, and the 'resident' and 'offshore' ecotypes, which feed on fish [33].

In February 2005, at the shoreline of Aidomari, Hokkaido prefecture, in the northern area of Japan, a pod of up to twelve killer whales became trapped on drifting ice floes and died after approximately one week (Figure 1). They were the transit ecotype and had eaten mainly seals and squid as determined from the digestive residues in their stomachs [27]. We obtained muscle and liver samples from nine dead killer whales including three calves and three lactating females (Aidomari killer whale (AKW) samples). We thereafter obtained three killer whale samples stranded in 2010 and 2015 from the Stranding Network of Hokkaido (SNH10055, SNH10057, and SNH12015, see Table 1), and reported the analytical data of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values and Hg concentrations in the combined killer whale samples [34]. In this previous study, we could not find the $\delta^{15}N$-enriched peak and apparent trends in the $\delta^{13}C$ and $\delta^{18}O$ values during the lactation period because of the small number of calf samples, but these data suggested that the $\delta^{13}C$ and $\delta^{15}N$ levels were higher in liver tissues than in muscle tissues from mature animals but were similar in these tissues from calves.

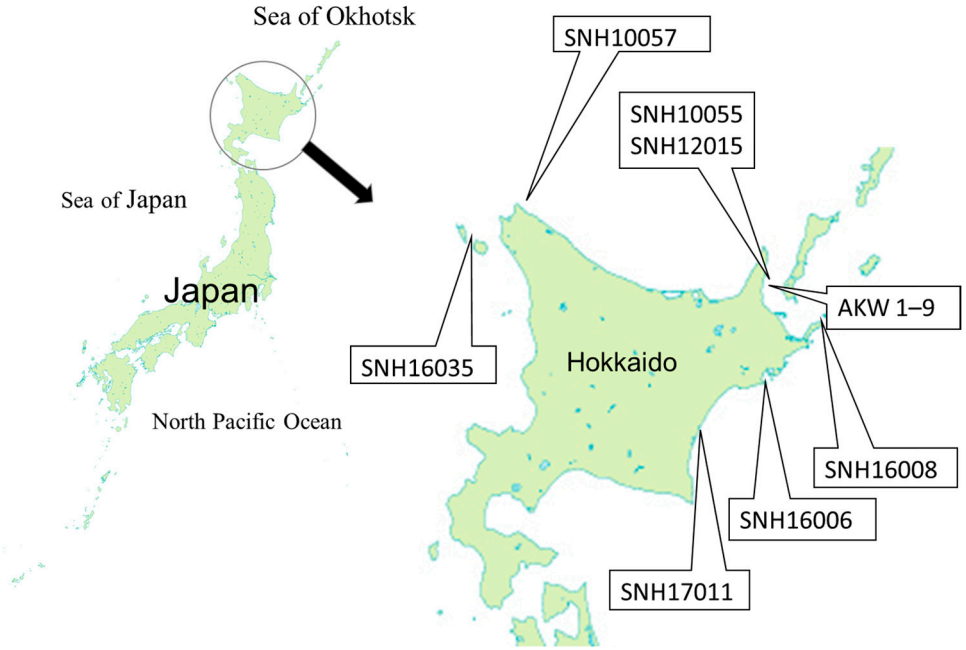


**Figure 1.** Map showing the stranding locations of AKW and SNH killer whales.

**Table 1.** Stable isotope ratios of carbon, nitrogen and oxygen and mercury concentrations in muscle and liver of killer whales stranded along the coast of Hokkaido, Japan. Data from SNH16006, SNH16035, SNH16008 and SNH17011 were added to previous data (Endo et al. [34]).

| | | | | Muscle | | | | Liver | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Body Length (m) | Age (y) | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | $\delta^{18}O$ (‰) | Hg (μg/ wet g) | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | $\delta^{18}O$ (‰) | Hg (μg/ wet g) |
| SNH16006 * | Calf male | 2.2 | <1 | −18.2 | 17.8 | 16.3 | 0.22 | ND | ND | ND | ND |
| SNH16035 * | Calf female | 2.3 | <1 | −18.3 | 16.9 | 15.9 | 0.21 | −18.3 | 17.2 | 16.0 | 0.48 |
| AKW3 ** | Calf male [a] | 2.7 | <1 | −16.7 | 18.1 | 12.4 | 0.10 | −16.6 | 18.6 | 12.6 | 0.30 |
| AKW8 ** | Calf female [b] | 2.7 | <1 | −16.9 | 18.1 | 12.3 | 0.08 | −16.7 | 18.7 | 13.4 | 0.50 |
| AKW7 ** | Calf female [c] | 3.0 | <1 | −16.8 | 18.2 | 11.9 | 0.07 | −16.6 | 18.7 | 13.3 | 0.30 |
| SNH12015 ** | Weaning calf [d] | 3.8 | 3 | −19.3 | 15.1 | 15.5 | 0.52 | −19.3 | 15.4 | 17.6 | 2.36 |
| AKW2 ** | Mature female [e] | 5.6 | 13 | −17.2 | 16.0 | 13.1 | 1.27 | −16.8 | 18.2 | 13.0 | 57.4 |
| SNH16008 * | Mature female | 5.7 | ND | −17.5 | 15.8 | 14.2 | 1.68 | ND | ND | ND | ND |
| SNH10055 ** | Mature female | 5.8 | ND | −18.2 | 16.5 | 15.0 | 2.45 | −18.0 | 18.0 | 14.4 | 107.6 |
| AKW6 ** | Mature female [f] | 6.0 | 17 | −17.1 | 16.4 | 12.7 | 1.06 | −16.8 | 19.2 | 13.7 | 38.0 |
| AKW9 ** | Mature female [g] | 6.5 | 29 | −17.2 | 16.5 | 12.0 | 1.26 | −16.7 | 19.2 | 13.4 | 62.4 |
| AKW4 ** | Mature female | 6.6 | 24 | −17.1 | 16.8 | 12.7 | 1.25 | −15.9 | 19.1 | 11.7 | 55.4 |
| AKW5 ** | Mature female | 6.9 | 59 | −17.0 | 16.4 | 12.5 | 1.30 | −16.3 | 18.6 | 12.0 | 97.8 |
| SNH10057 ** | Mature male | 7.0 | ND | −18.0 | 15.9 | 15.0 | 3.24 | −17.1 | 17.3 | 17.2 | 53.3 |
| SNH17011 * | Mature male | 7.1 | ND | −17.7 | 16.3 | 13.5 | 2.03 | −17.0 | 18.5 | 13.7 | 41.0 |
| AKW1 ** | Mature male | 7.7 | 34 | −16.9 | 17.0 | 13.1 | 1.46 | −16.5 | 18.8 | 12.7 | 35.1 |

N.D.; not detected; [a] Suckling, no mother among AKW. [b] Suckling, calf of AKW9. [c] Suckling, calf of AKW6. [d] Corrected from the juvenile female and age 2 (Endo et al. [34]) to the weaning calf and age 3. [e] Lactating, no calf among AKW. [f] Lactating, mother of AKW7. [g] Lactating, mother of AKW8; * Current study ** Endo et al. [34].

In the present study, we quantified the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values and the Hg concentrations in other killer whales stranded along the Hokkaido coast in 2016 and 2017 (SNH samples of two calves and two mature animals) after the previous study [34] for verification and further exploration. In addition, we quantified the $\delta^{18}O$ values in the remaining muscle samples of common minke whales in which we had quantified the $\delta^{13}C$ and $\delta^{15}N$ values before [6] to compare with the change of $\delta^{18}O$ values in killer whales. On the basis of these results, we studied the BL at the onset of weaning using the calculated $\delta^{15}N$-enriched peak in calf muscles; investigated whether the $\delta^{13}C$- and $\delta^{18}O$-enriched peaks are found in calf muscles; and compared the difference in $\delta^{13}C$ values, $\delta^{15}N$ value, and $\delta^{18}O$ values between liver and muscle tissues ($\delta^{13}C_{liver\text{-}muscle}$, $\delta^{15}N_{liver\text{-}muscle}$, and $\delta^{18}O_{liver\text{-}muscle}$, respectively) in calves with those in mature animals. Finally, we discussed placental transport of mercury to neonates, and compared hepatic mercury levels in mature killer whales with those previously reported in other odontocetes.

## 2. Materials and Methods

### 2.1. Samples of Killer and Common Minke Whales

Muscle samples from killer whale calves (SNH 16006 and SNH 16035), a mature male animal (SNH 16008), and a mature female animal (SNH 17011) stranded on the coast of Hokkaido, Japan in 2016 and 2017 were obtained from SNH (http://www.kujira110.com/ accessed on 30 March 2024). Liver samples from one calf (SNH 16006) and both mature whales (SNH 16008 and SNH 17011) were also obtained from SNH. These samples were quantified with the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values and the mercury concentrations. All killer whale data from the previous and current studies are summarized in Table 1, and stranding locations from both studies are presented in Figure 1. The ecotypes of all SNH whales in both studies are unidentified.

The calves with BLs of 2.2 m (SNH 16006) and 2.3 m (SNH 16035) were considered neonates because the BLs of newborns are reported to be 2.1 m [35] and 2.3 m [36]. Three calves with BLs of 2.7 m (AKW 3 and AKW 8) and 3.0 m (AKW 7) are estimated to be approximately 3 months old [34]. In two calves, milk was found in their stomachs, whereas the stomach of the third calf was empty. We believe that the three calves died immediately before or at the start of weaning, as the shortest BL of calves in which solid materials are found in the stomach is 2.6 m [37]. We believe that the animal with BL of 3.8 m (SNH 12015) is approximately 3 years old [38], and it was categorized into the late stage of

weaning because the weaning is reported to completely cease at BL of 4.3 m [37] and age of 4 years [39], although our previous study misstated its age as 2 years [34].

Muscle samples from common minke whales ($n$ = 20), in which the $\delta^{13}C$ and $\delta^{15}N$ values and the Hg concentrations had been previously quantified ([6], Table 2), were quantified the $\delta^{18}O$ values.

**Table 2.** Stable isotope ratio of oxygen in muscle of common minke whales stranded in Hokkaido, Japan.

| Samples | BL (m) | $\delta^{18}O$ (‰) | $\delta^{13}C$ * (‰) | $\delta^{15}N$ * (‰) | Hg * (μg/wet g) |
|---|---|---|---|---|---|
| 1 | 2.6 (C) | 13.0 | −9.1 | 11.2 | 0.03 |
| 2 | 3.0 (C) | 13.8 | −19.1 | 12.1 | 0.01 |
| 3 | 3.9 (C) | 12.2 | −18.8 | 13.7 | 0.04 |
| 4 | 4.1 (C) | 13.4 | −18.5 | 13.0 | 0.01 |
| 5 | 4.1 (C) | 13.2 | −19.0 | 13.4 | 0.02 |
| 6 | 4.4 (C) | 12.9 | −19.6 | 12.0 | 0.06 |
| 7 | 4.5 (C) | 13.1 | −19.7 | 13.8 | 0.02 |
| 8 | 4.6 (C) | 11.9 | −20.0 | 12.7 | 0.01 |
| 9 | 4.7 (C) | 13.0 | −19.6 | 12.4 | 0.07 |
| 10 | 4.8 (C) | 12.0 | −19.0 | 12.8 | 0.02 |
| 11 | 4.9 (C) | 12.0 | −19.2 | 11.4 | 0.06 |
| 12 | 5.0 (C) | 12.8 | −18.3 | 12.9 | 0.03 |
| 13 | 7.2 (M) | 11.9 | −19.0 | 11.5 | 0.12 |
| 14 | 7.4 (M) | 12.8 | −18.4 | 10.8 | 0.11 |
| 15 | 7.6 (F) | 12.5 | −18.7 | 12.3 | 0.14 |
| 16 | 7.7 (M) | 12.8 | −18.6 | 11.8 | 0.13 |
| 17 | 7.7 (F) | 13.0 | −18.9 | 12.2 | 0.15 |
| 18 | 7.9 (F) | 11.8 | −18.9 | 12.4 | 0.07 |
| 19 | 9.5 (M) | 12.0 | −18.9 | 11.5 | 0.17 |
| 20 | 10.2 (F) | 11.8 | −19.2 | 12.4 | 0.18 |

* The $\delta^{13}C$ and $\delta^{15}N$ values and Hg concentration in muscle were quoted from the previous report (Endo et al. [6]). C; calf, M; male mature animal, F; female mature animal.

The samples from killer whales and common minke whales were stored at −20 °C until chemical analysis.

### 2.2. Chemical Analyses

Lipids were removed from dried muscle and liver samples by chloroform/methanol extraction [40]. The extraction was performed at least three times and repeated until the color of the extraction solvent became clear. Samples were then analyzed for C, N, and O isotopes.

The $^{13}C$ and $^{15}N$ levels in the muscle and liver samples from killer whales, the $^{18}O$ levels in the muscle and liver samples from the killer whales, as well as in the muscle samples from common minke whales, were analyzed by an isotope-ratio mass spectrometry (Delta V PLUS or Delta V Advantage, Thermo Fisher Scientific, Tokyo, Japan) as reported previously [6,7,41].

As reported previously [6], total mercury (Hg) concentrations in the muscle and liver samples were quantified using a flameless atomic absorption spectrophotometer (Hiranuma Sangyo Co., Ltd., HG-310, Ibaraki, Japan) after digestion of samples by a mixture of $HNO_3$, $H_2SO_4$, and $HClO_4$. The Hg concentrations in muscle and liver samples were expressed on a wet weight basis, and the determination limit of Hg was approximately 0.01 μg/wet g.

### 2.3. Statistical Analyses

We investigated whether the relationship between BL and analytical data ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values and Hg concentrations) could be fitted by a linear or exponential function using JMP (SAS Institute Japan Ltd., Tokyo, Japan, version 14.3). The data were analyzed

by Student's *t*-test and presented as the mean ± SD. The level of significance was set at $p < 0.05$.

## 3. Results

### *3.1. Changes in $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ Values and Hg Concentrations during and after Lactation in Killer Whales*

Mature males had longer BLs (7.0–7.7 m, $n = 3$) than mature females (5.6–6.9 m, $n = 7$) (Table 1), and these ranges were consistent with the reported BLs of killer whales of mature males (6–8 m) and mature females (5–7 m), respectively [42]. The relationships of BL with $\delta^{13}C$, $\delta^{15}N$, or $\delta^{18}O$ values in muscle samples from calves and mature animals are shown in Figure 2.

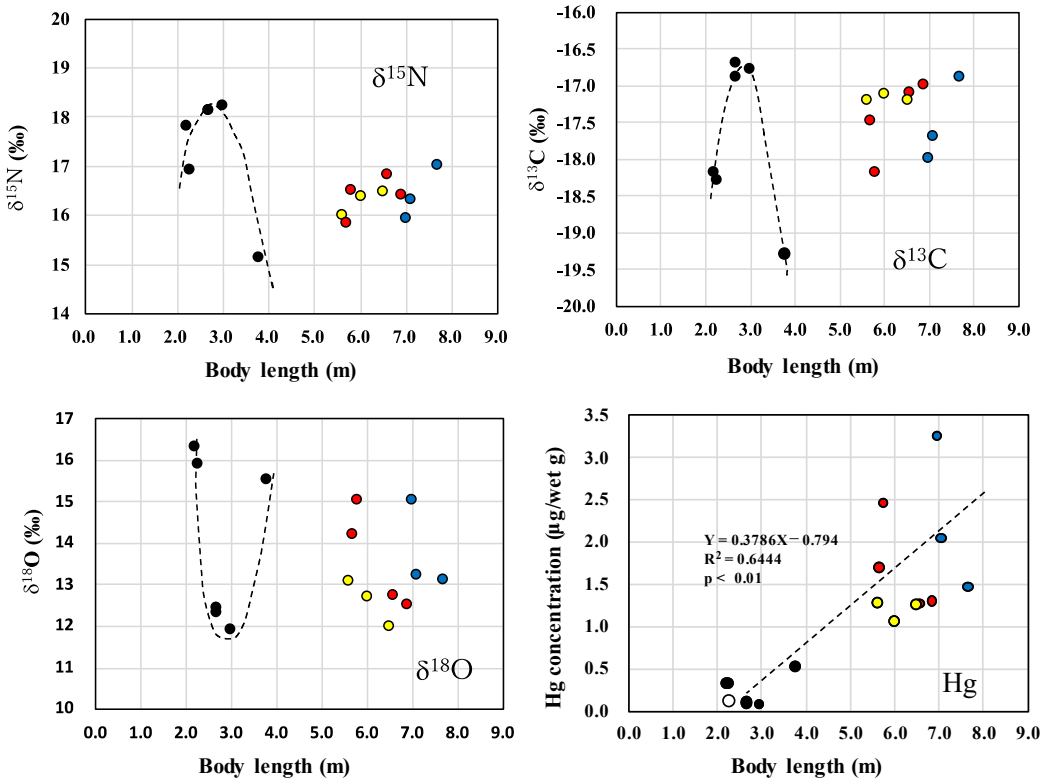


**Figure 2.** Relationship between body length and either $\delta^{15}N$ values, $\delta^{13}C$ values, $\delta^{18}O$ values or Hg concentrations in the muscle of calves (●), lactating female (●), non-lactating females (●) and mature males (●) of killer whales.

The $\delta^{15}N$ values from calves ($n = 6$) were fitted to a quadratic function (F = 8.0816, $R^2 = 0.84345$, $p = 0.0619$) although not significantly. The calculated $\delta^{15}N$-enriched peak (convex upward) was located at BL of 2.7 m and $\delta^{15}N$ value of 18.2‰. Similarly, the $\delta^{13}C$ values from calves fitted to a significantly quadratic function (F = 43.7615, $R^2 = 0.9668$, $p = 0.0060$), with the $\delta^{13}C$-enrichedpeak at BL of 2.9 m and $\delta^{13}C$ value of −16.7‰. The $\delta^{18}O$ values from calves were significantly fitted to a quadratic function (F = 91.3808, $R^2 = 0.98385$, $p = 0.0021$), but this function exhibited a convex downward peak ($\delta^{18}O$-depleted peak) at BL of 3.0 m and $\delta^{18}O$ value of 11.7‰.

As observed for muscle samples shown in Figure 2, the $\delta^{15}N$, $\delta^{13}C$, and $\delta^{18}O$ values in the liver samples from calves ($n = 5$) were significantly fitted to quadratic functions. As data are not shown in figure, the $\delta^{15}N$-enriched peak was located at BL of 2.9 m and $\delta^{15}N$ value of 18.8‰ (F = 705.41, $R^2 = 0.9986$, $p = 0.0014$), and the $\delta^{13}C$-enriched peak was located at BL of 2.9 m and $\delta^{13}C$ value of −16.5‰ (F = 143.64, $R^2 = 0.9930$, $p = 0.0069$). Meanwhile, the $\delta^{18}O$-depleted peak was located at BL of 2.9 m and $\delta^{18}O$ value of 12.9‰ (F = 23.406,

$R^2$ = 0.9590, $p$ = 0.0410). These BLs of the $\delta^{15}N$, $\delta^{13}C$, and $\delta^{18}O$ peaks calculated in liver samples (2.9 m) were extremely close to those calculated in muscle samples (2.7–3.0 m).

The $\delta^{13}C$ and $\delta^{15}N$ values for muscle samples in the largest calf at the late stage of weaning (SNH 12015) were the lowest among all killer whales, whereas the $\delta^{18}O$ value in the largest calf was higher than that in all mature whales.

The $\delta^{13}C$ and $\delta^{15}N$ values for muscle samples in mature males increased with increasing BL, whereas no or slight increases in these values were found in both lactating and non-lactating females. The $\delta^{13}C$ and $\delta^{15}N$ levels in lactating ($n$ = 3) and non-lactating females ($n$ = 4) were similar, being $-17.2 \pm 0.1$ and $-17.5 \pm 0.5$‰, respectively, for the $\delta^{13}C$ values and $16.3 \pm 0.3$ and $16.4 \pm 0.5$‰, respectively, for the $\delta^{15}N$ values. Conversely, the $\delta^{18}O$ levels in mature males, lactating females, and non-lactating females tended to decrease with increasing BL, and the $\delta^{18}O$ level was slightly lower in lactating females ($12.6 \pm 0.6$‰, $n$ = 3) than in non-lactating females ($13.6 \pm 1.2$‰, $n$ = 4) and mature males ($13.9 \pm 1.0$‰, $n$ = 3).

The Hg concentrations in muscle samples increased with increasing BL (F = 26.7529, $R^2$ = 0.6564, $p$ = 0.001), although large variation was found in mature males ($n$ = 3) and the Hg concentrations were slightly higher in small calves (0.22 and 0.21 μg/wet g in SNH 16006 and SNH 16035 samples, respectively) than in large calves (0.10, 0.07, and 0.08 μg/wet g in AKW 3, AKW 7, and AKW 8 samples, respectively). The Hg concentrations in lactating females ($1.40 \pm 0.24$ μg/wet g, $n$ = 3) were similar to these in non-lactating females ($1.52 \pm 0.62$ μg/wet g, $n$ = 4). Conversely, the increasing trend of Hg concentrations in liver samples due to increasing BL was unclear because of the large variation in Hg concentrations in the mature animals ($60.9 \pm 25.7$ μg/wet g, 35.1–107.6 μg/wet g, $n$ = 9) (not shown in figure). All Hg concentrations exceeded low-risk threshold for marine mammal health effects (16 μg/wet g), and two Hg concentrations exceeded high-risk threshold (83 μg/wet g) [43].

BLs, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of AKW samples from mature animals (transient type, $n$ = 6) were compared with those of SNH samples from mature animals (type is unidentified, $n$ = 4). The BLs were $6.1 \pm 0.1$ m and $6.1 \pm 0.8$ m, the $\delta^{13}C$ values were $-17.1 \pm 0.1$‰ and $-17.8 \pm 0.3$‰, the $\delta^{15}N$ values were $16.5 \pm 0.3$‰ and $16.1 \pm 0.3$‰, and the $\delta^{18}O$ values were $12.7 \pm 0.4$‰ and $14.4 \pm 0.7$‰, which were similar except for the $\delta^{18}O$ values. On the other hand, the Hg concentrations in muscle and liver samples of AKW were $1.27 \pm 0.13$ μg/wet g ($n$ = 6) and $12.75 \pm 0.78$ μg/wet g ($n$ = 6), respectively, slightly lower than those in muscle samples ($2.35 \pm 0.67$ μg/wet g, $n$ = 4) and liver samples ($15.12 \pm 1.85$ μg/wet g, $n$ = 3) of SNH.

### *3.2. Comparison of $\delta^{13}C_{liver\text{-}muscle}$, $\delta^{15}N_{liver\text{-}muscle}$, and $\delta^{18}O_{liver\text{-}muscle}$ Levels in Calves and Mature Animals*

We compared the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ levels in muscle samples with those levels in the liver samples, respectively (Table 3).

**Table 3.** Stable isotope ratios of carbon, nitrogen, and oxygen in muscle and liver samples of calves and mature animals from killer whales.

| | | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | $\delta^{18}O$ (‰) |
|---|---|---|---|---|
| Calves | Muscle ($n$ = 6) | $-17.7 \pm 1.1$ | $17.4 \pm 1.2$ | $14.1 \pm 2.1$ |
| | Liver ($n$ = 5) | $-17.5 \pm 1.2$ | $17.7 \pm 1.4$ | $14.6 \pm 2.1$ |
| Mature animals | Muscle ($n$ =10) | $-17.4 \pm 0.4$ | $16.4 \pm 0.4$ | $13.4 \pm 1.0$ |
| | Liver ($n$ = 9) | $-16.8 \pm 0.6$ | $18.5 \pm 0.6$ * | $13.5 \pm 1.6$ |

* Significantly different from muscle samples ($p < 0.01$).

In mature animals, the $\delta^{13}C$ and $\delta^{15}N$ levels were higher in liver samples ($-16.8 \pm 0.6$ and $18.5 \pm 0.6$‰, respectively) than in muscle samples ($-17.4 \pm 0.4$ and $16.4 \pm 0.4$‰, respectively), but only $\delta^{15}N$ levels were significantly different between tissues. On the other hand, no differences in $\delta^{13}C$ and $\delta^{15}N$ levels were observed between muscle and

liver samples from calves. On the contrary, the $\delta^{18}O$ levels were similar between liver and muscle samples from both calves and mature whales, although these levels were slightly lower in mature animals than in calves.

We investigated the relationship between BL and the differences in $\delta^{13}C$, $\delta^{15}N$, or $\delta^{18}O$ values between liver and muscle samples (Figure 3). The $\delta^{15}N_{liver\text{-}muscle}$ values were significantly larger ($t_{12} = 7.525$, $p < 0.01$) in mature animals (2.12 ± 0.48) than in calves (0.44 ± 0.13), and the $\delta^{13}C_{liver\text{-}muscle}$ values were also significantly larger ($t_{12} = 3.296$, $p < 0.01$) in mature animals (0.59 ± 0.32) than in calves (0.10 ± 0.10). Although the $\delta^{15}N_{liver\text{-}muscle}$ and $\delta^{13}C_{liver\text{-}muscle}$ values in calves were small, these values likely had small peaks, as in the case of the $\delta^{15}N$ and $\delta^{13}C$ values (Figure 2). The trends of $\delta^{15}N_{liver\text{-}muscle}$ and $\delta^{13}C_{liver\text{-}muscle}$ values associated with increasing BL in mature animals were unclear. By contrast, the $\delta^{18}O_{liver\text{-}muscle}$ values in calves (0.98 ± 0.84) increased linearly with increasing BL ($F = 13.7243$, $R^2 = 0.821$, $p = 0.0342$), whereas no particular trend was observed for the $\delta^{18}O_{liver\text{-}muscle}$ values in mature animals (0.28 ± 1.07).

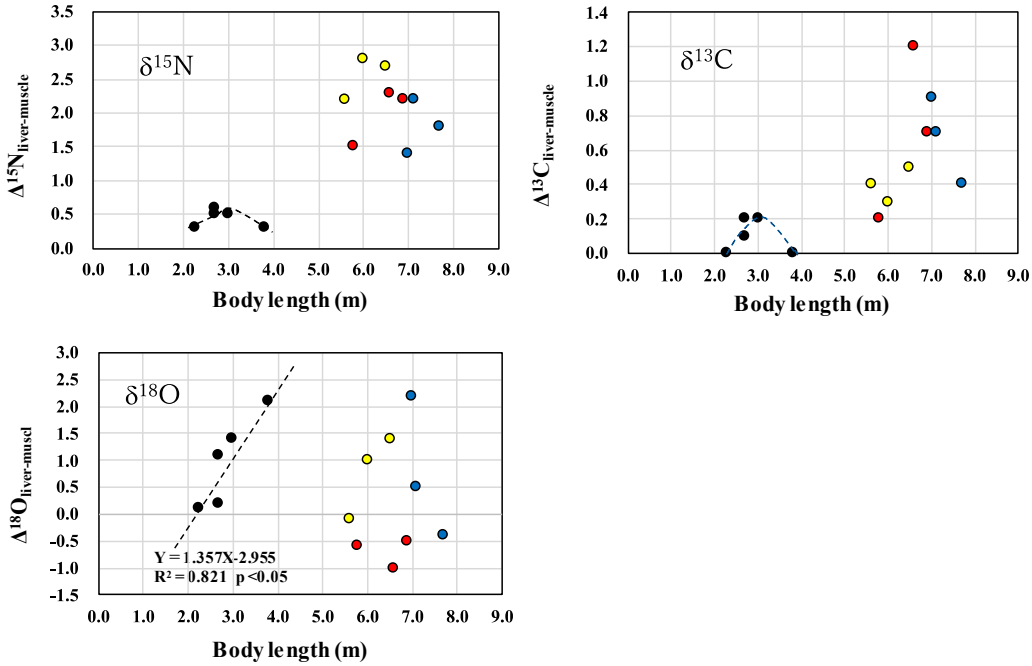


**Figure 3.** Differences in $\delta^{15}N$ values, $\delta^{13}C$ values and $\delta^{18}O$ values between liver and muscle samples of killer whale calves (●), lactating female (●), non-lactating females (●), and mature males (●).

### *3.3. Changes in $\delta^{18}O$ Values during Lactation in Common Minke Whales*

We quantified $\delta^{18}O$ values in muscle samples from common minke whales (Figure 4) to compare the change in $\delta^{18}O$ values in killer whales (Figure 2). We show the $\delta^{15}N$-enriched peak at BL of 4.0 m and the variation in $\delta^{13}C$ values in common minke whale calves in Figure 4, which was quoted from the previous report [6]. No sex-related differences were found in BL, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values and Hg concentrations between calves and mature animals.

The $\delta^{18}O$ values were 12.8 ± 0.6‰ ($n$ =12) in common minke whale calves, and these values tended to decrease throughout the lactation period ($F = 3.7185$, $R^2 = 0.27106$, $p = 0.0826$), as in the case of killer whale calves excluding the largest calf (Figure 2). By contrast, no particular changes were found in the $\delta^{18}O$ values from mature whales (12.3 ± 0.5‰, $n = 8$). The $\delta^{18}O$-enriched peak likely exists between the large calf and small mature animal.

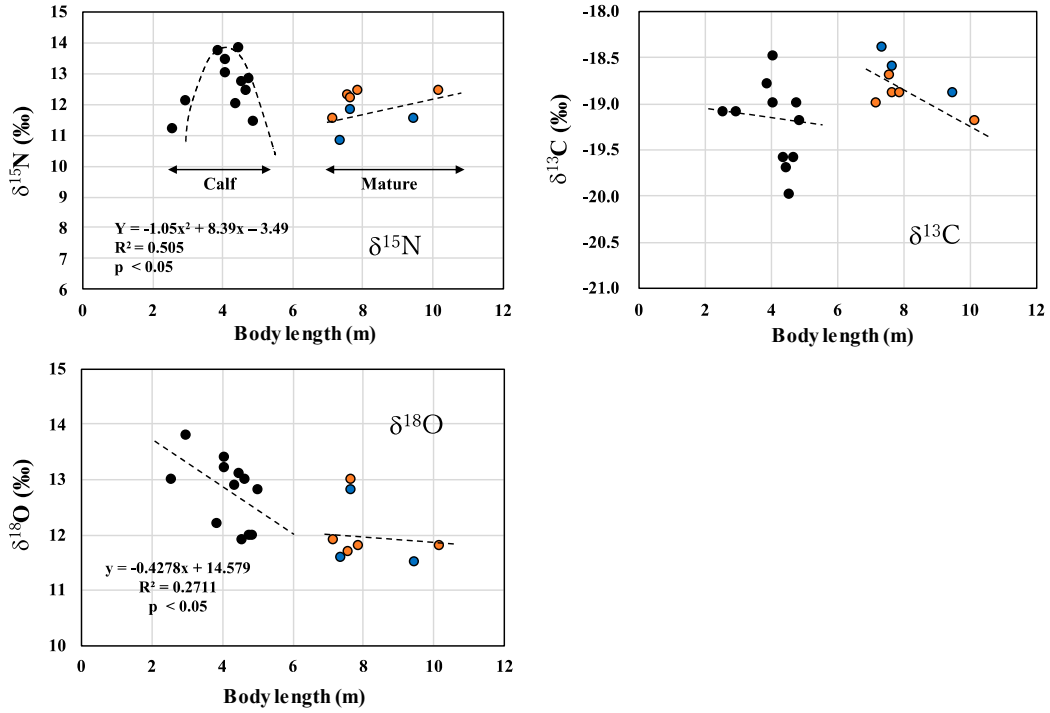


**Figure 4.** Relationship between body length and either $\delta^{15}N$ values, $\delta^{13}C$ values or $\delta^{18}O$ values in the muscle samples of common minke whale calves (●), mature males (●), and mature females (●). The upper figures showing $\delta^{15}N$ and $\delta^{13}C$ values were reprinted from Endo et al. [6] with permission from Aquatic Mammals.

## 4. Discussion

We studied the changes in $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ values, and Hg concentrations in muscle and liver tissues during and after lactation of killer whales.

The $\delta^{15}N$-enriched peak due to lactation was found in the current study of killer whale muscles (Figure 2). The increase in $\delta^{15}N$ values across the peak could represent the extensive nursing of $\delta^{15}N$-enriched milk, and the decrease in $\delta^{15}N$ values from the peak could represent the onset of weaning [1,2,4–7]. The BLs at the onset of weaning calculated from the $\delta^{15}N$ peak of 2.7 m in muscle samples (Figure 2) and 2.9 m in liver samples were consistent with the actual BLs at the onset weaning of 2.6 and 3.3 m reported in wild killer whales [37]. Meanwhile, the BL and the age at the complete cessation of weaning were reported to be approximately 4.3 m [37] and 4 years [39], respectively. Unfortunately, we could not estimate this BL because our analysis included only one weaning calf sample (SNH12015) and no juvenile samples.

As with the $\delta^{15}N$-enriched peak, the $\delta^{13}C$-enriched peaks attributable to lactation were found in muscle (Figure 2) and liver samples, with both peaks occurring at the BL of 2.9 m. The $\delta^{13}C$-enriched peak during the lactation period has been observed in human scalp hairs [2] and bowhead whale and Dall's porpoise muscles [1]; this peak was not found in common minke whale muscles (Figure 4) and humpback whale muscles [7]. Cetacean milk contains high concentrations of $\delta^{13}C$-depleted lipids and moderate concentrations of $\delta^{13}C$-enriched proteins, and lipid concentrations vary among species and during the lactation period [8,10]. The $\delta^{13}C$-enriched peak found in killer whales (Figure 2), bowhead whales, and Dall's porpoises [1] might result from the suckling of milk containing $\delta^{13}C$-enriched proteins and relatively lower concentrations of $\delta^{13}C$-depleted lipids, whereas the lack of $\delta^{13}C$ peak or no trend of $\delta^{13}C$ change found in common minke and humpback whales may be due to the large variation of $\delta^{13}C$-depleted lipid concentrations in milk [4,14].

The BLs of the $\delta^{18}O$-depleted peak of 3.0 and 2.9 m calculated from muscle samples (Figure 2) and liver samples, respectively, were extremely close to the BLs of the $\delta^{15}N$- and $\delta^{13}C$-enriched peaks. Thus, the BLs of the $\delta^{18}O$-depleted peak could be related to the

onset of weaning. A similar $\delta^{18}O$-depleted peak is likely to exist between the large calf and small mature animal (juvenile) of common minke whales (Figure 4) and humpback whales [7]. Suckling appears to decrease the $\delta^{18}O$ levels of calf tissues, and the onset of weaning may increase the $\delta^{18}O$ levels, seemingly because of lower $\delta^{18}O$ levels in milk than in calf muscles and in weaning foods. Interestingly, a large enrichment in $\delta^{18}O$ values with wide variability was reported in the deciduous teeth enamel formed during the first 6 months of breastfeeding in humans [44].

No studies have been reported on $\delta^{18}O$ levels in the milk of marine mammals. Available information on the $\delta^{18}O$ levels in milk has been obtained from terrestrial animals, for which the main source of oxygen in milk is drinking water, and cow's milk water is enriched in $\delta^{18}O$ by about 3‰ compared to drinking water (−9.2 to −0.02‰) [45]. The $\delta^{18}O$ values in the seawaters the stranded killer whales inhabited were reported to range from −0.1 to −0.01‰ in the Okhotsk Sea [46] and from −2.0 to 0.1‰ in the North Pacific Oceans around Japan [47]. Based on these reports, we estimate the $\delta^{18}O$ values in killer whale milk to be around 1–3‰, which supports our assumption that the $\delta^{18}O$ level in milk is lower than in muscle of calves. Analyses of $\delta^{18}O$ levels in milk and weaning foods are needed to clarify the $\delta^{18}O$-depleted peak and $\delta^{18}O$-enriched peak in killer whales (Figure 2).

The isotopic half-life ($T_{1/2}$) for C and N in terrestrial mammalian muscles (1–3 months) is markedly longer than that of the more metabolically active tissue of the liver (3–7 days) [48,49], and $T_{1/2}$ could decrease generally with a decrease in animal body mass and with increases in metabolic and growth rates [50,51]. Thus, we believe that the isotopic turnover rates of N and C in muscle tissue are much faster in calves than in mature animals, and therefore there is little time lag between the actual onset weaning and that calculated from the calf muscles (Figure 2). We previously estimated the BL at the onset of weaning in common minke whales [6] and humpback whales [7] using the $\delta^{15}N$-enriched peaks in their muscle samples, which are close to their actual BLs at the onset of weaning. Curve fitting of $\delta^{15}N$ values in calf muscle samples appears to be a powerful method for estimating the onset of weaning.

By contrast, little information is available on the turnover rate of O in marine mammals. The origins of O in milk could be mainly derived from water [45], whereas those of C and N in milk could be mainly derived from proteins, lipids, and carbohydrates [8,9]. The different patterns of the $\delta^{18}O$ values and the $\delta^{13}C$ and $\delta^{15}N$ values in calves (Figures 2 and 4) could be attributable to their different origins in milk.

It is known that the $\delta^{15}N$ levels are generally higher in liver tissue than in muscle tissue from mature animals [18,52–54], and the higher values in liver tissue are thought to reflect the higher metabolic turnover rates in liver tissue. Consistent with this, the $\delta^{15}N$ levels as well as the $\delta^{13}C$ levels were higher in liver tissue than in muscle tissue of mature animals (Table 2). Conversely, $\delta^{15}N$ and $\delta^{13}C$ values were similar between calf liver and muscle tissues, suggesting that the turnover rates of N and C between these tissues are similar in calves, which are rapidly growing animals. By contrast, no difference in $\delta^{18}O$ values was found between liver and muscle tissues in both calves and mature animals, whereas the $\delta^{18}O$ value in both tissues was slightly higher in calves than in mature animals and the $\delta^{18}O_{liver\text{-}muscle}$ values in calves increased with increasing BL (Figure 3). These differences between the $\delta^{18}O$ values and the $\delta^{13}C$ and $\delta^{15}N$ values could be attributed to the different origins of O and C and N and the different evolution of their turnover rates with growth. Further studies are necessary to investigate the different patterns of the $\delta^{13}C$ and $\delta^{15}N$ values and the $\delta^{18}O$ value between liver and muscle tissues and between the calves and mature animals.

The dentin growth layer in teeth can be used to investigate the continuous annual changes in the values of $\delta^{13}C$ and $\delta^{15}N$, although this analysis cannot investigate any changes occurring within the first year such as the $\delta^{15}N$- and $\delta^{13}C$-enriched peaks and $\delta^{18}O$-depleted peak in muscle tissues (Figure 2) and liver samples. Newsome et al. [39] analyzed the dentin growth layers of killer whale teeth and reported an approximate 2.5‰ decrease in $\delta^{15}N$ value due to the weaning, which occurs mainly during the first 3 years, and an approximate 1.5‰ of increase in $\delta^{15}N$ value thereafter due to the ontogenetic

increase in trophic levels. The decrease (2.5‰) and subsequent increase in $\delta^{15}N$ values (1.5‰) reported in the dentin growth layer corresponded to the present finding of a 3.0‰ increase in $\delta^{15}N$ values (range of maximum and minimum) in calves and 2.5‰ increase in $\delta^{15}N$ value from the largest calf to the largest mature animal (Figure 2). We reported previously the peaks of $\delta^{15}N$ and $\delta^{13}C$ values during lactation period and the ontogenetic slight increase in $\delta^{15}N$ of mature animals in the muscle samples from Dall's porpoises and bowhead whales [1].

The Hg is preferentially accumulated in the liver of marine mammals [27,28,55]. In agreement, the Hg concentrations in liver samples from mature killer whales were 60.9 ± 25.6 μg/wet g ($n$ = 9), and two Hg concentrations exceeded 83 μg/wet g, which is the high-risk threshold for marine mammal health effects [43]. Available information on the Hg concentrations in livers and $\delta^{15}N$ values in muscles of marine mammals stranded or incidentally caught off the coast of Hokkaido, which may be preyed upon by the transient ecotype of killer whales, are summarized in Table 4. The Hg concentrations and $\delta^{15}N$ values in killer whales were markedly higher than those in the other mammals, particularly common minke whales (baleen whale).

**Table 4.** Comparison of mercury concentrations in liver and $\delta^{15}N$ values in muscle of cetaceans inhabiting waters around Hokkaido.

| Species | Hg concentration in Liver (μg/wet g) | $\delta^{15}N$ in Muscle (‰) |
|---|---|---|
| Killer whale (mature animals) of this study | 60.9 ± 25.6 ($n$ = 9) [a] | 16.4 ± 0.4 ($n$ = 10) [a] |
| all animals of this study | 39.4 ± 36.0 ($n$ = 16) [a] | 16.7 ± 0.9 ($n$ = 16) [a] |
| Dall's porpoises (*Phocoenoides dalli*) | 8.62 ± 10.60 ($n$ = 52) [b] | 13.1 ± 0.9 ($n$ = 56) [b] |
| Harbor porpoises (*Phocoena phocoena*) | 6.80 ± 14.9 ($n$ = 45) [b] | 13.2 ± 1.0 ($n$ = 49) [b] |
| Spotted seals (*Phoca largha*) | 1.93 and 0.34 [b] | 14.5 and 15.1 [b] |
| Harbor seals (*Phoca vitulina*) | 2.96 ± 3.26 ($n$ = 32) [b] | 15.6 ± 0.52 ($n$ = 32) [b] |
| Common minke whales (*Balaenoptera acutorostrata*) | 0.103 ± 0.100 ($n$ = 8) [b] | 12.3 ± 0.8 ($n$ = 20) [c] |

[a] Current study, [b] Our unpublished data, [c] Endo et al. [6].

$\delta^{15}N$ and Hg levels were compared between two ecotypes of killer whales off Norway, fish-eaters and seal-eaters. Skin $\delta^{15}N$ levels were markedly higher in the seal-eaters (12.6 ± 0.3‰) than in the fish-eaters (11.7 ± 0.2‰) [56], and the Hg concentrations in the skin were about twice higher in the seal-eaters than in the fish-eaters (Andvik et al. 2020). In the current study, the $\delta^{15}N$ levels as well as the $\delta^{13}C$ and BL levels in mature animals from SNH (ecotype unidentified) approximated those from AKW samples (transient ecotype), with slightly higher Hg concentrations in muscle and liver tissues. This suggests that the SNH samples are likely to include the samples taken from transient ecotypes.

The Hg concentrations in muscle samples from small calves (SNH 16006 and SNH 16033) were slightly higher than those of large calves (AKW 3, AKW 7, and AKW 8). Similar phenomena have been reported in the calves of striped dolphins [57,58] and humpback whales [7]. The higher Hg concentrations in small calves (neonates) could be explained by the transfer of methylmercury across the placenta, and the lower Hg concentrations in large calves can be explained by the suckling of milk containing trace concentrations of Hg [57,59] and the growth dilution effect [57].

The marine mammal-eating type of killer whale could have higher Hg contamination and $\delta^{15}N$ levels than the fish-eating type [21,56]. However, these comparisons have not been investigated between both types of calves. In the current study, the Hg concentrations in muscle samples were higher in small calves (SNH samples, ecotype unidentified) than in large calves (AKW samples, transient ecotype), whereas the $\delta^{15}N$ levels were lower in small calves than in large calves. Further studies focusing on the Hg load across placenta and the $\delta^{15}N$ levels are needed to increase the number of ecotype-identified calves.

## 5. Conclusions

The $\delta^{15}N$- and $\delta^{13}C$-enriched peaks were found in muscle and liver samples from killer whale calves because of extensive nursing and subsequent weaning.

The $\delta^{18}O$-depleted peaks were found in muscle and liver samples from calves, probably because of the consumption of $\delta^{18}O$-depleted milk and weaning foods. The decrease in $\delta^{18}O$ values during the lactation period was found in common minke whale calves, similar to killer whale calves.

The $\delta^{13}C$ and $\delta^{15}N$ values were higher in liver samples than in muscle samples of mature killer whales. By contrast, the $\delta^{13}C$ and $\delta^{15}N$ values in calves were similar between liver and muscle tissues, probably because of the higher metabolic rate of calves, which are rapidly growing animals.

The Hg concentrations in muscle tissues were slightly higher in small calves than in large calves, probably due to the Hg transfer across placenta. The Hg concentrations in two liver samples from mature animals exceeded the high-risk threshold for marine mammal health effects (82 μg/wet g).

**Author Contributions:** T.E.: Conceptualization, Investigation, Data curation, Methodology, Writing—original draft, Writing—review and editing. O.K.: Methodology, Data curation. M.T.: Methodology, Data curation. Y.F.: Formal analysis, Writing—review and editing. K.H.: Funding acquisition, Writing—review and editing. Y.K.: Methodology, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by JSPS KAKENHI (grant number 20K12188, K.H.).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** We would like to thank the Stranding Network Hokkaido (SNH) for providing samples and information on the stranded whales studied in this study. We would like to thank Aquatic Mammals for granting permission to use Figure 4 from Endo et al. [6].

**Conflicts of Interest:** The authors declare that there are no conflicts of interest.

## References

1. Endo, T.; Kobayashi, M. Typical changes in carbon and nitrogen stable isotope ratios and mercury concentration during the lactation of marine mammals. In *Marine Mammals*; Kaoud, H.A.E., Ed.; IntechOpen: London, UK, 2022; ISBN 978-1-80355-490-7.
2. Fuller, B.; Fuller, J.; Harris, D.; Hedges, R. Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios. *Am. J. Phys. Anthr.* **2006**, *129*, 279–293. [CrossRef] [PubMed]
3. Newsome, S.D.; Koch, P.L.; Etnier, M.A.; Aurioles-Gamboa, D. Using Carbon and nitrogen isotope values to investigate maternal strategies in northeast pacific otariids. *Mar. Mammal Sci.* **2006**, *22*, 556–572. [CrossRef]
4. Borrell, A.; Gómez-Campos, E.; Aguilar, A. Influence of Reproduction on Stable-Isotope Ratios: Nitrogen and Carbon Isotope Discrimination between Mothers, Fetuses, and Milk in the Fin Whale, a Capital Breeder. *Physiol. Biochem. Zool.* **2016**, *89*, 41–50. [CrossRef] [PubMed]
5. Vales, D.; Cardona, L.; García, N.; Zenteno, L.; Crespo, E. Ontogenetic dietary changes in male South American fur seals Arctocephalus australis in Patagonia. *Mar. Ecol. Prog. Ser.* **2015**, *525*, 245–260. [CrossRef]
6. Endo, T.; Kimura, O.; Terasaki, M.; Kobayashi, M. Body length, stable carbon, and nitrogen isotope ratios and mercury levels in common minke whales (*Balaenoptera acutorostrata*) stranded along the coast of Hokkaido, Japan. *Aquat. Mamm.* **2021**, *47*, 86–95. [CrossRef]
7. Endo, T.; Terasaki, M.; Kimura, O. 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. *J. Veter.-Sci. Med.* **2022**, *10*, 1–10. [CrossRef]
8. Oftedal, O.T. Lactation in Whales and Dolphins: Evidence of Divergence Between Baleen- and Toothed-Species. *J. Mammary Gland. Biol. Neoplasia* **1997**, *2*, 205–230. [CrossRef] [PubMed]
9. Tsutaya, T.; Yoneda, M. Reconstruction of breastfeeding and weaning practices using stable isotope and trace element analyses: A review. *Am. J. Phys. Anthr.* **2015**, *156*, 2–21. [CrossRef]
10. Oftedal, O.T. Milk of marine mammals. In *Encyclopedia of Dairy Sciences*, 2nd ed.; Fuquay, J.W., Ed.; Academic Press: Cambridge, MA, USA, 2011; pp. 563–580. [CrossRef]

11. Kalish, J.M. $^{13}C$ and $^{18}O$ isotopic disequilibria in fish otoliths: Metabolic and kinetic effects. *Mar. Ecol. Prog. Ser.* **1991**, *75*, 191–203. [CrossRef]
12. Clementz, M.T.; Koch, P.L. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. *Oecologia* **2001**, *129*, 461–472. [CrossRef]
13. Liu, A.G.S.C.; Seiffert, E.R.; Simons, E.L. Stable isotope evidence for an amphibious phase in early proboscidean evolution. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5786–5791. [CrossRef]
14. Newsome, S.D.; Clementz, M.T.; Koch, P.L. Using stable isotope biogeochemistry to study marine mammal ecology. *Mar. Mammal Sci.* **2010**, *26*, 509–572. [CrossRef]
15. Lin, H.-Y.; Shiao, J.-C.; Chen, Y.-G.; Iizuka, Y. Ontogenetic vertical migration of grenadiers revealed by otolith microstructures and stable isotopic composition. *Deep. Sea Res. Part I Oceanogr. Res. Pap.* **2012**, *61*, 123–130. [CrossRef]
16. Matthews, C.J.D.; Longstaffe, F.J.; Ferguson, S.H. Dentine oxygen isotopes ($\delta^{18}O$) as a proxy for odontocete distributions and movements. *Ecol. Evol.* **2016**, *6*, 4643–4653. [CrossRef]
17. Matthews, C.J.D.; Longstaffe, F.J.; Lawson, J.W.; Ferguson, S.H. Distributions of Arctic and Northwest Atlantic killer whales inferred from oxygen isotopes. *Sci. Rep.* **2021**, *11*, 6739. [CrossRef]
18. Camin, F.; Bontempo, L.; Perini, M.; Piasentier, E. Stable Isotope Ratio Analysis for Assessing the Authenticity of Food of Animal Origin. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 868–877. [CrossRef]
19. Yoshida, N.; Miyazaki, N. Oxygen isotope correlation of cetacean bone phosphate with environmental water. *J. Geophys. Res. Ocean.* **1991**, *96*, 815–820. [CrossRef]
20. Wright, L.E.; Schwarcz, H.P. Stable carbon and oxygen isotopes in human tooth enamel: Identifying breastfeeding and weaning in prehistory. *Am. J. Physiol. Anthropol.* **1998**, *106*, 1–18. [CrossRef]
21. Andvik, C.; Jourdain, E.; Ruus, A.; Lyche, J.L.; Karoliussen, R.; Borgå, K. Preying on seals pushes killer whales from Norway above pollution effects thresholds. *Sci. Rep.* **2020**, *10*, 11888. [CrossRef]
22. Power, M.; Klein, G.M.; Guiguer, K.R.R.A.; Kwan, M.K.H. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. *J. Appl. Ecol.* **2002**, *39*, 819–830. [CrossRef]
23. Chouvelon, T.; Spitz, J.; Caurant, F.; Mèndez-Fernandez, P.; Autier, J.; Lassus-Débat, A.; Chappuis, A.; Bustamante, P. Enhanced bioaccumulation of mercury in deep-sea fauna from the Bay of Biscay (north-east Atlantic) in relation to trophic positions identified by analysis of carbon and nitrogen stable isotopes. *Deep. Sea Res. Part I Oceanogr. Res. Pap.* **2012**, *65*, 113–124. [CrossRef]
24. Chouvelon, T.; Caurant, F.; Cherel, Y.; Simon-Bouhet, B.; Spitz, J.; Bustamante, P. Species- and size-related patterns in stable isotopes and mercury concentrations in fish help refine marine ecosystem indicators and provide evidence for distinct management units for hake in the Northeast Atlantic. *ICES J. Mar. Sci.* **2014**, *71*, 1073–1087. [CrossRef]
25. Pethybridge, H.; Butler, E.; Cossa, D.; Daley, R.; Boudou, A. Trophic structure and biomagnification of mercury in an assemblage of deepwater chondrichthyans from southeastern Australia. *Mar. Ecol. Prog. Ser.* **2012**, *451*, 163–174. [CrossRef]
26. Honda, K. Contamination of heavy metals in marine mammals. In *Biology of Marine Mammals*; Miyazaki, N., Kasuya, T., Eds.; Scientist Inc.: Tokyo, Japan, 1990; pp. 242–253.
27. Endo, T.; Kimura, O.; Hisamichi, Y.; Minoshima, Y.; Haraguchi, K. Age-dependent accumulation of heavy metals in a pod of killer whales (*Orcinus orca*) stranded in the northern area of Japan. *Chemosphere* **2007**, *67*, 51–59. [CrossRef] [PubMed]
28. Endo, T.; Hisamichi, Y.; Kimura, O.; Haraguchi, K.; Baker, C.S. Contamination levels of mercury and cadmium in melon-headed whales (*Peponocephala electra*) from a mass stranding on the Japanese coast. *Sci. Total. Environ.* **2008**, *401*, 73–80. [CrossRef] [PubMed]
29. Sakamoto, M.; Itai, T.; Murata, K. Effects of Prenatal Methylmercury Exposure: From Minamata Disease to Environmental Health Studies. *Nippon. Eiseigaku Zasshi Jpn. J. Hyg.* **2017**, *72*, 140–148. [CrossRef] [PubMed]
30. Nielsen, M.L.K.; Ellis, S.; Towers, J.R.; Doniol-Valcroze, T.; Franks, D.W.; Cant, M.A.; Weiss, M.N.; Johnstone, R.A.; Balcomb, K.C., 3rd; Ellifrit, D.K.; et al. A long postreproductive life span is a shared trait among genetically distinct killer whale populations. *Ecol. Evol.* **2021**, *11*, 9123–9136. [CrossRef] [PubMed]
31. Ciner, B.; Wang, Y.; Parker, W. Oxygen isotopic variations in modern cetacean teeth and bones: Implications for ecological, paleoecological, and paleoclimatic studies. *Sci. Bull.* **2016**, *61*, 92–104. [CrossRef]
32. Morin, P.A.; Archer, F.I.; Foote, A.D.; Vilstrup, J.; Allen, E.E.; Wade, P.; Durban, J.; Parsons, K.; Pitman, R.; Li, L.; et al. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Res.* **2010**, *20*, 908–916. [CrossRef]
33. Mitani, Y.; Kita, Y.F.; Saino, S.; Yoshioka, M.; Ohizumi, H.; Nakahara, F. Mitochondrial DNA haplotypes of killer whales around Hokkaido, Japan. *Mammal Study* **2021**, *46*, 205–211. [CrossRef]
34. Endo, T.; Kimura, O.; Sato, R.; Kobayashi, M.; Matsuda, A.; Matsuishi, T.; Haraguchi, K. Stable isotope ratios of carbon, nitrogen and oxygen in killer whales (*Orcinus orca*) stranded on the coast of Hokkaido, Japan. *Mar. Pollut. Bull.* **2014**, *86*, 238–243. [CrossRef] [PubMed]
35. Kastelein, R.A.; Kershaw, J.; Berghout, E.; Wiepkema, P.R. Food consumption and suckling in Killer whales. *Int. Zoo Yearb.* **2003**, *38*, 204–218. [CrossRef]
36. Kasuya, T. *Biology and Conservation of Small Cetaceans around Japan*; University of Tokyo Press: Tokyo, Japan, 2019; ISBN 978-4-13-060238-9.
37. Heyning, J.E. Presence of solid food in a young calf killer whale (*Orcinus orca*). *Mar. Mamm. Sci.* **1988**, *4*, 68–71. [CrossRef]

38. Clark, S.T.; Odell, D.K.; Lacinak, C.T. Aspects of growth in captive killer whales (*Orcinus orca*). *Mar. Mammal Sci.* **2000**, *16*, 110–123. [CrossRef]
39. Newsome, S.; Etnier, M.; Monson, D.; Fogel, M. Retrospective characterization of ontogenetic shifts in killer whale diets via δ13C and $\delta^{15}$N analysis of teeth. *Mar. Ecol. Prog. Ser.* **2009**, *374*, 229–242. [CrossRef]
40. Logan, J.M.; Lutcavage, M.E. A comparison of carbon and nitrogen stable isotope ratios of fish tissues following lipid extractions with non-polar and traditional chloroform/methanol solvent systems. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 1081–1086. [CrossRef] [PubMed]
41. Ogasawara, H.; Hayasaka, M.; Maemoto, A.; Furukawa, S.; Ito, T.; Kimura, O.; Endo, T. Stable isotope ratios of carbon, nitrogen and selenium concentration in the scalp hair of Crohn's disease patients who ingested the elemental diet Elental®. *Rapid Commun. Mass Spectrom.* **2018**, *33*, 41–48. [CrossRef] [PubMed]
42. Baird, R.W. *Killer Whales of the World: Natural History and Conservation*; Voyager Press: McGregor, MN, USA, 2002.
43. Dietz, R.; Letcher, R.J.; Desforges, J.-P.; Eulaers, I.; Sonne, C.; Wilson, S.; Andersen-Ranberg, E.; Basu, N.; Barst, B.D.; Bustnes, J.O.; et al. Current state of knowledge on biological effects from contaminants on arctic wildlife and fish. *Sci. Total. Environ.* **2019**, *696*, 133792. [CrossRef]
44. de Armas, Y.C.; Mavridou, A.-M.; Domínguez, J.G.; Hanson, K.; Laffoon, J. Tracking breastfeeding and weaning practices in ancient populations by combining carbon, nitrogen and oxygen stable isotopes from multiple non-adult tissues. *PLoS ONE* **2022**, *17*, e0262435. [CrossRef]
45. O'Sullivan, R.; Schmidt, O.; Monahan, F.J. Stable isotope ratio analysis for the authentication of milk and dairy ingredients: A review. *Int. Dairy J.* **2021**, *126*, 105268. [CrossRef]
46. Yamamoto, M.; Tanaka, N.; Tsunogai, S. Okhotsk Sea intermediate water formation deduced from oxygen isotope systematics. *J. Geophys. Res. Oceans* **2001**, *106*, 31075–31084. [CrossRef]
47. Oda, M.; Tetsu, T.; Sakai, S.; Ishimura, T. Discrimination of the migration for Japanese sardine in Pacific stock by using mi-croscale stable isotopic analytical technique. *Bull. Jpn. Scoc. Fish Oceanogr.* **2016**, *80*, 48–55.
48. Boecklen, W.J.; Yarnes, C.T.; Cook, B.A.; James, A.C. On the Use of Stable Isotopes in Trophic Ecology. *Annu. Rev. Ecol. Evol. Syst.* **2011**, *42*, 411–440. [CrossRef]
49. O'Brien, D.M. Stable Isotope Ratios as Biomarkers of Diet for Health Research. *Annu. Rev. Nutr.* **2015**, *35*, 565–594. [CrossRef] [PubMed]
50. Trueman, C.N.; McGill, R.A.R.; Guyard, P.H. The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals. An experimental study with Atlantic salmon (*Salmo salar*). *Rapid Commun. Mass Spectrom.* **2005**, *19*, 3239–3247. [CrossRef] [PubMed]
51. Zanden, M.J.; Clayton, M.K.; Moody, E.K.; Solomon, C.T.; Weidel, B.C. Stable Isotope Turnover and Half-Life in Animal Tissues: A Literature Synthesis. *PLoS ONE* **2015**, *10*, e0116182. [CrossRef] [PubMed]
52. Caut, S.; Angulo, E.; Courchamp, F. Variation in discrimination factors ($\Delta^{15}$N and $\Delta^{13}$C): The effect of diet isotopic values and applications for diet reconstruction. *J. Appl. Ecol.* **2009**, *46*, 443–453. [CrossRef]
53. Drago, M.; Franco-Trecu, V.; Cardona, L.; Inchausti, P. Diet-to-female and female-to-pup isotopic discrimination in South American sea lions. *Rapid Commun. Mass Spectrom.* **2015**, *29*, 1513–1520. [CrossRef] [PubMed]
54. Borrell, A.; Abad-Oliva, N.; Gómez-Campos, E.; Giménez, J.; Aguilar, A. Discrimination of stable isotopes in fin whale tissues and application to diet assessment in cetaceans. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 1596–1602. [CrossRef]
55. Honda, K.; Tatsukawa, R.; Itano, K.; Miyazaki, N.; Fujiyama, T. Heavy metal concentrations in muscle, liver and kidney tissue of striped dolphin, Stenella coeruleoalba, and their variations with body length, weight, age and sex. *Agric. Biol. Chem.* **1983**, *47*, 1219–1228. [CrossRef]
56. Jourdain, E.; Andvik, C.; Karoliussen, R.; Ruus, A.; Vongraven, D.; Borgå, K. Isotopic niche differs between seal and fish-eating killer whales (*Orcinus orca*) in northern Norway. *Ecol. Evol.* **2020**, *10*, 4115–4127. [CrossRef] [PubMed]
57. Itano, K.; Kawai, S.; Miyazaki, N.; Tatsukawa, R.; Fujiyama, T. Mercury and selenium levels at the fetal and suckling stages of striped dolphin, Stenella coeruleoalba. *Agric. Biol. Chem.* **1984**, *48*, 1691–1698. [CrossRef]
58. Endo, T.; Kimura, O.; Terasaki, M.; Nakagun, S.; Kato, Y.; Fujii, Y.; Haraguchi, K.; Baker, C.S. Carbon, nitrogen, and oxygen stable isotope ratios of striped dolphins and short-finned pilot whales stranded in Hokkaido, northern Japan, compared with those of other cetaceans stranded and hunted in Japan. *Isot. Environ. Health Stud.* **2023**, *59*, 230–247. [CrossRef] [PubMed]
59. Caon, G.; Secchi, E.R.; Capp, E.; Kucharski, L.C. Milk composition of franciscana dolphin (*Pontoporia blainvillei*) from Rio Grande do Sul, southern Brazil. *J. Mar. Biol. Assoc. United Kingd.* **2008**, *88*, 1099–1101. [CrossRef]