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Uptake and Elimination of Trace Metals in Shells of Abalones *Haliotis* spp.

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Recent progress in biomonitoring techniques using molluscan shell has contributed to the evaluation of trace metal levels in the coastal environment. It has been clearly demonstrated that trace metal concentrations in molluscan shells parallel the trace metal concentrations in the ambient water (Gordon et al. 1970; Al-Thaqafi and White 1991; Hirao et al. 1994; Pitts et al. 1994; Hockett et al. 1997). Direct assessment of water quality is difficult for many reasons. Trace metal concentrations in seawater are often so low that chemical preconcentration is necessary for analysis and clean sample collection and handling techniques must be employed. Therefore, molluscan shell may have a great potential as a convenient tool for assessing the condition of the coastal environment.

Molluscan shell is believed to be grown in annual increments (Brouseau 1978; MacDonald and Thomas 1980; Tanaka and Tanaka 1980). Analysis of one annual growth increment should represent a time-integrated average of conditions experienced by the organism during its active growing season. Furthermore, it may also reveal the influence of the growth stage of the organism on trace metal concentrations in the shell. In order to examine the interaction between the organism and variations in ambient water quality, and to develop the organisms as a tool for biomonitoring, it is important to understand the variations in concentration and distribution of trace metals in the animals shell in relation to its growth stage, and to determine the behaviour of both essential and non-essential trace metals in the organism.

In comparison with molluscan shell as a biomonitoring indicator for estimation of the coastal environment, little is known about the influence of internal factors upon the concentration of trace elements in the shell, including aspects such as age, body size, sex, reproduction, and diet. Most of the previous studies considering those factors have estimated mainly the soft tissues (Boyden 1974; Lobel and Wright 1982; Fischer 1983; Swaileh and Adelung 1994). However, such studies concerning the internal factors in shells should also be a prerequisite for reliable biomonitoring.

The abalone *Haliotis* is a common marine gastropod distributed worldwide along the coastal regions (Ino 1952). It is relatively long-lived relative to other coastal

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gastropods and its annual growth is represented by a distinct ring structure (Ino 1952; Tanaka and Tanaka 1980). Accordingly, it might act as an effective coastal biomonitoring indicator (Stewart and Schulz- Baldes 1976; Hirao et al. 1994).

The main objectives of the present study were to reveal the relationship between trace metal (cadmium, copper, lead, manganese and zinc) and major metal (calcium, magnesium and strontium) concentrations in shells and shell length and age in the abalone collected from Japanese coastal regions. We also determined the above-mentioned metals concentrations in shells of the same growth stage (age) collected from various coastal regions of Japan. The results formed a basis for the use of molluscan shells as a biomonitoring indicator.

MATERIALS AND METHODS

Haliotis discus of different sizes (3.9 to 15.6 cm) was collected at the coast of Amatsu Kominato, Chiba Prefecture, Japan, on 24 August 1994 (Fig. 1). *Haliotis discus hannai* of different sizes (7.3 to 12.4 cm) were collected at the coast of Onagawa, Miyagi Prefecture, Japan, between September and November 1994. *Haliotis discus* and *Haliotis discus hannai* in a similar developmental stage (5 years old) were collected at the coasts of Noto, Ishikawa Prefecture, Mugi, Tokushima Prefecture and Nomozaki, Nagasaki Prefecture, Japan, between September and November 1994, and Okujiri, Hokkaido, Japan, in June 1995, respectively. The abalone sampled was frozen until dissection. Total lengths were measured to the nearest 0.1 mm, and age was determined according to Tanaka and Tanaka (1980). The whole soft part was dissected from each shell using a stainless steel knife in a clean room. A total of 47 shells were used for the present study. The shells were cleaned of extraneous organisms and materials, using a nylon brush and stainless steel knife, and were then warmed at 60 °C for 2 hours with a 40 % solution of 30 v/v hydrogen peroxide. The cleaned shells were dried to a constant weight at 110 °C, and then the outermost growth rings were cut away with a stainless steel saw and finely crushed in an alumina mortar.

The samples (0.5 - 2.0 g) were dissolved carefully in 20 ml of concentrated nitric acid, and then each solution was evaporated to dryness. The dry residue was converted into chlorides by evaporation in 15 ml of concentrated hydrochloric acid. Detailed procedures and criterion of trace (Cd, Cu, Pb, Mn and Zn) and major (Ca, Mg and Sr) metals analyses followed previous study (Arai et al. 2002). A standard reference material, argillaceous limestone (NIST SRM 1c), and internal reference material consisting of Cd, Cu, Mn, Pb, and Zn-spiked calcium carbonate, were included in the analysis as quality control samples. The recovery range of metals in those materials was 94 - 103 %.

RESULTS AND DISCUSSION

Close negative linear relationships were apparent between shell length and Cd and Pb concentrations in shells of *Haliotis discus hannai* collected from Onagawa and *H. discus* collected from Amatsu Kominato (Fisher's Z-transformation, p <

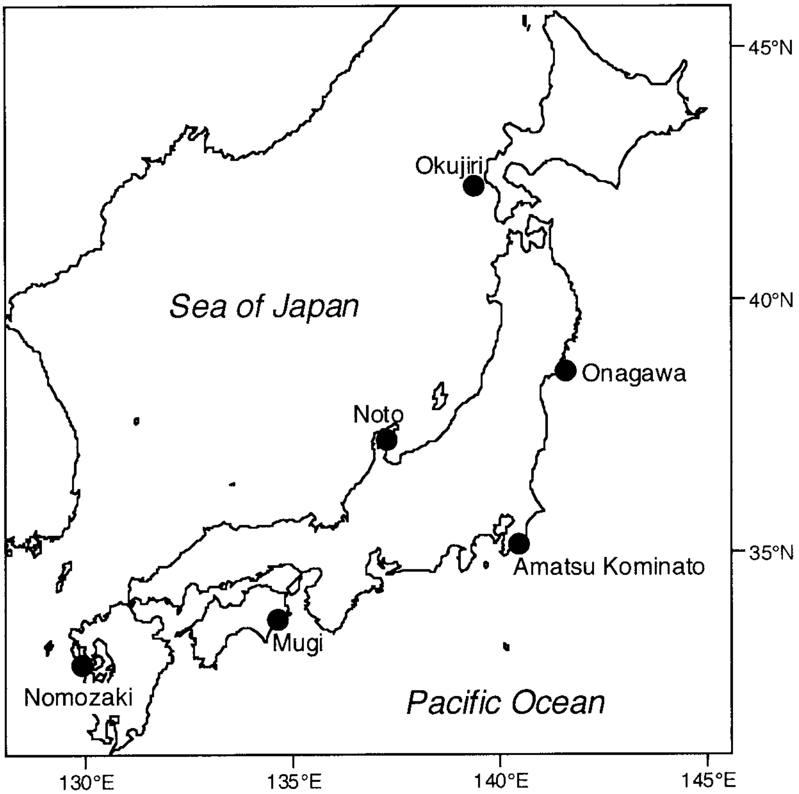

Figure 1. Map showing sampling locations (●).

0.0001) (Fig. 2). Significant differences in regression slopes in those metals occurred between the 2 abalone groups (ANCOVA, $p < 0.0001$). Cd and Pb concentrations in *H. discus hannai* shells from Onagawa were 0.019 μg/g and 0.076 μg/g, respectively, in 7.7 cm size (3 years old), and decreased gradually with increases in shell length to 0.007 μg/g and 0.017 μg/g, respectively, in 12.3 cm size animals (6 years old). Cd and Pb concentrations in *H. discus* shells from Amatsu Kominato gradually decreased also, from 0.021μg/g and 0.11 μg/g, respectively, in 3.9 cm size animals (0 years old), to 0.011 μg/g and 0.051 μg/g, respectively, in 15.6 cm size animals (8 years old). Concentrations of Zn, Mn and Cu in the shells of *H. discus* from Amatsu Kominato increased significantly with increases in shell length (Fisher's Z-transformation, $p < 0.005$), while no significant difference was apparent in metal concentrations of *H. discus hannai* shell from Onagawa with increases in shell length (Fisher's Z-transformation, $p > 0.1$). Zn, Mn, and Cu concentrations in *H. discus* shell from Amatsu Kominato were 0.86 μg/g, 1.04 μg/g, and 0.10 μg/g, respectively, in 3.9 cm size animals (0 years old) and increased gradually with increases in shell length, to 1.95 μg/g, 3.22 μg/g and 0.24 μg/g in 15.6 cm size animals (8 years in age). No close linear relationships were apparent between shell length and Ca, Sr, and Mg

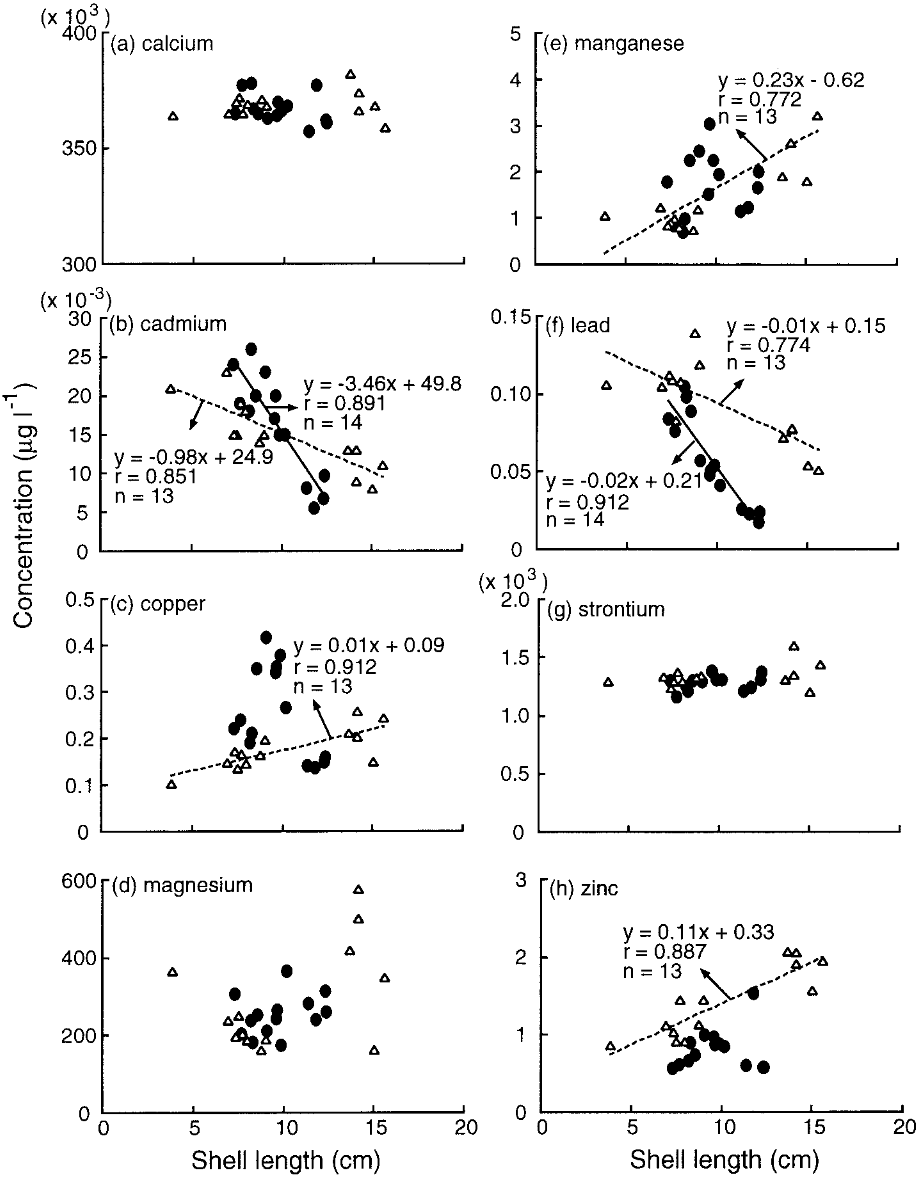

Figure 2. Relationships between concentrations of calcium (a), cadmium (b), copper (c),magnesium (d), manganese (e), lead (f), strontium (g), and zinc (h) in shell and shell length of abalone. ● : *H. discus hannai* (Onagawa), ▵ : *H. discus* (Amatsu Kominato).

concentrations in shells of those 2 abalone groups (Fisher's Z-transformation, $p > 0.1$) (Fig. 2).

The relationships between trace metals (Cd, Cu, Mn, Pb, and Zn) concentrations in the abalone shell and the shell length clearly showed that those concentrations varied accompanying growth. The larger individuals contained less Cd and Pb than did those of smaller animals, while, Cu, Mn, and Zn, the former contained more than the latter. It has been also reported that lead concentration decreased gradually with increases in shell length (age) in *Haliotis discus* and *H. sieboldii* (Hirao et al. 1994). Ca, the most major elemental component in the molluscan shell, showed a constant concentration independent of individual growth, similar to the changes in the concentrations of Mg and Sr accompanying the growth. Abalone, which inhabit the same locations within coastal areas, might experience similar ambient environmental conditions, e. g., water temperature, salinity, water chemistry, photo-periodicity, and diet, in spite of the difference in the growth stage. The composition of the most current annual-growth shell, which was influenced by the various metal concentrations in seawater, might be constant regardless of the various growth stages of abalone in each area. Accordingly, these observations suggest that the uptake or the elimination of trace metal concentrations (Cu, Mn, and Zn, and Cd and Pb, respectively) accompany the growth of the abalone shells in each coastal area.

Abalone may have a metabolic strategy, which discriminates between essential metals and non-essential metals, and it may be expressed to a greater degree in larger specimens than the smaller one in terms of growth. In general, Cu, Mn, and Zn are believed to be the essential metals that can be regulated by organisms, while, Cd and Pb are believed to be the non-essential metals (Clark 1992; Swaileh and Adelung 1994). The urgency of rapid shell development during the rapid growth stage of the abalones early life requires that Ca absorption in the stomach or intestines minimize discrimination between the essential and the non-essential metals, resulting in the "overlooking" of those metals that accompany Ca. As the abalone grows larger and the rate of shell formation decreases, discrimination against those metals in favor of Ca will become increasingly effective, reaching a plateau condition with the slower absorption rates. Under those conditions, Cd and Pb concentrations in newly grown shell might decrease gradually as the organism moves into adulthood, while Cu, Mn, and Zn concentrations in the shell might increase gradually.

Different species of abalone, *Haliotis discus hannai* from Onagawa and *H. discus* from Amatsu Kominato, showed clear differences in the regression slopes of the relationship between trace metal concentrations and shell length. The fundamental physiological differences that distinguish the separate species may control the differences observed for those metals uptake and elimination as the animal grows. The same phenomenon was found to occur between *Haliotis discuss* and *H. sieboldii* shells in regard to lead (Hirao et al. 1994). On the other hand, differences in the temperatures of their habitats might play an important role in the uptake and elimination of the trace metals into their shells in each species. *H.*

discus hannai generally inhabits water less than 12 °C, and *H. discus* lives in water warmer than 12 °C (Ino 1952). It has been reported that differences in environmental temperature influenced the rate and amount of uptake and elimination of trace metals in molluscan tissues (Ikuta 1972). The same phenomenon might also affect the uptake and elimination of trace metals in the molluscan shell. Nevertheless, no study has been performed regarding the uptake and elimination of trace metals in the molluscan shell under environments of different temperatures. In order to explore these phenomena, cultivation experiments giving the different temperature environment need to be performed. These results all lead to the conclusion that precise biomonitoring in order to estimate trace metal levels in coastal environments using molluscan shell requires the standardization of the growth stages of each species.

Trace metal concentrations in abalone shell of the same growth stage (5 years old) collected from six sites (Okujiri, Onagawa, Amatsu Kominato, Noto, Mugi and Nomozaki) varied markedly among those sites (Fig. 3). Cd concentrations, averaging 4×10^{-3} to 19×10^{-3} µg/g among the sites, and the range of Cd concentrations was much greater in the shell from Okujiri than in those from the other sites. A significant difference was seen between Okujiri and Mugi (ANOVA, $p < 0.05$). Cu concentrations, averaged from 0.15 to 0.28 µg/g among the sites, with no significant difference being observed (ANOVA, $p > 0.1$). Mn concentrations, averaging 0.82 to 2.08 µg/g among the sites, and the range of Mn concentrations was much greater in shells from Mugi than in those from other sites. Significant differences were seen between Noto and Mugi (ANOVA, $p < 0.05$), and between Mugi and Nomozaki (ANOVA, $p < 0.05$). Pb concentrations, averaging 0.017 to 0.119 µg/g among those sites, and the range of Pb concentrations was much greater in shells from Amatsu Kominato than in those from other sites. Significant differences were seen between Onagawa and Amatsu Kominato (ANOVA, $p < 0.05$), and between Amatsu Kominato and Mugi (ANOVA, $p < 0.01$). Zn concentrations, averaging 0.58 to 2.47 µg/g among those sites, as well as the range of Zn concentrations was much greater in shells from Mugi than in those from other sites. Significant differences existed between Okujiri and Mugi (ANOVA, $p < 0.005$), between Onagawa and Mugi (ANOVA, $p < 0.005$), between Noto and Mugi (ANOVA, $p < 0.05$), and between Mugi and Nomozaki (ANOVA, $p < 0.005$). Ca and Mg concentrations averaged 359×10^3 to 369×10^3µg/g and 190 to 425 µg/g, respectively, among those sites, while no significant differences were seen among those sites regarding those metals (ANOVA, $p > 0.1$). Sr was the next most abundant metal, with concentrations averaging $1.50 \times 10^3$to 1.23×10^3µg/g among those sites, while no significant difference existed among those sites regarding that metal (ANOVA, $p > 0.1$).

The differences of Pb concentrations recorded in the abalone shells of the same species from different locations were found to be the result of the differences in lead level in their environments (Hirao et al. 1994). Specimens from Okujiri and Onagawa were *Haliotis discus hannai*, and those of Amatsu Kominato, Noto, Mugi, and Nomozaki were *Haliotis discus*. These abalones have similar diets and inhabit similar temperature zones in the coastal environment (Ino 1952).

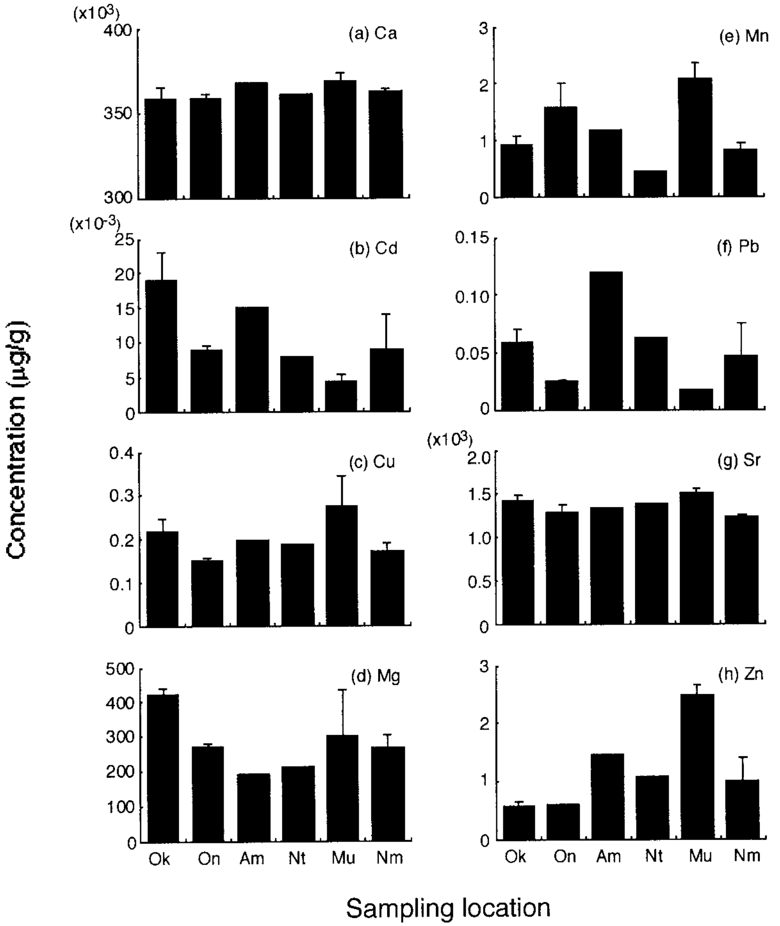

Figure 3. Concentrations of calcium (a), cadmium (b), copper (c), magnesium (d), manganese (e), lead (f), strontium (g), and zinc (h) in abalone shell in the same growth stage (5 years old) collected from the coasts of Okujiri (Ok) in Hokkaido, Onagawa (On) in Miyagi Prefecture, Amatsu-Kominato (Am) in Chiba Prefecture, Noto (Nt) in Ishikawa Prefecture, Mugi (Mu) in Tokushima Prefecture, and Nomozaki (Nm) in Nagasaki Prefecture, Japan.

Therefore, differences in trace metal concentrations were seen in the abalone shells in the same growth stage which were presumed to reflect the differences in environmental trace metals concentrations among the sites in which each species was found.

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