

## Monitoring Trace Elements in Coastal Waters Using Sardine as a Bioindicator

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**Abstract**—Monitoring trace elements including heavy and rare metals in coastal areas is important to understand their behavior and anthropogenic impacts on different environments. We selected sardine species as a bioindicator for monitoring trace elements because of their wide distribution in the coastal waters. Comparison of trace element concentrations between Japanese sardine from Japan (JS) and Pacific sardine from the U.S. (PS) revealed that the elemental concentrations like Mg, Cr, Zn, As, Sr, In, Pb and Bi were generally higher in JS than in PS. Levels of Li, Rb, Ba and Tl in JS showed negative correlations with the fork length and positive correlations with  $\delta^{13}\text{C}$ . Patterns of correlations among trace element concentrations in sardines, such as the alkaline earth elements, period 4 elements, period 6 elements and toxic elements (As, Cd, Hg and Pb) were different between JS and PS. It was reported that the highest consumption of Sr for industries is in Japan. On the other hand, As in the environment is mainly associated with natural sources. Therefore, anthropogenic impacts and/or natural inputs affect trace element concentrations in sardines, in addition to their physicochemical properties such as radius and electron shell of these elements.

Keywords: trace elements, bioindicator, sardine, coastal areas, North Pacific

### INTRODUCTION

Coastal areas are adjacent to terrestrial and marine environments and are the mixing zones of many trace elements from various sources such as natural and anthropogenic inputs. Trace elements including heavy and rare metals originally exist in natural sources; nevertheless anthropogenic inputs to aquatic systems such as those from mining activities, fuel combustion and wastewater are increasing (Nriagu and Pacyna, 1988; Pacyna and Pacyna, 2001; Zhou *et al.*, 2008). Monitoring trace elements, therefore, is needed to understand their contamination status, emission sources and environmental behavior. Several studies reported that concentrations of trace elements in fish are useful to understand their environmental distribution (Birungi *et al.*, 2007; Kojadinovic *et al.*, 2007). In the present study, sardines were used as bioindicators for trace

element monitoring because of their wide global distribution in the coastal waters (Chavez *et al.*, 2003).

The objective of this study was to test whether sardines can be used as bioindicators to understand the environmental levels of trace elements. In addition, nitrogen and carbon stable isotopes were analyzed to elucidate the spatial ecological differences such as trophic levels and carbon sources to sardines. In this paper, we evaluated the distribution of trace elements in coastal waters of the North Pacific, especially Japanese and the U.S. coastal waters, from the viewpoints of environmental chemistry and biogeochemistry using two species of sardines, *Sardinops melanosticus* (hereinafter Japanese sardine (JS)) and *Sardinops sagax* (hereinafter Pacific sardine (PS)). JS is distributed in coastal east-Asia and PS is distributed in Indian and Pacific oceans.

## MATERIALS AND METHODS

Japanese sardines (JS) and Pacific sardines (PS) were purchased from fish markets. Japanese sardine ( $n = 10 \times 4$  locations) were collected from Iwate, Miyagi, Fukushima and Kochi prefectures, Japan. Pacific sardine ( $n = 10 \times 2$  locations) were collected from the Washington and California States, USA. Muscle tissue was used for chemical analysis.

### *Trace elements*

Samples were freeze-dried and then digested in a microwave oven using nitric acid in a polytetrafluoroethylene vial. Concentration of 24 trace elements (Li, Mg, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Se, Rb, Sr, Mo, Ag, Cd, In, Sn, Sb, Cs, Ba, Tl, Pb and Bi) were measured by inductively coupled plasma mass spectrometer (ICP-MS; Hewlett-Packard, HP-4500, Avondale, PA, USA). Total Hg concentration was determined by cold vapor atomic absorption spectrometer (CV-AAS; HG-3000, Sanso, Tsukuba, Japan coupled with Shimadzu AA 680 AAS, Kyoto, Japan). For total As measurement, samples were digested in Kjeldahl flask using acid mixture (nitric acid, sulfuric acid and perchloric acid - 1:1:2) and then determined by hydride generation atomic absorption spectrometer (HG-AAS; Model Shimadzu HVG-1, Kyoto, Japan).

### *Stable isotopes*

Samples were dried for 24 hours at 60°C and powdered and loaded into tin cups. Stable carbon and nitrogen isotopes were measured using isotope ratio mass spectrometer (IR-MS; SerCon ANCA-GSL, the United Kingdom).  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were expressed as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where X =  $^{13}\text{C}$  or  $^{15}\text{N}$  and R =  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ .

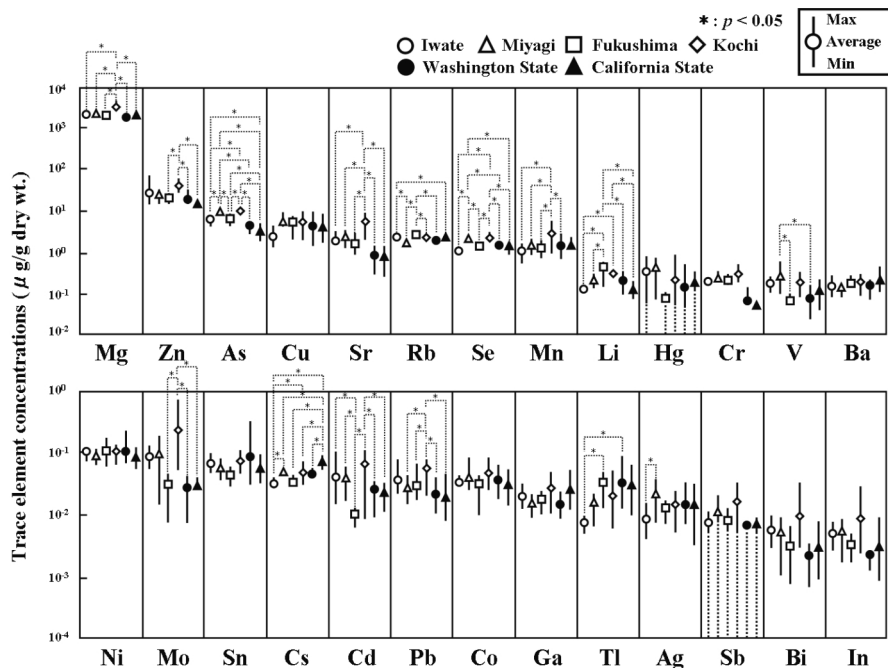


Fig. 1. Trace element concentrations in sardines collected from Japan and the U.S. (The dot line indicates concentrations in some sardine were below detection limits.)

### Statistical analyses

Spearman correlation coefficient was used to measure the strength of association between trace element concentrations, fork length and stable isotopes. Scheffe method was used for the detection of these spatial differences. A  $p$  value of less than 0.05 was considered to indicate statistical significance. Statistical analyses were executed by the programs SPSS version 12.0 for windows.

## RESULTS AND DISCUSSION

### Trace element concentrations in sardines

Concentrations of essential elements such as Mg, Cu, Zn and Se were generally high in all the sardines (Fig. 1). Mean concentrations of Mg were the highest of all the elements determined in both JS ( $2210 \pm 564 \mu\text{g g}^{-1}$  dry weight) and PS ( $1740 \pm 328 \mu\text{g g}^{-1}$  dry weight). Magnesium is a constituent of chlorophyll and the high Mg concentrations in sardines of the present study might have been derived from phytoplankton, a major constituent of their diet. Levels of As were also relatively high in all sardines, with mean values of  $7.5 \pm 2.3 \mu\text{g g}^{-1}$  dw in JS and  $3.9 \pm 1.2 \mu\text{g g}^{-1}$  dw in PS. It was reported that As concentrations are higher in marine fish than in freshwater fish (Schaeffer *et al.*, 2006). The levels of As

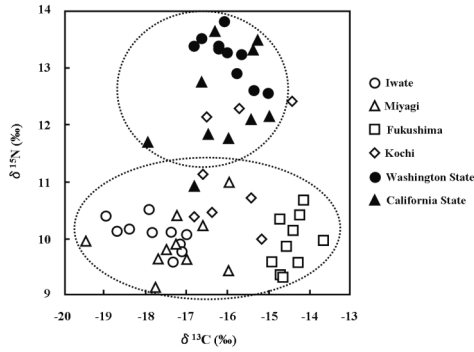


Fig. 2. C/N map of sardine collected from Japan and the U.S.

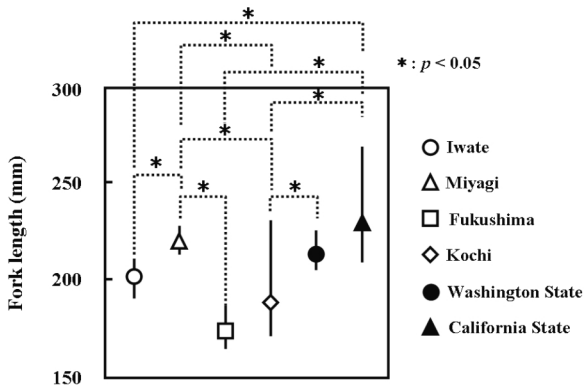


Fig. 3. Fork length in Japanese sardine and Pacific sardine.

observed in sardines of the present study were similar to that in other marine fish such as mackerel (*Scomber japonicus*;  $7.6 \pm 0.9 \mu\text{g g}^{-1}$  dw), black sea bream (*Acanthopagrus schlegelii*;  $10.8 \pm 1.1 \mu\text{g g}^{-1}$  dw) from Japan (Hirata *et al.*, 2006) and white croaker (*Pennahia argentata*;  $5.5 \pm 1.9 \mu\text{g g}^{-1}$  dw) from California (Maedor *et al.*, 2004). It has been reported that As in fish muscle is mainly found as arsenobetaine, which is non-toxic and the most stable form (Francesconi *et al.*, 1989; Shiomi *et al.*, 1996; Amlund *et al.*, 2006; Hirata *et al.*, 2006). However, inorganic As is toxic for all living organisms (Shiomi, 1994; Boyle *et al.*, 2008). To evaluate its possible effect on human health, chemical speciation of As in sardines is necessary. Mean concentrations of rare metals such as Ga ( $0.019 \pm 0.009 \mu\text{g g}^{-1}$  dw in JS and  $0.018 \pm 0.010 \mu\text{g g}^{-1}$  dw in PS), In ( $0.005 \pm 0.005 \mu\text{g g}^{-1}$  dw in JS and  $0.003 \pm 0.002 \mu\text{g g}^{-1}$  dw in PS), Sb ( $0.01 \pm 0.01 \mu\text{g g}^{-1}$  dw in JS and  $0.01 \pm 0.001 \mu\text{g g}^{-1}$  dw in PS), Tl ( $0.018 \pm 0.013 \mu\text{g g}^{-1}$  dw in JS and  $0.029 \pm 0.019 \mu\text{g g}^{-1}$  dw in PS) and Bi ( $0.006 \pm 0.006 \mu\text{g g}^{-1}$  dw in JS and  $0.003 \pm 0.002$

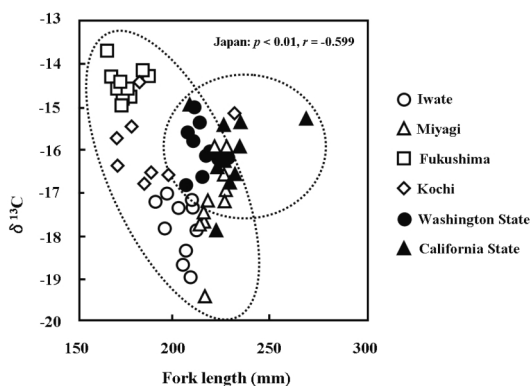


Fig. 4. Relationships between fork length and  $\delta^{13}\text{C}$  in Japanese sardine and Pacific sardine.

$\mu\text{g g}^{-1}$  dw in PS) were generally low in all the sardines (Fig. 1). Information on these rare metal concentrations in marine fish is limited. The levels of these rare metals were similar to that in the marine fish collected from Malaysia (Agusa *et al.*, 2005). These results may indicate less input to coastal areas of the present study or low bioavailability of these elements to sardines.

Figure 1 shows the comparison of the concentrations of trace elements in sardines collected from Japan and the U.S. Trace elements such as Mg, Cr, Zn, As, Sr, In, Pb and Bi were higher in sardines from Japan than from the U.S. On the other hand, elements like Li, Mn, Mo, Cd and Tl showed spatial variation within Japan. Such spatial difference in the trace element levels may relate to factors such as growth stage, variable species of diets and background levels between sampling locations or sardine species. In the later sections, we discussed relationships between trace element levels in sardines and these factors.

#### *Spatial differences of stable isotopes and growth stage in sardines*

We examined the spatial differences of stable isotopes,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in sardines (Fig. 2).  $\delta^{15}\text{N}$  values in sardines were higher in PS than in JS (Fig. 2). It was reported that off California has high production due to upwelling waters from the California Current as shown by the higher nitrate concentrations in seawater (Hurst and Bruland, 2008). Therefore, higher  $\delta^{15}\text{N}$  in fish might be from the upwelling waters, which is the major source of nitrate from deep water rather than through nitrogen fixation by phytoplankton.  $\delta^{13}\text{C}$  values and fork length in sardines varied, especially in Japan (Figs. 2 and 3). Negative correlation ( $p < 0.01$ ,  $r = -0.599$ ) between fork length and  $\delta^{13}\text{C}$  was found in sardines collected from Japan (Fig. 4). This relationship between fork length and  $\delta^{13}\text{C}$  in sardines suggest a possible change in their diet (Cartes *et al.*, 2007) with growth and/or location. To elucidate this hypothesis more clearly, we need to analyze stable isotope ratios of phytoplankton in future works.

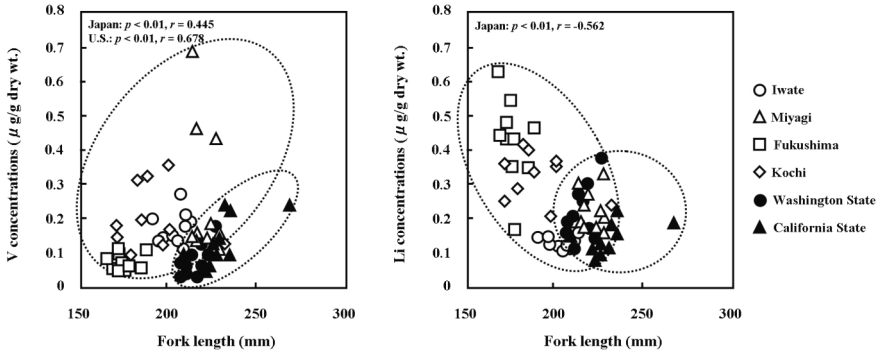


Fig. 5. Relationships between fork length and trace element concentrations in sardine.

### *Relationships between trace elements concentrations and some biological factors*

Positive correlations were observed between V and Cs concentrations and fork length in both JS and PS (Fig. 5) indicating linear bioconcentrations of V and Cs with growth in sardines. However, it was reported that other elements such as Se, Cd and Hg in other marine fish showed positive correlations with their respective size distributions (Branco *et al.*, 2007; Kojadinovic *et al.*, 2007). Se, Cd and Hg concentrations in the present study did not show positive correlations with fork length. Therefore, it is possible that the trace elements like Se, Cd and Hg accumulations in sardines was affected by their background levels such as natural sources and anthropogenic inputs. Negative correlations were observed between Li, Rb, Ba and Tl concentrations and fork length in JS (Fig. 5). Dilution of these elements with growth may be one of the reasons for this. In addition, positive correlations were observed between these element concentrations and  $\delta^{13}\text{C}$  in JS. Thus, it can be suggested that Li, Rb, Ba and Tl accumulations in sardine collected from Japan depended on their growth stages and/or spatially different carbon sources. From these results, it can be presumed that the elemental concentrations in sardines are not reflecting the growth stage and/or carbon sources but the background levels in the two sampling locations.

### *Correlations among trace element concentrations in sardines*

As for relationships among trace element concentrations in sardines, positive correlations were observed between alkaline earth elements (e.g. Sr–Ba,  $p < 0.01$ ), period 4 elements (e.g. Cu–As,  $p < 0.05$ ) and period 6 elements (e.g. Ba–Pb, Japanese sardine:  $p < 0.01$  Pacific sardine:  $p < 0.05$ ) (Fig. 6). When negative correlations were observed between period 5 elements (Rb–Cd,  $p < 0.05$ ) only in JS, positive correlations between alkali metals (Rb–Cs,  $p < 0.01$ ) and period 5 elements (e.g. Ag–In,  $p < 0.01$ ) were observed only in PS. These correlations among trace element concentrations in sardines suggest that the physicochemical properties such as ion radius and electron shell of trace elements may influence

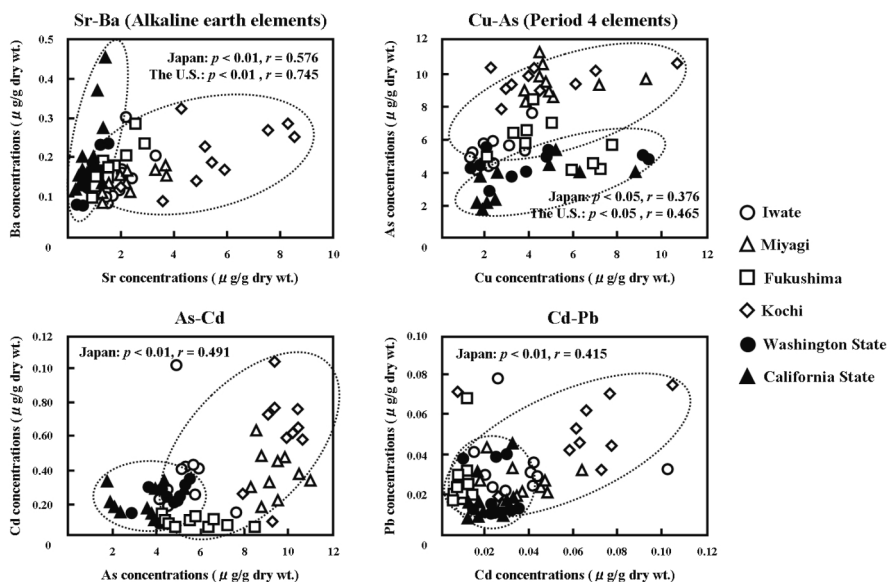


Fig. 6. Correlation of trace elements in Japanese sardine and Pacific sardine.

their accumulation patterns. However, site-specific patterns were also observed in JS and PS (Fig. 6). For example, Sr levels in JS were higher than in PS. It was estimated that the highest consumption of Sr for industries is in Japan (JOGMEC, 2005, 2008). Thus, it can be suggested that differences in the accumulation patterns of Sr–Ba between JS and PS is not only due to the similarity of biochemical process with Ca but also *via* anthropogenic impacts of Sr. As shown in Fig. 6, As levels in JS were higher than PS. Arsenic in the environment is mainly associated with natural sources such as sulfide minerals and volcanoes (Bissen and Frimmel, 2003). In addition, the smelting of Cu is one of the important anthropogenic As sources (Bissen and Frimmel, 2003). It was reported that Cu consumption for industries was higher in Asia than in North America (JOGMEC, 2006). Therefore, high ratio of As to Cu in JS may reflect the extent of Cu consumption in Asia in addition to the high background levels of As in the area derived through high volcanic activities. Same levels of Cu observed both in JS and PS plausibly indicates the homeostasis of Cu, an essential element.

Relationships among toxic elements like As, Cd, Hg and Pb also showed positive correlations in JS, and these elements were found relatively high in Kochi, Japan (Fig. 6). These correlations among toxic elements in JS indicate possibly same exposure sources and the concern over human health risk by dietary consumptions of these elements, especially in Japan. To understand the environmental effects and human effects by these toxic metals in detail, we have to measure the chemical forms of As and Hg in sardines because the toxicities of these two elements depend on the forms (organic or inorganic) in which they

occur in the environment. In future studies, more accurate risk assessment of the toxic chemical forms should be carried out by using PTWI (Provisional Tolerable Weekly Intake) value.

## CONCLUSIONS

Trace element concentrations in sardine showed spatial variation. Li, V, Rb, Cs, Ba and Tl concentrations were related to growth stages in sardines and lower  $\delta^{13}\text{C}$  carbon sources. Accumulation patterns of trace elements including toxic elements such as As and Hg were different among the sampling sites. This result may reflect the effects of natural behavior like As levels and anthropogenic impacts like Sr levels in addition to physicochemical properties of trace elements in fish. To make more accurate risk assessment analysis of the chemical speciation of As and Hg, calculation of the maximum safe weekly consumption is needed in the future.

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