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A Comparative Study on Temporal Trends of Trace Elements in Harbor Porpoise (*Phocoena phocoena*) from Coastal Waters of North Japan

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Abstract—Increasing human population and industrial activities on a global scale led to the release of many trace elements into the environment. Marine mammals have high potential to accumulate some trace elements, especially Hg, because of their long life span and higher trophic levels in marine food chains. In this study, we examined accumulation features and temporal trends of trace element levels in the liver and brain of harbor porpoise (*Phocoena phocoena*). Total Hg and O-Hg in harbor porpoise were lower than in other dolphins. The reason for this difference may be attributed to the different feeding habits and body size. In order to evaluate the temporal trend of trace element levels after correction for the effect of body size, multiple linear regression analysis was applied. Total Hg and O-Hg levels in harbor porpoise were almost constant since 1985 to 2010. This result is consistent with previous studies reporting that Hg concentration in coastal waters of Japan has been constant since 1986. Concentrations of Li, Al, Ca, Cr, Cu, Ga, As, Sr and Ba in harbor porpoise have significantly increased since 1985. Monitoring the trends of these elements in coastal waters of North Japan should be conducted.

Keywords: trace elements, mercury (Hg), rare metals, harbor porpoise, temporal trends

INTRODUCTION

Increasing human population and industrial activities on a global scale led to the release of many trace elements into the environment (Nriagu, 1996). For example, several reports suggested that emission of Hg is increasing in the Asian region by more than 50% and Hg level in sea water has increased from 1987 (average 0.58

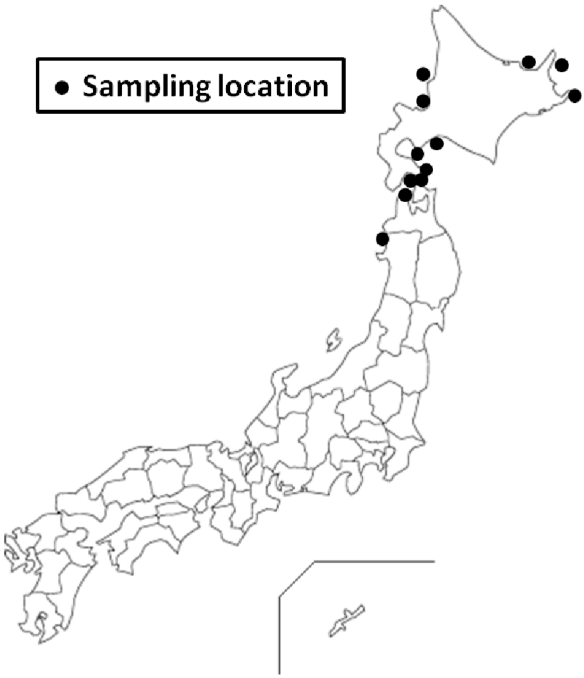

Fig. 1. Sampling locations of harbor porpoises in this study.

± 0.37 fM) to 2006 (average 1.14 ± 0.38 fM) in North Pacific Ocean (Pacyna *et al.*, 2006; Sunderland *et al.*, 2009). Demands and world productions of rare earth elements have also increased in recent years (JOGMEC, 2010). It is important to figure out the temporal trends of trace elements in various environmental and biological media in the point of the monitoring for the toxic trace elements and the appropriate management for rare metals. Marine mammals have high potential to accumulate various trace elements, especially Hg, because of their long life span and the highest trophic level in marine food chains (Capelli *et al.*, 2000; Kunito *et al.*, 2004). Hence, there have been some concerns regarding possible adverse effects of trace elements on marine mammals (Kunito *et al.*, 2004). Harbor porpoise (*Phocoena phocoena*) which lives in coastal waters of northern Japan can be a useful indicator of trace element contamination in that area. In this study, we examined accumulation features and temporal trends of trace element levels in the liver and brain of harbor porpoise.

MATERIALS AND METHODS

Sample collection

Forty five liver and 30 brain samples of harbor porpoise were collected from

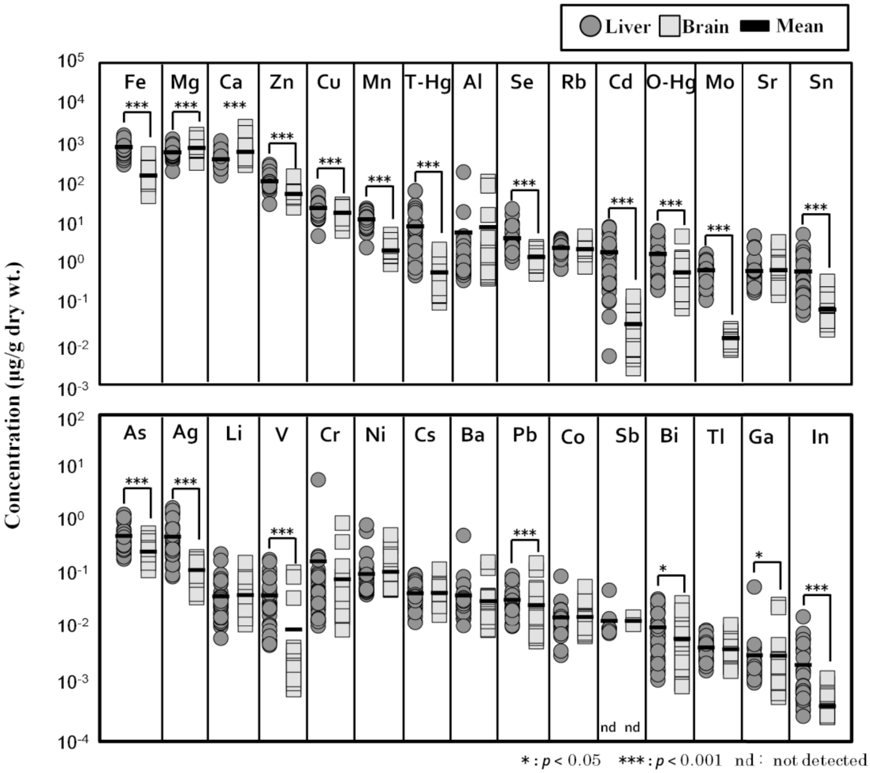

Fig. 2. Trace element concentrations in liver and brain of harbor porpoises.

specimens stranded or captured around North Japan since 1985 to 2010 (Fig. 1). Liver and brain samples were stored at Environmental Specimen Bank (*es*-BANK) of Ehime University at –25°C until chemical analysis.

Chemical analysis

Trace elements in liver and brain were measured according to the method described by Ikemoto *et al.* (2004) with slight modification. All tissues were dried at 80°C for 12 h and homogenized. After weighing 0.20 g of the dried sample into a Teflon vial, 3.5 ml of HNO_3 was added and the sample was pre-digested at room temperature for 16 hours. The sample was then digested in a closed microwave digestion system. Twenty eight trace elements (Li, Mg, Al. Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Ag, Cd, In, Sn, Sb, Cs, Ba, Tl, Pb and Bi) were measured by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500, Agilent Technologies, Japan). Total-Hg (T-Hg) concentrations were measured by cold vapor atomic absorption spectrometry (CV-AAS) (HG-450, Hiranuma, Japan). For the analysis of organic Hg (O-Hg), sample was mixed with

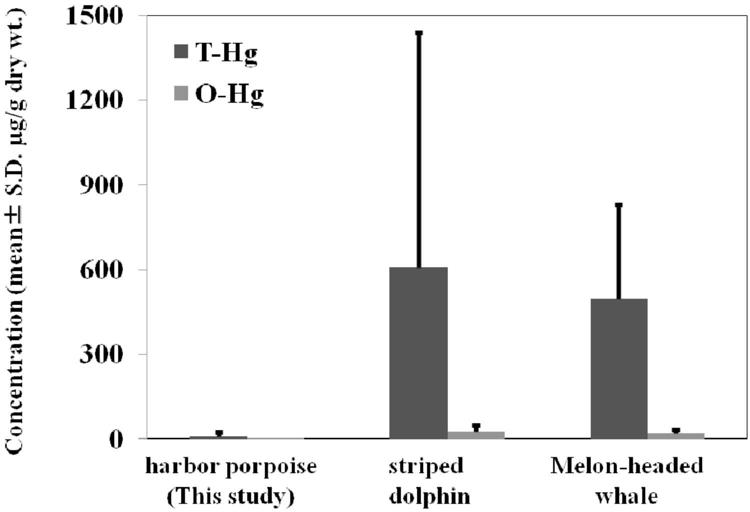

Fig. 3. Total Hg and O-Hg concentrations in liver of harbor porpoises and other cetaceans (literature data).

copper sulfate and acidic sodium bromide to release O-Hg, followed by extraction of O-Hg into toluene phase (40 minutes). Sodium thiosulfate solution was added to the toluene phase. A sub-sample of the sodium thiosulfate solution was digested with HNO_3. Then O-Hg concentrations were determined by CV-AAS. Standard reference materials, SRM1556b (National Institute of Standards & Technology, Gaithersburg, MD, USA) and DOLT-4 (National Research Council Canada, Ottawa, ON, Canada) were used to assess the accuracy of the analysis. Recoveries of all the elements ranged from 80% to 100% of the certified values.

RESULTS AND DISCUSSION

Trace elements distribution in liver and brain

Of all trace elements measured, Fe concentration was the highest in the liver, whereas Mg level was the highest in the brain (Fig. 2). Most of the trace elements measured in this study (i.e., Fe, Zn, Cu, Mn, T-Hg, Se, Cd, O-Hg, Sn, As, Ag, V, Pb, Bi, Ga and In) showed higher concentration in liver than brain ($p < 0.05$). Only two elements, Mg and Ca, were higher in brain than that of liver ($p < 0.001$) because these elements are major nerve transmitters (Frausto Da Silva and Williams, 1991).

Accumulation of Hg

Generally, marine mammals accumulate high levels of Hg in their bodies. T-Hg concentration of liver in the harbor porpoise was 8.58 ± 13.96 μg/g dry wt.,

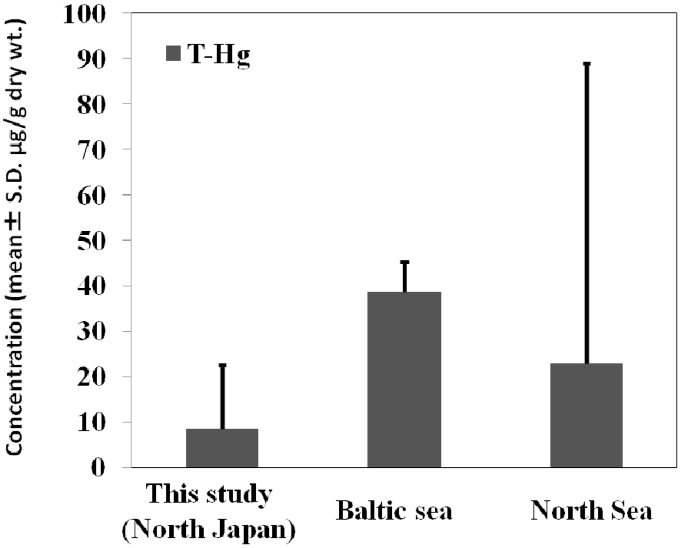

Fig. 4. Total Hg concentrations in liver of harbor porpoises from different areas (literature data).

whereas O-Hg concentration was 1.78 ± 1.58 μg/g dry wt. Comparing these concentrations to other dolphins, T-Hg and O-Hg in harbor porpoise were lower than striped dolphin (*Stenella coeruleoalba*) and melon-headed whale (*Peponocephala electra*) (Capelli *et al.*, 2000; Hirata *et al.*, 2010) (Fig. 3). One reason for this difference in Hg levels may be attributed to the effect of body size because body size of harbor porpoise (average 160 cm) is smaller than those of striped dolphin (average 250 cm) and melon-headed whale (average 270 cm). Additionally, melon-headed whale eats cephalopods mainly in relative to other two dolphins (Hirata *et al.*, 2010). Hg levels in fish are relatively higher than that in cephalopods (Agusa *et al.*, 2005). Therefore the difference of the feeding behavior might affect slightly higher Hg levels in striped dolphin than in melon-headed whale.

When looking at the regional difference, T-Hg levels of harbor porpoises in North Japan was lower than in harbor porpoises in Baltic Sea and North Sea (Siebert *et al.*, 1999; Das *et al.*, 2004) (Fig. 4). These differences suggested that pollution level of Hg in North Japan has been moderate so far compared to the Baltic Sea and North Sea.

It is well known that hepatic levels of Hg are positively correlated with Se, and formation of Hg-Se compounds is a possible detoxification pathway (Martoja and Berry, 1980; Nigro and Leonzio, 1996; Ikemoto *et al.*, 2004). In this study, a significant positive correlation was found between Hg and Se in the liver of harbor porpoises ($r = 0.93$, $p < 0.001$). However, the co-accumulation pattern of Se and Hg at *ca.* 1:1 molar ratio was apparent only in the harbor porpoises with

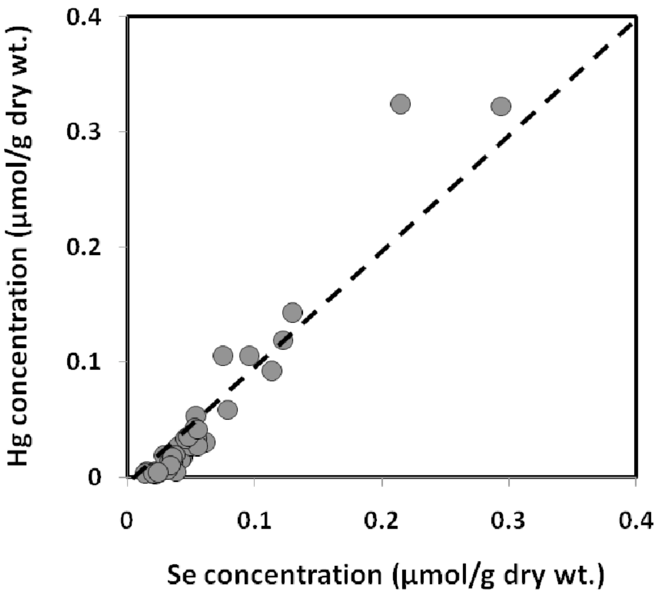

Fig. 5. Relationship between concentrations of Se and Hg in liver of harbor porpoises.

high hepatic T-Hg concentrations (>10 μg/g) (Fig. 5). Beyond 10 μg/g dry wt. (0.05 μmol/g dry wt.), the molar ratio becomes 1:1 suggesting that Hg-Se compounds are formed in the case of the accumulation of Hg > 10 μg/g dry wt. Consequently, it seems that accumulation of Hg in the liver of harbor porpoise is caused by the same mechanism as in other marine mammals, despite a generally lower hepatic Hg concentration in this species.

Temporal trends

Since some trace element levels (V, Co, Mo, Cd, T-Hg, O-Hg, Tl) in the liver of harbor porpoise showed positive correlation with body length ($p < 0.05$), multiple linear regression analysis was applied to evaluate temporal trends of trace element levels after correction for the effect of body size. The result of this analysis indicated that total Hg and O-Hg levels did not significantly increase with year during 1985 to 2010 (T-Hg: $p = 0.88$, O-Hg: $p = 0.61$) (Fig. 6). This result is consistent with other previous studies reporting that Hg concentration in coastal waters of Japan has been constant since 1986 (Japan Meteorological Agency, 2010). However, such trend is different from that in open sea as seen in melon-headed whales, which showed statistically significant increase of Hg levels since 1982 (Hirata *et al.*, 2010). Generally, anthropogenic contaminants are released to coastal region, then spread to open ocean. In the case of no

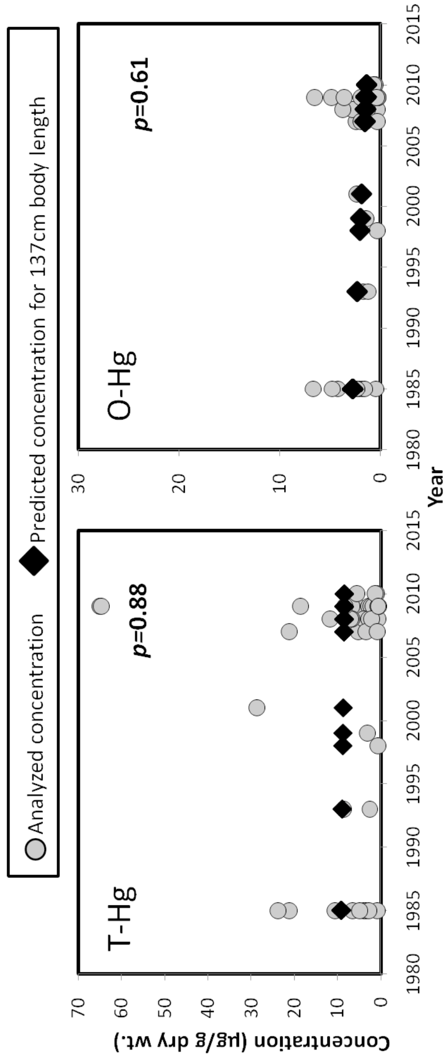

Fig. 6. Temporal trends of T-Hg and O-Hg in liver of harbor porpoises.

temporal increase of Hg in North Japan and significant increase in open sea, it can be suggested that the release of Hg from Japan is still limited and there should be some other important sources of Hg released to the ocean.

Of the other trace elements, significant increase with year were observed for hepatic Lithium, Al, Ca, Cr, Cu, Ga, As, Sr and Ba of harbor porpoise from 1985 to 2003. According to the statistical data by JOGMEC, demands and consumption of rare metals have increased in recent years (JOGMEC, 2010). Therefore, we must monitor the trends of these elements in coastal waters of North Japan. Melon-headed whale was employed to monitor the temporal increase of rare metals in open sea, and significant increase of hepatic V, Cr, Co, Cu, Zn, Sr, Mo, Cd, Cs and Bi were observed from 1982 to 2003 (Hirata *et al.*, 2010). These results indicated that many trace elements showed different temporal trends in harbor porpoise and melon-headed whale. Consequently, the contamination status of trace elements is variable for dolphin species according to the type of elements and the distance from their source.

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