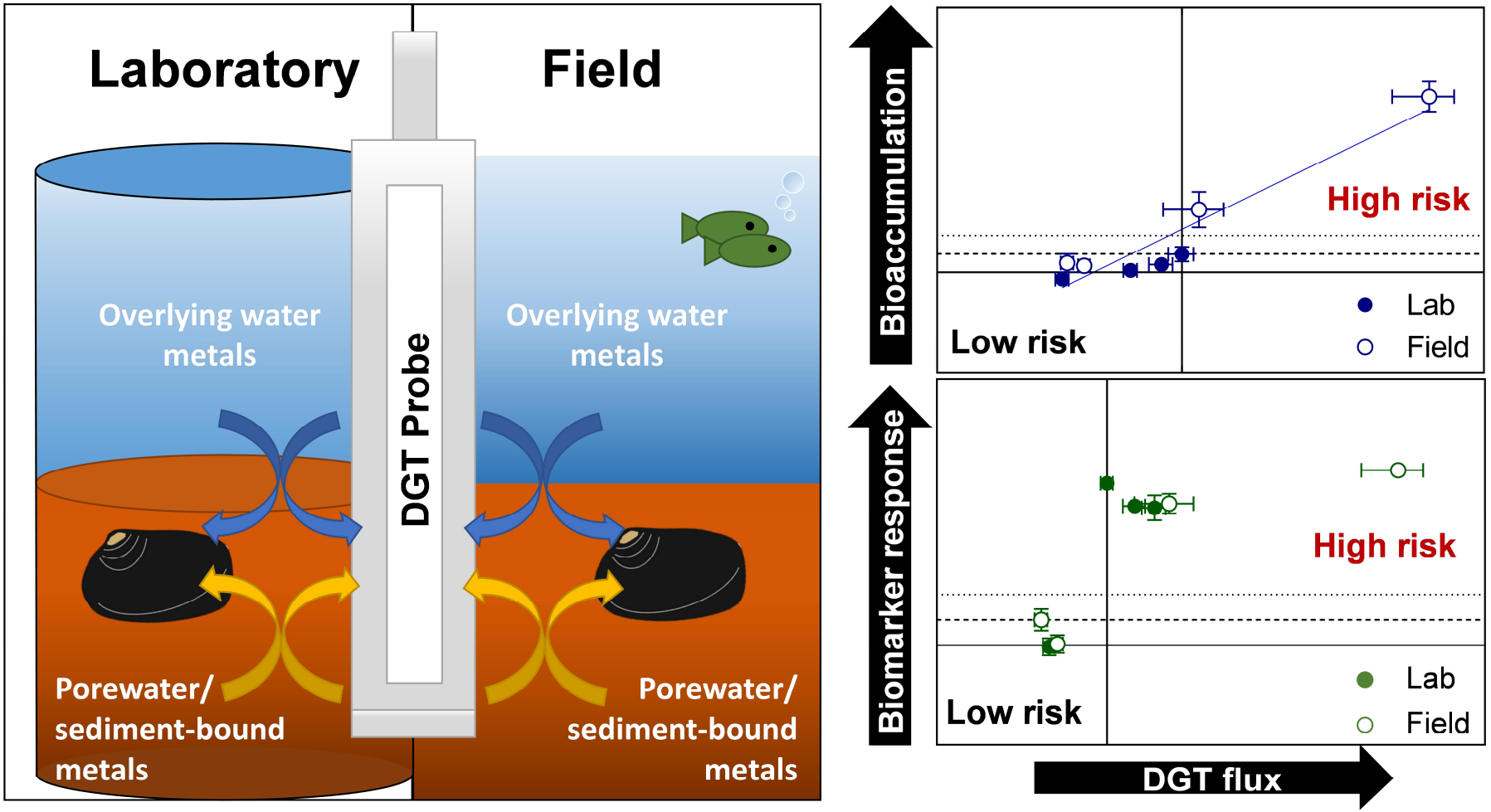
Field and laboratory evaluation of DGT for predicting metal bioaccumulation and toxicity in the freshwater bivalve **Hyridella australis** exposed to contaminated sediments


Laboratory
Field
Overlying water metals
Overlying water metals
DGT Probe
Porewater/ sediment-bound metals
Porewater/ sediment-bound metals
Bioaccumulation
High risk
Low risk
Lab
Field
Biomarker response
High risk
Low risk
Lab
Field
DGT flux

Running title: Field and laboratory evaluation of DGT for predicting metal bioaccumulation and toxicity in the freshwater bivalve *Hyridella australis* exposed to contaminated sediments

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Rationale: Assessing the risks to ecosystems posed by contaminants is not simple for many environmental matrices. For sediments, there is a need for better techniques for *in situ* assessment of contaminant bioavailability. Analysis of pore waters, acid volatile sulfide (AVS), simultaneously extracted metals (SEM), and organic carbon concentrations are frequently used to evaluate and assist in predicting contaminant bioavailability in sediments. Where concentrations of bioavailable contaminants are assessed to exist near or above guideline levels, bioassays are usually performed to evaluate whether toxic effects may result from contaminant exposure. For metal contaminants, chemical analyses have been useful for predicting metal toxicity in some sediments (e.g. AVS-SEM relationships), however the predictions for more oxidized surface sediments can be quite poor. This is attributed to a broader range of factors influencing metal bioavailability including variability in phases that are easily oxidized or reduced (e.g. AVS and Fe(II)). In addition, laboratory-based bioassays may provide inadequate predictions of metal bioavailability and toxicity due to their inability to adequately replicate field exposure conditions. Thus, techniques capable of providing reliable *in situ* assessments of metal bioavailability and toxicity are needed. In this study, the performance of the diffusive gradients in thin films (DGT) technique for predicting metal bioavailability and sublethal toxicity in sediments was assessed in both the laboratory and field. Strong predictions of bioaccumulation and toxicity were obtained using DGT metal fluxes irrespective of the type of sediment and exposure (i.e., laboratory and field), potentially enabling the derivation of DGT-based threshold values that may be measured *in situ* during sediment quality assessments.

Article Type: Research article

Field and laboratory evaluation of DGT for predicting metal bioaccumulation and toxicity in the freshwater bivalve *Hyridella australis* exposed to contaminated sediments

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ABSTRACT. The diffusive gradients in thin films (DGT) technique has shown to be a useful tool for predicting metal bioavailability and toxicity in sediments, however, links between DGT measurements and biological responses have often relied on laboratory-based exposures and further field evaluations are required. In this study, DGT probes were deployed in metal-contaminated (Cd, Pb, Zn) sediments to evaluate relationships between bioaccumulation by the freshwater bivalve *Hyridella australis* and DGT-metal fluxes under both laboratory and field conditions. The DGT-metal flux measured across the sediment/water interface (±1 cm) was useful for predicting significant cadmium and zinc bioaccumulation, irrespective of the type of sediment and exposure. A greater DGT-Zn flux measured in the field was consistent with significantly higher zinc bioaccumulation, highlighting the importance of performing metal bioavailability assessments *in situ*. In addition, DGT fluxes were useful for predicting the potential risk of sub-lethal toxicity (i.e., lipid peroxidation and lysosomal membrane damage). Due to its ability to account for multiple metal exposures, DGT better predicted bioaccumulation and toxicity than particulate metal concentrations in sediments. These results provide further evidence supporting the applicability of the DGT technique as a monitoring tool for sediment quality assessment.

Keywords: metals; bioavailability; biomarkers; bivalves; bioaccumulation; sediment quality assessment

Major findings:

DGT was useful for predicting bioaccumulation and sublethal toxicity to organisms exposed to metal contaminated sediments irrespective of type of sediment and exposure

1. INTRODUCTION

The comparison of total metal concentrations with sediment quality guideline values (SQG) is a common first tier in frameworks used for assessing the risks posed by metal contaminated sediments.[1,2] When a measured metal concentration exceeds a SQG value, then further investigations are generally required, and often the next step involves considering the bioavailability of the metal contaminants. For metals, analysis of concentrations in pore water, dilute-acid extractable forms, and concentrations of major metal binding phases such as sulfide, organic matter and iron and manganese oxide phases (e.g. through analyses of acid volatile sulfide (AVS), and organic carbon (OC)) are frequently used to evaluate and predict the metal bioavailability.[3–7] Where concentrations of bioavailable metals are determined to exceed SQG values, biological assessment methods are usually used to directly assess the risks of toxicity effects resulting from metal exposure.

While use of multiple lines of evidence (LOEs) is an important component of sediment quality assessment,[2,4,8,9] the requirement to include a LOE for toxicity in assessments adds significantly to assessment costs, and for many environments appropriate toxicity test methods (e.g., relevant species or endpoints) do not yet exist.[10] Consequently, methods that predict the risk of adverse effects comprising physical and chemical measurements continue to be sought to inform the initial phase of assessments.

Within surface sediments and the bioturbated zones that are subject to fluctuating dissolved oxygen concentrations, redox-processes strongly influence partitioning of metals among the different solid phases and control metal bioavailability.[11–14] Because of the diversity of metal-binding phases,[4,15] their differing affinities for metals,[6,12,16–18] and the dynamics of metal-partitioning that alter metal bioavailability and effects to organisms,[11–14] the creation of a universally applicable method for reliably predicting metal bioavailability in sediments remains elusive.

With the intent of providing a method for assessing metal bioavailability that does not need to consider the forms and concentrations of the many metal-binding phases present in the sediments, the diffusive gradient in thin films (DGT) technique is increasingly being applied.[19–27] The concept of using DGT to predict metal bioavailability is relatively simple, where higher levels of DGT-labile metals (reported as DGT-induced metal fluxes or concentrations) are taken to indicate a greater risk of metal exposure and adverse effects to benthic invertebrates.[20,22,23,27] The DGT technique provides time-integrated measurements of some of the metal fractions that are most relevant for bioavailability assessments, such as

dissolved metal species present in the pore water, and labile metals that are readily released from the solid phase of the sediment due to weak binding (Zhang et al., 1995).[28]

Another significant challenge for assessments of metal bioavailability is dealing with differences between observations made in the laboratory and those made in the field.[29,30] While laboratory-based measurements made on sediment collected from the field often provide important information for sediment quality assessments, frequently there is a significant mismatch in exposure conditions between the laboratory and field, and this can lead to considerable differences in the assessment of risk predicted for metal contaminated sediments.[12,31,32] Metal bioavailability is easily disturbed during sediment sampling and storage prior to testing,[14,33] and the use of *in situ* techniques such as DGT is expected to provide a more representative measure of concentrations of labile and bioavailable metals existing in the field when compared to measurements made using techniques applied in the laboratory.[23,26,34]

In this study, the ability of the DGT technique to provide measurements that can be used to predict metal bioaccumulation and sub-lethal toxicity to the bivalve *Hyridella australis* was tested under laboratory and field conditions. A mining-affected river showing a gradient of metal concentrations was identified as a suitable site for conducting investigations.[35] Relationships between biological responses and the DGT-induced metal fluxes were assessed in relation to the application of DGT as a suitable tool for environmental monitoring.

2. MATERIALS AND METHODS

2.1. General methods. All glass and plastic-ware used for analyses were new and cleaned by soaking in 10% HNO_3 (v/v) (BDH, AnalaR) for ≥24 h, followed by thorough rinsing with ultrapure water (18.2 MΩ·cm). For analytes above trace concentrations, no acid cleaning was undertaken. All chemicals were analytical reagent grade or equivalent analytical purity. Total recoverable metal (TRM) concentrations in sediments were analyzed following low-pressure microwave-assisted aqua regia digestion (3:1 v/v HNO_3:HCl, CEM Mars 5). Dilute-acid extractable metals (AEM, 60 min 1 M HCl digestion) and acid-volatile sulfide (AVS) concentrations were determined according to methods previously described by Simpson et al.[36] Metal concentrations in biological tissues were analysed following the procedure described by Baldwin et al.[37] Briefly, the soft tissue mass (approximately 0.07 g of tissue or certified reference materials, NIST 1566b, oyster tissue) was freeze-dried and finely ground before microwave-assisted (CEM MDS-2000, USA) extraction in nitric acid

(Suprapur® Nitric Acid, Merck, Germany) for 2 min at 630 W, 2 min at 0 W and 45 min at 315 W. Discrete overlying water samples were collected for analysis of dissolved metal concentrations. Samples were filtered (0.45 µm) and acidified (1% HNO_3 v/v) immediately after collection in the laboratory or within 24 h when collected from the field. Field samples were collected on days 0 and 28 of the exposure, while laboratory samples were collected on a weekly basis. All digests and water samples were analysed by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Varian 730-ES) and by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500ce). All sediment analyses were performed in triplicate and recoveries of certified material (ERM-CC0018, NIST 1566b) were between 90 and 110% of the expected value.

2.2. Test media and organisms. Sediments were collected from four sites along the Molonglo River at different distances from a mining complex located at Captains Flat, near Canberra, ACT, Australia, at a depth comprised between 0 and 10 cm. In each of the sites, a suitable location was identified based on the ease of cages and DGT probes deployment (i.e., water depth < 1m). Three metal-contaminated and one reference site were identified along the Molonglo River based on past studies.[38] The reference sediment was collected at approximately 8 km upstream from the mine (S1), and three contaminated sediments at approximately 45 (S2), 28 (S3), and 9 km (S4) downstream from the mine site (Table 1). Sediment sub-samples for chemical analyses (~500 g) were sieved (2 mm), homogenized and stored in the dark at 4º C until use. River water was collected from each location, filtered (0.45 µm) and stored at room temperature. The freshwater bivalve *Hyridella australis* (5.5 ± 0.5 cm) was collected from Nepean River, near Menangle, south-west of Sydney, NSW, Australia.

2.3. Bivalve bioassay. Bioaccumulation in *H. australis* was assessed by exposing bivalves to control (S1) and contaminated sediments (S2, S3, S4) over a period of 28 days. The laboratory bioassay was carried out in plastic aquariums (12 L) containing ~2 kg of sediment and 10 L of filtered (0.45 µm) river water. The exposure media (sediments and waters) were equilibrated for 2 days at room temperature (22-25 ºC) before addition of bivalves. Overlying water was aerated using air flow controllers to maintain 80-100% air-saturation during this pre-exposure phase and during the bioassays. In the field bioassay, sediments were placed in plastic mesh cages (35 × 30 × 10 cm^3) which allowed sufficient head-space for bivalve movement, adequate water circulation and prevented predation (see

Supporting Information (SI)). During the laboratory bioassay, 50% of the overlying water was renewed in all laboratory treatments every 3 days and the bivalves were fed twice a week using unicellular green algae *Nannochloropsis* (Nanno 3600, Instant algae, USA) at 1% (v/w) of total body mass, while no additional food was provided in the field bioassay. Both laboratory and field bioassays were performed in triplicate. At test commencement, six bivalves were placed on the sediment surface of the three treatment replicates (laboratory and field) and allowed to bury. The final bivalve density was approximately 0.8 and 1.2 bivalve/L of sediment for laboratory and field experiments, respectively. In the field bioassay, a mesh lid was placed on the top of the cages, secured in place using plastic cable ties and then placed in the same location as sample collection. Water quality parameters (temperature, pH, dissolved oxygen, and conductivity) were monitored at the beginning, middle and end of test in each of the locations (Table S1 of the SI). After 28 days, organisms were retrieved, depurated in clean fresh water for 24 h, and dissected prior to analysis.

Five organisms per replicate were utilized for measuring metal concentrations in whole tissues and one organism for enzymatic assays (biomarker responses). Hepatopancreas tissues were used for total antioxidant capacity (TAOC), lipid peroxidation (MDA), and lysosomal membrane stability assays. Tissues were thawed and homogenized on ice in 500 µL of a 5 mM potassium phosphate buffer (0.9% w/v sodium chloride and 0.1% w/v glucose, pH 7.4 (1:5, w/v)), sonicated on ice for 15 s, and centrifuged for 15 min at 4°C. TAOC and MDA assays were carried out on supernatants immediately after tissue processing. Sub-samples of tissue lysates were stored at −80°C for protein analyses that were completed on the following day. The procedures used for TAOC, MDA, and lysosomal membrane stability assays are described elsewhere.[39]

2.4. Diffusive gradients in thin films. Plastic planar DGT probes (24 cm × 4 cm × 0.5 cm, with an open window of 1.8 cm × 15 cm) were purchased from DGT Research (http://www.dgtresearch.com/). DGT probes were loaded with Chelex® binding gel (0.4 mm thickness) topped with a polyacrylamide diffusive gel (0.8 mm thickness) and a 0.45 µm pore size (100 µm thickness) polysulfone filter membrane.[28] Gel and resin preparation and handling were performed in an AURA SD4 Laminar Flow Cabinet following the procedures recommended by DGT Research (Lancaster, UK). Before deployment, probes were conditioned overnight using a 0.12 M NaCl solution of ultrapure water continuously bubbled with nitrogen gas, then maintained under an inert gas atmosphere until deployment (<4 h).

In the laboratory bioassays, one DGT probe was deployed in each of the three test chamber replicates. In the field, three DGT probes were deployed directly in the sediment near the cages used for the *in situ* bioassay (height limitations prevented probe deployment within cages). DGT probes were deployed in two separate events, in the first and third week of the bioassays (laboratory deployment: day 4 and 16 for sediments S1 and S2 and day 2 and 16 for sediments S3 and S4; field deployment: day 7 and 21 for sediments S1 and S3 and day 2 and 16 for sediments S2 and S4). After probe retrieval, a hole in the sediment bed was generally observed. This unlikely caused any significant disturbance in the sediment or in the overlying water. In the second deployment, care was taken to deploy probes at an adequate distance from the previous deployment point (~10-20 cm).

After a 24-h deployment, probes were removed and thoroughly rinsed with ultrapure water, placed in acid-washed plastic bags and stored at 4°C until analysis. Probes were disassembled and the Chelex-resin sliced at 1 and 3 cm above the sediment water interface (SWI), and 1, 2 and 4 cm below the SWI. In laboratory chambers, the maximum depth achievable for DGT measurements was -3 cm, and when necessary, the slicing procedure was adjusted according to the available depth (-2 and -4 cm for S1 and S4, respectively - first deployment). Resin slices were extracted with 1 M HNO_3, diluted 10-fold with ultrapure water and analysed by ICP-MS (Agilent 7500ce). The measured concentration in the extract was used to calculate the mass per unit area ($\mu g/m^2$) in the resin gel and divided by the deployment time to obtain DGT-labile metal fluxes ($\mu g/h/m^2$). For quality assurance, laboratory and field blank probes were analyzed; blank probes were exposed to the air and then rinsed with the same ultrapure water used for cleaning probes upon retrieval.

2.5. Data analysis. Statistical analyses were performed using the software R 3.1.2 (×64). Differences in metal bioaccumulation rates between organisms exposed to different treatments (S1, S2, S3, S4) and exposure conditions (laboratory and field) were investigated using two-way analysis of variance (ANOVA) with interactions between type of sediment and type of exposure, followed by Tukey's test. Data were tested for normality of residuals distribution (Shapiro-Wilk's test) and for homogeneity of variance (Levene test) prior to hypothesis testing. When the data did not follow a normal distribution, Kruskal-Wallis test followed by Wilcoxon–Mann–Whitney test were performed. Unless otherwise stated, the level of significance α was 0.05. To account for the varying toxicity expected to be caused by the different metals, exposure concentrations and fluxes (dissolved metal concentrations, DGT fluxes and AEM concentrations) were converted to quotients by dividing each metal

exposure concentration or flux by its corresponding guideline value,[40] and then summing the quotients in the case of more than one metal. For AEM, the guideline value was the corresponding SQG value[2] and, for DGT fluxes and dissolved metal concentrations, the guideline values were the corresponding water quality guidelines values (WQG).[41] According to this approach, DGT fluxes, dissolved metal concentrations, and AEM concentrations were converted to WQG value-based DGT_{SWI} fluxes ($DGT_{WQG\text{-}SWI}$), WQG value-based toxic units (TU), and SQG value-based quotients (SQG-Q), respectively.

3. RESULTS AND DISCUSSION

3.1. Sediment chemical and physical properties. General physico-chemical characteristics (particle size, TOC, AVS) and TRM and AEM concentrations (Cd, Pb and Zn) in field-collected sediment are shown in Table 1, with a greater range of metal and metalloid concentrations shown in Table S1 of the Supporting Information (SI). All sediments collected from sites located downstream of the mine site exhibited significant metal contamination, with SQG values being exceeded for lead in sediment S4, cadmium in S2 and S4, and zinc in S2, S3 and S4 (Table 1). In the reference sediment S1, metal concentrations were well below the SQG values (Table S2 of the SI). TOC concentrations were 1.4, 1.6, 4.3, 1.7% for site S1, S2, S3 and S4, respectively. AVS concentrations of 1.7±0.6 and 6.6±1.0 µmol/g were measured in sediment S2 and S4, respectively, whereas for S1 and S3, concentrations were <0.5 µmol/g (Table 1). The more labile sulfide phases (e.g. FeS and MnS) that comprise much of the AVS strongly influence the metal speciation and bioavailability in sediments as they react to form insoluble metal-sulfide compounds. AVS and simultaneously-extractable metal (SEM) concentrations (SEM is equivalent to 1 M HCl AEM) are used by equilibrium partitioning approaches (EqP) to identify increased environmental risk when molar concentrations of SEM exceed AVS in the sediment, where higher organic carbon (OC) concentrations also lower the bioavailability of SEM in excess of AVS (Burgess et al. 2013).[5] Based on calculations of SEM-AVS and (SEM-AVS)/f_{OC} (where f_{OC} is the fraction of OC), all contaminated sediments (S2, S3 and S4) indicated potential risk of toxicity to benthic organisms (Table 1).

3.2. Metal profiles in sediments and overlying waters by DGT. Concentrations in blank extracts from DGT-probes were generally low for all metals except for zinc. The blank Cu, Mn, Ni and Pb concentrations were estimated to contribute an equivalent flux <0.2 µg/h/m^2, whereas cadmium and lead contributed <0.005 and <4 µg/h/m^2, respectively.

Significant zinc contamination was found and estimated to contribute for an equivalent flux of 63±40 µg/h/m^2 (mean ± SD).

Fe(II) and Mn(II) profiles in sediments and overlying waters are shown in Figure 1. Increasing DGT-Fe and -Mn fluxes observed within 2 cm below the SWI indicated the presence of the sub-oxic transition zone, where both bacterial-assisted reduction of insoluble iron and manganese oxyhydroxides phases to soluble reduced forms (Fe(II) and Mn(II)), and the oxidation of iron and manganese sulfides to oxyhydroxides, may take place.[22,23,42]

Small but consistent variations in DGT-Cu profiles measured in the laboratory may indicate potential mobilization of this metal at approximately 0.5 cm below the SWI (Figure 1). Similarly, release of DGT-Pb was also observed at this depth in sediment S4. This was attributed to the reductive dissolution of the iron and manganese solid phases,[22,43] and the oxidation of metal sulfides.[44,45] The peaks in DGT metal profiles may also be associated with locations with the greatest rate of degradation of organic matter, resulting in an accompanying release of metals to the pore water.[46,47]

3.3. Differences in DGT-measured metal fluxes between laboratory and field deployments. The location within the sediment profiles and magnitude of the iron and manganese fluxes measured by DGT were similar for laboratory and field deployments for sediments S1 and S2, whereas sediment S3 showed greater iron and manganese fluxes in field deployments and sediment S4 greater iron fluxes in laboratory deployments (Figure 1, with greater detail in Figure S1 of the SI). In sediment S3, DGT-Fe fluxes in the sediment pore waters of the field deployments were approximately 5 times greater than those from the laboratory. Similarly, greater DGT-Mn fluxes were measured in sediment S3 during field deployments in both the sediment pore water (630±370 and 1900±440 µg/h/m^2, for laboratory and field deployments, respectively) and overlying water (16±10 and 840±100 µg/h/m^2, for laboratory and field deployments, respectively). This may be due to the higher oxygen concentrations measured in the overlying water of the laboratory-exposed sediments (i.e., oxygen saturation in the field was ~50-60%, Table S2 of the SI), which may have resulted in greater rates of oxidation of Fe(II) and Mn(II) in both the pore water and overlying water.[14,33,48] As a consequence, porewater iron and manganese concentrations in the laboratory-exposed sediment may have become lower due to a significantly greater rate of precipitation of iron oxyhydroxides occurring as a result of oxygen penetration in the sediment.[42] A different trend was observed for iron in sediment S4, where greater DGT-Fe

fluxes were measured in the sediment pore waters under laboratory conditions (7600±2600 and 2800±2200 µg/h/m^2 for laboratory and field deployments, respectively).

Despite the difference in particulate zinc concentrations (Table 1), similar DGT-Zn fluxes were measured in all laboratory-exposed sediments. In contrast, considerable differences between sites were observed in the field, with a 10 and 100-fold greater DGT-Zn flux measured in sediment S3 and S4, respectively, as compared to S1 and S2 (Figure 1). The DGT-Zn flux was greater in laboratory deployments for sediment S2 (48-53 and 97-209 µg/h/m^2 for field and laboratory deployments, respectively) and in field deployments for sediment S1 (36-51 and 53-87 µg/h/m^2 for laboratory and field deployments, respectively).

DGT-Cu and Pb fluxes appeared to be slightly lower in field deployments, except for sediment S4 which exhibited a greater DGT-Cu flux under field conditions (Figure 1). DGT-Ni fluxes were similar between laboratory and field deployments in sediments S3 and S2, but considerably greater in sediment S4 (and S1 in the sediment pore water) in the field. DGT-Cd fluxes were also considerably larger in field deployments for sediment S4 (2.4 µg/h/m^2), whereas in the other sediments fluxes were <0.3 µg/h/m^2.

Overall, little differences in metal fluxes measured by DGT were observed between laboratory and field deployments in the less contaminated sediments (S1 and S2), and considerably greater fluxes were observed for Cd, Ni and Zn for the two more contaminated sites located the nearer to the mine site (S3 and S4).

3.4. Metal bioaccumulation by the bivalve. Significant bioaccumulation in *H. australis* was observed only for cadmium and zinc. In the laboratory, cadmium bioaccumulation in bivalves exposed to contaminated sediments (S2, S3, S4) exceeded that of organisms exposed to the reference sediment (S1) ($p<0.05$) (Table 1), consistent with the higher particulate cadmium concentrations found in the contaminated sediments (Table 1). Under field conditions, only bivalves exposed to S4 exhibited higher cadmium bioaccumulation than organisms exposed to the reference site (S1) ($p<0.001$), consistent with S4 having the highest level of cadmium contamination. When comparing cadmium tissue concentrations of bivalves exposed to laboratory vs field conditions, bioaccumulation of bivalves deployed in S4 in the field was considerably greater than that of bivalves exposed to the same sediment under laboratory conditions ($p<0.001$), while, in contrast, lower cadmium bioaccumulation was observed in field-deployed bivalves for site S2 and S3 ($p<0.01$) (Figure S2 of the SI). These results indicate a significant difference in cadmium exposure between laboratory and field exposure.

Despite the considerable difference in zinc concentrations found between reference and contaminated sediments (Table 1), no significant differences ($p>0.05$) in zinc bioaccumulation were observed between bivalves exposed to these sediments under laboratory conditions. AEM/TRM ratios between 0.87-0.89 were measured in contaminated sediments, suggesting that only a minor but fairly constant portion of sediment-bound zinc was likely to be in highly mineralized and more inert forms. The similar level of zinc bioaccumulation measured in all the laboratory exposures suggests that zinc tissue concentrations in *H. australis* were not being influenced by the different particulate zinc concentrations. However, significantly greater zinc bioaccumulation was observed in field exposures at sites S3 and S4 ($p<0.001$) compared to the reference site. As the sediments in the respective field and laboratory exposures were the same, this indicates that other factors were influencing the bioaccumulation of zinc. This was confirmed by the strong correlations between dissolved zinc concentrations in overlying waters and bioaccumulation of this metal (Figure 2, additional regression parameters provided in Table S3), indicating a strong contribution from the dissolved phase to the exposure of this bivalve.

3.5. Use of the DGT fluxes for assessing potential for metal bioaccumulation.

The magnitude of the DGT-Cd and -Zn fluxes (average of first and second deployments) were generally consistent with the magnitude of their bioaccumulation by *H. australis* exposed to different treatments (S1, S2, S3, S4) and test conditions (laboratory and field). Generally low DGT-Cu and DGT-Pb fluxes measured in reference (S1) and contaminated (S2, S3, S4) sediments were consistent with low copper and lead bioaccumulation in bivalves exposed to these sediments (Figure 1, Table 1). In the field, the considerably larger DGT-Cd fluxes in S4, and DGT-Zn fluxes in S3 and S4, were consistent with significantly higher concentrations of cadmium and zinc accumulated in the bivalve tissues (Figures 2 and S3). For less contaminated sediments S1 and S2, DGT-metal fluxes in laboratory and field deployments were low (<0.1, <1.5, <2 and <200 $\mu g/h/m^2$ for Cu, Cd, Pb and Zn, respectively) (Figure 1) and within the range of DGT-metal fluxes measured in sediments shown to cause low toxicity to the amphipod *Melita plumulosa* and low bioaccumulation to the bivalve *Tellina deltoidalis*.[22,23,42]

The DGT flux measurements in the overlying water, and sediment pore water near the SWI and in deeper sediments, provide information on metal exposure routes for the bivalve. Previous studies have shown that DGT-metal fluxes measured at the SWI were useful for predicting toxicity to the amphipod *M. plumulosa*[22] and bioaccumulation and toxicity to the

bivalve *T. deltoidalis.*[20,23,42] *H. australis* is a filter-feeding bivalve which has been shown to accumulate metals in response to exposure to contaminated sediments.[49–51] Although exposure pathways for metal uptake have not been directly investigated for this species, previous studies indicated that exposure to the dissolved phase may be a major route of uptake for *H. australis*.[49,50] Since the bivalve may be exposed to metals present in the overlying waters and pore waters (including resupply of DGT-labile metals from the sediment phase that are not measured by direct porewater analyses), relationships between bioaccumulation and DGT-Cd and DGT-Zn fluxes were investigated using fluxes measured in (i) the water column (between 0 and 3 cm above the SWI, DGT_{OLW}), (ii) at the SWI (±1 cm, DGT_{SWI}) and (iii) in the pore water (between 0 and -3 or -4 cm below the SWI, DGT_{SED}) (Figure S3, additional regression parameters provided in Table S3 of the SI).

Strong correlations were found between bioaccumulation and both the log_DGT_{OLW}-Zn flux (R = 0.964) and the log_DGT_{SWI}-Zn flux (R = 0.951), whereas weaker correlations were obtained using corresponding fluxes measured in the sediment pore water (R = 0.820) (Figure S3 of the SI). These strong correlations are consistent with exposure to dissolved zinc being the major route for bioaccumulation for this bivalve. In a companion paper, Marasinghe Wadige et al.[52] showed that the majority of zinc accumulated in the organisms investigated in this study was found in the gills. Linear regression analysis obtained by plotting zinc concentrations measured in the gills against the log_DGT_{SWI}-Zn, log_DGT_{OLW}-Zn, and log_DGT_{SED}-Zn flux indicated strong correlation between DGT measurements and bioaccumulation in gills (Figure S4, additional regression parameters provided in Table S3 of the SI). The better fit obtained using DGT fluxes measured at the SWI (R = 0.960) and in the overlying water (R = 0.941) suggested a predominant exposure of *H. australis* to DGT-labile zinc present in the overlying water rather than DGT-labile zinc released in the bulk sediment (R = 0.883).

Poor relationships were found between cadmium bioaccumulation and DGT-Cd measurements, likely due to the smaller range of cadmium concentrations measured in sediments and overlying water, which resulted in relatively low bioaccumulation of cadmium for all bivalves expect for those exposed to S4 in the field (Figure S2 of the SI).

Overall, predictions of bioaccumulation obtained using the DGT-Zn flux measured in the overlying water and at the SWI were very similar. Previous research has shown that the DGT-metal flux measured at the SWI was useful for predicting bioaccumulation and toxic effects to benthic organisms.[22,23,42] To allow the comparison with this work, only DGT

measurements performed at the SWI (± 1 cm) will be further discussed for the interpretation of bioaccumulation and toxic effects to *H. australis*.

3.6. Comparison between predictions of zinc bioaccumulation based on exposure measurements to particulate zinc in sediments, dissolved zinc in overlying water, and the DGT-Zn flux. Due to the overall low bioaccumulation of cadmium, copper, and lead observed in exposed organisms (Table 1), comparisons of metal exposure and bioaccumulation are discussed only for zinc.

Total and dilute-acid extractable zinc concentrations (TR-Zn and AE-Zn) were highly correlated, and therefore the exposure from particulate zinc only considers AE-Zn. The AE-Zn concentrations provided strong predictions of bioaccumulation in bivalves in the separate laboratory (R = 0.965) and field (R = 0.910) exposures (Figure 2). However, when laboratory and field data were analyzed together, AE-Zn concentrations provided poor predictions of bioaccumulation (R = 0.624) (Figure 2). This was likely due to a much greater contribution of dissolved zinc to the exposure of organisms in the field, as indicated by dissolved zinc concentrations and the DGT measurements performed at the SWI and in the overlying water (Figures 2 and S2 of the SI).

Among the three approaches used to evaluate the risk of excessive zinc bioaccumulation in *H. australis*, the best predictions were obtained using the log_DGT_{SWI}-Zn flux (R = 0.951) and dissolved zinc concentrations (R = 0.940), whereas poor predictions were obtained using AEM concentrations (R = 0.624). TRM and AEM concentrations were measured in sub-samples collected in the field. While TRM concentrations were not expected to change between laboratory and field experiments, AEM may have differed due to alteration of the sediment chemical properties caused by sediment collection and homogenization.[33] Thus, such artifacts may have potentially affected the exposure of organisms to particulate zinc. However, both the DGT flux and dissolved concentrations indicated strong links between dissolved zinc and bioaccumulation (Figures 2 and S2 of the SI), suggesting that bivalves were mainly exposed to forms of zinc that were not inherently measured by the AEM method. This is consistent with the filter-feeding behavior of the organisms and also supported by the greatest concentration of metals found in gills which was accurately predicted by the DGT_{SWI}-Zn and the DGT_{OLW}-Zn flux (Figure S4 of the SI).

In a previous study, we used a quadrant approach to assist with the identification of DGT-based threshold values for predicting significant risk of metal bioaccumulation in bivalves.[23] This approach allowed identification of the DGT_{SWI}-Zn flux above which

bioaccumulation of zinc in bivalves was consistently higher than the mean metal concentration measured in control organisms plus 1 standard deviation (s) (Figure 2). Based on this approach, both dissolved metal concentrations and the DGT_{SWI}-Zn flux clearly distinguished sites and exposures that caused significant bioaccumulation (i.e., bioaccumulation > mean metal concentration measured in control organisms plus 1 s) (top-left quadrant) from those that did not cause significant bioaccumulation (bottom-right quadrant). In contrast, AEM or TRM concentrations did not provide a clear separation between sites and exposures that caused different bioaccumulation (figures not shown). Based on the quadrant approach, a DGT_{SWI}-Zn flux of 220 µg/h/m^2 was found suitable as a potential threshold value for predicting the risk of significant bioaccumulation of zinc (e.g., low risk when DGT-Zn flux was below 220 µg/h/m^2), irrespective of sediment type and exposure (Figure 2). This level of DGT-Zn flux was slightly greater than that found in previous work indicating significant risk of bioaccumulation of zinc in the epibenthic bivalve *Tellina deltoidalis* in the range 130-160 µg/h/m^2.[23,42] Considering the difference in size and exposure routes of the species investigated, DGT predictions of zinc bioaccumulation obtained in the different studies fell in a relatively narrow range, suggesting that the DGT technique could be used for deriving useful threshold values for sediment quality assessment purposes.

3.7. Use of DGT flux data to predict toxicity. In the companion paper, Marasinghe Wadige et al.[52] performed a series of enzymatic assays and sub-cellular distribution analysis to evaluate sub-lethal effects induced by the varying sediment types and exposures. In particular, significantly reduced lysosomal membrane stability, higher lipid peroxidation (malondialdehyde (MDA)), and lower total antioxidant capacity (TAOC) were found in organisms exposed to contaminated sediments in comparison to organisms exposed to reference sediments. In the present study, these data were combined with AEM concentrations, dissolved metal concentrations (dM) measured in discrete overlying water samples, and the DGT flux in order to investigate dose-response relationships useful for predicting the observed toxic effects. The performance of the three different techniques for assessing the potential risk of sub-lethal toxicity associated with metal exposure was assessed using the quadrant approach described in the previous section. To account for the varying toxicity expected to be caused by the different metals, dissolved metal concentrations, DGT_{SWI} fluxes and AEM concentrations were converted to WQG value-based toxic units (TU), WQG value-based DGT_{SWI} fluxes ($DGT_{WQG\text{-}SWI}$), and SQG value-based quotients

(SQG-Q), respectively (details provided in the Materials and Methods section). Based on the guideline quotient approach, onset of significantly lower lysosomal stability was similarly predicted by quotients based on AEM and $DGT_{WQG\text{-}SWI}$ fluxes (Figure 3), although the SQG-Q plot featured a data point in the top-left quadrant, indicating a weaker ability of this approach to separate toxic from non-toxic exposures. No significant trends were observed for TOAC, most likely due to the overall little differences in effects detected by this biomarker among the various exposures (Figure 3). TAOC appeared to decrease in response to increasing SQG-Qs and $DGT_{WQG\text{-}SWI}$ fluxes, consistently with increased oxidative stress induced by exposure to metals.[49,51,53] However, slightly lower TAOC was observed in laboratory-exposed organisms compared to field-exposed organisms,[52] in contrast with the greater exposure to cadmium and zinc in the field (S3 and S4) predicted by the DGT flux and dissolved concentrations (Figures1, 2 and S3). As a consequence, uncertainty still remain as to whether changes in TAOC may have been linked to exposure to metals or due to other factors.

Lipid peroxidation generally increased with increasing SQG-Qs, TUs, and $DGT_{WQG\text{-}SWI}$ fluxes (Figure 3), however, according to the quadrant approach, no suitable threshold values could be derived using SQG-Qs and TUs. In contrast, the $DGT_{WQG\text{-}SWI}$ flux was useful for separating toxic from non-toxic exposures (Figure 3), although a greater toxicity range may have contributed to a better evaluation of the performance of the three different approaches. $DGT_{WQG\text{-}SWI}$ fluxes of 9 and 19 $\mu g_{WQG}/h/m^2$ were identified as suitable threshold values for predicting potential risk of lysosomal destabilization and lipid peroxidation, respectively (Figure 3). These values were of similar magnitude to those indicating potential sub-lethal effects to the amphipod *M. plumulosa* for a $DGT_{WQG\text{-}SWI}$ flux greater than 17 $\mu g_{WQG}/h/m^2$.[22]

4. CONCLUSIONS

Overall, the findings of this study indicate that differences between laboratory and field-based bioassays may result in significantly different predictions of risk due to the inability of laboratory bioassays to recreate realistic exposure conditions. Among the three approaches investigated, the DGT-metal flux and dissolved metal concentrations appeared to provide more reliable predictions of metal exposure of *H. australis*, consistent with the filter feeding behavior of this bivalve. While measuring dissolved metal concentrations in discrete water samples is less labor-intensive than performing DGT measurements, for deposit-feeding organisms such as *T. deltoidalis*, exposure to particulate metals and release of metals at the

SWI may significantly contribute to benthic organisms' exposure.[23] Therefore, measurements that may be representative of both exposure to sediment and overlying water, such as DGT, could be useful for a more comprehensive evaluation of the risk posed by metal contaminants to benthic organisms. Strong relationships between the DGT-Zn flux and bioaccumulation were found irrespective of the different chemical and physical properties of the sediments (Table 1) and type of exposure (laboratory and field). Similarly, the $DGT_{WQG\text{-}SWI}$ flux was also useful for predicting sub-lethal effects to the bivalve. The DGT-based threshold values identified in this study may act as possible threshold values above which the risk of zinc bioaccumulation and sub-lethal effects caused by exposure to metal mixtures may be expected to become significant. These results suggest that the DGT technique has the potential to be used as a tool for *in situ* monitoring of metal bioavailability in contaminated sediments, and may improve this line of evidence when applied for sediment quality and risk assessment purposes.

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Supporting information

Plastic cages (2 cm mesh) used to for the *in situ* bioassay. Metal concentrations in the test sediments. Water quality parameters measured during the field bioassay.

Comparison between DGT-Fe and -Mn vertical profiles measured in the first and second deployment in laboratory-exposed sediments. Relationships between bioaccumulation and the DGT-Zn flux measured in the overlying water (OLW) (between 0 and 3 cm above the SWI), at the SWI (± 1 cm), and in the pore water (SED) between 0 and -2.5 and between 0 and -3 cm below the SWI for laboratory and field deployment, respectively. Relationships between bioaccumulation and the DGT-Cd flux measured in the overlying water (OLW) (between 0

and 3 cm above SWI), at the SWI (± 1 cm), and in the pore water (SED) between 0 and 2.5 and between 0 and 3 cm below the SWI for laboratory and filed deployment, respectively. Relationships between bioaccumulation in the gills of *H. australis* and the DGT-Zn flux measured in the overlying water (OLW) (between 0 and 3 cm above the SWI), at the SWI (± 1 cm), and in the pore water (SED) between 0 and 2.5 and between 0 and 3 cm below the SWI for laboratory and field deployment, respectively.

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725 Gigas, Leading to Mitochondrial Disruption and Oxidative Stress. *Aquat. Toxicol.* **2014**,
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727

728
729 **Table 1. Physical and chemical properties of sediments and bioaccumulation in *H. australis*.**

	Total recoverable metals (TRM), mg/kg				Tissue concentration in *H. australis* (µg/g) - laboratory				AVS	Particle size
Sediment	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn	µmol/g	< 63 µm, %
S1 (reference)	<1	10.0±0.3	16±1	95±2	0.08±0.02	4.6±0.2	2.9±0.3	258±8	<0.5	31±2.7
S2	3.2±0.1*	26.9±0.9	47.2±0.3	910±2*	0.17±0.01ab	5.0±0.4	2.6±0.3	281±7	1.7±0.6	48±0.4
S3	1.2±0.1	26.2±0.3	33±1	840±10*	0.32±0.01ab	5.23±0.3	2.8±0.4	297±12b	<0.5	96±0.4
S4	5.3±0.1*	55.1±0.5	130±1*	2100±18*	0.31±0.01ab	5.0±0.4	3.4±0.3	325±20b	6.6±0.7	43±0.4
	Dilute-acid extractable metals (AEM), mg/kg				Tissue concentration in *H. australis* (µg/g) - field				SEM-AVS	TOC
Sediment	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn	µmol/g	%
S1 (reference)	<1	5.9±0.1	8.9±0.2	58±3	0.07±0.02	5.5±0.5	2.7±0.2	294±15	1	1.4
S2	2.6±0.3*	4.8±0.3	37±2	790±14*	0.07±0.02b	5.8±0.6	3.6±0.9	301±25	11	1.6
S3	1.0±0.1	9.0±0.2	23±1	750±33*	0.08±0.03b	6.2±0.4	4.5±0.9	447±48ab	12	4.3
S4	4.5±0.2*	5.4±0.3	106±5*	1800±80*	1.47±0.14ab	5.1±0.6	3.4±0.3	756±42ab	22	1.7
SQG values	1.5	65	50	200					0	

730 AVS = acid-volatile sulfide; SEM-AVS = the molar difference, where SEM is equivalent to AEM. TOC = total organic carbon and % <63 µm
731 refers to the percentage (by weight) of fine sediment particles. All concentration are mean ± SE (*n*=3). Concentrations for a greater range of
732 metals and metalloids are provided in the Table S1. Bioaccumulation data are mean ± SE (*n*=15, dry mass). The asterisk denotes concentrations
733 exceeding the sediment quality guideline values (SQG).[1] The letters 'a' and 'b' indicate statistically different bioaccumulation (see main text)
734 between bivalves exposed to the reference (S1) and contaminated sediments (S2, S3, S4), and between bivalves exposed to laboratory and field
735 conditions, respectively.
736

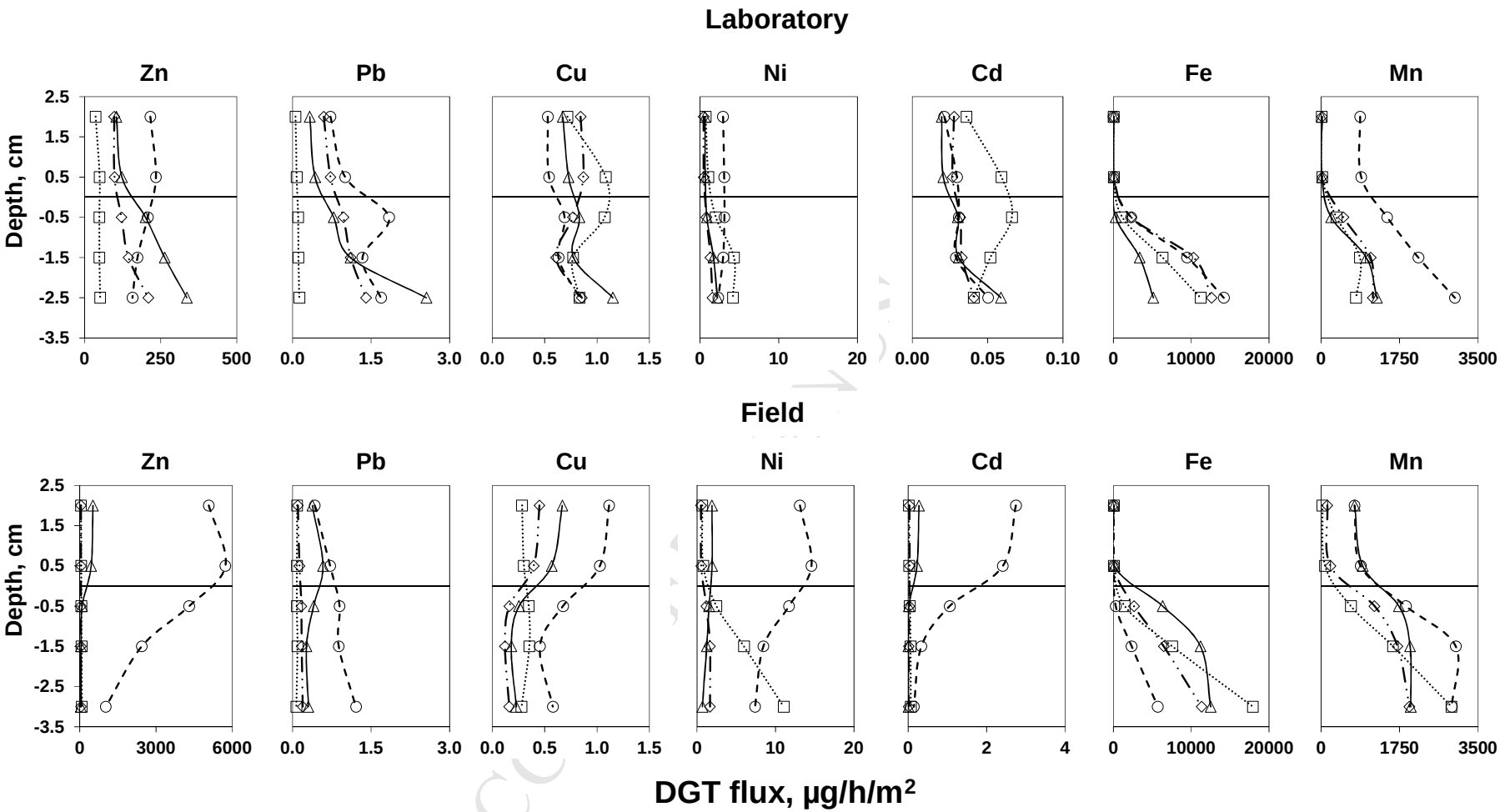
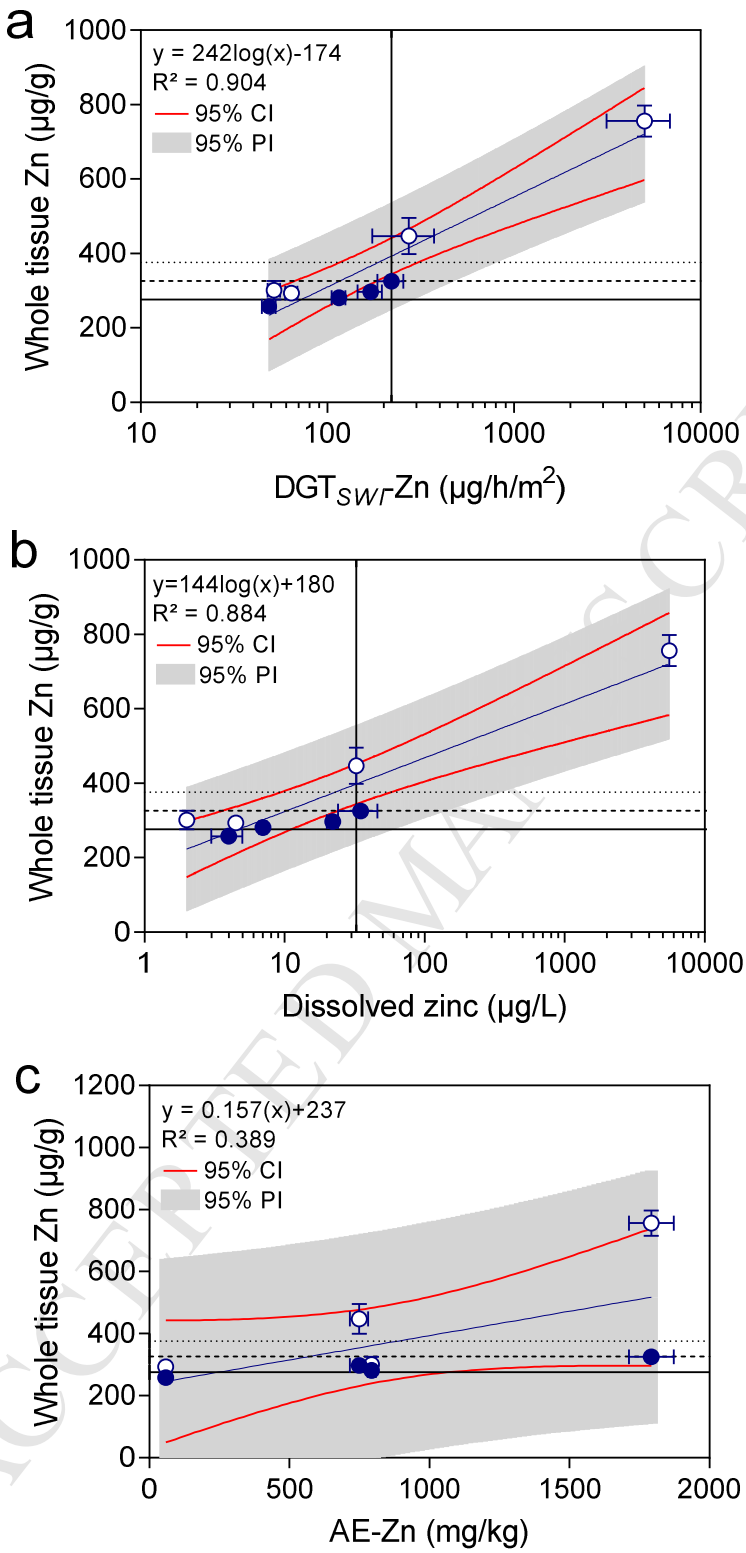


Figure 1. DGT profiles of Zn, Pb, Cu, Ni and Cd measured in the different sediments (S1(□), S2(◇), S3(△), S4(○),) in the laboratory and field. Data points are average values of six replicates from the first and second deployments (standard deviations were ~25 and ~30% of mean values for laboratory and field deployments, respectively). Connecting lines are for visual aid only.



741

742 **Figure 2.** Comparison between predictions of bioaccumulation in *H. australis* obtained using
743 (a) log_DGT$_{SWI}$-Zn flux, (b) dissolved zinc concentrations, (c) dilute-acid extractable
744 (particulate) AE-Zn concentrations, and. Filled and open circles refer to laboratory and field
745 deployments, respectively. Shaded areas and red lines represent the 95% prediction intervals
746 (PI) and 95% confidence intervals (CI), respectively. The solid horizontal and vertical lines

747 separate the data into four quadrants: the solid horizontal line represents the mean
748 concentration found in bivalves exposed to control sediments (average of laboratory and
749 field), whereas the dashed and dotted horizontal lines are the mean concentration measured in
750 bivalves exposed to control sediments plus 1s and 2s, respectively; the continuous vertical
751 line corresponds to the dissolved zinc concentration, AE-Zn concentration, or DGT-Zn flux
752 above which bioaccumulation was consistently higher than the controls mean plus 1s. Data
753 points are means ± SE (n=2, 3, 6, and 15 for dissolved zinc concentrations, AE-Zn
754 concentrations, log_DGT_{SWI}-Zn fluxes and whole tissue zinc concentrations in *H. australis*,
755 respectively).

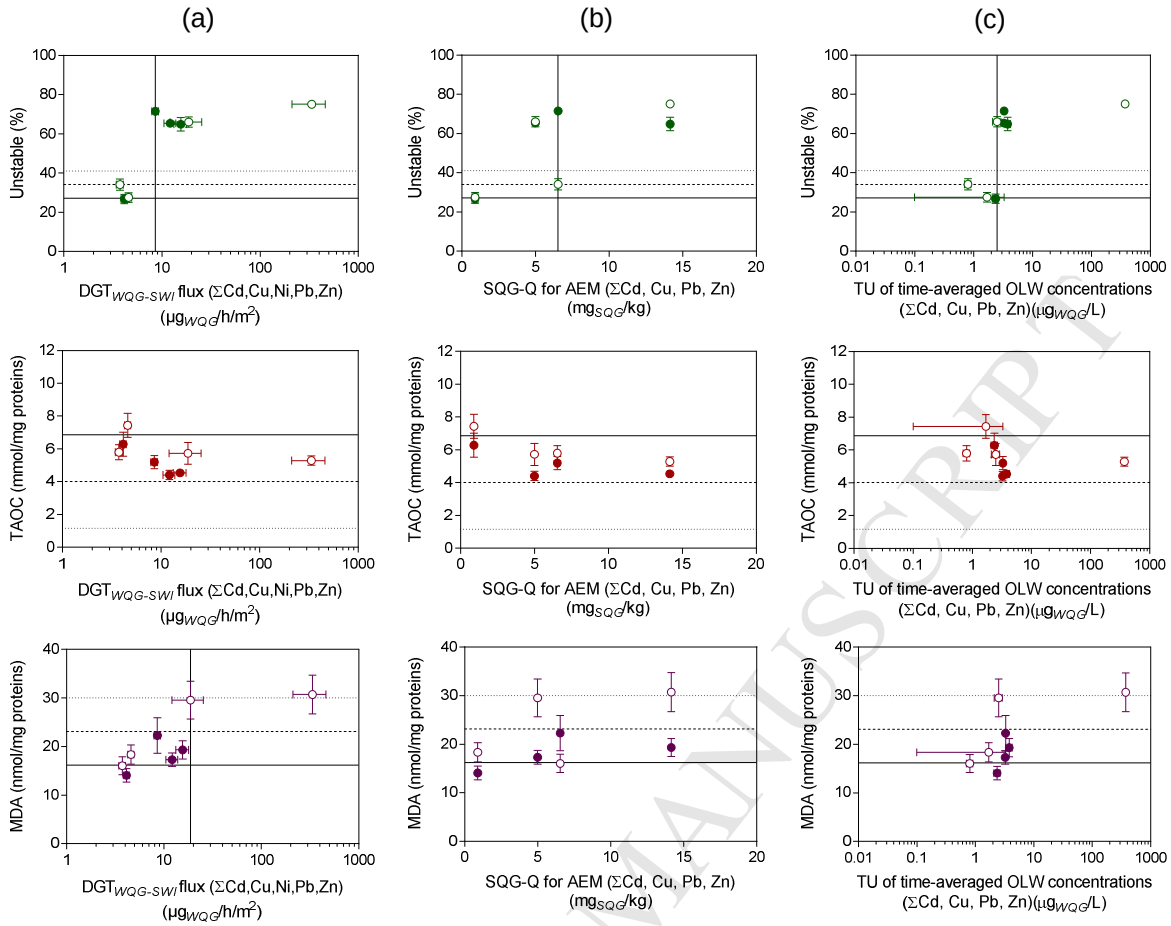


Figure 3. Comparison between predictions of stress biomarker levels in *H. australis* obtained using (a) DGT_{SWI} flux normalized to WQG threshold values, (b) SQG-Q for AEM concentrations, and (c) TU of time averaged OLW concentrations. Filled and open circles refer to laboratory and field deployments, respectively. The solid horizontal and vertical lines separate the data into four quadrants: the solid horizontal line represents the mean concentration found in bivalves exposed to control sediments (average of laboratory and field), whereas the dashed and dotted horizontal lines are the mean concentrations measured in bivalves exposed to control sediments plus 1s and 2s, respectively; the continuous vertical line corresponds to the DGT_{SWI} flux, SQG-Q concentration or TU concentrations above which bioaccumulation was consistently higher than the controls mean plus 1s.

- Laboratory and field sediment bioassays provided different predictions of risk
- The exposure of organisms to metals differed between laboratory and field bioassays
- DGT predicted the risk of bioaccumulation and toxicity irrespective of the exposure
- *In situ* evaluation of metal bioavailability may improve sediment quality assessment