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Teeth as biomonitors of selenium concentrations in tissues of beluga whales (*Delphinapterus leucas*)

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ABSTRACT

Selenium (Se) is an essential element which has been shown to play an important role in protecting marine mammals against the toxic effects of mercury (Hg) and other metals. It has been suggested that metal concentration in marine mammal teeth can potentially be used as bioindicators for body burden. The objective of this study was to investigate the relationship between Se concentrations in beluga (*Delphinapterus leucas*) teeth and those previously measured in soft tissues (liver, kidney, muscle and muktuk). Tooth Hg concentrations are also measured, and the relationships between Se and Hg in teeth and soft tissues are examined. Se in the teeth of beluga was measured using hydride generation atomic fluorescence spectrometry (HG-AFS) and Hg in beluga teeth was measured by cold-vapour atomic absorption. Tooth Se concentrations ranged from 108 ng/g to 245 ng/g dry weight, and tooth Hg concentrations ranged from 10 to 189 ng/g dry weight. In the soft tissues, Se concentrations were highest in the liver, followed by kidney, muktuk, and muscle. There were significant correlations between tooth Se concentrations and animal age, tooth Se and liver and muscle Se, and between liver Se and animal age. The molar ratio of Hg:Se in the liver was found to be 0.70. This study is the first to measure Se in the teeth of a marine mammal species, and HG-AFS is found to be an effective technique for determining Se in beluga teeth. Tooth Se can be used as predictor for liver and muscle Se, although these relationships may be strongly influenced by the association of Se with Hg in marine mammal tissues. This study contributes to an increased understanding of the storage and metabolism of Se in marine mammals.

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1. Introduction

Predatory marine mammals often exhibit higher levels of metal contaminants, such as mercury (Hg), than their terres-

trial counterparts (Wagemann et al., 1996; Woshner et al., 2001; Lockhart et al., 2005). This occurs because Hg is methylated much more effectively in aquatic environments than in terrestrial. In addition, marine mammals generally occupy a

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high-trophic position in the marine ecosystem (marine trophic chains are generally longer than terrestrial chains) and they have long life spans, resulting in high Hg exposure to top predators over long periods of time (Ikemoto et al., 2004). Despite the elevated Hg concentrations observed, which are particularly high in the liver, there have been very few cases of observed metal intoxication in high-trophic level marine mammals (Cuvin-Aralar and Furness, 1991). This suggests the existence of an effective metal detoxification mechanism in these marine mammals.

There is substantial evidence that selenium (Se) plays an important role in protecting marine mammals against the toxic effects of Hg and other metals. This detoxification mechanism may involve metals such as silver, copper, cadmium and lead (Becker et al., 1995; Ikemoto et al., 2004); however, the relationship has been best characterized between Se and Hg. Although the exact mechanisms of this interaction are not known, it has been observed that the end product of the detoxification of organic Hg is the formation of a stable and inert complex with selenium, called tiemannite (mercuric selenide, HgSe) (Martoja and Berry, 1980; Cuvin-Aralar and Furness, 1991; Becker et al., 1995). This inert complex binds the two elements in an equimolar ratio when high levels of Hg are present, and a significant correlation of Hg and Se in the liver of marine mammals has been reported in many studies (Koeman et al., 1973; Hansen et al., 1990; Wagemann et al., 1990; Dietz et al., 2000; Woshner et al., 2001).

Metals, such as Hg and Se, are incorporated either into the crystalline apatite structure or the protein fraction of teeth (Ando et al., 2005). Hg has been measured in the teeth of beluga whales (*Delphinapterus leucas*), and has been found to be a good biomonitor of soft tissue Hg levels (Outridge et al., 2000). Because teeth are not modified or reabsorbed, long-term changes in trace element body burden can be monitored, and preserved tooth samples can be used to give information about metal contamination in the past. Although Se has been measured in the teeth of humans and rats (Hadjimarkoes and Bonhorst, 1959; Shearer, 1975), it has never been measured in a marine mammal species. Considering the important detoxification role of Se in marine mammals, there is interest in characterizing the relationship between tooth Se and soft tissue Se in a marine mammal species. The objective of this study was to investigate the relationship between Se concentrations in beluga teeth and those previously reported in other soft tissues. We measured Se concentrations in the teeth of beluga whales collected in northern Canada. Tooth Hg concentrations were also measured, and the relationships between Se and Hg in teeth, between Se in beluga teeth and soft tissues, and between Hg in teeth and soft tissues were examined.

2. Methods

2.1. Sample collection and preparation

Belugas were harvested from the Mackenzie Delta, Northwest Territories, in 1996 and 2002, as part of an annual traditional Inuit hunt. The soft tissues (liver, muscle, muktuk and kidney) were sampled and analyzed for Hg and Se as part of a long-term assessment program into contaminants in the Canadian Arctic (AMAP, 2005; Lockhart et al., 2005). Briefly, Hg was measured by

cold-vapour atomic absorption after digestion with a mixture of sulphuric and nitric acids at 180 °C and Se was measured by hydride generation atomic absorption method after digestion with a mixture of nitric, sulphuric and perchloric acids (Lockhart et al., 2005). Soft tissue data for some of the harvested animals were made available for this study by Fisheries and Oceans Canada (G. Stern, Freshwater Institute, Winnipeg, Manitoba, personal communication). The second and fifth right mandibular teeth of each animal were removed for aging purposes. Beluga teeth are composed of an inner core of dentine, surrounded by thick cementum (enamel is absent). Teeth grow in incremental layers, which are deposited in both cementum and dentine on a biannual basis; these layers are used to age individuals (Brodie et al., 1990).

For this study, teeth were split vertically, from root to crown, using a Dremel high speed rotary saw with interchangeable bits. Care was taken to ensure that each half preserved all growth layers in cementum and dentine. This is critical for both aging and trace element analysis, since it ensures that all growth layers are included in age determination and that the lifetime accumulation of trace elements is accounted for. One half-tooth was sent to a Fisheries and Oceans Canada laboratory for aging, which was conducted by mounting each half-tooth in epoxy resin blocks, thin-sectioning, and counting growth layers (Outridge et al., 2000). The other half-tooth was used for Se and Hg measurement.

Using a Dremel tool, the inner dentine layer was removed and discarded from each half-tooth, leaving only the cementum material. Because one of our main objectives is to compare Hg and Se concentrations in these teeth, and Hg accumulation in teeth occurs mainly in cementum (Outridge et al., 2000), Se was likewise only determined on cementum. Cementum sections were cleaned with a rotary grinder to remove organic tissue or other contaminating material on the outer surface of the tooth. Cross sections were taken from the thickest part of the cementum sections (ensuring equal representation of all growth layers). Samples were dipped in 10% HNO₃ for 20 s, then rinsed several times in distilled deionized water and left to air-dry in a fumehood. Dried samples were weighed and stored in sterile sampling bags in preparation for digestion and analysis. Sample weights were between 0.2 and 0.4 g. The samples were not ground to powder form to reduce the risk of contamination.

A microwave digestion was performed using a mixture of HNO₃ and H₂O₂. Each sample was placed in a microwave digestion vial with 8.0 mL concentrated HNO₃ (ACS grade) and 2.0 mL H₂O₂ (30%, ACS grade). The samples were heated in a microwave (Milestone Microwave Laboratory System model Ethos SEL with HPR-1000/10S rotor) using the following digestion program: 1) 0–85 °C, 4 min; 2) 85–145 °C, 10 min; 3) 145–210 °C, 6 min; 4) maintain 210 °C, 10 min; 5) vent, 20 min. No pre-reduction step was conducted, since previous tests had shown that this step was unnecessary, most likely due to the largely inorganic nature of the matrix. The digests were diluted to a final volume of 25 mL, using distilled deionized water.

2.2. Analysis

Total Se was determined by hydride generation atomic fluorescence spectrometry (HG-AFS; model PSA 10.055 Millennium Excalibur). Analytical quality was controlled using the

standard reference material (SRM) no. 1486 bonemeal (National Institute of Standards and Technology [NIST], USA), which was the available SRM with a matrix most similar to that of teeth. Although this SRM is not certified for Se, the measured Se concentration was 0.13 $\mu\text{g/g}$. In addition, analytical quality was controlled by analyzing all samples in duplicate, and measuring a digest blank, a SRM and a standard spike recovery for every eight digested tooth samples. The potential for a difference in matrix effect between the bonemeal SRM and the tooth samples was tested by spiking four replicates of a bonemeal digest, a tooth sample digest, and a blank acid digest with 0.0, 0.1, 0.2, and 0.4 $\mu\text{g Se/L}$.

Total Hg in the teeth was determined by cold-vapour atomic absorption (CVAA; model CETAC QuickTrace M-6000 Mercury Analyzer), using the digests prepared for Se analysis. Analytical quality was controlled by measuring random samples in duplicate, measuring several digest blanks, and measuring an oyster tissue SRM (NIST, no. 1566b) every five samples. The oyster tissue SRM contains a certified concentration of 37.1 ± 1.3 ng Hg/g. Bonemeal SRM digests were analyzed along with tooth samples, but since this SRM contains no information value for Hg, it was not used for external quality control.

2.3. Statistical analyses

Data on Se concentrations satisfied statistical assumptions of normality and heteroscedasticity. Linear regression and correlation analysis were conducted between tooth Se concentrations and soft tissue Se concentrations, between tooth and soft tissue Se concentrations and tooth age estimates, and between tooth Se and tooth Hg concentrations. Subsequently, age was included as an additional predictor of tooth Se concentration using multiple regression analysis. Sex was not considered to be a factor, since almost all (90%) of the harvested animals were male. A p value of <0.05 was considered significant. All statistical analyses were performed using the program SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Analytical quality

The measured standard reference material Se values were consistently above the information value, with an average accuracy of 115%, and a precision of 7.0% relative stan-

Table 1 – Analytical data quality (accuracy and precision) for selenium in bonemeal standard reference material and for mercury in oyster tissue standard reference material (ng/g)

SRM	N	measured mean \pm SD	% relative SD	NIST value	Accuracy (% of info value)
Bonemeal (NIST 1486)	8	148 ± 10.4	7.0	130	115
Oyster tissue (NIST 1566b)	4	38.2 ± 1.9	4.9	37.1	103

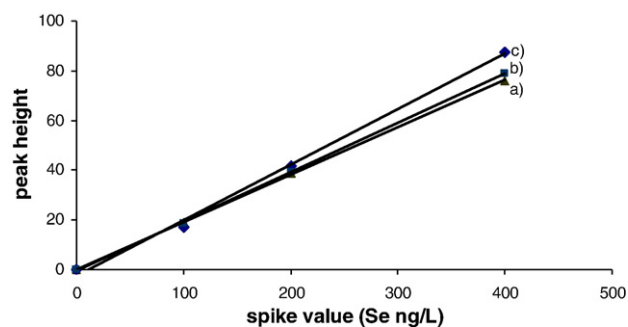


Fig. 1 – Peak height measurements of replicates spiked with 0.0, 0.1, 0.2, and 0.4 $\mu\text{g Se/L}$ for (a) sample blanks (mean of 4 separate runs); (b) tooth sample digest; (c) bonemeal digest. Solid line represents trendline.

dard deviation (Table 1). Repeatability of duplicate samples was $\pm 5.3\%$.

No significant matrix effect was detected using HG-AFS. The slopes of the graphs representing the value of spiked tooth sample replicates and corresponding peak height measurements (slope=0.199) were within the standard deviation of the mean slope of four separate measurement runs of the spiked blank replicates (with corresponding peak height measurement) (mean slope=0.207, standard deviation=0.008) (Fig. 1). The slope of the spiked bonemeal replicates was slightly above this standard deviation (slope=0.223). These absences of matrix effects justify the use of external calibration method.

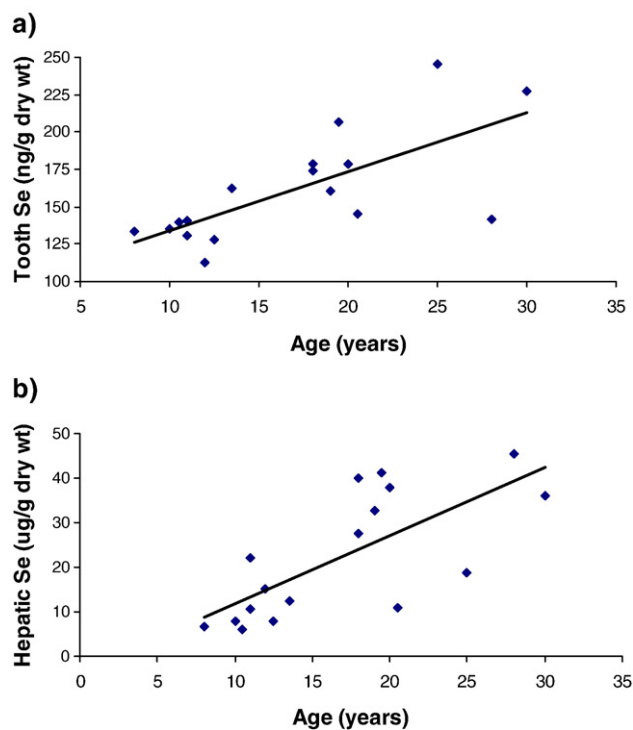


Fig. 2 – Age-dependent concentrations of selenium in (a) teeth and (b) liver of beluga harvested from the Mackenzie Delta. Solid lines represent linear regressions.

Table 2 – Mean, standard deviation and range of selenium concentrations in beluga teeth and soft tissue, harvested from the Mackenzie Delta ($\mu\text{g/g}$ dry weight^a or $\mu\text{g/g}$ wet weight^b, N=20*)

Tissue	Tooth ^a	Liver ^a	Muscle ^b	Muktuk ^b	Kidney ^a
Mean	0.16	20.65	0.38	3.82	4.86
Standard deviation	0.04	13.50	0.06	0.97	1.75
Range	0.11–0.25	6.17–45.52	0.30–0.50	2.33–5.96	2.06–8.99

*Except for kidney, where N=12.

The measured Hg values in the oyster tissue SRM were based on a series of four replicate samples. The average accuracy was 103%, and the precision was 4.9% relative standard deviation (Table 1).

3.2. Tooth selenium

Tooth concentrations ranged from 108 ng Se/g to 245 ng Se/g. There was a linear correlation of tooth Se with animal age (Fig. 2a; $r^2=0.50$, $p<0.05$) and liver Se with animal age (Fig. 2b; $r^2=0.52$, $p<0.001$). Age was not a significant factor in the Se concentrations of the other soft tissues.

3.3. Comparison of selenium in teeth and soft tissues

In the soft tissues, Se concentrations were highest in the liver, followed by kidney, muktuk, and muscle (Table 2). Simple linear regression showed that tooth Se is significantly related

Table 3 – Coefficients of determination (r^2) for linear regression between selenium in beluga teeth, liver, muscle, muktuk, and kidney, harvested from the Mackenzie Delta

Tissue type	Kidney	Muktuk	Liver	Muscle	Tooth
Kidney	–	0.28	0.09	0.77*	0.06
Muktuk	–	–	0.01	0.10	0.03
Liver	–	–	–	0.21*	0.29*
Muscle	–	–	–	–	0.36*
Tooth	–	–	–	–	–

* Significant at $p<0.05$.

to Se concentrations in liver and muscle, but not in muktuk or kidney (Fig. 3a, b, c and d; Table 3). The linear regression equations predicting tooth Se (in ng/g dry weight), from soft tissue Se (in $\mu\text{g/g}$ wet weight or dry weight depending on tissue; see Table 2) are:

$$\text{Tooth Se} = 1.45 \times \text{liver Se} + 126.6$$

$$\text{Tooth Se} = 352 \times \text{muscle Se} + 21.3.$$

When age is included as a co-variate in the analysis of the relationship between tooth Se and soft tissue Se, tooth Se becomes significantly related to muktuk Se and age, but the model remains non-significant for kidney. It appears that age accounts for most of the co-accumulation of Se in teeth and the soft tissues (liver, muscle and muktuk) (Table 4). The

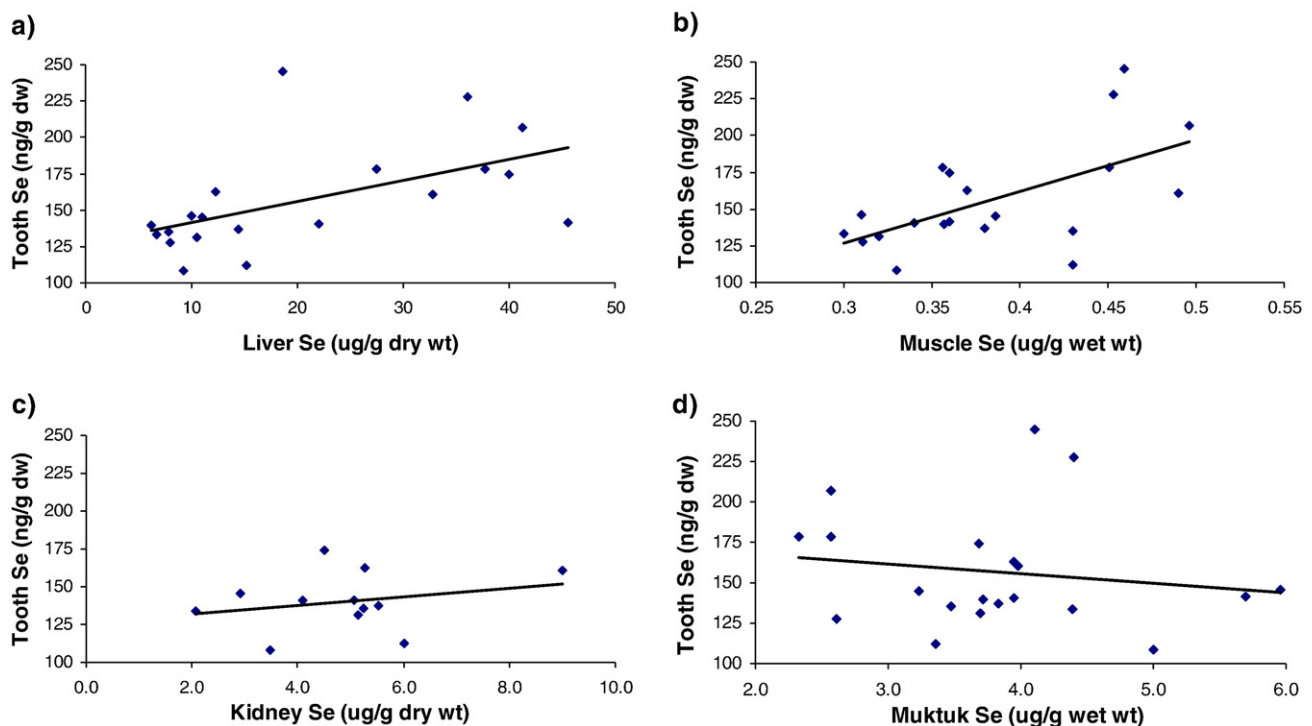


Fig. 3 – Relationship between concentrations of selenium in beluga tooth and (a) liver, (b) muscle, (c) kidney, and (d) muktuk. Solid lines represent linear regressions (see equations in text for liver and muscle).

Table 4 – Coefficients of determination (r^2) and p values for the relationships between selenium in teeth, soft tissue (liver and muscle) Se concentrations, and animal age, from beluga harvested in the Mackenzie Delta

Model summary	Soft tissue		
	Liver	Muscle	Muktuk
r^2 of model	0.496	0.574	0.546
Significance of model (p)	0.008*	0.003*	0.004*
Significance of age variable (p)	0.020*	0.014*	0.001*
Significance of soft tissue variable (p)	0.945	0.132	0.232

*Significant at $p < 0.05$.

multiple regression equations predicting tooth Se (in ng/g dry weight), from soft tissue Se (in $\mu\text{g/g}$ wet weight or dry weight depending on tissue; see Table 2), and age in years are:

$$\text{Tooth Se} = -0.50 * \text{liver Se} + 4.03 * \text{age} + 94.4$$

$$\text{Tooth Se} = 186.2 * \text{muscle Se} + 3.11 * \text{age} + 35.7$$

$$\text{Tooth Se} = -10.30 * \text{muktuk} + 4.32 * \text{age} + 125.7.$$

3.4. Tooth mercury

Concentrations of Hg in teeth ranged from 10.0 to 188.6 ng/g, with a mean of 70.9 ng Hg/g (Table 5). There was no significant correlation between tooth Hg concentrations and tooth Se concentrations ($r^2=0.11$; $p=0.16$). Linear regression also showed that animal age is not a significant factor in the accumulation of Hg in beluga teeth in this study ($r^2=0.04$; $p=0.45$). However, when age is included as a co-variate in a multiple regression analysis of tooth Se and tooth Hg, the overall regression model becomes significant ($r^2=0.51$, $p=0.01$). In this model, age accounts for most of the accumulation of tooth Se ($p=0.005$), and the correlation with tooth Hg remains non-significant ($p=0.466$).

3.5. Mercury in soft tissues

In the soft tissues, measured Hg concentrations were highest in the liver, followed by kidney, muscle and muktuk (Table 5). The concentrations of total Hg measured in the liver were all above 10 nmol/g (2 $\mu\text{g/g}$), which is considered to be the point at which most Hg in the liver is present as inorganic Hg in association with Se (Dietz et al., 1990, 2000). The mean liver MeHg concentration was 2.56 $\mu\text{g/g}$, with an average of 10.6% of the total Hg in the liver present as MeHg. The mean molar Hg:Se ratio in the liver was 0.70 ($r^2=0.84$; $p<0.001$). Linear regression showed that the mean molar liver Hg:Se ratio was significantly correlated with tooth Hg concentrations ($r^2=0.37$; $p=0.007$), but not with tooth Se concentrations ($r^2=0.09$; $p=0.21$). Age was a significant factor in hepatic total Hg concentrations ($r^2=0.52$; $p=0.002$), but not in hepatic Hg:Se molar ratio ($r^2=0.07$; $p=0.33$).

The tooth–soft tissue relationships described by Outridge et al. (2000) were re-tested using the data gathered for this

study. In this study, tooth Hg was significantly associated with Hg in the liver ($r^2=0.42$; $p=0.004$), muscle ($r^2=0.32$; $p=0.015$) and kidney ($r^2=0.62$; $p=0.002$), but not in muktuk ($r^2=0.21$; $p=0.056$). The equations described by Outridge et al. (2000) are within the 95% confidence intervals of the tooth–soft tissue relationships tested here for liver and kidney, but not for muscle and muktuk.

4. Discussion

This study showed that hydride generation atomic fluorescence spectrometry is an effective technique for measuring Se in beluga teeth. Fluorimetric methods have been used in the past to measure Se in hard tissues in rats and humans (e.g. Johnson and Shearer, 1979), but radioactive techniques such as neutron activation analysis and gamma spectrometry have been used more frequently (Nixon and Myers, 1970; Retief et al., 1974, 1976). Atomic fluorescence spectrometry represents a relatively inexpensive and precise technique for measuring Se in mammal teeth.

Selenium concentrations in teeth are significantly related to Se in liver and muscle, with 29% and 36%, respectively, of variation in tooth Se accounted for by the soft tissue Se. These results indicate that tooth Se can be used as a predictor for Se in liver and muscle. On the other hand, our results showed that tooth Se concentrations were not significantly related to kidney and muktuk Se, suggesting that tooth Se alone is a poor predictor of Se in kidney and muktuk. The relationships between tooth Se and Se in these four soft tissues are not as strong as those found for tooth Hg and Hg in the same soft tissues in this study or described by Outridge et al. (2000). Outridge et al. (2000) found that Hg levels in all four soft tissues were significantly correlated with tooth Hg, with soft tissue Hg explaining 54% to 66% of variance in tooth Hg. This study found that Hg levels in liver, kidney and muscle are significantly correlated with tooth Hg, but this relationship was not significant for muktuk Hg. The tooth–soft tissue relationship tested in this study for muscle, kidney and liver accounted for 32% to 62% of the variance of Hg in teeth. These percentages were lower than those described by Outridge et al. (2000), but higher than the relationships tested between tooth Se and soft tissue Se.

The difference between tooth and soft tissue relationships for Se and Hg may be explained by the different roles that these two elements play in the body. Selenium is an essential element for all animals, and is found throughout the body as

Table 5 – Mean, standard deviation and range of total mercury concentrations in beluga teeth and soft tissue, harvested from the Mackenzie Delta (ng/g dry weight^a, $\mu\text{g/g}$ dry weight^b or $\mu\text{g/g}$ wet weight^f, $N=20$)

Tissue	Tooth ^a	Liver ^b	Muscle ^c	Muktuk ^c	Kidney ^b
Mean	70.92	40.17	1.66	0.93	8.14
Standard deviation	45.35	32.78	0.61	0.35	3.47
Range	10.01– 188.61	3.36– 96.9	0.79– 3.36	0.30–1.73	2.20–13.0

^aExcept for kidney, where $N=14$.

selenoprotein compounds. Approximately 35 different selenoproteins have been isolated from animal cells, and are particularly important as anti-oxidants (Raymond and Ralston, 2004). Selenium is incorporated into two of the 22 primary amino acids: selenomethionine and selenocysteine, the latter of which is integrated into proteins contributing to necessary biological functions. Selenium accumulation in storage organs, such as the liver or kidneys, occurs when Se is present at concentrations which exceed metabolic requirements (Mackey et al., 1996; Holben and Smith, 1999). Conversely, Hg serves no functional purpose in the animal body. It is present solely as a contaminant, and may therefore be more likely to be concentrated in storage organs. This may explain the stronger correlations of Hg levels between storage organs (teeth and soft tissues), relative to Se.

The significant correlation between tooth Se and liver Se concentrations probably occurs because Se was also found to accumulate with age in both liver and teeth, and because the liver is the main storage organ for Se and for Hg (Outridge et al., 2000). Multiple regression analysis using liver Se and age as independent variables to predict tooth Se concentrations showed that age accounted for most of the accumulation of Se in teeth (see Table 4). Thus, although tooth Se may be used as a biomonitor of liver Se concentrations in a sample of multi-aged individuals, it does not indicate whether individuals of a given age class have high or low levels of liver Se. Metabolic studies in humans, other mammals, and fish indicate that the liver acts in the storage, metabolism, and elimination of Se (Thomson and Stewart, 1974; Hilton et al., 1982; Behne and Wolters, 1983). In addition, in marine mammals the deposition of tiemannite (mercuric selenide) crystals in the liver, as an end product of methylmercury detoxification, contributes to the accumulation of Se in this organ, when Hg levels are sufficiently elevated.

In this study, liver Se concentrations were found to range from 6.2 to 45.2 $\mu\text{g Se/g}$ (Table 2). This is within the ranges of values previously reported for beluga by other studies (Hansen et al., 1990; Mackey et al., 1996; Woshner et al., 2001). It is likely that the liver Se concentrations reported in these studies all occur in the context of high Hg load, since, like all predatory marine mammals, belugas often demonstrate high liver Hg levels, even in uncontaminated environments (Outridge et al., 2002). It is therefore likely that these levels are representative of “normal” liver Se levels. Similarly, it is probable that the concentrations measured in this study reflect an association of Se with inorganic Hg in the liver, since the measured total Hg concentrations in this organ are high enough (above 2 $\mu\text{g/g}$; see Table 5) that the majority of the Hg is present in an inorganic form as part of mercuric selenide crystals (Koeman et al., 1975; Dietz et al., 1990; Hansen et al., 1990; Dietz et al., 2000). In this study, the mean molar Hg:Se ratio in the liver was 0.70, which is below the 1:1 equimolar ratio observed by Koeman et al. (1973, 1975). However, many subsequent studies have found molar Hg:Se ratios below 1 in cetacean livers (Hansen et al., 1990; Krone et al., 1999; Outridge et al., 2000; Woshner et al., 2001).

Unlike in the liver, where Se acts as part of a detoxifying mechanism when Hg concentrations are elevated, the Se present in muscle plays an important metabolic role independent of Hg levels in this tissue. The association between

tooth Se and muscle Se may therefore reflect the metabolic function of this element. From a physiological perspective, Se is largely associated with proteins in animal tissues, and muscle tissue is an important organ for selenoprotein activity and metabolism. Selenoprotein W, for example, plays a role in muscle metabolism (Holben and Smith, 1999; Gu et al., 2000; Whanger, 2000), while glutathione peroxidases and other selenoproteins act as anti-oxidants in muscle cells. In addition, Se is stored in muscle tissues as part of the amino acid selenomethionine (Butler et al., 1990; Raymond and Ralston, 2004). The association between muscle Se levels and those of tooth Se may reflect an accumulation of Se in teeth and muscle as selenomethionine when the availability of Se in the body exceeds physiological requirements. However, multiple regression analysis using muscle Se and age as co-variables to predict tooth Se concentrations showed that age was a better predictor of tooth Se accumulation than muscle Se concentrations, even though a simple linear regression did not find muscle Se concentrations to be significantly correlated with age. In this case, co-accumulation of Se in beluga teeth and muscle may occur because Se is deposited into the teeth and the muscle tissues at a rate proportional to blood concentrations.

In marine mammals, including beluga, the highest concentrations of Se are generally found in the liver, followed by the kidneys and muscle (Dietz et al., 1996; Bustamante et al., 2003, 2004). The soft tissue Se concentrations discussed in this study are in agreement with these previous findings, with the range of muktuk Se concentrations between kidney and muscle Se ranges. However, extremely high Se levels have been found in the skin (muktuk) of harbour porpoises (*Phocoena phocoena*) (Paludan-Muller et al., 1993; Dietz et al., 2000). Paludan-Muller et al. (1993) suggest that these elevated levels indicate that the skin may function as part of an excretion mechanism of excess Se. Similarly elevated muktuk Se levels have not been found in other species, but it has been suggested that the moulting of the epidermis contributes to the excretion of Hg and Se in beluga (Wagemann et al., 1995; Woshner et al., 2001). Wagemann et al. (1996) found that Se was 10 times higher in beluga muktuk than in muscle, and the results from this study give a similar ratio (Table 2). Hansen et al. (1990) found no significant correlations in Se concentrations between liver, kidney and muscle in beluga, and Paludan-Muller et al. (1993) found none between liver, kidney, muscle and muktuk in harbour porpoises. This study, however, found significant correlations between muscle Se and kidney Se and between muscle Se and liver Se.

There is a relative strong association between age and Se accumulation in beluga teeth. This accumulation occurs because teeth are stable hard tissues and the Se that is deposited over time remains in the teeth as an animal gets older. Age is also a significant factor in liver Se, with half of the variance in liver Se explained by animal age. This age-dependent accumulation is likely due to the association of Se with Hg in the liver, since the liver Hg concentrations reported in this study are sufficiently high to prompt the formation of inert tiemannite. Liver Hg levels are strongly associated with age in marine mammals (Hansen et al., 1990; Becker et al., 1995; Mackey et al., 1996; Wagemann et al., 1996; Lockhart et al., 2005), and the 1:1 ratio of Hg and Se frequently found in

the liver of marine mammals when liver Hg levels are sufficiently high suggests that most of the Se in the liver is in association with inorganic Hg. Unlike Hg, which accumulates with age in most marine mammal soft tissues (Wagemann et al., 1998), the age accumulation of Se is tissue specific. Age accumulation of Se in tissues other than liver is inconsistent, and Se is generally not found to increase with age to the same degree in kidney, muscle and other soft tissues as in the liver (Hansen et al., 1990; Wagemann et al., 1990; Paludan-Muller et al., 1993; Dietz et al., 1996). In this study we did not find Se to accumulate significantly with age in kidney, muscle and muktuk.

This study did not find a significant linear correlation between tooth Se and tooth Hg concentrations. Age alone was also not found to be a significant factor in the accumulation of Hg in teeth, although previous research had found tooth Hg to be significantly linearly correlated with age (Outridge et al., 2000). Multiple regression, however, shows that tooth Hg and age together do contribute to significant variation in tooth Se concentrations, though most of that variation is explained by the significant correlation between age and tooth Se. Considering the strong interaction between Hg and Se in the liver, the accumulation with age of both liver Hg and Se, the significant correlation between liver Se and tooth Se found in this study, the significant correlation between liver Hg and tooth Hg found in previous research (Outridge et al., 2000), and the interaction between tooth Se, tooth Hg and animal age may be a reflection of the age-dependent accumulation of inert tiemannite in the liver of beluga.

It is unclear what form of Se is stored in beluga teeth. Shearer (1975) found that Se incorporates into the protein fraction of rat teeth, generally in association with cysteine and methionine. That study found that the majority of Se in enamel and dentine was associated with protein fractions, not with the inorganic hydroxyapatite fraction of these tissues. Ando et al. (2005) suggested, however, that in marine mammals most trace elements are incorporated into the inorganic hydroxyapatite structure of the teeth. Since cementum is mostly composed of inorganic material, Se in beluga teeth may be found mainly in the inorganic apatite matrix. However, cementum does contain an organic component, composed mainly of collagen (Hillson, 2005), so the incorporation of Se into the protein fraction of beluga teeth remains a possibility. It is also unclear how high levels of Hg in the bodies of marine mammals might affect the incorporation of Se in hard tissues. First, it is not known whether Se and Hg are stored in association with one another in marine mammal teeth. If they are, it is possible that Se is entering teeth already in association with Hg, or that the two elements form an association after both have already been incorporated into the tooth structure. If Se and Hg are stored in the teeth in the form of inorganic tiemannite, this compound is more likely to be found in the inorganic matrix, rather than the protein fractions of teeth.

There are several factors that contribute to potential variance in the determination of tooth Se. First, there are likely to be variations in Se concentrations between teeth in the same jaw, as was found by Outridge et al. (2000) for Hg in beluga teeth. It is felt, however, that these variations do not distort the results in any particular direction, since the

differences in measured Hg in teeth in the same jaw were not consistent across multiple animals. Secondly, the deposition of Se may not be homogenous within each tooth. This uncertainty was addressed by consistently removing samples from the same position from all teeth sampled.

5. Conclusion

This study is the first to measure Se in the teeth of a marine mammal species. HG-AFS was found to be an effective technique for determining Se in beluga teeth. Tooth Se can be used as a predictor for liver and muscle Se, though these relationships are not as strong as those for Hg in teeth and soft tissues. The ability to calculate estimates of soft tissue Se and Hg concentrations from measured tooth concentrations could be useful in situations where soft tissues are not available. For example, archaeological beluga tooth samples can be used to estimate pre-historic trends in trace element accumulation (Kinghorn et al., 2006; Outridge et al., 2000, 2002). Considering the important role that Se plays in the detoxification of Hg in marine mammals, the distribution of Se in the bodies of marine mammals can provide information about this protective mechanism. This study contributes to an increased understanding of the storage and metabolism of Se in marine mammals.

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