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Subcellular controls of silver biokinetics in the green mussel *Perna viridis* from two hydrographic zones

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ABSTRACT: The roles of subcellular distribution on silver (Ag) accumulation and elimination in the green mussel *Perna viridis* collected from 2 hydrographical zones (harbour vs. estuarine) in Hong Kong during 3 different seasons were investigated. Ag concentration and biokinetics varied widely between the 2 zones, but were less variable among the different seasons (pre-wet, wet and dry). The harbour population had a 2- to 6-fold higher Ag tissue concentration than the estuarine population. The insoluble fraction was the predominant pool for Ag subcellular distribution in the green mussels. In both the pre-wet and wet seasons, a lower Ag tissue concentration was associated with the insoluble fraction in the estuarine population as compared to the harbour population. Ag uptake from the aqueous phase was faster in the estuarine population during all seasons, but no significant relationship between Ag uptake and salinity was found in this study. Dietary uptake of Ag was similar in both populations, although there was a slight difference in assimilation from diatoms and natural seston. Efflux of Ag was 2 times faster in the estuarine population during the wet and dry seasons, which may partially explain the lower Ag tissue burden in the mussels. These results demonstrated a close correlation between Ag kinetics and its subcellular distribution. The percentage of Ag in the heat-sensitive proteins correlated positively to the influx of dissolved Ag and Ag efflux, but negatively to the dietary assimilation. On the other hand, a higher percentage of Ag in the insoluble fraction was related to a higher dissolved uptake rate constant and a lower assimilation. Thus, the role of the individual subcellular fraction in the uptake was exposure pathway-specific. This study highlights the importance of subcellular Ag partitioning in controlling its accumulation and detoxification in bivalves.

KEY WORDS: Uptake · Efflux · Mussels · Hydrographical zones · Hong Kong

INTRODUCTION

Aquatic invertebrates accumulate varying concentrations of trace metals in their tissues. The natural variability of metal concentrations in bivalves outside of anthropogenic influence has created an on-going puzzle for environmental regulators (O'Connor 2002). Trace-metal bioaccumulation in aquatic organisms is controlled by numerous geochemical, physiological and ecological factors, such as food composition and quantity, gut passage time and acidity, pumping activities and salinity (reviewed in Wang 2003). Such complexity has presented a major obstacle in interpreting body metal concentrations in marine invertebrates, which are frequently employed as biomonitors. There is now a tremendous interest in understanding the mechanistic differences of trace-metal bioaccumulation among different populations of marine bivalves, which may help in the interpretation of data generated by biomonitoring programs.

Ke & Wang (2001) compared metal accumulation (Cd, Se, and Zn) in the Hong Kong estuarine oyster *Crassostrea rivularis* with that in the coastal oyster *Saccostrea glomerata.* The estuarine oysters have high

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metal influx from the dietary and aqueous phases, which was, however, balanced by a higher metal efflux rate than in the coastal oysters. The roles of metal uptake and loss kinetics in the metal accumulation of these 2 populations were difficult to determine, because the metal concentrations in the oysters were not measured in that study. Blackmore & Wang (2003) determined the metal concentrations (Cd, Cr, Se and Zn) in the mussel *Perna viridis* collected from 2 hydrographical zones in Hong Kong. Mussels from the 2 sites were then acclimated to different salinities in the laboratory. The metal influx from the dissolved phase in both mussel populations generally increased with a decrease in salinity, but the metal dietary assimilation was unaffected by the salinity variation. This study provided information on the effects of salinity on metal uptake and on the potential role of physiological factors in metal accumulation, but the efflux rate was not concurrently considered.

The hydrography of Hong Kong's coastal waters is complex. Within a narrow geographical distance, there are contrasting hydrological conditions that vary seasonally. The estuarine waters of Hong Kong are affected primarily by the Pearl River Delta, which drains a large area of southern China in the summer months. In winter, this influence is replaced by the Kuroshio Current, which is characterised by high salinity (34.4 to 35 psu) and high temperature (26 to 29°C). Due to the special hydrography of the area, marine invertebrates may have different metal accumulation strategies within a narrow region. The green mussel *Perna viridis* is widespread around the waters of Hong Kong, including both the estuarine side characterised by low salinity during the summer season and the harbour side characterised by high salinity throughout the seasons. Thus, it is of substantial interest to understand the differences in metal uptake and loss kinetics among regional populations of mussels, which may lead to different body metal concentrations.

The 'intracellular speciation' of metals in marine invertebrates has generated much interest (Mason & Jenkins 1995, Rainbow 2002). Recent studies have employed differential centrifugation to quantity the subcellular metal partitioning in marine animals, as well as their potential transfer to higher trophic levels such as marine shrimps and gastropods (Wallace & Luoma 2003, Wallace et al. 2003, Vijver et al. 2004, Cheung & Wang 2005). Whereas numerous studies have been conducted on the controls of metal 'speciation' in the environment (e.g. metal partitioning in phytoplankton cytoplasm and metal geochemical speciation in sediment), no study has addressed whether there is any potential link between the metal biokinetics and subcellular distribution in any marine organisms.

Aquatic organisms are very sensitive to silver (Ag) (Ratte 1999). Although the Ag concentration is often low in the environment, dietary exposure to Ag at environmentally realistic concentrations may cause toxicity to marine bivalves (Hornberger et al. 2000). In Hong Kong, Ag is one of the major metal contaminants, especially in marine sediments, due to the significant input of industrial activities. Shi et al. (2003) first demonstrated that the accumulation of Ag in the Hong Kong green mussels was affected by Ag preexposure. The redistribution of Ag in the cellular components caused a significant change in Ag biokinetics in the mussels, but field evidence is sought to verify such laboratory findings. On the other hand, field results showed that green mussels collected from the harbour zones of Hong Kong contain a much higher Ag concentration than those from the estuarine zones, despite the fact that there was no obvious difference in the environmental concentration of Ag at these 2 locations. Therefore, in this study, the biokinetics of Ag and the role of subcellular distribution in Ag accumulation and loss in these 2 populations of green mussels were examined. The mussels from 2 hydrographical zones in 3 seasons (pre-wet, wet, dry) were collected to obtain a variation of Ag accumulation. The aqueous and dietary phases of Ag uptake and Ag efflux in the mussels were concurrently examined; they were then correlated with its accumulation and subcellular distribution in the mussels. To our knowledge, this study represents the first correlating the Ag kinetics and Ag subcellular distribution in marine bivalves.

MATERIALS AND METHODS

Collection of mussels and seawater. *Perna viridis* (average shell length: 2.5 to 3 cm, mean dry weight: 0.25 g) were collected during May (pre-wet season, end of spring), August (wet season, mid-summer) and December (dry season, mid-winter) from 2 field sites, Ma Liu Shui pier, Tolo Harbour (a semi-enclosed harbour site), and Tung Chung, Lantau (an estuarine site), in Hong Kong (Fig. 1). After transport to the laboratory, the mussels were maintained in seawater and the salinity was adjusted to be the same as the local habitat where the mussels were collected. The diatom *Thalassiosira pseudonana* was fed to the mussels at a ration of about 2% of the mussels' body tissue dry weight per day. Individual tissues and shells of 5 mussels from each site were dissected and weighed, and the condition index was calculated as the ratio of soft tissue dry weight to shell dry weight multiplied by 100.

Each time mussels were collected, the *in situ* water temperature and salinity were recorded. Water samples from both sites were also collected for chloro-

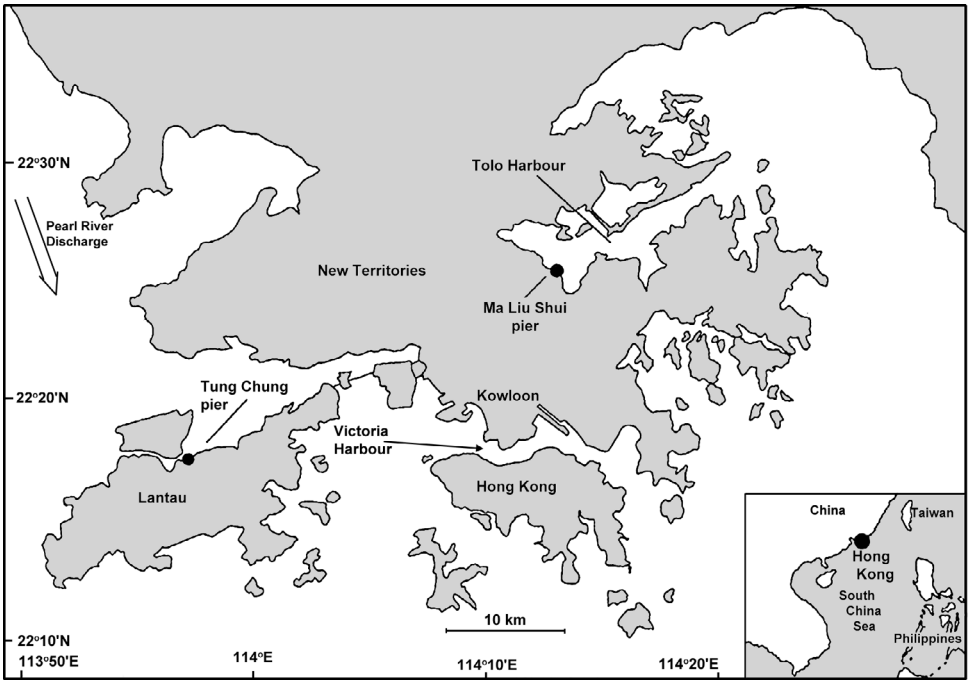

Fig. 1. Map of Hong Kong coastal waters that shows the collection sites of *Perna viridis*, Ma Liu Shui (harbour site) and Tung Chung (estuarine site)

phyll *a* (chl *a*) and total suspended solid (TSS) determination and for conducting the Ag dissolved uptake experiments in the laboratory (described 2 subsections below). Chl *a* was analysed by a fluorometer (TD-700, Turner Designs), and concentration was obtained from the standard curve of chl *a* extracted from the alga *Anacystis nidulans* (Sigma). TSS was determined by filtering 1 l of seawater through a pre-weighed glass fibre filter (GF/F, Whatman, pore size: ~0.8 µm) and rinsing with 0.5 M ammonium formate (CH_5NO_2). The filter was dried at 80°C for 1 d and weighed. We also attempted to quantify the dissolved Ag concentrations in the 2 locations using trace metal clean techniques; however, the dissolved Ag concentrations were all below the detection limits (5 ng l^{-1}).

Tissue Ag concentration and subcellular Ag distribution. Total tissue Ag concentrations in the green mussels were analysed in 5 individuals from each site, using previously described methods (Blackmore & Wang 2002, Ng & Wang 2004). The soft tissue of the mussels was acid-digested, and the digests were analysed by inductively coupled plasma–mass spectroscopy (ICP–MS, Perkin-Elmer, Elan 6000). Measurements were checked with oyster tissue standard (1566a, National Institute of Standards and Technology, Gaithersburg, Maryland), with recovery >90%. Soft tissue Ag concentrations are expressed as micrograms per gram dry weight.

Five individuals were dissected for analysis of the subcellular Ag distribution using the method described by Wallace & Luoma (2003) and Ng & Wang (2004). The tissue was homogenised and centrifuged into subcellular insoluble and soluble fractions. The insoluble fraction comprised the tissue fragments, cellular debris and organelles. The soluble fraction was divided into metallothionein-like protein (MTLP, heat-stable) and the heat-sensitive protein (HSP). In this study, only 3 fractions were analysed, whereas in several previous studies (Wallace & Luoma 2003, Wallace et al. 2003, Cheung & Wang 2005), a total of 5 fractions were analysed (i.e. the insoluble fraction was further divided into cellular debris, metal-rich granule and organelles). The insoluble fraction was not further separated in our study primarily because it was difficult to separate some of the small membrane-bound, metal-rich granules and granules in the early stage of deposition in the lysosomes without supplementary microscopic and biochemical analyses. The percentage of Ag distributed in each fraction was calculated and used throughout the study to interpret the distribution of Ag among subcellular fractions and how it was correlated with the Ag kinetics.

Clearance rate, Ag uptake and Ag efflux. The clearance rate was used to compare any physiological difference in pumping activities between the 2 populations of mussels, and was quantified using a well-established method (Blackmore & Wang 2002, Shi et al. 2003). The clearance rates of 8 individuals were determined, and the rate of each individual was calculated from the mean of 2 consecutive measurements (0 to 20 and 20 to 40 min).

Ag uptake by the mussels was determined from the dissolved and dietary phases. Seawater collected from the site of mussel population was used for the dissolved uptake experiment. During the experiments, 8 mussels were individually placed into 200 ml of ^{110m}Ag-radiolabelled seawater (in $AgNO_3$, 7.4 kBq l^{-1}, corresponding to 60 ng l^{-1}) for 1 h. Afterwards, they were taken out, rinsed with nonradioactive water, and the soft tissue was dissected for radioanalysis. The tissue was dried at 80°C for 1 d, after which the dry weight was determined. The dissolved uptake rate constant (k_u, l g^{-1} d^{-1}) was calculated as the rate of Ag taken up per gram of dry weight of tissue divided by the concentration of dissolved Ag in the medium. The radioactivity was measured by a gamma counter (Wallac 1480 Wizard 3′, Perkin Elmer) at 643 keV. The counting time was adjusted for counting error <5%.

The assimilation efficiency (AE, %) of Ag from the dietary phase was determined in the 2 populations of mussels. Both the natural seston (NS) and the diatom *Thalassiosira pseudonana* were used as food sources for the mussels, resulting in 4 treatments (harbour–diatom; harbour–NS; estuarine–diatom; estuarine–NS) in this experiment. The natural seston was collected by filtering 2 l of seawater from the field, first through a 60 µm mesh to remove the large particles in the water, then filtered through a 3 µm polycarbonate membrane. Then they were radiolabelled with 37 kBq ^{110m}Ag for 2 d. *T. pseudonana* was labelled with ^{110m}Ag (7.4 kBq) in 200 ml filtered seawater enriched with *f*/20 levels of nutrients, but without EDTA, for 1 d. The radiolabelled NS or diatoms were collected by filtration through a 3 µm polycarbonate membrane and resuspended into 12 ml of filtered seawater before use. Seven mussels from each treatment were put into the filtered seawater and allowed to feed the radiolabelled food at a concentration of about 1 mg l^{-1}, which was added at 2 intervals of 10 min each. The radioactivity of each mussel was subsequently counted, and 5 mussels with the highest counts were selected for continuous depuration of Ag. The mussels were placed in a 10 l aquarium with circulating aerated seawater. The diatom *T. pseudonana* was fed to the mussels at a 2% dry weight ration every day. Faecal pellets were collected frequently during the experiment to minimise desorption of the radiotracers from the faecal pellets to the seawater and subsequent accumulation by the mussels. The radioactivity of the mussels was monitored at 3, 6, 9, 12, 24, 48, 60 and 72 h. The AE was determined as the percentage of initial radioactivity retained in the mussels after 60 h of depuration, using the established methods for green mussels (Chong & Wang 2000, Blackmore & Wang 2002, Shi et al. 2003).

The Ag efflux rate was determined by the depuration of radiolabelled Ag in the mussels for 30 d, using previously described methods (Wang et al. 1996, Ke & Wang 2002). Twelve individual mussels were placed in 2 l of filtered seawater with aeration. The diatoms *Thalassiosira pseudonana* (7.4 kBq ^{110m}Ag in 500 ml filtered water) were radiolabelled as described above and fed to the mussels for 2 h. Mussels were then transferred to nonradioactive water and fed with unlabelled diatom food. The mussels were fed under this condition (i.e. 2 h radioactive feeding each day) for a total of 6 d, and then depurated in a similar set-up as in the AE experiment. The seawater of the aquarium was changed every 2 d. The radioactivity of the mussels was counted regularly for 30 d, and corrected for radioactive decay. Samples of mussels were taken on Days 0, 15 and 30, and dissected for radioactivity analysis of the digestive glands, soft tissue and shells. The percentage of Ag retained was plotted over time, and the efflux rate constant (k_e, d^{-1}) was calculated from the slope of the regression between the natural log of the percentage of Ag retained and the time.

Statistical analyses. Data were checked for normality and homogenous variation. All the percentage data were arcsine-transformed before statistical analyses. Student's *t*-tests detected the difference between populations, and 1-way ANOVA tested the differences in AE from 4 groups of mussels from 2 locations. Tukey's HSD multiple comparison tests were used to detect the differences between groups when $p < 0.05$. Linear regression analysis was used to test the correlations between subcellular Ag distribution and each Ag uptake or efflux parameter. The level of significance for all tests was p (or α) = 0.05.

Table 1. Physical parameters of the water at the harbour and estuarine sites of Hong Kong in different seasons

	Pre-wet		Wet		Dry	
	Harbour	Estuarine	Harbour	Estuarine	Harbour	Estuarine
Salinity (psu)	30	22	30	15	33	35
Temperature (°C)	29	28	30	30	18	18
Chl *a* (µg l^{-1})	7.4	1.1	0.7	0.4	0.8	0.5
Total suspended solid (mg l^{-1})	24.0	6.0	5.2	3.5	4.8	8.7

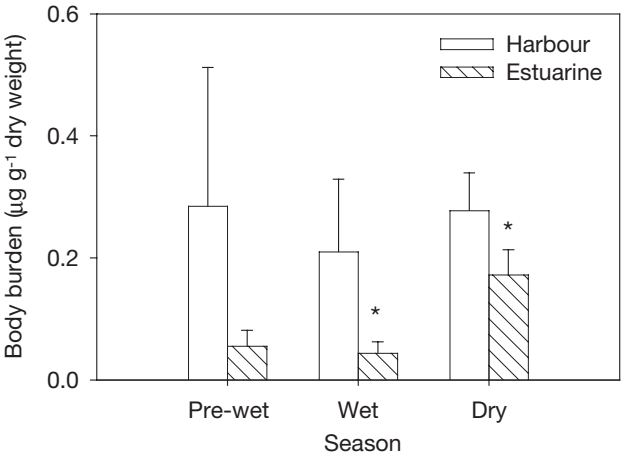

Fig. 2. *Perna viridis*. Seasonal variation in Ag concentration (µg g^{-1} dry weight) in the soft tissue of the green mussels at Ma Liu Shui (harbour site) and Tung Chung (estuarine site) (mean ± SD, n = 5). Asterisk indicates significant difference at $p < 0.05$ between populations of mussels in each season by the Student's *t*-test

RESULTS

The temperatures were similar between sites in all seasons (Table 1). The salinity at the estuarine site was lower than that at the harbour site during the pre-wet and wet seasons, but they were comparable during the dry season (winter). Lowest salinity recorded for the estuarine site was 15 psu during the wet seasons. The condition indices of the mussels were: 12.2 ± 2.7 (harbour) and 10.0 ± 1.3 (estuarine) in the pre-wet season; 12.8 ± 3.1 (harbour) and 10.3 ± 1.6 (estuarine) in the wet season; and 10.0 ± 1.3 (harbour) and 8.4 ± 0.9 (estuarine) in the dry season. The average condition of the mussels at the harbour site was better than that at the estuarine site in all seasons, but was slightly worse during the dry season. However, the condition index of the mussels did not vary significantly between seasons and locations of mussels ($p > 0.05$).

The Ag concentration in the soft tissue of mussels varied seasonally only at the estuarine site, with a higher Ag concentration in the dry season (Fig. 2). When comparing the locations, mussels at the harbour site had a higher Ag accumulation (2- to 6-fold) than those at the estuarine site ($p = 0.09$, $p = 0.03$ and $p = 0.01$, in the pre-wet, wet and dry seasons, respectively). The subcellular Ag distribution was, in general, similar among seasons and between the locations (Fig. 3). Most Ag was distributed in the insoluble fraction (60 to 80%), with ~5 to 20% in the MTLP and HSP fractions. Ag partition was different when compared among fractions between the estuarine and harbour populations. In both the pre-wet and wet seasons,

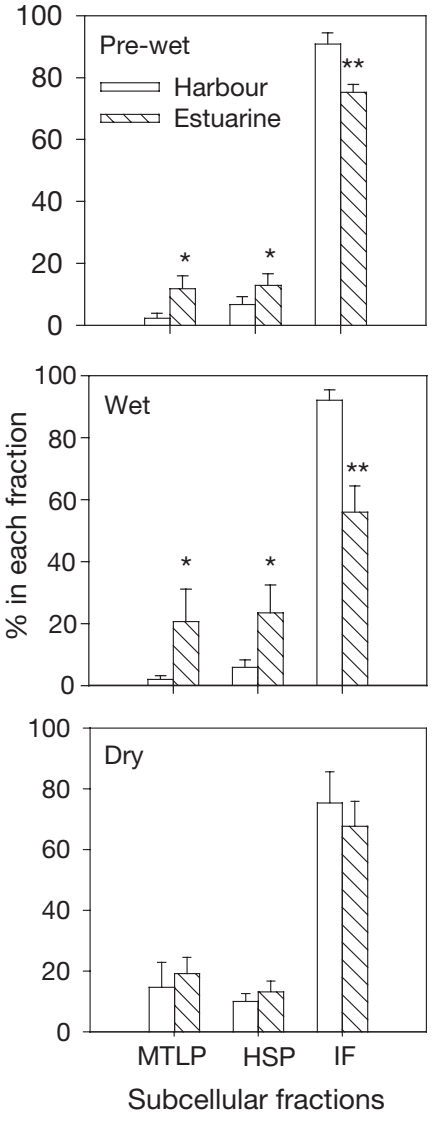

Fig. 3. *Perna viridis*. Subcellular Ag distribution (%) in the green mussels in different seasons (mean ± SD, n = 5). Asterisks indicate significant differences at $p < 0.05$ (*) and $p < 0.01$ (**), between populations of mussels in each season by Student's *t*-tests. MTLP: metallothionein-like protein; HSP: heat-sensitive protein; IF: insoluble fraction

higher Ag was found in the MTLP and HSP ($p < 0.05$), whereas lower Ag was distributed in the insoluble fraction ($p < 0.01$) of the estuarine population than in that of the harbour population.

The clearance rate of the mussels (*Perna viridis*) from the harbour site was similar across seasons (3 to 5 l g^{-1} h^{-1}) (Table 2), but mussels from the estuarine site varied, and sometimes the clearance rate (>10 l g^{-1} h^{-1}) was greater than that for the harbour population (wet season: $p < 0.001$; dry season: $p = 0.01$).

The uptake of dissolved Ag varied across seasons in the estuarine population (Table 2). Generally, Ag

Table 2. *Perna viridis*. Summary of the clearance rates and the uptake and efflux kinetics of the mussels at the harbour and estuarine sites in Hong Kong, in different seasons. Mean ± SD (clearance rate: n = 8; dissolved uptake: n = 8; assimilation efficiency: n = 5; efflux: n = 12). Asterisks indicate $p \leq 0.01$ (**) compared within the same metal between the sites, analysed by Student's *t*-tests. AE: assimilation efficiency; NS: natural seston; k_u: dissolved uptake rate constant; k_e: efflux rate constant

	Pre-wet		Wet		Dry	
	Harbour	Estuarine	Harbour	Estuarine	Harbour	Estuarine
Clearance rate (l g^{-1} h^{-1})	5.9 ± 0.9	6.3 ± 0.7	3.2 ± 1.0	12.6 ± 2.5**	2.6 ± 1.2	12.1 ± 4.5**
AE–diatom (%)	31.6 ± 23.0	19.2 ± 6.5	25.0 ± 5.7	12.7 ± 2.1**	24.7 ± 6.2	21.6 ± 3.9
AE–NS (%)	12.5 ± 3.4	8.9 ± 2.9	18.6 ± 3.8	17.6 ± 8.5	18.7 ± 5.5	26.4 ± 6.6
k_u (l g^{-1} d^{-1})	0.638 ± 0.214	3.964 ± 0.942**	2.191 ± 0.482	8.212 ± 1.258**	2.368 ± 1.032	5.380 ± 1.273**
k_e (d^{-1})	0.050 ± 0.006	0.064 ± 0.017	0.039 ± 0.011	0.087 ± 0.007**	0.032 ± 0.010	0.062 ± 0.014**

uptake from the dissolved phase was faster in the estuarine population during all seasons ($p < 0.001$). There was no significant correlation between the dissolved uptake of Ag and salinity when all seasons and both populations were plotted together ($r^2 = 0.39$, $p = 0.18$). Furthermore, when all 3 seasons and 2 locations were combined, Ag uptake from the dissolved phase had a negative relationship with its subcellular distribution in the insoluble fraction (Fig. 4). A higher Ag k_u was related to a higher percentage in HSP ($p = 0.003$) and a slightly higher percentage in MTLP ($p = 0.04$, Fig. 4).

The depuration of Ag following pulse ingestion of radiolabelled seston and diatoms is shown in Fig. 5. Assimilation of Ag from ingested diatoms was generally similar during different seasons and for both populations (Table 2, Fig. 5). However, the Ag AE of the diatom by the estuarine population was 13% lower than that by the harbour population in the wet season ($p = 0.01$). The AEs of Ag from natural seston also varied during different seasons (9 to 26%), but were not different between the 2 populations of mussels ($p > 0.05$). By combining all different seasons and 2 locations, the relationships between the subcellular distribution of Ag and the dietary uptake were opposite that of the dissolved uptake (Fig. 6). A higher AE was correlated with a smaller percentage in HSP ($p = 0.01$) and a higher percentage in IF ($p = 0.03$). However, these relationships were mainly dependent on results of the estuarine population in the wet season that showed a significant difference from the harbour population. No significant correlation between the Ag AE and the Ag distribution in the MTLP was evident.

The total Ag retained in the mussels (including the soft tissue and shell) over the efflux period is shown in Fig. 7. The Ag retained in the shell accounted for 26 ± 10% (estuarine population) and 15 ± 5% (harbour population) of the total Ag in the mussels. The elimination of Ag after 6 d of dietary uptake was divided into an initial faster and then a slower compartment (Fig. 7). Generally, depuration was faster between Days 1 and 7. The depuration rate after 7 d in the slower exchanging compartment was only used for the calculation of the efflux rate constant (k_e, Table 2). There was no seasonal variation in the efflux rate of Ag in either population, but Ag was depurated faster in the estuarine population in the wet ($p < 0.001$) and dry seasons ($p = 0.002$) (Table 2, Fig. 7). In this experiment, Ag efflux as high as 0.09 d^{-1} was measured. The biological retention half-life ($t_{1/2}$) was 14 to 23 d and 8 to 12 d for the harbour and estuarine populations, respectively. A higher percentage of Ag associated with HSP was related to a faster efflux rate (Fig. 8, $p = 0.02$). No significant correlation between the Ag efflux and its distribution in the insoluble fraction or MTLP fraction was detected.

The relationships between the Ag biokinetics and the Ag body concentration in the green mussels were further correlated by combining 3 different seasons and 2 locations. The Ag AE was positively related to Ag body burden, but the relationship was unlikely to appear if 1 highly variable set of AE data (pre-wet season, harbour population) was not considered. k_u and k_e were both negatively related to Ag body burden in the mussels (Fig. 9, $p \leq 0.05$).

DISCUSSION

Hong Kong has an interesting hydrography and physical water parameters vary considerably between the harbour and estuarine zones. The salinity at the estuarine site was often lower than that at the harbour site, especially during the discharge of freshwater from the Pearl River in summer (May to August) during the wet season. In addition, phytoplankton abundance and suspended solids in the seawater fluctuated between the sites and the seasons. Mussels *Perna viridis* from both sites had distinct differences in Ag concentration (a 6-fold maximum difference). There are limited field data on Ag concentration in green mussels in Hong Kong. Chan et al. (1990) reported that Ag body concentrations in the green mussels from Hong Kong

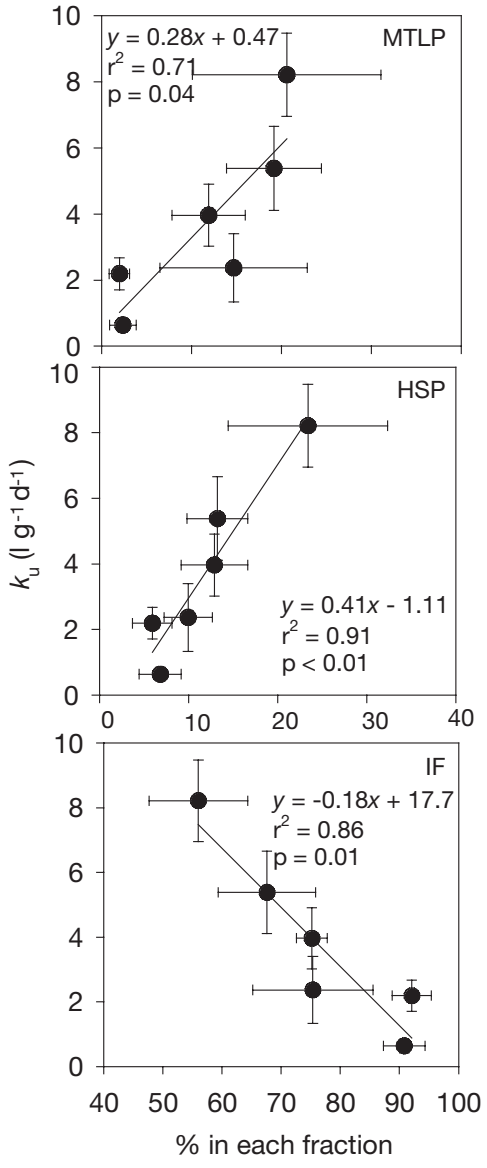

Fig. 4. *Perna viridis*. Correlations between the Ag dissolved uptake rate constant (k_u, l g^{-1} d^{-1}) and the subcellular distribution in the green mussels. Data were pooled from all seasons and fitted with the linear regression model. r^2: regression coefficient; for other abbreviations, see Fig. 3

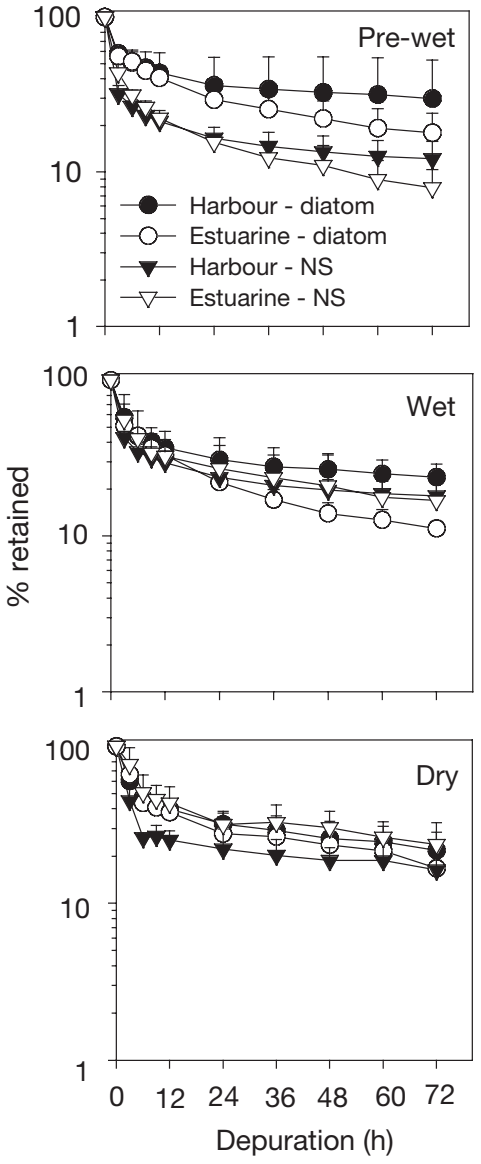

Fig. 5. *Perna viridis*. Percentage of Ag (%) retained following pulse feeding of radiolabelled food to the green mussels in different seasons (mean ± SD, n = 5). NS: natural seston

ranged between 0.05 and 1.5 µg g^{-1}, which was comparable to these measurements.

In general, the Ag concentration in the green mussels varies more extensively in different regions of Hong Kong than among seasons. The spatial variation may not be due only to the different salinities of seawater, as this difference was only distinctly observed in the wet season, but also to the variation of Ag biokinetics consistently found in all seasons. Spatial and temporal differences in metal concentrations have been observed previously in bivalves (Amiard et al. 1986, Chu et al. 1990, Bordin et al. 1992). Metal concentrations in bivalves from unpolluted sources were often higher in winter and lower in spring or summer, which may be related to the gonad maturation and spawning of the bivalves. However, a similar pattern was only observed in the estuarine population. Some studies have also suggested that body weight may affect Ag concentration in bivalves (inverse relationship, e.g. Mouneyrac et al. 1998), but it seems not to contribute to the variation in Ag concentration in this study, because the condition index of the mussels was, in general, similar between locations and among seasons.

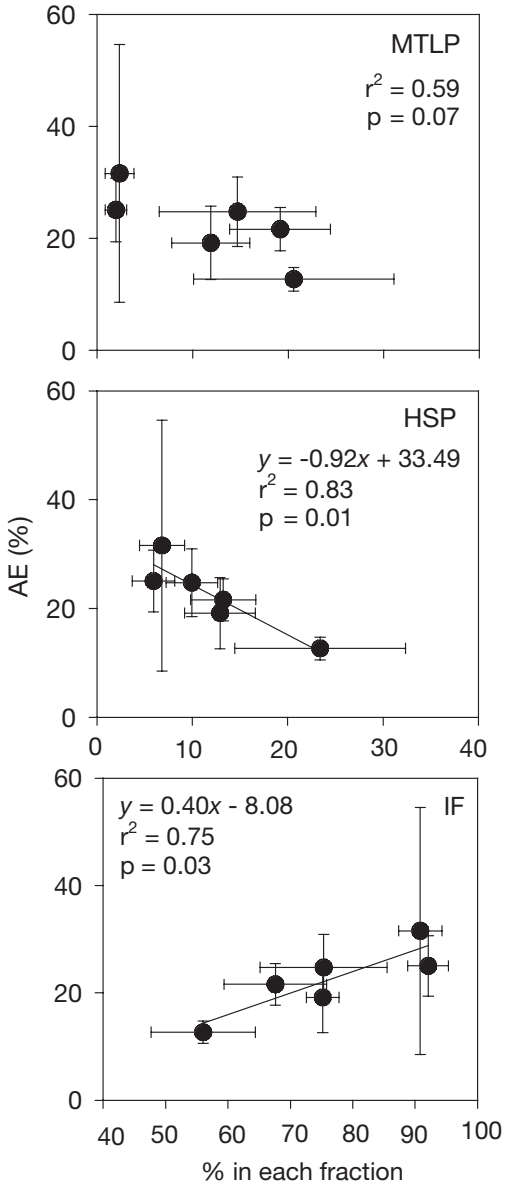

Fig. 6. *Perna viridis*. Correlations between the Ag assimilation efficiency (AE) and the subcellular distribution in the green mussels. Data were fitted with the linear regression model. r^2: regression coefficient; for other abbreviations, see Fig. 3

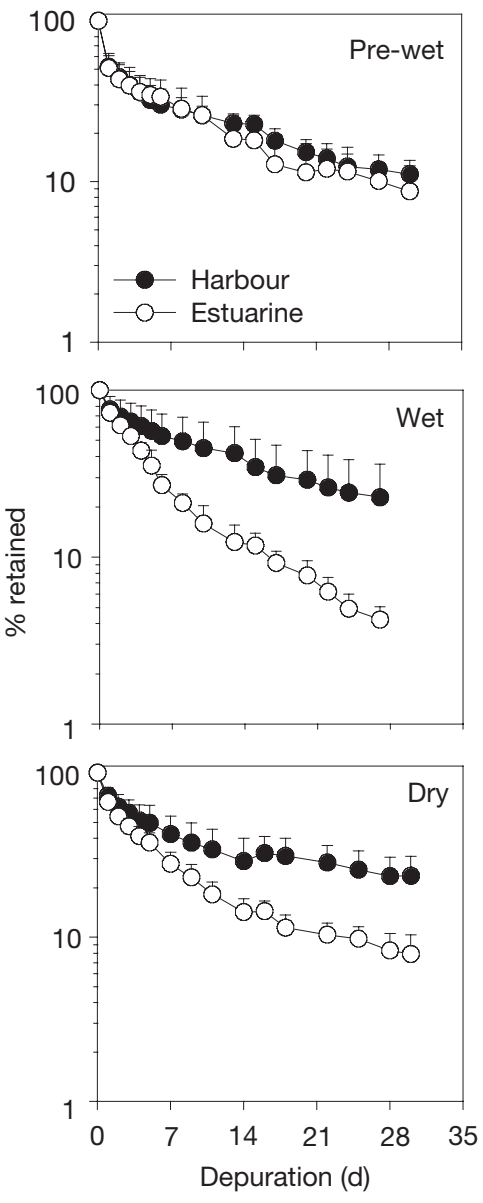

Fig. 7. *Perna viridis*. Percentage of Ag (%) retained by green mussels following 6 d of feeding on radiolabelled food in different seasons (mean ± SD, n = 12). The efflux rate constant (k_e) was calculated from the slope of the regression between the natural log of the percentage of Ag retained from 7 d and the time

Previous laboratory or field studies have often focused on metal partitioning in bivalves during or after metal exposure (Evtushenko et al. 1986, Giguere et al. 2003, Wallace et al. 2003). In the clam *Potamorcorbula amurensis*, after 14 d of exposure, MTLP was found to play an important role in detoxifying Cd, while most Zn was bound to organelles, but in *Macoma balthica*, Cd and Zn partitioned similarly (Wallace et al. 2003). Cd was stored in metal-rich granules, and Zn was stored in both metal-rich granules and metallothioneins. These previous limited studies implied that the subcellular distribution in marine bivalves was very dynamic and species-specific. In this study, despite the variation in total Ag body concentration, Ag partition into the subcellular fractions was similar within the same species of mussels from different habitats. Ag appears to be detoxified mainly in the insoluble fraction (>80%) of the harbour population. Shi & Wang (2004) showed that ~60% of Ag was in organelles and 30% in Ag-rich granules in Ag-exposed green mussels. Ag may accumulate as an insoluble sulphide complex, which is very stable, in

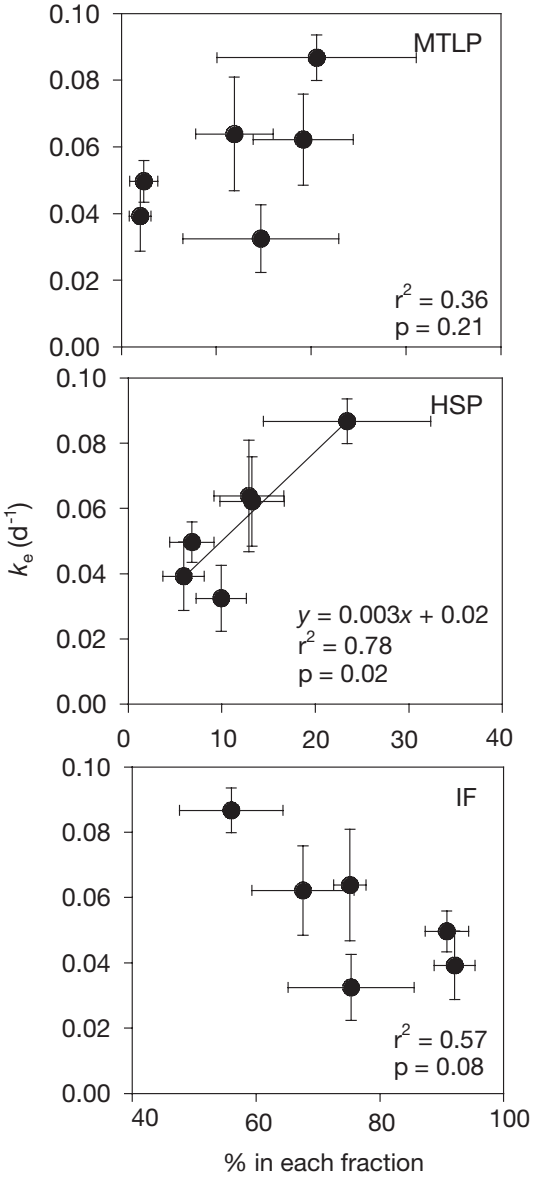

Fig. 8. *Perna viridis*. Correlations between the Ag efflux rate constant (k_e, d^{-1}) and the subcellular distribution in the green mussels. Data were fitted with the linear regression model. r^2: regression coefficient; for other abbreviations, see Fig. 3

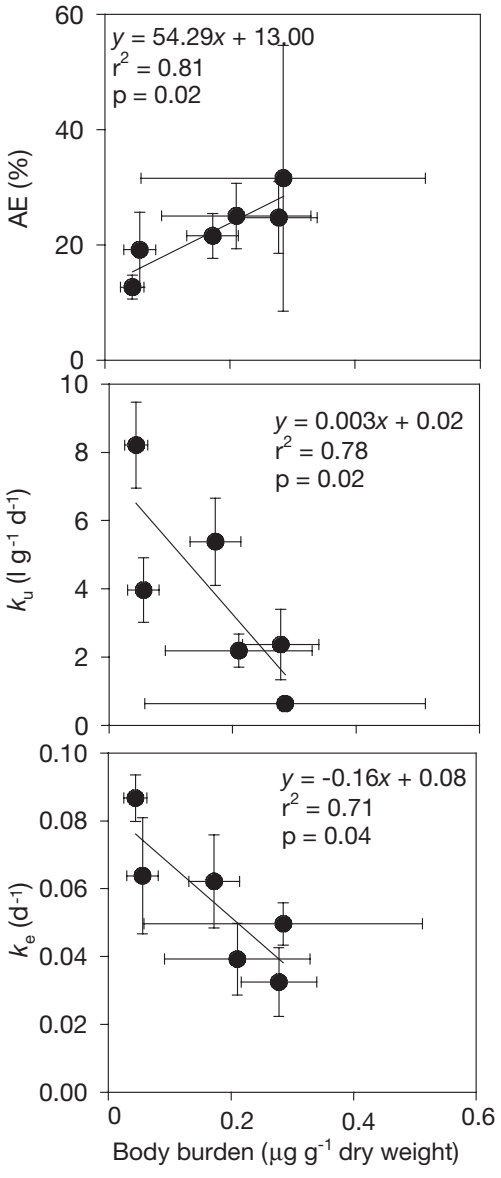

Fig. 9. *Perna viridis*. Correlations between Ag uptake (k_u) and loss kinetics (k_e) and body accumulation (AE) in the green mussels. Data were fitted with the linear regression model. r^2: regression coefficient

mussels (Fisher & Wang 1998). The estuarine population had relatively more Ag in the MTLP (2- to 4-fold); thus, MTLP may also share some detoxification roles.

These results demonstrated variation in Ag uptake from the dissolved phase between the populations. Ag geochemical speciation may be more strongly affected by salinity at low ranges (e.g. 5 to 20 psu) compared to intermediate ranges (e.g. 20 to 30 psu) (Ke & Wang 2001). In the pre-wet season, the salinity at the estuarine site (22 psu) may not be low enough to increase dissolved uptake rate significantly compared to the harbour site (30 psu). During the wet season, the higher Ag uptake in the estuarine population was probably a result of an increasing free-Ag-ion concentration with a decreasing chloride complexation and presumably a change in membrane permeability (Campbell 1995, Blackmore & Wang 2003). In the dry season, however, Ag uptake was higher in the estuarine population despite the comparable salinities at both sites. There is evidence to suggest that other chemical forms of Ag, besides the free ions, are also involved in its biological uptake (Engel et al. 1981).

Physiological factors, such as the clearance rate, may not account for the variation in the Ag uptake rate between the populations. For example, clearance rates of both populations in the pre-wet season were comparable, but the k_u of Ag was different between the populations. A previous study also suggested that clearance rate did not explain the intra-species variation in metal uptake in marine bivalves, since the metal uptake was not diffusion limited (Wang 2001). In addition, neither gill size nor apparent water permeability was significantly different between the populations from the 2 distinct hydrographic zones (Blackmore & Wang 2003).

Assimilation from the natural seston may provide relevant information on Ag uptake from food sources in the field. The natural seston often consists of diverse biological and inorganic particles, including algal cells and resuspended sediment. Previous studies found that the Ag AEs from the natural seston were generally in the lower range of AEs measured for diverse phytoplankton foods (Wang & Fisher 1996). In this study, the AEs of Ag from the natural seston were comparable (except the pre-wet season) to the AEs from the diatoms; thus, its AE was less dependent on food quality. However, it was possible that most of the radiolabelled Ag was associated with the phytoplankton during the radiolabelling of natural seston (since there was growth of the standing phytoplankton); thus, the difference in terms of food quality between the radiolabelled natural seston and diatoms may be small. Food quality may be an important factor in the AE of other metals, such as Cd (Wang & Fisher 1996), which was often lower from the natural seston than from pure diatoms. Wong & Wang (2003) showed that the AEs of Cd from diatoms and sediments varied by a factor of 1.5 to 4.2 in the green mussels. The AE of Cd also varied 2.3-fold (11 to 25%) among 5 phytoplankton diets and 1 natural seston (Chong & Wang 2000). In general, there was no seasonal or inter-population variation in Ag AEs (diatom or natural seston), even when there was variation in salinity and were possible differences in food quality between the locations. Blackmore & Wang (2003) also demonstrated that AEs of Cd, Cr and Zn were not affected by salinity.

Ag uptake from the dissolved and dietary phases had opposing or different relationships (few or no relationships in the dietary uptake) with its subcellular distribution, implying that detoxification was different between the 2 Ag exposure routes. This study provides the first significant correlation between the uptake kinetics and the subcellular distribution of Ag. Such results may have important implications for further understanding the control of Ag intracellular speciation in bivalves in metal accumulation from the natural environment. The significantly positive correlation between the dissolved Ag uptake and its partitioning in the MTLP and HSP fractions suggested that MTLP is important for Ag detoxification during dissolved exposure and that faster Ag uptake causes a higher toxicity to the cellular components. In contrast, Ag assimilation from the dietary phase was positively related to Ag partitioning in the insoluble fraction and negatively related to the Ag distribution in HSP, suggesting that the assimilated Ag may largely be associated with the insoluble fraction for detoxification. Furthermore, the Ag AE was found positively related to a high Ag body burden, whereas the Ag dissolved uptake was negatively correlated with a high Ag body burden in the mussels. These measurements in the field-collected green mussels were rather remarkably comparable to the observations in the laboratory pre-exposed mussels (Shi et al. 2003). Shi et al. (2003) demonstrated that after the green mussels were exposed to Ag at an elevated body concentration, the Ag AE increased, whereas the dissolved uptake decreased with increasing Ag tissue concentration up to 3.2 µg g^{-1}.

Previous studies showed that efflux rates of metals in the bivalves are relatively constant (e.g. 0.01 to 0.03 d^{-1} in clams and mussels) and do not vary considerably under natural conditions (reviewed in Wang 2003) or under different metal exposure conditions (Blackmore & Wang 2002, Shi & Wang 2004). These results indicated that there were inter-population differences in the efflux rates of the green mussels. Ag was eliminated 2 times faster in the estuarine population than in the harbour population. A faster efflux was significantly related to a higher fraction of Ag partitioned in the HSP, implying that the percentage of Ag binding to some subcellular fractions may result in the differences in the Ag efflux rates in the 2 populations. Ag from food may bind with metal-rich granules as an Ag sulphide complex (Berthet et al. 1992). The higher Ag associated with HSP in the estuarine population may cause a faster efflux in order to protect the tissue from toxic effects. Conversely, a higher Ag associated with HSP results in a lower percentage in IF, causing a slower removal by the mussels. Interestingly, Ke & Wang (2001) also demonstrated a faster efflux of Cd, Se and Zn (2.6- to 4.4-fold) in the estuarine oyster *Crassostrea rivularis* than the coastal oyster *Saccostrea glomerata*. In addition, the negative correlation between the Ag efflux and the Ag body burden was consistent with the observations of Shi et al. (2003) on the laboratory pre-exposed green mussels. When the Ag body burden was elevated to 3.2 µg g^{-1}, there was essentially little efflux of Ag from the mussel tissue due to the strong binding of Ag with the sulphide complex.

Estuarine and harbour mussels have contrasting Ag accumulation strategies. The harbour population accumulated more Ag than the estuarine population, and this pattern was found almost consistently in all seasons. Both Ag geochemistry and metal physiology (e.g. Ag uptake and loss kinetics) may explain the observed differences in the body burdens between the populations. It is possible to predict the likely Ag concentrations in green mussels based on the measurements of Ag biokinetics. Since the background dissolved Ag concentration was below the detection limit, a few assumptions were made in the predictions. First, the dissolved Ag concentration was assumed to be 5 ng l^{-1}, a typical concentration observed in unpolluted coastal environments (Flegal et al. 1991). Second, a typical Ag partition coefficient (Fisher et al. 2000) was used and the Ag dissolved concentration to calculate the likely Ag concentration in the ingested particles by the mussels. The kinetic bioaccumulation equation described in Wang et al. (1996) was then used to calculate the likely Ag concentration in the mussels. The calculation indicated that the predicted Ag concentrations in the harbour mussels (0.10 to 0.53 µg g^{-1}) were indeed very close to the measured Ag concentrations in mussels (0.21 to 0.29 µg g^{-1}). For the estuarine location, however, the predicted Ag concentrations (0.37 to 0.55 µg g^{-1}) were about 2.8- to 10-fold higher than the actual measurements (0.056 to 0.172 µg g^{-1}). Such overestimation was probably due to the very high dissolved uptake of this population and the high dissolved Ag concentration used in this modelling calculation.

To conclude, this study suggests that a difference in Ag biokinetics may partially account for the inter-population difference in Ag bioaccumulation in green mussels. Such differences were possibly related to the different subcellular partitioning in the mussels. It demonstrated that control of the influx and efflux of Ag in the green mussels and the role of each subcellular fraction is exposure-route-dependent. This study may be helpful in understanding the Ag bioaccumulation in different populations of marine bivalves collected from different environments. Given the importance of marine bivalves as biological monitors around different harbour and estuarine regions, both at national and international scales, differences in their biokinetics clearly need to be understood before a sound interpretation of Ag pollution history and Ag bioavailability can be conducted.

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