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Short communication

Trace-element analysis of Steller sea lion (*Eumetopias jubatus*) teeth using a scanning X-ray analytical microscope

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Marine mammals are top predators in marine ecosystems and bioaccumulate various trace elements that pass through the food web (Miles and Hills 1994; Wagemann and Stewart 1994; Dietz et al. 1996). Trace elements include both essential and non-essential metals. Iron (Fe) and zinc (Zn) are essential metals that occur widely and abundantly in living organs. The concentrations at which they occur in an animal are affected not only by the diet, age, nutritional stress but also health status of the animal. In mammals, it is possible to examine element accumulation using their teeth. During dentine formation, trace elements are removed from dietary and environmental sources, and incorporated into the crystalline apatite structure of the teeth. Here, unlike in bones, they are not subject to reabsorption or remodeling, so they can be used to examine an animal's life history. Ando et al. (2005) found significant long-term differences in Zn/calcium (Ca) and Fe/Ca ratios from 1968 to 1999 by particle-induced X-ray emission analysis (PIXE). And Zn/Ca and Fe/Ca ratios were higher in younger males than older males, but showed no trend with age in females. There are question about fluctuation of these metals within each animal. In this study, we examined the canine teeth of Steller sea lions (*Eumetopias jubatus*) to assess their action and the fluctuation of essential metals of that occur in the teeth with their each life history.

Materials and methods

Three male and three female Steller sea lions were collected off the coast of Hokkaido, Japan in 1997–1999 (Table 1). These samples store at Faculty of Fisheries, Hokkaido University. We selected these 6 samples used in Ando et al. (2005), which are near by average concentration of Fe, Zn and Ca. One upper canine from each specimen sawn in half longitudinally to determine age, and then cut into a 1-cm-thick piece for analysis (Fig. 1). New dentine form inside and vertical side the teeth near

Table 1. Summary specimens of Steller sea lions used in this study.

Specimen #	Location	Month of collection	Sex	Age (years)
97019	Shakotan, Hokkaido	Feb. 1997	M	4
98101	Shakotan, Hokkaido	Feb. 1998	M	5
98107	Shakotan, Hokkaido	Mar. 1998	M	7
98020	Rausu, east Hokkaido	Jan. 1998	F	5
99001	Rausu, east Hokkaido	Jan. 1999	F	6
97011	Rausu, east Hokkaido	Feb. 1997	F	8

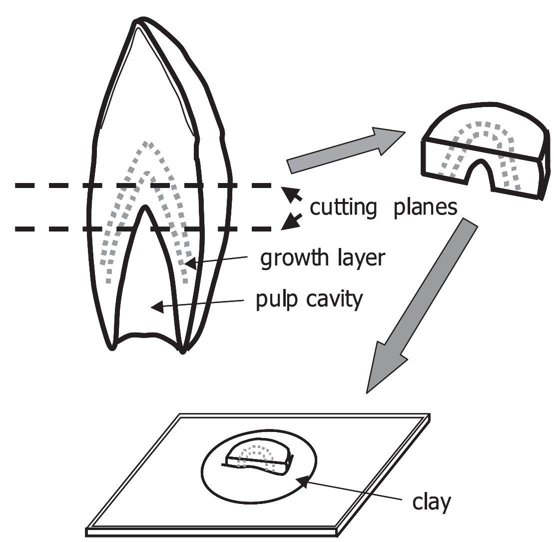

Fig. 1. Figure showing how tooth samples were prepared for analysis. Each tooth was first cut in half from top to bottom. A this thin cross section was then taken through the tooth for analysis.

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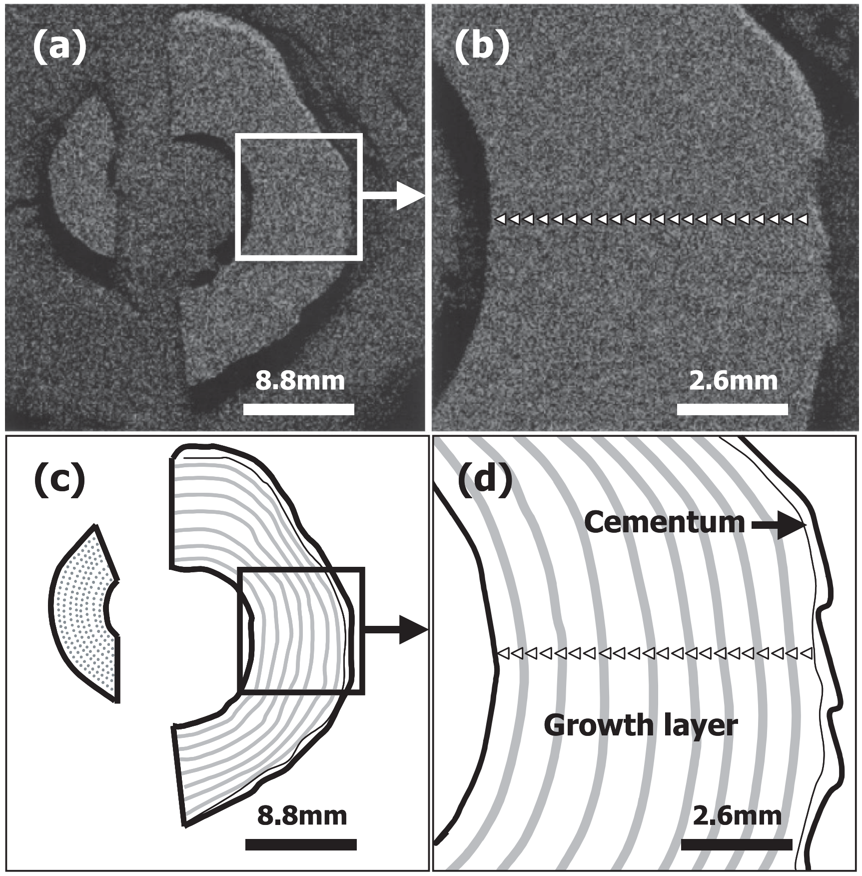

Fig. 2. (a) Photo of cut tooth samples from specimens #98107 (male, 7 years old, right) and #97011 (female, 8years old, left). (b) Enlarged view of sample from specimen #98107 showing points where samples were analyzed using scanning microscope. (c) Image illustration of cut tooth samples from specimens #98107 and #97011. (d) Enlarged view illustration of sample from specimen #98107 showing points where samples were analyzed using scanning microscope.

the pulp cavity. We suppose the tooth of neck include dentine accumulated during 0–1 year-old and also most recently accumulated, so used this part of dentine. Samples were cleaned with 99.5% ethanol to remove surface contamination.

Trace elements of dentine were analyzed at the National Research Institute of Far Seas Fisheries, Japan using a scanning X-ray analytical microscope (SXAM, Horiba XGT-2700). This type of microscope has been used to make mineral maps of a rock slab (Michibayashi et al. 1999). We applied this analysis, it is possible to determine the quantitative distribution of major elements within sample. Each tooth sample was set on a clay scanning table (Fig. 1), and excitation X-rays focused by a guide tube (100 µm diameter) were radiated onto the surface of the sample through a window of organic thin film. Fluorescence X-ray photons were observed by a high-purity silicon detector, and the energy was measured by comparison with the energy range of the selected fluorescence line of each element. The count number of fluorescence X-rays of each element was stored as a digital image that consisted of 256 × 256 pixels (Fig. 2a, c). In this study, iron (Fe) and zinc (Zn) were each measured as a ratio to the amount of calcium (Ca) in the tooth. We expected to identify growth layers by Ca or phosphorus (P) because tooth dentine is composed mainly hydroxyapatite crystals $[Ca_{10}(PO_4)_6(OH)_2]$. We could not identify it from scanning images. So teeth

Table 2. Measurement intervals and numbers of scanning point in each sample.

Specimen #	Measurement intervals (mm)	Scanning points
97019	0.36	11
98101	0.38	12
98107	0.35	21
98020	0.36	13
99001	0.45	12
97011	0.20	19

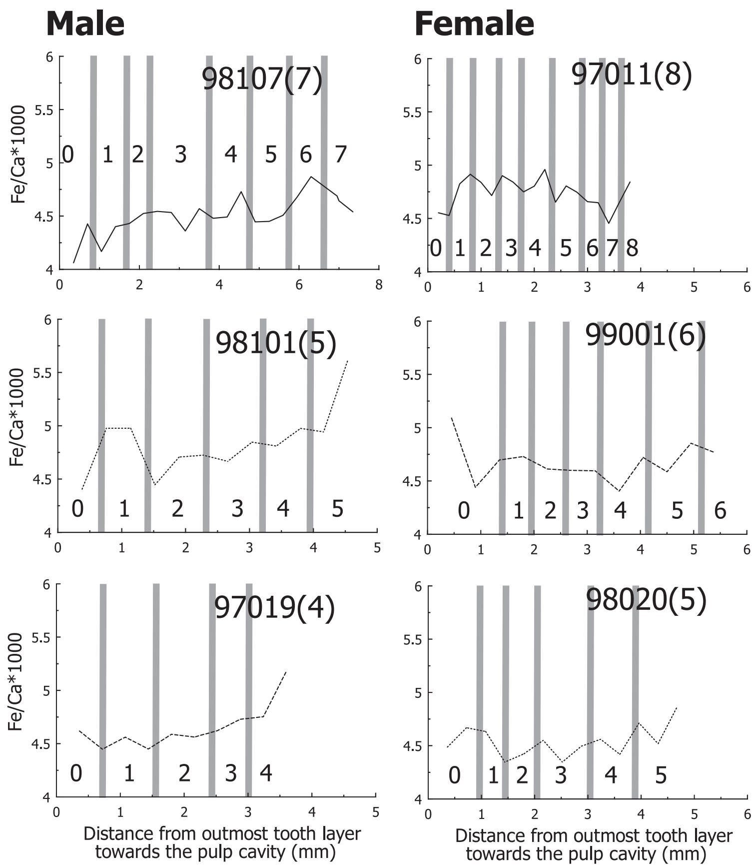

Fig. 3. Patterns of Fe/Ca ratios with growth. Numbers within parentheses indicate each specimen's age (years). Gray vertical line indicate each growth layer. Numbers between growth layers means age of each animal.

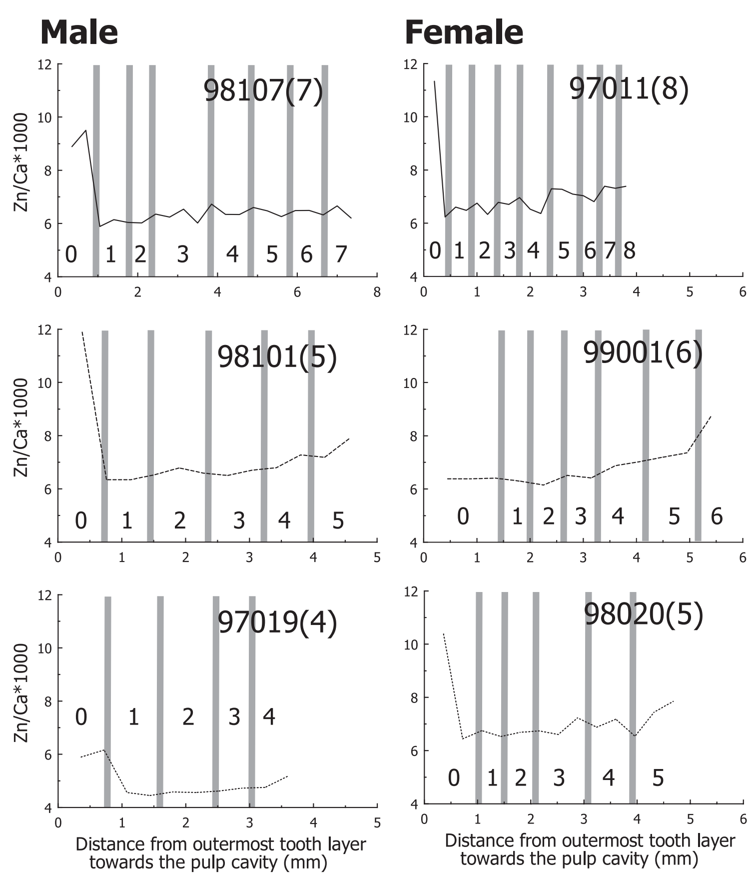

Fig. 4. Patterns of Zn/Ca ratios with growth. Numbers within parentheses indicate each specimen's age (years). Gray vertical line indicate each growth layer. Numbers between growth layers means age of each animal.

were scanned at many points from the outer layer to the inner pulp cavity (Fig. 2b, d). Table 2 showed the measurement intervals and numbers of scanning point in each sample. New growth layers form near the pulp cavity, so inner layers in the teeth formed later than outer layers. To compare with age, we measured the layer length between outmost and inner using digital caliper and microscope.

Results and discussion

As shown in Fig. 2a, there is sexual difference of the tooth size between male and female, and male tooth is apparently larger than female one (Isono 1998). Fe/Ca and Zn/Ca ratios varied considerably from the outer layer to the pulp cavity. The Fe/Ca ratio gradually increased with growth in males, but remained stable in females (Fig. 3). Isono (1998) showed that the growth patterns of various body parts differ between males and females. Males continue to grow until about 10 years of age, however females tend to stop growing when they reach 5 years of age. Changes in Fe/Ca accumulation might be related to physiological changes that occur with age. In addition, females can begin producing young at 2–3 years of age (Winship et al. 2001; Hoelzel 2002), and a developing fetus might affect the element accumulation in a pregnant female. In previous studies, the accumulation of Fe in dugong tusk was suggested to be related to the breeding state of the animal (Edmonds et al. 1997). Similarly, the accumulation of Fe in Steller sea lion females might be related to their breeding state (i.e., if they are pregnant).

The Zn/Ca ratio in both males and females tended to be high in the young and then suddenly decrease (Fig. 4). This pattern might have been due to their nutritional condition. Steller sea lion pups remain dependent on their mothers for food until near the end of their first or second year (Riedman 1990). Ando et al. (2005) described that the Zn/Ca ratio in the teeth of 19 Steller sea lions older than 1 year showed no clear pattern with age. The decrease we observed that occurred in the young might have been associated with their weaning.

These essential metals could be affected by the physiological condition of individuals, but the reason for the differences observed with age is very complicated. To clarify the interaction between trace metals and growth, more biological information on trace metal accumulation in teeth is needed.

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