

Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically available metal (TAM) in the brine shrimp *Artemia franciscana*

David R. Seebaugh^{1,2}, William G. Wallace^{1,*}

¹Center for Environmental Science, College of Staten Island, 65-310, City University of New York, 2800 Victory Boulevard, Staten Island, New York 10314, USA

²Present address: Department of Biology, Graduate School and University Center, City University of New York, 365 Fifth Avenue, New York 10016, USA

ABSTRACT: Studies have shown that the trophic transfer of certain metals in aquatic systems may be controlled by the internal distribution of metal within prey and that this distribution may be influenced by detoxification mechanisms (i.e. metals bound to metal-binding proteins [metallothioneins, MT] may be more available to predators than metals associated with insoluble cellular constituents). The purpose of this investigation was to examine the interactive effects of Cd and Zn exposure on the accumulation and subcellular distribution of these metals in the brine shrimp *Artemia franciscana*. Particular attention was given to the partitioning of metals to a subcellular compartment containing heat-stable proteins (HSP) (e.g. MT), heat-denatured proteins (HDP) (e.g. 'enzymes') and organelles, here considered as trophically available metal (TAM). Adult *A. franciscana* were exposed for 3 d to Cd (control, 1, 89 or 445 μM and Zn-control) or Zn (control, 1, 89 or 445 μM and Cd-control) through solution using radioisotopes (¹⁰⁹Cd and ⁶⁵Zn) as tracers of stable metals. Following exposure, various operationally defined subcellular fractions were obtained. Increased binding of Cd to HSP resulted in increased partitioning of Cd to the TAM compartment (i.e. TAM-Cd% increased from 57 to 80 % over the range of Cd exposures). This increase in TAM was greater than proportional at the 1 μM Cd exposure concentration and could result in a 'bioenhancement' of Cd trophic transfer to predators. Exposure to elevated concentrations of Cd (89 and 445 μM) also resulted in a ~50% suppression in Zn accumulation by *A. franciscana*. Upon increasing Zn exposure, a shift in the subcellular partitioning of Zn among TAM fractions (from HSP to HDP and organelles) maintained a TAM-Zn% of ~65%. As a verification of the TAM concept, a direct relationship was observed between the partitioning of Cd and Zn to the TAM compartment of *A. franciscana* exposed to the Cd-control/Zn-control treatment and absorption of these metals by the grass shrimp *Palaemonetes pugio*.

KEY WORDS: Trophically available metal · TAM · Subcellular partitioning · Metallothioneins · MT · Bioenhancement · *Artemia franciscana* · Cadmium · Zinc

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Environmental exposure to metal pollutants can exert numerous toxic effects on individuals and may have community-wide consequences (Sullivan et al. 1983, Klerks & Levinton 1989a, Wallace et al. 2000). In aquatic ecosystems, metals (e.g. Cd, Cr, Ag, Se, Zn, and methylmercury) can be accumulated by biota and transferred to predators (Riisgård & Hansen 1990,

Fisher & Reinfelder 1995, Wallace et al. 1998, Ni et al. 2000). Since the ingestion of metal-contaminated food can serve as a source of metals to predators and can result in sublethal toxicity, understanding the mechanisms that influence metal trophic transfer is a critical step in the management of metal-contaminated ecosystems (Wang et al. 1999, Ni et al. 2000, Wallace et al. 2000, Fisher & Hook 2002, Wallace & Luoma 2003).

*Corresponding author. Email: wallace@postbox.csi.cuny.edu

One poorly understood process in the cycling of metals in aquatic systems is the relationship between tissue burdens in prey and metal trophic transfer. It has been suggested that metal assimilation by predators may be related to the internal partitioning of metals within prey (Reinfelder & Fisher 1994, Wallace & Lopez 1996, Wang et al. 1999, Baines et al. 2002). For example, studies have shown that metals associated with cytosolic proteins and organelles are readily available to predators, while metals bound to cell walls and metal-rich concretions are less available (Reinfelder & Fisher 1994, Wang & Fisher 1996, Wallace & Lopez 1997, Wallace et al. 1998). Since internal metal distributions can be modulated by detoxification, these mechanisms may ultimately influence the transfer of metal to predators (Nott & Nicolaidou 1989, 1990, Wallace & Lopez 1997, Wallace et al. 1998, 2003, Wallace & Luoma 2003).

A principal metal detoxification strategy in aquatic organisms involves the synthesis of metal-binding proteins (metallothioneins, MT) (Roesijadi 1981, 1992). MT are inducible, heat-stable, low molecular weight, metal-binding proteins that play an important role in the regulation of essential metals (e.g. Zn and Cu) and in the sequestration of non-essential metals (e.g. Cd and inorganic Hg) (Cherian & Goyer 1978, George 1982, Kagi & Schaffer 1988, Roesijadi 1992). These proteins may also serve to transport metals to sites of metal-rich granule precipitation (George 1982, 1983). The induction of MT can result from exposure to metals through solution and food, and has been shown to result in high metal body burdens (Klerks & Bartholomew 1991, Roesijadi 1992, del Ramo et al. 1993, Wallace et al. 2000). MT induction can also lead to increased metal tolerance and may play a role in genetic resistance (Klerks & Levinton 1989b, Klerks & Bartholomew 1991). The quantification of MT in field-collected organisms may also serve as a bioindicator of metal pollution in aquatic ecosystems, although recent studies have questioned the validity of this type of bioassay (Hennig 1986, Mouneyrac et al. 2001, 2002). Wallace & Luoma (2003) have suggested that increased partitioning of metal to a subcellular compartment presumed to contain trophically available metal (TAM) may act to 'bioenhance' metal transfer along aquatic food chains and may be related to the concentration of metal associated with MT. MT-driven 'bioenhancement' of metal trophic transfer may, in part, account for the variable assimilation efficiencies observed in predators ingesting different metal-contaminated prey (Ni et al. 2000).

Cd is a borderline (marginal Class B), non-essential metal that can displace essential metal ions and interfere with the functional characteristics of proteins because of its high affinity for sulphhydryl groups

(Nieboer & Richardson 1980, Mason & Jenkins 1995). Zn is a borderline (with Class A characteristics), essential metal that plays a role in the function of several enzymes (Nieboer & Richardson 1980, Mason & Jenkins 1995). Although both metals induce MT, Cd has been shown to be more effective than Zn in inducing synthesis in a number of organisms, including a strain of *Artemia parthenogenetica* (Webb 1987, Martínez et al. 1999).

Artemia sp. has been found to produce Cd- and Zn-binding isoforms of a heat-stable, low molecular weight, metal-binding protein that have been isolated from dormant cysts as well as 24 and 48 h old nauplii (Acey et al. 1989). Cd-binding MT have also been isolated from adult *Artemia* spp. following exposure to Cd through solution (Martínez et al. 1991, 1999, del Ramo et al. 1993, 1995).

Compared to other crustaceans, *Artemia* spp. are able to tolerate high levels of Cd, although there is a wide range of Cd sensitivities among species (i.e. 24 h lethal concentration, LC_{50} , values range from ~830 μ M [*A. franciscana*] to ~2500 μ M Cd [*A. persimilis*]) (Eisler 1971, Jennings & Rainbow 1979b, Sarabia et al. 2002b). *Artemia* spp. can accumulate Cd through solution and food, and exposure interferes with emergence and hatching and may impact reproduction (Jennings & Rainbow 1979b, Bagshaw et al. 1986, Rafiee et al. 1986, Sarabia et al. 2002a). The cyst shell of *Artemia* spp. appears to be impermeable to Cd, which may limit exposure during early stages of development (Thall & Acey 1985, Rafiee et al. 1986). Exposure to Zn through solution has also been shown to interfere with larval development (Bagshaw et al. 1986).

Due to its widespread availability and ease of laboratory maintenance, *Artemia franciscana* was chosen as a test organism to investigate the influence of Cd and Zn exposure on the accumulation of metal and the potential for metal transfer to predators (Dhont & Sorgeloos 2002). Additionally, these small crustaceans are used routinely in studies examining the toxicity and trophic transfer of metals, as well as in aquaculture, and are important prey for fishes and a variety of other crustaceans, including *Palaemonetes* spp. (Persoone & Castritsi-Catharios 1989, Petrucci et al. 1995, Ni et al. 2000, Wallace et al. 2000, Dhont & Sorgeloos 2002, Van Stappen 2002).

The purpose of this investigation was to examine the interactive effects of Cd and Zn exposure on the accumulation and subcellular distribution of metals in the brine shrimp *Artemia franciscana*. Since whole tissue metal concentrations within prey may not accurately reflect metal availability to predators, a subcellular compartment containing trophically available metal TAM (heat-stable proteins [e.g. MT], heat-denatured proteins [e.g. 'enzymes'] and organelles) was con-

structed as a means to estimate the potential for Cd and Zn trophic transfer to predators of *A. franciscana* (Wallace & Luoma 2003).

MATERIALS AND METHODS

Maintenance of *Artemia franciscana*. Adult *A. franciscana*, obtained from a commercial supplier (Newman's Fish Food), were maintained in a glass aquarium containing 76 l of aerated seawater (20 ppt, 21–22°C) (Instant Ocean®, Aquarium Systems) for 3 to 5 d prior to use in experiments. The aquarium was equipped with a 500 µm mesh wall that partitioned the tank into 2 chambers (4:1 by volume). Water filtration was provided by a Penguin® Bio-Wheel 125 filter (Marineland) equipped with a modified inlet siphon that extended into the smaller of the 2 chambers, with overflow dripping into the larger chamber containing *A. franciscana*. This system maintained the water quality of the holding tank, yet prevented the entrapment of brine shrimp within the filter. *A. franciscana* were fed daily on ground OSI® Spirulina fish flakes (OSI Marine Laboratory). Filtration was suspended for 4 h following feeding. *A. franciscana* were not fed for 24 h prior to use in experiments.

Exposure of *Artemia franciscana* to Cd and Zn. For each treatment, ~2000 *A. franciscana* (~11 g wet wt), were exposed for 3 d to various concentrations of Cd and Zn in 4 l polycarbonate bottles containing 3 l of filtered artificial seawater (0.4 µm filter, 20 ppt, 21–22°C) (~0.66 shrimp ml⁻¹). During exposure, aeration was provided by a 1 ml (35 cm long) glass pipette tip that was connected to an electric air pump via flexible aquarium tubing. Exposure treatments were as follows: a Cd-series comprising control, 1, 89 or 445 µM Cd and Zn-control and a Zn-series comprising control, 1, 89 or 445 µM Zn and Cd-control, yielding a total of 7 treatments. Exposure solutions were prepared by adding appropriate amounts of stock metal solutions (1000 mg l⁻¹ Cd or Zn) and radiotracers (¹⁰⁹Cd and ⁶⁵Zn) to filtered artificial seawater (0.4 µm, 20 ppt) (Instant Ocean® dissolved in NANOpure® deionized water). Control concentrations of Cd and Zn in the artificial seawater were 0.012 mg l⁻¹ (~0.1 µM Cd and ~0.2 µM Zn) or less, based on information provided by Aquarium Systems.

While the estimated concentrations of Cd and Zn in the control exposures (and Zn in the 1 µM Zn/Cd-control exposure) were higher than would be expected in pristine, natural seawater, they were below maximum concentration criteria (0.36 µM for Cd and 1.38 µM for Zn) for marine systems (US EPA 1999, 2001). The 1 µM Cd exposure was ~2.8× the maximum Cd concentration permitted in saltwater and may be

considered environmentally realistic in metal-impacted ecosystems (US EPA 2001). The highest Cd and Zn exposures (i.e. 89 and 445 µM) were included in this study to facilitate comparison with previous studies of metal accumulation by *Artemia* spp. (Jennings & Rainbow 1979b, Blust et al. 1995).

Stock metal solutions were prepared from reagent-grade CdCl₂ or ZnCl₂ and filtered seawater (0.4 µm, 20 ppt). Radioisotopes (¹⁰⁹CdCl₂ and ⁶⁵ZnCl₂, both in 0.5 M HCl) were obtained from Isotope Products. Acidification of exposure solutions due to the addition of radiotracers was offset by 0.1 N NaOH. Radioactivities for each treatment were 2.22 × 10² kBq l⁻¹ for ¹⁰⁹Cd and 7.40 × 10¹ kBq l⁻¹ for ⁶⁵Zn and were verified through radioanalysis of 5 ml samples. Final specific activities among the treatments ranged from 2.03 × 10⁻⁵ to 7.23 × 10⁻² µg kBq⁻¹ for Cd and 5.22 × 10⁻⁵ to 1.12 × 10⁻¹ µg kBq⁻¹ for Zn.

Following exposure, the contents of each bottle were transferred to a 63 µm nylon screen using a peristaltic pump (Masterflex®, Cole Parmer Instrument) fitted with 1 cm-diameter flexible tubing. Brine shrimp were not damaged as they passed through the pump mechanism. *Artemia franciscana* were rinsed 3 times with artificial seawater (20 ppt), separated into batches of ~180 individuals (~1 g wet wt) and stored frozen (-80°C) in 20 ml glass scintillation vials.

Subcellular fractionation. In order to characterize the subcellular distribution of Cd and Zn in *Artemia franciscana*, brine shrimp from each treatment were subjected to subcellular fractionation and tissue digestion to obtain 5 operationally defined subcellular fractions (see below): heat-stable proteins, HSP (e.g. MT), heat-denatured proteins, HDP (e.g. 'enzymes'), organelles, 'insoluble' components and cellular debris (Wallace et al. 1998, Wallace & Luoma 2003). A subcellular compartment containing TAM (HSP, HDP and organelles) was also reconstructed as a means to estimate the potential for Cd and Zn transfer to predators of *A. franciscana* (Wallace & Luoma 2003).

Samples of *Artemia franciscana* from each treatment (n = 4) were thawed, dabbed dry with a Kimwipe®, wet-weighed and homogenized on ice in 3.3 ml of cold 20 mM Tris buffer (pH 7.6) using a Polytron® (Kinematica) tissue homogenizer. The mass of homogenized tissue was typically ~0.25 g wet wt; however, larger quantities (~0.75 to ~1.3 g wet wt) were used for some treatments (i.e. 89 and 445 µM Zn-series [Cd-control]) in order to compensate for weak ⁶⁵Zn signals.

Following homogenization, tissue samples were centrifuged at 500 × g (15 min at 4°C). The pellet was resuspended in 3.0 ml of Tris buffer and heated at 100°C for 2 min. An equal volume of 1 N NaOH was then added and the suspension was heated at 65°C for 1 h. This suspension was centrifuged at 4500 × g

(15 min at 4°C) and the supernatant, containing dissolved cellular debris, was removed. The resulting pellet was resuspended in 6.0 ml of 1 N NaOH and centrifuged a second time (4500 × *g*). Supernatants were combined with previously collected dissolved cellular debris. The remaining pellets, containing exoskeleton and other 'insoluble' components, were collected (Silverman et al. 1983, Wallace & Luoma 2003, Wallace et al. 2003).

The 500 × *g* supernatants were centrifuged at 100 000 × *g* (1 h at 4°C) to produce a pellet containing organelles. The supernatant was heated at 80°C for 10 min and then cooled on ice for 1 h (Bebiano et al. 1992). The heat-treated cytosol was centrifuged at 38 000 × *g* (30 min at 4°C) to pelletize HDP (e.g. 'enzymes'). HSP (e.g. MT) remained in the supernatant. Once isolated, subcellular fractions were transferred to 20 ml scintillation vials and analyzed for ¹⁰⁹Cd and ⁶⁵Zn as described below.

Absorption efficiency (AE) analysis. Grass shrimp *Palaemonetes pugio* (~2.5 cm in length) were collected by dip net from Great Kills Park, Gateway National Recreation Area, Staten Island, New York, and were maintained in aerated seawater (20 ppt, 21–22°C) for 4 d prior to use in absorption efficiency experiments. *P. pugio* were fed daily on OSI® Spirulina fish flakes, but were not fed for 48 h prior to feeding on *Artemia franciscana*. *P. pugio* (n = 5) were placed in a 1000 ml plastic beaker containing 500 ml of seawater (20 ppt) and allowed to feed on ~120 ¹⁰⁹Cd- and ⁶⁵Zn-labeled *A. franciscana* (exposed to the Cd-control/Zn-control treatment). Grass shrimp promptly ingested the presented tissue (<15 min).

Following the ingestion of *Artemia franciscana* tissue, *Palaemonetes pugio* were rinsed with clean seawater, placed in 20 ml glass scintillation vials containing 10 ml of seawater (20 ppt) and radioanalyzed for ¹⁰⁹Cd and ⁶⁵Zn. *P. pugio* were then placed in individual mesh-lined chambers contained within an aquarium (20 ppt, 21–22°C), where they depurated ingested tissue for 5 d (Wallace et al. 1998, Wallace & Luoma 2003). During depuration, *P. pugio* were fed daily on Tetraamin® fish flakes (Tetra Sales). ¹⁰⁹Cd and ⁶⁵Zn activities in the aquarium water were monitored daily through radioanalysis of 5 ml samples and were found to be negligible. *P. pugio* were removed from the aquarium periodically and radioanalyzed for ¹⁰⁹Cd and ⁶⁵Zn. Linear regressions were fit to the physiological loss components of each retention curve (time = 48 to 120 h) and the corresponding y-intercepts were used to estimate ¹⁰⁹Cd and ⁶⁵Zn absorption efficiencies (AE–Cd% and AE–Zn%) (Wallace et al. 1998, Wang & Fisher 1999).

Radioanalysis and tissue-burden calculations. All samples were analyzed for ¹⁰⁹Cd and ⁶⁵Zn by deter-

mining photon emissions (at 88 and 1115 keV, respectively) in a Wallac Wizard™ 1480 automatic gamma counter equipped with a 7.6 cm NaI crystal (Wallac Oy). Counting times for *Artemia franciscana* tissues (whole body and subcellular fractions) (1 to 10 min) and for live *Palaemonetes pugio* (10 to 20 min) were adjusted to maintain propagated counting errors of 5% or less. Radioanalysis data and specific activities of exposure solutions were used to calculate the concentrations of Cd and Zn associated with whole tissues and subcellular fractions of *A. franciscana*. Due to losses of radioactivity (typically <15%) that accrued throughout the fractionation process, percentage distributions of metal among subcellular fractions were calculated based on the total radioactivity recovered subsequent to fractionation. Following Wallace & Luoma (2003), a subcellular compartment containing TAM was reconstructed by combining the percentages, or concentrations, of Cd and Zn associated with HSP (e.g. MT), HDP (e.g. 'enzymes') and organelles (i.e. TAM – Metal% = HSP% + HDP% + organelles%; TAM – [Metal] = [HSP] + [HDP] + [organelles]).

Statistical analysis. Tissue-burden data sets for *Artemia franciscana* were transformed (log₁₀ for concentration data, arcsine for percentage data) and normality was verified using the Shapiro-Wilk's *W*-test. Treatment effects were analyzed using 1-way analysis of variance (Sokal & Rohlf 1981). Differences between means were compared using the Scheffé test and homogeneity of variances were analyzed using Levene's test. In cases when data sets did not meet assumptions of analysis of variance, Kruskal-Wallis ANOVA and Mann-Whitney *U*-tests were performed (Sokal & Rohlf 1981). All statistical analyses were performed using STATISTICA Version 5.1 (Statsoft).

RESULTS

Cd-series (Zn-control): tissue concentrations and subcellular partitioning

Whole body tissue concentrations of Cd in *Artemia franciscana* increased over the range of Cd exposures (Fig. 1a). At the level of 1 μM Cd (~10× control), the concentration of Cd in *A. franciscana* was ~18× times greater than that of the control treatment, suggesting greater than linear accumulation of Cd (1.8×) with respect to exposure concentration. Accumulation of Cd over the range of the Cd-series exposures, however, was not maintained at this level. For example, a 5-fold increase in Cd exposure concentration (from 89 to 445 μM Cd) resulted in only an ~2.3-fold increase in whole-tissue concentration (Fig. 1a). Exposure of *A. franciscana* to elevated concentrations of Cd (89 and

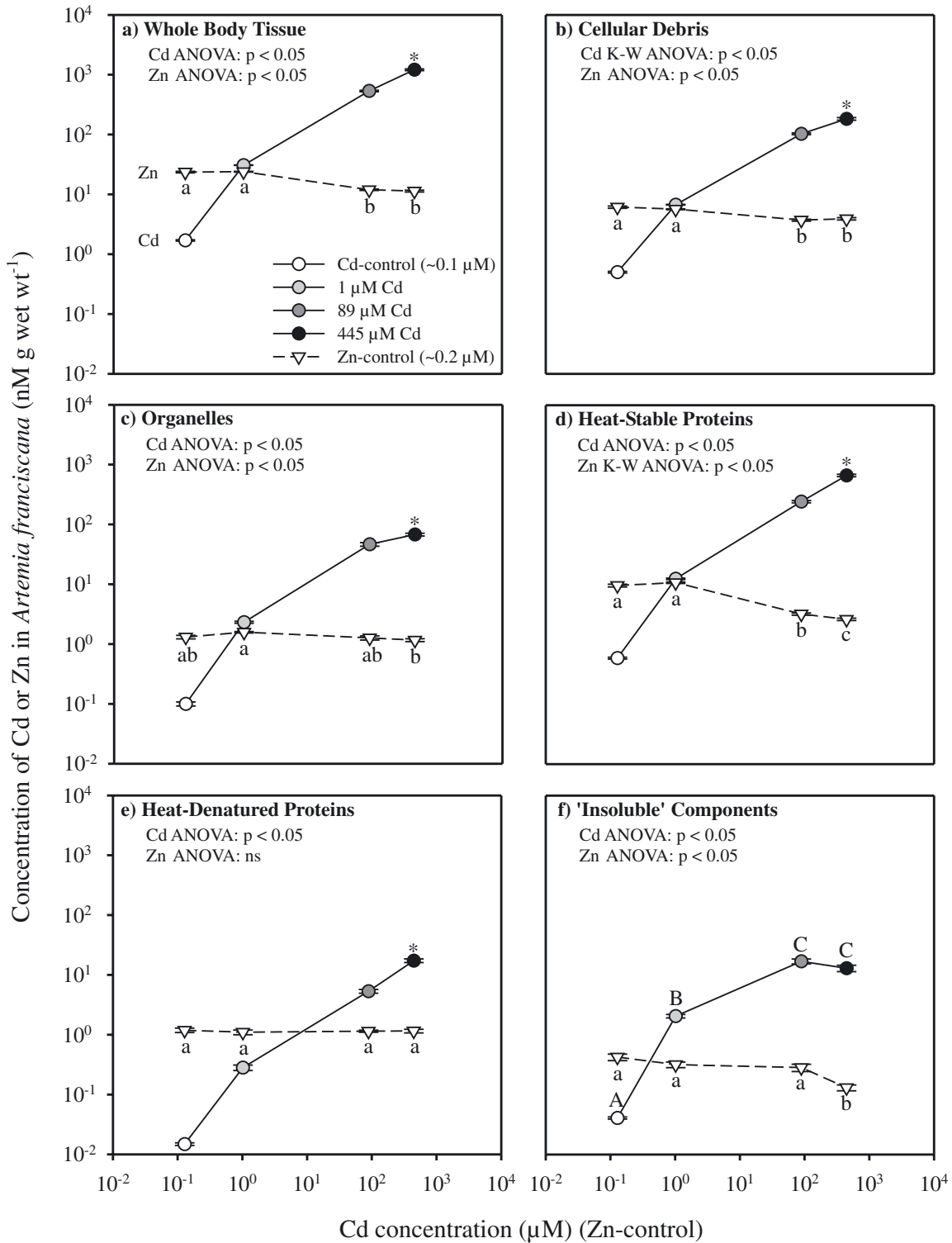


Fig. 1. *Artemia franciscana*. Tissue burdens following Cd-series (Zn-control) exposures, showing Cd and Zn concentrations in (a) whole body tissue and (b-f) subcellular fractions (n = 4; mean ± SE; log-plots) following 3 d aqueous exposure to ~0.1 (control), 1, 89 or 445 μM Cd (log-plots), all with a control concentration of Zn (~0.2 μM). Subcellular fractions were obtained through homogenization, differential centrifugation and tissue-digestion. Results from standard or Kruskal-Wallis (K-W) ANOVA are shown in each graph. Different letters indicate significant differences ($p < 0.05$) among treatment means for Cd (A-C) and Zn (a-c) (Scheffé test for ANOVA data sets; Mann-Whitney *U*-test for K-W data sets). *All mean Cd comparisons significantly different ($p < 0.05$), ns: not significant

445 μM) resulted in an ~50% suppression of whole body tissue Zn concentrations (Fig. 1a).

The pattern of whole body tissue Cd concentrations in *Artemia franciscana* is reflected in the partitioning of Cd to HSP (e.g. MT), HDP (e.g. 'enzymes') and cellular debris (Fig. 1b,d,e). Partitioning of Cd to 'insoluble' components reached saturation at the level of 89 μM , while partitioning to the organelles appeared to approach saturation at the highest Cd levels (Fig. 1c,f). The pattern of whole body tissue Zn concentrations in *A. franciscana* (Fig. 1a) was mirrored by the partitioning of Zn to HSP and cellular debris, as both fractions displayed suppressed Zn concentrations at the highest Cd exposures (Fig. 1b,d). The concentration of Zn bound to constituents within the 'insoluble' fraction was also suppressed at the highest Cd exposure (445 μM Cd) (Fig. 1f). The partitioning of Zn to HDP and organelles was largely unresponsive to increasing Cd (Fig. 1c,e).

Zn-series (Cd-control): tissue concentrations and subcellular partitioning

Whole body tissue concentrations of Zn in *Artemia franciscana* did not reach saturation (Fig. 2a). The only notable influence of increased Zn exposure on Cd accumulation in *A. franciscana* was an elevated whole body tissue concentration at 89 μM Zn (Fig. 2a). The subcellular partitioning of Zn in *A. franciscana* among most of the subcellular fractions matches, in general, the pattern of whole body tissue Zn concentrations (Fig. 2b–f). Although not apparent from whole body tissue Cd concentrations (Fig. 2a), increasing Zn exposure had an influence on the partitioning of Cd to all subcellular fractions (Fig. 2b–f). Most notably, there were increases in the concentrations of Cd bound to organelles and 'insoluble' components at the highest Zn concentrations (Fig. 2c,f). The concentration of Cd associated with HSP was elevated in the presence of 1 μM Zn and suppressed in the presence of 445 μM Zn (Fig. 2d). There was also an increase in the partitioning of Cd to HDP at 89 μM (Fig. 2e). Partitioning of Cd to cellular debris oscillated under the influence of increasing Zn (Fig. 2b).

Cd-series (Zn-control): percentage subcellular distributions

For the Cd-control/Zn-control treatment, the HSP fraction of *Artemia franciscana* was found to contain the largest proportion of accumulated Cd (~47%) (Fig. 3c). Partitioning to the other fractions followed the pattern: cellular debris (~40%) > organelles (~8%)

> 'insoluble' components (~3%) > HDP (~1%) (Fig. 3a,b,d,e). The percentage of Cd partitioned to HSP in *A. franciscana* increased with increasing Cd exposure, while the importance of cellular debris decreased (Fig. 3a,c). The percentage of Cd bound to organelles in *A. franciscana* was highest for the 89 μM treatment (~11%) (Fig. 3b). Partitioning to the 'insoluble' components increased at an exposure of 1 μM and decreased with increasing Cd exposure (Fig. 3e).

The HSP fraction in *Artemia franciscana* from the Cd-control/Zn-control treatment contained the majority of accumulated Zn (~52%) (Fig. 3c). Partitioning to the other fractions followed the pattern: cellular debris (~33%) > organelles ~ HDP (~7%) > 'insoluble' components (~2%) (Fig. 3a,b,d,e). The percentage of Zn bound to HSP decreased sharply (to ~31%) in the presence of Cd concentrations greater than 1 μM (Fig. 3c). At the highest exposures, Zn partitioning increased to ~41% for cellular debris, ~13% for organelles and ~12% for HDP (Fig. 3a,b,d). Partitioning of Zn to the 'insoluble' fraction was generally unresponsive to increasing Cd exposure (Fig. 3e).

Zn-series (Cd-control): percentage subcellular distributions

The partitioning of Zn among the various subcellular fractions was influenced by increasing Zn exposure. For example, the percentage of Zn bound to HSP decreased steadily (from ~52 to ~21%) over the range of increasing Zn (Fig. 4c). Partitioning of Zn to organelles exhibited a commensurate increase over the same range (~7 to ~34%) (Fig. 4b). The percentage of Zn bound to cellular debris oscillated throughout the range of Zn exposures, while partitioning to 'insoluble' components in *Artemia franciscana* reached a plateau at an exposure of 89 μM Zn (Fig. 4a,e). The HDP fraction exhibited increased partitioning of Zn at the highest exposures (Fig. 4d).

Over the range of Zn exposures (Cd-control), the percentage of Cd in *Artemia franciscana* bound to the organelles was similar to that for Zn (Fig. 4b). Partitioning of Cd to cellular debris varied with Zn exposure, and there was little change in partitioning to HDP (Fig. 4a,d). The percentage of Cd bound to HSP increased at 1 μM Zn (to ~63%) and then decreased (to ~30%) for the remaining exposures (Fig. 4c).

Trophically available metal (TAM)

A reconstruction of a subcellular compartment presumed to contain TAM (i.e. the summation of metal associated with HSP [e.g. MT], HDP [e.g. 'enzymes']

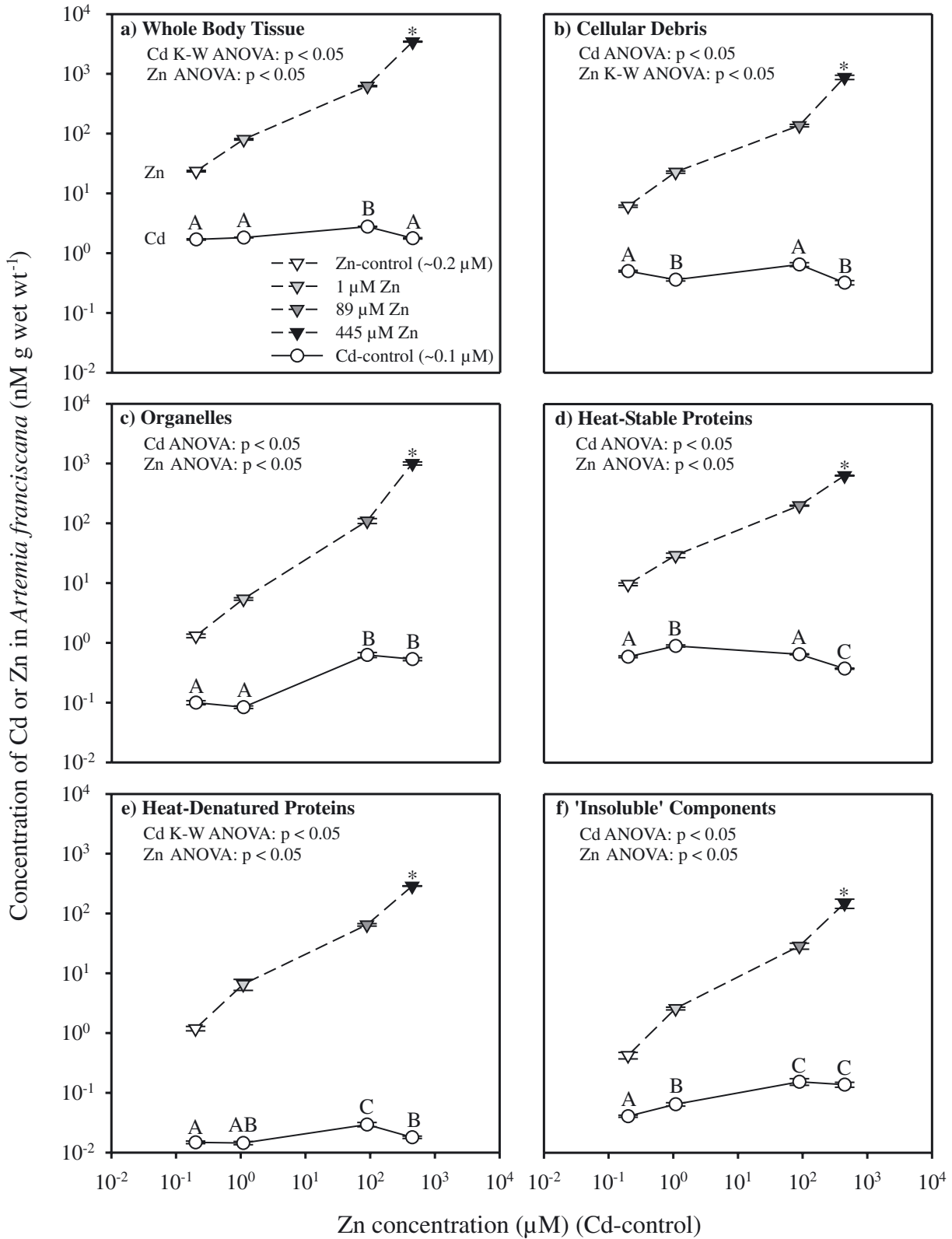


Fig. 2. *Artemia franciscana*. Tissue burdens following Zn-series (Cd-control) exposures, showing Cd and Zn concentrations in (a) whole body tissue and (b–f) subcellular fractions ($n = 4$; mean \pm SE; log-plots) in following 3 d aqueous exposure to ~ 0.2 (control), 1, 89 or 445 μM Zn (log-plots), all with a control concentration of Cd ($\sim 0.1 \mu\text{M}$). *All mean Zn comparisons significantly different ($p < 0.05$); further details as in Fig. 1

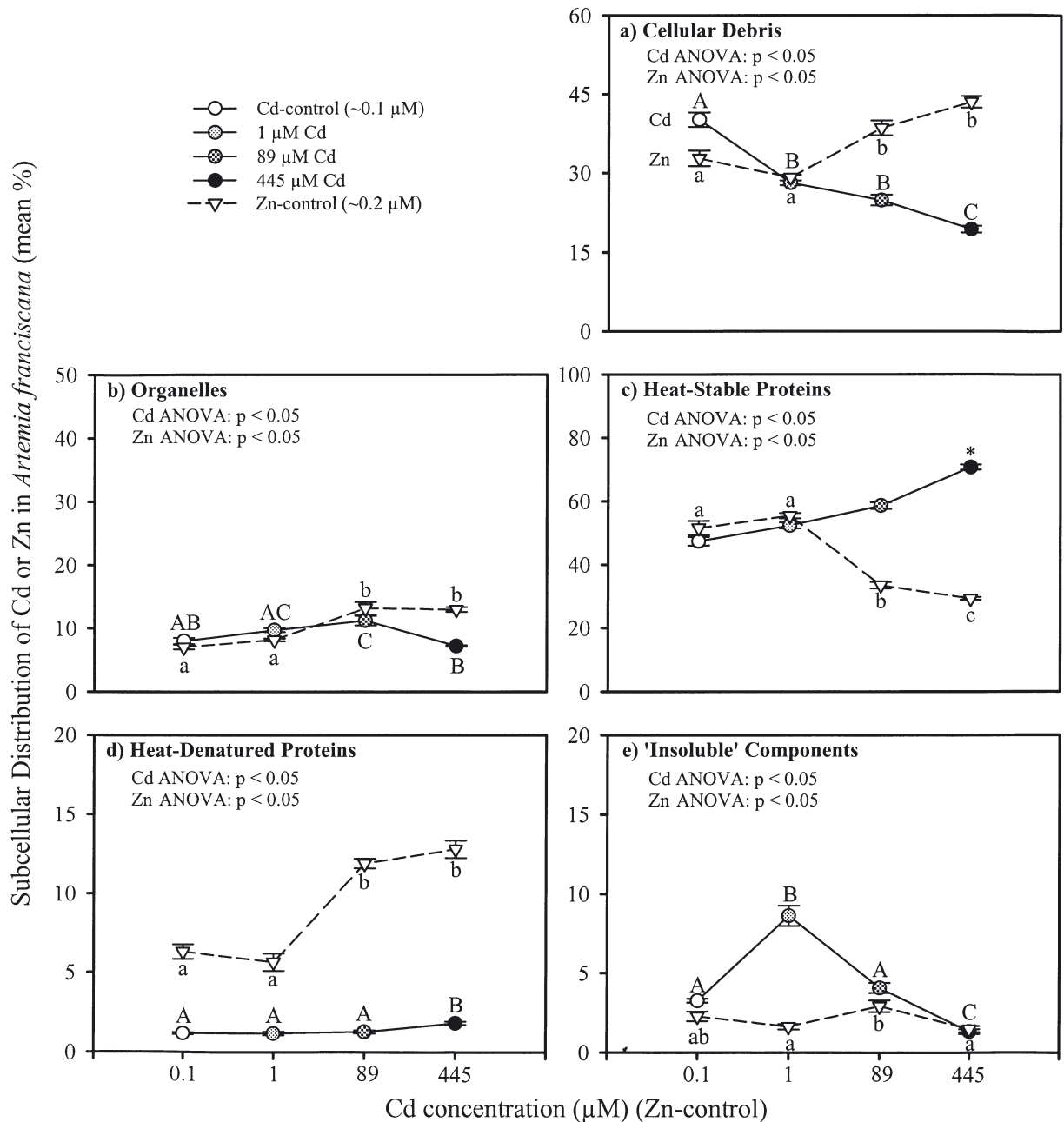


Fig. 3. *Artemia franciscana*. Subcellular distributions following Cd-series (Zn-control) exposures, showing percentage Cd and Zn in subcellular fractions ($n = 4$; mean \pm SE) following 3 d aqueous exposure to ~0.1 (control), 1, 89 or 445 μM Cd, all with a control concentration of Zn (~0.2 μM). Results from standard ANOVA are shown in each graph. Different letters indicate significant differences ($p < 0.05$) among treatment means for Cd (A–C) and Zn (a–c) (Scheffé test). Further details as in Fig. 1

and organelles: Wallace & Luoma 2003) revealed a number of interesting patterns in the potential for Cd and Zn trophic transfer to predators of *Artemia franciscana*. For example, upon increasing Cd exposure (i.e. Cd-series [Zn-control] treatments), TAM-Cd% in *A. franciscana* increased from ~57 to ~80% due to increased partitioning of Cd to HSP (Fig. 5a). Although the partitioning of Zn to HSP in *A. franciscana* de-

creased by ~23% upon increasing Cd exposure, a shift in the partitioning of Zn to other TAM fractions (HDP and organelles) resulted in a reduction in TAM-Zn% of only ~12% (Fig. 5b).

In terms of Cd and Zn concentrations in *Artemia franciscana* potentially available for trophic transfer among the Cd-series treatments (i.e. TAM-[Cd] and TAM-[Zn]), it was found that TAM-[Cd] did not

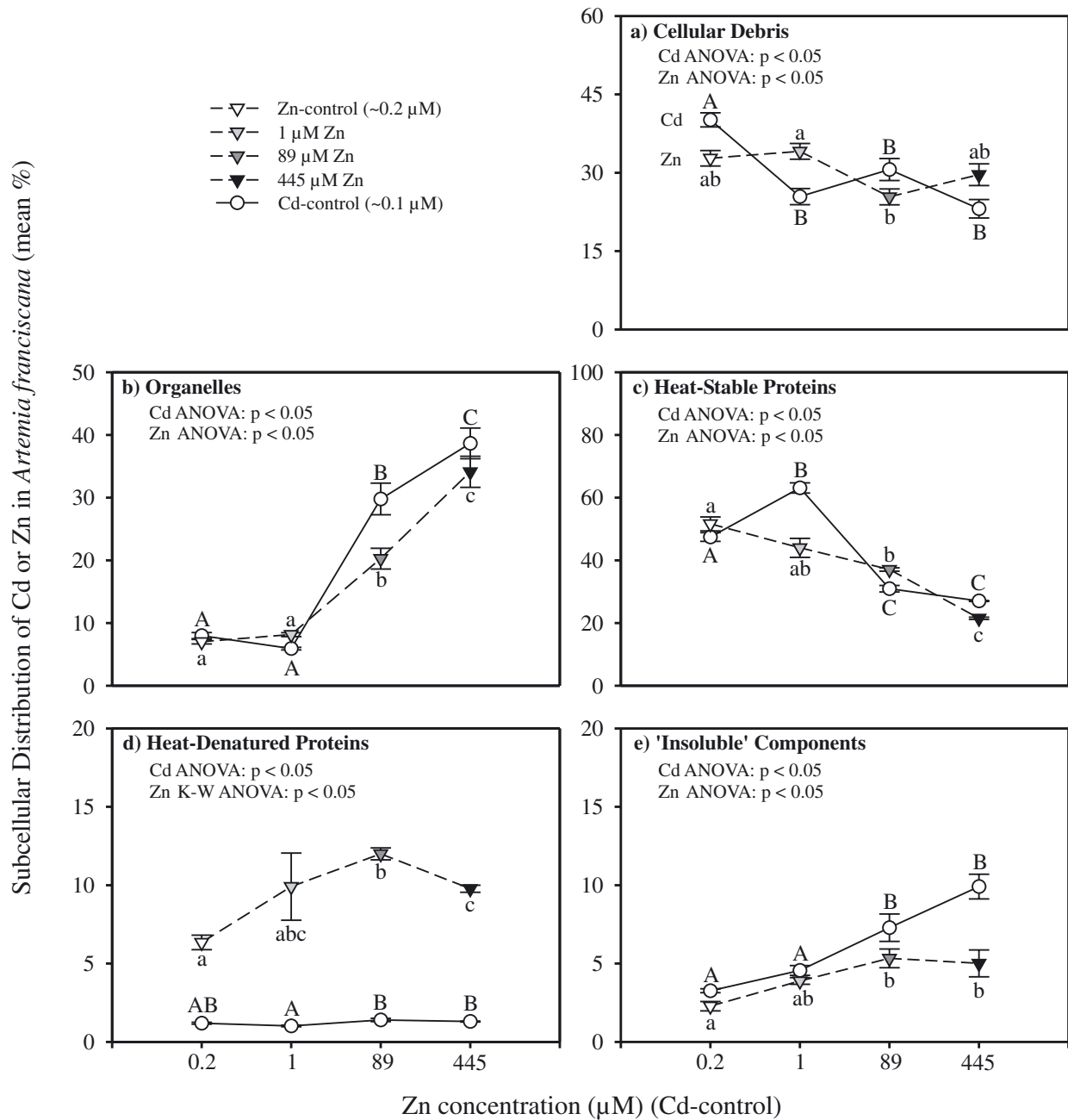


Fig. 4. *Artemia franciscana*. Subcellular distributions following Zn-series (Cd-control) exposures, showing percentage of Cd and Zn in subcellular fractions ($n = 4$; mean \pm SE) following 3 d aqueous exposure to ~0.2 (control), 1, 89 or 445 μM Zn, all with a control concentration of Cd (~0.1 μM). Further details as in Fig. 1

increase linearly with increasing Cd exposure (Fig. 5a: top of graph). For example, an increase in exposure concentration from 0.1 μM Cd (control) to 1 μM Cd (a 10-fold increase in exposure) resulted in a ~21-fold increase in TAM-[Cd] (from ~0.71 to ~15.1 nM g wet wt^{-1}). This increase in TAM-[Cd] was ~2.1 \times greater than would be predicted had partitioning to TAM been linear with exposure concentration. At the highest Cd

exposures, TAM-[Cd] was ~0.47 \times and ~0.23 \times greater than would be predicted, indicating that the increase in TAM was less than proportional to exposure concentration (Fig. 5a: top of graph). *A. franciscana* exposed to the Cd-control/Zn-control treatment had a TAM-[Zn] of ~12.14 nM g wet wt^{-1} . Despite an equal exposure to Zn (i.e. control), TAM-[Zn] decreased to ~5.63 nM g wet wt^{-1} in the presence of 89 μM Cd and

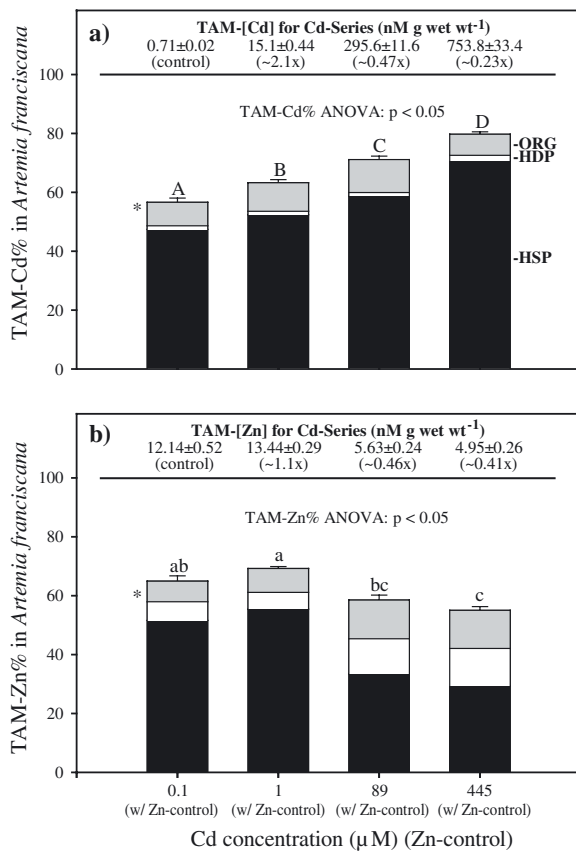


Fig. 5. *Artemia franciscana*. Subcellular partitioning as trophically available metal (TAM) following Cd-series (Zn-control) exposures, showing percentage (n = 4; mean ± SE) of Cd and Zn associated with a subcellular compartment consisting of (a) TAM-Cd% and (b) TAM-Zn%, following 3 d aqueous exposure to ~0.1 (control), 1, 89 or 445 µM Cd, all with a control concentration of Zn (~0.2 µM). Distribution of metals among subcellular fractions that contribute to the TAM compartment (i.e. heat-stable proteins: HSP; heat-denatured proteins: HDP; and organelles) was taken from Fig. 3. Different letters above bars indicate significant differences (p < 0.05) among treatment means for Cd (A–D) and Zn (a–c) (Scheffé test). Concentrations of metal associated with the TAM compartment (i.e. TAM-[Cd] or TAM-[Zn]), calculated as summation of metal associated with HSP, HDP, and organelles, for the various treatments are shown at top of each graph (n = 4; mean ± SE), where values in parentheses indicate difference in TAM-[metal], compared to control and normalized to increase in exposure concentration. *Adjacent to control-exposure histograms indicates absorption efficiencies, AE-Cd% or AE-Zn%, observed for *Palaeomonetes pugio* fed *A. franciscana* from this treatment (data from Fig. 7)

to ~4.95 nM g wet wt⁻¹ in the presence of 445 µM Cd (Fig. 5b: top of graph).

For the Zn-series (Cd-control) treatments, TAM-Cd% in *Artemia franciscana* oscillated between ~57 and ~70% due to a shift in the partitioning of Cd from HSP to organelles (Fig. 6a). Although there was a similar shift in the subcellular partitioning of Zn, TAM-Zn% was main-

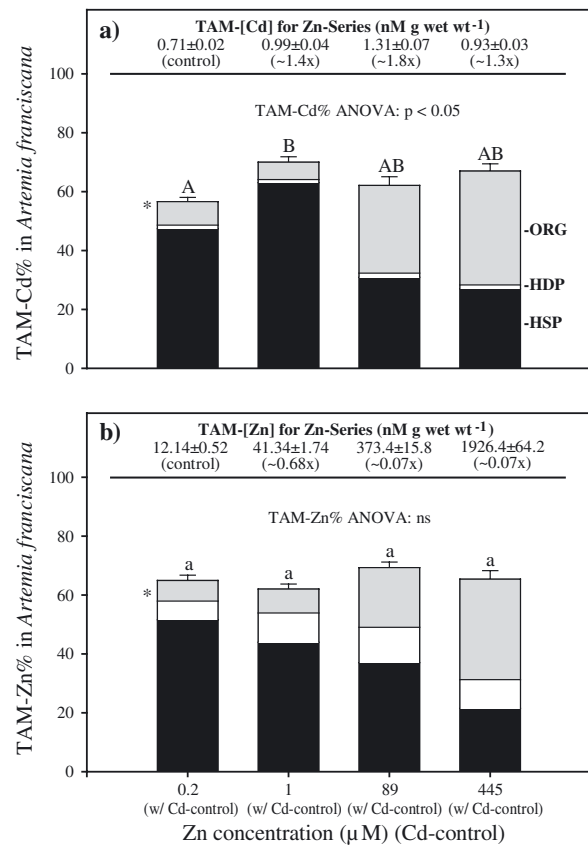


Fig. 6. *Artemia franciscana*. Subcellular partitioning as TAM following Zn-series (Cd-control) exposures, showing percentage (n = 4; mean ± SE) of Cd and Zn associated with subcellular compartment consisting of (a) TAM-Cd% and (b) TAM-Zn% following 3 d aqueous exposure to ~0.2 (control), 1, 89 or 445 µM Zn, all with a control concentration of Cd (~0.1 µM). Distribution of metals among subcellular fractions that contribute to TAM compartment was taken from Fig. 4. Further details as in Fig. 5

tained at ~65% over the range of Zn concentrations (Fig. 6b). TAM-[Cd] in *A. franciscana* among the Zn-series exposures (Cd-control) ranged between ~0.71 and ~1.31 nM g wet wt⁻¹ (Fig. 6a: top of graph). Although TAM-[Zn] did increase with increasing Zn exposure, this increase was less than proportional between control and 89 µM Zn (Fig. 6b: top of graph). TAM-[Zn], however, did increase linearly with exposure concentration between 89 and 445 µM Zn (Fig. 6b: top of graph).

Relationship between TAM% in *Artemia franciscana* and AE% by a predator

AE-Cd% and AE-Zn% were determined for *Palaeomonetes pugio* fed *Artemia franciscana* exposed to the Cd-control/Zn-control treatment. Depuration of ¹⁰⁹Cd and ⁶⁵Zn by *P. pugio* was characterized by a 2-compo-

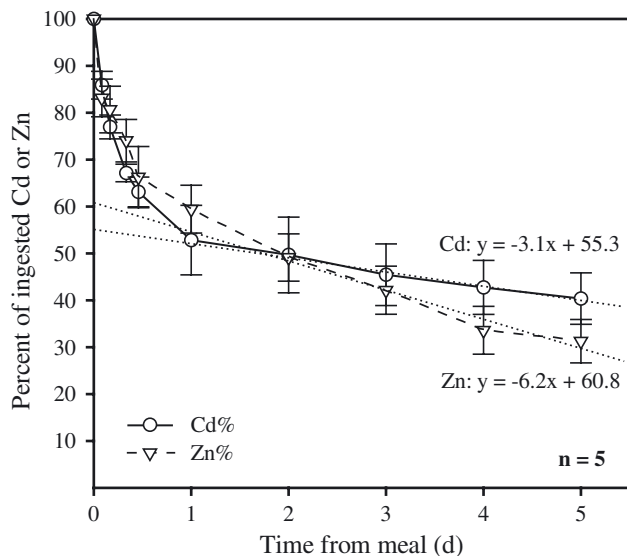


Fig. 7. *Palaemonetes pugio*. Time course of retention of ^{109}Cd and ^{65}Zn ($n = 5$; mean \pm SE) by *P. pugio* fed *Artemia franciscana* exposed to Cd-control/Zn-control treatment. Linear regressions (dotted lines) were fit to physiological loss-components of the retention curves (i.e. $t > 24$ h) and the corresponding y -intercepts were used to estimate ^{109}Cd and ^{65}Zn absorption efficiencies

ment loss, with an initial rapid loss of unassimilated metal due to the release of radiolabeled fecal strands (Fig. 7) (Wallace & Lopez 1997, Wallace et al. 1998). Comparison of the slopes of the linear regressions suggests that the physiological loss of Zn occurred at approximately twice the loss rate of Cd (~ 6 vs $\sim 3\%$ d^{-1}) (Fig. 7). Estimates of metal absorption efficiency using the y -intercept method revealed an AE-Cd% of $\sim 55\%$ and an AE-Zn% of $\sim 61\%$ (Wallace et al. 1998, Wang & Fisher 1999). These values are in good agreement with the percentages of Cd (TAM-Cd% $\sim 57\%$) and Zn (TAM-Zn% $\sim 65\%$) predicted to be trophically available from *A. franciscana* exposed to control levels of Cd and Zn (Figs. 5 & 6).

DISCUSSION

Studies investigating the subcellular partitioning of metals have often focused on relating internal metal processing to potential toxicity, tolerance and resistance (George 1983, Jenkins & Mason 1988, Klerks & Bartholomew 1991, Wallace et al. 2000). Several investigators, however, have recently highlighted the need to consider the role of subcellular partitioning in controlling metal trophic transfer in aquatic systems (Wallace & Lopez 1996, Fisher & Wang 1998, Wallace et al. 1998, Ni et al. 2000, Ettajani et al. 2001, Wallace & Luoma 2003). The purpose of the present study was to

examine the accumulation and subcellular distributions of Cd and Zn in *Artemia franciscana*, with particular focus on the partitioning of metal to a subcellular compartment presumed to contain trophically available metal (Wallace & Luoma 2003). Due to the potential effects of metal-metal interactions within *A. franciscana*, the experimental design also considered the influence of increasing Cd or Zn exposure on the accumulation and partitioning of the other metal (i.e. Zn or Cd).

Accumulation and subcellular distribution of Cd (Cd-series; Zn-control)

Cd accumulation in *Artemia franciscana* did not reach saturation at concentrations at or below $445 \mu\text{M}$ Cd. This is consistent with work by Blust et al. (1995), whereby Cd accumulation by adult *A. franciscana* increased with Cd exposure concentration and reached saturation at concentrations greater than $800 \mu\text{M}$. Although Cd was associated with all subcellular fractions, the greatest increase in partitioning for the $1 \mu\text{M}$ Cd exposure was observed for the 'insoluble' fraction (~ 50 -fold increase vs. ~ 13 - to ~ 23 -fold increases for the other fractions) and may have resulted from increased adsorption of Cd to the exoskeletal surfaces of *A. franciscana* (Jennings & Rainbow 1979a, Robinson et al. 2002).

The high partitioning of Cd to HSP ($\sim 47\%$ of the total Cd burden) in *Artemia franciscana* at a control concentration of Cd ($\sim 0.1 \mu\text{M}$) suggests that heat-stable, metal-binding proteins (i.e. MT) may act to protect metal-sensitive components (i.e. 'enzymes' and organelles), the combined partitioning of which was only $\sim 9\%$ (Brown & Parsons 1978, Cherian & Goyer 1978, Roesijadi 1992, Wallace et al. 2003). The greater tolerance to Cd among *Artemia* spp. compared to other crustaceans may be due to enhanced storage of Cd to MT and/or low ion permeability coincident with a life history involving hypersaline conditions (Eisler 1971, del Ramo et al. 1995, Rainbow 1998, Martínez et al. 1999, Sarabia et al. 2002b, Van Stappen 2002). The association between the quantification of Cd bound to MT and Cd detoxification/tolerance must be interpreted with caution, however, since metal enrichment of the HSP fraction may also be an artifact of heat-denaturation (Bragigand & Berthet 2003). The only other subcellular fraction containing a substantial portion of Cd in controls was cellular debris ($\sim 40\%$). The importance of cellular debris in terms of metal toxicity and detoxification, however, remains unclear (Wallace et al. 2003).

An increase in the percentage of Cd partitioned to HSP (from ~ 47 to $\sim 71\%$) was observed over the range

of Cd exposures, with commensurate decreases in the partitioning of Cd to the cellular debris and 'insoluble' components. In terms of subcellular Cd concentrations, levels of Cd bound to the organelles and 'insoluble' fractions approached or reached saturation at the highest Cd exposures (89 and 445 μM). Saturation within the 'insoluble' fraction (which contains exoskeleton and other insoluble components) at these high Cd exposures may be related to the maximum adsorption potential of the *Artemia franciscana* exoskeleton for Cd (Robinson et al. 2002, Wallace et al. 2003).

In terms of potential toxicity of Cd to *Artemia franciscana*, the increase in the concentration of Cd bound to HDP (e.g. 'enzymes') at the highest Cd exposures would be expected to interfere with critical cellular processes (Brown & Parsons 1978, Hennig 1986, Roesijadi 1992, Wallace et al. 2000). For example, Rafiee et al. (1986) demonstrated that tubulin extracted from *A. franciscana* larvae precipitates upon exposure to 10 μM Cd, but that the microtubule assembly is not inhibited at lower concentrations. Disruption of this potential target (which contains critical sulphhydryl groups) by Cd would likely interfere with microtubule-dependent cellular processes and result in toxicity (Ludueña et al. 1985, Rafiee et al. 1986).

Accumulation and subcellular distribution of Zn (Zn-series; Cd-control)

Zn accumulation by *Artemia franciscana* did not reach saturation over the range of Zn exposures. The portion of Zn partitioned to HSP in controls ($\sim 0.2 \mu\text{M}$ Zn) accounted for $\sim 52\%$ of the total Zn tissue burden in *A. franciscana*, while the combined partitioning to HDP and organelles was only $\sim 13\%$. This suggests a role for metal-binding proteins within the HSP fraction (e.g. MT) in the regulation of metabolically available Zn in *A. franciscana* (Engel & Brouwer 1987, Roseijadi 1992, Mason & Jenkins 1995). The percentage of Zn partitioned to HSP decreased (from ~ 52 to 21%) with increasing Zn exposure and was accompanied by increased partitioning to organelles (from ~ 7 to $\sim 32\%$). Partitioning to HDP was also elevated at the highest Zn exposures (89 and 445 μM). This shift from the reliance on HSP (e.g. MT) for the storage of Zn to metal-sensitive cellular components may have implications for Zn toxicity in *A. franciscana* (Roesijadi 1992, Wallace et al. 2003).

Effects of metal-metal interactions on accumulation and subcellular distribution

Interactive effects between metals have been shown to influence accumulation and toxicity in several crus-

taceans, including *Artemia franciscana* (Nugegoda & Rainbow 1995, Bat et al. 1998, Rainbow et al. 2000, Hadjispyrou et al. 2001, Barata et al. 2002). Very few studies, however, have considered the effects of metal-metal interactions at the subcellular level. In the present study, several interesting Cd-Zn interactions were observed in the accumulation and subcellular distribution of these metals in *A. franciscana*.

Since Zn exposure did not vary among the Cd-series treatments, a curve with a slope of zero (0) would be expected for all Zn tissue burdens (i.e. whole body tissue and subcellular fractions) in the absence of any Cd-Zn interactions. Increased exposure to Cd (89 and 445 μM), however, suppressed whole body tissue accumulation of Zn in *Artemia franciscana* ($\sim 50\%$), which was reflected in the partitioning of Zn to HSP (i.e. the reduction in the concentration of Zn to bound to this fraction accounts for $\sim 71\%$ of the decrease in total tissue Zn concentration). A similar suppression of whole-tissue Zn accumulation resulting from exposure to Cd has been observed in larval prawns (Deveineau & Amiard-Triquet 1985). In terms of percentages, Cd associated with HSP in *A. franciscana* increased (from ~ 47 to $\sim 71\%$, a 24% increase) with a corresponding reduction in the partitioning of Zn to this fraction (from ~ 52 to $\sim 29\%$, a 23% decrease). Reduced partitioning of Zn to HSP in the presence of high Cd may be attributable to (1) displacement of Zn by Cd ions, and/or (2) the higher capacity of Cd (relative to Zn) to induce MT in *Artemia* spp. (del Ramo et al. 1995, Mason & Jenkins 1995, Martínez et al. 1999).

Despite indications that increasing Cd exposure influenced the partitioning of Zn to other fractions, these changes were not as remarkable as those described above for the HSP fraction. For example, reduced partitioning of Zn to cellular debris in the presence of high Cd (445 μM) accounted for $\sim 20\%$ of the decrease in whole-tissue Zn concentrations. The concentration of Zn bound to the 'insoluble' fraction was also reduced at the highest Cd concentration (445 μM) and may have been due to interactive effects (e.g. competition for binding sites) on metal adsorption to the *Artemia franciscana* exoskeleton (Robinson et al. 2002). Zn availability for enzymatic and other intracellular processes in *A. franciscana*, however, may not be affected by increasing Cd exposure, since there was little or no variation in the partitioning of Zn to HDP (e.g. 'enzymes') and organelles over the range of Cd exposures (Depledge & Rainbow 1990, Wallace et al. 2003). This may suggest the existence of a mechanism for Zn homeostasis in *Artemia* spp. (Martínez et al. 1999).

In terms of the influence of increasing Zn exposure on the uptake and subcellular partitioning of Cd in *Artemia franciscana*, there were several notable

Cd–Zn interactions. For example, Cd accumulation was enhanced (~1.6-fold) in the presence of 89 μM Zn. This excess Cd was bound to organelles and ‘insoluble’ components and, to a lesser extent, HDP. The concentration of Cd partitioned to the HSP fraction at the 89 μM Zn exposure was similar to that at control levels of Zn. At the highest Zn exposure (445 μM), Cd concentrations in *A. franciscana* returned to control levels as partitioning of Cd to the HSP, HDP and cellular debris fractions decreased. Partitioning to organelles and ‘insoluble’ components remained saturated. Although increased accumulation of Cd in the presence of increasing Zn exposure may be important in terms of the potential for metal trophic transfer (see ‘Conclusions’), repartitioning of Cd from HSP to metal-sensitive HDP (e.g. ‘enzymes’) and organelles may also have implications for Cd toxicity (Silverberg 1976, Brown et al. 1977, Viarengo et al. 1987, Wallace et al. 2000, 2003).

Compartmentalization of Cd and Zn as TAM

Although there were concentration- and interaction-dependent variations in the accumulation and subcellular distributions of Cd and Zn, the implications for metal toxicity in *Artemia franciscana* are uncertain. The partitioning of metals to a subcellular compartment containing TAM, however, was clearly impacted by these changes. The percentage of Cd potentially available to predators (i.e. TAM-Cd%) increased with increasing exposure of *A. franciscana* to Cd and was due to increased partitioning of Cd to HSP (e.g. MT). While whole body tissue Cd concentrations did not increase linearly over the entire range of Cd exposures, there was greater than linear accumulation of TAM-[Cd] in *A. franciscana* that were exposed to 1 μM Cd (Zn-control). This greater than linear increase in the potential availability of Cd to predators of *A. franciscana* may be considered a ‘bioenhancement’ of TAM (Wallace & Luoma 2003). The observation of a ‘bioenhancement’ of TAM-[Cd] for the 1 μM Cd level of exposure may be of particular significance, as this concentration of Cd is only ~2.8 \times the maximum permitted dissolved Cd concentration in marine systems (US EPA 2001). Wallace et al. (2000) demonstrated an MT-driven ‘bioenhancement’ of Cd in *Palaemonetes pugio* that were subjected to increased dietary exposure to Cd. Despite a shift in the subcellular partitioning of Zn among TAM fractions (from HSP to HDP and organelles), TAM-Zn% in *A. franciscana* was constant over the range of the Zn-series (Cd-control) exposures. A ‘bioenhancement’ of TAM-[Zn] was, therefore, not observed for any of these treatments.

Metal–metal interactions within *Artemia franciscana* were also important in terms of influencing TAM-[Cd] and TAM-[Zn]. For instance, TAM-[Cd] was elevated in the presence of high levels of Zn (1, 89 and 445 μM), while TAM-[Zn] was suppressed at the highest Cd concentrations (89 and 445 μM). Interactive effects on the partitioning of metal to the TAM compartment may have important implications for consumers of prey that are subjected to mixed-metal exposures, and requires additional study.

Relationship between TAM% in *Artemia franciscana* and AE% in a predator

Cd and Zn absorption by *Palaemonetes pugio* was directly related to the partitioning of Cd and Zn to the TAM compartment in *Artemia franciscana* exposed to the Cd-control/Zn-control treatment. This provides a further validation of the TAM concept (i.e. that metal associated with HSP, HDP and organelles may represent bioavailable metal from the diet) (Wallace & Luoma 2003). A similar (~1:1) relationship was established between TAM-Cd% in oligochaetes and bivalves and AE-Cd% in grass shrimp (i.e. *P. pugio* and *Palaemon macrodactylus*) (Wallace & Lopez 1997, Wallace & Luoma 2003). The present study, however, is the first to establish a relationship between the subcellular partitioning of Zn in prey and trophic transfer to a predator. The analysis of TAM may therefore serve as a sensitive measure of Cd and Zn bioavailability in prey and may be used to estimate the ‘dose’ of these metals in the diets of some predators (Wallace et al. 2000, Wallace & Luoma 2003). TAM%-AE% relationships for other predators (e.g. fishes), however, may be expected to vary due to differences in digestive physiology (Reinfelder et al. 1998, Ni et al. 2000).

CONCLUSIONS

Dietary exposure to metals can be an important source of toxicity, and sublethal effects can occur at metal concentrations that fall within regulatory guidelines (Wallace et al. 2000, Fisher & Hook 2002). The present work has demonstrated that increased Cd exposure through solution and increased partitioning to a subcellular fraction containing heat-stable, metal-binding proteins (e.g. MT) can result in a ‘bioenhancement’ in the partitioning of Cd to a subcellular compartment containing TAM.

In metal-impacted ecosystems, reliance on MT for the detoxification of metal may have the effect of increasing the potential for metal transfer to predators (Wallace et al. 2000, 2003, Wallace & Luoma 2003).

Organisms that rely on strategies other than MT for metal detoxification (e.g. metal-rich granules), however, would be expected to have reduced concentrations of metal associated with the TAM compartment (Nott & Nicolaidou 1989, 1990, Wallace et al. 1998, Wallace & Luoma 2003). The threat of dietary metal exposure to a predator (i.e. TAM in prey) therefore appears to be influenced by the primary mode of metal detoxification within prey (Nott & Nicolaidou 1990, Wallace & Luoma 2003).

The quantification of TAM in tissues of ecologically and economically important species that inhabit metal-impacted environments may serve as an indicator of the potential for metal trophic transfer and associated toxicity to predators within these systems (Wallace & Luoma 2003). TAM may also be incorporated into models that are designed to predict the transfer of metals through aquatic food chains (Thomann 1981).

Acknowledgements. This work was supported by a PSC-CUNY Research Award (#6427800-33) to W.G.W. Access to radiation facilities and environmental chambers was provided by the Department of Biology at the College of Staten Island and is greatly appreciated. *Palaemonetes pugio* were collected from Great Kills Park under permit from the Division of Natural Resources, Gateway National Recreation Area, National Park Service. The authors would like to thank P. S. Rainbow and 3 anonymous reviewers for valuable comments on earlier versions of this manuscript. M. H. Perez assisted in the maintenance of experimental animals. This manuscript represents contribution 0304 from the Center for Environmental Science, College of Staten Island, City University of New York.

LITERATURE CITED

- Acey RA, Yoshida BN, Edep ME (1989) Metalloproteins in developing *Artemia*. In: Warner AH, MacRae TH, Bagshaw JC (eds) Cell and molecular biology of *Artemia* development. Plenum Press, New York, p 203–219
- Bagshaw JC, Rafiee P, Matthews CO, MacRae TH (1986) Cadmium and zinc reversibly arrest development of *Artemia* larvae. Bull Environ Contam Toxicol 37:289–296
- Baines SB, Fisher NS, Stewart R (2002) Assimilation and retention of selenium and other trace elements from crustacean food by juvenile striped bass (*Monroe saxatilis*). Limnol Oceanogr 47:646–655
- Barata C, Markich SJ, Baird DJ, Taylor G, Soares AMVM (2002) Genetic variability in sublethal tolerance to mixtures of cadmium and zinc in clones of *Daphnia magna* Straus. Aquat Toxicol 60:85–99
- Bat L, Raffaelli D, Marr IL (1998) The accumulation of copper, zinc and cadmium by the amphipod *Corophium volutator* (Pallas). J Exp Mar Biol Ecol 223:167–184
- Bebianno MJ, Langston WJ, Simkiss K (1992) Metallothionein induction in *Littorina littorea* (Mollusca: Prosobranchia) on exposure to cadmium. J Mar Biol Assoc UK 72:329–342
- Blust R, Baillieul M, Declair W (1995) Effect of total cadmium and organic complexing on the uptake of cadmium by the brine shrimp, *Artemia franciscana*. Mar Biol 123:65–73
- Bragigand V, Berthet B (2003) Some methodological aspects of metallothionein evaluation. Comp Biochem Physiol A 134:55–61
- Brown DA, Parsons TR (1978) Relationship between cytoplasmic distribution of mercury and toxic effects to zooplankton and chum salmon (*Oncarhynchus keta*) exposed to mercury in a controlled ecosystem. J Fish Res Board Can 35:880–884
- Brown DA, Bawden CA, Chatel KW, Parsons TR (1977) The wildlife community of Iona Island jetty, Vancouver, BC, and heavy-metal pollution effects. Environ Conserv 4: 213–216
- Cherian MG, Goyer RA (1978) Metallothioneins and their role in the metabolism and toxicity of metals. Life Sci Part I 23:1–10
- del Ramo J, Martínez M, Pastor A, Torreblanca A, Díaz-Mayans J (1993) Effect of cadmium pre-exposure in cadmium accumulation by brine shrimp *Artemia*: involvement of low-molecular-weight cadmium-binding ligands. Mar Environ Res 35:29–33
- del Ramo J, Torreblanca A, Martínez M, Pastor A, Díaz-Mayans J (1995) Quantification of cadmium-induced metallothionein in crustaceans by the silver-saturation method. Mar Environ Res 39:121–125
- Depledge MH, Rainbow PS (1990) Models of regulation and accumulation of trace metals in marine invertebrates. Comp Biochem Physiol C 97:1–7
- Devineau J, Amiard-Triquet C (1985) Patterns of bioaccumulation of an essential trace element (zinc) and a pollutant metal (cadmium) in larvae of the prawn *Palaemon serratus*. Mar Biol 86:139–143
- Dhont J, Sorgeloos P (2002) Applications of *Artemia*. In: Abatzopoulos TJ, Beardmore JA, Clegg JS, Sorgeloos P (eds) *Artemia*: basic and applied biology. Kluwer Academic Publishers, Dordrecht, p 251–277
- Eisler R (1971) Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. J Fish Res Board Can 28:1225–1234
- Engel DW, Brouwer M (1987) Metal regulation and molting in the blue crab, *Callinectes sapidus*: metallothionein function in metal metabolism. Biol Bull (Woods Hole) 173: 239–251
- Ettajani H, Berthet B, Amiard JC, Chevolut L (2001) Determination of cadmium partitioning in microalgae and oysters: contribution to the assessment of trophic transfer. Arch Environ Contam Toxicol 40:209–221
- Fisher NS, Hook SE (2002) Toxicology tests with aquatic animals need to consider the trophic transfer of metals. Toxicology 181-182:531–536
- Fisher NS, Reinfelder JS (1995) The trophic transfer of metals in marine systems. In: Tessier A, Turner DR (eds) Metal speciation and bioavailability in aquatic systems. John Wiley & Sons, New York, p 363–406
- Fisher NS, Wang WX (1998) Trophic transfer of silver to marine herbivores: a review of recent studies. Environ Toxicol Chem 17:562–571
- George SG (1982) Subcellular accumulation and detoxication of metals in aquatic animals. In: Vernberg WB, Calabrese A, Thurberg FP, Vernberg FJ (eds) Physiological mechanisms of marine pollutant toxicity. Academic Press, New York, p 3–52
- George SG (1983) Heavy metal detoxification in *Mytilus* kidney—an *in vitro* study of Cd- and Zn-binding to isolated tertiary lysosomes. Comp Biochem Physiol C 76:59–65
- Hadjispyrou S, Kungolos A, Anagnostopoulos A (2001) Toxicity, bioaccumulation, and interactive effects of organotin, cadmium and chromium on *Artemia franciscana*. Ecotoxicol Environ Saf 49:179–186

- Hennig HFKO (1986) Metal-binding proteins as metal pollution indicators. *Environ Health Perspect* 65:175–187
- Jenkins KD, Mason AZ (1988) Relationship between subcellular distributions of cadmium and perturbations in reproduction in the polychaete *Neanthes arenaceodonta*. *Aquat Toxicol* 12:229–244
- Jennings JR, Rainbow PS (1979a) Studies on the uptake of cadmium by the crab *Carcinus maenas* in the laboratory. I. Accumulation from seawater and a food source. *Mar Biol* 50:131–139
- Jennings JR, Rainbow PS (1979b) Accumulation of cadmium by *Artemia salina*. *Mar Biol* 51:47–53
- Kagi JHR, Schaffer A (1988) Biochemistry of metallothionein. *Biochemistry* 27:8509–8515
- Klerks PL, Bartholomew PR (1991) Cadmium accumulation and detoxification in a Cd-resistant population of the oligochaete *Limnodrilus hoffmeisteri*. *Aquat Toxicol* 19:97–112
- Klerks PL, Levinton JS (1989a) Effects of heavy metals in a polluted aquatic ecosystem. In: Levin SA, Harwell MA, Kelley JR, Kimball KD (eds) *Ecotoxicology: problems and approaches*. Springer-Verlag, New York, p 41–67
- Klerks PL, Levinton JS (1989b) Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site. *Biol Bull (Woods Hole)* 176:135–141
- Ludueña RF, Roach MC, MacRae TH, Langford GM (1985) *N,N'*-ethylene-bis (iodoacetamide) as a probe for structural and functional characteristics of brine shrimp, squid, and bovine tubulins. *Can J Biochem Cell Biol* 63:439–447
- Martínez M, del Ramo J, Torreblanca A, Díaz-Mayans J, Pastor A (1991) Presence of Cd-binding proteins in pre-exposed and not pre-exposed cadmium brine shrimp *Artemia*. *Toxicol Environ Chem* 31-32:417–424
- Martínez M, del Ramo J, Torreblanca A, Díaz-Mayans J (1999) Effect of cadmium exposure on zinc levels in the brine shrimp *Artemia parthenogenetica*. *Aquaculture* 172:315–325
- Mason AZ, Jenkins KD (1995) Metal detoxification in aquatic organisms. In: Tessier A, Turner DR (eds) *Metal speciation and bioavailability in aquatic systems*. John Wiley & Sons, New York, p 479–608
- Mouneyrac C, Amiard-Triquet C, Amiard JC, Rainbow PS (2001) Comparison of metallothionein concentrations and tissue distribution of trace metals in crabs (*Pachygrapsus marmoratus*) from a metal-rich estuary, in and out of the reproductive season. *Comp Biochem Physiol* 129C:193–209
- Mouneyrac C, Amiard JC, Amiard-Triquet C, Cottier A, Rainbow PS, Smith BD (2002) Partitioning of accumulated trace metals in the talitrid amphipod crustacean *Orchestia gammarellus*: a cautionary tale on the use of metallothionein-like proteins as biomarkers. *Aquat Toxicol* 57:225–242
- Ni IH, Wang WX, Tam YK (2000) Transfer of Cd, Cr and Zn from zooplankton prey to mudskipper *Periophthalmus cantonensis* and glassy *Ambassis urotaenia* fishes. *Mar Ecol Prog Ser* 194:203–210
- Nieboer E, Richardson DHS (1980) The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. *Environ Pollut Ser B Chem Phys* 1:3–26
- Nott JA, Nicolaidou A (1989) Metals in gastropods—metabolism and bioreduction. *Mar Environ Res* 28:201–205
- Nott JA, Nicolaidou A (1990) Transfer of detoxification along marine food chains. *J Mar Biol Assoc UK* 70:905–912
- Nugegoda D, Rainbow PS (1995) The uptake of dissolved zinc and cadmium by the decapod crustacean *Palaemon elegans*. *Mar Pollut Bull* 31:460–463
- Persoon G, Castritsi-Catharios J (1989) A simple bioassay with *Artemia* larvae to determine the acute toxicity of antifouling paints. *Water Res* 23:893–897
- Petrucci F, Caimi S, Mura G, Caroli S (1995) *Artemia* as a bioindicator of environmental contamination by trace elements. *Microchem J* 51:181–186
- Rafiee P, Matthews CO, Bagshaw JC, MacRae TH (1986) Reversible arrest of *Artemia* development by cadmium. *Can J Zool* 64:1633–1641
- Rainbow PS (1998) Phylogeny of trace metal accumulation in crustaceans. In: Langston WJ, Bebianno M (eds) *Metal metabolism in aquatic environments*. Chapman & Hall, London, p 285–319
- Rainbow PS, Amiard-Triquet C, Amiard JC, Smith BD, Langston WJ (2000) Observations on the interaction of zinc and cadmium uptake rates in crustaceans (amphipods and crabs) from coastal sites in UK and France differentially enriched with trace metals. *Aquat Toxicol* 50:189–204
- Reinfelder JR, Fisher NS (1994) The assimilation of elements ingested by marine planktonic bivalve larvae. *Limnol Oceanogr* 39:12–20
- Reinfelder JR, Fisher NS, Luoma SN, Nichols JW, Wang WX (1998) Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. *Sci Total Environ* 219:117–135
- Riisgård HU, Hansen S (1990) Biomagnification of mercury in a marine grazing food-chain: algal cells *Phaeodactylum tricorutum*, mussels *Mytilus edulis*, and flounders *Platichthys flesus* studied by means of a stepwise-reduction–CVAA method. *Mar Ecol Prog Ser* 62:259–270
- Robinson KA, Baird DJ, Wrona FJ (2002) Surface metal adsorption on zooplankton carapaces: implications for exposure and effects in consumer organisms. *Environ Pollut* 122:159–167
- Roesijadi G (1981) The significance of low molecular weight, metallothionein-like proteins in marine invertebrates: current status. *Mar Environ Res* 4:167–179
- Roesijadi G (1992) Metallothioneins in metal regulation and toxicity in aquatic animals. *Aquat Toxicol* 22:81–114
- Sarabia R, del Ramo J, Díaz-Mayans J, Torreblanca A (2002a) Development and reproductive effects of low cadmium concentration on *Artemia parthenogenetica*. *Mar Environ Res* 54:633–643
- Sarabia R, del Ramo J, Varo I, Díaz-Mayans J, Torreblanca A (2002b) Comparing the acute response to cadmium toxicity of nauplii from different populations of *Artemia*. *Environ Toxicol Chem* 21:437–444
- Silverberg BA (1976) Cadmium-induced ultrastructural changes in mitochondria of freshwater green algae. *Phycologia* 15:155–159
- Silverman H, Steffens WL, Dietz TH (1983) Calcium concretions in the gills of freshwater mussel serve as a calcium reservoir during periods of hypoxia. *J Exp Biol* 227:177–189
- Sokal RR, Rohlf FJ (1981) *Biometry. The principles and practice of statistics in biological research*, 2nd edn. WH Freeman & Co, New York
- Sullivan BK, Buskey E, Miller DC, Ritacco PJ (1983) Effects of copper and cadmium on growth, swimming and predator avoidance in *Eurytemora affinis* (Copepoda). *Mar Biol* 10:299–306
- Thall A, Acey R (1985) Cadmium binding proteins in developing *Artemia*. *Fed Proc* 44:1461
- Thomann RV (1981) Equilibrium model of fate of microcontaminants in diverse aquatic food chains. *Can J Fish Aquat Sci* 38:280–296

- US EPA (US Environmental Protection Agency) (1999) National recommended water quality criteria—correction. EPA-822-Z-99-001, April 1999
- US EPA (US Environmental Protection Agency) (2001) 2001 update of ambient water quality criteria for cadmium. EPA-822-R-01-001. April 2001
- Van Stappen G (2002) Zoogeography. In: Abatzopoulos TJ, Beardmore JA, Clegg JS, Sorgeloos P (eds) *Artemia*: basic and applied biology. Kluwer Academic Publishers, Dordrecht, p 171–224
- Viarengo A, Moore MN, Mancinelli G, Mazzucotelli A, Pipe RK, Farrar SV (1987) Metallothioneins and lysosomes in metal toxicity and accumulation in marine mussels: the effect of cadmium in the presence and absence of phenanthrene. *Mar Biol* 94:251–257
- Wallace WG, Lopez GR (1996) Relationship between the subcellular cadmium distribution in prey and cadmium trophic transfer to a predator. *Estuaries* 19:923–930
- Wallace WG, Lopez GR (1997) Bioavailability of biologically sequestered cadmium and the implications of metal detoxification. *Mar Ecol Prog Ser* 147:149–157
- Wallace WG, Luoma SN (2003) Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). *Mar Ecol Prog Ser* 257: 125–137
- Wallace WG, Lopez GR, Levinton JS (1998) Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. *Mar Ecol Prog Ser* 172: 225–237
- Wallace WG, Hoexum Brouwer TM, Brouwer M, Lopez GR (2000) Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. *Environ Toxicol Chem* 19:962–971
- Wallace WG, Lee BG, Luoma SN (2003) Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). *Mar Ecol Prog Ser* 249:183–197
- Wang WX, Fisher NS (1996) Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. *Limnol Oceanogr* 41:197–207
- Wang WX, Fisher NS (1999) Assimilation efficiencies of chemical contaminants in aquatic invertebrates: a synthesis. *Environ Toxicol Chem* 18:2034–2045
- Wang WX, Qiu JW, Qian PY (1999) The trophic transfer of Cd, Cr, and Se in the barnacle *Balanus amphitrite* from planktonic food. *Mar Ecol Prog Ser* 187:191–201
- Webb M (1987) Toxicological significance of metallothionein. *Exper Suppl* 52:109–134

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

*Submitted: April 24, 2003; Accepted: December 9, 2003
Proofs received from author(s): April 22, 2004*