COMPARISON OF METAL UPTAKE RATE AND ABSORPTION EFFICIENCY IN MARINE BIVALVES

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(Received 5 July 2000; Accepted 7 November 2000)

Abstract—Recent studies have quantified extensively metal assimilation efficiency from ingested food sources in aquatic invertebrates. Metal absorption efficiency (α) from the dissolved phase is analogous to metal assimilation efficiency, but it remains poorly defined and quantified. In this study, the α of four trace metals [Cd, Cr(VI), Se(IV), and Zn] was determined in three species of marine bivalves (green mussel [Perna viridis], black mussel [Septifer virgatus], and clam [Ruditapes philippinarum]). Individual bivalves were first measured for their clearance rates, followed by measurements of the metal influx rate, after which the metal α and the uptake rate constant (Ku) were then computed. Among the four metals considered, the highest Ku and α were found for Zn, followed by Cd > Cr(VI) > Se(IV). The Ku values were comparable between the two mussels but were 1.8- to 3.3-fold lower in the clams. Interspecific difference in metal Ku was strongly related to, but intraspecific difference in Ku was not affected by, the bivalve’s clearance rate. Interspecific difference in metal α was smaller than the metal Ku and was independent of the clearance rate, whereas the intraspecific difference in metal α correlated with the individual variations of the clearance rate. Within each bivalve species, a significant negative correlation was found between the metal α and the clearance rate, implying that an individual pumping a greater amount of water was coupled to a lower α. Significant correlation between the α of four metals was also documented in all three species of bivalves. Thus, metal bioavailability from the aqueous phase was directly related to the physiological conditions of the animals. Both the aqueous chemistry and the physiology of the animals can be important in affecting metal bioavailability from the dissolved phase.

Keywords—Metals  Bivalves  Absorption efficiency  Uptake rate

INTRODUCTION

Metal uptake by marine bivalves has been extensively investigated since the 1970s. Most studies have been concerned with the influences of environmental (e.g., temperature, salinity, oxygen concentration, seasons), biological (e.g., body size, sex, biochemical binding), and chemical conditions (e.g., metal concentrations, metal speciation, metal–metal interaction) on metal accumulation [1–7]. These extensive interests largely resulted from development of the concept of the Mussel Watch Program in the 1970s, which employed mussels or other sedentary bivalves to monitor environmental pollution in coastal and estuarine waters [8–10]. An underlying assumption of the biomonitoring program is that metal concentrations in bivalves reflect ambient metal bioavailability. Direct measurements of metal concentrations in biomonitor are also biologically relevant and feasible for toxicological interpretation. Although the biological and physicochemical factors potentially influencing metal uptake in marine bivalves are relatively well known, knowledge regarding the physiological controls of metal absorption from the dissolved phase is very limited. Processes controlling the inter- and intraspecific differences in metal uptake also remain less well defined.

During the past few years, interest in the dietary exposure of marine bivalves to metals has increased [11–13]. A bioenergetic-based kinetic bioaccumulation model has demonstrated that dietary exposure can represent a major pathway by which metals are accumulated in marine bivalves. Recent progress in delineating exposure pathways has largely resulted from realistic measurement of metal assimilation efficiency (AE) from ingested food sources, as well as from the identification of important physiological and geochemical processes controlling metal uptake in these animals. Assimilation efficiency is considered to be the first-order physiological parameter quantifying metal bioavailability from ingested food sources and can now be routinely measured in suspension-feeding bivalves under diverse biological and environmental conditions. When combined with the metal concentration of the ingested food particles and the ingestion rate of the animals, the metal AE can be used to quantify the influx rate of metals into the animals, which can then be used to predict metal concentration in the animals under steady-state conditions with the metal efflux rate [12,14].

Although the bioenergetic-based kinetic model provides an important tool for understanding metal bioaccumulation and bioavailability in aquatic animals [14,15], a few of the parameters defined in the model are not well understood. In contrast to metal AE from ingested food, metal absorption efficiency from the dissolved phase (α) is very poorly defined. In previous modeling studies [12], metal influx rate from the dissolved phase was calculated from the metal uptake rate constant (Ku), which was estimated based on laboratory measurement of the relationship between the metal influx rate and the metal concentration in the dissolved phase. Furthermore, metal Ku is a product of α and the clearance rate of animals. Metal α, defined as the fraction of metal absorbed by the bivalves from a volume of water they pump, is analogous to AE and can be considered as a first-order physiological process quantifying metal bioavailability from the dissolved phase. Quantification of metal α requires simultaneous measurement of the metal influx rate and the pumping rate of the animals. Because no direct method

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is available to quantify $\alpha$, the variability of, and the factors affecting, this parameter are largely unknown.

Many field studies have indicated considerable intra- and interspecific differences in metal concentrations of marine bivalves [10]. Notable variation of the metal uptake rate and bivalve clearance rate has also been documented by many laboratory studies [16,17]. Variations in the clearance rate among different individual bivalves provide an excellent opportunity for testing the hypothesis regarding the dependence of metal $K_u$ and $\alpha$ on the clearance rate. Whereas previous studies have estimated the metal $\alpha$ in a few marine and freshwater bivalves [12,17,18], the physiological controls of this parameter remain essentially unknown. In this study, metal $\alpha$ and $K_u$ in three species of marine bivalves have been quantified and compared. The influences of the bivalve’s clearance rate on their inter- and intraspecific variabilities were also examined.

**MATERIALS AND METHODS**

**Bivalves and metals**

Three species of marine bivalves (two mussels and one clam) common in Hong Kong coastal waters were examined. Among the three species of bivalves, the green mussel (*Perna viridis*) was collected from Lantau Island, Hong Kong; the black mussel (*Septifer virgatus*) from Clear Water Bay, Hong Kong; and the clam (*Ruditapes philippinarum*) from Tolo Harbour, Hong Kong. The shell length of the bivalves ranged from 3.5 to 4.0 cm. The dry tissue weights were 0.22 to 0.47 g for *P. viridis*, 0.23 to 0.44 g for *S. virgatus*, and 0.17 to 0.40 g for *R. philippinarum*. After the bivalves had been brought back to the laboratory, they were cleaned of epibionts and maintained at 22°C and 30 ppt seawater for 10 d before the experimental measurements. All experiments were conducted at this temperature and salinity. During the acclimation period, the bivalves were fed continuously with the diatom *Thalassiosira pseudonana*.

Four metals were examined in this study: Cd, Cr(VI), Se(IV), and Zn. The choice of these metals largely resulted from the availability of radiotracers as well as the concern for their environmental impact in Hong Kong coastal waters [19]. Radiotracers $^{106}$Cd, $^{51}$Cr(VI), $^{75}$Se(IV), and $^{65}$Zn were used to trace the uptake of their respective stable metals. The radioisotopes were obtained from NEN Research Products (Boston, MA, USA) [$^{106}$Cd, $^{51}$Cr(VI), and $^{65}$Zn] or Livermore National Laboratory (Los Alamos, NM, USA) [$^{75}$Se(IV)]. For Cr and Se, we quantified the uptake of anionic chromate [Cr(VI)] and selenite [Se(IV)]. Results of previous studies have indicated that their uptake rates in marine bivalves are higher than the uptake of Cr(III) and selenate, respectively, from the dissolved phases [11,20].

**Clearance rate of bivalves**

According to a simple kinetic model, metal influx rate can be calculated [12,14,21] as

$$I = K_u \times C_w = \alpha \times CR \times C_w$$  \hspace{1cm} (1)

where $I$ is the metal influx rate (ng·g$^{-1}$·h$^{-1}$), $K_u$ is the uptake rate constant (L·g$^{-1}$·h$^{-1}$), $C_w$ is the metal concentration in the dissolved phase (ng·L$^{-1}$), $\alpha$ is the metal absorption efficiency, and $CR$ is the bivalve’s clearance rate (L·g$^{-1}$·h$^{-1}$). Thus, $K_u$ and $\alpha$ can be quantified, respectively, by the following equations:

$$K_u = I/C_w$$  \hspace{1cm} (2)

$$\alpha = I/(CR \times C_w)$$  \hspace{1cm} (3)

The clearance rate of each individual bivalve was measured before the influx rate measurements and was quantified using the indirect method as described by Widdows [16] and by Wang and Dei [17]. Briefly, individual bivalves were placed in 2 L of filtered seawater (<0.2 μm). The diatom *T. pseudonana* at the exponential growing phase was filtered to remove the algal metabolites and excess nutrients and then resuspended in the feeding beakers at a cell density of 10,000 cells/ml$^{-1}$. The cell suspension was homogenized by a magnetic stirrer. At time intervals (every 20 min for mussels and every 30 min for clams), a 15-ml water sample was taken for cell density measurement by a Coulter Counter (Beckman Coulter, Fullerton, CA, USA). The clearance rate ($CR$) was calculated as

$$CR = (\ln C_t - \ln C_o) \times V/t$$  \hspace{1cm} (4)

where $C_o$ and $C_t$ are the cell density at time zero and time $t$, respectively; $t$ is the duration of feeding; and $V$ is the volume of water. The average clearance rate was calculated based on the two consecutive time-point measurements (e.g., 40-min measurement for mussels and 60-min measurement for clams). Eight individuals were measured concurrently. A total of 18 individual green mussels and 20 individual black mussels and clams were measured for their clearance rates.

**Metal influx rate and absorption efficiency**

After the measurement of individual clearance rate, the animals were immediately placed in 400 ml of filtered (0.2 μm) seawater containing radiotracers and stable metals (18 nM Cd, 38 nM Cr(VI), 25 nM Se(IV), and 77 nM Zn). These metal concentrations were 5- to 20-fold greater than the typical metal concentrations in estuarine waters [22,23]. The metals were added as a mixture, because results of previous studies indicated no interaction of metal uptake at these concentrations [17,24]. Radioisotope additions were 1.85 kBq L$^{-1}$ (0.22 nM) for $^{106}$Cd, 14.8 kBq L$^{-1}$ (0.03 nM) for $^{51}$Cr, 7.4 kBq L$^{-1}$ (0.19 nM) for $^{75}$Se, and 3.7 kBq L$^{-1}$ (0.44 nM) for $^{65}$Zn. Radioisotopes and stable metals were equilibrated overnight before the uptake experiments. Immediately before the uptake experiments, an aliquot of water was taken for measurement of radioactivity.

Mussels and clams were exposed to stable plus radioactively labeled metals for 1 and 2 h, respectively. Results of previous kinetic studies have demonstrated that metal uptake in these bivalves exhibits a linear pattern over time of exposure, suggesting that metals are probably internalized and that the initial surface sorption does not contribute to the overall metal accumulation in the animals [17,25]. The water was gently stirred at time intervals during the exposure period to minimize the concentration gradient because of metal uptake. Feces produced during the exposure period were also removed to minimize sorption of radioisotopes onto the egested feces. At the end of the exposure period, an aliquot of water was taken again for radioactivity measurements. Results indicated a negligible decrease of radioactivity in the water medium because of accumulation in the bivalves (e.g., 4% for Cd, 2% for Cr, 1.5% for Se, and 14% for Zn). The fraction of metals associated with the particulate phase (>0.2 μm) was also determined, and the majority of metals (e.g., 97%) was confirmed to be in the dissolved phase during the uptake period. After
Comparison of metal uptake among bivalves

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Fig. 1. Influx rate of metals in three marine bivalves (Perna viridis, Septifer virgatus, and Ruditapes philippinarum) as a function of the clearance rate of the animals. Each dot represents an individual bivalve.

Table 1. The uptake rate constant and absorption efficiency of metals from the dissolved phase in three species of bivalves

<table>
<thead>
<tr>
<th>Metal</th>
<th>Perna viridis</th>
<th>Septifer virgatus</th>
<th>Ruditapes philippinarum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake rate constant (L g⁻¹ d⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.182 ± 0.035</td>
<td>0.180 ± 0.053</td>
<td>0.054 ± 0.013</td>
</tr>
<tr>
<td>Cr(VI)</td>
<td>0.037 ± 0.012</td>
<td>0.041 ± 0.008</td>
<td>0.020 ± 0.006</td>
</tr>
<tr>
<td>Se(IV)</td>
<td>0.019 ± 0.004</td>
<td>0.031 ± 0.007</td>
<td>0.009 ± 0.002</td>
</tr>
<tr>
<td>Zn</td>
<td>0.483 ± 0.051</td>
<td>0.350 ± 0.076</td>
<td>0.191 ± 0.029</td>
</tr>
<tr>
<td>Absorption efficiency (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.105 ± 0.036</td>
<td>0.025 ± 0.022</td>
<td>0.111 ± 0.079</td>
</tr>
<tr>
<td>Cr(VI)</td>
<td>0.021 ± 0.008</td>
<td>0.062 ± 0.064</td>
<td>0.041 ± 0.025</td>
</tr>
<tr>
<td>Se(IV)</td>
<td>0.011 ± 0.003</td>
<td>0.045 ± 0.042</td>
<td>0.019 ± 0.014</td>
</tr>
<tr>
<td>Zn</td>
<td>0.273 ± 0.061</td>
<td>0.510 ± 0.507</td>
<td>0.387 ± 0.221</td>
</tr>
</tbody>
</table>

a Values are mean ± standard deviation (n = 18–20).

The calculated influx rate of metals in individual bivalves as a function of the clearance rate is shown in Figure 1. In general, no significant relationship was found between the metal uptake rate and the animal’s clearance rate. Considerable variation of the clearance rate and the metal uptake rate was found, however, among the 18 to 20 experimental individuals. For example, the clearance rate varied by 2.1-, 10.6-, and 7.4-fold, respectively, for P. viridis, S. virgatus, and R. philippinarum, and the influx rate of all metals varied by 1.5- to 2.8-fold, 2.0- to 2.8-fold, and 1.7- to 3.2-fold, respectively. Metal influx rates were generally lower in the clams than in the two mussels but were comparable between the two mussel species. The $K_u$ values, as calculated from Equation 2, are shown in Table 1. Because $K_u$ is a function of the metal uptake rate divided by the metal concentration in the dissolved phase (Eqn. 2), it was also not significantly related to the clearance rate of individual bivalve. The $K_u$ value was lowest for Se(IV) and highest for Zn. Both Cd and Cr(VI) were intermediate between Se(IV) and Zn. Among the three bivalve species, $K_u$ was comparable between the two mussel species but was lower in the clams. The relative ratio of the $K_u$ was rather constant among the three bivalves examined in this study (5.8–9.7: (1.3–2.3):1:(11.2–25.6) for Cd:Cr(VI):Se(IV):Zn).

A significant log-log negative relationship was found between $\alpha$ and the clearance rate of the bivalves (Fig. 2). The degree to which $\alpha$ was affected by the clearance rate appeared to be comparable among the three bivalve species and the four metals examined. The power coefficients describing such a relationship ranged between −0.77 and −1.35 and, under most
certain circumstances, were close to -1.0, suggesting that $\alpha$ was negatively dependent on the clearance rate. The $\alpha$ was highest for Zn, followed by Cd > Cr > Se (Table 1). The mean $\alpha$ was calculated from the replicated individual bivalves, although it was recognized that the clearance rate varied greatly among different individuals. As low as 0.01% of metal $\alpha$ was found for Se(IV) in the green mussels, and the highest metal $\alpha$ was approximately 0.5%, for Zn in the black mussels. Greater variation of $\alpha$ compared with $K_u$ was found among the experimental individuals, especially in the black mussels and clams. For example, $\alpha$ varied by an order of magnitude among different experimental individuals in the black mussels and the clams. This difference appeared to be largely explained by the difference in the clearance rate of animals. In contrast to metal $K_u$, no evidence was found that the relative ratio of metal $\alpha$ was constant among the three bivalve species.

Because a large number of individuals were measured in this study, it was possible to examine the relationship of the uptake rate among different metals by linear regression. A significant positive relationship was found in metal influx rates among the four metals in the black mussel and between Se and Cd in the clam (Fig. 3). No significant relationship was found for other metals in other species of bivalves. A significant relationship of $\alpha$ was, however, documented for all metals in the three species of bivalves examined, except for Se and Cr in P. viridis (Fig. 4).

**DISCUSSION**

**Metal uptake rate constants in bivalves**

Metal influx rate is a function of metal $\alpha$, $C_w$, and clearance rate. Results of previous studies have shown that the influx rate is directly proportional to $C_w$, an important basis for the use of marine bivalves as biomonitor of ambient metal bioavailability [12,17,26]. When modeling metal bioaccumulation in marine bivalves, $K_u$ has been employed and assumed to represent the relative metal bioavailability from the aqueous phase. In most previous studies, $K_u$ was calculated from the metal influx rates measured at different $C_w$ values [11,12]. Alternatively, it can be estimated from the metal influx rate as quantified at one metal ambient concentration (Eqn. 2). In the latter approach, $K_u$ is relatively independent of $C_w$ and, thus, is applicable to a wide range of ambient metal concentrations [12]. For metals such as Zn, which may be regulated by marine bivalves, however, $K_u$ may be slightly inversely related to $C_w$ [12,26] The $K_u$ is also a function of environmental variability (e.g., salinity) and biological conditions (e.g., the bivalve’s body size) [17,27].

Our measurements of the metal $K_u$ are comparable to those in a few previous studies of the same bivalve species [17,25]. For example, $K_u$, as estimated from the relationship between influx rate and $C_w$, was 0.206, 0.039, and 0.637 L g$^{-1}$ d$^{-1}$ in the green mussels and 0.064, 0.032, and 0.125 L g$^{-1}$ d$^{-1}$ in the clams for Cd, Cr(VI), and Zn, respectively [25]. In the black mussels, $K_u$ was 0.286, 0.085, 0.031, and 0.460 L g$^{-1}$ d$^{-1}$ for Cd, Cr(VI), Se(IV), and Zn, respectively [17]. Thus, variation of metal $K_u$ values among different batches of experiments is rather small. Considerable variation of $K_u$ values was, nevertheless, documented among different metals. As found in previous studies, the uptake rate was highest for metals (e.g., Cd and Zn) that require facilitated transport in which SH-containing ligands are involved in metal binding [24]. Anionic metals [e.g., Cr(VI) and Se(IV)] are generally taken up at a slower rate, probably because of competition with major anions in seawater [28-30].

Interspecific differences in metal $K_u$ values presumably relate to the differences in clearance rate among different bivalve species. Figure 5 summarizes the relationship between metal $K_u$ and clearance rate in eight species of marine bivalves.
ied to date. For the bivalve species Macoma balthica and Potamocorbula amurensis, $K_u$ was quantified without simultaneous measurement of the bivalve’s clearance rate [11,24,26]. The reported clearance rates of bivalves of a similar size were taken from the literature ([11,20,24,26,27,31,32]. Data for two oysters, Crassostrea virgata and Saccostrea glomerata, are from C. Ke and W.-X. Wang (unpublished data). The standard deviations of the $K_u$ values and clearance rates are also presented whenever possible. A. Perna viridis. B. Sepiifer virgatus. C. Rudtapes philippinarum. D. M. edulis. E. M. balthica. F. P. amurensis. G. C. virgata. H. S. glomerata.

Fig. 5. Relationship between the metal uptake rate constant ($K_u$) and the clearance rate of different species of bivalves. For Mytilus edulis, Macoma balthica, and Potamocorbula amurensis, data are from [11,20,24,26]. The reported clearance rates of bivalves of a similar size were taken from the literature ([11,20,24,26]. Data for two oysters, Crassostrea virgata and Saccostrea glomerata, are from C. Ke and W.-X. Wang (unpublished data). The standard deviations of the $K_u$ values and clearance rates are also presented whenever possible.

An increase in the animal’s pumping activity was coupled to a decrease in the efficiency at which a metal was absorbed across the biological membrane. Because $\alpha$ was dependent on the clearance rate, the overall metal influx rate or $K_u$ was not strongly related to the intraspecific difference in clearance rate. Conversely, absorption across the membrane may be controlled by the $K_u$, which, in turn, was not affected by a change in clearance rate. Thus, a smaller fraction of metals was absorbed across the membrane with increasing pumping rate, because the $K_u$ was maintained constant. In this study, all metals in the three bivalves appeared to be similarly affected by the clearance rate of the animals, and the power coefficient of such relationship was close to $-1$. Consistently, metal AE from ingested algae was inversely related to the ingestion rate of the marine mussel $M. edulis$ [33]. However, clearance rate appeared to have a greater influence on metal $\alpha$ compared to the influence of ingestion rate on metal AE. For example, metal AE was only moderately affected by a change in ingestion rate (e.g., a $1.2-2.0$-fold decrease in the AEs of Cd, Se, and Zn with an increase in the mussel’s ingestion rate of 15-fold). In this study, $\alpha$ decreased by approximately an order of magnitude with a 10-fold increase in the clearance rate among different individuals.

In contrast to the intraspecific difference, $\alpha$ was not strongly related to the clearance rate among different species of bivalves (data not shown). However, comparison of $\alpha$ values among different bivalves can be inherently influenced by the intraspecific variation of clearance rate. For example, Chong and Wang [25] showed that the metal $\alpha$ was three- to eightfold lower in the clam $R. philippinarum$ than in the mussel $P. viridis$, whereas these values were somewhat comparable between these two species as measured in the present study. The clearance rates in the clams measured by Chong and Wang were also higher than those measured in this study.

Recent studies have extensively investigated the variability of metal assimilation in aquatic invertebrates [13]. In contrast, very little knowledge regarding the variability of metal $\alpha$ exists. Table 2 summarizes the factors that may potentially affect metal $\alpha$ from the aqueous phase and metal AE from the ingested food source. Both parameters appeared to be similarly affected by various physiological and geochemical factors and can be considered analogous in predicting metal bioavailability from the aqueous and dietary phases. To our knowledge, the influence of metal concentration on $\alpha$ has not been specifically examined. Because $K_u$ was not notably affected by metal concentration (except for Zn, which can be partially regulated by many bivalves), it can be postulated that $\alpha$ is probably not considerably affected by the ambient metal concentration, at

Table 2. Comparison of factors potentially affecting metal assimilation efficiency from the ingested food source and metal absorption efficiency from the dissolved phase

<table>
<thead>
<tr>
<th>Factors</th>
<th>Assimilation efficiency (food)</th>
<th>Absorption efficiency (water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bivalve feeding physiology and biology</td>
<td>Ingestion rate</td>
<td>Clearance rate</td>
</tr>
<tr>
<td></td>
<td>Gut passage time</td>
<td>Gill surface area</td>
</tr>
<tr>
<td></td>
<td>Digestive partitioning</td>
<td>Gill permeability</td>
</tr>
<tr>
<td>Environmental quality</td>
<td>Body size (small effect)</td>
<td>Body size (small effect)</td>
</tr>
<tr>
<td></td>
<td>Food quality</td>
<td>Salinity</td>
</tr>
<tr>
<td></td>
<td>Food quantity</td>
<td>Osmolality</td>
</tr>
<tr>
<td></td>
<td>Season</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>Metal geochemistry</td>
<td>Phase speciation (e.g., cytosolic, sulfide)</td>
<td>Speciation (e.g., free-ion activity)</td>
</tr>
<tr>
<td></td>
<td>Concentration (small effect)</td>
<td>Concentration (small effect)</td>
</tr>
<tr>
<td></td>
<td>Metal–metal interaction</td>
<td>Metal–metal interaction</td>
</tr>
</tbody>
</table>
least until it reaches a level high enough to reduce the clearance rate of the bivalves. Results of previous studies also demonstrated the influence of metal speciation [7,17,34], salinity [35–37], and dissolved organic carbon concentration [12] on metal uptake rate. Because the clearance rate of bivalves was not related to these environmental conditions, metal $\alpha$ may be similarly affected by these physicochemical factors. A further challenge would be to directly quantify the $\alpha$ of various metal species in the bivalves.

The metal uptake rate was also greatly dependent on the bivalve’s body size [17,26,27]. In contrast, when the size dependence of the clearance rate was considered, the calculated $\alpha$ was only slightly affected by the body size. Wang and Dei [17] indicated that the $\alpha$ of Cr(VI) and Se(IV) in the black mussels was independent of, but that the $\alpha$ of Cd and Zn was weakly related to, the body size. Wang and Fisher [27] found that the $\alpha$ of metals in the common mussel (M. edulis) was somewhere comparable among the three body sizes (shell length, 1.5–5.0 cm). Similarly, Chong and Wang [25] concluded that $\alpha$ was not greatly affected by a change in body size of P. viridis and R. philippinarum. For example, with an increase in body size from 0.05 to 1.5 g (i.e., a 30-fold difference), the $\alpha$ of Cd, Cr, and Zn only decreased by 1.6-, 3.9-, and 1.2-fold in the mussels and by 1.6-, 1.2-, and 1.3-fold in the clams, respectively. Metal AE from ingested phytoplankton in the mussel M. edulis was also independent of body size [27].

Given the dependence of metal $\alpha$ on the clearance rate of animals, a comparison of metal bioavailability based on $\alpha$ measurements may be limited. Metal bioavailability defines the fraction that is potentially available for biological uptake [38]. Such a definition is generally constrained by the control by metal chemistry, and it does not incorporate the physiological influence on metal uptake. Great intraspecific variation of $\alpha$ also renders it difficult to directly incorporate into the bioenergetic-based kinetic model for prediction of metal concentration and bioavailability in marine bivalves. In these previous modeling studies, only $K_u$, which is a function of $\alpha$ and the clearance rate, was incorporated into the model to quantify metal aqueous uptake. Because $K_u$ was relatively independent of the clearance rate of the animals, it may exclude the potential influence of the individual variability of CR on metal uptake.

Metal $\alpha$, as measured in this and in previous studies, was much lower than the metal AE from ingested food sources. For example, $\alpha$ was generally less than 0.5% for Cd and Zn in the three bivalves and less than 0.06% for the two anionic metals (Cr and Se). By contrast, the AEs of Cd and Zn in these bivalves ranged between 11 and 55% [39] and were several orders of magnitude higher than the metal $\alpha$ from the aqueous phase. Nevertheless, the bivalves can pump a considerable amount of water, and uptake from the aqueous phase is likely to contribute substantially to the total metal accumulation in bivalves. Recently, Chong and Wang [25] modeled the exposure pathways of Cd and Zn in the green mussel (P. viridis) and the clam (R. philippinarum). The modeling results indicated that both dissolved uptake and food ingestion contributed equally to the overall Cd and Zn accumulation in the mussels. For clams, Zn accumulation was dominated by ingestion from food particles, and food ingestion contributed more than 50% of Cd accumulation under most circumstances. Dominance of dietary exposure of Cd and Zn in the clams primarily resulted from the low CR and high AE of these metals in the clams. Conversely, an equal contribution of aqueous metal and dietary metal in the green mussels was largely attributed to the high $K_u$ resulting from the high clearance rate of the animals, despite the fact that the $\alpha$ was much lower compared with the AE.

**Relationship of uptake among metals**

In this study, direct coupling of different metal influx rates (or $K_u$ values) was only found in the black mussel and not in the other two bivalves. Wang and Dei [17] found a similar coupling of influx of Cd and Zn in the black mussel, but they found no relationship between Cd and Cr(VI) or Se(IV), or between Cr(VI) and Se(IV), in that study. A direct coupling may imply that these metals are transported via the same pathway. In fact, a few studies have indicated that Cd and Zn are probably taken up by marine bivalves through facilitated transport [17,24,40,41]. The uncoupling of Cd and Zn influx in green mussels and clams was rather unexpected in this study. However, recent studies have also indicated that metal transport may be species specific. For example, the Ca channel was involved in Cd and Zn uptake in the common mussel (M. edulis) [24,42] and oyster gills (Crassostrea virginica) [41], but not in the clam (Macoma balthica) [24] and the black mussel [17].

In contrast to the metal uptake rate, direct coupling of $\alpha$ was found for four metals in three species of bivalves. Such a coupling may, however, be inherently caused by the dependence of $\alpha$ on the clearance rate of the bivalves. For example, an increase in clearance rate of the animals may lead to a decrease in the $\alpha$ of all metals, resulting in a significant correlation of $\alpha$ among the four metals. Thus, further studies are necessary to examine the coupling of metal absorption by minimizing the inherent influence of bivalve clearance rate on metal absorption.

In summary, the present study demonstrated that the interspecific difference in metal uptake rate was largely related to, whereas the interspecific difference in metal $\alpha$ was not affected by, the difference in bivalve clearance rates. In contrast, the intraspecific difference in metal uptake rate was not directly related to different clearance rates among different individuals, largely because of the dependence of metal $\alpha$ on the clearance rate. Within each bivalve species, $\alpha$ was greatly affected by the volume of water pumped by the animals. Because of the direct influence of pumping physiology on metal $\alpha$, the $K_u$ value, which incorporates both $\alpha$ and clearance, is probably a better parameter to be employed in the bioenergetic-based kinetic model for prediction of metal concentration in the animals. This study provides strong evidence that bivalve physiology should be considered when studying and predicting metal bioavailability from the ambient environment. It is well known that metal chemistry, particularly metal speciation, plays a critical role in metal bioavailability from the aqueous phase [34], but physiological processes such as the pumping physiology may be equally important to our understanding of metal bioavailability in marine bivalves.

**Acknowledgement**—I thank Robert Dei and Caihuan Ke for their technical assistance. Thanks are also due to Philip Rainbow and Nicholas Fisher for their helpful discussions and comments on this work and to the two anonymous reviewers for their many constructive comments, which improved the presentation of this work. This study was supported by a Research Grant Council/Competitive Earmarked Research Grant (HKUST6137/99M) to W.-X. Wang.
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REFERENCES


