

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

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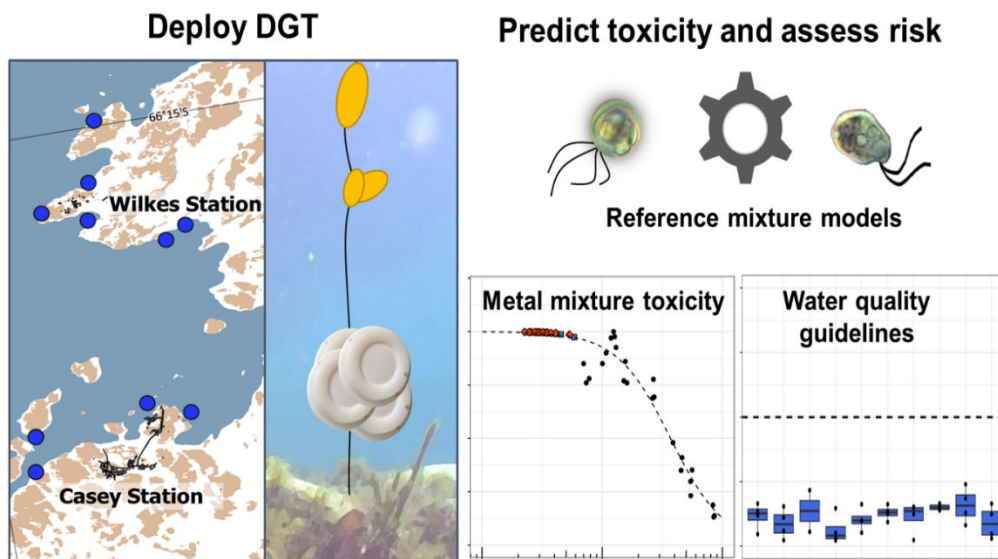
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11 **Graphical abstract**



12

13 **Keywords**

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

16 **Abstract**

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

34 Introduction

35 Exploration, tourism, and scientific research activities have left a potentially toxic legacy of
36 organic and inorganic contamination in localized areas of Antarctica particularly around
37 research stations¹. The majority of these research stations are built on scarce coastal ice-
38 free rocky areas, which represent approximately 6,000 km² of the Antarctic continent^{2,3}. An
39 estimated total of 53 stations are in operation in these areas, with many more abandoned
40 and decommissioned stations, waste sites, and field camps present⁴. Contaminants such as
41 cadmium, copper, nickel, lead, and zinc from these sites are mobilized to the coastal
42 environment through processes of dissolution and/or particle entrainment from melt waters
43 running through the sites during the summer season^{5,6}. This has already been shown to
44 affect the near-shore marine ecosystem^{7,8}, and is expected to worsen with increasing
45 temperatures associated with climate change⁹.

46 The Protocol on Environmental Protection to the Antarctic Treaty defines the need for
47 environmental monitoring, impact assessments, and the remediation of historical waste¹⁰.
48 The unique environmental conditions and ecosystems in Antarctica necessitate the
49 validation of environmental management techniques commonly used in temperate or
50 tropical environments¹¹. Recent examples have included toxicity assessments using
51 Antarctic organisms¹² or the use of contaminant monitoring tools¹³.

52 Diffusive gradients in thin-films (DGT) is an *in situ* passive sampling technique that has been
53 used in Antarctica to assess labile metal concentrations in marine waters^{6,14-16}. While DGT is
54 predominately used to measure a labile fraction of metal contaminants in the environment
55¹⁴, it has recently being used to predict the toxicity and bioavailability of contaminants to
56 benthic organisms¹⁷⁻¹⁹. The ease of use, ability to provide a time-averaged measure of a
57 labile metal fraction, low cost, and simultaneous detection of multiple contaminants makes
58 DGT samplers an attractive option for environmental monitoring, especially in remote and
59 harsh environments. However, their application to the Antarctic marine environment and
60 practicality to environmental managers is still unclear⁶.

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

64 environmental toxicology: independent action (IA) and concentration addition (CA)²⁰. Both
65 provide ways of integrating expected toxicities from individual components of the mixture
66 using toxicity thresholds, such as EC₁₀ or EC₅₀ values, derived from single-metal exposures. In
67 lieu of toxicity thresholds, water quality guidelines (WQGVs) may be used to calculate a risk
68 quotient to describe the risk of contaminant mixtures^{18,21}. WQGVs are ideally calculated
69 using a cumulative probability distribution of chronic toxicity endpoints (based on dissolved
70 metal concentrations) to derive guideline concentrations which are likely to protect a
71 defined proportion of species²².

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

78 **Methods**

79 ***Sampling locations***

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

86 ***DGT synthesis and field deployment***

87 DGT pistons with a Chelex-100 binding layer were prepared following the procedures
88 recommended by DGT Research (Lancaster, UK) as outlined by Davison²⁴. The binding resin
89 was a 0.4 mm thick polyacrylamide gel laden with Chelex-100 (Bio-Rad, mesh 200-400).
90 During the binding resin synthesis, Chelex-100 beads concentrated at the bottom of the gel
91 by gravitational settling. This concentrated side was placed towards the window of the DGT,
92 in contact with the 0.8 mm thick polyacrylamide diffusive layer. A 0.13 mm thick, 0.45 μm

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

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

101 Moorings were created by connecting a mesh bag filled with locally collected rocks (devoid
102 of moss or lichens) to hard-plastic buoys (2 large and 1 small) with synthetic rope. The
103 moorings were approximately 5 m in length and designed to ensure the two large buoys
104 remained ~2 m under the water, with only the small buoy rising to the surface. This design
105 was used with a goal to prevent snagging of the moorings on ice floes or ice bergs. However,
106 two moorings were lost (presumably by iceberg ensnarement) and some others were found
107 10-50 m from their original deployed locations.

108 Prior to deployment, DGT baskets were attached to the mooring using plastic cable ties.
109 Moorings were deployed to a site after the depth was confirmed to be between 3 and 5 m.
110 Irrespective of the final deployment depth, DGT samplers were positioned 1 m above the
111 rock bag. Samplers were deployed for between 22 and 37 days (Fig. 1), between December
112 27, 2017 and February 11, 2018. Dates for deployment were limited by sea ice which
113 prohibited the deployment of inflatable rubber boats from the station wharf during the
114 2017/18 summer season at Casey Station.

115 Following the period of deployment, moorings were retrieved and DGT devices were
116 recovered from the cages, rinsed with ultrapure water, and returned to the station
117 laboratories. DGT devices were disassembled and the binding resin placed in 1 mL of 1 M
118 HNO₃ (Suprapur grade, Merck Millipore) for ≥12 h on an orbital shaker. The resulting eluents
119 were diluted to a final concentration of 0.2% HNO₃ and stored at 4 °C until analyzed in
120 Australia. Field blank DGT samplers were treated in the same manner as deployed DGT
121 samplers (i.e. attached to polypropylene baskets and stored moist at 4 °C until eluted), and
122 were used to calculate DGT limits of detection.

123 ***Seawater sampling and measurements at DGT deployment sites***

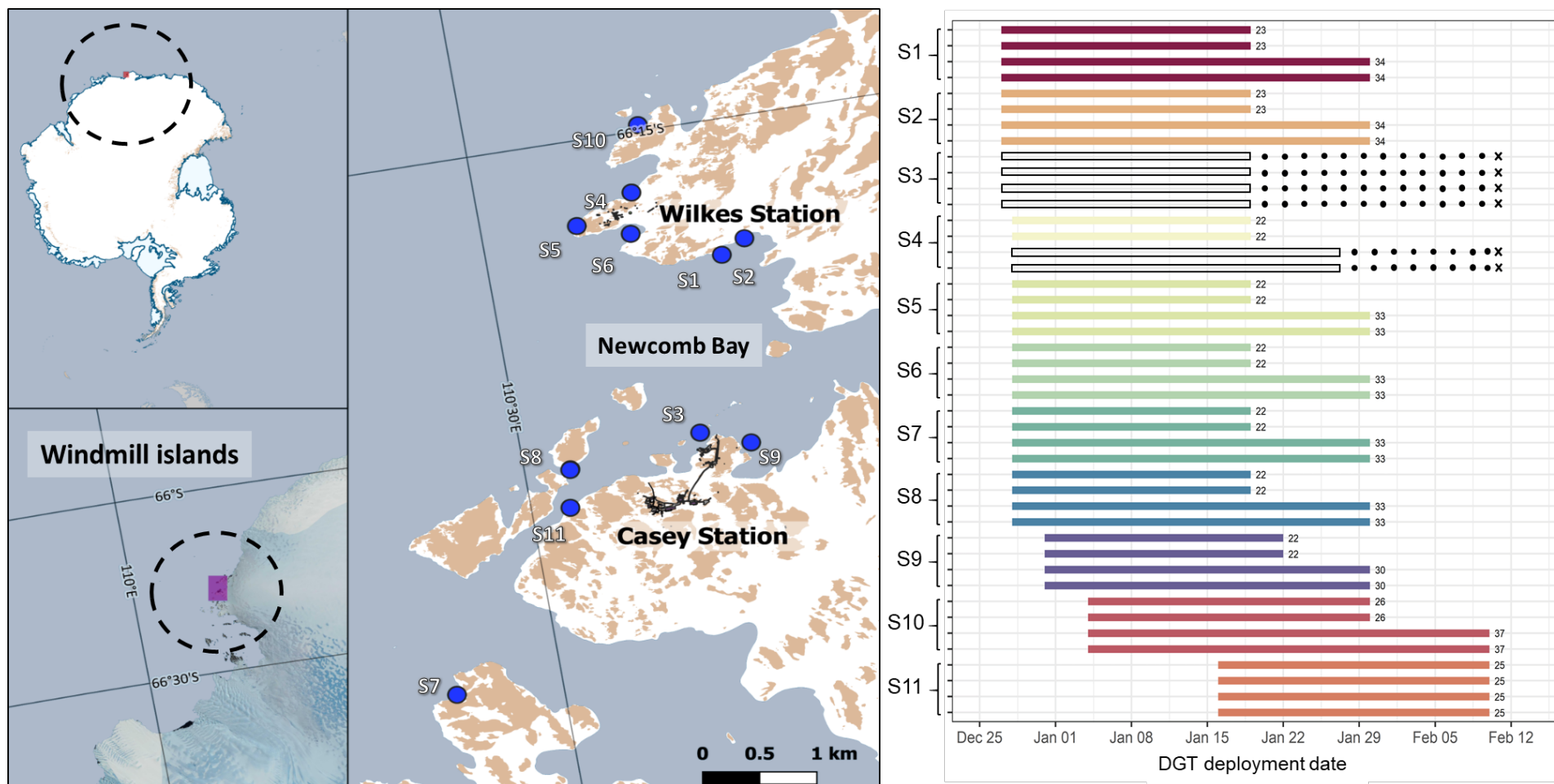
124 Seawater was sampled at deployment sites from an inflatable rubber boat. All samples were
125 taken at a depth of approximately 30 to 50 cm below the surface to avoid the layer of less
126 saline seawater that develops on the sea surface from sea-ice melt. Physicochemical
127 parameters of seawater samples including salinity (PSU), dissolved oxygen (mg L^{-1}), and pH
128 were measured directly in the seawater using a Professional Plus Multiparameter
129 Instrument (YSI, USA), calibrated as per manufacturer's instruction on the day of sampling.
130 Seawater for metal analysis was sampled from the boat. Approximately 20 mL was
131 withdrawn into a plastic syringe and filtered to $0.45 \mu\text{m}$ (polyethersulfone membrane,
132 Sartorius). To rinse the filter unit the first 10 mL of seawater was discarded and the second
133 10 mL filtered directly into acid-washed plastic vials. These samples were acidified to 0.2%
134 HNO_3 in Antarctica and returned to Australia for metal analysis.

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

139 ***Metal analysis***

140 Metal analysis was conducted in Australia by inductively coupled plasma - atomic emission
141 spectrometry (ICP-AES, Varian 730-ES) or ICP – mass spectroscopy (ICP-MS, Agilent 7900)
142 where lower detection limits were needed, using matrix-matched calibration standards (i.e.
143 ultrapure water for DGT eluents or seawater for seawater grab samples). A multi-element
144 standard (QCS27; Analytical West Inc.) was used as a drift standard to correct for
145 measurement suppression over time, particularly for samples in a seawater matrix. Metal
146 detection limits of the ICP-AES were $0.1 \mu\text{g Cd L}^{-1}$, $1.0 \mu\text{g Cu L}^{-1}$, $0.3 \mu\text{g Ni L}^{-1}$, $1.0 \mu\text{g Pb L}^{-1}$,
147 and $0.1 \mu\text{g Zn L}^{-1}$ (Supplementary Table 1). Two certified reference materials were analyzed
148 to validate instrument measurements, TM-24.4 (lot 0916) and TMDA-64.3 (lot 0317)
149 (National Research Council, Canada) and had recoveries of between 91 and 110 %
150 (Supplementary Table 1). Non-deployed DGT were used as field blanks and to calculate DGT
151 limits of detection (Supplementary Table 2) which were then used to calculate method
152 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.



155

156 **Figure 1. Locations and deployment durations of diffusive gradients in thin-films (DGT) around Casey and Wilkes stations in the Newcomb**
 157 **Bay area, Windmill Islands, East Antarctica**^{25,26}. Colored bars represent the deployment dates for each DGT at each site. Numbers at the end
 158 **of bars indicate total days of deployment. Lost moorings are represented by an unfilled bar (indicating the dates its location was known),**
 159 **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 ($\mu\text{g L}^{-1}$), 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 metals²⁷. The DGT-labile concentration (C_{DGT} , in units of ng mL^{-1} but will be reported in the
168 equivalent units of $\mu\text{g L}^{-1}$) 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 $\times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ 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 piston²⁷.

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 exists³¹. 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 models³², 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**
$$y_{IA} = 100 * \prod_{i=1}^n \frac{1}{1 + \left(\frac{x_i}{EC_{10i} * 9^{\beta_i}} \right)^{\beta_i}}$$

185

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

187 ***Determination of risk quotients***

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

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

192 where WQGV_x represents the default guideline value for x% species protection at the 99%
 193 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
 194 Ni L⁻¹, 2.2 and 4.4 µg Pb L⁻¹, and 7 and 15 µg Zn L⁻¹, respectively.

195

196 **Results**

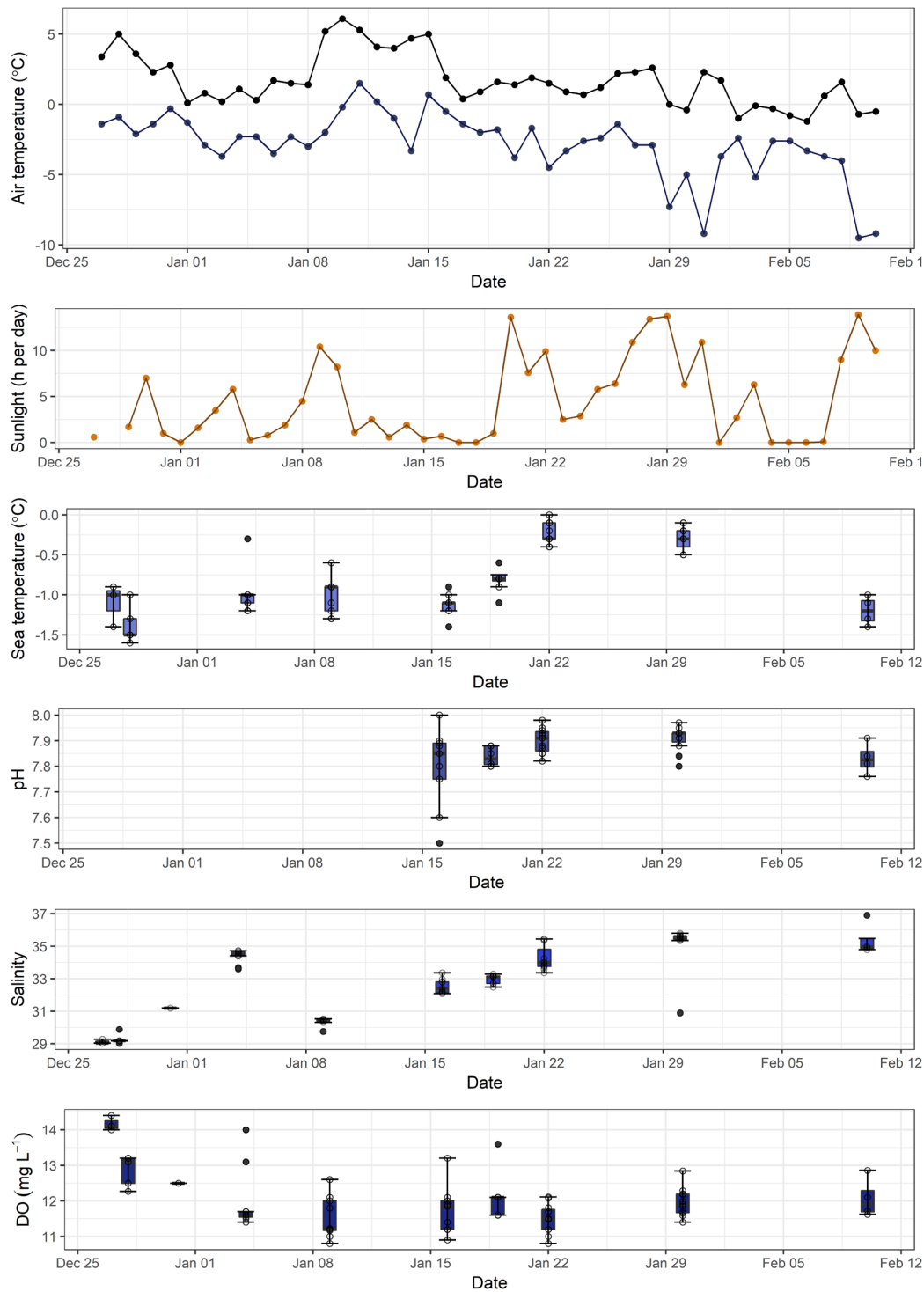
197 ***Physicochemical properties of seawater and weather observations at deployment sites***

198 The climate for the deployment period through the mid-summer period at Casey station
 199 was characterized by: sea temperature rising from -1.2 °C in December to -0.2 °C in the
 200 middle of January, then decreasing to -1.2 °C by February; dissolved oxygen concentration
 201 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
 202 ppt. The physicochemical properties of the near-shore marine waters and weather
 203 observations, averaged across all sites, are given in Fig. 2. All measurements were within the
 204 expected range for the Antarctic near-shore marine environment in Newcomb Bay, based
 205 on previous studies.

206 ***Metal concentrations in seawater at deployment sites***

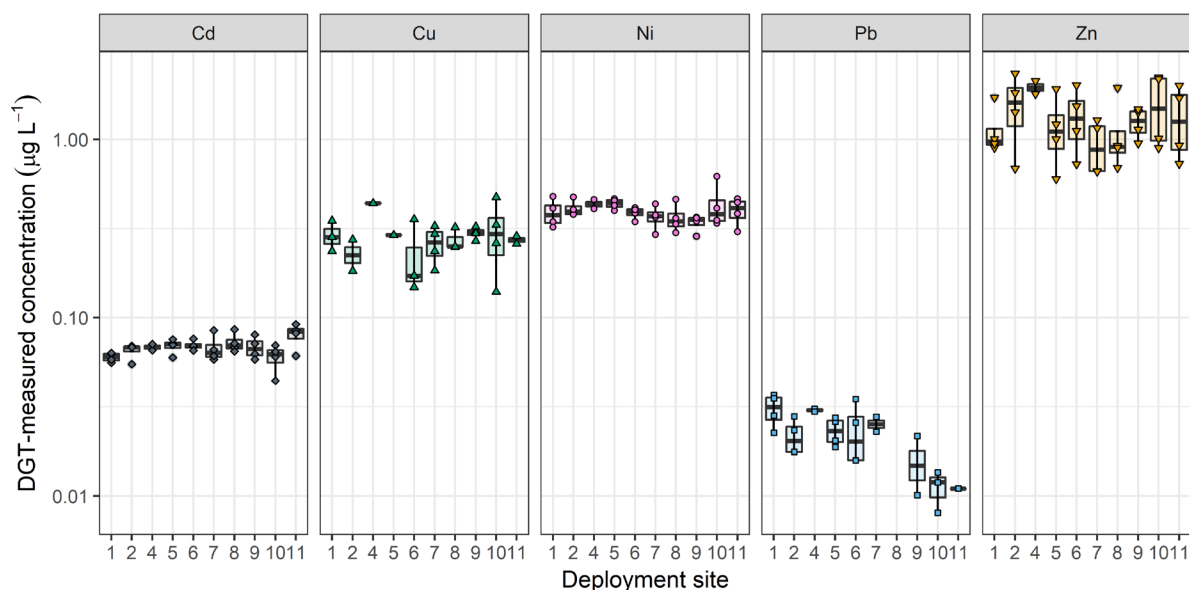
207 Dissolved metal concentrations in seawater taken at deployment sites were typically below
 208 instrument detection limits (see Methods section) throughout the deployment period. Some
 209 sites had nickel and cadmium concentrations around 0.1 µg L⁻¹, and zinc concentrations up
 210 to 10 µg L⁻¹;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

213 DGT-labile concentrations from deployment sites were low and typical of clean near-shore
214 marine environments in Antarctica ^{14,33}. Lead was the only metal below detection limits
215 ($<0.2 \mu\text{g L}^{-1}$, method detection limit for DGT deployments in these conditions, times, and
216 within the instrument's limit of detection of $1.0 \mu\text{g Pb L}^{-1}$), and so was analyzed by ICP-MS.
217 The average DGT-labile concentrations (mean \pm standard deviation, with range of
218 measurements in brackets) for all sites were: cadmium $0.07 \pm 0.01 \mu\text{g L}^{-1}$ ($0.04 - 0.09 \mu\text{g L}^{-1}$),
219 copper $0.28 \pm 0.08 \mu\text{g L}^{-1}$ ($0.14 - 0.47 \mu\text{g L}^{-1}$), nickel $0.39 \pm 0.07 \mu\text{g L}^{-1}$ ($0.29 - 0.62 \mu\text{g L}^{-1}$),
220 lead 0.022 ± 0.008 ($0.008 - 0.037 \mu\text{g L}^{-1}$), and zinc $1.3 \pm 0.5 \mu\text{g L}^{-1}$ ($0.6 - 2.3 \mu\text{g L}^{-1}$) (Fig. 3,
221 Table 1).



222

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



232
 233 **Figure 3. DGT-labile concentrations of cadmium, copper, nickel, lead, and zinc from 20- to**
 234 **37-d deployments in the near-shore marine environment around Casey and Wilkes**
 235 **stations, East Antarctica. Error bars represent 1.5 times the interquartile range with**
 236 **outliers as black dots outside the range.**

237

238

239 **Table 1. DGT-labile concentrations of metals from deployments in the near-shore marine**
 240 **environment around Casey and Wilkes station, East Antarctica, compared to previously**
 241 **reported measurements in nearshore and open ocean environments in Antarctica. Values**
 242 **are mean \pm standard deviation ($\mu\text{g L}^{-1}$). Measurements from the literature are averaged**
 243 **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 \pm 0.01	0.20 \pm 0.04	0.0031 \pm 0.0005	0.05 \pm 0.02
Cu	0.28 \pm 0.08	~1.7 ^b	0.04 \pm 0.03	0.012 \pm 0.02
Ni	0.39 \pm 0.07	~1.6 ^b	0.033 \pm 0.004	0.034 \pm 0.03
Pb	0.022 \pm 0.008	0.43 \pm 0.05	0.016 \pm 0.007	0.003 \pm 0.002
Zn	1.3 \pm 0.5	2.0 \pm 0.41	0.10 \pm 0.06	0.26 \pm 0.09

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

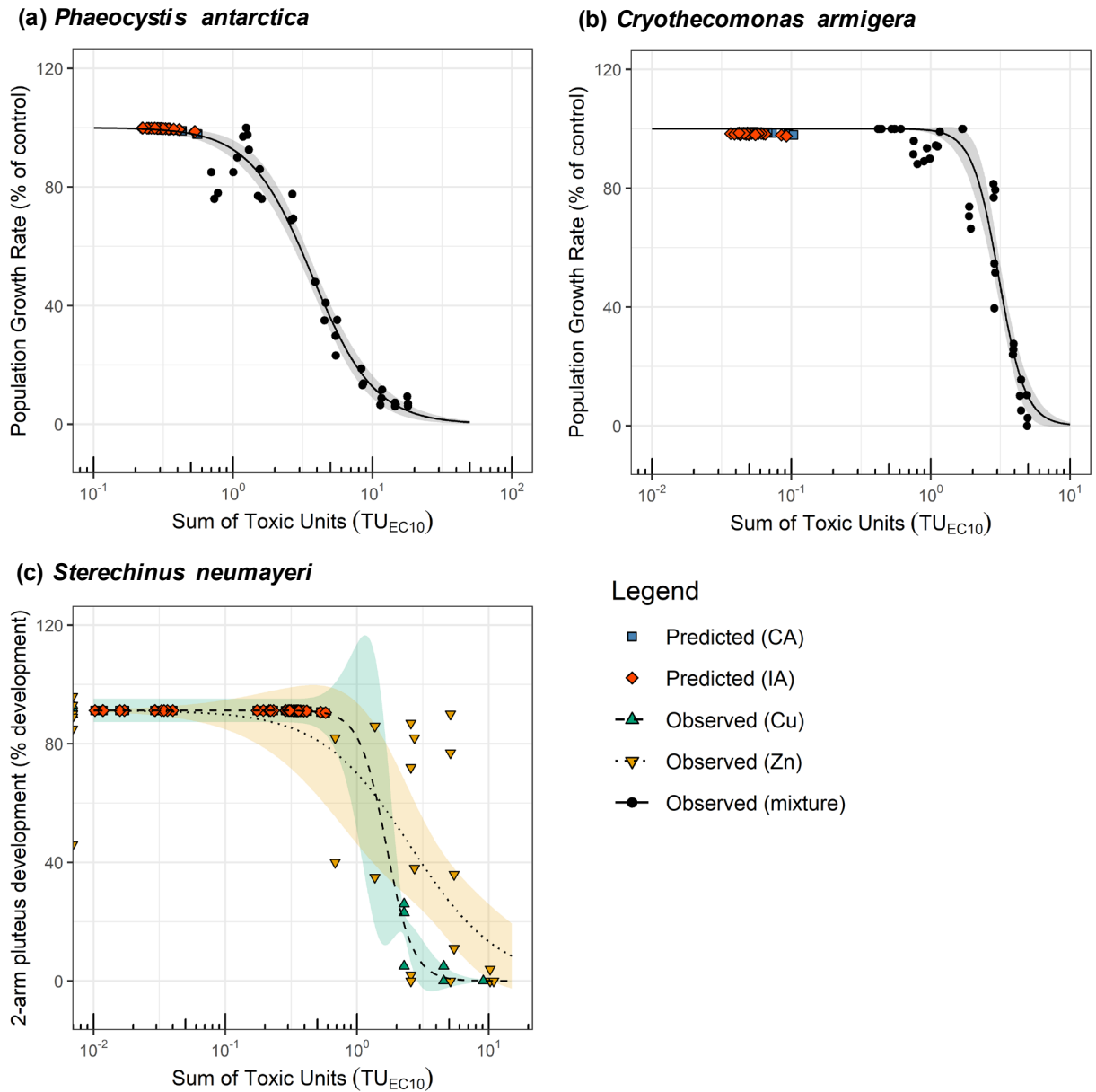
246 b. Approximated from Figure 3 of Cabrita et al. ¹⁴

247 **Predicted risk to Antarctic organisms**

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

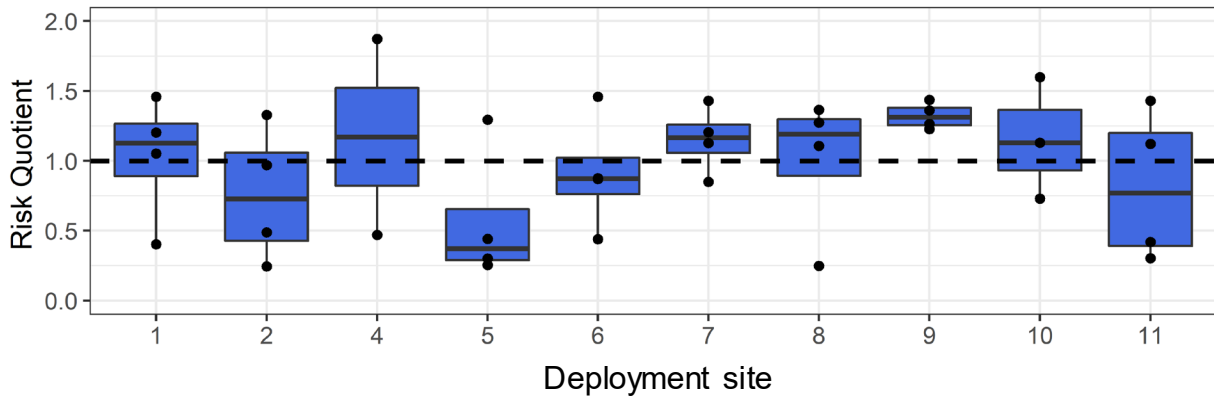
254 No single DGT-labile metal concentration exceeded the EC10 or EC50 values for metal
255 exposure to any Antarctica species that have been assessed in previous studies^{12,29–31,36–38}.
256 However, DGT-labile copper concentrations (which ranged from 0.14 – 0.47 $\mu\text{g L}^{-1}$, Fig. 3)
257 were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

262 The risk quotients for 99 and 95% species protection at each site were calculated based on
263 DGT-labile cadmium, copper, nickel, lead, and zinc metal concentrations (Fig. 5). Quotient
264 values >1 imply a risk of toxicity from the metals to 5% or 1% of species, when applying the
265 respective WQGVs²². No site had metal concentrations that exceeded the risk quotient
266 based on 95% guideline values; however, all sites had at least one DGT sampler which had
267 sufficient metal to exceed the quotient based on the 99% guideline values (Fig. 5).

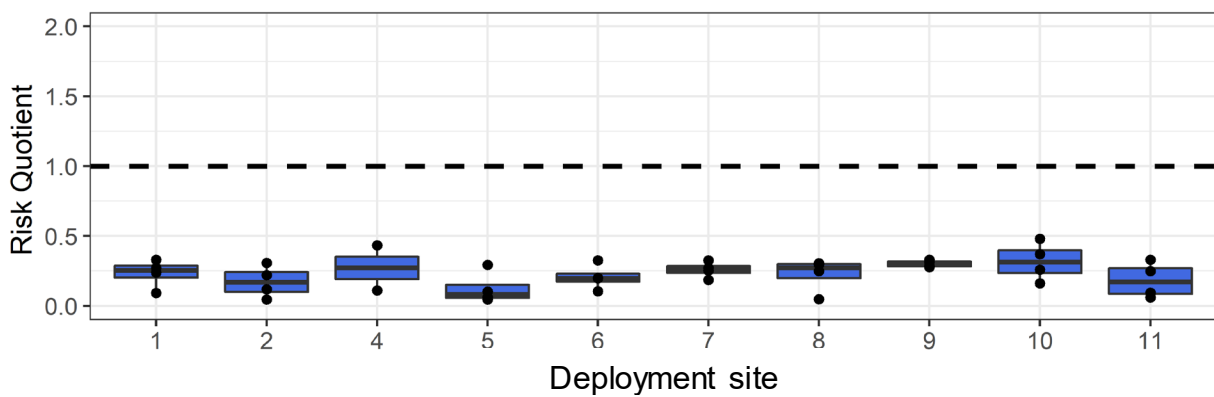


270 **Figure 4. Predicted metal-mixture toxicity from field-measured DGT-labile metal**
 271 **concentrations (Fig.3) to the Antarctic microalgae (a) *Phaeocystis antarctica* and (b)**
 272 ***Cryothecomonas armigera*, and the Antarctic echinoderm (c) *Sterechinus neumayeri*.**
 273 **Predictions are compared against observed toxicities from previously conducted**
 274 **laboratory studies with metal mixtures (a²⁹, b³⁰) or single metal exposures (c³¹) overlaid**
 275 **with 3-parameter log-logistic model fits (black lines) and 95% confidence intervals**
 276 **(ribbons). DGT-labile metals used in prediction modeling include cadmium, copper, nickel,**
 277 **lead and zinc for (a) and (b) and copper and zinc for (c) which reflects the availability of**
 278 **single-metal toxicity data. Data shown are from all sites as there were no differences**
 279 **between metal concentrations at each site.**

(a) Default WQGVs for 99% species protection



(b) Default WQGVs for 95% species protection



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

285 **Discussion**

286 ***Metal concentrations***

287 Concentrations of all metals in Antarctic near-shore waters in this study were elevated
288 compared to previous measurements in Antarctica in pristine environments representative
289 of coastal shelf waters³⁵ and near-shore environments with inputs from glacial melt³³, but
290 were lower than near-shore marine waters immediately adjacent to a research station on
291 King George Island¹⁴ (Table 1). There were only minor differences in DGT-labile cadmium,
292 copper, nickel, lead, or zinc concentrations between deployment sites (Fig. 3). This suggests
293 that the source of the metals is diffuse and geogenic in origin. In Antarctica, this could be
294 from sediment resuspension³⁹, sea ice melting⁴⁰, volcanic or hydrothermal inputs⁴¹, or
295 deposition from terrestrial ice melt and the weathering of rocks⁴².

296 Interestingly, no elevation in metal contaminants were observed from sites near penguin
297 colonies, which have been proposed to be a major source of metals on the West Antarctic
298 Fildes Peninsula and Ardley island⁴³. In this study, four sites (Sites 4, 8, 10, and 11) were in
299 close proximity to large Adélie penguin colonies, and had large volumes of melt stream
300 runoff from the colonies to the marine sites (personal observation). This could imply that
301 the metal contaminants were insoluble, remain complexed in guano, or were bound to
302 other organic carbon rendering them less labile⁴⁴.

303 ***Predicted toxicity***

304 For most Antarctic marine organisms, including the microalgae and echinoid examined in
305 this study, copper is the most toxic metal investigated. Copper was the only metal found to
306 possibly be a risk to marine organisms in this area, with DGT-labile concentrations near
307 concentrations likely to cause a toxic response to the sea urchin *S. neumayeri* (EC50 of 1.4
308 $\mu\text{g L}^{-1}$, King and Riddle³¹).

309 However, there was no difference in copper concentrations between sites (Fig. 3),
310 suggesting a geogenic rather than anthropogenic source. This is consistent with previous
311 findings of dissolved copper concentrations in Antarctic near-shore marine waters, both
312 near research stations such as at Ardley Cove, King George Island¹⁴ or Brown Bay, East
313 Antarctica¹⁵ and away from research stations, such as O'Brien Bay, East Antarctica¹⁵ or

314 Marion Cove, King George Island (up to $0.1 \mu\text{g Cu L}^{-1}$)³³, which is more representative of
315 background copper concentrations in Southern Ocean surface waters⁴⁵.

316 The approach to predicting metal-mixture toxicity, based on independent action or
317 concentration addition reference models, was applied to two microalgal species, *P.*
318 *antarctica* and *C. armigera* and the echinoderm *S. neuymayeri*, Fig. 4, but the response of
319 other species could be predicted using these same methods if the parameters needed for
320 the mixture models are reported⁴⁶.

321 ***Practicalities of using DGT samplers in the Antarctic marine environment***

322 DGT sampler deployment to the near-shore marine environment in Antarctica was not
323 without its challenges. Some biofouling of the DGT windows was apparent after
324 approximately 21 d (Supplementary information S3). This was unexpected, as previous
325 studies in Antarctica using DGT have not reported biofouling^{6,14}, even at deployment times
326 of 29 d¹⁵. Biofouling is dependent on the biological productivity of the receiving
327 environment, which in the Antarctic nearshore ecosystem is highly seasonal and subject to
328 summer microalgae blooms. If extensive biofouling occurs on a sampler the diffusion of
329 some metals may be inhibited while others could be promoted depending on the relative
330 binding affinities for the iminodiacetic acid functional groups of the Chelex-100 binding resin
331 compared to the biofilm on the DGT window⁴⁷.

332 The impact of biofouling is difficult to quantify, and so measurements from field deployed
333 DGT with extensive biofouling must be interpreted with caution⁴⁸. In this study, DGT
334 samplers were retrieved at two time points from Sites 1, 2, 5, 6, 7, 8, 9, and 10 (Fig. 1). A
335 comparison of metal concentrations in DGTs between these time points did not suggest that
336 biofouling caused changes to the measured metal concentrations. This comparison,
337 however, assumes similar seawater conditions and labile metal concentrations which we
338 believe likely given the large overlap (i.e. >21 d) in deployment durations and the lack of
339 temporal and spatial trends in metal concentrations between sites in this study.

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

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

347 On disassembly of the DGT it was noticed that the diffusion gel had shrunk by approximately
348 2-3 mm. This effect was not able to be recreated in the lab at similar temperatures so was
349 attributed to rapid dehydration of the polyacrylamide gel from the dry Antarctic air.

350 ***Considerations on the assessment of contaminant risk***

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

358 The approach outlined in this study can be used by environmental managers to assess the
359 risk of contaminants in the Antarctic nearshore marine environment. This may be a useful
360 inclusion in environmental impact assessments⁴⁹, for triaging contaminated sites for
361 remediation⁵⁰, or in making decisions about the protection of cultural heritage that may be
362 causing environmental harm⁵¹.

363 Care should be taken around applying national environmental quality standards to regions
364 they are not designed for, as they are based on response of endemic organisms and may
365 have specific caveats about environmental conditions that need to be considered. However,
366 as interim measures in lieu of Antarctic specific guidelines, Australian and New Zealand
367 WQGVs have previously been applied in Antarctic contamination assessments^{5,6,11,23,52,53}.

368 This study showed that the Australian and New Zealand WQGV for 99% species protection
369 for copper is unsuitable for the Antarctic nearshore marine ecosystem because the copper
370 concentrations, which were likely geogenic in origin (see the Metal concentrations section),
371 were largely equivalent to the default WQGV of 0.3 µg L⁻¹²². There is also some general
372 contention about the applicability of the 99% species protection level because of high
373 uncertainty in their derivation⁵⁴. 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 assessments⁵⁶, including dietary
379 exposure via particulate matter⁵³, phytoplankton^{14,57}, or other organisms⁵⁸. 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 zinc⁵⁷. 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 microalgae⁵⁹.

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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406 **Supporting information**

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

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

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1 **Supporting information**

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

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- 12 • Pages: 7
- 13 • Tables: 4
- 14 • Figures: 2

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17 **Supplementary Table 1**

18 Instrument limits of detection and certified reference material (CRM) analysis using TMDA-64.4 and TM 24.4 (NRC, Canada). All values are in
 19 $\mu\text{g L}^{-1}$ unless otherwise stated.

	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Sr	V	Zn
Metal (wavelength)	328.068	214.439	228.615	205.560	324.754	258.588	257.610	231.604	220.353	407.771	292.401	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

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22 **Supplementary Table 2**

23 DGT blank concentrations and calculated DGT LOD values used in method detection limit
 24 calculations. All values are in $\mu\text{g L}^{-1}$ 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, $\mu\text{g L}^{-1}$)	-0.05	0.2	-0.2	-0.6	-0.1
Standard deviation (#5-9, $\mu\text{g L}^{-1}$)	0.04	0.2	0.2	0.5	0.006
LOD calculations^c					
3x Standard deviation ($\mu\text{g L}^{-1}$)	0.1	0.6	0.5	1.4	0.1
Theoretical elution concentration ($\mu\text{g L}^{-1}$) ^a	4	20	14	44	4
Theoretical mass on resin (μg) ^b	0.006	0.027	0.020	0.062	0.006

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

26 ^b Calculated using Equation 1

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

30 **Supplementary Table 3**

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

Metal	D_i ($\times 10^6 \text{ s}^{-1} \text{ cm}^2$)	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

34 ^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
 35 undeployed DGT measured concentrations, and MDL is the method detection limit for the devices based on the LOD at a
 36 deployment time of 22 and 37 days.

37 **Supplementary Table 4.**

38 Model parameters for concentration-response relationships of metal toxicity for the Antarctic test species *Phaeocystis antarctica*,
 39 *Cryothecomonas armigera*, and *Sterechinus neumayeri*. Models used were a 4-parameter log-logistic model (LL4) and a mixed-effect 4-
 40 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.

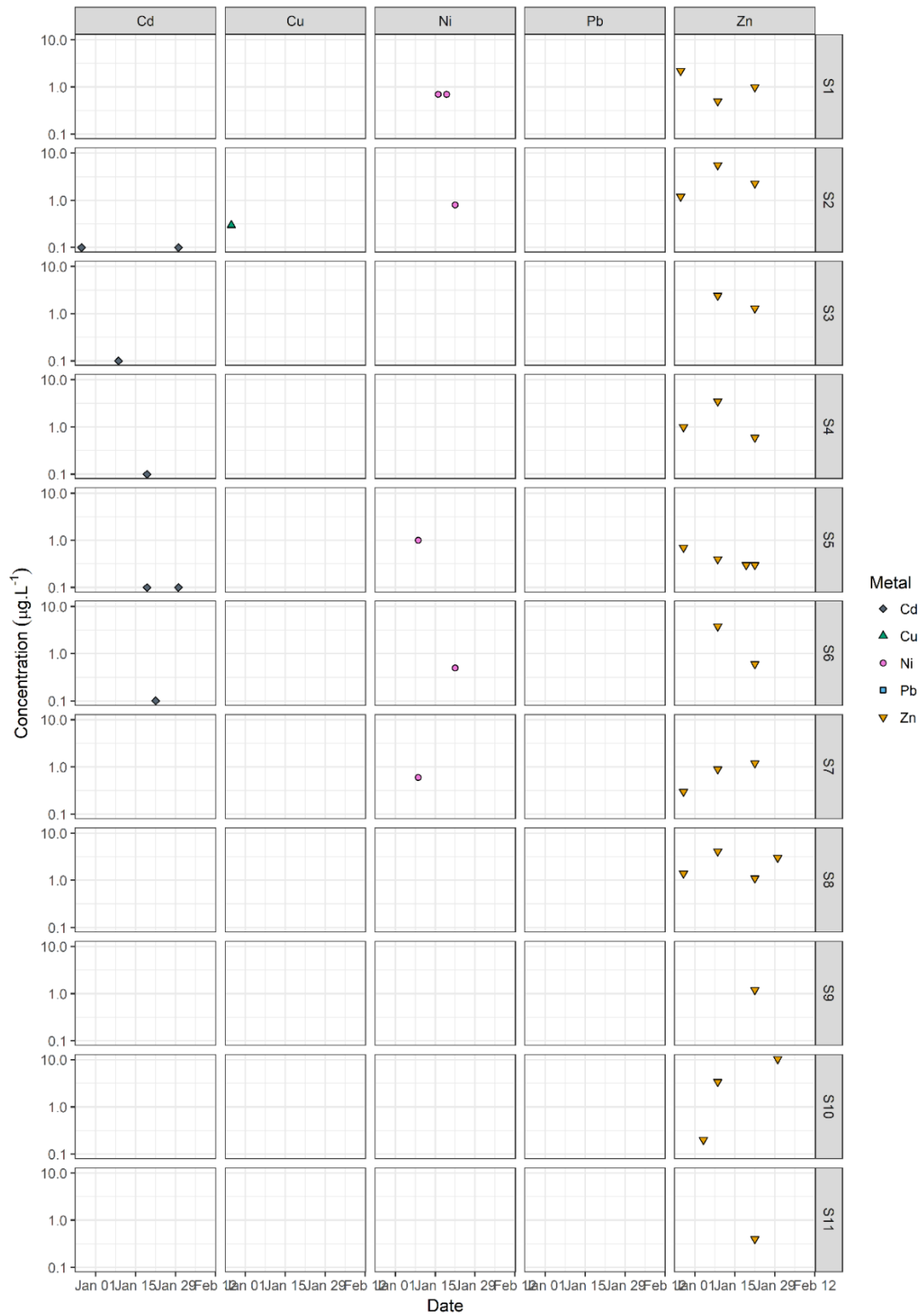
Antarctic species	Endpoint	Test duration (days)	Metal	Number of treatments	Model	10% effect concentration	Inflection point (e)	Slope (b)	Upper limit (c, % response)	Lower limit (d, % response)*	Source data	
<i>Phaeocystis antarctica</i>	Population growth rate	10	Cd	48	LL4	220 ± 50	1700 ± 200	1.1 ± 0.1	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		
<i>Cryothecomonas armigera</i>	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		
<i>Sterechinus neumayeri</i>	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		

41 *indicates that the model parameter was fixed at this value

42

43 **Supplementary Figure 1.**

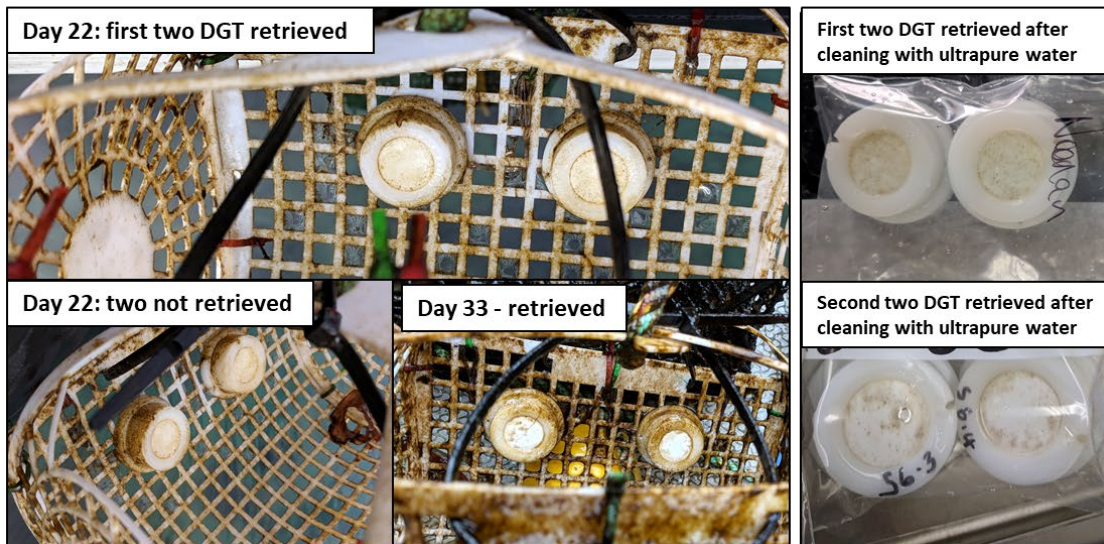
44 Measured dissolved metal concentrations in seawater at DGT deployment sites (all site data
 45 combined for each date and metal). The absence of points indicates measurements below
 46 detection limits (see Methods Section or Supplementary Table 1 for limits of detection).



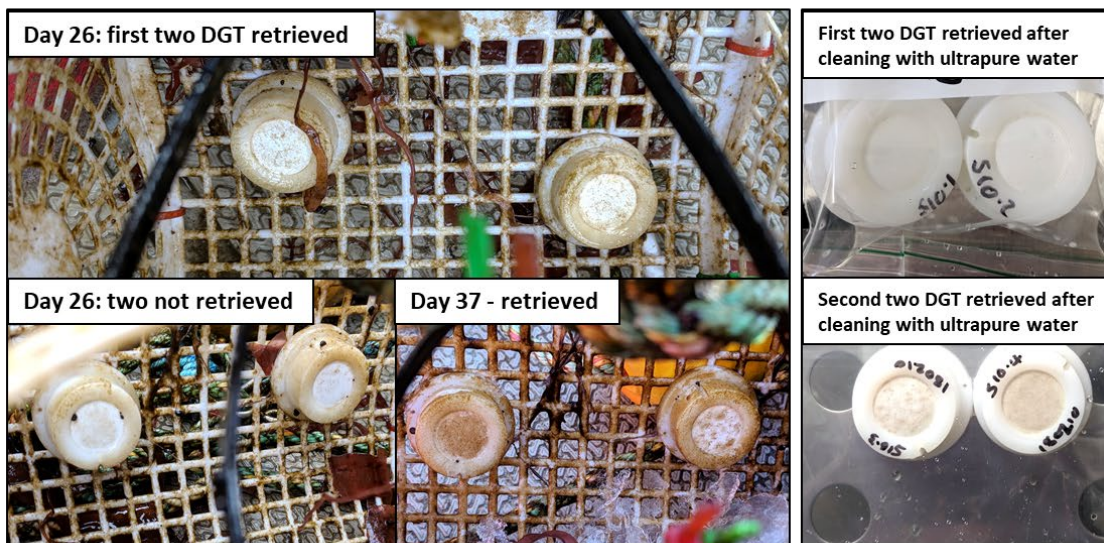
47 **Supplementary Figure 2**

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

Site 6



Site 10



Site 1

Site 2

Site 8



52 **Supporting information references**

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