Supplemental materials

Table 1. Salt additions for solutions of water with varying hardness. Values are given in mg L^{-1} of DI water.

Type	$NaCO_3$ (mg/L)	$CaSO_4 \cdot 2H_2O$ (mg/L)	$MgSO_4$ (mg/L)	KCl (mg/L)
Very soft	12	7.5	7.5	0.5
Very soft + Ca	12	240	7.5	0.5
Moderately hard	96	60	60	4
Very hard	384	240	240	16

Table 2. Taxonomic data and summary information for species used. Mass is given as the mean ± standard deviation of the wet weights in µg.

Order:family	*Genus species*	Experimental variables	[Mn] (µg L^{-1})	Exposure Duration (hours)	Mass (µg)
Ephemeroptera:Ephemerellidae	*Ephemerella dorothea*	Accumulation rates	25.6	8	4.4 ± 0.5
		k_e	25.6	240	4.4 ± 0.5
		Ca competition	24.2	24	4.0 ± 1.3
	Drunella cornutella	Accumulation rates	25.6	8	6.0 ± 1.5
		k_e	25.6	240	6.0 ± 1.5
	Drunella cornuta	Ca competition	24.2	24	4.5 ± 1.4
Ephemeroptera:Heptageniidae	*Maccaffertium pudicum*	Accumulation rates	25.6	8	15.4 ± 7.0
		k_e	25.6	240	15.4 ± 7.0
		Ca competition	24.2	24	21.5 ± 10.0
	Maccaffertium modestum	Adsorbed oxide quantification	100	96	16.4 ± 3.3
		Thiols	100	96	38.9 ± 7.7
	Maccaffertium spp.	Adsorbed oxide quantification	100	96	40.0 ± 19.5
		Subcellular fractionation	100	96	40.0 ± 19.5
	Epeorus vitreus	Accumulation rates	25.6	8	17.8 ± 6.5
Ephemeroptera:Leptophlebiidae	*Leptophlebiid* spp.	Adsorbed oxide quantification	100	96	5.5 ± 3.0
Ephemeroptera:Isonychiidae	*Isonychia* spp.	Adsorbed oxide quantification	100	96	26.8 ± 11.0
Trichoptera:Hydropsychidae	*Hydropsyche betteni*	V_{max} and K_m	0.26 - 400	24	35.8 ± 5.5
		Thiols	5 - 500	96	52.6 ± 11.8
	Cheumatopsyche spp.	Adsorbed oxide quantification	100	96	6.4 ± 2.3
		Subcellular fractionation	100	96	6.4 ± 2.3
		Thiols	100	96	54.8 ± 5.0
	Diplectrona modesta	Adsorbed oxide quantification	100	96	19.0 ± 8.2
		Subcellular fractionation	100	96	19.0 ± 8.2
Trichoptera:Rhyacophilidae	*Rhyacophila fuscula*	Adsorbed oxide quantification	100	96	25.2 ± 9.8
Plecoptera:Perlidae	*Acroneuria carolinensis*	Accumulation rates	25.6	8	29.1 ± 13.5
		k_e	25.6	240	29.1 ± 13.5
		Adsorbed oxide quantification	100	96	123.8 ± 78.1
		Subcellular fractionation	100	96	123.8 ± 78.1
	Acroneuria abnormis	Adsorbed oxide quantification	100	96	114.1 ± 61.1
		Subcellular fractionation	100	96	114.1 ± 61.1

	Acroneuria spp.	Ca competition	24.2	24	38.0 ± 43.9
Plecoptera:Perlodidae	*Malirekus hastatus*	Adsorbed oxide quantification	100	96	103.7 ± 17.5
		Subcellular fractionation	100	96	103.7 ± 17.5
Plecoptera:Pteronarcyidae	*Pteronarcys* sp.	Accumulation rates	25.6	8	184.6 ± 18.9
		k_e	25.6	240	184.6 ± 18.9
Diptera:Tipulidae	*Hexatoma* sp.	Accumulation rates	25.6	8	97.2 ± 14.2

Methods

Manganese efflux

The acquisition of sufficient radioactivity in larvae exposed to relatively low (0.1864 μM) and high (7.281 μM) Mn concentrations allowed us to test the premise that efflux rate constants are independent of tissue concentrations. Following 24 hours of exposure to these concentrations, *H. betteni* larvae were added to individual beakers containing 500 mL of clean reconstituted soft water. The animals and their surrounding water were assayed daily for radioactivity for 10 days. The water was changed on day four of depuration. The efflux rate constant (k_e) was determined using the equation:

$$C_t = C_i \times e^{-ket}$$

Where:

C_i = Mn concentration in the animal at time 0 (μg Mn g^{-1} wet weight)

C_t = Mn concentration in the animal at time t (μg Mn g^{-1} wet weight)

k_e = efflux rate constant (day^{-1})

t = time in days

The same procedure was used to assess efflux in six species used for uptake experiments (*Ephemerella dorothea*, *Acroneuria carolinensis*, *Drunella cornutella*, *Maccaffertium pudicum*, *Pteronarcys sp*). Insects were individually placed into approximately 400 mL of uncontaminated ASW for depuration as described above, with water changes at 3 day intervals. Animals were fed periphyton from day 2-7. After ten days, the animals were frozen and stored at -20°C.

Ascorbate Rinse to Remove Mn Oxides

At the end of the exposure period, animals were counted and rinsed thoroughly with 0.1M ascorbate, which is known to reduce metal oxides (such as iron oxide) at much lower concentrations (*1*). After the ascorbate rinse, the animals were then rinsed with 0.05M EDTA to remove the reduced Mn, and were finally washed with deionized water. The use of Leucoberbelin Blue, which changes color upon contact with Mn oxides, confirmed that this rinsing process successfully removed Mn oxides from the cuticles (*2*).

Molting Mn loss from field samples

We were able to obtain Mn tissue concentrations from US Department of Energy (Oak Ridge) and Tennessee Valley Authority (TVA) biologists from field collected larvae and adult mayflies (*Hexegenia sp.*) from two field sites – the Clinch and Emory Rivers in Tennessee. During the field sampling process, Oak Ridge and TVA scientists archived shed exuvia (sub-imago to imago molt), which they subsequently sent to us for analysis by ICP-MS.

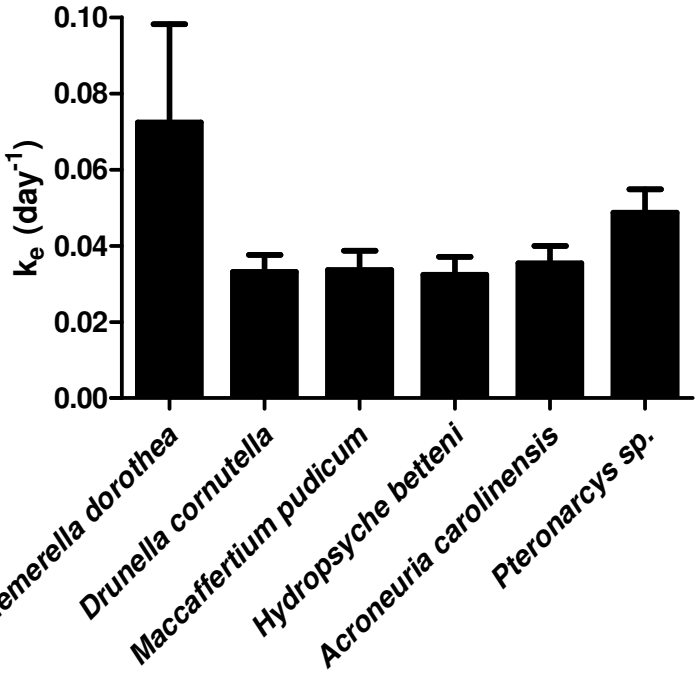

Figure 1. Mean (± standard deviation) efflux rate constants, k_e (day^{-1}) for six species of aquatic insects loaded with Mn and depurated in clean water for ten days.

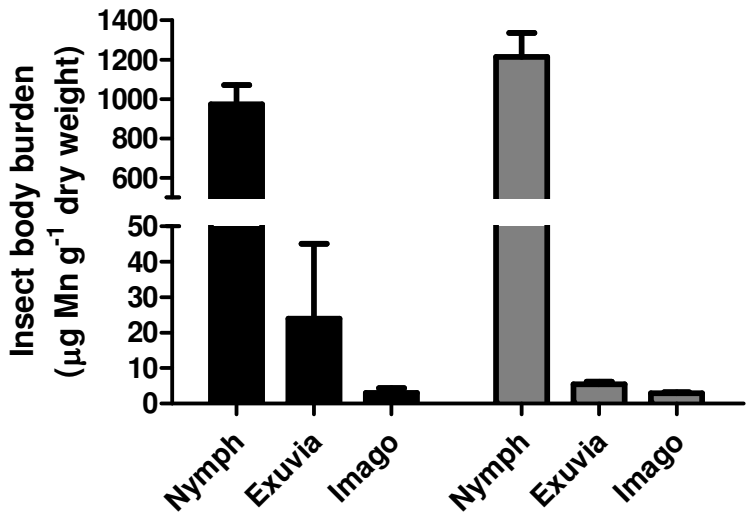

Figure 2. Mean (± standard deviation) manganese body burdens (µg Mn g^{-1} dry weight) of mayfly nymphs, imagos, and exuvia from the sub-imago to imago molt collected from two sites in Tennessee. Black bars indicate samples from Clinch River; grey bars represent Little Emory River.

Literature Cited

(1) Larsen, O. and Postma, D. Kinetics of reductive bulk dissolution of lepidocrocite, ferrihydrite, and goethite. *Geochim. Cosmochim. Acta*. **2001**, 65 (9), 1367-1379.

(2) Krumbein, W. E. and Altmann, H. J. New Method for Detection and Enumeration of Manganese Oxidizing and Reducing Microorganisms. *Helgol. Wiss. Meeresunters*. **1973**, 25 (2-3), 347-356.