

1 **Title:** Geographic and bathymetric comparisons of trace metal concentrations (Cd, Cu, Fe, Mn, and  
2 Zn) in deep-sea Lysianassoid amphipods from abyssal and hadal depths across the Pacific Ocean.

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9 **Running Head:** Trace metals in deep-sea amphipods

10 **Abstract**

11 Spatial patterns in trace metal (Cd, Cu, Fe, Mn, and Zn) bioavailability were analysed in deep-sea  
12 lysianassoid amphipods (*Eurythenes gryllus* and *Hirondellea* spp) from three subduction trenches;  
13 the Izu-Bonin (8000-9000 m), Kermadec (3000-10,000 m) and Peru-Chile trenches (4500-6000 m).  
14 Geographical differences in metal concentrations were evident. Iron and Mn concentrations were  
15 higher in *H. gigas* from the Izu-Bonin Trench compared to the *H. dubia* from the Kermadec Trench.  
16 Copper and Cd were higher in the *E. gryllus* in the Peru-Chile Trench compared to Kermadec Trench.  
17 There were significant interactions between trench and depth for a number of metals. This was  
18 evident in the tissues of the genus *Hirondellea* where there was an interaction between trench and  
19 depth for Cu and Zn. Both these metals were found in higher concentrations at approximately 8000  
20 m in the Izu-Bonin Trench compared to the same depth in the Kermadec Trench. At deeper depths,  
21 however, the opposite occurred. An interaction between trench and depth also occurred for Fe and  
22 Mn in *E. gryllus* where Fe and Mn were found in higher concentrations at approximately 4500m in  
23 the Kermadec Trench compared to the Peru-Chile Trench but the opposite was true at deeper  
24 depths. This indicated that the relation between metals and depth were not consistent over the  
25 depth ranges sampled. Cadmium in *H. dubia* from Kermadec Trench was the only metal that  
26 decreased in concentration across the depths sampled whereas Mn and Zn increased in  
27 concentrations with depth within this species and trench. The high concentrations of Cd within these  
28 amphipods suggested that the Cd-anomaly observed in polar amphipods could potentially be  
29 extended to deep-sea amphipods. Furthermore the low levels of Cu in *E. gryllus* may indicate Cu-  
30 deficiencies. These results indicated a complex relationship between depth and trench and metal

31 concentrations in amphipods with Fe, Mn and Zn largely reflecting the concentrations in the  
32 environment.

33 **Keywords:** biomonitoring; deep sea; trace metals; hadal; amphipod; manganese; iron; Pacific Ocean;  
34 Kermadec; Izu-Bonin; trench.

### 35 **1. Introduction**

36 Amphipods are extremely widespread in their geographical distributions, occurring in habitats from  
37 the intertidal to hadal depths (> 6000 m). They have been extensively used for monitoring the  
38 bioavailability of metal concentrations in coastal environments (Fairey *et al.*, 2001; Long *et al.*, 1995;  
39 Mearns *et al.*, 1986; Rainbow, 1995) because they accumulate metals as insoluble forms (e.g.  
40 granules) with limited excretion (Rainbow, 2002; Rainbow *et al.*, 1998). Numerous studies have  
41 shown that metal concentrations in amphipods reflect the bioavailability of metals from their  
42 habitat, which may become elevated by physiological, localised enrichment natural or anthropogenic  
43 sources (Moore *et al.*, 1991; Rainbow, 1995; Rainbow *et al.*, 2000). The result is that amphipod body  
44 tissues often reflect bioavailability of trace metals within their habitat better than other regulatory  
45 species such as decapod crustaceans (Marsden and Rainbow, 2004) and are used as biomonitors for  
46 examining spatial and temporal variations in trace metal bioavailability (Rainbow, 2002; Rainbow *et al.*  
47 *et al.*, 1998). However, we know very little about natural variability of deep-sea amphipods and  
48 whether they could be used in the future to monitor anthropogenic impacts in the deep sea.

49 Marine sediments are considered the final receptor of metals released from terrestrial and  
50 anthropogenic sources (Eggleton and Thomas, 2004; Roberts, 2012). In coastal systems, metals are  
51 introduced into the marine environment from rivers through terrestrial runoff as a result of a natural  
52 erosion processes (Rainbow, 1985). Riverine input of naturally occurring metals are the dominant  
53 source of metal concentrations within coastal surface waters (Matsunaga *et al.*, 1998), which  
54 subsequently feed deeper waters through downslope transport from decomposition of particulate  
55 organic matter (POM) (Saitoh *et al.*, 2008). Within open ocean pelagic and deep-sea habitats, metals  
56 can be released into the water via remineralisation of faecal pellets, fluids from hydrothermal vent  
57 systems, weathering of mineral deposits, sedimentary diagenic recycling of metals and the input of  
58 aeolian material (e.g. dust) which binds to POM that subsequently sinks (Bennett *et al.*, 2008; Chu *et al.*  
59 *et al.*, 2006; German *et al.*, 2010; Grand *et al.*, 2015; Hauton *et al.*, 2017; Koschinsky *et al.*, 2003;  
60 Laglera *et al.*, 2017; Ratnarajah *et al.*, 2014; Yucel *et al.*, 2011).

61 Anthropogenic influences can exaggerate natural levels of metal within the deep sea through the  
62 supply of POM that has accumulated metals from surface waters. A future anthropogenic threat, is a

63 potential for deep-sea mining (DSM). DSM will target seafloor massive sulfides, polymetallic nodules  
64 or rich-cobalt crusts. During the mineral extraction and processing phases, there is the potential for  
65 releasing toxic mixtures of metals into the environment in various different physical forms either  
66 through sediment plumes or newly dissolved material (Fallon *et al.*, 2017; Hauton *et al.*, 2017).  
67 These metals may pose a risk to deep-sea fauna depending on their bioavailability (Hauton *et al.*,  
68 2017). Assessing risk requires an understanding of the natural variability of metals within any given  
69 area and for any biomonitoring species. It is likely that any DSM impacts will occur over large spatial  
70 scales (Glover and Smith, 2003). Therefore, it is important to understand natural and spatial  
71 variability in metal concentrations from which to gauge risk. However, at present there is a paucity  
72 of information on natural metal levels in deep-sea fauna not associated with hydrothermal vents,  
73 which needs to be addressed.

74 Lysianassoid amphipods have the potential to be used for biomonitoring in a similar way to shallow  
75 water talitrid amphipods because of their vast geographic and bathymetric distribution. In abyssal  
76 and hadal habitats, the lysianassoid amphipods *Eurythenes gryllus* (Lichtenstein in Mandt 1822) and  
77 *Hirondellea* sp. are the dominant amphipod species (Charmasson and Calmet, 1987; Eustace *et al.*,  
78 2016; Lacey *et al.*, 2016). Conventional experimentation on potential biomonitoring species that  
79 requires live specimens to establish baseline and excess trace metal concentrations are logistically  
80 very difficult to undertake in abyssal and hadal environments because of decompression mortality  
81 when individuals are recovered at the surface (Yayanos, 2009). However, a sufficiently large spatial  
82 data set which contains the same species or genus allows testing for trace metal variation over large  
83 geographic and bathymetric scales. The Pacific Ocean offers such possibilities to examine trends of  
84 distance and by depth, with 165 million km<sup>2</sup> of largely abyssal depths (3000 – 6000 m) as well as 23  
85 out of the world's 27 ocean trenches that range from 6000 to nearly 11,000 m depth (Jamieson,  
86 2015). The aim of this study was to assess spatial variability in metal concentrations in Lysianassoid  
87 amphipods from abyssal and hadal depths from in and around three subduction trenches (Izu-Bonin,  
88 Kermadec and Peru-Chile trenches) in the Pacific Ocean. The cosmopolitan *E. gryllus* was chosen for  
89 samples collected at less than 6200 m in the Kermadec and Peru-Chile trenches. *Hirondellea dubia*  
90 (Dahl, 1959) (Kermadec Trench) and *H. gigas* (Birstein and Vinogradov, 1955) (Izu-Bonin Trench) are  
91 the dominant Lysianassoid in their respective trenches. They are extremely similar from a  
92 behavioural, functional and genetic basis (Lacey *et al.*, 2016; Ritchie *et al.*, 2015). Specifically, we  
93 have investigated whether metal concentrations in amphipods: (1) vary between trenches; (2) if  
94 there are relationships between tissue metal concentrations and depth; and (3) whether specific  
95 metals found within the amphipods co-varied.

## 96 **2. Materials and Methods**

### 97 **2.1. Sampling**

98 Samples were collected using a free-fall, full ocean depth rated lander vehicle. The lander remained  
99 on the seafloor for 10.5 to 13.5 hours. Connected to the lander were three invertebrate funnel traps  
100 (30 cm in length, 6 cm diameter that housed openings 2.5 cm diameter). The traps were baited with  
101 approximately 100 g of mackerel enclosed within a mesh allowing the release of an odour plume.  
102 Samples were landed on deck, and immediately preserved in 70% ethanol.

### 103 **2.2. Study Site and Species**

104 Samples were collected from three subduction trenches formed at tectonic convergence zones (Fig.  
105 1): the Izu-Bonin trench, lying beneath the North-West Pacific; the Kermadec trench, situated  
106 beneath the South West Pacific; and the Peru-Chile Trench, located off the west coast of South  
107 America in the South-East Pacific. *Hirondellea gigas* were collected from the Izu-Bonin Trench at  
108 8172 m and 9316 m. *Hirondellea dubia* were collected from the Kermadec Trench at 6999 m, 8148  
109 m, 9053 m and 9908 m. *Eurythenes gryllus* were collected from the Kermadec trench at 3268 m,  
110 4519 m and 5242 m as well as the Peru-Chile Trench at 4602 m, 5329 m and 6173 m.

### 111 **2.3. Trace Metal Analysis**

112 All animals were analysed as whole body samples. Each replicate pooled together individuals to  
113 generate sufficient dry weight for trace metal analysis. Samples were freeze dried for 74 hours. The  
114 samples were homogenised using a pestle and mortar. Up to four replicates were produced for each  
115 depth at each location. However, at some locations and depths there was insufficient tissue to  
116 generate four replicates. For example, *E. gryllus* samples from the Kermadec Trench at 3268 m only  
117 supplied enough material to generate one replicate and at 4519 m only generated enough material  
118 to create three replicates.

119 For each replicate, 0.15 g of dry material was digested in 5 ml of concentrated nitric acid (70%) using  
120 a CEM Mars 6 microwave digester within Teflon vessels. The samples were heated to 200 °C for 35  
121 min. The majority of samples were fully digested at the end of the microwave digester cycle.  
122 However, two samples contained suspended particles. All samples were then diluted to 25 ml with  
123 distilled water, before being filtered with Whatman 41 to remove any residual undigested material.  
124 Method blanks were undertaken at the same time as digesting the dried amphipod tissue. These  
125 contained no dried tissue in order to establish if there was any potential contamination. Samples of

126 TORT-2 Lobster Hepatopancreas Certified Reference Material (National Research Council, Canada)  
127 were analysed (Table 1) to establish the efficiency of the digestion technique. The results were  
128 agreed to be satisfactory for Cd, Cu, Fe, Mn, and Zn. A Varian Vista-MPX CCD Simultaneous ICP-OES  
129 was used to measure the metal concentrations in the blanks, TORT-2 material and amphipod  
130 samples. All metal concentrations are given in dry weight.

## 131 **2.4. Statistical Analysis**

132 Generalised Linear Models (GLMs) were used to investigate the relationship between metal  
133 concentration and depth or geographical location. The geographical analysis compared: (1) the  
134 metal concentrations of *H. gigas* and *H. dubia* from the Izu-Bonin and Kermadec Trenches  
135 (respectively); and (2) *E. gryllus* metal concentrations between the Kermadec and Peru-Chile  
136 Trenches. For each of these comparisons, only individuals that were collected from similar depth  
137 ranges were used in the analysis. The model residuals were inspected using QQ-plots to check that  
138 they were consistent with the model assumptions. In cases where the QQ-plots indicated that the  
139 residuals were not normally distributed then those metal data were log transformed and re-  
140 analysed. In only a single case were the model assumptions not met. In this case a Kruskal-Wallis test  
141 was used followed by *post-hoc* Nemenyi Test with Tukey distance approximation for independent  
142 samples. The relationship between two metals within the amphipods was tested with a Spearman  
143 rank correlation. All analysis was undertaken in R version 3.4.1 using the following packages dplyr,  
144 ggplot2, PMCMR and car.

## 145 **3. Results**

### 146 **3.1. Geographical differences in metal concentrations**

147 Spatial differences in metal concentrations occurred in the genus *Hirondellea*, at comparable depth  
148 ranges, in samples collected from the Izu-Bonin (8172 m to 9316 m) and Kermadec trenches (8148 m  
149 to 9053 m). Mn (GLM:  $t = -10.71$ ,  $p < 0.01$ ) and Fe (GLM:  $t = -4.76$ ,  $p < 0.01$ ) were found to be  
150 significantly higher in *H. gigas* from the Izu-Bonin Trench than *H. dubia* from the Kermadec Trench  
151 whereas the opposite was the case for Cd (GLM:  $t = 2.80$ ,  $p < 0.05$ ). There was a significant  
152 interaction between sampling depth and trench for Cu (GLM:  $t = 4.82$ ,  $p < 0.01$ ) and Zn (GLM:  $t =$   
153  $8.50$ ,  $p < 0.01$ ), which indicated that the relationship between depth and metal concentrations  
154 differed in these two trenches. There were trench specific differences in metal concentrations with  
155 higher concentrations of Cu (GLM:  $t = -4.72$ ,  $p < 0.01$ ) and Zn (GLM:  $t = -8.69$ ,  $p < 0.01$ ) in *H. gigas* in  
156 the Izu-Bonin Trench compared to *H. dubia* from the Kermadec Trench at the shallow site but the  
157 opposite was the case at the deep site.

158 Spatial differences in the metal concentrations in *Eurythenes gryllus*' tissues were compared  
159 between the Kermadec (4519 m to 5242 m) and Peru-Chile (4602 m to 5329 m) trenches (Fig. 3). For  
160 Mn and Fe, there was a significant interaction between depth and trench (GLM:  $t = -10.29$ ,  $p < 0.01$ ;  $t$   
161  $= 3.02$ ,  $p < 0.05$ , respectively). Both Mn and Fe were found in greater concentrations at  
162 approximately 4500 m in the Kermadec Trench, whereas the opposite was true at the deeper  
163 location. In the Peru-Chile Trench, Cu (GLM:  $t = 2.60$ ,  $p < 0.05$ ) and Cd (GLM:  $t = 2.83$ ,  $p < 0.05$ ) were  
164 present in higher concentrations compared to the Kermadec Trench. There were no differences  
165 between the two trenches for Zn (GLM:  $t = 0.93$ ,  $p > 0.05$ ).

### 166 **3.2. Relationship between metal concentrations and depth**

167 *Hirondella gigas* from the Izu-Bonin Trench showed a significant increase in Mn (GLM:  $t = 6.02$ ,  $p <$   
168  $0.01$ ) and Fe (GLM:  $t = 2.86$ ,  $p < 0.05$ ) with depth whereas Zn decreased with depth (GLM:  $t = -5.95$ ,  $p$   
169  $< 0.01$ ) (Fig. 2). Neither Cu (GLM:  $t = -1.29$ ,  $p = 0.24$ ) or Cd (GLM:  $t = 0.97$ ,  $p = 0.37$ ) showed any  
170 relationship with depth.

171 There were depth related trends in metal concentrations of *H. dubia* from the Kermadec Trench. The  
172 concentration of Mn in tissues of *H. dubia* was greater at the deeper sites than the shallowest site  
173 (GLM:  $t = 7.83$ ,  $p < 0.01$ ;  $t = 18.31$ ,  $p < 0.01$ ;  $t = 11.54$ ,  $p < 0.01$ ). Fe showed an initial decrease in  
174 tissue concentration relative to the shallowest site (GLM:  $t = -2.26$ ,  $P < 0.05$ ) before increasing in  
175 concentrations at 9053 m (GLM:  $t = 7.18$ ,  $p < 0.01$ ) compared to 6999m. There was no difference in  
176 Fe concentrations between 6999 m and 9908 m (GLM:  $t = 1.27$ ,  $p = 0.23$ ). Zinc tissue concentrations  
177 showed significant differences among the depths (Kruskal-Wallis test:  $\chi^2 = 13.26$ ,  $df = 3$ ,  $p < 0.01$ )  
178 with *H. dubia* Zn tissue concentrations being much higher at 9053 m than either 6999 m (Nemenyi  
179 Test:  $p < 0.01$ ) or 8148 m (Nemenyi Test:  $p < 0.05$ ). Copper concentrations varied with depth  
180 (Kruskal-Wallis test:  $\chi^2 = 12.73$ ,  $df = 3$ ,  $p < 0.01$ ) but not in a similar way to Mn, Fe and Zn. In *H. dubia*,  
181 Cu concentrations were significantly greater at 6999 m (Nemenyi Test:  $p < 0.05$ ) and 9053 m  
182 (Nemenyi Test:  $p < 0.01$ ) compared to 8148 m but there were no differences among 6999 m, 9053 m  
183 and 9908 m (Nemenyi Test:  $p > 0.05$ ). Cadmium was the only metal to have a large decrease in  
184 between 6999 m and 8148 m (GLM:  $t = -18.46$ ,  $p < 0.01$ ), 9053 m (GLM:  $t = -16.95$ ,  $p < 0.01$ ) and  
185 9908 m (GLM:  $t = -20.60$ ,  $p < 0.01$ ) with little variation observed in the metal concentration deeper  
186 than 8148 m.

187 *Eurythenes gryllus* was collected from three depths in the Kermadec Trench ( $< 6000$  m). Both Mn  
188 (GLM:  $t = 5.50$ ,  $p < 0.01$ ) and Fe (GLM:  $t = 4.06$ ,  $p < 0.01$ ) significantly increased in concentration  
189 between 3268 m and 4519 m and then decreased in concentration between 4519 m and 5242 m so

190 that there were no differences in Mn (GLM:  $t = -0.31$ ,  $p = 0.77$ ) and Fe (GLM:  $t = -0.80$ ,  $p = 0.46$ )  
191 concentrations between 3268 m and 5242 m sites. Cd also significantly increased in the  
192 concentration between 3268 m and 4519 m (GLM:  $t = 3.64$ ,  $p < 0.05$ ) and remained significantly  
193 higher at 5242 m than 3268 m (GLM:  $t = 2.83$ ,  $p < 0.05$ ). There were no differences in Cu  
194 concentrations between 3268 m and 4519 m (GLM:  $t = 1.39$ ,  $p = 0.22$ ) but Cu concentrations were  
195 significantly lower at 5242 m than 3268 m (GLM:  $t = -3.03$ ,  $p < 0.05$ ). The concentration of Zn did not  
196 differ between 3268 m and 4519 m (GLM:  $t = 0.40$ ,  $p = 0.73$ ) nor 3268 m and 5242 m (GLM:  $t = 0.19$ ,  
197  $p = 0.86$ ).

198 *Eurythenes gryllus* was also collected from three depths in the Peru-Chile but over a different range  
199 to the Kermadec Trench. Manganese (GLM:  $t = -0.80$ ,  $p = 0.46$ ;  $t = 0.08$ ,  $p = 0.94$ ), Fe (GLM:  $t = -0.16$ ,  $p$   
200  $= 0.88$ ;  $t = 0.58$ ,  $p = 0.59$ ), Cu (GLM:  $t = -1.68$ ,  $p = 0.15$ ;  $t = -0.51$ ,  $p = 0.63$ ) and Cd (GLM:  $t = -1.22$ ;  $p$   
201  $= 0.30$ ;  $t = 0.40$ ,  $p = 0.70$ ) did not show significant differences in metal concentrations between 4602 m  
202 and the other two deeper sites at 5329 m and 6173 m. The only metal to show any depth related  
203 trends in metal concentrations was Zn where there was a decrease in metal concentrations between  
204 4602 m and 6173 m (GLM:  $t = -4.56$ ,  $p < 0.01$ ) but no differences between 4602 m and 5329 m (GLM:  
205  $t = 1.71$ ,  $p = 0.15$ ).

### 206 **3.3 Co-varying relationships between metals within amphipods**

207 There was some evidence of co-varying patterns in metal concentrations within the amphipods. Fe  
208 and Mn were positively correlated in the tissues of *H. gigas* ( $\rho = 0.98$ ,  $p < 0.01$ ), *H. dubia* ( $\rho =$   
209  $0.74$ ,  $p < 0.01$ ) and *E. gryllus* ( $\rho = 0.98$ ,  $p < 0.01$ ) from the Izu-Bonin and Kermadec trenches,  
210 respectively (Figures 4-6). *Eurythenes gryllus* from the Peru-Chile Trench was the only location with  
211 no significant relationship between these two metals. This may have been the a result of the low  
212 sample size given there appeared to be a positive relationship (Figure 7). Copper also appeared to  
213 co-vary with a number of metals but there was no consistent pattern among the metals or trenches.  
214 There was a positive relationship between Cu and Fe in *H. dubia* ( $\rho = 0.71$ ,  $p < 0.01$ ) and *E. gryllus*  
215 ( $\rho = 0.71$ ,  $p = 0.058$ ) in the Kermadec Trench (Figure 5 and 6) but this was not seen in the  
216 amphipods from the Izu-Bonin or Peru-Chile trenches (Figures 4 and 7). *Eurythenes gryllus* from the  
217 Kermadec Trench also had a positive relationship between Cu and Mn ( $\rho = 0.76$ ,  $p < 0.05$ ). Zn and  
218 Fe ( $\rho = 0.78$ ,  $p < 0.01$ ) and Zn and Mn ( $\rho = 0.95$ ,  $p < 0.01$ ) were also positively related in *H. dubia*  
219 from the deeper sites in the Kermadec Trench. Cd and Cu were positively related in *H. dubia* ( $\rho =$   
220  $0.54$ ,  $p < 0.05$ ; Figure 5) and in *E. gryllus* from the Peru-Chile Trench ( $\rho = 0.90$ ,  $p < 0.01$ ; Figure 7).

## 221 **4. Discussion**

222 The metal concentrations of the three deep-sea amphipods are all in the range of those observed in  
223 coastal and polar regions (Cipro *et al.*, 2018; Fialkowski *et al.*, 2009; Keil *et al.*, 2008; Marsden *et al.*,  
224 2003; Moore *et al.*, 1991; Rainbow *et al.*, 1993; Rainbow *et al.*, 1998; Rainbow *et al.*, 1989; Zauke *et*  
225 *al.*, 1996). Cadmium in coastal and polar regions ranges between 0.8 to 51.0  $\mu\text{g g}^{-1}$  and 0.3 to 73.4  $\mu\text{g}$   
226  $\text{g}^{-1}$ , respectively (Cipro *et al.*, 2018; Keil *et al.*, 2008; Ugolini *et al.*, 2004; Zauke *et al.*, 1996). The Cd  
227 concentrations in *H. dubia* (6999m Kermadec Trench), and *E. gryllus* (4602m and 6173m, Peru-Chile  
228 Trench) appear to be at the higher end of the spectrum for Cd concentrations ( $> 15 \mu\text{g g}^{-1}$  dry  
229 weight) in amphipods. High Cd concentrations have been observed in polar decapods and  
230 amphipods, which is termed the Cd-anomaly (Bargagli *et al.*, 1996; Keil *et al.*, 2008; Petri and Zauke,  
231 1993; Ritterhoff and Zauke, 1997). This is potentially because of Cu deficiencies within these  
232 organisms that results in increased up-take of Cd because of indiscriminate uptake mechanisms  
233 between Cd and Cu (Petri and Zauke, 1993).

234 A similar hypothesis has been put forward for high concentrations of Cd and potential Cu  
235 deficiencies observed in deep-sea crustaceans (Prowe *et al.*, 2006; Ridout *et al.*, 1985; White and  
236 Rainbow, 1987). The concentrations of Cu in this study (10 to 45  $\mu\text{g g}^{-1}$ ) are at the lower end of the  
237 spectrum for polar (5 to 261  $\mu\text{g g}^{-1}$ ) and coastal (14 to 342  $\mu\text{g g}^{-1}$ ) amphipods (Keil *et al.*, 2008;  
238 Rainbow *et al.*, 1993; Rainbow *et al.*, 1989; Zauke *et al.*, 1996). When the metal concentrations of Cu  
239 are considered along with Cd concentrations, the Cu concentrations within *E. gryllus*, *H. dubia* and *H.*  
240 *gigas* are close to or less than the 30 to 40  $\mu\text{g g}^{-1}$  threshold for the enzymatic and metabolic  
241 requirements for decapods (Rainbow, 1993b). This range needs to be considered as an estimate  
242 given it was calculated based on temperate shallow water decapods (Rainbow, 1993a). The  
243 concentrations of Cu in *E. gryllus* from the Kermadec and Peru-Chile Trench are all below 25  $\mu\text{g g}^{-1}$ ,  
244 which potentially indicates that they are Cu deficient. The underlying mechanism why these values  
245 are so low is not clear. Copper can decrease in deep-sea crustaceans in times of nutritional stress  
246 (McAllen *et al.*, 2005), which given the deep sea is a low food environment, Cu-deficiencies or low  
247 concentrations within these amphipods may be also related to food availability. Therefore, low Cu  
248 concentrations may be related to species-specific physiological mechanisms rather than depth-  
249 specific adaptations. This is because higher concentrations of Cu were found in *Hirondellea* spp at  
250 greater depths than *E. gryllus* from the shallower sites. Although, *H. gigas* within the Izu-Bonin  
251 Trench at 9316 m and *H. dubia* specimens from the Kermadec Trench at 8148 m were below 30 to  
252 40  $\mu\text{g g}^{-1}$  Cu which may indicate that these species are at the threshold of Cu-deficiency.

253 The concentrations of Fe and Mn were similar to those found in coastal regions (Fe, 47 to 1103  $\mu\text{g g}^{-1}$ ;  
254 Mn, 12 to 111  $\mu\text{g g}^{-1}$ ) (Fialkowski *et al.*, 2009; Rainbow *et al.*, 1998; Ugolini *et al.*, 2004). However,



255 data for polar regions is still lacking. The highest recorded values of Fe (1103  $\mu\text{g g}^{-1}$ , Dulas Bay,  
256 Wales) and Mn (~70 to 111  $\mu\text{g g}^{-1}$ , Poland and Italy) in coastal amphipod occur in areas with high  
257 pollution (Fialkowski *et al.*, 2009; Rainbow *et al.*, 1998). Excluding the extreme Fe concentration  
258 (1103  $\mu\text{g g}^{-1}$ ) from Dulas Bay, the Fe and Mn concentrations in *H. gigas* (Izu-Bonin Trench) appear to  
259 be at the higher end of the spectrum for amphipods.

260 To our knowledge, the only published records of metal concentrations in deep-sea amphipods are  
261 those from benthopelagic amphipods, putatively identified as *E. gryllus*, collected from the Peru  
262 Basin Mn nodule field (Koschinsky *et al.*, 2003). This highlights the paucity of information on regional  
263 variation in metal concentrations in deep-sea habitats. The values of these benthopelagic amphipods  
264 were high compared with coastal species (Koschinsky *et al.*, 2003). Koschinsky *et al.* (2003)  
265 suggested that the naturally high metal concentrations relative to coastal amphipods should be  
266 treated with caution because it could be a result of varying metal bioaccumulation pathways among  
267 amphipods. However, these benthopelagic amphipods from the Peru Basin had Cu (53 to 85  $\mu\text{g g}^{-1}$ )  
268 and Mn (168 to 628  $\mu\text{g g}^{-1}$ ) concentrations (Koschinsky *et al.*, 2003) much higher than the three  
269 species within this study while Fe (304 to 837  $\mu\text{g g}^{-1}$ ) and Zn (165 to 181  $\mu\text{g g}^{-1}$ ) concentrations were  
270 at the higher end of the spectrum compared to hadal species. This would suggest that there are  
271 spatial differences in the bioavailability of metals in the deep sea that may be related to regional  
272 biogeochemical processes.

#### 273 **4.1. Geographical Differences in Metal Concentrations**

274 Concentrations of Fe and Mn in amphipods were considerably higher in the Izu-Bonin than the  
275 Kermadec trench, for those that were caught at comparable depths. The difference in the Fe and Mn  
276 concentrations of the trench amphipods may be related to a series of environmental factors and  
277 proximity to land. Dissolved Fe and Mn concentrations in the water column are higher in the Izu-  
278 Bonin Trench and surrounding water masses than in the Kermadec Trench (eGEOTRACES Electronic  
279 Atlas, 2018; Ellwood *et al.*, 2018; Nishioka and Obata, 2017; Obata *et al.*, 2017). This is a result of a  
280 combination of processes that have influenced the geochemistry of the water column including  
281 hydrothermal activity and terrestrial input. Hydrothermal fluids are enriched in Fe and Mn relative to  
282 seawater and are key inputs of these metals to the deep ocean (Bennett *et al.*, 2008; Chu *et al.*,  
283 2006; Gamo *et al.*, 1988; Resing *et al.*, 2015). The Izu-Bonin tectonic region is approximately 25  
284 million years older than the Kermadec region (Brandl *et al.*, 2017; de Ronde *et al.*, 2001; de Ronde *et al.*  
285 *et al.*, 2007; Honda *et al.*, 2007). This would equate to a greater volume of metal rich hydrothermal  
286 fluids entering and influencing the seawater geochemistry as well as forming large expanses of  
287 seafloor Mn-Fe crusts (Usui and Nishimura, 1992; Usui and Lasby, 1998). Terrestrial sources of Fe

288 and Mn from riverine inputs and aeolian sources are also potentially important for the shelf sea  
289 areas (Saitoh *et al.*, 2008) in proximity to the Izu-Bonin Trench. Fe and Mn respiration within Sagami  
290 Bay, Japan is extremely high when compared to other geographical areas, with a high percentage of  
291 total benthic carbon mineralization coming from the respiration of Fe and Mn, sourced from  
292 Japanese soil (unpublished data; Pers. Comms. Ronnie Glud). The high Fe and Mn concentrations  
293 enabling high levels of carbon mineralization are as a result of natural input into the marine  
294 environment via terrestrial runoff. High Fe and Mn bioavailability are often correlated with increased  
295 organic carbon content in the sediments (Hyun *et al.*, 2017; Rainbow *et al.*, 1998). These sources  
296 may result in increased levels of downslope transport of the shelf sediments containing metals  
297 (Nozaki and Ohta, 1993). The overall indication is that there is potentially more bioavailable Fe and  
298 Mn present in the Izu-Bonin Trench compared to the Kermadec Trench.

299 There were no clear differences in the concentrations Cd, Cu and Zn for the species of *Hirondellea*  
300 between the Izu-Bonin and Kermadec trenches. The concentration of Cu in *H. gigas* was found in  
301 higher concentrations at the shallower depth in the Izu-Bonin Trench than *H. dubia* from the  
302 Kermadec Trench whereas the opposite was true for Zn. Dissolved Cu and Zn in the Izu-Bonin Trench  
303 and deep western Pacific Ocean have similar concentrations to the Kermadec Trench (eGEOTRACES  
304 Electronic Atlas, 2018; Kim *et al.*, 2015; Kim *et al.*, 2017; Obata *et al.*, 2017; Takano *et al.*, 2014). The  
305 depth related differences may be the result of either regional resuspension of sedimented material  
306 or localised water column remineralisation processes. However, even though there are geographical  
307 differences in the dissolved metals concentrations in the water column, there may be additional  
308 biological and physiological mechanisms that result in the geographical differences observed in  
309 these metals.

310 Although the Kermadec and Peru-Chile trenches are separated by approximately 10,000 km of  
311 seafloor (Fig. 1), there was no overall difference in the concentrations of Fe and Mn within *E. gryllus*.  
312 Differences in Fe and Mn concentrations were observed at specific depths. This would indicate that  
313 potentially depth specific geochemical and environmental factors are dictating metal concentrations  
314 in the amphipods. Dissolved Fe and Mn concentrations in the Peru-Chile Trench water column are  
315 higher than found in the Kermadec Trench (eGEOTRACES Electronic Atlas, 2018; Ellwood *et al.*, 2018;  
316 Resing *et al.*, 2015), which was reflected in *E. gryllus* tissue with the exception of samples collected  
317 at approximately 4500 m. There is a large plume of dissolved Fe and Mn between 1000-3000m in the  
318 Peru-Chile Trench (John *et al.*, in press-b; Marsay *et al.*, in press). The Fe plume may be related to a  
319 combination of reversible scavenging between particulate and dissolved Fe and pore water Fe (John  
320 *et al.*, in press-b; Marsay *et al.*, in press). Sediment cores taken from the Peru margin indicate low

321 levels of porewater Mn (Boning *et al.*, 2004), which may be related to low sedimentation rates as a  
322 result of the Mn cycling in the water column oxygen minimum zone (Boning *et al.*, 2004). Even  
323 though the mechanisms are not clear as to why there is an increased level of dissolved Fe and Mn in  
324 the water column around the Peru-Chile Trench, the salient point is that these metals are present at  
325 higher concentrations in the water column and *E. gryllus* tissue in the Peru-Chile Trench region  
326 compared to the Kermadec Trench. An exception were the samples from at approximately 4500 m.  
327 This would indicate Fe and Mn are more bioavailable to the *E. gryllus* in the Peru-Chile Trench.  
328 Copper and Cd concentrations were higher in *E. gryllus* from the Peru-Chile Trench than Kermadec  
329 Trench. Both dissolved Cu and Cd are found in higher concentrations in the Peru-Chile Trench than  
330 the Kermadec Trench, which appears to be reflected in the amphipods (eGEOTRACES Electronic  
331 Atlas, 2018; John *et al.*, in press-a). Although as mentioned earlier, Cd and Cu levels may also be  
332 related to physiological factors which might be influencing the uptake and concentration of these  
333 metals. Zinc concentrations in the amphipods did not differ between the Peru-Chile and Kermadec  
334 trenches and neither does dissolved Zn in the water column.

#### 335 **4.2. Relationships between depth and metals and covarying metal concentrations in amphipods**

336 There were no continuous increases or decreases in metal concentrations with depth for *H. gigas*, *H.*  
337 *dubia* and *E. gryllus*. The exceptions was Cd in *H. dubia* from the Kermadec Trench, which decreased  
338 with depth while Mn and Zn showed an overall increase in concentrations with depth. Instead, there  
339 were differences in metal concentrations at certain depths, which suggested that metal  
340 concentrations are related to site specific bioavailability in the environment. Hadal depth trenches  
341 are dynamic environments with high water column sedimentation rates and the potential for large-  
342 scale sediment movement based on gravitational forces or seismic activity (Itou *et al.*, 2000; Mather  
343 *et al.*, 2014; Nozaki and Ohta, 1993; Oguri *et al.*, 2013; Stewart and Jamieson, 2018). The complex  
344 topography within trenches includes trough, plains and ridges associated with the flanks of the  
345 trench (Stewart and Jamieson, 2018). These features may collect sediments, which could potentially  
346 influence local bioavailability of metals either through changes in the redox conditions of sediments,  
347 binding of metals with organic matter substrates or through resuspension of particles in the water  
348 column (Cantwell *et al.*, 2002; Eggleton and Thomas, 2004; Hyun *et al.*, 2017; Rainbow *et al.*, 1998;  
349 Saulnier and Mucci, 2000).

350 Sediment disturbance has the potential to influence the bioavailability of metals in the environment  
351 (Eggleton and Thomas, 2004; Koschinsky *et al.*, 2003; Rainbow *et al.*, 1998; Roberts, 2012).  
352 Koschinsky *et al.* (2003) provide the only data on metal concentrations in deep-sea amphipods.  
353 Benthopelagic amphipods collected from a Mn nodule field within the Peru Basin were found to

354 have more than double the Fe and nearly quadrupled the Mn concentrations after being disturbed  
355 by experimental deep-sea mining practices, whereas Cu and Zn levels remained relatively similar  
356 (Koschinsky *et al.*, 2003). The mechanism by which the Fe and Mn increased by such large  
357 concentrations was not discussed (Koschinsky *et al.*, 2003); though it is likely to be related to a  
358 combination of active membrane facilitated transport, particle associated ingestion via a food source  
359 or passive diffusion across the animal surface (Eggleton and Thomas, 2004). Given the highly  
360 dynamic nature of sediment movement in trenches, it is likely that amphipod metal concentrations  
361 will potentially vary to some extent on sediment deposition patterns and level of organic content  
362 within the sediment.

363 There was some evidence of co-varying patterns in metal concentrations. Fe and Mn in the tissues of  
364 amphipods positively co-varied within in all three species but only in two out of the three trenches.  
365 Iron and Mn did not co-vary in the *E. gryllus* from the Peru-Chile Trench but the lack of an  
366 association may be due to small sample size. The relationship between Fe and Mn suggests similar  
367 controls in metal accumulation regardless of depth. Dissolved Mn concentrations in the water  
368 column follow a scavenged-type vertical distribution which generally decreases with depth and then  
369 increases near the seafloor (Landing and Bruland, 1987). The redox sensitive nature of Mn often  
370 results in Mn-maxima occurring in association with oxygen minimum zones (Klinkhammer and  
371 Bender, 1980; Landing and Bruland, 1987). Although Mn concentrations appear to be elevated at the  
372 oxyclines at the oxygen minimum zone boundaries in the Peru-Chile margin (Vedamati *et al.*, 2015).  
373 Hydrothermally active regions also supply Mn and Fe to deep-water (Bennett *et al.*, 2008; de Ronde  
374 *et al.*, 2001; Usui and Lasby, 1998), which can influence seawater chemistry over tens to thousands  
375 of kilometres (Klinkhammer and Bender, 1980; Resing *et al.*, 2015). It is unlikely that the site-specific  
376 amphipod concentrations are related to depth specific hydrothermal activity from a point source but  
377 more likely related to a combination of long-term hydrothermalism, the downslope transport of  
378 metalliferous sediments, Fe-Mn crusts or regional sediment diagenetic processes influencing metal  
379 fluxes at the sediment-water interface.

380 Cadmium, Cu and Zn exhibited interesting behaviours in the amphipods in relation to depth and co-  
381 varied with other elements. Dissolved Cd and Zn both have nutrient type profiles in the water  
382 column, which result in depleted concentrations in surface water relative to deeper water where  
383 these metals are oxidised and remineralised (Bruland and Lohan, 2003). Below approximately 3000  
384 m in the Pacific Ocean, dissolved Zn concentrations in the water column remain relative stable down  
385 to 10,000 m (Bruland, 1980; John *et al.*, in press-a; Nozaki *et al.*, 1998; Obata *et al.*, 2017). The Zn  
386 concentrations in *E. gryllus* in the Kermadec Trench remained relatively constant with depth as did

387 *H. dubia* apart from a sharp increase in concentrations at approximately 9000 m. In *H. dubia*, Zn also  
388 co-varied with Mn and Fe in the Kermadec Trench. It is not clear what caused such a large increase in  
389 Zn concentrations at this site or why Zn co-varied with Mn and Fe. In contrast, Cu has a hybrid profile  
390 which means that its distribution in the water column is strongly governed by recycling and  
391 scavenging processes (Bruland and Lohan, 2003). Dissolved Cu concentrations have been found to  
392 increase with depth from 2000-8000 m (Bruland, 1980; Nozaki *et al.*, 1998). The high variability in Cu  
393 concentrations among depths observed in the amphipods indicated that there is no clear increase in  
394 Cu with depth but that other factors were potentially governing its concentrations in these  
395 amphipods.

## 396 **5. Conclusions**

397 The concentrations of trace metals found within the tissues of lysianassid amphipods within the Izu-  
398 Bonin, Kermadec and Peru-Chile trenches appear to be consistent with data from coastal regions but  
399 concentrations were smaller than those found within the Peru Basin Mn nodule field. The high  
400 concentrations of Cd and low concentrations of Cu indicated that these metals may be more  
401 governed by physiological mechanisms rather than background metal levels. Cadmium and Cu  
402 concentrations in the amphipods may be indicative of the Cd-anomaly and Cu-deficiencies observed  
403 in other deep-sea and polar crustaceans (Keil *et al.*, 2008; Petri and Zauke, 1993; Prowe *et al.*, 2006).  
404 The elevated concentrations of Fe, Mn and Cu within the Peru Basin appear to be linked to the  
405 proximity to a Mn nodule field and disturbance of the sediment (Koschinsky *et al.*, 2003). This  
406 highlights that regional geochemical processes potentially drive the bioavailability of these metals in  
407 amphipods. This was further observed in the geographical differences in metal concentrations  
408 among the trenches which largely reflected the levels of dissolved metals in the water column. The  
409 highly variable relationship between metal concentration with depth also suggest that localised  
410 processes were influencing metal bioavailability, which may be related to the disturbance and  
411 redistribution of sediment within the trenches and potentially food availability. However, a more  
412 integrated sampling strategy that involves collection of sediment and water along with amphipods  
413 may shed better light on the relationship between depth and metal concentrations in amphipods.  
414 The results of this study indicate that deep-sea amphipods are potentially indicator species for  
415 assessing broad-scale variability in bioavailability of Fe, Mn and potentially Zn in the deep-sea  
416 environment but more experimental work is required to understand Cd and Cu concentrations.

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## 425 **References**

- 426 Bargagli, R., Nelli, L., Ancora, S., Focardi, S., 1996. Elevated cadmium accumulation in marine  
427 organisms from Terra Nova Bay (Antarctica). *Polar Biology* 16 (7), 513-520.
- 428 Bennett, S.A., Achterberg, E.P., Connelly, D.P., Statham, P.J., Fones, G.R., German, C.R., 2008. The  
429 distribution and stabilisation of dissolved Fe in deep-sea hydrothermal plumes. *Earth and Planetary  
430 Science Letters* 270 (3-4), 157-167.
- 431 Boning, P., Brumsack, H.J., Bottcher, M.E., Schnetger, B., Kriete, C., Kallmeyer, J., Borchers, S.L.,  
432 2004. Geochemistry of Peruvian near-surface sediments. *Geochimica et Cosmochimica Acta* 68 (21),  
433 4429-4451.
- 434 Brandl, P.A., Hamada, M., Arculus, R.J., Johnson, K., Marsaglia, K.M., Savov, I.P., Ishizuka, O., Li, H.,  
435 2017. The arc arises: The links between volcanic output, arc evolution and melt composition. *Earth  
436 and Planetary Science Letters* 461, 73-84.
- 437 Bruland, K.W., 1980. Oceanographic distributions of cadmium, zinc, nickel, and copper in the North  
438 Pacific. *Earth and Planetary Science Letters* 47 (2), 176-198.
- 439 Bruland, K.W., Lohan, M.C., 2003. 6.02 - Controls of Trace Metals in Seawater A2 - Holland, Heinrich  
440 D. In: Turekian, K.K. (Ed.), *Treatise on Geochemistry*. Pergamon, Oxford, pp. 23-47.
- 441 Cantwell, M.G., Burgess, R.M., Kester, D.R., 2002. Release and phase partitioning of metals from  
442 anoxic estuarine sediments during periods of simulated resuspension. *Environmental science &  
443 technology* 36 (24), 5328-5334.
- 444 Charmasson, S.S., Calmet, D.P., 1987. Distribution of scavenging Lysianassidae amphipods  
445 *Eurythenes gryllus* in the Northeast Atlantic- comparisons with studies held in the Pacifici. *Deep-Sea  
446 Research Part a-Oceanographic Research Papers* 34 (9), 1509-1523.
- 447 Chu, N.C., Johnson, C.M., Beard, B.L., German, C.R., Nesbitt, R.W., Frank, M., Bohn, M., Kubik, P.W.,  
448 Usui, A., Graham, I., 2006. Evidence for hydrothermal venting in Fe isotope compositions of the deep  
449 Pacific Ocean through time. *Earth and Planetary Science Letters* 245 (1), 202-217.

450 Cipro, C.V.Z., Cherel, Y., Bocher, P., Caurant, F., Miramand, P., Bustamante, P., 2018. Trace elements  
451 in invertebrates and fish from Kerguelen waters, southern Indian Ocean. *Polar Biology* 41 (1), 175-  
452 191.

453 de Ronde, C.E.J., Baker, E.T., Massoth, G.J., Lupton, J.E., Wright, I.C., Feely, R.A., Greene, R.R., 2001.  
454 Intra-oceanic subduction-related hydrothermal venting, Kermadec volcanic arc, New Zealand. *Earth  
455 and Planetary Science Letters* 193 (3), 359-369.

456 de Ronde, C.E.J., Baker, E.T., Massoth, G.J., Lupton, J.E., Wright, I.C., Sparks, R.J., Bannister, S.C.,  
457 Reyners, M.E., Walker, S.L., Greene, R.R., Ishibashi, J., Faure, K., Resing, J.A., Lebon, G.T., 2007.  
458 Submarine hydrothermal activity along the mid-Kermadec Arc, New Zealand: Large-scale effects on  
459 venting. *Geochemistry, Geophysics, Geosystems* 8, Q07007.

460 eGEOTRACES Electronic Atlas, 2018.  
461 <http://www.egeotraces.org/?group=Dissolved%20Trace%20Elements,variable=SELECT%20A%20TRA>  
462 CER.

463 Eggleton, J., Thomas, K.V., 2004. A review of factors affecting the release and bioavailability of  
464 contaminants during sediment disturbance events. *Environment International* 30 (7), 973-980.

465 Ellwood, M.J., Bowie, A.R., Baker, A., Gault-Ringold, M., Hassler, C., Law, C.S., Maher, W., Marriner,  
466 A., Nodder, S., Sander, S., Stevens, C., Townsend, A., van der Merwe, P., Woodward, E.M.S., Wuttig,  
467 K., Boyd, P.W., 2018. Insights into the biogeochemical cycling of iron, nitrate and phosphate across a  
468 5300 km South Pacific zonal section (153°E-150°W). *Global Biogeochemical Cycles* 32 (2), 187-207.

469 Eustace, R.M., Ritchie, H., Kilgallen, N.M., Piertney, S.B., Jamieson, A.J., 2016. Morphological and  
470 ontogenetic stratification of abyssal and hadal *Eurythenes gryllus sensu lato* (Amphipoda:  
471 Lysianassoidea) from the Peru-Chile Trench. *Deep-Sea Research Part I-Oceanographic Research  
472 Papers* 109, 91-98.

473 Fairey, R., Long, E.R., Roberts, C.A., Anderson, B.S., Phillips, B.M., Hunt, J.W., Puckett, H.R., Wilson,  
474 C.J., 2001. An evaluation of methods for calculating mean sediment quality guideline quotients as  
475 indicators of contamination and acute toxicity to amphipods by chemical mixtures. *Environmental  
476 Toxicology and Chemistry* 20 (10), 2276-2286.

477 Fallon, E.K., Petersen, S., Brooker, R.A., Scott, T.B., 2017. Oxidative dissolution of hydrothermal  
478 mixed-sulphide ore: An assessment of current knowledge in relation to seafloor massive sulphide  
479 mining. *Ore Geology Reviews* 86 (Supplement C), 309-337.

480 Fialkowski, W., Calosi, P., Dahlke, S., Dietrich, A., Moore, P.G., Olenin, S., Persson, L.E., Smith, B.D.,  
481 Spegys, M., Rainbow, P.S., 2009. The sandhopper *Talitrus saltator* (Crustacea: Amphipoda) as a  
482 biomonitor of trace metal bioavailabilities in European coastal waters. *Marine Pollution Bulletin* 58  
483 (1), 39-44.

484 Gamo, T., Ishibashi, J.-I., Shitashima, K., Kinoshita, M., Watanabe, M., Nakayama, E., Sohrin, Y., Kim,  
485 E.-S., Masuzawa, T., Fuji, K., 1988. Anomalies of bottom CH<sub>4</sub> and trace metal concentrations  
486 associated with high heat flow at the *Calyptogena* community off Hatsu-shima Island, Sagami Bay,  
487 Japan: a preliminary report of Tansei Maru KT-88-1 cruise Leg-1. *Geochemical Journal* 22, 215-230.

488 German, C.R., Thurnherr, A.M., Knoery, J., Charlou, J.L., Jean-Baptiste, P., Edmonds, H.N., 2010. Heat,  
489 volume and chemical fluxes from submarine venting: A synthesis of results from the Rainbow  
490 hydrothermal field, 36 degrees N MAR. *Deep-Sea Research Part I-Oceanographic Research Papers* 57  
491 (4), 518-527.

492 Glover, A.G., Smith, C.R., 2003. The deep-sea floor ecosystem: current status and prospects of  
493 anthropogenic change by the year 2025. *Environmental Conservation* 30 (3), 219-241.

494 Grand, M.M., Measures, C.I., Hatta, M., Morton, P.L., Barrett, P., Milne, A., Resing, J.A., Landing,  
495 W.M., 2015. The impact of circulation and dust deposition in controlling the distributions of  
496 dissolved Fe and Al in the south Indian subtropical gyre. *Marine Chemistry* 176, 110-125.

497 Hauton, C., Brown, A., Thatje, S., Mestre, N.C., Bebianno, M.J., Martins, I., Bettencourt, R., Canals,  
498 M., Sanchez-Vidal, A., Shillito, B., Ravaux, J., Zbinden, M., Duperron, S., Mevenkamp, L., Vanreusel,  
499 A., Gambi, C., Dell'Anno, A., Danovaro, R., Gunn, V., Weaver, P., 2017. Identifying toxic impacts of  
500 metals potentially released during deep-sea mining - a synthesis of the challenges to quantifying  
501 risk. *Frontiers in Marine Science* 4 (368).

502 Honda, S., Yoshida, T., Aoike, K., 2007. Spatial and temporal evolution of arc volcanism in the  
503 northeast Honshu and Izu-Bonin Arcs: Evidence of small-scale convection under the island arc?  
504 *Island Arc* 16 (2), 214-223.

505 Hyun, J.H., Kim, S.H., Mok, J.S., Cho, H., Lee, T., Vandieken, V., Thamdrup, B., 2017. Manganese and  
506 iron reduction dominate organic carbon oxidation in surface sediments of the deep Ulleung Basin,  
507 East Sea. *Biogeosciences* 14 (4), 941-958.

508 Itou, M., Matsumura, I., Noriki, S., 2000. A large flux of particulate matter in the deep Japan Trench  
509 observed just after the 1994 Sanriku-Oki earthquake. *Deep-Sea Research Part I-Oceanographic*  
510 *Research Papers* 47 (10), 1987-1998.

511 Jamieson, A.J., 2015. *The Hadal Zone: live in the deepest oceans*. Cambridge University Press,  
512 Cambridge.

513 John, S.G., Helgoe, J., Townsend, E., in press-a. Biogeochemical cycling of Zn and Cd and their stable  
514 isotopes in the Eastern Tropical South Pacific. *Marine Chemistry*.

515 John, S.G., Helgoe, J., Townsend, E., Weber, T., DeVries, T., Tagliabue, A., Moore, K., Lam, P., Marsay,  
516 C.M., Till, C., in press-b. Biogeochemical cycling of Fe and Fe stable isotopes in the Eastern Tropical  
517 South Pacific. *Marine Chemistry*.



518 Keil, S., De Broyer, C., Zauke, G.-P., 2008. Significance and interspecific variability of accumulated  
519 trace metal concentrations in Antarctic benthic crustaceans. *International Review of Hydrobiology*  
520 93 (1), 106-126.

521 Kim, T., Obata, H., Kondo, Y., Ogawa, H., Gamo, T., 2015. Distribution and speciation of dissolved zinc  
522 in the western North Pacific and its adjacent seas. *Marine Chemistry* 173, 330-341.

523 Kim, T., Obata, H., Nishioka, J., Gamo, T., 2017. Distribution of Dissolved Zinc in the Western and  
524 Central Subarctic North Pacific. *Global Biogeochemical Cycles* 31 (9), 1454-1468.

525 Klinkhammer, G.P., Bender, M.L., 1980. The distribution of manganese in the Pacific Ocean. *Earth*  
526 *and Planetary Science Letters* 46 (3), 361-384.

527 Koschinsky, A., Borowski, C., Halbach, P., 2003. Reactions of the heavy metal cycle to industrial  
528 activities in the deep sea: an ecological assessment. *International Review of Hydrobiology* 88 (1),  
529 102-127.

530 Lacey, N.C., Rowden, A.A., Clark, M.R., Kilgallen, N.M., Linley, T., Mayor, D.J., Jamieson, A.J., 2016.  
531 Community structure and diversity of scavenging amphipods from bathyal to hadal depths in three  
532 South Pacific Trenches. *Deep-Sea Research Part I-Oceanographic Research Papers* 111, 121-137.

533 Laglera, L.M., Tovar-Sánchez, A., Iversen, M.H., González, H.E., Naik, H., Mangesh, G., Assmy, P.,  
534 Klaas, C., Mazzocchi, M.G., Montresor, M., Naqvi, S.W.A., Smetacek, V., Wolf-Gladrow, D.A., 2017.  
535 Iron partitioning during LOHAFEX: Copepod grazing as a major driver for iron recycling in the  
536 Southern Ocean. *Marine Chemistry* 196, 148-161.

537 Landing, W.M., Bruland, K.W., 1987. The contrasting biogeochemistry of iron and manganese in the  
538 Pacific Ocean. *Geochimica et Cosmochimica Acta* 51 (1), 29-43.

539 Long, E.R., Macdonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects  
540 within ranges of chemical concentrations in marine and estuarine sediments. *Environmental*  
541 *Management* 19 (1), 81-97.

542 Marsay, C.M., Lam, P.J., Heller, M.I., Lee, J.-M., John, S.G., in press. Distribution and isotopic  
543 signature of ligand-leachable particulate iron along the GEOTRACES GP16 East Pacific Zonal Transect.  
544 *Marine Chemistry*.

545 Marsden, I.D., Rainbow, P.S., 2004. Does the accumulation of trace metals in crustaceans affect their  
546 ecology - the amphipod example? *Journal of Experimental Marine Biology and Ecology* 300 (1-2),  
547 373-408.

548 Marsden, I.D., Rainbow, P.S., Smith, B.D., 2003. Trace metal concentrations in two New Zealand  
549 talitrid amphipods: effects of gender and reproductive state and implications for biomonitoring.  
550 *Journal of Experimental Marine Biology and Ecology* 290 (1), 93-113.

551 Mather, A.E., Hartley, A.J., Griffiths, J.S., 2014. The giant coastal landslides of Northern Chile:  
552 Tectonic and climate interactions on a classic convergent plate margin. *Earth and Planetary Science*  
553 *Letters* 388, 249-256.

554 Matsunaga, K., Nishioka, J., Kuma, K., Toya, K., Suzuki, Y., 1998. Riverine input of bioavailable iron  
555 supporting phytoplankton growth in Kesenuma Bay (Japan). *Water Research* 32 (11), 3436-3442.

556 McAllen, R., Taylor, A., Freel, J., 2005. Seasonal variation in the ionic and protein content of  
557 haemolymph from seven deep-sea decapod genera from the Northeast Atlantic Ocean. *Deep Sea*  
558 *Research Part I: Oceanographic Research Papers* 52 (11), 2017-2028.

559 Mearns, A.J., Swartz, R.C., Cummins, J.M., Dinnel, P.A., Plesha, P., Chapman, P.M., 1986. Inter-  
560 laboratory comparison of a sediment toxicity test using the marine amphipod, *Rhepoxynius abronius*.  
561 *Marine Environmental Research* 19 (1), 13-37.

562 Moore, P.G., Rainbow, P.S., Hayes, E., 1991. The beach-hopper *Orchestira gammarellus* (Crustacea,  
563 Ampipoda) as a biomonitor for copper and zinc - North Sea trials. *Science of the Total Environment*  
564 106 (3), 221-238.

565 Nishioka, J., Obata, H., 2017. Dissolved iron distribution in the western and central subarctic Pacific:  
566 HNLC water formation and biogeochemical processes. *Limnology and Oceanography* 62 (5), 2004-  
567 2022.

568 Nozaki, Y., Ohta, Y., 1993. Rapid and frequent turbidite accumulation in the bottom of the Izu-  
569 Ogasawara Trench- chemical and radiochemical evidence. *Earth and Planetary Science Letters* 120  
570 (3-4), 345-360.

571 Nozaki, Y., Yamada, M., Nakanishi, T., Nagaya, Y., Nakamura, K., Shitashima, K., Tsubota, H., 1998.  
572 The distribution of radionuclides and some trace metals in the water columns of the Japan and Bonin  
573 trenches. *Oceanologica Acta* 21 (3), 469-484.

574 Obata, H., Nishioka, J., Kim, T., Norisuye, K., Takeda, S., Wakuta, Y., Gamo, T., 2017. Dissolved iron  
575 and zinc in Sagami Bay and the Izu-Ogasawara Trench. *Journal of Oceanography* 73 (3), 333-344.

576 Oguri, K., Kawamura, K., Sakaguchi, A., Toyofuku, T., Kasaya, T., Murayama, M., Fujikura, K., Glud,  
577 R.N., Kitazato, H., 2013. Hadal disturbance in the Japan Trench induced by the 2011 Tohoku-Oki  
578 Earthquake. *Scientific Reports* 3, 6.

579 Petri, G., Zauke, G.P., 1993. Trace metals in crustaceans in the Antarctic Ocean. *Ambio* 22 (8), 529-  
580 536.

581 Prowe, F., Kirf, M., Zauke, G.P., 2006. Heavy metals in crustaceans from the Iberian deep sea plain.  
582 *Scientia Marina* 70 (2), 271-279.

583 Rainbow, P.S., 1985. The biology of heavy metals in the sea. *International Journal of Environmental*  
584 *Studies* 25 (3), 195-211.

585 Rainbow, P.S., 1993a. THE SIGNIFICANCE OF TRACE-METAL CONCENTRATIONS IN MARINE-  
586 INVERTEBRATES. Lewis Publishers Inc, Boca Raton.

587 Rainbow, P.S., 1993b. The significance of trace metal concentrations in marine invertebrates. Lewis  
588 Publishers Inc, Boca Raton.

589 Rainbow, P.S., 1995. Biomonitoring of heavy metal availability in the marine environment. Marine  
590 Pollution Bulletin 31 (4-12), 183-192.

591 Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what?  
592 Environmental Pollution 120 (3), 497-507.

593 Rainbow, P.S., Amiard-Triquet, C., Amiard, J.C., Smith, B.D., Langston, W.J., 2000. Observations on  
594 the interaction of zinc and cadmium uptake rates in crustaceans (amphipods and crabs) from coastal  
595 sites in UK and France differentially enriched with trace metals. Aquatic Toxicology 50 (3), 189-204.

596 Rainbow, P.S., Emson, R.H., Smith, B.D., Moore, P.G., Mladenov, P.V., 1993. Talitrid amphipods as  
597 biomonitors of trace metals near Dunedin, New Zealand. New Zealand Journal of Marine and  
598 Freshwater Research 27 (2), 201-207.

599 Rainbow, P.S., Fialkowski, W., Smith, B.D., 1998. The sandhopper *Talitrus saltator* as a trace metal  
600 biomonitor in the Gulf of Gdansk, Poland. Marine Pollution Bulletin 36 (3), 193-200.

601 Rainbow, P.S., Moore, P.G., Watson, D., 1989. Talitrid amphipods (Crustacea) as biomonitors for  
602 copper and zinc. Estuarine Coastal and Shelf Science 28 (6), 567-582.

603 Ratnarajah, L., Bowie, A.R., Lannuzel, D., Meiners, K.M., Nicol, S., 2014. The biogeochemical role of  
604 baleen whales and krill in Southern Ocean nutrient cycling. Plos One 9 (12), 18.

605 Resing, J.A., Sedwick, P.N., German, C.R., Jenkins, W.J., Moffett, J.W., Sohst, B.M., Tagliabue, A.,  
606 2015. Basin-scale transport of hydrothermal dissolved metals across the South Pacific Ocean. Nature  
607 523, 200.

608 Ridout, P.S., Willcocks, A.D., Morris, R.J., White, S.L., Rainbow, P.S., 1985. Concentrations of Mn, Fe,  
609 Cu, Zn and Cd in the mesopelagic decapod *Systemaspis debilis* from the east Atlantic Ocean. Marine  
610 Biology 87 (3), 285-288.

611 Ritchie, H., Janamieson, A.J., Piertney, S.B., 2015. Phylogenetic relationships among hadal  
612 amphipods of the Superfamily Lysianassoidea: Implications for taxonomy and biogeography. Deep-  
613 Sea Research Part I-Oceanographic Research Papers 105, 119-131.

614 Ritterhoff, J., Zauke, G.-P., 1997. Trace metals in field samples of zooplankton from the Fram Strait  
615 and the Greenland Sea. Science of the Total Environment 199 (3), 255-270.

616 Roberts, D.A., 2012. Causes and ecological effects of resuspended contaminated sediments (RCS) in  
617 marine environments. Environment International 40, 230-243.

618 Saitoh, Y., Kuma, K., Isoda, Y., Kuroda, H., Matsuura, H., Wagawa, T., Takata, H., Kobayashi, N.,  
619 Nagao, S., Nakatsuka, T., 2008. Processes influencing iron distribution in the coastal waters of the  
620 Tsugaru Strait, Japan. *Journal of Oceanography* 64 (6), 815-830.

621 Saulnier, I., Mucci, A., 2000. Trace metal remobilization following the resuspension of estuarine  
622 sediments: Saguenay Fjord, Canada. *Applied Geochemistry* 15 (2), 191-210.

623 Stewart, H.A., Jamieson, A.J., 2018. Habitat heterogeneity of hadal trenches: Considerations and  
624 implications for future studies. *Progress in Oceanography* 161, 47-65.

625 Takano, S., Tanimizu, M., Hirata, T., Sohrin, Y., 2014. Isotopic constraints on biogeochemical cycling  
626 of copper in the ocean. *Nature Communications* 5, 5663.

627 Ugolini, A., Borghini, F., Calosi, P., Bazzicalupo, M., Chelazzi, G., Focardi, S., 2004. Mediterranean  
628 *Talitrus saltator* (Crustacea, Amphipoda) as a biomonitor of heavy metals contamination. *Marine*  
629 *Pollution Bulletin* 48 (5-6), 526-532.

630 Usui, A., Nishimura, A., 1992. Submersible observations of hydrothermal manganese deposits on the  
631 Kaikata Seamount, Izu-Ogasawara (Bonin) Arc. *Marine Geology* 106 (3), 203-216.

632 Usui, A.U., Lasby, G.P.G., 1998. Submarine hydrothermal manganese deposits in the Izu–Bonin–  
633 Mariana arc: An overview. *Island Arc* 7 (3), 422-431.

634 Vedamati, J., Chan, C., Moffett, J.W., 2015. Distribution of dissolved manganese in the Peruvian  
635 Upwelling and Oxygen Minimum Zone. *Geochimica et Cosmochimica Acta* 156, 222-240.

636 White, S.L., Rainbow, P.S., 1987. Heavy metal concentrations and size effects in the mesopelagic  
637 decapod crustacean *Systellaspis debilis*. *Marine Ecology Progress Series* 37 (2-3), 147-151.

638 Yayanos, A.A., 2009. Recovery of Live Amphipods at Over 102 MPa from the Challenger Deep.  
639 *Marine Technology Society Journal* 43 (5), 132-136.

640 Yucel, M., Gartman, A., Chan, C.S., Luther, G.W., 2011. Hydrothermal vents as a kinetically stable  
641 source of iron-sulphide-bearing nanoparticles to the ocean. *Nature Geoscience* 4 (6), 367-371.

642 Zauke, G.P., Krause, M., Weber, A., 1996. Trace metals in mesozooplankton of the North Sea:  
643 Concentrations in different taxa and preliminary results on bioaccumulation in copepod collectives  
644 (*Calanus finmarchicus C-helgolandicus*). *Internationale Revue Der Gesamten Hydrobiologie* 81 (1),  
645 141-160.

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652 Table 1. Comparisons of mean ( $\pm$  95% CL,  $n = 4$ ) measured and certified metal concentrations ( $\mu\text{g g}^{-1}$ )  
 653 in Certified Reference Material (TORT-2 Lobster Hepatopancreas, NRC, Canada).

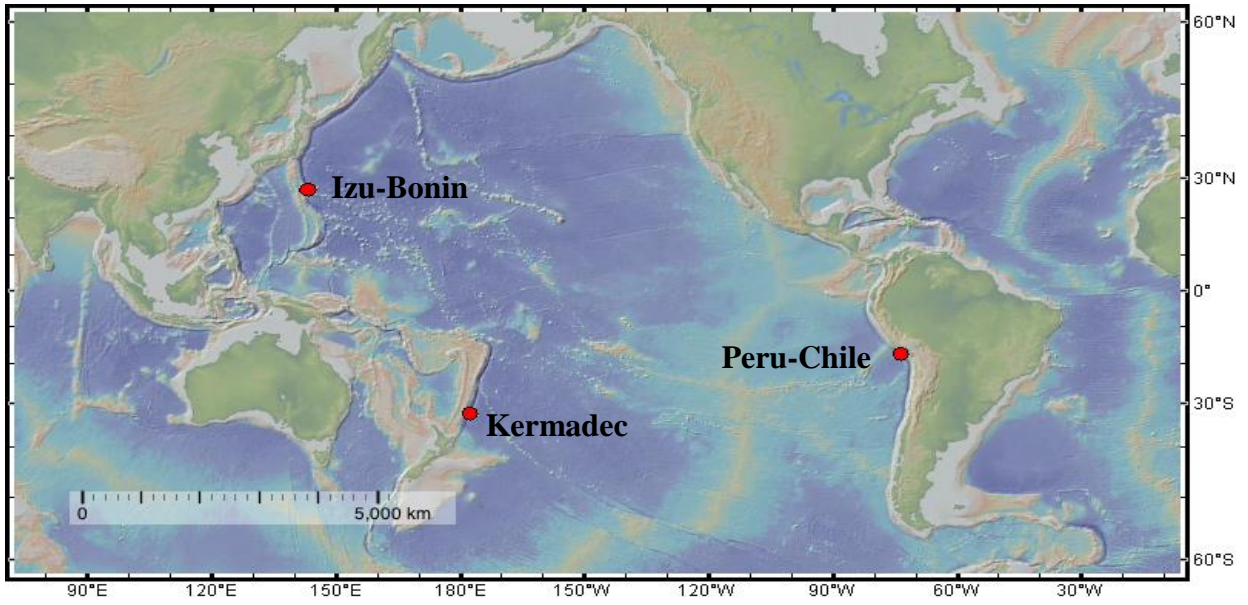
Metal	Measured Concentration	Certified Concentration	Percentage recovery
Manganese (Mn)	$11.6 \pm 0.6$	$13.6 \pm 1.2$	85.2%
Iron (Fe)	$88.15 \pm 3.3$	$105 \pm 13$	83.9%
Copper (Cu)	$88.02 \pm 3.3$	$106 \pm 10$	83.0%
Zinc (Zn)	$193.28 \pm 10.1$	$180 \pm 6$	107%
Cadmium (Cd)	$23.12 \pm 1.0$	$26.7 \pm 0.6$	86.6%

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677 Table 2. Mean cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) concentrations  
 678 ( $\mu\text{g g}^{-1}$  dry weight) in benthopelagic amphipods collected from the Izu-Bonin, Kermadec and Peru-  
 679 Chile trenches at depths ranging from 3268 m to 9908 m. Standard deviations are in parentheses.

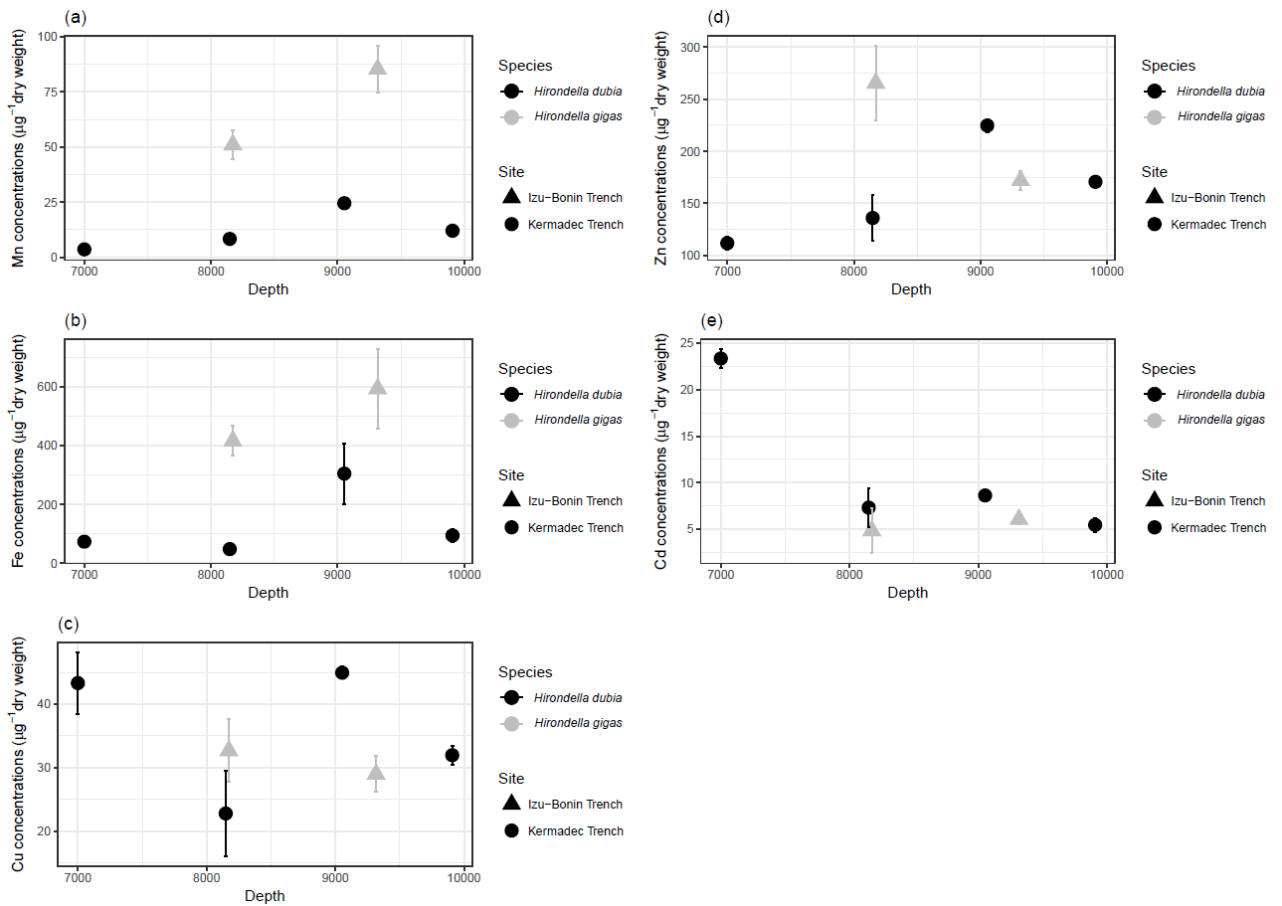
Species	Location	Depth	Number	Cd	Cu	Fe	Mn	Zn
<i>Hirondellea gigas</i>	Izu-Bonin Trench	8172	4	4.84 (2.44)	32.67 (4.97)	415.50 (50.6)	51.04 (6.39)	265.48 (35.66)
		9316	4	6.06 (0.51)	28.98 (2.84)	592.81 (132.29)	85.34 (10.54)	172 (9.15)
<i>Hirondellea dubia</i>	Kermadec Trench	6999	4	23.35 (1.01)	43.31 (4.85)	73.41 (14.16)	3.58 (0.33)	111.81 (6.63)
		8148	4	7.32 (2.1)	22.77 (6.78)	48.17 (14.25)	8.32 (1.92)	136.02 (21.92)
		9053	4	8.63 (0.08)	44.94 (0.94)	304.25 (103)	24.51 (1.65)	224.71 (6.27)
		9908	4	5.45 (0.76)	31.95 (1.54)	94.44 (22.3)	12.03 (1.05)	170.69 (3.63)
<i>Eurythenes gryllus</i>	Kermadec Trench	3268	1	1.44	17.90	105.62	2.99	187.33
		4519	3	3.52 (0.24)	21.5 (3.42)	243.84 (23.97)	14.84 (2.92)	198.2 (37.68)
		5242	4	3.01 (0.61)	10.34 (0.74)	91.75 (19.09)	2.35 (0.36)	192.86 (14.19)
<i>Eurythenes gryllus</i>	Peru-Chile Trench	4602	2	15.56 (15.55)	24.73 (10.29)	167.3 (19.61)	7.18 (0.65)	188.43 (3.59)
		5329	2	6.19 (3.13)	15.21 (1.63)	146.92 (57.43)	5.69 (0.14)	230.2 (17.8)
		6173	4	18.25 (3.84)	22.23 (4.13)	230.11 (156.75)	7.32 (2.38)	120.27 (17.13)

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Figure 1. Locations of the sampling sites within the Izu-Bonin Trench, Kermadec Trench and Peru-Chile Trench situated within the Pacific Ocean.



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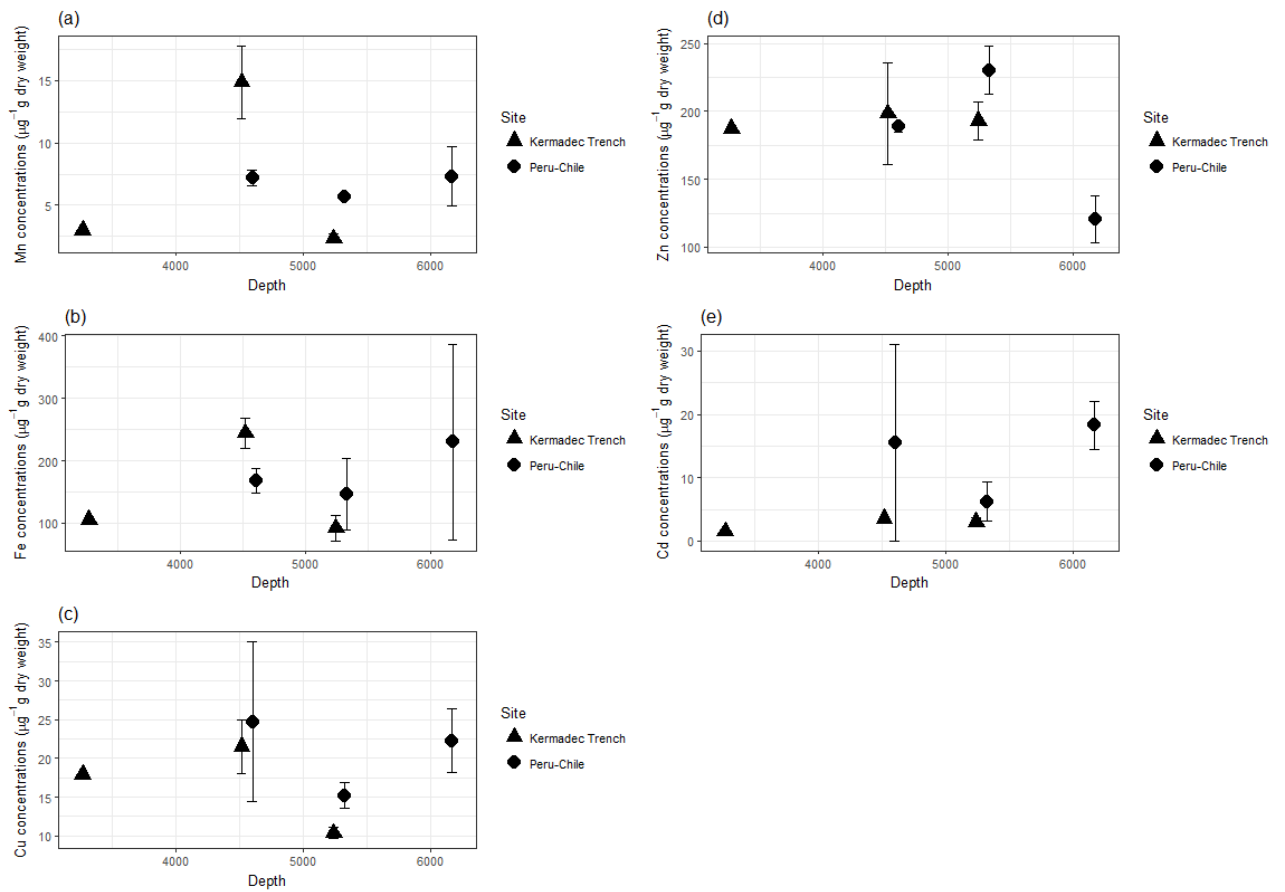
692 Figure 2. Mean concentrations  $\pm$  SD of Mn (a), Fe (b), Cu (e), Zn (d) and Cd (e) of the hadal  
 693 amphipods *Hirondelella gigas* from the Izu-Bonin Trench and *H. dubia* from the Kermadec Trench.

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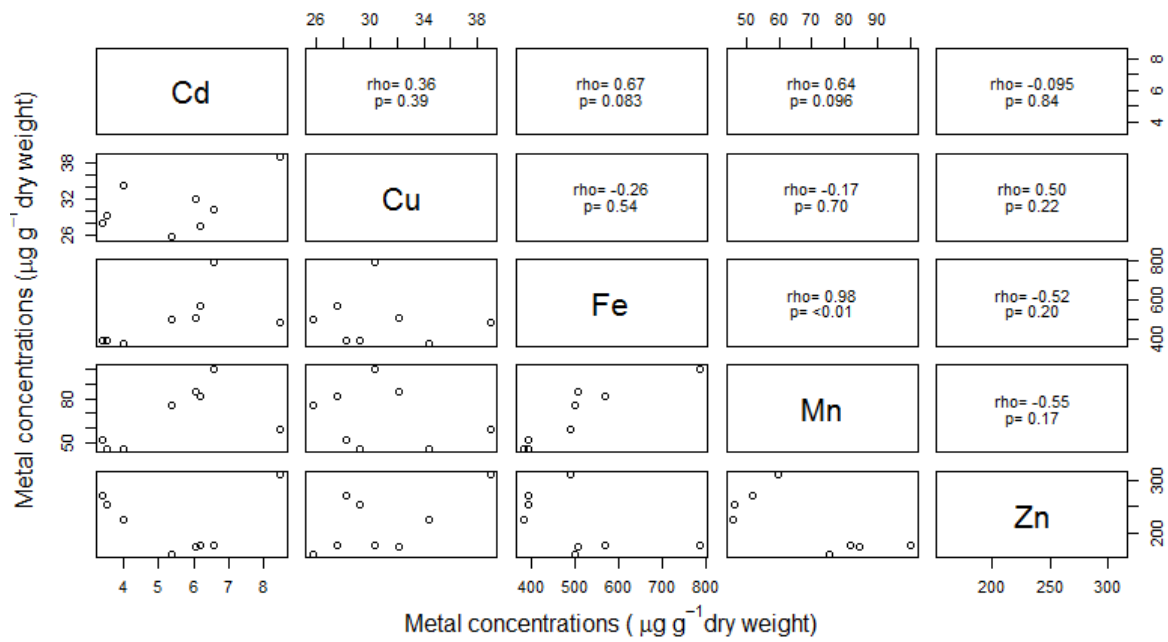




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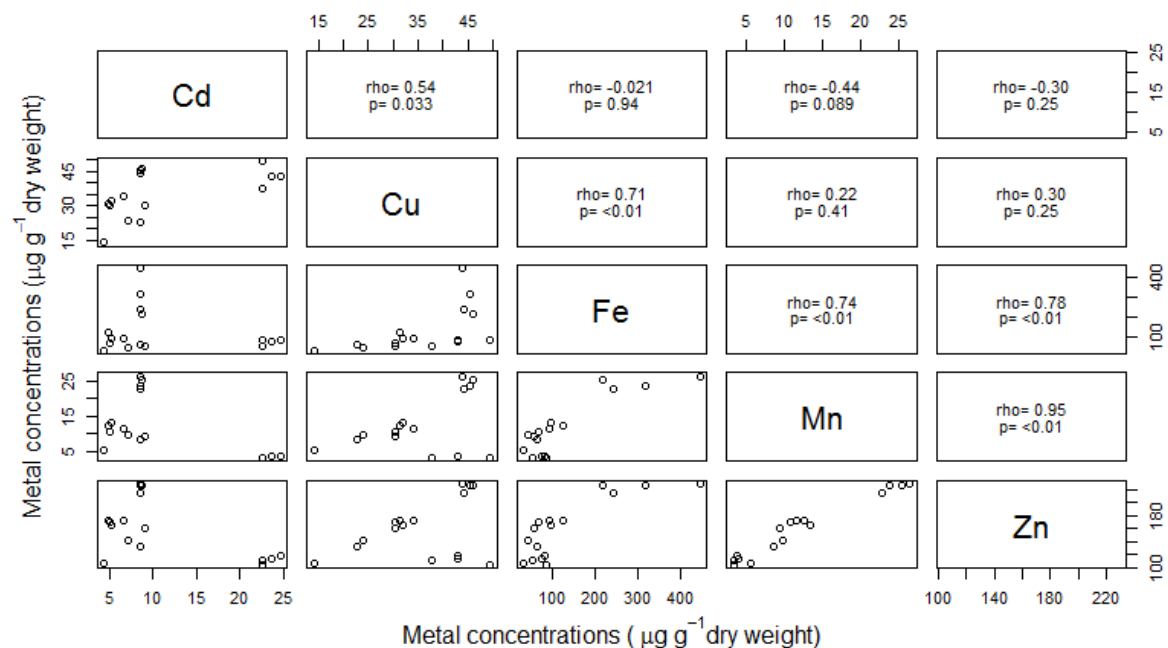
699 Figure 3. Mean concentrations  $\pm$  SD of Mn (a), Fe (b), Cu (e), Zn (d) and Cd (e) of the abyssal  
700 amphipod *Eurythenes gryllus* from the Kermadec and Peru-Chile trenches.

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718 Figure 4: The relationships between pairs of metals within *Hirondelea gigas* from the Izu-Bonin  
 719 Trench. The results of the Spearman rank correlation are in above the diagonal line containing the  
 720 abbreviation of the metals (cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn)).

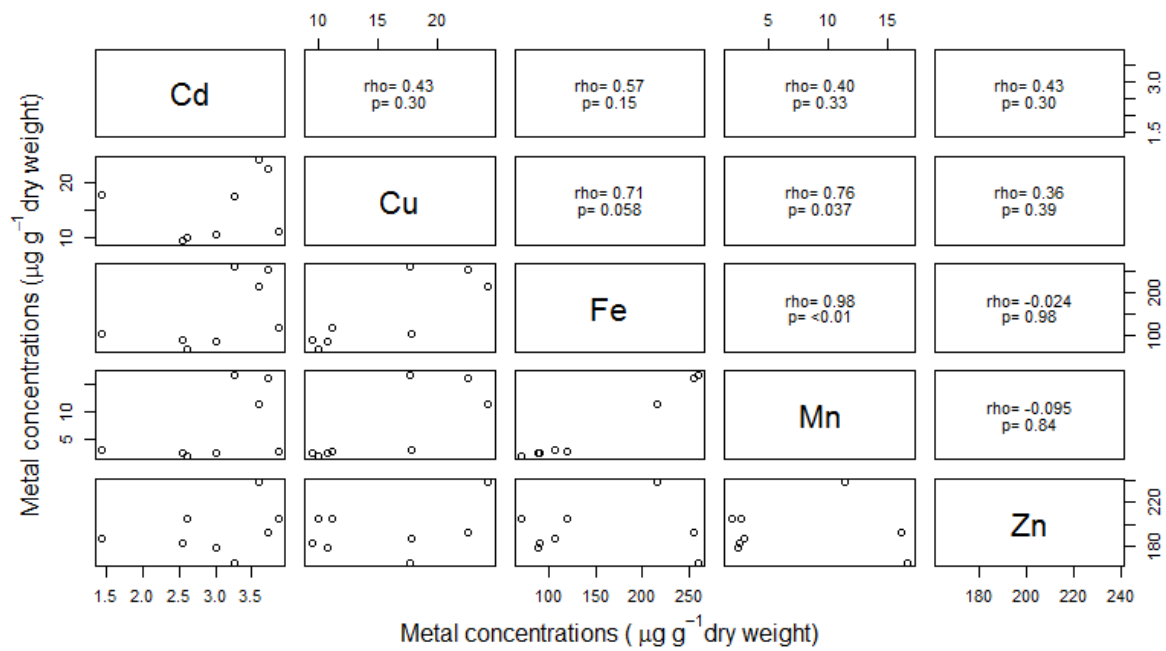


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722 Figure 5: The relationships between pairs of metals within *Hirondelea dubia* from the Kermadec  
 723 Trench. The results of the Spearman rank correlation are in above the diagonal line containing the  
 724 abbreviation of the metals (cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn)).

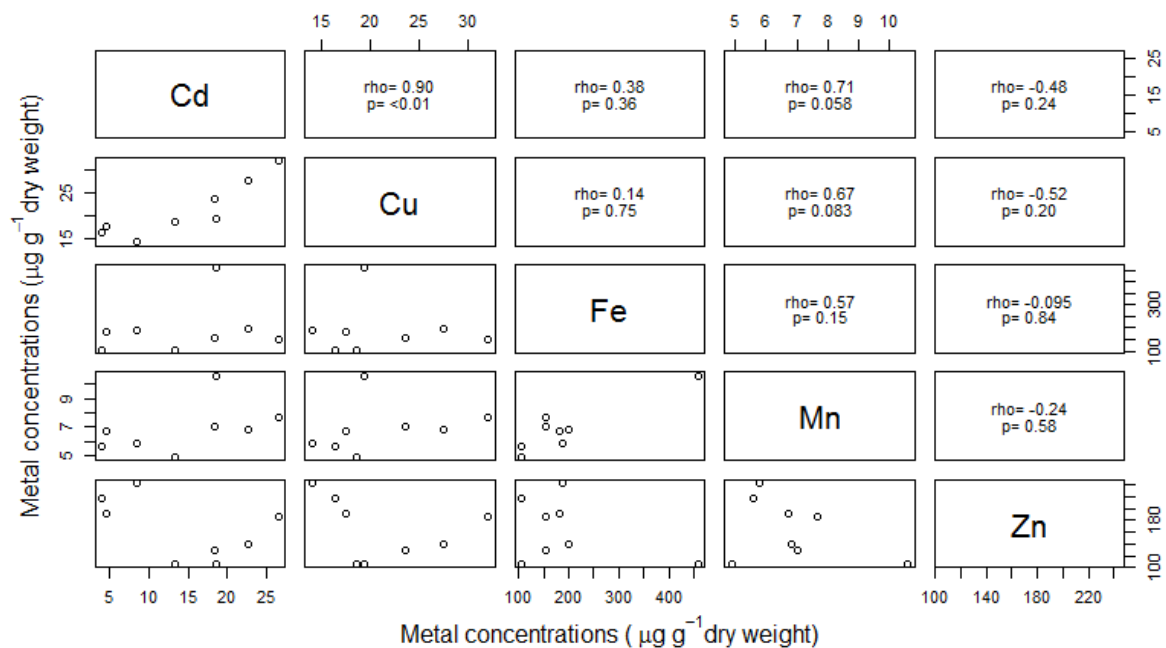
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728 Figure 6: The relationships between pairs of metals within *Eurythenes gryllus* from the Kermadec  
 729 Trench. The results of the Spearman rank correlation are in above the diagonal line containing the  
 730 abbreviation of the metals (cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn)).



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732 Figure 7: The relationships between pairs of metals within *Eurythenes gryllus* from the Peru-Chile  
 733 Trench. The results of the Spearman rank correlation are in above the diagonal line containing the  
 734 abbreviation of the metals (cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn)).