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Assessing the Risk of Metals and Their Mixtures in the Antarctic Nearshore Marine Environment with Diffusive Gradients in Thin-Films

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Robust environmental assessments and contaminant monitoring in Antarctic near-shore marine environments need new techniques to overcome challenges presented by a highly dynamic environment. This study outlines an approach for contaminant monitoring and risk assessment in Antarctic marine conditions using diffusive gradients in thin-films (DGT) coupled to regionally specific ecotoxicology data and environmental quality standards. This is demonstrated in a field study where DGT samplers were deployed in the near-shore marine environment of East Antarctica around the operational Casey station and the abandoned Wilkes station to measure the time-averaged biologically available fraction of metal contaminants. The incorporation of DGT-labile concentrations to reference toxicity mixture models for three Antarctic organisms predicted low toxic effects (<5% effect to the growth or development of each organism). The comparison of metal concentrations to the Australian and New Zealand default water quality guideline values (WQGVs) showed no marine site exceeding the WQGVs for 95% species protection. However, all sites exceeded the 99% WQGVs due to copper concentrations that are likely of geogenic origin (i.e., not from anthropogenic sources). This study provides evidence supporting the use of the DGT technique to monitor contaminants and assess their environmental risk in the near-shore marine environment of Antarctica.

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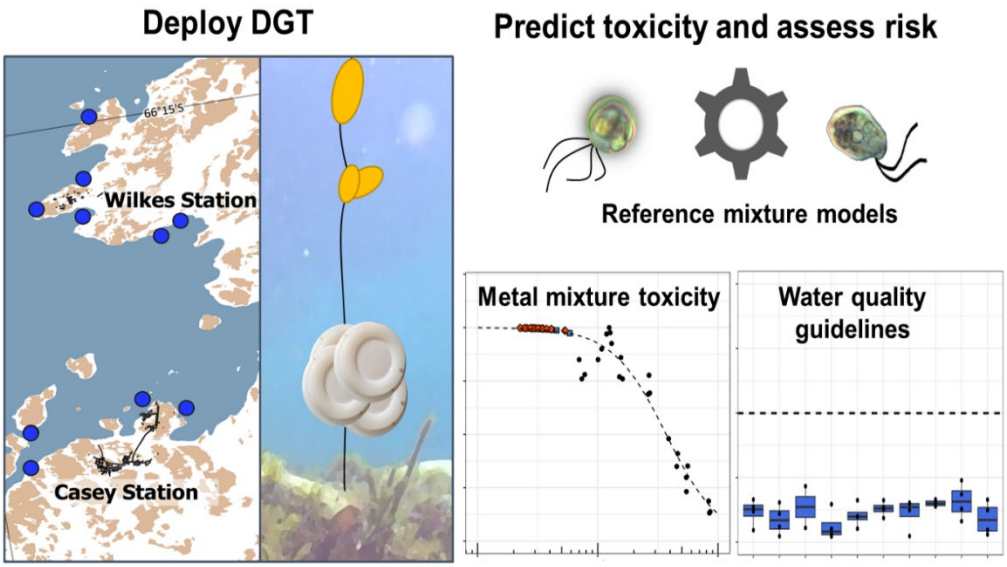
Graphical abstract



Keywords

Environmental management, trace element, metal bioavailability, toxicity modeling, *Phaeocystis*

Abstract

Robust environmental assessments and contaminant monitoring in Antarctic near-shore marine environments need new techniques to overcome challenges presented by a highly dynamic environment. This study outlines an approach for contaminant monitoring and risk assessment in Antarctic marine conditions using diffusive gradients in thin-films (DGT) coupled to regionally-specific ecotoxicology data and environmental quality standards. This is demonstrated in a field study where DGT samplers were deployed in the near-shore marine environment of East Antarctica around the operational Casey station and the abandoned Wilkes station to measure the time-averaged biologically available fraction of metal contaminants. The incorporation of DGT-labile concentrations to reference toxicity mixture models for three Antarctic organisms predicted low toxic effects (<5% effect to the growth or development of each organism). The comparison of metal concentrations to the Australian and New Zealand default water quality guideline values (WQGVs) showed no marine site exceeding the WQGVs for 95% species protection. However, all sites exceeded the 99% WQGVs due to copper concentrations that are likely of geogenic origin (i.e. not from anthropogenic sources). This study provides evidence supporting the use of the DGT technique to monitor contaminants and assessing their environmental risk in the near-shore marine environment of Antarctica.

Introduction

Exploration, tourism, and scientific research activities have left a potentially toxic legacy of organic and inorganic contamination in localized areas of Antarctica particularly around research stations [1]. The majority of these research stations are built on scarce coastal ice-free rocky areas, which represent approximately 6,000 km^2 of the Antarctic continent [2,3]. An estimated total of 53 stations are in operation in these areas, with many more abandoned and decommissioned stations, waste sites, and field camps present [4]. Contaminants such as cadmium, copper, nickel, lead, and zinc from these sites are mobilized to the coastal environment through processes of dissolution and/or particle entrainment from melt waters running through the sites during the summer season [5,6]. This has already been shown to affect the near-shore marine ecosystem [7,8], and is expected to worsen with increasing temperatures associated with climate change [9].

The Protocol on Environmental Protection to the Antarctic Treaty defines the need for environmental monitoring, impact assessments, and the remediation of historical waste [10]. The unique environmental conditions and ecosystems in Antarctica necessitate the validation of environmental management techniques commonly used in temperate or tropical environments [11]. Recent examples have included toxicity assessments using Antarctic organisms [12] or the use of contaminant monitoring tools [13].

Diffusive gradients in thin-films (DGT) is an *in situ* passive sampling technique that has been used in Antarctica to assess labile metal concentrations in marine waters [6,14–16]. While DGT is predominately used to measure a labile fraction of metal contaminants in the environment [14], it has recently being used to predict the toxicity and bioavailability of contaminants to benthic organisms [17–19]. The ease of use, ability to provide a time-averaged measure of a labile metal fraction, low cost, and simultaneous detection of multiple contaminants makes DGT samplers an attractive option for environmental monitoring, especially in remote and harsh environments. However, their application to the Antarctic marine environment and practicality to environmental managers is still unclear [6].

The use of DGT as monitoring tools with reference models to integrate the risk of contaminant mixtures provides a novel method of *in situ* contaminant assessment in the Antarctic marine environment. Two reference models of mixture toxicity are widely used in

environmental toxicology: independent action (IA) and concentration addition (CA) [20]. Both provide ways of integrating expected toxicities from individual components of the mixture using toxicity thresholds, such as EC_{10} or EC_{50} values, derived from single-metal exposures. In lieu of toxicity thresholds, water quality guidelines (WQGVs) may be used to calculate a risk quotient to describe the risk of contaminant mixtures [18,21]. WQGVs are ideally calculated using a cumulative probability distribution of chronic toxicity endpoints (based on dissolved metal concentrations) to derive guideline concentrations which are likely to protect a defined proportion of species [22].

This field study aims to demonstrate the applicability of DGT for environmental monitoring and risk assessment in Antarctic marine environments. The process outlined couples DGT-labile concentrations from *in-situ* Antarctic field deployments with existing ecotoxicological data for Antarctic organisms and Australian national environmental quality standards to predict the risk of mixtures of cadmium, copper, nickel, lead, and zinc from anthropogenic activities to Antarctic near-shore marine environments.

Methods

Sampling locations

Eleven sites in Newcomb Bay in the Windmill Islands region of East Antarctica, were selected for sampling and DGT sampler deployment (Fig. 1). Their proximity to sites of anthropogenic disturbance, including the abandoned Wilkes station and the operational Casey station, the surrounding environment type, and meltwater flows based on personal observation and reports from Fryirs et al. [23], were considerations in identifying deployment sites to provide a range of potentially impacted and control sites.

DGT synthesis and field deployment

DGT pistons with a Chelex-100 binding layer were prepared following the procedures recommended by DGT Research (Lancaster, UK) as outlined by Davison [24]. The binding resin was a 0.4 mm thick polyacrylamide gel laden with Chelex-100 (Bio-Rad, mesh 200-400). During the binding resin synthesis, Chelex-100 beads concentrated at the bottom of the gel by gravitational settling. This concentrated side was placed towards the window of the DGT, in contact with the 0.8 mm thick polyacrylamide diffusive layer. A 0.13 mm thick, 0.45 µm

pore size polyethersulfone filter paper was placed on top of the diffusive layer. The three layers were sandwiched on the piston base by a housing with a 2 cm diameter window. Prior to deployment, assembled pistons were conditioned for 24 h in a 0.12 M NaCl solution (Suprapur, Merck Millipore). Prepared DGT samplers were stored moist in low-density polyethylene bags at 4 °C for up to 3 months before use.

In Antarctica, four DGT samplers were attached to acid-washed polypropylene baskets with nylon thread. The prepared DGT baskets were stored moist in low-density polyethylene bags at 4 °C until deployment.

Moorings were created by connecting a mesh bag filled with locally collected rocks (devoid of moss or lichens) to hard-plastic buoys (2 large and 1 small) with synthetic rope. The moorings were approximately 5 m in length and designed to ensure the two large buoys remained ~2 m under the water, with only the small buoy rising to the surface. This design was used with a goal to prevent snagging of the moorings on ice floes or ice bergs. However, two moorings were lost (presumably by iceberg ensnarement) and some others were found 10-50 m from their original deployed locations.

Prior to deployment, DGT baskets were attached to the mooring using plastic cable ties. Moorings were deployed to a site after the depth was confirmed to be between 3 and 5 m. Irrespective of the final deployment depth, DGT samplers were positioned 1 m above the rock bag. Samplers were deployed for between 22 and 37 days (Fig. 1), between December 27, 2017 and February 11, 2018. Dates for deployment were limited by sea ice which prohibited the deployment of inflatable rubber boats from the station wharf during the 2017/18 summer season at Casey Station.

Following the period of deployment, moorings were retrieved and DGT devices were recovered from the cages, rinsed with ultrapure water, and returned to the station laboratories. DGT devices were disassembled and the binding resin placed in 1 mL of 1 M HNO_3 (Suprapur grade, Merck Millipore) for ≥12 h on an orbital shaker. The resulting eluents were diluted to a final concentration of 0.2% HNO_3 and stored at 4 °C until analyzed in Australia. Field blank DGT samplers were treated in the same manner as deployed DGT samplers (i.e. attached to polypropylene baskets and stored moist at 4 °C until eluted), and were used to calculate DGT limits of detection.

Seawater sampling and measurements at DGT deployment sites

Seawater was sampled at deployment sites from an inflatable rubber boat. All samples were taken at a depth of approximately 30 to 50 cm below the surface to avoid the layer of less saline seawater that develops on the sea surface from sea-ice melt. Physicochemical parameters of seawater samples including salinity (PSU), dissolved oxygen (mg L^{-1}), and pH were measured directly in the seawater using a Professional Plus Multiparameter Instrument (YSI, USA), calibrated as per manufacturer's instruction on the day of sampling. Seawater for metal analysis was sampled from the boat. Approximately 20 mL was withdrawn into a plastic syringe and filtered to 0.45 µm (polyethersulfone membrane, Sartorius). To rinse the filter unit the first 10 mL of seawater was discarded and the second 10 mL filtered directly into acid-washed plastic vials. These samples were acidified to 0.2% HNO_3 in Antarctica and returned to Australia for metal analysis.

Physical weather observations on each sampling day including hours of sunlight and maximum and minimum air temperatures were obtained from recordings published by the Australian Bureau of Meteorology's automatic weather station (AWS 300017) at Casey station.

Metal analysis

Metal analysis was conducted in Australia by inductively coupled plasma - atomic emission spectrometry (ICP-AES, Varian 730-ES) or ICP – mass spectroscopy (ICP-MS, Agilent 7900) where lower detection limits were needed, using matrix-matched calibration standards (i.e. ultrapure water for DGT eluents or seawater for seawater grab samples). A multi-element standard (QCS27; Analytical West Inc.) was used as a drift standard to correct for measurement suppression over time, particularly for samples in a seawater matrix. Metal detection limits of the ICP-AES were 0.1 µg Cd L^{-1}, 1.0 µg Cu L^{-1}, 0.3 µg Ni L^{-1}, 1.0 µg Pb L^{-1}, and 0.1 µg Zn L^{-1} (Supplementary Table 1). Two certified reference materials were analyzed to validate instrument measurements, TM-24.4 (lot 0916) and TMDA-64.3 (lot 0317) (National Research Council, Canada) and had recoveries of between 91 and 110 % (Supplementary Table 1). Non-deployed DGT were used as field blanks and to calculate DGT limits of detection (Supplementary Table 2) which were then used to calculate method detection limits (Supplementary Table 3), here defined as the minimum concentration

153 detectable in receiving waters given the deployment conditions and detections limits of this
154 study.

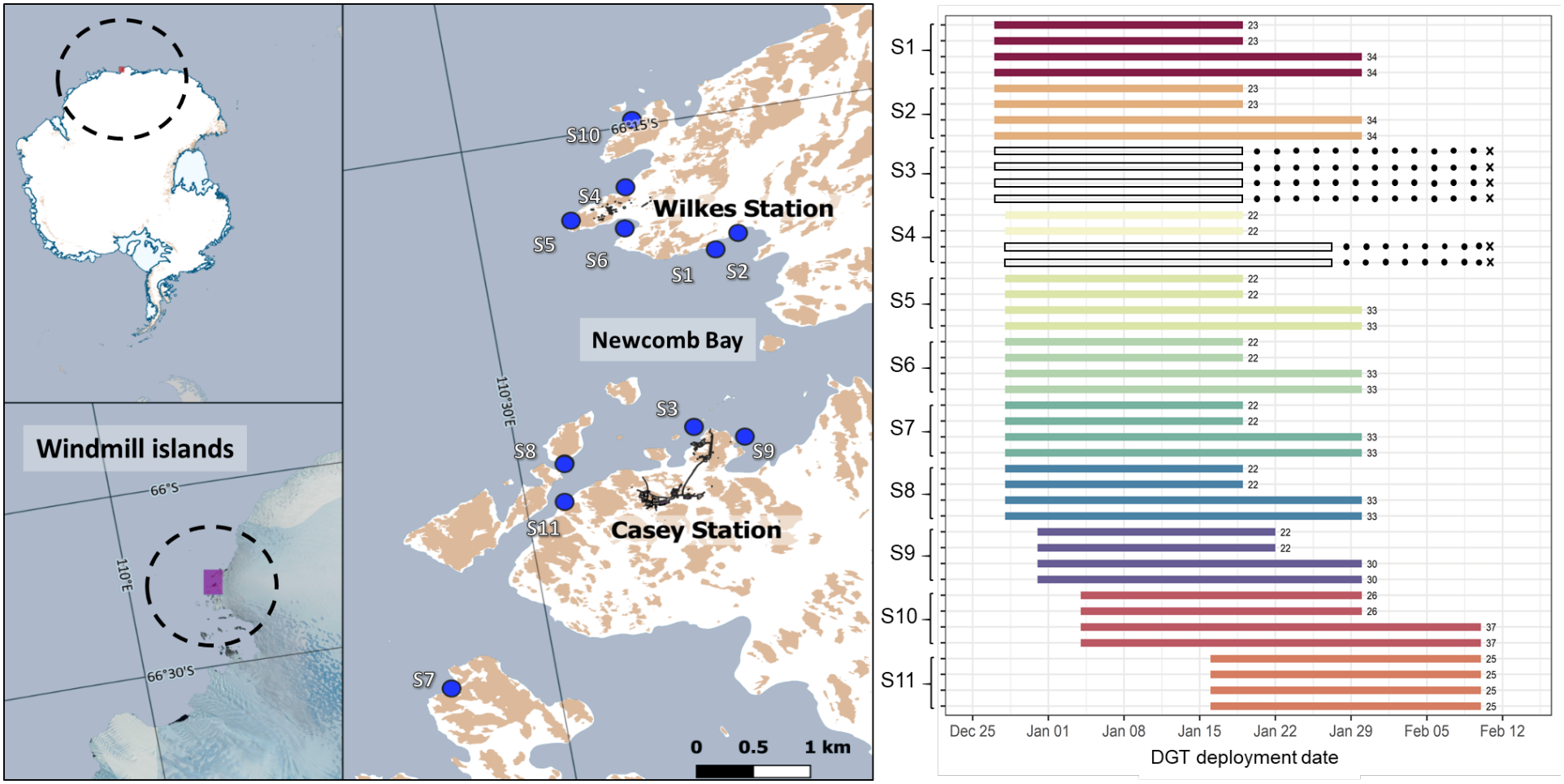


Figure 1. Locations and deployment durations of diffusive gradients in thin-films (DGT) around Casey and Wilkes stations in the Newcomb Bay area, Windmill Islands, East Antarctica [25,26]. Colored bars represent the deployment dates for each DGT at each site. Numbers at the end of bars indicate total days of deployment. Lost moorings are represented by an unfilled bar (indicating the dates its location was known), followed by dots indicating the period it was missing. The search for the lost moorings was terminated on the date indicated by the x.

160 ***Data analysis***

161 ***Predicting toxicity using DGT-labile concentrations***

162 The measured metal concentrations from DGT eluents were converted to a mass of metal
163 (M_i, in ng) accumulated to the binding resin by Equation 1.

164 **Equation 1** $$M_i = \frac{C_e\,(V_e + V_{gel})}{f_e}$$

165 where C_e is the concentration of metal *i* in the eluent (µg L^{-1}), V_e and V_{gel} are the volumes
166 (mL) of the eluent and gel, respectively, and f_e is the elution factor, which was 0.8 for all
167 metals [27]. The DGT-labile concentration (C_{DGT}, in units of ng mL^{-1} but will be reported in the
168 equivalent units of µg L^{-1}) was then determined by Equation 2:

169 **Equation 2** $$C_{DGT} = \frac{M_i\,\Delta g}{D_i\,t\,A}$$

170 where D_i is the diffusion coefficient of metal i (in units of $x10^{-6}$ cm^2 s^{-1} at 1 °C) given by
171 Koppel et al.[28], M_i is mass (ng) accumulated over time t (s), Δg is the thickness of the overall
172 diffusion layer (0.093 cm, the combined thickness of the diffusive gel and filter membrane,
173 assuming negligible water diffusion layer thickness), and A (3.14 cm^2) is the surface area of
174 the exposed window of the DGT piston [27].

175 DGT-labile concentrations were used in mixture models to determine DGT-predicted
176 toxicities. This was conducted for two common Antarctic marine microalgae for which
177 cadmium, copper, nickel, lead, and zinc single-metal toxicity data exist [29,30] and an Antarctic
178 echinoderm for which copper and zinc single-metal toxicity data exists [31]. The slope (β_i) and
179 EC_{10i} of single-metal log-logistic concentration-response curves was used to parameterize
180 the independent action (IA, Equation 3) and concentration addition (CA, Equation 4) toxicity
181 mixture models [32], where x_i is the DGT-labile metal concentration and y is the predicted
182 population growth rate inhibition for metal i. The parameters used in these models for each
183 species are provided in Supplementary Table 4.

184 **Equation 3** $$y_{IA} = 100 * \prod_{i=1}^{n} \frac{1}{1+\left(\frac{x_i}{EC10_i * 9^{\frac{1}{\beta_i}}}\right)^{\beta_i}}$$

185

Equation 4 $$\sum_{i=1}^{n} \frac{x_i}{(EC10_i * 9^{\frac{1}{\beta i}}) * (\frac{100 - y_{CA}}{y_{CA}})^{\frac{1}{\beta i}}} = 1$$

Determination of risk quotients

To assist in interpreting the risk of C_{DGT} derived concentrations, a risk quotient approach was adopted using the default Australian and New Zealand marine WQGVs for 99 and 95% species protection [22]. The risk quotient was defined for each site by Equation 5:

Equation 5 $$\sum_{i=1}^{n} \frac{C_{DGT}}{WQGV_x}$$

where $WQGV_x$ represents the default guideline value for x% species protection at the 99% and 95% levels for metal i, which are 0.7 and 5.5 µg Cd L^{-1}, 0.3 and 1.3 µg Cu L^{-1}, 7 and 70 µg Ni L^{-1}, 2.2 and 4.4 µg Pb L^{-1}, and 7 and 15 µg Zn L^{-1}, respectively.

Results

Physicochemical properties of seawater and weather observations at deployment sites

The climate for the deployment period through the mid-summer period at Casey station was characterized by: sea temperature rising from -1.2 °C in December to -0.2 °C in the middle of January, then decreasing to -1.2 °C by February; dissolved oxygen concentration decreasing from 14 mg L^{-1} to 12 mg L^{-1}; pH of 7.8 to 7.9; and salinity increasing from 29 to 35 ppt. The physicochemical properties of the near-shore marine waters and weather observations, averaged across all sites, are given in Fig. 2. All measurements were within the expected range for the Antarctic near-shore marine environment in Newcomb Bay, based on previous studies.

Metal concentrations in seawater at deployment sites

Dissolved metal concentrations in seawater taken at deployment sites were typically below instrument detection limits (see Methods section) throughout the deployment period. Some sites had nickel and cadmium concentrations around 0.1 µg L^{-1}, and zinc concentrations up to 10 µg L^{-1}; however, there were no clear temporal or spatial trends explaining these concentrations which could be explained by contamination from the gloves used in the inflatable boat (Supplementary Figure 1).

DGT-labile concentrations from deployment sites were low and typical of clean near-shore marine environments in Antarctica [14,33]. Lead was the only metal below detection limits (<0.2 μg L^{-1}, method detection limit for DGT deployments in these conditions, times, and within the instrument's limit of detection of 1.0 μg Pb L^{-1}), and so was analyzed by ICP-MS. The average DGT-labile concentrations (mean ± standard deviation, with range of measurements in brackets) for all sites were: cadmium 0.07 ± 0.01 μg L^{-1} (0.04 – 0.09 μg L^{-1}), copper 0.28 ± 0.08 μg L^{-1} (0.14 – 0.47 μg L^{-1}), nickel 0.39 ± 0.07 μg L^{-1} (0.29 – 0.62 μg L^{-1}), lead 0.022 ± 0.008 (0.008 – 0.037 μg L^{-1}), and zinc 1.3 ± 0.5 μg L^{-1} (0.6 – 2.3 μg L^{-1}) (Fig. 3, Table 1).

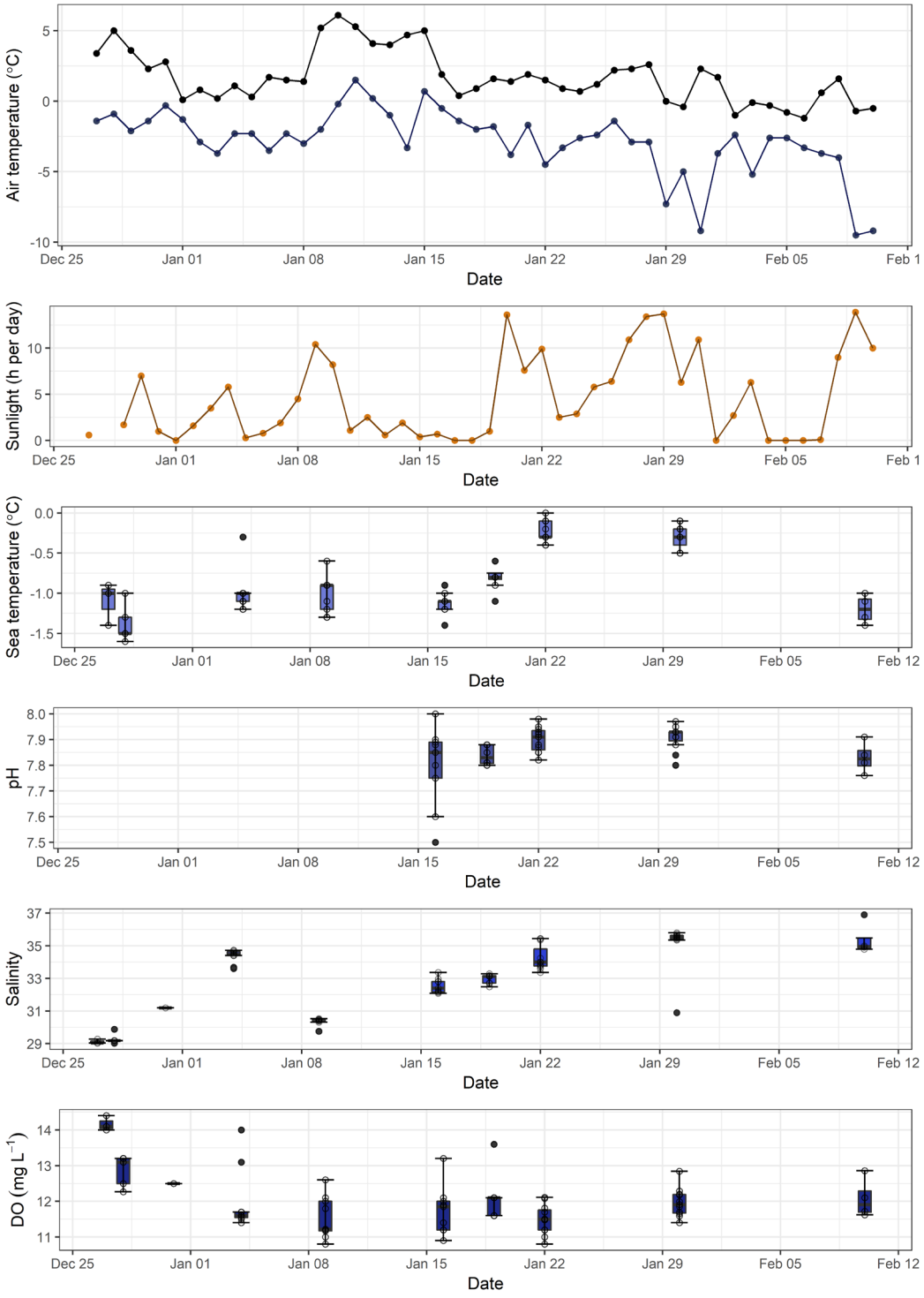


Figure 2. Physicochemical conditions of seawater and weather observations at DGT deployment sites throughout the deployment period. Sunlight photo period and air temperature (day minimum and maximum) data taken from the Australian Bureau of Meteorology's automatic weather station (AWS 300017) at Casey station [34]. Error bars represent 1.5 times the interquartile range with outliers as black dots outside the range. Observations from December 31, 2017 were taken through a drilled hole in the sea ice at a depth of approximately 1.8 m. This may be more representative of sea ice conditions than open seawater. An issue with the calibration of the pH probe prevented reliable measurements before January 15, 2018.

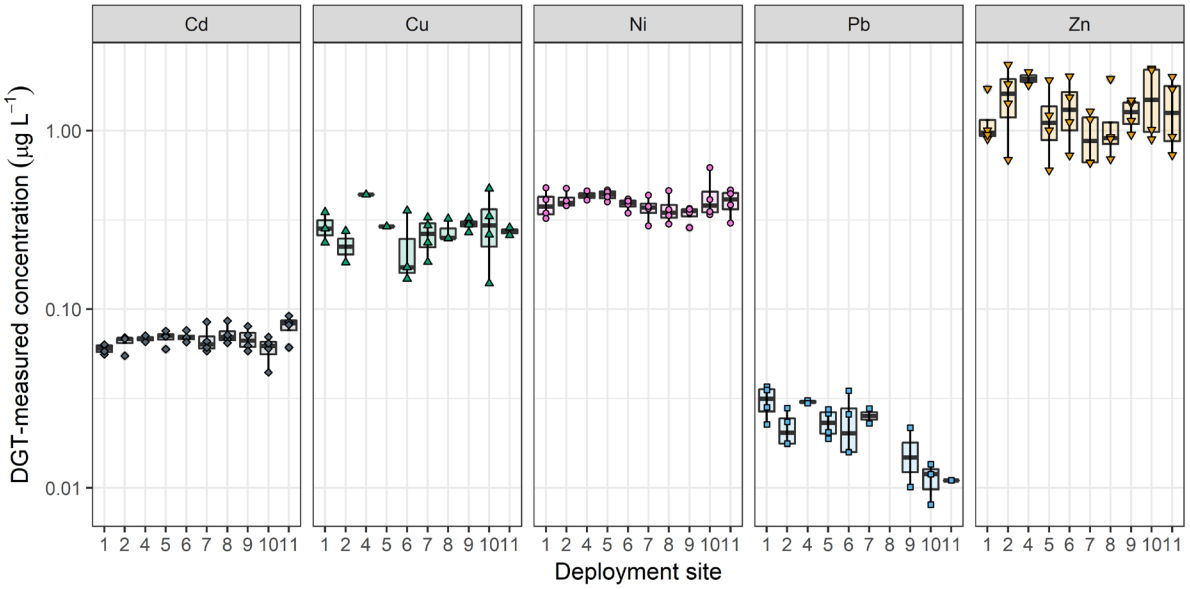


Figure 3. DGT-labile concentrations of cadmium, copper, nickel, lead, and zinc from 20- to 37-d deployments in the near-shore marine environment around Casey and Wilkes stations, East Antarctica. Error bars represent 1.5 times the interquartile range with outliers as black dots outside the range.

Table 1. DGT-labile concentrations of metals from deployments in the near-shore marine environment around Casey and Wilkes station, East Antarctica, compared to previously reported measurements in nearshore and open ocean environments in Antarctica. Values are mean ± standard deviation (µg L^{-1}). Measurements from the literature are averaged where samples from multiple sites were analyzed.

	This study	Cabrita et al. [14]	Kim et al. [33]	Sañudo-Wilhelmy et al. [35]
Location	Newcomb Bay, East Antarctica	Ardley Cove, King George Island	Marion Cove, King George Island	Weddell Sea, Antarctic Peninsula
Sampling method	Chelex-100 DGT	Chelex-100 DGT	Preconcentration to Chelex-100	Preconcentration to APDC/DDDC[a]
Cd	0.07 ± 0.01	0.20 ± 0.04	0.0031 ± 0.0005	0.05 ± 0.02
Cu	0.28 ± 0.08	~1.7[b]	0.04 ± 0.03	0.012 ± 0.02
Ni	0.39 ± 0.07	~1.6[b]	0.033± 0.004	0.034 ± 0.03
Pb	0.022 ± 0.008	0.43 ± 0.05	0.016 ± 0.007	0.003 ± 0.002
Zn	1.3 ± 0.5	2.0 ± 0.41	0.10 ± 0.06	0.26 ± 0.09

a. APDC and DDDC = ammonium pyrrolidine dithiocarbamate and diethylammonium diethyldithiocarbamate

b. Approximated from Figure 3 of Cabrita et al. [14]

Predicted risk to Antarctic organisms

DGT-labile cadmium, copper, nickel, and zinc concentrations were used to predict toxicity to two microalgae, *Phaeocystis antarctica* and *Cryothecomonas armigera* and the echinoderm *Sterechinus neumayeri* using EC10 estimates and slope parameters for the individual metals cadmium, copper, nickel, lead, and zinc (Supplementary Table 4). Toxicity mixture modeling predicted low toxicities to all species, with predicted effects of <5% across all sites for all organisms (Fig. 4).

No single DGT-labile metal concentration exceeded the EC10 or EC50 values for metal exposure to any Antarctica species that have been assessed in previous studies [12,29–31,36–38]. However, DGT-labile copper concentrations (which ranged from 0.14 – 0.47 µg L^{-1}, Fig. 3) were close to the EC10 and EC50 (0.9 and 1.4 µg L^{-1}, respectively) for 23-d larval development inhibition to the echinoid *S. neumayeri* (King and Riddle, 2001). However, these estimates have a high degree of uncertainty due to limited data and poor concentration-response fits (Fig 4. c) which is typical of the challenges undertaking chronic toxicity tests in Antarctica with indigenous species.

The risk quotients for 99 and 95% species protection at each site were calculated based on DGT-labile cadmium, copper, nickel, lead, and zinc metal concentrations (Fig. 5). Quotient values >1 imply a risk of toxicity from the metals to 5% or 1% of species, when applying the respective WQGVs [22]. No site had metal concentrations that exceeded the risk quotient based on 95% guideline values; however, all sites had at least one DGT sampler which had sufficient metal to exceed the quotient based on the 99% guideline values (Fig. 5).

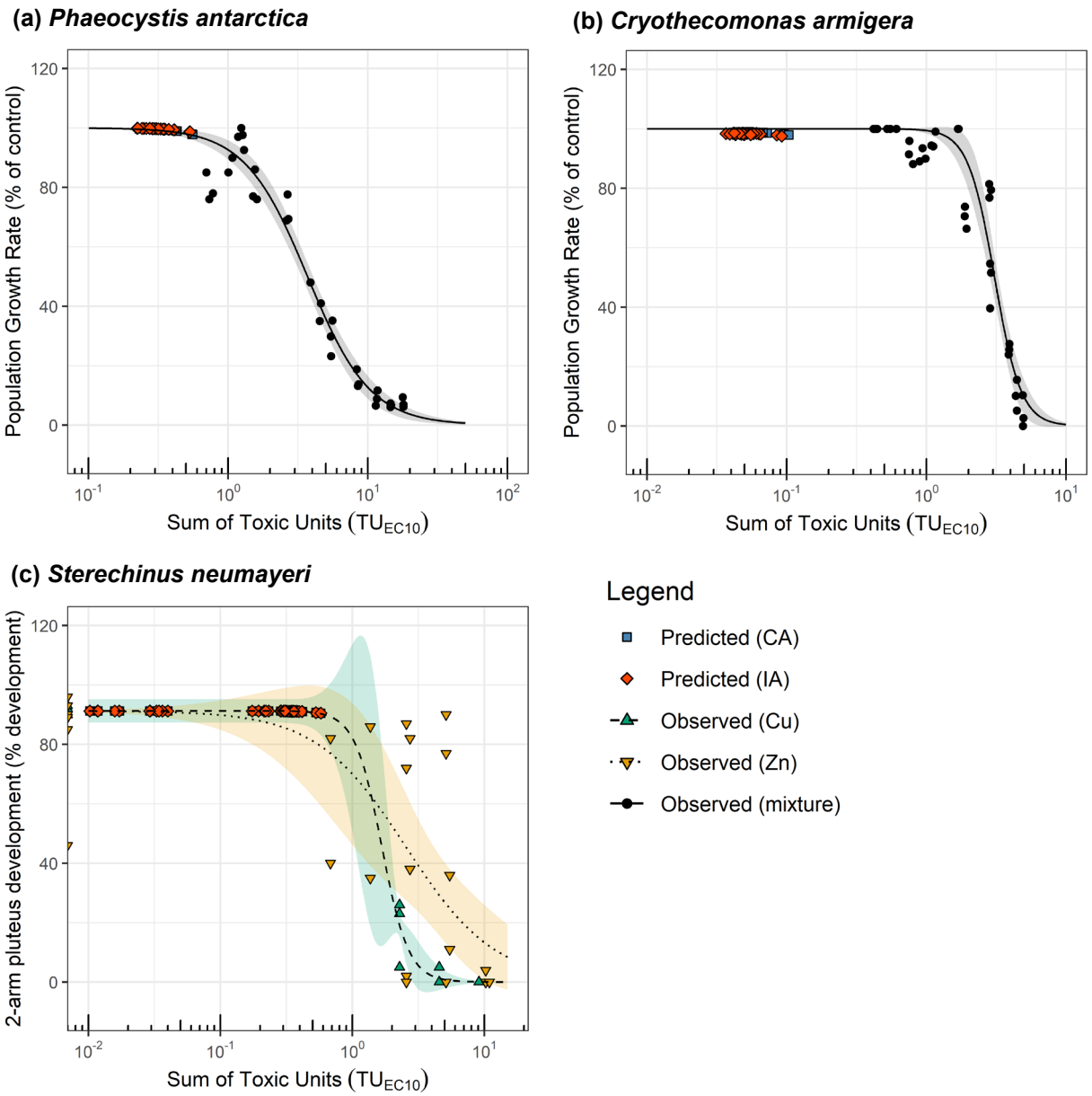


Figure 4. Predicted metal-mixture toxicity from field-measured DGT-labile metal concentrations (Fig.3) to the Antarctic microalgae (a) *Phaeocystis antarctica* and (b) *Cryothecomonas armigera*, and the Antarctic echinoderm (c) *Sterechinus neumayeri*. Predictions are compared against observed toxicities from previously conducted laboratory studies with metal mixtures (a[29], b[30]) or single metal exposures (c[31]) overlaid with 3-parameter log-logistic model fits (black lines) and 95% confidence intervals (ribbons). DGT-labile metals used in prediction modeling include cadmium, copper, nickel, lead and zinc for (a) and (b) and copper and zinc for (c) which reflects the availability of single-metal toxicity data. Data shown are from all sites as there were no differences between metal concentrations at each site.

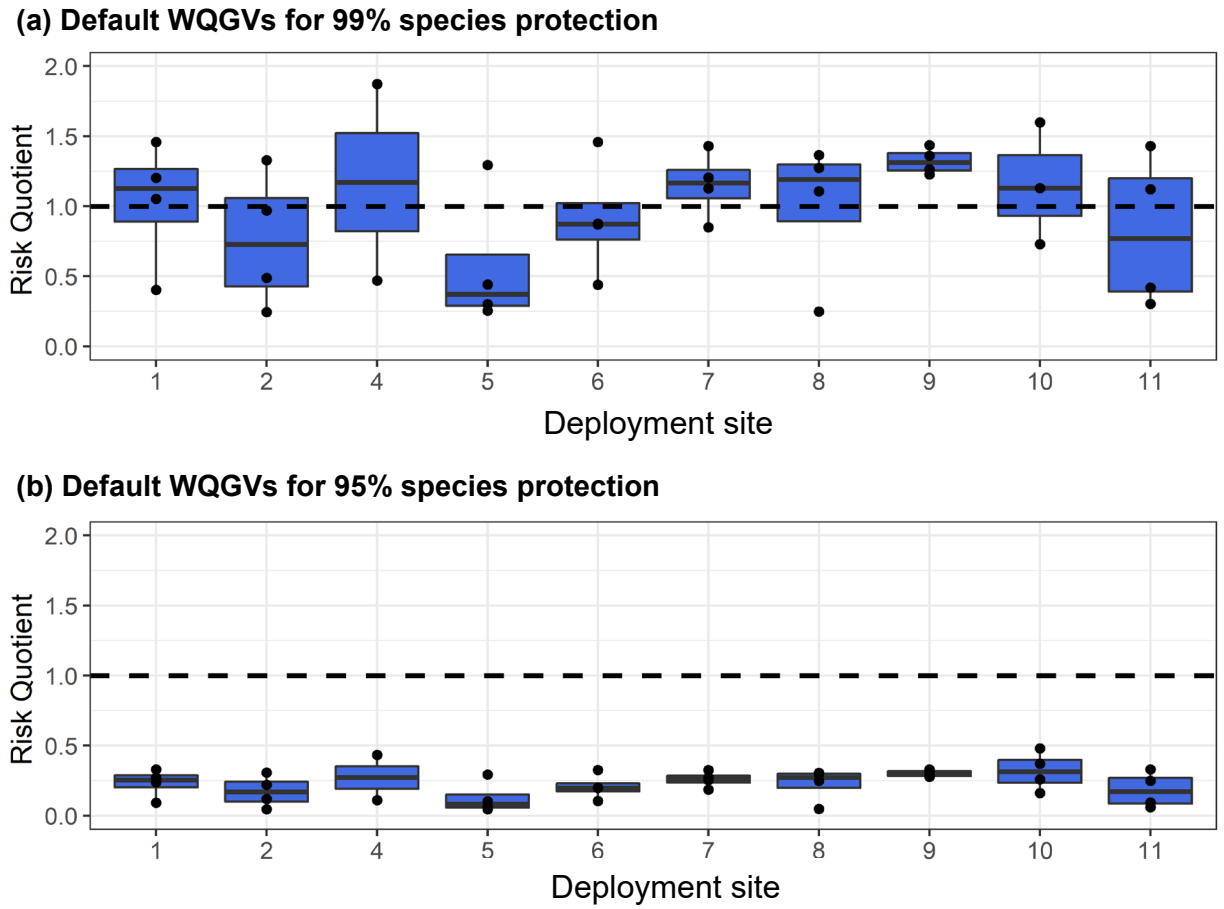


Figure 5. Predicted risk to the marine ecosystem based on the Australian and New Zealand default water quality guideline values (WQGVs) for (a) 99% and (b) 95% species protection [22]. Dotted line indicates a risk quotient of 1, values above which indicate exceedance of WQGVs. Points represent the risk quotient determined from metal concentrations measured by a single DGT sampler deployed at its respective deployment site.

Discussion

Metal concentrations

Concentrations of all metals in Antarctic near-shore waters in this study were elevated compared to previous measurements in Antarctica in pristine environments representative of coastal shelf waters [35] and near-shore environments with inputs from glacial melt [33], but were lower than near-shore marine waters immediately adjacent to a research station on King George Island [14] (Table 1). There were only minor differences in DGT-labile cadmium, copper, nickel, lead, or zinc concentrations between deployment sites (Fig. 3). This suggests that the source of the metals is diffuse and geogenic in origin. In Antarctica, this could be from sediment resuspension [39], sea ice melting [40], volcanic or hydrothermal inputs [41], or deposition from terrestrial ice melt and the weathering of rocks [42].

Interestingly, no elevation in metal contaminants were observed from sites near penguin colonies, which have been proposed to be a major source of metals on the West Antarctic Fildes Peninsula and Ardley island [43]. In this study, four sites (Sites 4, 8, 10, and 11) were in close proximity to large Adélie penguin colonies, and had large volumes of melt stream runoff from the colonies to the marine sites (personal observation). This could imply that the metal contaminants were insoluble, remain complexed in guano, or were bound to other organic carbon rendering them less labile [44].

Predicted toxicity

For most Antarctic marine organisms, including the microalgae and echinoid examined in this study, copper is the most toxic metal investigated. Copper was the only metal found to possibly be a risk to marine organisms in this area, with DGT-labile concentrations near concentrations likely to cause a toxic response to the sea urchin *S. neumayeri* (EC50 of 1.4 µg L^{-1}, King and Riddle [31]).

However, there was no difference in copper concentrations between sites (Fig. 3), suggesting a geogenic rather than anthropogenic source. This is consistent with previous findings of dissolved copper concentrations in Antarctic near-shore marine waters, both near research stations such as at Ardley Cove, King George Island [14] or Brown Bay, East Antarctica [15] and away from research stations, such as O'Brien Bay, East Antarctica [15] or

Marion Cove, King George Island (up to 0.1 µg Cu L^{-1}) [33], which is more representative of background copper concentrations in Southern Ocean surface waters [45].

The approach to predicting metal-mixture toxicity, based on independent action or concentration addition reference models, was applied to two microalgal species, *P. antarctica* and *C. armigera* and the echinoderm *S. neuymayeri*, Fig. 4, but the response of other species could be predicted using these same methods if the parameters needed for the mixture models are reported [46].

Practicalities of using DGT samplers in the Antarctic marine environment

DGT sampler deployment to the near-shore marine environment in Antarctica was not without its challenges. Some biofouling of the DGT windows was apparent after approximately 21 d (Supplementary information S3). This was unexpected, as previous studies in Antarctica using DGT have not reported biofouling [6,14], even at deployment times of 29 d [15]. Biofouling is dependent on the biological productivity of the receiving environment, which in the Antarctic nearshore ecosystem is highly seasonal and subject to summer microalgae blooms. If extensive biofouling occurs on a sampler the diffusion of some metals may be inhibited while others could be promoted depending on the relative binding affinities for the iminodiacetic acid functional groups of the Chelex-100 binding resin compared to the biofilm on the DGT window [47].

The impact of biofouling is difficult to quantify, and so measurements from field deployed DGT with extensive biofouling must be interpreted with caution [48]. In this study, DGT samplers were retrieved at two time points from Sites 1, 2, 5, 6, 7, 8, 9, and 10 (Fig. 1). A comparison of metal concentrations in DGTs between these time points did not suggest that biofouling caused changes to the measured metal concentrations. This comparison, however, assumes similar seawater conditions and labile metal concentrations which we believe likely given the large overlap (i.e. >21 d) in deployment durations and the lack of temporal and spatial trends in metal concentrations between sites in this study.

A minimum deployment period of 14 d is recommended by Koppel et al.[28] for the purposes of achieving minimum detection limits for environmental management (assuming the use of an ICP-AES for metals analysis). The results of this study suggest that metal-uptake to DGT is unlikely to be affected by biofouling for deployment times <21 d. Therefore, we recommend

a deployment period of 14-21 d for contaminant monitoring in the near-shore marine environment in Antarctica as a compromise between metal detection limits and minimizing interferences from biofouling.

On disassembly of the DGT it was noticed that the diffusion gel had shrunk by approximately 2-3 mm. This effect was not able to be recreated in the lab at similar temperatures so was attributed to rapid dehydration of the polyacrylamide gel from the dry Antarctic air.

Considerations on the assessment of contaminant risk

Without the ability to link the presence of contaminants to a measure of environmental harm, assessing environmental "impact" is difficult. This study demonstrates how DGT samplers are useful tools to assess time-averaged labile metal concentrations and consider the risk they pose to the marine environment. Linking DGT-labile concentrations with toxicity thresholds for known Antarctic marine organisms (including the use of reference mixture models) or national water quality standards provides a benchmark from which environmental impact can be assessed.

The approach outlined in this study can be used by environmental managers to assess the risk of contaminants in the Antarctic nearshore marine environment. This may be a useful inclusion in environmental impact assessments [49], for triaging contaminated sites for remediation [50], or in making decisions about the protection of cultural heritage that may be causing environmental harm [51].

Care should be taken around applying national environmental quality standards to regions they are not designed for, as they are based on response of endemic organisms and may have specific caveats about environmental conditions that need to be considered. However, as interim measures in lieu of Antarctic specific guidelines, Australian and New Zealand WQGVs have previously been applied in Antarctic contamination assessments[5,6,11,23,52,53]. This study showed that the Australian and New Zealand WQGV for 99% species protection for copper is unsuitable for the Antarctic nearshore marine ecosystem because the copper concentrations, which were likely geogenic in origin (see the Metal concentrations section), were largely equivalent to the default WQGV of 0.3 µg L^{-1} [22]. There is also some general contention about the applicability of the 99% species protection level because of high uncertainty in their derivation [54]. Nevertheless, this study shows that the WQGVs for 95%

374 species protection is suitable as an interim measure, particularly when considered against
375 the known toxicity thresholds for Antarctic marine organisms [29–31,36,55], at least until the
376 development of Antarctic-specific guidelines.

377 This study only assessed the risk of the dissolved labile metal fraction. Other routes of
378 exposure should also be considered in environmental risk assessments [56], including dietary
379 exposure via particulate matter [53], phytoplankton [14,57], or other organisms [58]. This may be
380 particularly important for microalgae as preliminary studies have shown that *P. antarctica*
381 and *C. armigera* are capable of accumulating potentially toxic concentrations of metals like
382 copper and zinc [57]. Other risks may exist, such as changes to dissolved organic carbon
383 production which has been shown to alter allelopathic potential in bloom-forming marine
384 microalgae [59].

385 Similarly, this study only investigated the risk of metals in the pelagic marine environment
386 but other sources of contaminants should be considered. The contaminated terrestrial sites
387 expected to leach metal contaminants to the nearshore marine environment are highly
388 weathered and partly remediated [6,23]. Previous assessments have found that contaminants
389 are also deposited to the benthic environment via sedimentation [8,60]. DGT samplers have
390 been used in temperate and tropical sediments to assess the bioavailable metal flux from
391 sediments to overlying waters, which has shown good agreement with toxicity and
392 bioaccumulation to benthic organisms [17–19,61]. While the metal concentrations in Antarctic
393 sediments and benthic organisms are commonly reported [60,62–66], the DGT technique
394 combined with sediment-based ecological or toxicology assessments may also provide a
395 way to translate the presence of a contaminant to its risk to the benthic ecosystem.

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Supporting information

Supporting information is available and includes 4 tables and 2 figures:

- Supplementary Table 1 - Instrument limits of detection (LOD) and certified reference material (CRM) analysis.
- Supplementary Table 2 - DGT blank concentrations and calculated DGT limits of detection.
- Supplementary Table 3 – DGT method detection limits for the deployment conditions, times, and instrument detection limits used in this study
- Supplementary Table 4 – Model parameters used in the toxicity mixture modeling for three Antarctic marine organisms.
- Supplementary Figure 1 - Seawater dissolved metal concentrations from grab sampling during the deployment period.
- Supplementary Figure 2 – Observed biofouling on the DGT-devices during their deployment to the Antarctic nearshore marine environment.

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Supporting information

Assessing the risk of metals and their mixtures in the Antarctic nearshore marine environment with diffusive gradients in thin-films

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Supplementary Table 1

Instrument limits of detection and certified reference material (CRM) analysis using TMDA-64.4 and TM 24.4 (NRC, Canada). All values are in µg L^{-1} unless otherwise stated.

Metal (wavelength)	**Ag 328.068**	**Cd 214.439**	**Co 228.615**	**Cr 205.560**	**Cu 324.754**	**Fe 258.588**	**Mn 257.610**	**Ni 231.604**	**Pb 220.353**	**Sr 407.771**	**V 292.401**	**Zn 213.857**
Instrument LOD	0.19	0.08	1.58	0.18	1.00	0.63	0.03	0.32	1.00	0.01	0.30	0.12
TMDA-64.4 measured	13	255	248	281	258	301	286	252	280	608	293	326
TMDA-64.4 reported	12.6	258	250	283	261	298	292	252	280	628	279	320
% of CRM	104	99	99	99	99	101	98	100	100	97	105	102
TM24.4 measured	9	4	7	5	7	16	8	5	5	110	8	30
TM24.4 reported	8.92	3.96	6.27	5	6.31	16	8.24	5.03	5.6	113	7	NA
% of CRM	101	105	106	101	107	97	98	98	91	98	110	NA

Supplementary Table 2

DGT blank concentrations and calculated DGT LOD values used in method detection limit calculations. All values are in µg L^{-1} unless otherwise stated.

Metal	Cd	Cu	Ni	Pb	Zn
Instrument LOD	0.1	1.0	0.3	1.0	0.1
DGT Blank 1	0.0	0.8	-0.2	-1.2	3.9
DGT Blank 2	-0.1	0.5	-0.2	-0.8	8.6
DGT Blank 3	0.2	9.7	0.0	-0.6	20.1
DGT Blank 4	0.0	0.8	-0.1	0.2	4.0
DGT Blank 5	0.0	0.1	-0.1	-1.0	-0.1
DGT Blank 6	-0.1	-0.1	-0.2	-1.0	-0.1
DGT Blank 7	-0.1	0.5	-0.1	-0.5	-0.2
DGT Blank 8	0.0	0.2	-0.4	0.1	-0.1
DGT Blank 9	0.0	0.2	-0.4	-0.4	-0.1
Average blank (#5-9, µg L^{-1})	-0.05	0.2	-0.2	-0.6	-0.1
Standard deviation (#5-9, µg L^{-1})	0.04	0.2	0.2	0.5	0.006
LOD calculations[c]					
3x Standard deviation (µg L^{-1})	0.1	0.6	0.5	1.4	0.1
Theoretical elution concentration (µg L^{-1})[a]	4	20	14	44	4
Theoretical mass on resin (µg)[b]	0.006	0.027	0.020	0.062	0.006

[a] Based on a dilution of 6.37% HNO_3 (i.e. 1 M) to 4 mL of 0.2 % acid content

[b] Calculated using Equation 1

[c] DGT Blank 1-4 measurements were excluded from the LOD calculations due to contamination. It remains unclear whether the contamination is from processes related to this study or another study which involved DGT deployed to soils following a deoxygenation process in nitrogen bubbled water.

Supplementary Table 3

Method detection limits (MDL) for the deployment conditions described in this study using detection limits of an ICP-AES given in Supplementary Table 1 and theoretical DGT LOD given in Supplementary Table 2[a].

Metal	D_i ($x10^6$ s^{-1} cm^2)	LOD (ng)	MDL (ng L^{-1}) at	
			22 days	37 days
Cd	2.4	5.9	39	23
Cu	2.1	27	207	123
Ni	2.2	20	140	83
Pb	2.6	62	375	223
Zn	2.1	5.7	42	25

[a] D_i is the diffusion coefficient for each metal [1], LOD is the limit of detection calculated as 3 times the standard deviation of undeployed DGT measured concentrations, and MDL is the method detection limit for the devices based on the LOD at a deployment time of 22 and 37 days.

Supplementary Table 4.

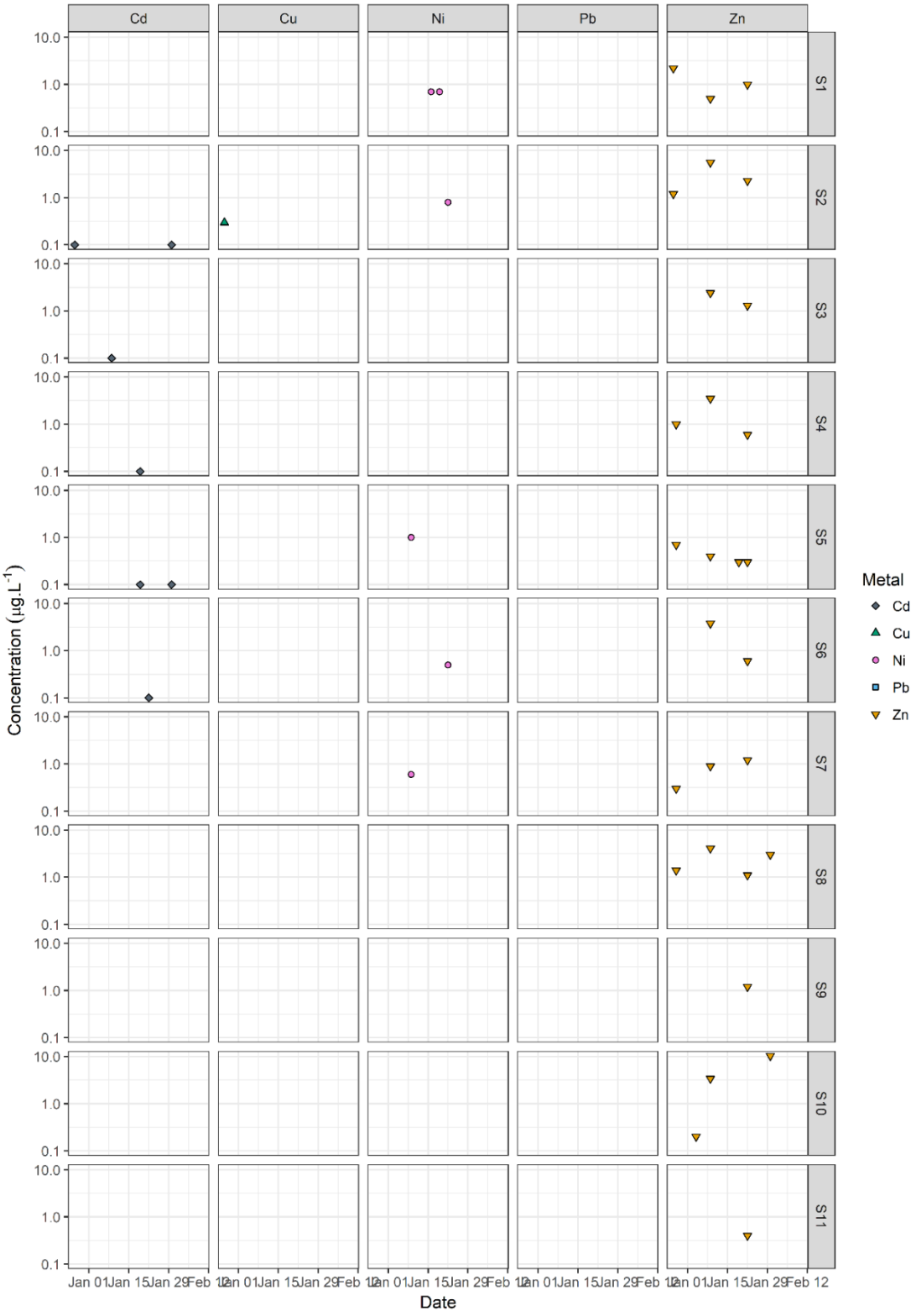
Model parameters for concentration-response relationships of metal toxicity for the Antarctic test species *Phaeocystis antarctica, Cryothecomonas armigera*, and *Sterechinus neumayeri*. Models used were a 4-parameter log-logistic model (LL4) and a mixed-effect 4-parameter log-logistic model (meLL4, see Gehard et al. [2] for a description of the model). Values are mean ± standard error in µg L^{-1}.

Antarctic species	Endpoint	Test duration (days)	Metal	Number of treatments	Model	10% effect concentration	Inflection point (e)	Slope (b)	Upper limit (c, % response)	Lower limit (d, % response)*	Source data
Phaeocystis antarctica	Population growth rate	10	Cd	48	LL4	220 ± 50	1700 ± 200	1.1 ± 0.1	100*	0	Gissi et al.[3]
			Cu	93	meLL4	2.8 ± 0.3	5.6 ± 0.2	3.0 ± 0.2	100	0	
			Ni	39	-	-	-	-	-	-	
			Pb	63	meLL4	150 ± 45	570 ± 60	1.7 ± 0.4	101 ± 4	0	
			Zn	39	meLL4	220 ± 70	1200 ± 100	1.7 ± 0.3	100	0	
Cryothecomonas armigera	Population growth rate	24	Cd	36	meLL4	400 ± 200	20000 ± 300000	0.4 ± 0.1	100 ± 1	0	Koppel et al.[4]
			Cu	75	meLL4	22 ± 2	63 ± 2	2.1 ± 0.2	99 ± 3	0	
			Ni	45	meLL4	1220 ± 60	1560 ± 30	9 ± 1	96 ± 2	0	
			Pb	45	LL4	150 ± 50	3000 ± 1000	0.7 ± 0.1	100*	0	
			Zn	39	meLL4	300 ± 100	30000 ± 20000	0.5 ± 0.1	101 ± 3	0	
Sterechinus neumayeri	23-day larval development to 2-arm pluteus stage	23	Cu	24	LL4	0.9 ± 0.4	1.5 ± 0.2	4.3 ± 2.2	91 ± 2	0	King and Riddle [5]
			Zn	32	LL4	56 ± 31	195 ± 44	1.8 ± 0.8	91 ± 2	0	

*indicates that the model parameter was fixed at this value

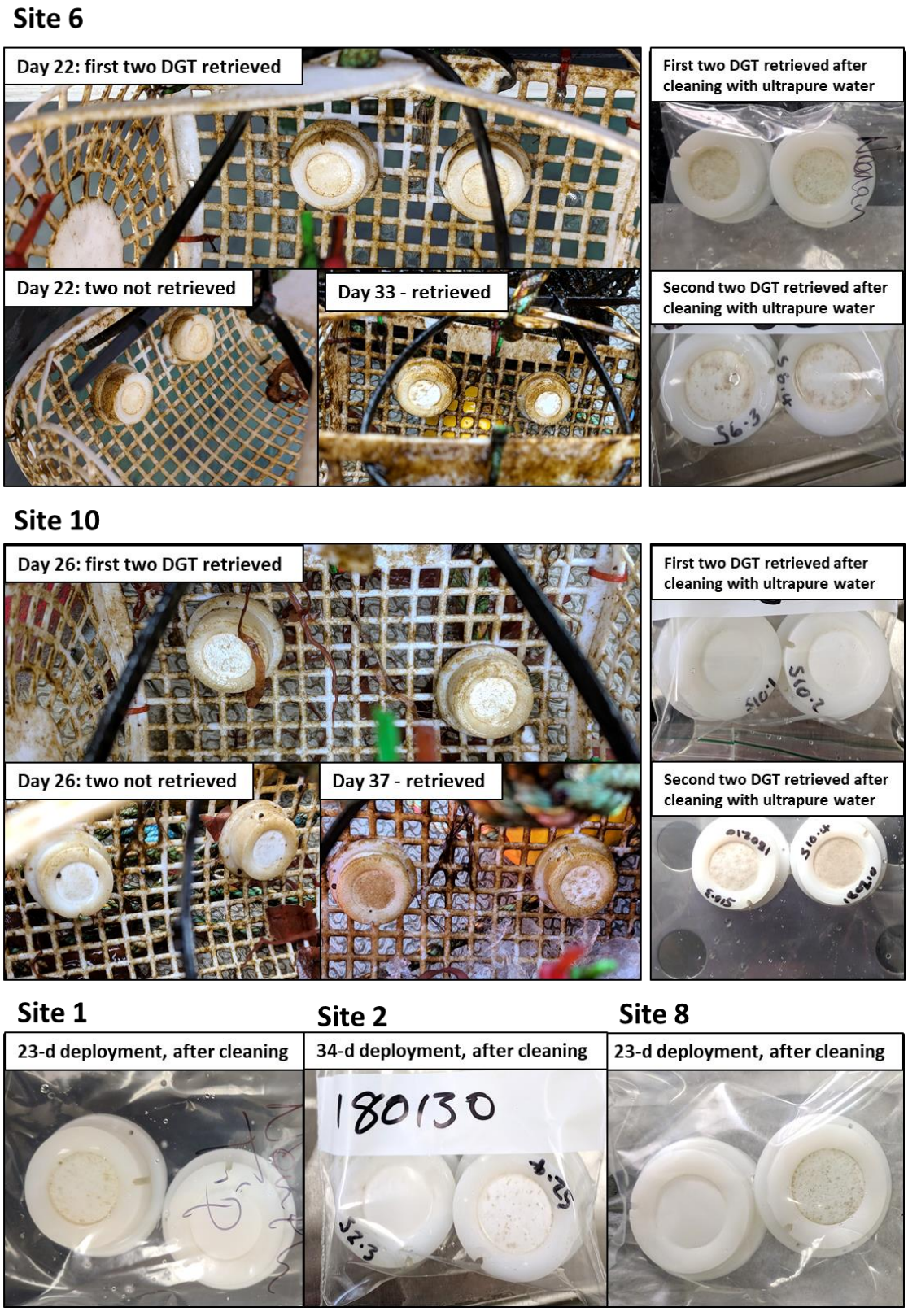
Supplementary Figure 1.

Measured dissolved metal concentrations in seawater at DGT deployment sites (all site data combined for each date and metal). The absence of points indicates measurements below detection limits (see Methods Section or Supplementary Table 1 for limits of detection).



Supplementary Figure 2

Representative examples of DGT sampler biofouling after seawater deployments. For most sites, two DGT were retrieved after deployment for 22 and 30 d (see Fig. 1 for deployment times).



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