SUPPORTING INFORMATION

Mercury (II) bioaccumulation and antioxidant physiology in four aquatic insects

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Determination of total mercury in water, periphyton and insects. Total mercury concentrations in water, periphyton and insects were analyzed using USEPA method 1631 revision E with a modified digestion procedure (17). Approximately 4 to 10 animals were combined (~0.2 g wet weight) per replicate (total 5 replicates) for each species. Insect samples and periphyton samples were freeze-dried and then digested in 4.5 ml of ultrapure 16M HNO_3 (OmniTrace Ultra, Merck, Darmstadt, Germany) and 1.5 ml of 18M H_2SO_4 (Leeman Labs Inc, West Chester, Pennsylvania, USA) in 50 ml Teflon digestion vessels using a microwave heating system (MarsXpress, CEM Inc, Mathews, North Carolina, USA). Samples (water, periphyton and insect) were then subjected to BrCl oxidation and $SnCl_2$ reduction. Insect and periphyton samples were diluted with 0.2 % HCl for total mercury analysis by flow-injection cold-vapor atomic fluorescence spectrophotometry (CVAFS) with a Leeman Laboratories Hydro AF Gold plus analyzer (Leeman Labs Inc, USA). Approximately 40 ml of filtered water samples were transferred directly to sample cups for total mercury analysis. The accuracy of total

mercury determination was evaluated by analysis of certified standard reference material from the National Institute of Standards and Technology (NIST Mussel 2976), method blanks (acid), spiked samples and calibration standards. Methods precision was determined to be within ± 6% (n=3) by replicate analysis of randomly selected insect samples. Our measured concentration (mean ± SD) of total Hg in NIST Mussel 2976 was 66.3 ± 0.4 ng g^{-1} dry wt (n = 3) (certified value: 61± 3.6, total recovery 108 ± 0.9%). Total Hg in method blanks were less than the lowest standard (0.5 ng L^{-1}) used for generating the standard curve (0.5 to 100 ng L^{-1}). Spiked samples had a recovery of 103 ± 1.2% (n = 3). Mercury levels in all the samples exceeded our method detection limit of 1.3 ng g^{-1} dry weight, calculated as 3 times the standard deviation of the method blank mass divided by the average sample mass.

Mercury in water, periphyton and aquatic insects. Total mercury in the filtered water samples (1.7 ± 0.2 ng L^{-1}), periphyton (36.6 ± 11.8 ng g^{-1} dry wt), and insects suggest that the source populations were derived from a system that while not heavily contaminated, is subject to some Hg inputs. *Chimarra* sp and *H. betteni* had total mercury body burdens of 60.6 ± 11.7 and 61.1 ± 23.4 ng g^{-1} dry weight respectively, which were not statistically ($P < 0.05$) different from *Isonychia* sp and *M. modestum* with 44.1 ± 13.1 and 38.1 ± 10.9 ng g^{-1} dry weight respectively.

Determination of non-enzymatic antioxidants. The analysis of N-ethylmaleimide stabilized cysteine and reduced glutathione (GSH), along with cystine and oxidized glutathione (GSSG) were performed by reversed phase liquid chromatography-mass spectrometry (LC-MS) methods. Insects were homogenized directly in 10 mM N-ethylmaleimide (Sigma Aldrich) containing reserpine (internal standard) at 1μM. Reaction of thiols with the N-ethylmaleimide was allowed to proceed for 1 hr at room temperature. Analysis was by LC-ESI-MS/MS on a Thermo LTQ ion trap mass spectrometer with Thermo Surveyor autosampler and HPLC. Briefly, a 5μl injection was made onto a Thermo Hypersil GOLD (150 x 2mm) column equilibrated in 97:3 A:B, where A = 50mM acetic acid in water, B = acetonitrile, flow rate = 250 μl min^{-1}. Compounds were eluted from the column by application of a 13 min linear gradient to 15:85 A:B. Ionization was by electrospray in positive ion mode, and detection of the compounds of interest was in MS/MS mode. Quantitation was by a processing method written within Xcalibur 2.0 software (Thermo), and a five-point calibration curve was created through calibration standards interspersed throughout the analysis sequence.

Analysis of algal plate scrapings. Microscopic analysis showed that periphyton on the plates was most composed of diatoms. The very common diatom species were: *Synedra*

nr. *berolinensis*, *Nitzschia* nr. *subacicularis*, *Navicula* sp., and *Melosira varians*. Less common diatoms were: *Diatoma vulgaris*, *Gomphonema* sp., *Fragilaria capucina*, *Hantzschia amphioxys* and *Cymbella (Encyonema)*. Bluegreens algae on the plates were *Oscillatoria* and *Phormidium*. Green algae on the plates were *Mougeotia*, *Ulothrix*, and *Klebsormidium*.