

## Chapter

# Typical Changes in Carbon and Nitrogen Stable Isotope Ratios and Mercury Concentration during the Lactation of Marine Mammals

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## Abstract

The increase and decrease in the  $\delta^{15}\text{N}$  values of offspring owing to the suckling of  $\delta^{15}\text{N}$ -enriched milk (nursing) and the feeding shift from milk to solid food (weaning), respectively, are thought to be common traits observed in mammals. However, there are a few studies on lactation in marine mammals, especially large whales, because samples of calf, lactating mother, and milk are difficult to obtain. In this chapter, we review the studies on reproduction of marine mammals using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values analyzed in several tissues and describe the typical changes reported to date in those values and Hg concentrations in offspring and milk during lactation. Next, we present data on ontogenetic changes in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  profiles and Hg concentration, especially focusing on the lactation period, in muscle samples of hunted bowhead whale, and stranded common minke whale (mysticetes), Dall's porpoise (odontocete), and the harbor seal (phocid). Finally, we compare the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in muscle samples of calves from common minke whale, Dall's porpoise, and killer whale and suggest that these values could be excellent proxies for maternal foraging habits and trophic levels.

**Keywords:** lactation, calf, pup, marine mammal, stable isotope of ratios, mercury

## 1. Introduction

Viviparity and lactation are the most important traits in mammals. The developing fetus is connected to the placenta via an umbilical cord and obtains nutrients through it. Milk is the most complete and natural food of mammals that offspring can consume during the early stages of their life, as it ensures proper nutrition and development. Two types of reproductive taxa have been proposed in mammals based on whether a pregnant and lactating female can catabolize the energy stored in the body to grow fetuses and produce milk [1–4]. Mammals who catabolize accumulated energy resources are called capital breeders, whereas income breeders use energy resources gained concurrently; mysticetes and true seals are generally classified as capital breeders, whereas odontocetes and fur seals are generally classified as income breeders.

Cetaceans give birth and suckle in inaccessible oceans, making it difficult to observe their reproduction, especially in large mysticete whales. Studies focused on the biochemistry and ecology of large whales have been mainly conducted using preserved samples from commercial whaling in the past, but there is little information on reproduction because of the restricted whaling for lactating females and calves. Owing to the international ban on whaling, new samples from large cetaceans are not easy to collect [5–7]. Currently, most studies on lactation and mother-to-offspring relations in marine mammals are conducted using pinnipeds as they give birth and nurse pups on land or ice in accessible areas; they are easy to observe and sample [4, 6–9].

Stable isotope ratios of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) are used exclusively to study the ecology and biochemistry of marine mammals, and most samples used in these studies are the muscle, liver, blood components, hair, whiskers, bones, skin, and teeth. However, the integrated terms of dietary information are different among these tissues because of different turnover rates [10, 11]. Some studies analyzed two tissues at different turnover rates from individual animals, that is, red blood cells and plasma samples, bone and muscle samples, and hair and plasma samples, to estimate the past and recent information on dietary ecology and biochemistry [12–16].

As the contamination of mercury (Hg) and cadmium (Cd) in milk is very low [17], these burdens in offspring are usually low. However, after weaning, Hg and Cd concentrations in some mammals tend to increase sharply, reflecting the feeding on fish and cephalopods [18–22]. In contrast, adult marine mammals located at high trophic positions are extremely contaminated with Hg [23, 24]. As Hg passes through the placenta, there is great concern regarding the neurotoxicological effects of Hg on infants of not only human populations but also marine mammals [25, 26]. As fetal samples of marine mammals are difficult to obtain, the Hg exposure of the fetus is indirectly estimated using the Hg concentrations in lanugo hair [16, 27–29] and in the red blood cells of neonates [16].

This chapter first describes the different reproduction strategies between mysticetes and odontocetes and between seals and fur seals, and then reviews the studies on lactation in marine mammals using the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures and Hg concentration. Next, we discuss our data on the ontogenetic profiles (focused on lactation) of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures and Hg concentration in muscle samples of stranded common minke whale (*Balaenoptera acutorostrata*; MW) (mysticete), Dall's porpoise (*Phocoenoides dalli*; DP) (odontocete), and Kurill harbor seals (*Phoca vitulina stejnegeri*, HS) (pinniped) analyzed in our laboratory. Finally, we compare the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in muscle samples of stranded calves from MW, DP, and killer whale (*Orcinus orca*; KW), and suggest that these values in calves could be excellent proxies for maternal foraging habits and trophic levels.

## 2. Lactation in marine mammals

### 2.1 Lactation and milk composition

Most baleen whales (mysticetes) are characterized by long migrations between the feeding and breeding grounds, and the storage of adequate energy for pregnancy and lactation, giving birth and lactating but fasting or eating relatively little [6]. Most baleen whale species have relatively brief lactation (nursing and weaning) periods of 5–7 months, except for bowhead whale (*Balaena mysticetus*, BW) [5, 6]. They produce

milk relatively low in water (40–50%), high in fat (lipids) (30–50%), and moderately high in protein (9–15%), relying on energy stored before parturition to support the rapid fattening and growth of their calves over a brief lactating period [6]. In contrast, most toothed whales, dolphins, and porpoises (odontocetes) have much more extensive lactations typically lasting 1–3 years, during which the mothers feed. Their milk is higher in water (60–70%) and lower in fat (10–30%) than that of mysticetes but has similar levels of protein (8–11%) [6]. Cetaceans with brief lactation periods tend to produce high-fat milk, investing heavily in offspring for a brief period compared to those with prolonged lactation periods, which tend to produce low-fat milk. The prolonged weaning period in odontocetes may be related to the learning of predatory and social skills, whereas mysticetes, which feed on swarms of zooplankton and schools of small fish, do not need such learning.

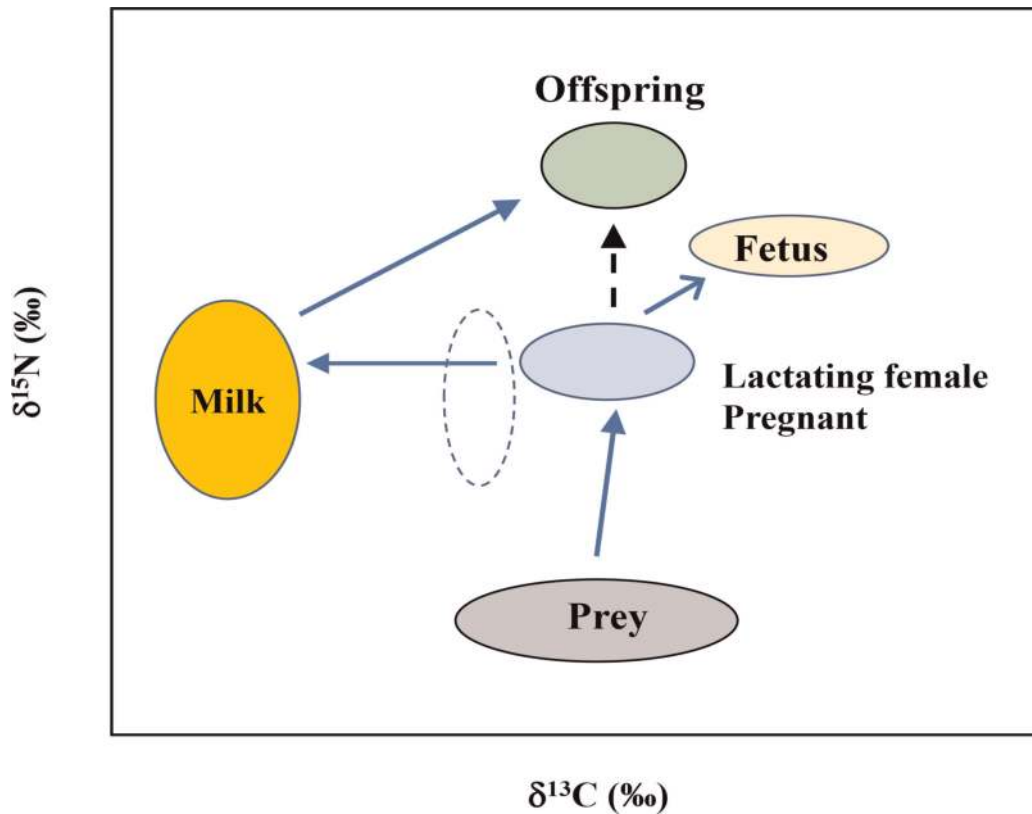
The mysticete-odontocete contrast is similar to the contrast between the true seals (family Phocidae) and the fur seal (family Otariidae) [4, 6–8, 30], although both families are piscivores. Most phocid mothers fast during the lactation period rely on energy stored before parturition, which is generally less than 2 months in duration, and pups are usually weaned abruptly. In contrast, otariid mothers feed throughout lactation (except for the initial perinatal period), which may last 1–2 years, and most otariid species are weaned gradually. Furthermore, the milk of phocids is more energy-rich (higher fat concentration) than that of otariids. The different reproduction strategies between mysticetes and odontocetes and between phocids and otariids can be explained by the different breeding strategies as capital and income breeders [1–4].

Weaning involves the transition from nutritional dependence on milk to solid food, and milk and solid food can often be found in the stomachs of gradually weaned offspring. Body lengths (BL) in weaned cetaceans have been reported to be correlated with maternal BL [31].

## 2.2 Carbon and nitrogen stable isotope ratios

Stable isotope analyses of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) are useful tools for obtaining information on feeding ecology. The  $\delta^{15}\text{N}$  value shows a stepwise increase with increases in the trophic level through a food chain, whereas the  $\delta^{13}\text{C}$  value is used to estimate the relative contribution to the diet of potential primary sources [11, 32, 33]. Significant increases of  $\sim 3\text{‰}$  in the  $\delta^{15}\text{N}$  value have been shown to occur between predators and their prey, whereas the trophic fractionation for  $\delta^{13}\text{C}$  values is smaller than that for  $\delta^{15}\text{N}$  values, averaging approximately  $1\text{‰}$  [11, 32–35] (**Figure 1**). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  profiles are used in studies of habitat preferences such as pelagic *vs.* benthic, and nearshore *vs.* offshore *vs.* estuarine [33], as well as the geographical differences of inhabitants [11, 32, 36, 37]. Furthermore, mother-to-offspring transfer of nutrients owing to lactation in marine mammals has been preferentially investigated using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures. Higher trophic levels ( $\delta^{15}\text{N}$  values) in nursing offspring than those in their mothers are widely observed in terrestrial and marine mammals [3, 11, 32, 33, 38–40], as the trophic level of milk is higher than that of foods which the mother feeds on (**Figure 1**). In contrast, the  $\delta^{13}\text{C}$  value of offspring does not show a clear-cut pattern because of the large variation in  $^{13}\text{C}$ -depleted lipids (fat) concentration in the milk they suckled [3, 33, 40].

As the  $\delta^{13}\text{C}$  values of lipids in mammals are typically 5–7% lower than those of tissue proteins or carbohydrates [11], most studies have analyzed lipid-extracted tissues to remove the potential confounding effect of variation in tissue lipid content on the  $\delta^{13}\text{C}$  values [11, 32, 41]. Consistent with this, the  $\delta^{13}\text{C}$  values in lipid-extracted



**Figure 1.** Diagram showing the relationships among lactating female, milk and offspring and between pregnant and fetus by stable isotope ratios of carbon and nitrogen. The dashed ellipse indicates the lipid extracted milk.

milk (shown as a dotted ellipse of milk protein in **Figure 1**) were approximately 5–6% higher than those in milk containing lipids [3, 9, 42, 43].

### 2.3 Isotopic discrimination and half-life in tissues

As mentioned above, the enrichment of  $\delta^{15}\text{N}$  values between whole predator and prey ( $\Delta^{15}\text{N}_{\text{predator-prey}}$ ) is  $\sim 3\text{‰}$  [3, 11, 32–35]. However, it is practically impossible to measure the whole body  $\delta^{15}\text{N}$  value in large animals; the  $\delta^{15}\text{N}$  value in a tissue is usually substituted for the  $\delta^{15}\text{N}$  value of the whole body, despite the  $\delta^{15}\text{N}$  value in each tissue is different. For instance, the highest  $\delta^{15}\text{N}$  value among seven tissues of fin whales (*Balaenoptera physalus*; FW) was the brain ( $11.4 \pm 0.36\text{‰}$ ), the middle was muscle ( $9.88 \pm 0.58\text{‰}$ ), and the lowest was the bone protein ( $9.19 \pm 0.71\text{‰}$ ) of with a difference of  $\sim 2.2\text{‰}$  [44]. The following is the order of  $\delta^{15}\text{N}$  values in tissues that are widely observed [34, 35, 39, 44–46].

*brain > plasma (serum) > liver > muscle = hair (fur) = skin = baleen > red blood cells.*

Thus, the  $\Delta^{15}\text{N}_{\text{predator-prey}}$  values calculated for each tissue are different. Among these tissues, the  $\delta^{15}\text{N}$  value of muscle tissue may closely reflect that of the whole body, as the mass of muscle tissue is the highest in the body (for example,  $\sim 30\%$  in humans) [11, 32].

Tissue turnover rates are important for understanding the timeframe of dietary information preserved by the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures in a given tissue. Tissue turnover should be closely linked to protein turnover, as the tissues typically used for isotopic analyses (hair, muscle, plasma/serum, red blood cells, bone collagen, etc.) primarily comprise proteins, with protein turnover being the most rapid in the liver and plasma/serum, followed by that in the muscle, and slow in red blood cells and bone collagen [11, 32, 47].

The tissue turnover rate in animals generally decreases with body size; for example, the time until 50% replacement of red blood cells was 35 days in humans, but 114 days in beef cattle [11]. Furthermore, the tissue turnover rate in animals is rapid in growing animals, as also in metabolically active tissues [48, 49]. Therefore, as we previously implied, the turnover rate of muscle in MW calves may be markedly faster than that in MS adults, with little time lag between the feeding shift from milk to solid food and the change in  $\delta^{15}\text{N}$  signature in the calf muscle [40].

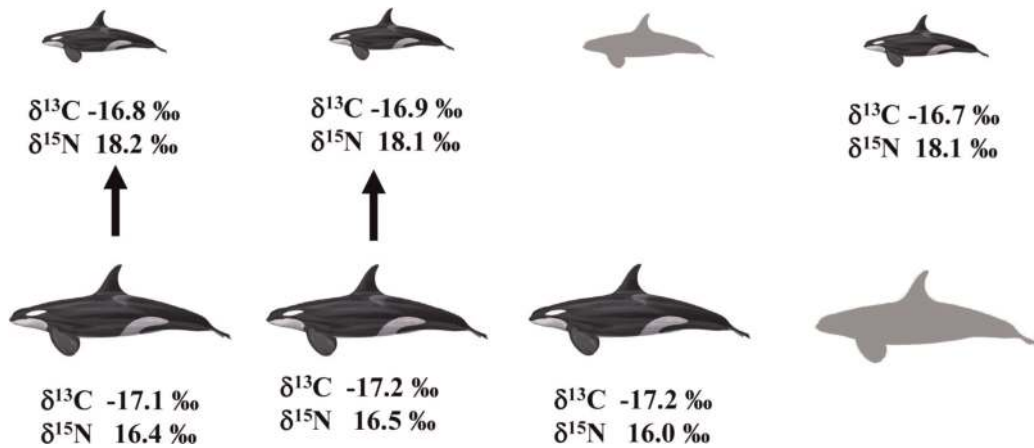
Unlike the blood, liver, and kidney, the hair, baleen plates, and whiskers do not undergo turnover; past information is preserved without being erased. Hair and baleen plates are reported to grow continuously, at a rate of approximately 1 cm/month for humans [11] and 12.9–20 cm/year for Balaenopteridae species [50], respectively; thus, sampling close to the roots of hair, baleen plates, and whiskers will record the more recent information ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values), whereas sampling farther along those will record the information in the past [1, 51–54]. In contrast, the dentin growth layer in teeth allows us to investigate the annual changes in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures in beluga whales (*Delphinapterus leucas*) [55], KW, and sperm whales (*Physeter microcephalus*) [33]. Studies using the dentin layer have reported a decrease in the  $\delta^{15}\text{N}$  profile because of weaning, with little information on nursing that occurs in a brief period of less than one year. Aubail et al. [56] analyzed not only the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values but also the Hg concentration in the dentin layers of ringed seals (*Phoca hispida*) and reported a high Hg concentration owing to the placental transfer of Hg and high  $\delta^{15}\text{N}$  values owing to nursing in the first layer (first year) of dentin.

Bones are well-preserved samples, but they can only be obtained from cadavers. Because of their slow turnover rate, it is suitable for the study of prolonged lactation in animals. Jansen et al. [14] and Vales et al. [57] investigated the ontogenetic dietary changes in harbor porpoises (*Phocoena phocoena*) and South American fur seals (*Arctocephalus australis*) using bone samples. However, the time lag between the weaning and the  $\delta^{15}\text{N}$  signature in the bone should be considered when analyzing the results.

Most blood and milk samples used for the studies on lactation and feeding ecology of marine mammals were obtained from pinnipeds because they are easy to handle and can be sampled blood from pup and mother pairs, and milk and pup tissue pairs [9, 13, 43], in addition to lanugo hair and whisker samples, which is not possible in large whales.

#### 2.4 Isotopic relationships among lactating female, milk, and offspring and between pregnant female and fetus: strategies of income and capital breeders

Figure 2 shows the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in the muscle samples of KWs that were mass-stranded in Hokkaido. Nine corpses, including three lactating mothers and three calves at few months in age, (including two pairs of lactating mothers and calves), were recovered [58, 59]. Stranded lactating mothers could starve for several days before death, whereas milk was found in the stomach of the calf corpses.



**Figure 2.** Stable isotope ratios of carbon and nitrogen in muscle of killer whales from 3 calves and 3 lactating females mass-stranded in Hokkaido. Genetic analysis revealed two pairs of mother-calf relationship. See **Table 1**.

The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in the muscle samples were similar between the three calves and the three lactating mothers. The  $\Delta^{15}\text{N}_{\text{offspring-mother}}$  values of two pairs of whales were 1.7‰ and 1.6‰, and the  $\Delta^{13}\text{C}_{\text{offspring-mother}}$  values of two pairs were slightly positive 0.3‰ and 0.3‰ (**Figure 2**). To the best of our knowledge, the  $\Delta^{15}\text{N}_{\text{offspring-mother}}$  values in muscle samples (1.7‰ and 1.6‰) have not yet been reported in cetaceans within nature; however, these values were consistent with those calculated from blood and milk samples of pinniped pairs [9, 39, 60] and captive mammalian pairs [61].  $\Delta^{15}\text{N}_{\text{offspring-mother}}$  in KWs (1.7‰ and 1.6‰) were smaller than those reported for  $\Delta^{15}\text{N}_{\text{predator-prey}}$  (~3‰), as the lactation of KW calves was at an early stage of nursing before the  $\delta^{15}\text{N}$ -enriched peak (**Figures 3–5**).

In contrast, the  $\Delta^{15}\text{N}_{\text{offspring-mother}}$  values in the liver samples were slightly negative (−0.5‰ and −0.5‰) (**Table 1**). These phenomena may be explained by the faster

	Remark	Muscle			Liver			Blubber	
		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Hg <sup>a</sup>	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Hg <sup>a</sup>	PCB <sup>b, c</sup>	<i>p,p'</i> -DDE <sup>c</sup>
AKW6	Lactating female	−17.1	16.4	1.27	−16.8	19.2	38.0	42.3	109
AKW7	Calf of AKW6	−16.8	18.2	0.07	−16.6	18.7	0.30	68.2	237
AKW9	Lactating female	−17.2	16.5	1.26	−16.7	19.2	62.4	26.2	50.5
AKW8	Calf of AKW9	−16.9	18.1	0.08	−16.7	18.7	0.50	42.3	98.9
AKW2	Lactating female (no calf among AKW pod)	−17.2	16.0	1.26	−16.8	18.2	57.4	31.4	68.6
AKW3	Calf (no mother among AKW pod)	−16.7	18.1	0.10	−16.6	18.6	0.30	41.6	112

<sup>a</sup>total mercury concentration (µg/wet g).

<sup>b</sup>sum of 12 PCB isomers.

<sup>c</sup>(mg/lipid wet g).

From Endo et al. [58], and Haraguchi et al. [62].

**Table 1.**

Analytical results of stable isotope ratios of carbon and nitrogen and mercury concentrations in muscle and liver and PCB and *p,p'*-DDE concentrations in blubber of killer whales mass-stranded in Hokkaido (AKW pod).

turnover rate of nitrogen in the liver than in the muscle [10, 47], and the increase in maternal  $\delta^{15}\text{N}$  values by starvation [63]. The Hg concentrations in the muscle and liver samples of lactating mothers were markedly higher than those of the calves, and this trend was particularly pronounced in the liver samples. In contrast to Hg, polychlorinated biphenyl (PCB), and 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (*p,p'*-DEE) concentrations in blubber samples were significantly higher in calves than in lactating mothers because of the suckling of contaminated milk containing lipophilic compounds of PCB and *p,p'*-DEE [62].

Two types of reproductive strategies have been proposed for mammals [1–4, 9]. In capital breeders, lactating mothers catabolize their tissues to grow fetuses and produce milk. The trophic levels ( $\delta^{15}\text{N}$  values) of fetuses and milk are higher than those of pregnant females and lactating mothers, respectively (**Figure 1**); that is, the fetus and nursing offspring consume their mother's tissues [32, 33]. In contrast, in income breeders, fetuses and milk should not be enriched in  $^{15}\text{N}$  during parturition and lactation, as resources ingested by the pregnant female and lactating mother will not have been incorporated into her own tissues, and these are directly routed into fetal tissue and milk production.

Considering nitrogen in more detail, capital breeders show positive  $\Delta^{15}\text{N}_{\text{milk-mother}}$  values: 2‰ in the northern elephant seal (*Mirounga angustirostris*) [13], 1‰ in polar bears (*Ursus maritimus*) [42], 0.5‰ in the southern right whale (*Eubalaena australis*) [38], and 0.3‰ in FW [3] (**Figure 1**). Contrary to the positive values in capital breeders, the  $\Delta^{15}\text{N}_{\text{milk-mother}}$  values of income breeders are negative:  $-1.0 \pm 0.5\%$  in nine income-breeder species [61],  $-0.8\%$  in Steller sea lion (*Eumetopias jubatus*) [43]  $-0.3\%$  in deer mice (*Peromyscus maniculatus*) [2], and  $-1.61\%$  and  $-1.48\%$  in two species of fur seals (*Arctocephalus gazella* and *Arctocephalus tropicalis*) [9]. However, Chilvers [64] recently reported a positive  $\Delta^{15}\text{N}_{\text{milk-mother}}$  value of 0.4‰ in the New Zealand sea lion (*Phocartos hookeri*), an income breeder, and suggested that the production of milk is not only from the diet but also through catabolizing of tissue. In contrast to milk, sampling of fetuses from marine mammals, especially cetaceans, is very rare, with only a single  $\Delta^{15}\text{N}_{\text{fetus-mother}}$  value of 1.6‰ reported in FW, a capital breeder [3].

## 2.5 Mercury and cadmium

As apex predators, odontocetes are exposed to high levels of pollutants, such as Hg and organochlorines, through feeding on contaminated fish [65–67]. In contrast, mysticetes, which feed lower on the food chain (zooplankton and small fish), typically have less significant exposure to pollutants. Cd accumulates in molluscs, particularly cephalopods, and accumulates in predators via not only marine food webs but also species-specific physiologic mechanisms [18, 19, 68]. Cd is likely to be distributed preferentially in the kidney rather than in the liver of marine mammals, whereas Hg tends to be distributed preferentially in the liver [18, 19, 21, 58]. A sharp increase in the Hg burden in the liver is thought to be a weaning proxy for fish eaters in marine mammals, whereas a sharp increase in the Cd burden in the kidney could be a weaning proxy for cephalopod eaters [22]. The degree of Hg burden in mysticetes is generally low, but the Hg burden in opportunistic feeders of mysticetes could reflect the amount of fish consumed [69].

Methyl Hg, produced within marine ecosystems, is a neurotoxicant that is transported across the blood-brain barrier and placental barrier [26]. There is great concern regarding the neurotoxicological effects of Hg on infants of the human

population and piscivores [26]. Because of the difficulty of sampling from fetal tissue, Hg exposure in the fetus is usually estimated using the Hg concentrations in the scalp hair of infants [26], lanugo hair [27–29], dentin [56], and red blood cells of neonates [16]. Rea et al. [28] reported a high level of Hg ( $> 40 \mu\text{g/g}$ ) as well as  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in the fur of sea lion pups, suggesting that their mothers ate fish contaminated with Hg at the high trophic position during the late gestation. Furthermore, the Hg concentration in red blood cells of suckling offspring could reflect the Hg burden in their fetus stage [26], as the majority of red blood cells are likely to be produced during late gestation [13, 61]. As hair is an excretory route for Hg, the Hg in hair is used for biomonitoring of Hg burden in human populations as well as pinnipeds, and in humans, scalp hair Hg is on average 250 times greater than that in whale blood [11]. Endo et al. [70] reported high Hg concentrations in the scalp hair of residents living in a whaling town and a positive correlation between the Hg concentrations and the  $\delta^{15}\text{N}$  values in their hair. Brookens et al. [71] and Hobson et al. [52] reported high Hg concentrations in the hair of Pacific harbor seals (*Phoca vitulina richardii*) and in the baleen plate of MWs and the correlations between Hg concentrations and  $\delta^{15}\text{N}$  values in these samples.

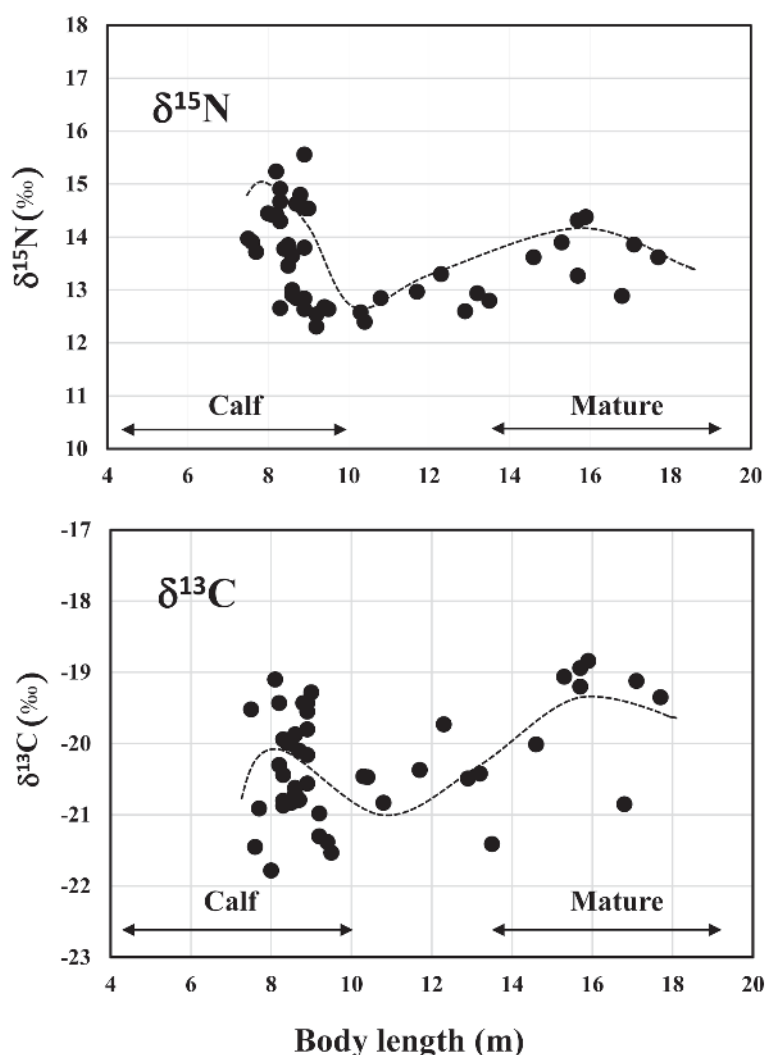
To the best of our knowledge, the only study on Hg analyzed in the fetus of marine mammals was conducted by Itano et al. [23] using hunted striped dolphins (*Stenella coeruleoalba*), which is heavily contaminated with Hg among odontocetes inhabiting waters around Japan [24]. Itano et al. [23] reported an increase in the Hg burden in striped dolphin fetuses with the increase in pregnancy term. Comparing muscle Hg concentrations in fetuses, suckling offspring, and mature females, they reported values of  $0.900 \pm 0.310$ ,  $0.520 \pm 0.060$ , and  $11.100 \pm 0.300 \mu\text{g/wet g}$ , respectively. In addition, they reported trace levels of Hg in milk ( $26 \text{ ng/g}$ ) and high levels of Hg in the blood of adult females ( $1.200 \pm 0.200 \mu\text{g/g}$ ), which was 46 times higher than that in the milk. Thus, the Hg concentration in calves, which are contaminated with a high Hg burden through the placenta, is likely to be diluted by the growth dilution effect, even though a small amount of Hg could be taken from milk. In contrast, the Hg concentration in breast milk and blood in women at 6 weeks after delivery were  $0.6 \pm 0.4$  and  $2.2 \pm 1.9 \text{ ng/mL}$ , respectively, and that in blood was only  $\sim 3$  times higher [25]. No further studies on Hg transfer through the placenta and milk have been conducted in cetaceans.

## 2.6 Ontogenetic changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in bowhead whales

Large-scale ontogenetic studies on baleen whales, focusing on lactation using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures, have only been conducted in BW samples from native subsistence hunts [20, 72, 73]. The BWs are large baleen whales inhabiting the icy Arctic waters, and they are born at 4–5 m BL, weaned at  $\sim 10$  m BL, with sexual maturity at over 13.5 m BL [72, 74]. BWs feed on pelagic zooplankton and benthic amphipods, where the  $\delta^{13}\text{C}$  and Cd levels of benthic amphipods are lower and higher than those of zooplankton, respectively, and Hg is in trace concentrations [20].

**Figure 3** shows the ontogenetic changes in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures in the muscle samples of BWs by Lee et al. [72]. They analyzed the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of non-lipid-extracted muscle samples. Line smoothing shows the  $\delta^{15}\text{N}$ -enriched peak at  $\sim 8$  m BL and a  $\delta^{15}\text{N}$ -depleted peak at  $\sim 10$  m BL. A large study on BWs conducted thereafter by Horstmann-Dehn et al. [73] showed prominent  $\delta^{15}\text{N}$ -enriched peaks in muscle ( $n = 133$ ) and epidermis ( $n = 130$ ) samples of BWs at  $\sim 8$  m BL. The increase in  $\delta^{15}\text{N}$  values to the enriched peak ( $\sim 8$  m BL) could represent nursing, and this peak is likely to be the onset of weaning (shift from milk to solid foods). Furthermore, the BL





**Figure 3.** Ontogenetic changes of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures in muscle of bowhead whales. These figures were drawn based on the data from Table 5 ( $n = 47$ ) reported by Lee et al. [72] with permission. Two outliers were not drawn.

at the  $\delta^{15}\text{N}$ -depleted peak may represent BL at complete weaning in BWs, which coincides with the weaned BL at  $\sim 10$  m reported previously [72, 74]. After weaning, the  $\delta^{15}\text{N}$  values gradually increased and reached a plateau. The ontogenetic change in the  $\delta^{13}\text{C}$  profile was similar but more prominent than that of  $\delta^{15}\text{N}$  change; no correlation was found between the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. The prominent increase in post-weaning  $\delta^{13}\text{C}$  values may be related to the feeding on zooplankton and benthic amphipods, although the confounding effect of tissue lipid content on  $\delta^{13}\text{C}$  values should be considered: large differences are found between the lipid-extracted and non-extracted muscle samples when  $\delta^{13}\text{C}$  values are low [73]. Dehn et al. [20] reported trace levels of Hg in the muscle ( $0.02 \pm 0.01 \mu\text{g}/\text{wet g}$ ) and a moderate level of Cd in the kidney ( $15.08 \pm 14.94 \mu\text{g}/\text{g}$ ) of BWs, and these levels of Hg and Cd could be consistent with their habit of feeding on pelagic zooplankton and benthic amphipods. Despite a large number of BW samples, Lee et al. [72] and Horstmann-Dehn et al. [73] did not investigate sex-related differences in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  profiles of BW samples.

Dehn et al. [20] reported a marked depletion in  $\delta^{13}\text{C}$  values (between  $-24\text{‰}$  and  $-25\text{‰}$ ) in two fetus of BWs at  $\sim 1$  m and  $\sim 4$  m BL as compared to those in adults (approximately  $-20\text{‰}$ ), and these values are in the range of the  $\delta^{13}\text{C}$  values in milk found widely in marine mammals (between  $-24\text{‰}$  and  $-26\text{‰}$ ) [3, 9, 43, 64]. Dehn et al. [20] did not mention the  $\delta^{15}\text{N}$ -enrichment in the muscle of BW fetuses, but Horstmann-Dehn et al. [73] reported the enriched  $\delta^{15}\text{N}$  values in the epidermis of BW fetuses. Borrell et al. [3] reported higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in FW fetuses ( $-17.45 \pm 0.53\text{‰}$  and  $11.46 \pm 0.38\text{‰}$ ,  $n = 10$ ) than in lactating females ( $-18.06 \pm 0.22\text{‰}$ , and  $9.46 \pm 0.55\text{‰}$ ,  $n = 13$ ), although they did not mention the BL of the analyzed fetuses. More research on  $\Delta^{13}\text{C}_{\text{fetus-mother}}$  values in addition to  $\Delta^{15}\text{N}_{\text{fetus-mother}}$  values is needed to clarify the transport of nutrition from mother to fetus. The BW and FW are mysticetes and are classified as capital breeders.

### 3. Ontogenetic change in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures of marine mammals stranded in Hokkaido, Japan

#### 3.1 Stranding of marine mammals along the coast of Hokkaido, Japan

Hokkaido is the northernmost island in Japan and is surrounded by the North Pacific Ocean, the Sea of Japan, and the Sea of Okhotsk. Most stranded cetaceans in Hokkaido are odontocetes, such as DP, harbor porpoise (*Phocoena phocoena*), Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), and the mysticete MW. In this section, we describe the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  profiles in muscle samples of MW and DP calves, in addition to the muscle samples of Kurill harbor seals (*Phoca vitulina stejnegeri*; HS) stranded in Hokkaido. Furthermore, we compared the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in calf muscle samples from MW, DP and KW stranded in Hokkaido. We analyzed the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in the muscle samples after lipid extraction.

#### 3.2 Ontogenetic changes in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in common minke whales

**Figure 4** shows the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures with a focus on calves of MW [40]. MWs are mysticete and opportunistic feeders that change their prey items temporally and regionally; they feed on zooplankton and small fish. MW may strand during their migration [75].

A clear  $\delta^{15}\text{N}$ -enriched peak was found in MW calves ( $n = 12$ ), including newborn animals at 2.6 and 3.0 m BL. Similar to BWs (**Figure 3**), the increase in  $\delta^{15}\text{N}$  values toward this peak could represent nursing, and the decrease in  $\delta^{15}\text{N}$  values from this peak could represent weaning [40]. The  $\delta^{15}\text{N}$  values of calves fitted to a quadratic function ( $p < 0.05$ ), and this peak calculated by the fitted equation was 4.0 m BL and  $13.3\text{‰}$ , suggesting the onset weaning at  $\sim 4$  m BL. In addition, the BL of weaned animals was estimated at  $\sim 5$  m BL from the fitted equation and the average  $\delta^{15}\text{N}$  values of mature animals as  $11.9\text{‰}$ , which coincided with the reported BL of weaning [76]. Thus, the weaned BL of MWs (**Figure 4**) and BWs (**Figure 3**) estimated using  $\delta^{15}\text{N}$  profiles in muscle samples coincided with the weaned BL estimated from ecological and morphological studies. We believe that the  $\delta^{15}\text{N}$  value of the muscle from calves of mysticetes reflects the dietary shift quite rapidly with little time lag, because of the brief weaning period [5, 6] and the fast turnover rate of small and growing animals [48, 49].

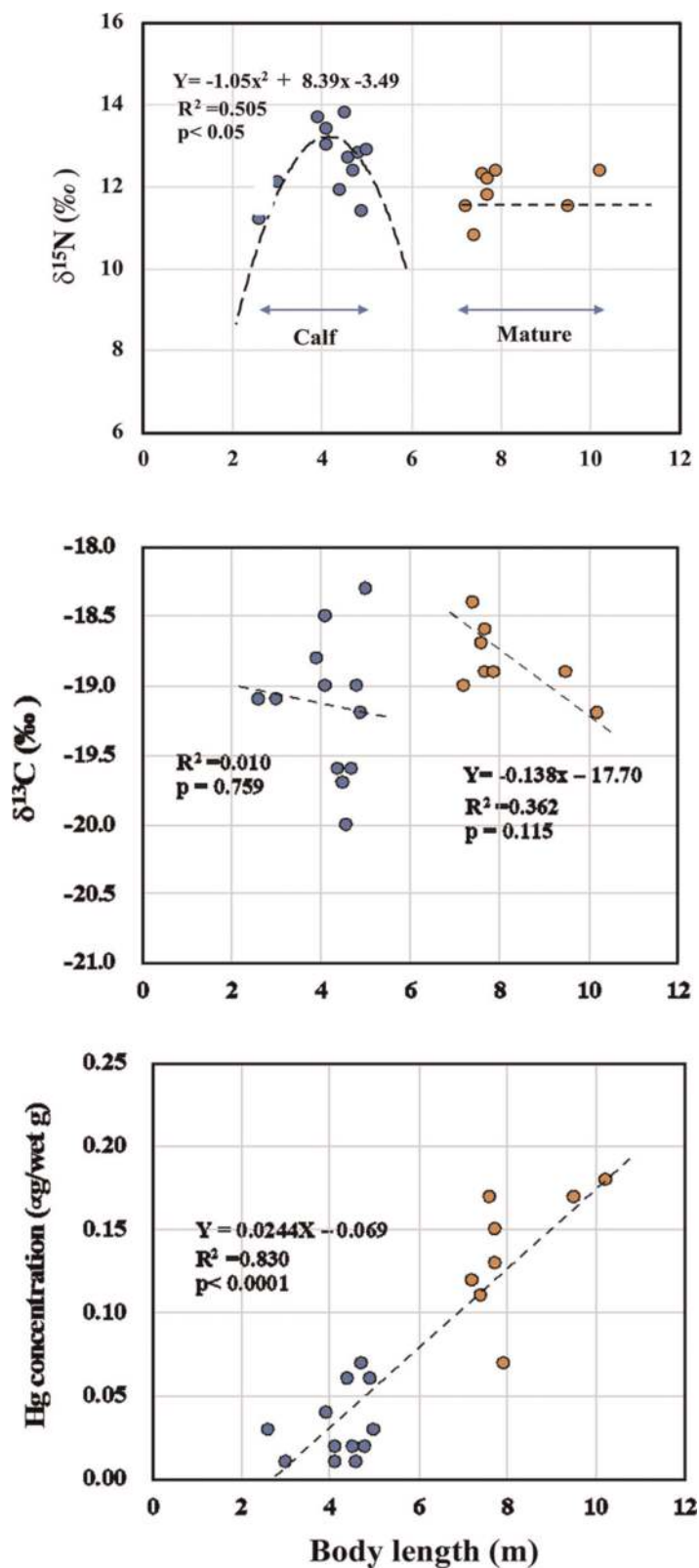


Figure 4.  
Ontogenetic changes of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures in muscle of common minke whale stranded in Hokkaido. This figure was reprinted from Endo et al. [40] with permission from Aquatic Mammals.

No particular pattern was found in  $\delta^{13}\text{C}$  values of MW calves, probably owing to the large variation in  $\delta^{13}\text{C}$ -depleted lipid concentrations in milk [3, 33, 42].

The Hg concentrations in immature (weaned) animals were apparently higher than those in MW calves because of the opportunistic eating of fish. Marked increases in Hg owing to weaning were found in liver samples of MWs (data not shown).

Studies on the ontogenetic changes in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in mysticetes were conducted by Borrell et al. [3] using muscle samples from FWs and by Mitani et al. [53] using baleen plate samples of MWs. However, the study on FWs did not include calf samples, and a clear  $\delta^{15}\text{N}$ -depleted peak related to weaning was not found among the few  $\delta^{15}\text{N}$ -depleted peaks observed in the baleen plate of MWs.

### 3.3 Ontogenetic changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in *Dalli*-type Dall's porpoises

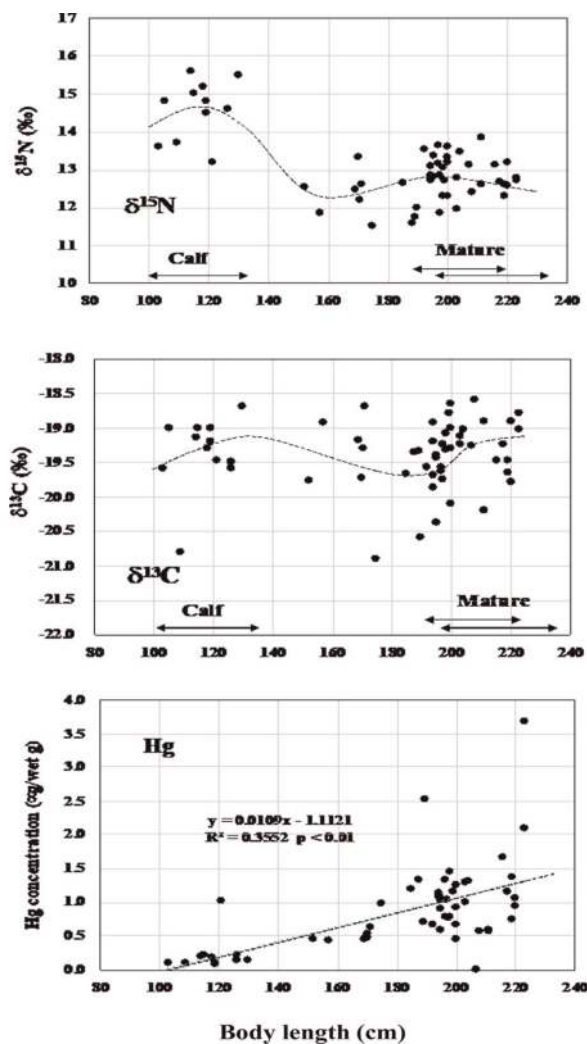
*Dalli*-type DP (odontocete) is widely distributed in the northern part of the North Pacific and move from south to north seasonally in the North Pacific Ocean [75]. According to Kasuya [77], the calves are born from August to September at 100 cm BL and nursed for approximately 2 years. In addition, sexual maturity is attained in males at the age of 7.9 years at 195.7 cm BL and in females at 6.8 years at 186.5 cm BL. Huang et al. [31] estimated the weaned BL of DPs to be 135 cm. We analyzed the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values and Hg concentrations of muscle samples of DPs stranded along the coast of Hokkaido.

An  $\delta^{15}\text{N}$ -enriched peak at  $\sim 115$  cm BL owing to lactation was found in the muscle samples of DPs (**Figure 5**), similar to that observed in the mysticetes BWs and MWs (**Figures 3** and **4**). The  $\delta^{15}\text{N}$  values following the peak gradually declined at  $\sim 160$  cm BL, then increased slightly and plateaued. The BL at the  $\delta^{15}\text{N}$ -enriched peak could imply the BL at the onset of weaning, although available information on the onset of weaning in DPs is lacking. The  $\delta^{15}\text{N}$ -depleted peak at  $\sim 160$  cm BL may be related to BL at complete weaning, although the reported BL of DP at weaning DP is 135 cm [31]. The DP sample shown in **Figure 5** was biased and did not include the DP samples at 130–150 cm BL at which weaning was reported to occur.

Although the  $\delta^{13}\text{C}$  values varied considerably, small  $\delta^{13}\text{C}$ -enriched and  $\delta^{13}\text{C}$ -depleted peaks were found at  $\sim 130$  cm BL and  $\sim 190$  cm BL, respectively, and the small  $\delta^{13}\text{C}$ -enriched peaks may be related to weaning. No correlation was found between the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of DPs, similar to that of the mysticetes (**Figures 3** and **4**). We believe that the large variability in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values between 180 and 230 cm BL may be due to sex-related differences, but this was not clear (males and females are not shown separately in **Figure 5**). Ontogenetic changes in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  profiles in DP samples (**Figure 5**) were similar to those in BW samples (**Figure 3**).

The Hg concentrations in DPs increased with increases in BL, and were markedly higher than those of MWs (**Figure 4**), reflecting the higher trophic position of DPs.

The ontogenetic signatures of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in KWs were investigated by Newsome et al. [33] using the dentin growth layer of teeth. They reported a decrease in  $\delta^{15}\text{N}$  values related to weaning in those dentins with no  $\delta^{15}\text{N}$ -enriched peaks related to nursing, and a gradual and continuous increase in  $\delta^{15}\text{N}$  values after weaning. This post-weaning increase in  $\delta^{15}\text{N}$  values in KWs is more prominent than that in DPs (**Figure 5**), ringed seals [56], and the beluga whales [55], and similar to that in harbor porpoises [14] and South American fur seals [57]. We believe that the increase in  $\delta^{15}\text{N}$  values after weaning may be correlated with the increase in trophic level of juvenile animals owing to growth.



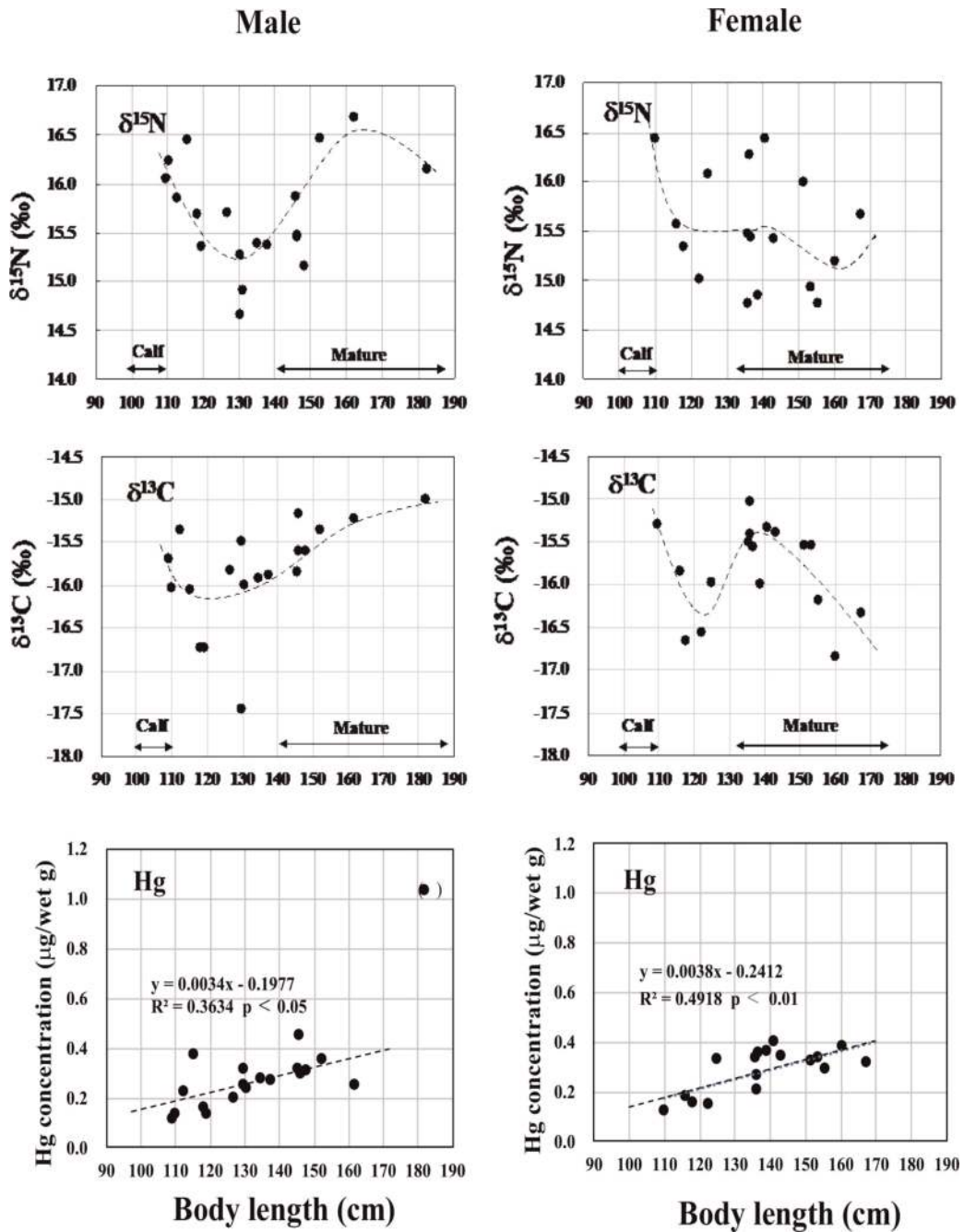
**Figure 5.**  
 Ontogenetic changes of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures and Hg concentration in muscle of Dall's porpoises stranded in Hokkaido, Japan.

### 3.4 Ontogenetic changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in kurill harbor seals

HSs are distributed on the eastern coastline of Hokkaido, Japan. According to Naito and Nishiwaki [78], HSs are born at 98 cm BL after a 9-month gestation period, and weaning occurs at approximately 4 weeks after birth at ~110 cm BL. It is unknown whether weaning occurs suddenly or gradually, but the weaning is likely rapid, considering the brief lactation period. Males attain sexual maturity at 140 cm BL (4 years) and females at 133 cm BL (3 years), and the maximum BL observed was 191 and 175 cm in males and females, respectively. HSs feed on prey in inshore areas, such as cephalopods, crustaceans, and small fish. The competition between HSs and coastal fisheries has recently become a serious problem in Hokkaido because of the increased number of HSs. Tissue samples were collected from stranded HSs in salmon set nets. **Figure 6** shows our data on HS males and females.

A  $\delta^{15}\text{N}$ -depleted peak was found at 120–130 cm BL of males, and the  $\delta^{15}\text{N}$  values increased thereafter, whereas a small  $\delta^{13}\text{C}$ -depleted peak was found at

120 cm BL, and  $\delta^{13}\text{C}$  values gradually increased thereafter. In contrast, a  $\delta^{15}\text{N}$ -depleted peak was observed in females, whereas the  $\delta^{13}\text{C}$ -depleted peak and following  $\delta^{13}\text{C}$ -enriched peak were clearly found at  $\sim 125$  and  $\sim 135$  cm BL, respectively. The small HSs at  $\sim 110$  cm BL, shown in **Figure 6**, could be just-weaned animals who might forage independently of their mothers, and be stranded in salmon set nets.



**Figure 6.** Ontogenetic changes of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures and Hg concentration in muscle of male and female harbor seals stranded in Hokkaido.

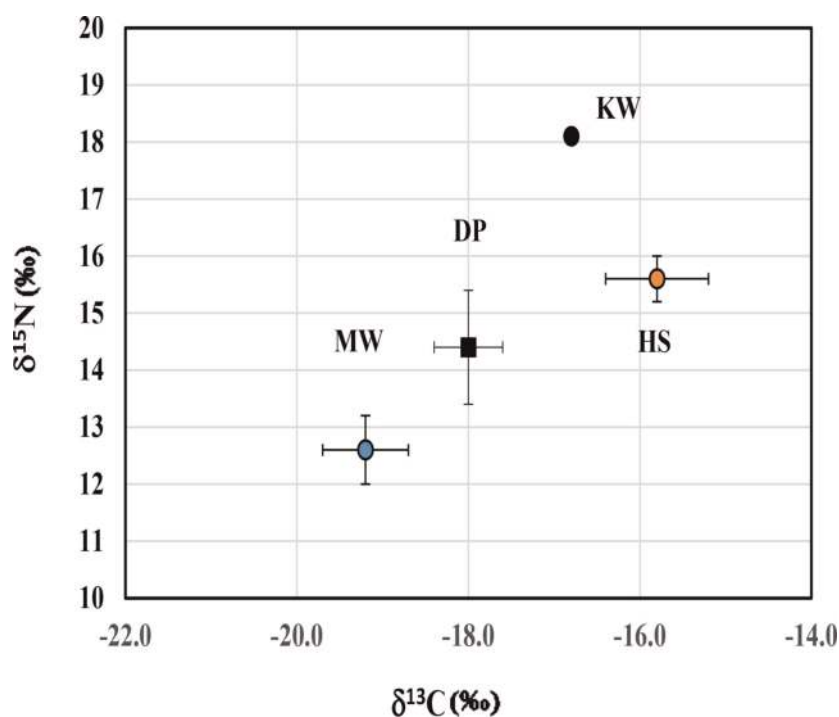
The  $\delta^{15}\text{N}$ -enriched peaks due to the lactation were not observed in HS samples, as our samples did not include nursing pups whose BL was less than 110 cm. Sharp decreases in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were found in male and female pups at 110–120 cm BL after weaning, which may reflect their weaning processes: The feeding shifts from milk to solid food and turnover rate of  $\delta^{15}\text{N}$  signature may be extremely first in HS muscle.

Sex-related differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  profiles were observed in the animals. Pregnancy and lactation of females may be the reasons for the differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures. Sex-related differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures were not found in the MWs (**Figure 4**) and were unclear in DPs (**Figure 5**), which migrate annually in the waters around Japan.

### 3.5 Isotopic segregation of calves of cetacean species stranded in Hokkaido

Stable isotope analyses of carbon and nitrogen in the tissues of suckling offspring, before weaning, when milk constitutes the entire diet, are increasingly used as proxies for maternal foraging habits and trophic levels [54, 60, 79]. This indirect approach is useful when sampling from females is difficult or has a high potential risk. In this section, we compare the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of stranded calves (nursing and weaning stages) from mysticete species (MW) and two odontocete species (DP and KW) inhabiting the waters around Hokkaido, Japan, and the North Pacific Ocean, to investigate whether these calves reflect their maternal foraging habits and trophic levels.

**Figure 7** shows the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of MW, DP, and KW calves stranded along the coast of Hokkaido. MW is opportunistic feeders that temporally and



**Figure 7.** Isotopic discrimination of calves of common minke whale, Dall's porpoise and killer whale stranded in Hokkaido. MW: Common minke whales (see **Figure 4**,  $n = 12$ ), DP: Dall's porpoises (see **Figure 5**,  $\text{BL} \geq 130$  cm,  $n = 11$ ), KW: killer whales (see **Table 1**,  $n = 3$ ), HS: harbor seals (see **Figure 6**,  $n = 32$ ).

regionally adapt to prey items (zooplankton and small fish), whereas KW and DP are odontocetes, and the KW is a cetacean located at the top of the marine food chain.

The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of KW calves and MW calves were the highest and lowest, respectively, and those values of DP calves were intermediate, reflecting the trophic positions of their mothers.

Stable isotope signatures of HSs (immature and mature animals shown in **Figure 6** (pinniped) were compared with those of MW, DP and KW calves (**Figure 7**). According to the inshore prey comprising cephalopods, crustaceans, and fish, the  $\delta^{13}\text{C}$  values of HSs were the highest among the marine mammals tested and  $\delta^{15}\text{N}$  values of HSs were higher than those of DP and MW calves.

#### **4. Conclusion**

The  $\delta^{15}\text{N}$ -enriched peaks owing to nursing and weaning were observed in BW, MW, and DP muscle samples, as shown in **Figures 3–5**. The time lag between the dietary shift from milk to solid food and the  $\delta^{15}\text{N}$  signature in muscle tissue is considered to be small in BWs, and MWs (mysticetes) and probably in DPs (odontocete). Thus, the  $\delta^{15}\text{N}$  signature in muscle tissues could serve as an excellent proxy for the lactation of calves.

Brief lactation (weaning) could result in a sharp decrease in  $\delta^{15}\text{N}$  values (**Figures 4 and 6**), whereas prolonged weaning may result in a gradual decrease (**Figure 5**).

The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the muscle of cetacean calves may serve as excellent proxies for maternal forging habits and trophic levels (**Figure 7**).

In marine mammals, the increase in Hg burden in calves could serve as a proxy for the weaning and eating of fish (**Figures 4–6**).

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