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Biomagnification of Arsenic Species in the Deep-sea Ecosystem of the Sagami Bay, Japan

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Abstract—Accumulation of arsenic (As) species and its biomagnification profile in the deep-sea environment are poorly understood. This study deals measured As compounds and stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) in deep-sea organisms collected from the Sagami Bay, Japan to understand the trophic transfer of As species. Arsenobetaine (AB) was the predominant organic As species in deep-sea organisms and the other As species were at low levels. However, the levels of residual As, trimethylarsine oxide (TMAO) varied among phyla of deep-sea organisms. Significant biomagnification of lipid-soluble As was observed in pelagic organisms. In contrast, total As, AB and tetramethylarsonium ion (TETRA) were biodiluted in pelagic organisms. To our knowledge, this is the first study on biomagnification of As species in the deep-sea ecosystem.

Keywords: arsenic species, stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N), trophic transfer, deep-sea ecosystem

INTRODUCTION

It is well known that arsenic (As) concentrations in marine organisms are higher than those in terrestrial organisms (Lunde, 1977). Toxicity and environmental behavior of As depend on its chemical form (Cullen and Reimer, 1989). In the marine ecosystem, inorganic As (arsenate and arsenite) is the predominant As species in seawater and sediment (Francesconi and Edmonds, 1998). Marine algae and animals mainly accumulate arsenosugars (ASs) and arsenobetaine (AB), respectively (Edmonds and Francesconi, 1987; Francesconi and Edmonds, 1998). Other As species such as monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), tetramethylarsonium ion (TETRA) and arsenocholine (AC) have been detected in various marine organisms as minor

constituents (e.g. Francesconi and Edmonds, 1998; Agusa *et al.*, 2008). However, in contrast to a large amount of information on As accumulation in coastal or oceanic environments, As speciation in the deep-sea (>200 m of water depth) organisms have been poorly reported because of the difficulty in sample collection. Although As compounds might be transformed to other As species through the marine food web (Edmonds and Francesconi, 1987), there is no study which quantitatively assessed trophic transfer of As species.

To understand the food web structure, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios have been used (Wada *et al.*, 1991). $\delta^{13}C$ and $\delta^{15}N$ are indicators of carbon source and trophic levels (TLs), respectively of organisms. Hence, trophic transfer of As species through the food web can be evaluated using these stable isotopes.

Here, we analyzed As compounds and stable isotopes in zooplankton, crustaceans and fish collected from the deep-water of the Sagami Bay, Japan and discussed biomagnification profile of As species. The Sagami Bay has 1600 m of water depth and rich in diversity of organisms. Thus, we selected this bay as suitable area to investigate the trophic transfer of As species.

MATERIALS AND METHODS

Samples

Fish (28 species), crustaceans (12 species) and zooplankton (mostly copepods) were collected from the Sagami Bay in June 2004. Fish and crustaceans were classified into demersal and pelagic species. These samples were stored at –25°C in the Environmental Specimen Bank (*es*-BANK), Ehime University, Japan (Tanabe, 2006), until analysis.

Analyses of total As and As compounds

Whole specimens were homogenized and freeze-dried. Total As concentration was measured following the method of Agusa *et al.* (2008) with slight modifications. After digestion with acid mixture (HNO_3:H_2SO_4:$HClO_4$ = 1:1:2, v/v) by heating, total As concentration was measured by a hydride generation-atomic absorption spectrometer (HG-AAS).

Lipid was extracted from the sample using hexane, then the hexane extract was digested in a microwave oven with HNO_3. Lipid-soluble As concentration in the hexane extract was determined by an inductively coupled plasma mass spectrometer (ICP-MS). The water-soluble As was extracted using a mixture of methanol/water (9:1, v/v) from the sample after hexane extraction of lipid-soluble As. Eight As compounds including arsenite, arsenate, MMA, DMA, AB, TMAO, TETRA and AC were measured by a high-performance liquid chromatograph (HPLC) coupled with ICP-MS. The residual As was determined by HG-AAS after acid digestion. The concentrations of total As and As compounds were represented in units of μg As g^{-1} on a dry weight basis. The $\sum$As is defined as sum of water-soluble As, lipid-soluble As and residual As.

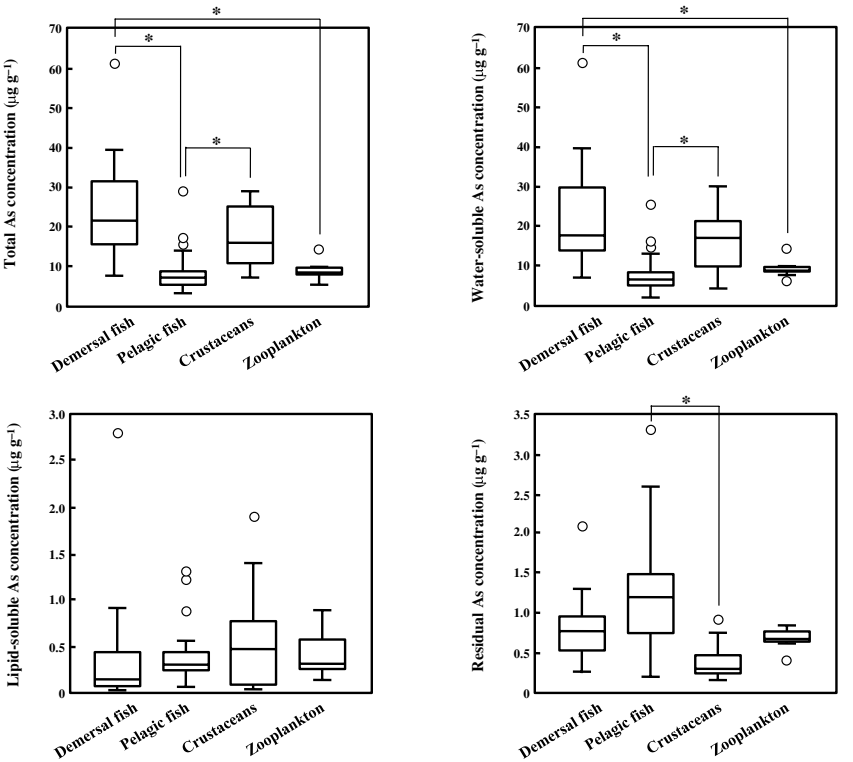

Fig. 1. Box plot of concentrations of total As, water and lipid-soluble As, and residual As in the deep-sea organisms. *: statistically significant difference at $p < 0.05$.

Analysis of stable isotope ratios

Muscle or whole body was dried at 60°C. Lipid was removed using chloroform and methanol (2:1, v/v) mixture. The isotope ratios were measured by an isotope ratio mass spectrometer (IR-MS). $\delta^{15}N$ and $\delta^{13}C$ in the samples were expressed as the deviation from standards in parts per thousand (‰) according to the following equation:

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

where X and R represent ^{13}C or ^{15}N and $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively.

RESULTS AND DISCUSSION

Accumulation of total As and As species

Total As concentration in demersal fish (mean ± S.D.; 23.6 ± 14.6 μg g^{-1})

was significantly ($p < 0.05$) higher than that in pelagic fish (8.50 ± 5.66 μg g^{-1}) and zooplankton (8.93 ± 2.70 μg g^{-1}) (Fig. 1). Additionally, total As concentration in crustaceans (17.0 ± 7.63 μg g^{-1}) was also significantly ($p < 0.05$) higher than that in pelagic fish (Fig. 1). Arsenic concentrations in feeds of demersal species such as crustaceans, gastropods, polychaete and cephalopods are generally found to be high (Lunde, 1977; Neff, 1997; Waring and Maher, 2005). Thus, the difference in concentration of As between demersal fish than pelagic organisms are likely the result of different feeding habits.

We analyzed As concentrations in three fractions, lipid-soluble As, water-soluble As and residual As. Residual As in pelagic fish (1.28 ± 0.70 μg g^{-1}) was significantly ($p < 0.05$) higher than in crustaceans (0.384 ± 0.228 μg g^{-1}) (Fig. 1). Proportion of residual As to $\sum$As was also high in pelagic fish ($20.7 \pm 11.7\%$). Arsenic in residual fraction in pelagic fish may be occurring upon binding to several proteins (Suzuki *et al.*, 2002; Hirano *et al.*, 2004). Concentration of As in lipid-soluble fraction did not vary much among deep-sea organisms (0.020–2.76 μg g^{-1}) (Fig. 1). Previous studies detected phosphatidyldimethylarsinic acid and DMA-containing sphingomyelin in squid liver (Ninh *et al.*, 2007) and DMA-containing fatty acids in cod liver oil (Rumpler *et al.*, 2008). These As compounds may be existing as lipid-soluble As in deep-sea organisms.

As observed in the case of total As, water-soluble As concentration was the highest in demersal fish (Fig. 1). Among the eight As species that can exist in water-soluble fraction, AB was the predominant As compound in many deep-sea organisms. Concentration of AB in demersal fish (22.2 ± 15.2 μg g^{-1}) was significantly ($p < 0.05$) higher than those in pelagic fish (5.21 ± 4.90 μg g^{-1}) and zooplankton (4.44 ± 2.36 μg g^{-1}). Additionally, AB concentration in crustaceans (13.6 ± 8.2 μg g^{-1}) was significantly ($p < 0.05$) higher than that in pelagic fish. Proportion of AB in water-soluble As was particularly high in demersal fish ($96.8 \pm 2.95\%$) and crustaceans ($88.3 \pm 14.7\%$). These results indicate that AB is the dominant As species in many deep-sea organisms as well as general marine crustaceans and fish (Edmonds and Francesconi, 1987; Francesconi and Edmonds, 1998).

The concentrations of other As species in the deep-sea organisms were generally low and this is similar to previous studies (Neff, 1997; Francesconi and Edmonds, 1998), suggesting that accumulation profiles of water-soluble As species in deep-sea organisms are the same as general marine animals. Species specific accumulation of TMAO was observed in a shrimp, *Aristeomorpha faliacea* and the level (5.65 μg g^{-1}) was higher than the concentration of AB (4.70 μg g^{-1}).

Trophic transfer of total As and As species

δ^{15}N typically increases about 3.4 times with every increase in TL within a food chain. To assess the trophic transfer of total As and As species, TLs of deep-sea organisms in each water depth (divided into surface-layer (0–200 m), meso-layer (200–700 m) and deep-layer (>700 m) based on the sampling depth and

ecological behavior of the organisms) were calculated by the following equation (Jæger *et al*., 2009).

$$TLs = (\delta^{15}N_{consumer} - \delta^{15}N_{zooplankton})/3.4 + 2$$

where, $\delta^{15}N$ value of zooplankton in each water depth is assumed as $\delta^{15}N_{zooplankton}$. Relationships between TLs and arsenate, arsenite and MMA were not discussed because these compounds were detected in less than 50% of the deep-sea organisms.

Among pelagic organisms, significant negative correlations were found for individual As species with TLs: total As: $p < 0.001$, $r = -0.521$, AB: $p < 0.001$, $r = -0.522$, TETRA: $p < 0.001$, $r = -0.570$, $\sum$As: $p < 0.01$, $r = -0.484$. These results indicate biodilution of total As and water-soluble As species like AB and TETRA in the pelagic species. TETRA also showed negative correlation with TLs including demersal fish ($p < 0.01$, $r = -0.380$). Other As species like DMA, AC and residual As were not significantly correlated with TLs in pelagic organisms. Hence, it can be presumed that these As species are not biomagnified in pelagic organisms. Generally, As species, except AB and AC, are not accumulated in fishes (Francesconi *et al*., 1989; Shiomi *et al*., 1996). In contrast, lipid-soluble As ($p < 0.01$, $r = 0.399$) was biomagnified in pelagic organisms. Biomagnification of lipid-soluble As may be explained by its high lipophilicity.

CONCLUSION

This study revealed the trophic transfer of As in deep-sea organisms, for the first time. Lipid-soluble As tended to be biomagnified in pelagic food web, whereas total As, AB and TETRA were biodiluted. Additionally, other As species such as DMA and AC showed neither biomagnification nor biodilution. Our data provide useful information for comprehensive understanding of trophic transfer of As in the marine ecosystem. Further study is needed to understand the factors controlling observed magnification/dilution trends, such as chemical characteristic and bioavailability of each As species.

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