Vol. 277: 135–145, 2004 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published August 16

Relationships between metallothioneins and metal accumulation in the whelk *Thais clavigera*

Graham Blackmore, Wen-Xiong Wang*

Department of Biology, The Hong Kong University of Science and Technology (HKUST), Clear Water Bay, Kowloon, Hong Kong SAR

ABSTRACT: Whelks *Thais clavigera* were collected from field populations containing different concentrations of metallothioneins (MTs) or were pre-exposed to aqueous or dietary Cd in the laboratory to result in differential body MT concentrations. Cd, Hg and Zn uptake from the dissolved phase, assimilation efficiency from the dietary phase, and body burden were subsequently quantified. MT concentrations in whelks collected from 11 sites in the coastal waters of Hong Kong were significantly correlated with Cd body concentrations, but were not correlated with Cu and Zn body concentrations. There was a clear relationship between the dissolved Cd exposure and the resultant MT levels in the animals, but no such relationship was found following dietary exposure to Cd. The assimilation of Cd and Hg increased in whelks originating from impacted field populations with higher MT levels and following inducement of MTs by pre-exposure to dissolved or dietary Cd. Zn assimilation was comparable in the field populations with different MT levels, but decreased in groups with higher MTs following pre-exposure to Cd, presumably due to the saturation of MTs by Cd binding, and thus less MT binding sites were available for Zn. MTs may thus play an important role in metal assimilation. In contrast to dietary assimilation, metal uptake from the dissolved phase in whelks was unaffected by MT body concentrations. Dissolved uptake appears to be largely a physico-chemical process and is little affected by the intracellular physiological changes made by the animals. Given the ubiquity of MTs in marine animals and the importance of dietary sources in metal accumulation, the influence of MT should be considered in interpreting body metal concentrations, and the use of MTs as biomarkers of metal contamination should be treated with caution.

KEY WORDS: Whelk · Metallothioneins · Metals · Pre-exposure · Assimilation · Biomonitoring

INTRODUCTION

Detection of biomarkers as measurable biochemical and/or physiological alterations may serve as an early warning when compared to other parameters such as growth or reproductive abnormalities, which may not be detected until it is too late to take countermeasures (Langston et al. 2002). Metallothioneins (MTs) are a group of low molecular weight, heat stable and cysteine-rich metal binding proteins which normally bind with metal ions, e.g. Cd^{2+}, Hg^{2+} and Zn^{2+}, and are present in many aquatic species (Roesijadi 1992). They play an important role in the storage and supply of essential metals such as Zn and Cu, which are used during protein synthesis, as well as the detoxification of non-essential metals such as Cd and Hg (Hamer 1986, Roesijadi 1992, 1996, Palmiter 1994, Langston et al. 1998). MT synthesis occurs under conditions of elevated metal concentrations, thereby binding excess cellular concentrations of metals. In mammals, Zn is the most potent inducer of MT synthesis (Palmiter 1994), whereas in marine bivalves Cd is a much stronger inducer of MTs than Zn (Langston et al. 1998). MTs have a short biological half-life (e.g. 25 d in the mussel *Mytilus edulis*) (Bebianno & Langston 1993) and concentrations will decrease accordingly once metal stress is reduced. MT concentrations are also generally correlated with metal concentrations (George & Olsson 1994), and as such MTs have been suggested as potential biomarkers for metal pollution

*Corresponding author. Email: wwang@ust.hk

(Roesijadi 1994). The influence of MT induction on metal bioaccumulation is not well known. To apply MT assays in environmental assessments, however, there must first be validation of the responses of the proposed monitoring species, given the diversity and function of MTs. Furthermore, metals are not the only factors affecting MT levels and expression. For example, temperature, salinity, reproduction state, nutritional state, age/size, and growth rate have all been shown to affect tissue MT concentrations (Olsson et al. 1987, Overnell et al. 1988, Mouneyrac et al. 1998, 2000, Leung & Furness 1999a,b, Legras et al. 2000, Leung et al. 2000). The high in sensitivity and a measurable response to metal exposure outweigh these shortcomings (Langston et al. 2002). Few studies have however focused on the induction of MTs in marine molluscs following exposure to metals via a dietary route (Blackmore & Wang 2002, Shi et al. 2003).

Little attention has focused on the effects of MTs on metal bioaccumulation. Blackmore & Wang (2002) documented that dietary assimilation of Cd, and under certain conditions, Zn, increased in mussels pre-exposed to Cd. This increase corresponded to an increase in the levels of metallothioneins-like proteins (MTLPs) in the mussels following exposure, suggesting a potential role of MTs in Cd assimilation. Shi et al. (2003) noted a similar increase in Ag assimilation in mussels following pre-exposure to Ag, although there was no concomitant increase in the proportion of MTLPs. Gully & Mason (1993) reported that both Cd and Cu were redistributed in the gills of the marine snail *Littorina littorea* following exposure to Cd. This was related to the affinity of each metal for MTs and the induction of MTs by Cd exposure. These limited studies imply that MTs can have a large effect on metal accumulation.

Interest in metal accumulation in marine molluscs originates from their use as biomonitors of metal pollution (Phillips & Rainbow 1993). It is important to understand any factors affecting body metal concentrations in biomonitors. Metal exposure can cause alterations in an organism's physiology and such changes can potentially affect metal accumulation (Wang 2002), thus there is a need to examine the evolutionary significance of trace metal contamination. MTs have been measured in many marine invertebrates (Roesijadi

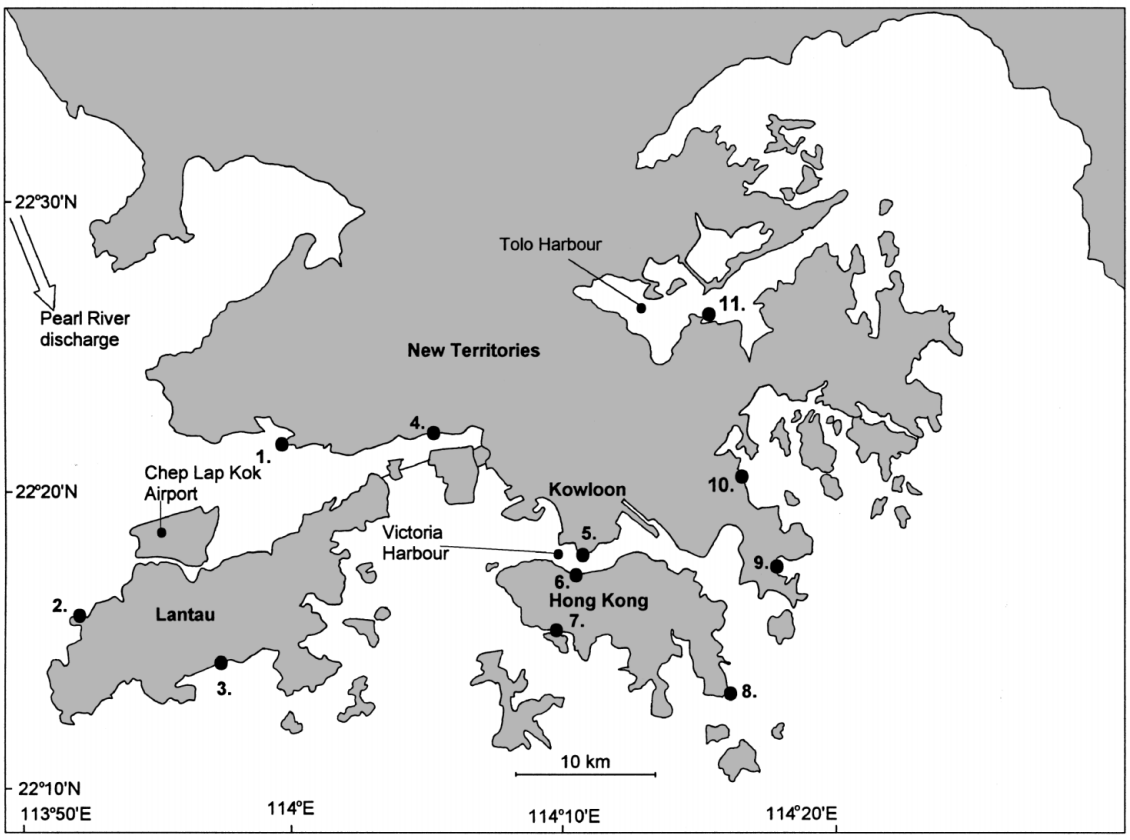

Fig. 1. Hong Kong sampling locations. 1: Tuen Mun, 2: Tai O, 3: South Lantau, 4: Tsing Yi, 5: Queen's Pier, 6: Tsim Sha Tsui, 7: Aberdeen, 8: Cape d'Aguilar, 9: Clear Water Bay, 10: University of Science and Technology (UST), and 11: Starfish Bay

1992) and may even be ubiquitous. MT body concentrations are affected by a range of natural and anthropogenic factors and importantly, as a metal binding protein, they may have a large effect on metal biology and consequently metal accumulation.

To our knowledge, no previous study has examined the influence of metallothionein concentration on metal biokinetics in field-collected marine invertebrates. In this study, we quantified the effect of different MT concentrations on Cd, Hg and Zn accumulation in the whelk *Thais clavigera*. This species has a high concentration of MTs especially when compared to bivalves (G. B. & W.X. W. unpubl. data), thus providing an excellent model to examine the interaction of MTs and metal bioavailability. Previous studies indicated that MTs have an affect on dietary intake of metals (Blackmore & Wang 2002, Shi et al. 2003) and that predatory snails have a dominance of dietary metal uptake (Wang & Ke 2002). We measured metal assimilation efficiency from ingested food and influx from the dissolved phase, and quantified metal body burdens and MT concentrations in whelks that had differential MT concentrations. Whelks with different MT concentrations were obtained from metal-enriched and clean field-populations and by exposure to aqueous and dietary Cd.

MATERIALS AND METHODS

Gastropods and exposure. The effects of MTs on metal accumulation were determined using the whelk *Thais clavigera* from field-populations with elevated MT concentrations and groups that were exposed to aqueous and dietary Cd to induce MT synthesis. Whelks *Thais clavigera* (shell length of 25 to 30 mm and tissue weight of ~0.2 g) were collected from 11 sites in the coastal waters of Hong Kong. The sites chosen (Fig. 1) represent a range of hydrographical and anthropogenic metal enrichment conditions (Blackmore 1998). Sites 1 (Tuen Mun), 2 (Tai O) and 3 (South Lantau) are heavily affected by the low salinity discharges of the Pearl River. Sites 8 (Cape d'Aguilar) and 9 (Clear Water Bay) are wave-exposed, receiving oceanic water from the South China Sea. The University of Science and Technology (UST, Site 10), located within Port Shelter, is more sheltered from the influence of the South China Sea than Sites 8 and 9 but is still far from major urban and industrial developments. The Victoria Harbor sites (Queen's Pier, 5, and Tsim Sha Tsui, 6), Aberdeen (Site 7) and Tsing Yi (Site 4) are at the historical center of the main urban and industrial development areas. Starfish Bay, Tolo Harbor (Site 11), is sheltered and is located on the edge of an area that was developed in the 1980s as industry translocated from the more central areas around Victoria Harbor.

Whelks, collected from piers and rocks, were placed in a clean polythene bag and transported to the laboratory where they were frozen at −80°C. The whelks were subsequently defrosted, but kept cold (<4°C), and the leiblein gland dissected out. The leiblein gland was chosen for MT analysis (methods described below) since it is a sensitive and important gland in MT synthesis (Leung & Furness 1999a). After the MT analysis, 2 populations (Starfish Bay and UST sites) were chosen on the basis of MT body concentrations (as indicated by leiblein gland MT concentration) and local abundance (i.e. ease of collection) for further bioaccumulation study. Starfish Bay whelks were chosen to represent a low MT concentration and UST whelks a high concentration.

In addition to the 2 field populations (Starfish Bay and UST) which were directly used for the bioaccumulation study, we also conducted laboratory Cd pre-exposure to examine the influences of MT induction on metal bioaccumulation. *Thais clavigera* collected from UST (Site 10) were used for the laboratory exposure experiments (Expt 1). They were exposed in groups to dissolved Cd at 50, 100 or 400 µg l^{-1} for 14 d. These concentrations, while environmentally unrealistic, were necessary in order to achieve higher Cd concentrations than the background tissue levels due to the low Cd uptake from the dissolved phase. The whelks were maintained in aerated seawater and kept at a constant temperature of 20°C. Another 3 groups were fed (ad libitum) Cd-enriched mussel *Perna viridis* digestive gland, oyster *Saccostrea cucullata* digestive gland, or the barnacle *Balanus amphitrite*. The prey had previously been exposed to 400 µg Cd l^{-1} for 7 d and then frozen at −80°C. The dissolved exposure and control (clean seawater collected from UST-nominal Cd concentration of 0.055 µg l^{-1}) groups were fed (ad libitum) with the barnacle *B. amphitrite*. After 14 d exposure, a portion of whelks were frozen at −80°C for MT determination (kidney, digestive gland, and leiblein gland) and whole body Cd concentration analysis. The remaining individuals from 3 groups, i.e. those exposed to dissolved 100 µg Cd l^{-1}, those fed Cd-enriched oyster *S. cucullata* digestive glands, and the control, were subsequently used in metal accumulation experiments. Cd and Zn influx from the dissolved phase, and their assimilation efficiencies from ingested food source, were determined in each group, using methods described below.

A second dissolved Cd pre-exposure experiment was conducted with *Thais clavigera* collected from Starfish Bay in order to investigate its influence on Hg assimilation (Expt 2). Whelks were exposed to dissolved Cd at 5, 20 or 100 µg l^{-1} for 14 d. A control group without a Cd spike was also included in the experiment. During the experimental period, whelks were

kept as outlined above. Following exposure, Hg assimilation efficiency and kidney MTs concentrations were determined in each group, using methods described below.

Metal uptake from the dissolved phase. Eight *Thais clavigera* from each group were placed individually into 200 ml of 0.22 µm filtered seawater. The water was spiked with stable metals and radiotracers (^{109}Cd and ^{65}Zn) which were equilibrated overnight. A range of metal concentrations were used in the metal uptake experiments to allow calculation of the uptake rate constant. The levels were 0.5, 2, 8 and 20 µg l^{-1} for Cd, and 2, 8, 20 and 100 µg l^{-1} for Zn. Radioisotope additions were 1.85 kBq l^{-1} for Cd and 3.7 kBq l^{-1} for Zn. The radioactivity of the exposure medium was measured at the start and end of the experiment. Depletion of dissolved Cd and Zn was assumed to be negligible since radioactivity was similar. Care was taken to keep the whelks submerged. Following 24 h exposure, whelks were dissected and the soft tissues radioassayed. The relatively long exposure (24 h) was necessary since previous experiments have shown that dissolved uptake in whelks was much slower as compared to bivalves (Leung & Furness 1999a). The tissues were then dried at 80°C and dry weights determined. Uptake rate were calculated as the amount of metal accumulated by the soft tissues of whelks divided by the exposure duration and standardized to nanogram dry weight per h (ng g^{-1} h^{-1}).

Metal assimilation efficiency. The oyster *Saccostrea cucullata* was radiolabeled following 14 d of aqueous exposure to 37 kBq of ^{109}Cd and 74 kBq of ^{65}Zn (Expt 1), or 37 kBq ^{203}Hg (Expt 2), after which time the prey was considered uniformly labeled. Previous work has shown that uptake from a bivalve prey by a predatory gastropod varies little following prey labeling either by aqueous or food (algae) exposure (Wang & Ke 2002). Oyster digestive glands were chosen as prey because the whelks fed readily on these when compared to other prey. Furthermore, oysters form a common component of the diet in the field (Blackmore 2000), where their digestive gland are selectively preyed upon. Digestive glands were dissected out of the radiolabeled oysters and placed individually in a tray of water. Whelks were then added individually and allowed to feed on the digestive glands for 1 h, after which they were rinsed in seawater and radioassayed. Whelks were then tagged and 10 individuals were placed in 3 l of seawater. Fecal pellets were collected and water was changed regularly (at least twice a day) to minimize desorption of radiotracers. Whelks were fed ad libitum on the barnacle *Balanus amphtrite*. Barnacles were chosen as food because of their small size (i.e. unconsumed tissue would not foul the water). The whelks were radioassayed at 3 to 12 h intervals over the 72 h depuration period. Assimilation efficiencies were determined as the percentage of initial radioactivity retained in *Thais clavigera* after 60 h.

MT determination. MTs were determined using a modified (Leung & Furness 1999a) silver saturation method (Scheuhammer & Cherian 1986). Soft tissues from 15 individual whelks were removed and their wet weight determined. The leiblein gland, kidney and digestive gland/gonad complex were dissected out. These tissues were chosen because they are important and sensitive tissues in MT induction (Leung & Furness 1999a). Care was taken to keep the samples at 4°C or below. The tissues were weighed and then homogenized in 4× vol. of cold 0.25 M sucrose buffer. The homogenate was centrifuged at 4°C at 20 000 × *g* for 20 min. The supernatant was separated from the pellet and frozen at –80°C until MT determination. The supernatant (0.3 ml) was later incubated with 1 ml of 10 µg ^{110m}Ag ml^{-1} (activity 0.18 kBq ml^{-1}) in 0.5 M glycine buffer at room temperature for 10 min. This step saturated the MT binding sites with Ag. Excess Ag was removed with the addition of 0.1 ml rabbit red blood cell haemolysate (Scheuhammer & Cherian 1991), followed by heat treatment (100°C for 5 min) and centrifugation (5 min at 1200 × *g*). Heat treatment caused precipitation of Ag bound to hemoglobin and other proteins but not the MTs, which are heat-stable. The addition of haemolysate, heat treatment and centrifugation was repeated twice more. The amount of Ag present in the final supernatant is proportional to the MT concentration and was determined following radioassaying for ^{110m}Ag. MT concentrations were calculated as 3.54× the Ag concentrations when fully saturated (1 µg Ag ml^{-1} is bound to 3.54 µg MT ml^{-1}) (Scheuhammer & Cherian 1986). All MT concentrations were expressed as µg g^{-1} wet weight tissue units.

Analytical measurements. Radioactivity was measured using a Wallac gamma counter. Spillover of radioisotopes was corrected and all counts were related to standards for each isotope and corrected for radioactive decay. The gamma emissions of ^{110m}Ag were determined at 658 keV, ^{109}Cd at 88 keV, ^{203}Hg at 279 keV, and ^{65}Zn at 1115 keV. Counting times in all samples were adjusted so that the propagated counting errors were typically <5%.

Whelks used to determine body metal concentrations were first depurated for 2 d in clean seawater and then frozen at –80 °C. At a later date, 15 whelks from each group or site were dissected and the soft tissues dried at 80°C to constant weight and then digested at 100°C in concentrated nitric acid. Dilutions were analyzed for Cd, Cu and Zn using Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS). Throughout the analyses, random checks were made using aliquots of a certified reference material (Standard

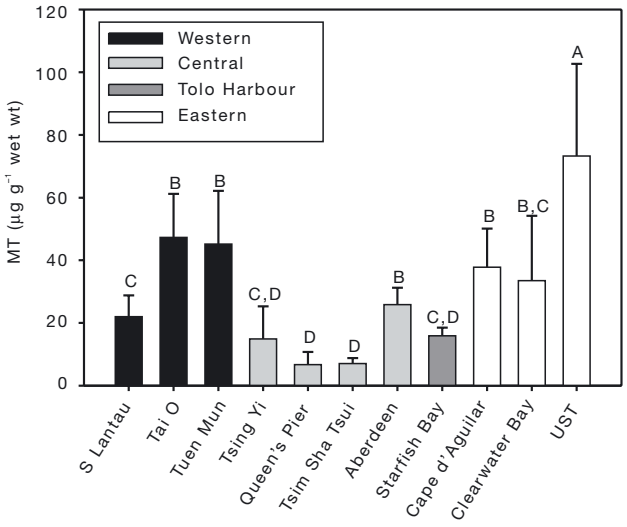

Fig. 2. *Thais clavigera*. Metallothionein (MT) concentrations in whelks collected from 11 sites in the coastal waters of Hong Kong. See Fig. 1 for sampling locations. Mean ± SD (n = 15). Sites sharing the same letter do not differ significantly ($p > 0.05$)

Reference Material 1566a Oyster tissue, National Institute of Standards and Technology, Gaitherburg, MD). Agreement was within 10%. All metal concentrations were expressed as µg g^{-1} dry weight.

Data were tested to confirm the assumptions of parametric tests and investigated using analysis of variance (ANOVA). Unplanned comparisons (post hoc tests) were carried out to establish the ranking with respect to MT concentrations by using multiple comparisons with Bonferroni's correction to determine significance using SAS 6.12.

RESULTS AND DISCUSSION

Relationships between MT and metal concentrations

The MT concentrations in the leiblein gland of *Thais clavigera* collected from around the coastal waters of Hong Kong are shown in Fig. 2. There was significant ($p < 0.01$) inter-site variability in the leiblein gland MT levels. Leiblein gland MT concentrations were highest in those individuals collected from UST and >10× lower in those collected from Queen's Pier, Victoria Harbour. The high MT concentration measured from UST was not accompanied with the relatively low dissolved Cd concentration at this site (0.5 nM), implying that other environmental conditions may be responsible for the MT induction (Leung et al. 2000). The MT levels measured in the present study are comparable to those measured in a temperate species of neogastropod, i.e. *Nucella lapillus* (Leung et al. 2000). Such levels were, however, much lower than those reported from cephalopod digestive glands (Bustamante et al. 2002), but similar to those found in *Littorina littorea* (Leung & Furness 1999b) and much higher than those found in mussels and clams (G.B. & W.X.W. unpubl. data). Fig. 3 shows the relationship between the MT concentrations and the Cd, Cu and Zn body burdens in *T. clavigera*. There was a significant relationship ($p = 0.006$) between Cd body concentrations and MTs but there was no such relationship for Cu ($p = 0.225$) and Zn ($p = 0.657$).

The quantified Cd concentration in the whelks in the control treatments (without Cd exposure) was 12.5 ± 1.6 µg g^{-1} dry weight. Following exposure to dissolved Cd, the Cd tissue burden increased to 22.6 ± 1.5 µg g^{-1}, 41.3 ± 1.4 µg g^{-1}, 130 ± 15.6 µg g^{-1}, in the Cd 50 µg l^{-1}, 100 µg l^{-1}, and 400 µg l^{-1} treatment respectively.

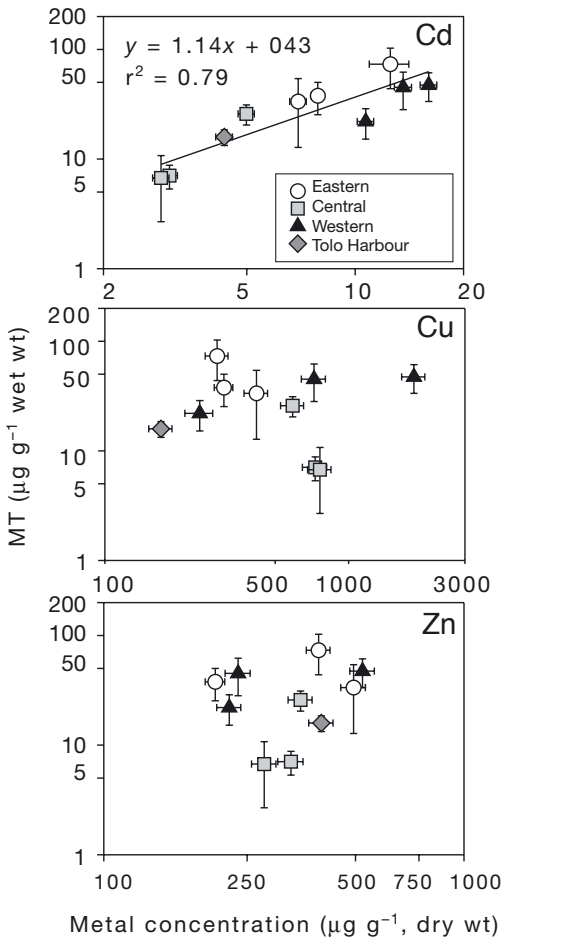

Fig. 3. *Thais clavigera*. Relationship between metallothionein (MT) concentrations and Cd, Cu and Zn body concentrations in whelks collected from 11 sites in the coastal waters of Hong Kong. Mean ± SD (n = 15)

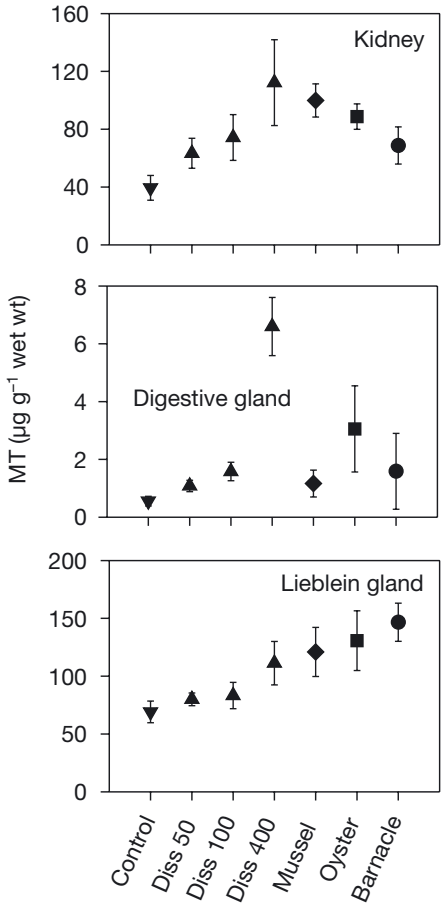

Fig. 4. *Thais clavigera*. Metallothionein (MT) concentrations in different tissues of the whelks following pre-exposure to different dissolved Cd concentrations and dietary Cd from different preys. Diss 50: dissolved Cd concentration of 50 µg l^{-1}; Diss 100: dissolved Cd concentration of 100 µg l^{-1}; Diss 400: dissolved Cd concentration of 400 µg l^{-1}. Mean ± SD (n = 15)

Increases in Cd tissue burden following dietary exposure to different prey appeared to be relatively smaller compared to the aqueous Cd exposure. The Cd tissue burden was 19.3 ± 1.6 µg g^{-1}, 15.6 ± 3.5 µg g^{-1}, 18.6 ± 1.7 µg g^{-1}, following feeding on barnacles, oyster digestive glands, and mussel digestive glands respectively. The MT concentrations in the kidney, leiblein gland and digestive gland of *Thais clavigera* (collected from UST) following 14 d of exposure to aqueous Cd or Cd-enriched food are shown in Fig. 4. In agreement with Fig. 2, the control MT concentrations in the leiblein gland were similar to those of the field-collected *T. clavigera* from UST. This suggests that a 14 d experimental exposure period had very little effect on MT concentrations and presumably MT synthesis in the control treatment. Following exposure to dissolved Cd and Cd-enriched food, MT concentrations increased 1.6 to 2.8× in the kidney, 1.9 to 11.9× in the digestive gland and 1.2 to 2.1× in the leiblein gland when compared to the control. MT concentrations were similar in the leiblein gland and kidney (63.4 to 120 µg g^{-1} wet weight) and were much higher (>30×) when compared to the digestive gland (1.08 to 6.60 µg g^{-1} wet weight). This result agrees with previous work on marine snails, which shows that the MT concentration in the kidney is much greater than in the digestive gland (Leung & Furness 1999a). Furthermore, MT concentrations in the leiblein gland, which is restricted to neogastropods, have been shown to be high and increase dramatically following exposure to Cd (Leung & Furness 1999a). This tissue is therefore considered an important and sensitive tissue for MT induction (Leung & Furness 1999a).

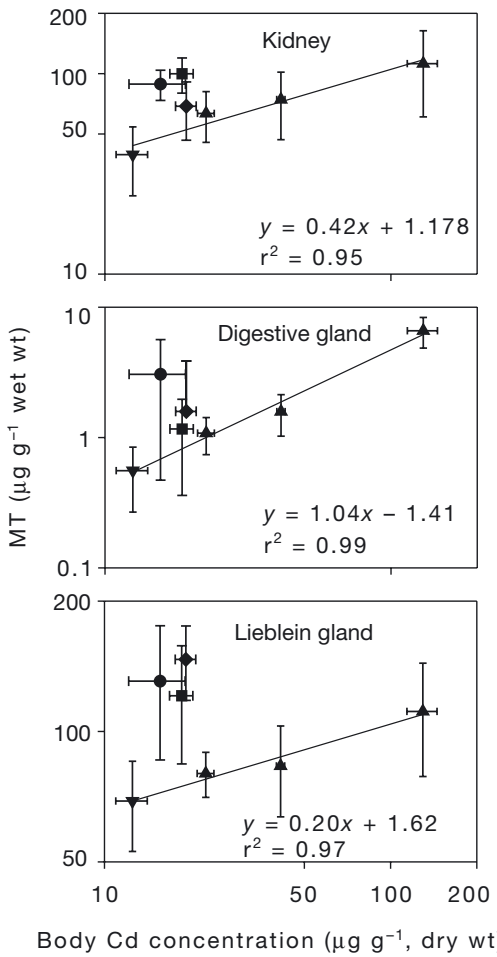

Fig. 5. *Thais clavigera*. Relationship between metallothionein (MT) concentrations and Cd body concentrations in whelks. Linear regression was performed for dissolved groups only. (▲) dissolved exposure; (▼) control; (■) fed on Cd-enriched oyster digestive gland; (●) fed on Cd-enriched barnacle; (◆) fed on Cd-enriched mussel digestive gland. Mean ± SD (n = 15)

In contrast to the results for dissolved Cd exposure, there was no clear pattern of MT concentrations following feeding upon Cd-enriched food (Fig. 4). For example, whelks that had been fed Cd-enriched *Perna viridis* digestive glands had the highest concentrations of MTs in the kidney but the lowest concentrations in the digestive gland, when compared to whelks fed on the other 2 food types. Those individuals that had been fed Cd-enriched *Balanus amphitrite* had the highest levels of MT in the leiblein gland but the lowest levels in the kidney. MT concentrations were ~40 to 90× lower in the digestive glands, when compared to the other 2 tissues. However, in contrast to the dissolved exposure individuals, MT levels were 1.2 to 2.1× higher in the leiblein gland when compared to the kidney. In general, MT concentrations following feeding upon Cd-enriched prey were comparable to those of the dissolved exposure in both the kidney (64.5 to 112 µg g^{-1} wet weight) and the digestive gland (1.08 to 6.60 µg g^{-1} wet weight), whereas MT concentrations were 1.5× greater in the leiblein gland after dietary exposure, presumably due to the important role this tissue plays in digestion.

There was a significant relationship between the MT concentration in all 3 tissues and the Cd body concentration following dissolved Cd exposure (Fig. 5). These results agree with our field-collected *Thais clavigera*, i.e. MT concentrations increase with Cd exposure, which is also reflected in accumulated body burdens. Similarly, George & Olsson (1994) concluded that MT concentrations are generally correlated with metal concentrations. In contrast, there was no clear pattern between Cd body concentration resulting from dietary exposure and MT concentration in any tissue (Fig. 5). MT concentrations for a given body Cd concentration were much higher following food exposure compared to the same body concentration derived from dissolved exposure (Fig. 5). Wang & Ke (2002) reported that for 2 predatory gastropods *Nassarius teretiusculus* and *Babylonia formasae habei*, intake of Cd and Zn is dominated by the dietary sources. Blackmore (2000) and Blackmore & Morton (2002) suggest that diet may be the most important source of both Cu and Zn to *T. clavigera*. This may help explain why there was no correlation between MT concentration and Cu and Zn body concentrations in the field-collected whelks (Fig. 2), since there was no clear relationship between dietary exposure and MT levels. *T. clavigera* can have significantly different body Cu and Zn concentrations depending on the resident prey community (Blackmore 2000). The presence or absence of strong net metal accumulators such as oysters and barnacles is particularly important (Blackmore 2000, Blackmore & Morton 2002). Thus *T. clavigera* Cu and Zn body concentrations often do not agree with the established contamination gradients (Rainbow & Blackmore 2001).

There was however a significant relationship between field MT levels and Cd body burdens. In Hong Kong, there was very little interspecific body Cd concentration variation in the potential prey items of *Thais clavigera* (Blackmore 1999), and body concentrations generally match dissolved metal bioavailabilities. Thus, body concentrations in the predator correspond well to dissolved bioavailabilities, even though diet is the major source of Cd (Bryan et al. 1983). Previous validation of MT as suitable biomarkers of metal contamination has focused on the dissolved exposure. The significance of dietary source for metal bioaccumulation has recently been recognized (Wang & Fisher 1999, Blackmore 2000, Wang 2002). This study further suggests that the route of metal uptake affects resultant MT concentrations. Clearly, this has important implications for the use of MT as a biomarker of metal pollution especially where the dietary source predominates.

Metal-dissolved uptake at different MT concentrations

The influx of Cd and Zn into whelks with differential MT concentrations is shown in Fig. 6. Cd influx in *Thais clavigera* (3.07 to 5.33 ng g^{-1} h^{-1}) was ~5× lower when compared to mussels *Perna viridis*, *Mytilus edulis*, *M. trossulus* and *M. galloprovincialis* (14.1 to 19.2 ng g^{-1} h^{-1}) at a dissolved Cd concentration of 2 µg l^{-1} (Blackmore & Wang 2003a). Similarly, Zn influx was ~4× lower in whelks (14.1 to 20.4 ng g^{-1} h^{-1}) when compared to mussels (37.1 to 50.5 ng g^{-1} h^{-1}) at a dissolved concentration of 8 µg l^{-1}. The relationships between metal influx and ambient Cd and Zn concentrations are shown in Table 1. For all treatments and both metals there was a significant ($p < 0.01$) log–log linear relationship between influx and dissolved concentration. The coefficients of this relationship were close to 1, indicating that uptake proceeded by absorption/facilitated transport and conformed to Freundlich isotherms. These results agree with similar literature for bivalve mussels (Wang & Fisher 1999).

Dissolved uptake rate by *Thais clavigera* varied little ($p > 0.05$) among laboratory treatments and between field populations. MT thus had no clear or consistent effect on the dissolved uptake of Cd or Zn. This result is again in agreement with works on bivalves (Blackmore & Wang 2002, 2003a). For example, dissolved uptake rate among mussel species (*Perna viridis*, *Mytilus* spp.) and among collection locations (England, China and

Table 1. *Thais clavigera*. Relationship between Cd and Zn influx (I_w, ng g^{-1} h^{-1}) and metal concentration (C_w, µg l^{-1}) in whelks with differential MT concentrations. log $I_w = a + b \log C_w$. Data are mean ± SD

Treatment	Cd			Zn		
	a	*b*	r^2	*a*	*b*	r^2
Field population						
UST (high MT)	0.18 ± 0.036	1.02 ± 0.047	0.94	0.47 ± 0.059	1.02 ± 0.046	0.94
SFB (low MT)	0.11 ± 0.041	1.06 ± 0.053	0.93	0.44 ± 0.059	1.02 ± 0.046	0.93
Post hoc		$p > 0.05$			$p > 0.05$	
Lab Cd pre-exposure						
Control	0.33 ± 0.038	0.98 ± 0.049	0.94	0.61 ± 0.049	0.98 ± 0.038	0.96
Dissolved Cd (100 µg l^{-1})	0.33 ± 0.041	1.01 ± 0.062	0.97	0.53 ± 0.075	1.01 ± 0.067	0.98
Cd-enriched diet	0.31 ± 0.030	1.01 ± 0.040	0.93	0.66 ± 0.042	0.93 ± 0.033	0.93
Post hoc		$p > 0.05$			$p > 0.05$	

USA) were largely similar despite differing soft tissue concentrations (Blackmore & Wang 2003a). Furthermore, Blackmore & Wang (2002) reported that Cd and Zn influx were similar in the green mussel *P. viridis* with elevated body Cd and MTLP concentrations and following Cd pre-exposure. Pre-exposure to Zn, however, resulted in a decrease in Cd and Zn influx but there was no increase in MTLP levels. Boisson et al. (1998) found that following chronic exposure to Ag in a contaminated estuary, the clam *Macoma balthica* accumulated Ag at a significantly lower rate than conspecifics collected from a clean estuary, but MT levels were not quantified in their study. In contrast to Cd exposure, Ag (Boisson et al. 1998, Shi et al. 2003) and Zn (Blackmore & Wang 2002) have been shown to trigger reductions in dissolved uptake in bivalves but do not have a clear concomitant effect on MT levels.

Metal assimilation at different MT concentrations

Depuration of Cd, Hg, and Zn in whelks is shown in Fig. 7. Metals were rapidly egested initially, after which much less was lost from the whelks. A higher fraction of Zn was egested as compared to Cd and Hg. These depuration patterns agree with data from other marine invertebrates (Chong & Wang 2000, Rainbow & Wang 2001). Assimilation efficiencies (AEs) were calculated as the amounts of radioactivity left in the organism at 60 h divided by the amount ingested, measured following the pulse radioactive feeding (Table 2). In contrast to the dissolved exposure, AEs were affected by differential MT concentrations. The assimilation of Cd and Zn by *Thais clavigera* was not significantly different (ANOVA: $p = 0.27$ for Cd and $p = 0.38$ for Zn) in whelks collected from Starfish Bay and UST, although Cd AEs were higher in the population (UST) with the greater MT body concentration. Cd AE was 12 to 14% greater (ANOVA: $p < 0.01$) in those individuals that had elevated MT body concentrations following exposure to aqueous and dietary Cd when compared to the controls. Hg assimilation was significantly higher (14 to 28%, $p < 0.01$) following dissolved Cd exposure and concomitant increases in MTs (Table 2) in a separate Cd pre-exposure experiment. In this experiment, the MT concentration in the kidney was 12.7 ± 3.7 µg g^{-1} in the control treatment, and this concentration increased to 15.1 ± 2.6, 31.4 ± 7.0, and 48.1 ± 20.0 µg g^{-1} in

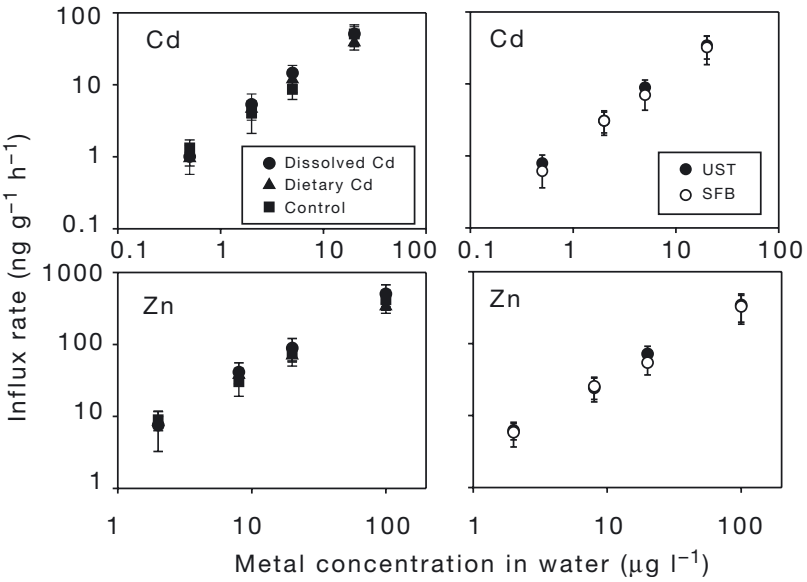

Fig. 6. *Thais clavigera*. Cd and Zn uptake rates in whelks with differential concentrations of metallothioneins following exposure to aqueous or dietary Cd (left panels) or originating from more or less impacted field populations (right panels). SFB: Starfish Bay, Site 11; UST: University of Science and Technology, Site 10. Mean ± SD (n = 8)

the 5, 20, and 100 µg l^{-1} Cd exposure treatments respectively. In contrast, the Zn AE showed the opposite pattern, i.e. assimilation in the groups with higher MT was significantly lower (ANOVA: $p < 0.01$: 79 to 88 %) compared to the controls. Previous studies have indicated that there was considerable variation between AEs obtained within each experiment (Wang 2002). Such variation in AEs may be explained by subtle differences in feeding rates, digestive physiology and condition of the prey.

The assimilation of both Cd and Hg in whelks was generally higher in individuals with a higher MT concentration. The induction of MTs may have provided more binding sites available for Cd and Hg sequestration. However, the overall induction capacity of MT, which may be metal-specific, remains unknown. The results for Zn were more complicated. Following laboratory exposure, there was a negative relationship between Zn AE and MT, but this pattern was reversed in the field populations. Following laboratory Cd exposure it is likely that any synthesized MTs become saturated by Cd binding compared to MT in control groups. Zn has a lower binding affinity for MT compared to Cd (Roesijadi 1996) and would thus be unable to displace Cd. Consequently, the reduction in intracellular Zn binding resulted in a lower assimilation by the whelks. In the field populations MTs may have been induced by a variety of factors and it is unlikely that MTs were saturated by a particular metal, thus Zn AE increased with increasing MT concentration.

Table 2. *Thais clavigera*. Cd, Hg and Zn assimilation efficiencies (AEs, %) in whelks with differential MT concentrations. Data are mean ± SD (n = 5). nm: not measured

Treatment	Cd	Hg	Zn
Field population			
UST (high MT)	90.9 ± 9.2	nm	68.2 ± 8.6
SFB (low MT)	85.5 ± 4.4	nm	62.7 ± 9.8
Post hoc	p = 0.27		p = 0.375
Lab Cd pre-exposure			
Expt 1			
Control (C)	74.0 ± 4.6	nm	66.3 ± 5.0
Dissolved Cd (100 µg l^{-1}) (D)	84.0 ± 4.2	nm	58.4 ± 5.0
Cd-enriched diet (F)	82.7 ± 3.7	nm	52.4 ± 5.8
Post hoc	p < 0.01: D = F > C		p < 0.01 C > D = F
Expt 2			
Control (C)	nm	72.0 ± 5.4	nm
Dissolved Cd (5 µg l^{-1}) (D5)	nm	79.8 ± 6.0	nm
Dissolved Cd (20 µg l^{-1}) (D20)	nm	81.2 ± 4.8	nm
Dissolved Cd (100 µg l^{-1}) (D100)	nm	92.3 ± 6.0	nm
Post hoc	p < 0.01: D100 > D20 = D5 = C		

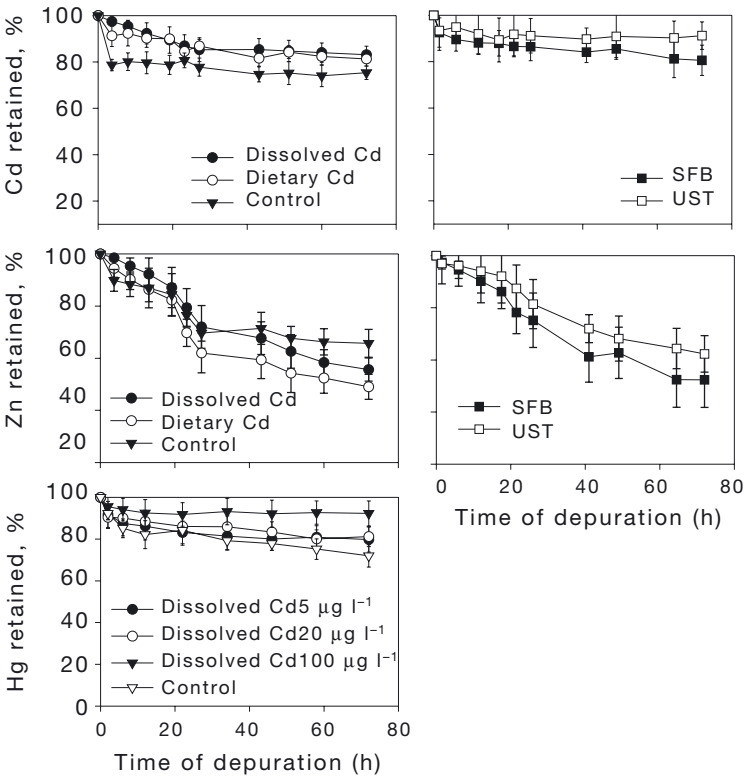

Fig. 7. *Thais clavigera*. Retention of Cd, Hg, and Zn in whelks with differential concentrations of metallothioneins following exposure to aqueous or dietary Cd (left panels) or originating from more or less impacted field populations (right panels) following a pulse ingestion of radiolabeled oyster *Saccostrea cucullata* digestive glands. SFB: Starfish Bay, Site 11; UST: University of Science and Technology, Site 10. Mean ± SD (n = 5)

Our results for metal AE were in contrast to dissolved metal influx, where no effect was noted in individuals with differential metal MT levels. Other studies have found that dissolved uptake is typically less sensitive to intracellular physiological changes (e.g. synthesis of MTs) (Blackmore & Wang 2002) and differential body metal burdens (Shi et al. 2003, Blackmore & Wang 2003a). Dissolved uptake is a passive process and is controlled largely by physico-chemical factors, e.g. changes in the free ion concentration. Physiology may, however, affect dissolved metal uptake particularly where membrane permeability has been changed (Rainbow 1995, Rainbow & Black 2002, Blackmore & Wang 2003b).

Our study suggests a link between MT levels and increased assimilation of metals by whelks from dietary sources, in particular for Cd and Hg. This is true for field populations

with differential MT levels and for whelks in which MTs were induced following laboratory exposure to Cd. It is interesting to note that there was a clear relationship between MT concentration and dissolved Cd exposure and Cd field body burdens. The importance of metal accumulation from dietary pathways in marine animals is becoming increasingly clear. There was, however, no apparent relationship between MT concentrations and body Cd concentrations following dietary exposure to Cd. Given the dominance of dietary metal uptake, the use of MTs as biomarkers of metal contamination should be treated with caution. Furthermore, relationships between dietary metal accumulation and biomarker expression remain to be further investigated.

Acknowledgements. We thank 4 anonymous reviewers for their useful comments on this work. This study was supported by a Competitive Earmarked Research Grant from the Hong Kong Research Grants Council (HKUST6097/02M) to W.X.W. G.B. was also supported by a postdoctoral fund from the Hong Kong University of Science and Technology.

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: October 20, 2003; Accepted: April 8, 2004
Proofs received from author(s): August 3, 2004