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# **Biodynamic modelling of the accumulation of Ag, Cd and Zn by the deposit-feeding polychaete: inter-population variability and a generalised predictive model**

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## Accepted Manuscript

Biodynamic modelling of the accumulation of Ag, Cd and Zn by the deposit-feeding polychaete *Nereis diversicolor*: inter-population variability and a generalised predictive model

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1 **Biodynamic modelling of the accumulation of Ag, Cd and Zn by the deposit-feeding**  
2 **polychaete *Nereis diversicolor*: inter-population variability and a generalised predictive**  
3 **model**

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42 19 **Abstract**

43  
44  
45  
46  
47 21 Biodynamic parameters of the ragworm *Nereis diversicolor* from southern Spain and south  
48  
49  
50 22 England were experimentally derived to assess the inter-population variability of  
51  
52 23 physiological parameters of the bioaccumulation of Ag, Cd and Zn from water and sediment.  
53  
54  
55 24 Although there were some limited variations, these were not consistent with the local metal  
56  
57 25 bioavailability nor with temperature changes. Incorporating the biodynamic parameters into a  
58  
59 26 defined biodynamic model, confirmed that sediment is the predominant source of Cd and Zn  
60  
61  
62  
63  
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65

27 accumulated by the worms, accounting in each case for 99% of the overall accumulated  
28 metals, whereas the contribution of dissolved Ag to the total accumulated by the worm  
29 increased from about 27 to about 53 % with increasing dissolved Ag concentration.  
30 Standardized values of metal-specific parameters were chosen to generate a generalised model  
31 to be extended to *N. diversicolor* populations across a wide geographical range from western  
32 Europe to North Africa. According to the assumptions of this model, predicted steady state  
33 concentrations of Cd and Zn in *N. diversicolor* were overestimated, those of Ag  
34 underestimated, but still comparable to independent field measurements. We conclude that  
35 species-specific physiological metal bioaccumulation parameters are relatively constant over  
36 large geographical distances, and a single generalised biodynamic model does have potential  
37 to predict accumulated Ag, Cd and Zn concentrations in this polychaete from a single  
38 sediment metal concentration.

39  
40 **Keywords:** *Nereis diversicolor*, cadmium, zinc, silver, biodynamic modelling, sediment

## 41 42 **1. Introduction**

43  
44 Estuarine sediments act as sinks for contaminant trace metals of anthropogenic origin  
45 and these toxic metals may be taken up by deposit-feeding invertebrates ingesting the  
46 sediment (Luoma and Rainbow, 2008). These sediment metals not only have the capacity to  
47 cause direct ecotoxicological effects on this invertebrate but may also enter coastal food  
48 chains leading to vertebrates such as fish, birds and even man, with the potential to cause  
49 indirect ecotoxicological effects. An understanding of the uptake and accumulation of toxic  
50 metals by estuarine deposit-feeding invertebrates is therefore key to understanding the  
51 environmental risk associated with the metal contamination of estuarine sediments.

52           Biodynamic modelling is now being used to deconstruct the routes of uptake and  
53 subsequent accumulation of trace metals by marine animals, and accumulated concentrations  
54 predicted by biodynamic modelling generally match field concentrations very well (Luoma  
55 and Rainbow, 2005, 2008). The principal advantages of the biodynamic modelling approach  
56 are that (i) geochemical and biological processes affecting the metal bioaccumulation are  
57 considered simultaneously, (ii) it accounts separately for the metal uptake from distinct  
58 exposure routes, (iii) trace metal accumulation biodynamics has the potential to link total  
59 uptake rate with expected toxicity in a given species (Simpson and King, 2005; Wang and  
60 Rainbow, 2008). Use of biodynamic modelling includes geochemical analysis of  
61 environmental metal concentrations and measurement of key physiological parameters for a  
62 particular species from a site, and explains the great variability of accumulated metal  
63 concentrations found in organisms (Luoma and Rainbow, 2005). The biodynamic model  
64 describes the steady state concentration of metal in an organism as the sum of the net  
65 accumulation from solution and from the diet, the latter now appreciated as being a major  
66 source of metal accumulation in many aquatic invertebrates (Luoma and Rainbow, 2005;  
67 Wang, 2002).

68           Biodynamic modelling has recently been applied to two estuarine deposit-feeding  
69 polychaetes, defining the relative importance of dissolved and dietary uptake routes and  
70 successfully predicting field accumulated concentrations – the lugworm *Arenicola marina*  
71 (Casado-Martinez et al., 2009a,b) and the common ragworm *Nereis diversicolor* (Rainbow et  
72 al., 2009a,b). In both polychaetes ingested sediment supplies almost all accumulated cadmium  
73 and zinc, while for silver dissolved metal is a significant source for uptake (Casado-Martinez  
74 et al., 2009a,b; Rainbow et al., 2009a,b).

75           As the application of biodynamic modelling becomes more widespread, further  
76 questions become relevant. The first such question asks how variable are the physiological

77 parameters defining metal accumulation between different populations of a species. These  
78 parameters are the uptake rate constant from the dissolved phase, the assimilation efficiency  
79 from the ingested particulate phase, and the efflux rate constants following uptake from water  
80 and diet (all measured in the laboratory typically using tracers), as well as the ingestion rate,  
81 and the growth rate constant of the species (often taken from the literature). Incorporation of  
82 these physiological parameters into the model, together with the dissolved and dietary metal  
83 concentrations determined in the field, provides site-specific predictions of metal  
84 accumulation in the relevant species at that site. So Rainbow et al. (2009a) explored  
85 variability in the above physiological parameters in populations of *N. diversicolor* from south  
86 England, concluding that, although there was some variability, the variation was limited and  
87 correlation with changes in local metal bioavailabilities were absent. In this study we  
88 investigate whether such conclusions still hold if populations of the worm from southern  
89 Spain are included in the comparison, with particular attention to the effect of raised  
90 temperature. We have therefore measured the physiological bioaccumulation parameters for  
91 Ag, Cd and Zn in *N. diversicolor* from Río San Pedro, Puente Zuazo and Trocadero (a  
92 reduced set of parameters in the latter case owing to limited numbers of worms available)  
93 from the region of Cadiz, Spain at 18°C, and in a population from the Blackwater estuary in  
94 England (Table 1), again at 18°C for comparison against the equivalent data collected for this  
95 latter population at 10°C by Rainbow et al. (2009a).

96 The subsequent question addressed is whether it is possible to choose standardised  
97 values of the relevant parameters that can be applied in a generalised biodynamic model of  
98 metal accumulation by *N. diversicolor* and allow accurate prediction of bioaccumulated  
99 concentrations in the worm across a wide range of western European and Moroccan estuaries  
100 of different degrees of contamination. Such a generalised model would allow prediction on a  
101 mechanistic basis of accumulated metal concentrations in the worm from a single sediment

102 concentration, and greatly facilitate any modelling of metal uptake, accumulation and  
103 potentially ecotoxicity in a key estuarine species (Gillet et al., 2008; Mouneyrac et al., 2009).

104

## 105 **2. Materials and methods**

106

### 107 *2.1 Collection*

108

109 Ragworms, *Nereis diversicolor*, (minimum length 3 cm) were collected by hand from  
110 the intertidal zones of three sites located in the Río San Pedro and Caño Sancti-Petri  
111 saltmarsh environment (SW Spain) and from the Blackwater estuary (SE England) in  
112 September 2007 (Table 1). Populations of *N. diversicolor* were selected from sites  
113 characterized by different degrees of contamination; Río San Pedro is a shallow tidal inlet  
114 with no known elevated metal concentrations; Puente Zuazo receives domestic effluents from  
115 a town of about 88,000 habitants; and Trocadero is situated near a shipyard. The Blackwater  
116 estuary is considered by Rainbow et al. (2009a,b) to be uncontaminated in comparison with  
117 the SW England sites used in their comparisons. Ragworms were transported to the laboratory  
118 in cool boxes in wet sediment from the collection site. In the laboratory worms were kept in  
119 sediment from the site covered by artificial seawater (TM: Tropic Marin, Tropicarium  
120 Buchschlag, Dreieich, Germany) at a salinity of 16 at 18°C. The artificial seawater was used  
121 to provide physico-chemical stability and replication for trace metal uptake studies (Rainbow,  
122 1997).

123

### 124 *2.2 Metal analyses*

125

126 Organism and sediment samples were also collected for stable metal analyses. Ten  
127 worms from each site were placed in artificial seawater to depurate the digestive tract for 2  
128 days. Worms were dried to constant weight at 60°C (Table 1) and digested in concentrated  
129 nitric acid (Suprapur, Merck) at 100°C, and made up to a known volume with double distilled  
130 water. The accuracy of the analyses for Cd and Zn (certified Ag concentrations not available)  
131 was checked by analysis of certified reference material (TORT-2, Lobster Hepatopancreas,  
132 National Research Council, Canada) and indicated good agreement (the recovery was 92-  
133 98%) between measured concentrations and the reference values. Sediment samples were  
134 dried at 60°C, digested in concentrated nitric acid at 100°C and made up to a known volume  
135 with double distilled water. Concentrations of Zn were determined by inductively coupled  
136 plasma optical emission spectrometer (Optima 2000 DV, Perkin Elmer), while the analyses of  
137 Cd and Ag were carried out by ICP-MS (Serie X7, Thermo elemental).

### 139 *2.3 Organic matter*

141 The organic matter content in the oxic surface sediment was determined by the loss of  
142 weight on ignition at 450°C for 48 hours.

### 144 *2.4 Radioisotopes*

146 Radioactive tracers  $^{109}\text{Cd}$  and  $^{65}\text{Zn}$  were obtained from Brookhaven National  
147 Laboratory, New York, USA, and  $^{110\text{m}}\text{Ag}$  from Riso National Laboratory, Denmark.  
148 Radioisotopes in live worms and faecal pellets in experiments were counted on an LKB  
149 Wallac 1480 Wizard gamma counter. A standard was used with each analysis to establish the



150 background radiation level and activity changes in samples due to radioactive decay during  
151 the experimental periods.

152

### 153 *2.5 Uptake from solution*

154

155 *Nereis diversicolor* from all four sites were exposed individually to radiolabelled Cd,  
156 while three populations (Blackwater estuary, Río San Pedro and Puente Zuazo) were also  
157 exposed to radiolabelled Ag and Zn, separately in acid-washed 100 ml beakers with 50 ml  
158 TM at 16 salinity at 18°C. Exposure concentrations were 0.17, 0.5, 1, 5  $\mu\text{g l}^{-1}$  for Cd; 0.92, 2,  
159 4, 6  $\mu\text{g l}^{-1}$  for Ag; and 10, 20, 40, 60  $\mu\text{g l}^{-1}$  for Zn. These concentrations were labelled with  
160 0.25  $\mu\text{Ci }^{109}\text{Cd}$ ,  $^{110\text{m}}\text{Ag l}^{-1}$  or 2.5  $\mu\text{Ci }^{65}\text{Zn l}^{-1}$ . All experimental beakers were soaked in the  
161 corresponding experimental solution for 3 days before experiments to saturate all adsorption  
162 sites. Worms were acclimated to experimental conditions in 50 ml of clean TM water  
163 individually for 3 days then exposed to fresh labelled metal solution. Counts were determined  
164 after 3 h (assessment of adsorption onto body surface) and on days 1, 2, 3 and 4 of the uptake  
165 phase. After each count worms were replaced to the corresponding beaker filled with the  
166 renewed metal solution in order to maintain the initial exposure concentration. After 4 days  
167 worms were replaced to 50 ml clean TM and counted after 8 h (loss of weakly bound metals)  
168 and on days 3, 5, 7, 10, 12, 14 of the depuration phase. At the end of the experiments worms  
169 were dried individually at 60°C to a constant weight.

170

### 171 *2.6 Uptake from ingested sediment*

172

173 In order to examine the dietary uptake, radiolabelled sediment as a food source was  
174 offered to each worm. To avoid any exposure to radiolabelled metal in the porewater, worms

175 were fed on labelled sediment packaged in gelatine as described by Rainbow et al. (2009a).  
176 For each metal separately, approximately 0.25 g of wet sediment from the site of collection in  
177 the Blackwater estuary was labelled with 2  $\mu\text{Ci}$  of radiotracer and left to stand at 4°C for at  
178 least 1 month. A gelatine solution was prepared (2.4 g gelatine (Sigma), in 15 ml TM at  
179 salinity of 16) under gentle heat with 0.25 ml cod liver oil (Seven Seas Health Care, Hull,  
180 UK) added as feeding stimulant. This solution was mixed with the labelled sediment and the  
181 resulting mixture solidified by cooling at 4 °C.

182 Six worms from three different sites (Blackwater estuary, Río San Pedro and Puente  
183 Zuazo) were transferred individually to small plastic boxes containing 50 ml of artificial sea  
184 water (salinity 16, 18°C) a few days before the start of experiments. During the acclimation  
185 worms were trained to accept packaged food by feeding unlabelled sediment treated as  
186 described above. Worms were then allowed to feed on a package of labelled sediment  
187 overnight. Sixteen hours after feeding each worm was counted for 30 seconds, this count  
188 representing 100% of ingested labelled metal content before defaecation. Worms were then  
189 placed individually in TM (salinity 16, 18°C) and were fed unlabelled sediment following the  
190 same procedure. Radioactivity contents in live worms and casts were determined at 8, 24, 32,  
191 48, 56 and 72h. After then worms were allowed to continue depuration of any metal and  
192 counted every two days during the following week. Worms were placed individually in plastic  
193 boxes containing acid-washed sand covered by the artificial sea water and counted every 2 or  
194 3 days for another week. The medium (TM water or acid-washed sand) was renewed after  
195 every count.

## 196 197 *2.7 Data analysis*

198

199 Parametric tests, such as regression analyses and analysis of variance (ANOVA)  
 200 followed by Tukey's HSD *post hoc* methods were performed by using STATISTICA  
 201 (Statsoft, version 6). Data expressed as percentages have been arcsine-transformed before  
 202 using statistics. Level of significance was established at  $p=0.05$ .

203

## 204 2.8 Biodynamic modelling of trace metal accumulation

205

206 The biodynamic model assumes that net bioaccumulation at steady state ( $C_{ss}$ ) in an  
 207 animal can be described by the following equation:

$$C_{ss} = (K_u \times C_w) / (K_{ew} + g) + (AE \times IR \times C_f) / (K_{ef} + g)$$

210

211 where  $K_u$  is the metal uptake rate constant from solution ( $l \text{ g}^{-1} \text{ d}^{-1}$ ),  $C_w$  is the metal  
 212 concentration in solution ( $\mu\text{g l}^{-1}$ ), AE is the assimilation efficiency (%) of the metal from  
 213 ingested food,  $C_f$  is the metal concentration in food ( $\mu\text{g g}^{-1}$ ), IR is the ingestion rate ( $\text{g g}^{-1} \text{ d}^{-1}$ ),  
 214  $g$  is the growth rate constant ( $\text{d}^{-1}$ ), and  $K_{ew}$  and  $K_{ef}$  are the efflux rate constants ( $\text{d}^{-1}$ ) after  
 215 uptake from solution and food respectively. In this study the ingested food is sediment and  $K_{ef}$   
 216 is expressed as  $K_{esed}$ .

217 Although living in burrows in the sediment, *Nereis diversicolor* strongly irrigates its  
 218 burrow with a current of oxygenated water from the overlying water column (Banta et al.,  
 219 1999, Kristensen, 2001), and water bathing the worm is from the water column and not pore  
 220 water in chemical equilibrium with the sediment particles. We have, therefore, followed  
 221 Casado-Martinez et al. (2009b) and Rainbow et al. (2009b) by not considering dissolved  
 222 concentrations of metals in pore water in equilibrium with sediment particles but used  
 223 overlying water dissolved metal concentrations in our modelling. Dissolved metal

224 concentrations are not available for the water column in the estuaries sampled and we have  
1  
2 225 therefore fallen back on ranges of dissolved concentrations reported in the literature (Luoma  
3  
4 226 and Rainbow, 2008). Dissolved concentration ranges extending from low concentrations  
5  
6  
7 227 typical of uncontaminated coastal waters to concentrations recognised as atypically high and  
8  
9 228 indicative of significant anthropogenic contamination in estuaries are: 0.006 to 0.03  $\mu\text{g Ag l}^{-1}$   
10  
11 229 (Smith and Flegal, 1993; Ranville and Flegal, 2005), and 0.01 to 0.10  $\mu\text{g Cd l}^{-1}$  and 0.3 to 5  
12  
13  
14 230  $\mu\text{g Zn l}^{-1}$  (both from Table 5.5, Luoma and Rainbow, 2008). We have no reason to consider  
15  
16  
17 231 that local dissolved concentrations in the water column at our sites are atypically high, and in  
18  
19 232 our modelling we have used 0.01  $\mu\text{g Ag l}^{-1}$ , 0.01  $\mu\text{g Cd l}^{-1}$  and 0.3  $\mu\text{g Zn l}^{-1}$ .

21  
22 233 The growth rate constant of *Nereis diversicolor* is mainly influenced by the type of  
23  
24 234 available food sources, and an artificial food supply normally results in a greater value.  
25  
26 235 Kristensen (1984) reported an average weight-specific growth rate constant of 0.005  $\text{d}^{-1}$  for a  
27  
28  
29 236 population of *N. diversicolor* in Norsminde Fjord, Denmark. Riisgard et al. (1996) found the  
30  
31  
32 237 growth rate constant to be in the range of 0.002-0.025  $\text{d}^{-1}$  in populations from Kertinge Nor,  
33  
34 238 Denmark, and 0.049-0.056  $\text{d}^{-1}$  when the worms were fed on algal cells or shrimp. Olivier et  
35  
36 239 al. (1996) recorded a growth rate constant of 0.0056 to 0.019  $\text{d}^{-1}$  for this species fed on plant  
37  
38  
39 240 material. In agreement with Rainbow et al. (2009b) who carried out a sensitivity analysis of  
40  
41 241 the effects on a biodynamic model of varying the growth rate constant of *N. diversicolor* from  
42  
43  
44 242 0.005 to 0.05  $\text{d}^{-1}$ , we have chosen to use a growth rate constant of 0.02  $\text{d}^{-1}$  in the models to be  
45  
46 243 employed here.

47  
48 244 Cammen (1980) has shown that the assimilation processes of deposit-feeding  
49  
50  
51 245 invertebrates, including polychaetes like *Arenicola marina* and *Nereis diversicolor*, are  
52  
53 246 focused on the organic matter component of the sediment. Indeed deposit-feeding  
54  
55  
56 247 invertebrates in general appear to maintain a rate of ingestion of organic matter which is  
57  
58 248 dependent on body weight but independent of the organic content of the ingested sediment,  
59  
60  
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62  
63  
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249 following the relationship  $C = 0.381W^{0.742}$  where C is the organic matter (mg) ingested per  
1  
2 250 organism per day and W the body dry weight (mg) of the organism (Cammen, 1980). Thus  
3  
4 251 there appears to be a constant organic matter ingestion rate for a given size animal, even  
5  
6  
7 252 though the rate of ingestion of total sediment will vary substantially. We have therefore  
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9  
10 253 followed the arguments of Casado-Martinez et al. (2009b) and Rainbow et al. (2009b), and  
11  
12 254 used the mean body weight of the worms to calculate the rate of ingestion of sediment-  
13  
14 255 associated organic matter using the above equation of Cammen (1980), also presenting the  
15  
16  
17 256 total sediment ingestion rate (Table 2)

19 257 We need also to estimate the relevant measure of metal concentration in the ingested  
20  
21  
22 258 sediment. Since we are using an ingestion rate of  $g_{OM} g_{organism}^{-1} d^{-1}$  in the determination of  
23  
24 259 uptake rates from food, then the concentration of metal in the sediment must be in similar  
25  
26  
27 260 units, and the most relevant measure is the metal concentration in the organic component of  
28  
29 261 the sediment. There is no direct method to separate metal associated with the organic  
30  
31  
32 262 components of sediment from that associated with inorganic ligands. One way to obtain the  
33  
34 263 correct units would be to divide the mass of metal in a gram of sediment by the mass of  
35  
36 264 organic matter. But this makes the false assumption that all sediment metal is associated with  
37  
38  
39 265 the organic matter, while it is well known that there will necessarily be metals associated with  
40  
41 266 inorganic components of oxidised sediment, not least manganese and iron oxide components  
42  
43  
44 267 (Luoma and Rainbow, 2008). Since Ag, Zn and Cd in the oxidised sediment are distributed  
45  
46 268 between organic and inorganic components (Luoma and Rainbow, 2008), it is not valid to  
47  
48  
49 269 assume that all metal in the sediment is associated with organic matter, nor is it valid to  
50  
51 270 assume that no metal is associated with sedimentary organic matter. The default assumption  
52  
53  
54 271 made by Casado-Martinez et al. (2009b) and Rainbow et al. (2009b) and followed here is that  
55  
56 272 organic and inorganic sedimentary components have a generally similar affinity for the  
57  
58 273 metals, and thus the same metal concentration (content per unit weight of that component);  
59  
60  
61  
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274 i.e. the metal is distributed relatively evenly among organic and inorganic binding sites in the  
1  
2 275 sediment. We accept that this is unlikely to be exactly correct (e.g. Luoma and Bryan, 1982),  
3  
4 276 but consider this to be a more valid generalization than the alternatives. Under such  
5  
6  
7 277 conditions, the metal concentration per unit mass of organic material is necessarily the same  
8  
9  
10 278 as the metal concentration per unit mass of total sediment. Our estimate of the metal  
11  
12 279 concentration of the organic component of the sediment is therefore the total metal sediment  
13  
14 280 concentration.

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16  
17 281

### 18 19 282 **3. Results**

20  
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22 283

#### 23 24 284 *3.1 Trace metal concentrations*

25  
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27 285

28  
29 286 The concentrations of Cd, Ag and Zn measured in oxic surface sediments and in  
30  
31 287 *Nereis diversicolor* originating from the four sites are summarized in Table 1. Sediment from  
32  
33  
34 288 the Blackwater estuary had the highest Cd concentration, whereas the Spanish sediments  
35  
36 289 exhibited similar concentrations. The bioaccumulated concentrations of Cd in the ragworms  
37  
38  
39 290 were significantly correlated with the local sediment concentrations ( $p < 0.001$ ). The highest  
40  
41 291 Ag concentration was found in sediment from Puente Zuazo and the highest Zn concentration  
42  
43  
44 292 in sediment from Trocadero, but in neither case was this reflected in the highest  
45  
46 293 bioaccumulated concentration in the local ragworms.

47  
48  
49 294

#### 50 51 295 *3.2 Uptake and depuration from solution*

52  
53  
54 296

55  
56 297 The uptake of labelled metal from solution into the body was plotted against the  
57  
58 298 exposure period for each exposure concentration (see Rainbow et al. (2009a) for example)

299 and the slopes of these regression lines (uptake rates) were then expressed as a function of the  
1  
2 300 dissolved exposure concentrations. The slope of this fitted line is defined as the uptake rate  
3  
4 301 constant ( $K_u$ ) of the radiolabelled metal (Table 3). Typically the uptake rate showed a  
5  
6  
7 302 significant linear increase ( $p < 0.05$ ) with increasing exposure concentrations except in the case  
8  
9  
10 303 of worms from the Blackwater estuary for Ag; the latter result may simply be an effect of  
11  
12 304 chance when working at the  $p = 0.05$  level of significance. In general, the highest absolute  
13  
14 305 uptake rates at a given dissolved molar concentration were recorded for dissolved Ag and the  
15  
16  
17 306 lowest for dissolved Cd, showing one order of magnitude difference.

19 307 As  $K_u$  is a unified measure of the uptake rate by a particular population of worms  
20  
21  
22 308 under the physicochemical conditions used in the tests (salinity 16, 18°C), it can be compared  
23  
24 309 between the populations (Rainbow et al., 2009a). Analysis of variance of Cd  $K_u$  showed  
25  
26 310 significant differences between the populations studied (Table 3); the Cd  $K_u$  of the Puente  
27  
28  
29 311 Zuazo population was significantly higher than those of the Blackwater and Trocadero  
30  
31 312 populations, and the latter population showed a significantly lower  $K_u$  than that observed in  
32  
33  
34 313 the worms from Río San Pedro. The uptake rate constant of Ag did not vary significantly  
35  
36 314 between the populations (Table 3). Only one comparison showed a significant difference  
37  
38  
39 315 between populations in the case of Zn  $K_u$ , between worms from the Blackwater estuary and  
40  
41 316 Puente Zuazo (Table 3).

44 317 The change in temperature from 10°C to 18°C did not significantly affect the uptake  
45  
46 318 rate constants of any of the three metals in worms from the Blackwater estuary (Table 3).

49 319 In the depuration phase, counts expressed as percentages of the final count after the  
50  
51 320 uptake phase were ln-transformed, and then plotted against the efflux time (see Rainbow et al.  
52  
53 321 (2009a) for example). The slope of the regression line is the efflux rate constant ( $K_{ew}$  in  $d^{-1}$ ) of  
54  
55  
56 322 the metal, and the biological retention half-life of the metal is calculated as 0.693 divided by  
57  
58 323 the  $K_{ew}$  (Table 3). Data points of the depuration phase were examined carefully and when two  
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324 compartments were detected, only the 'slow pool' was considered for the calculation of the  
1  
2 325 efflux rate constant.  
3

4 326 According to the efflux rate constants of radiolabelled metal after uptake from  
5  
6  
7 327 solution, Ag and Zn were excreted more rapidly than Cd by worms from each site (Table 3).  
8  
9 328 The slower depuration rate of Cd resulted in longer retention in the tissue (biological half-  
10  
11 329 lives varied between 31.2 and 76.5 d). Significant intersite differences in these efflux rate  
12  
13 330 constants were only found in the case of Zn, with worms from Puente Zuazo exhibiting a  
14  
15 331 significantly higher efflux rate constant than those from the Blackwater estuary (Table 3).  
16  
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18

19 332 For the Blackwater population, only in the case of silver did exposure to 18°C as  
20  
21 333 opposed to 10°C result in a significantly changed (in this case increased) efflux rate constant  
22  
23 334 (Table 3).  
24  
25  
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27 335

### 29 336 *3.3 Assimilation and depuration from food*

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31 337

32  
33  
34 338 The radioactive counts of the live animal after ingestion of the radiolabelled meal were  
35  
36 339 plotted against the time (see Rainbow et al. (2009) for example). The assimilation efficiency  
37  
38 340 (AE) was considered as the percentage of ingested labelled metal retained in the worm after  
39  
40 341 complete defaecation. After this initial rapid loss resulting from defaecation, the percentage of  
41  
42 342 the remaining radioactive metal declined very slowly or stayed at a constant level,  
43  
44 343 representing the 'slow pool' of assimilated metal. In most cases, unassimilated labelled metals  
45  
46 344 were eliminated by worms 3 to 4 days after the pulse-chase feeding. As above, counts  
47  
48 345 expressed as percentages of the initial count after feeding were then ln-transformed, and  
49  
50 346 plotted against the efflux time. The efflux rate constant of radiolabelled metal after uptake  
51  
52 347 from the labelled sediment ( $K_{\text{esed}}$ ) was calculated as the slope of the regression line of the  
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348 'slow pool'. The AE percentage was estimated by back extrapolation of this regression line to  
1  
2 349 intercept the y axis.  
3

4 350 Table 4 shows that assimilation efficiencies of *Nereis diversicolor* for Cd were highest  
5  
6  
7 351 in the population from Río San Pedro, significantly above that of the Blackwater estuary  
8  
9 352 population. In the case of Ag no significant intersite differences in the AEs were recorded, a  
10  
11  
12 353 result influenced by the low number of samples available (Table 4). No significant differences  
13  
14 354 in assimilation efficiencies for Zn were found between the populations (Table 4).  
15

16  
17 355 The change in temperature from 10°C to 18°C had no significant effect on the AE of  
18  
19 356 Cd and Zn of the Blackwater population, but there was a significant difference in the case of  
20  
21  
22 357 the Ag AE (Table 4).  
23

24 358 The efflux rate constants of each metal by *Nereis diversicolor* did not differ  
25  
26 359 significantly between the populations (Table 4). Retention of Cd after uptake from food was  
27  
28  
29 360 greater than observed for Zn in all populations, with a corresponding longer biological half  
30  
31 361 life of Cd (31.1-39.2 d) than of Zn (17.0-21.8 d) (Table 4).  
32  
33

34 362 The change in temperature from 10°C to 18°C had no significant effect on the efflux  
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36 363 rate constants of Cd and Ag for the Blackwater worms after uptake from ingested sediment,  
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38  
39 364 but there was a significant difference in the case of Zn (Table 4).  
40  
41 365

#### 42 43 366 *3.4 Comparison of efflux rate constants after uptake from solution or ingested sediment* 44 45

46 367  
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48 368 Efflux rate constants of radiolabelled Cd, Ag and Zn after uptake from solution or  
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51 369 ingested sediment were compared for each metal, and no significant difference was found in  
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53 370 each population at 18°C.  
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#### 56 371 57 58 372 *3.5 Biodynamic modelling* 59 60 61 62 63 64 65

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1  
2 374 The physiological parameters measured for each population (Tables 3 and 4) have  
3  
4  
5 375 been entered into a biodynamic model, together with the associated sediment metal  
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7 376 concentration (Table 1), and the estimated ingestion rate of the worms from each site  
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9  
10 377 expressed in terms of sedimentary organic matter (Table 2). As explained above, single values  
11  
12 378 have been entered for the growth rate constant ( $0.02d^{-1}$ ) and for the dissolved concentrations  
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14 379 of Cd ( $0.01 \mu g l^{-1}$ ), Ag ( $0.01 \mu g l^{-1}$ ) and Zn ( $0.3 \mu g l^{-1}$ ) which can be considered low. Steady  
15  
16 380 state bioaccumulated concentrations predicted by the biodynamic model for each population  
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18  
19 381 of worms are shown in Table 5.

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21  
22 382 The model shows that the sediment is the vastly predominant source of Cd and Zn,  
23  
24 383 accounting for more than 99% of the total Cd and Zn accumulated in the ragworms. In the  
25  
26 384 case of Ag, uptake from water contributed a significant proportion (24 to 33%) of the metal  
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28  
29 385 bioaccumulated.

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31 386 The predicted steady state metal concentrations in the worms from the different sites  
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33  
34 387 are compared with the measured concentrations in Table 6. The model with the present  
35  
36 388 assumptions overpredicted in all cases the accumulated concentration of Cd; it underpredicted  
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39 389 the Ag concentration of the Blackwater worms but gave good correspondence for Río San  
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41 390 Pedro and Puente Zuazo worms. Predictions for Zn bioaccumulation were also good.

391

## 4. Discussion

393

### 4.1 Biodynamic parameters

395

56 396 In this study, biodynamic accumulation parameters of *Nereis diversicolor* from the  
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58 397 different estuaries have been determined as population-specific coefficients, addressing the

398 question of how variable are the physiological parameters defining metal accumulation  
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2 399 between different populations of a species.  
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4  
5 400 One possible cause of variation may be the effect of previous field contamination  
6  
7 401 history on the uptake and elimination processes of the animals. Field measurements, however,  
8  
9 402 showed no extraordinary high concentrations of Cd, Ag and Zn either in sediment or in  
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11 403 ragworm at each site in this study, and we did not find any relationship between metal  
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13 404 bioavailability (as measured by accumulated concentration) and uptake rate constant in  
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15 405 ragworms from the four sites. Nevertheless, some intersite differences in some biodynamic  
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17 406 parameters were found.  
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21  
22 407 In the case of the mean of  $K_u$  of the three metals, the highest uptake rate constants  
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24 408 from solution were recorded for Ag followed by Zn and Cd in ragworms from the three  
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26 409 populations, similarly to the observations in the same species by Rainbow et al. (2009a). Our  
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28 410 results also agree with those observed in other marine polychaetes, such as in *Nereis succinea*  
29  
30 411 by Wang et al. (1999) or in the lugworm *Arenicola marina* by Casado-Martínez et al. (2009a).  
31  
32  
33 412 Trace metals are typically taken up from solution across the apical cell membrane of a surface  
34  
35 413 epithelial cell of an aquatic invertebrate via facilitated diffusion carried by a transporter  
36  
37 414 protein, the dissolved free metal ion representing the best model for the dissolved metal form  
38  
39 415 that binds to the protein (Luoma and Rainbow, 2008). The free cadmium ion may also cross  
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41 416 the membrane via calcium ion channels; zinc may similarly be transported by calcium  
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43 417 channels in vertebrates but not invertebrates (Luoma and Rainbow, 2008). In addition to the  
44  
45 418 possible binding of free silver ions to a transporter protein for facilitated diffusion, some of  
46  
47 419 these silver ions may also cross the membrane by trespassing in sodium channels. One  
48  
49 420 possible reason for the high uptake rate of dissolved silver by aquatic invertebrates is the fact  
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51 421 that the predominant form of Ag in brackish and marine water is the zero-charged, non-polar,  
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422 chloro-complex AgCl which can diffuse through the membrane, in addition to any other  
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2 423 routes of uptake (Luoma et al., 1995).  
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4 424 There were no significant inter-population differences in the efflux rate constants of  
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7 425 Cd and Ag after uptake from solution, but Puente Zuazo exhibited significantly enhanced  
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9  
10 426 efflux of Zn compared to worms from the Blackwater estuary. The regulation of total Zn body  
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12 427 burden by *N. diversicolor* has been documented (Amiard et al., 1987; Bryan and  
13  
14 428 Hummerstone, 1973; Bryan et al., 1980), as the worms have developed an efficient efflux  
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17 429 system to remove excess metal. It appears to be the case that in the worms from Puente  
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19 430 Zuazo, the higher uptake rate constant (Table 3) is compensated by the higher efflux rate  
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21  
22 431 constant (Table 3), to result in a final regulated body concentration that is not elevated (Table  
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24 432 1).  
25

26 433 According to Cammen (1980) deposit feeders ingest sediments at a rate of about twice  
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29 434 their body weight per day. Thus to model bioaccumulation it is essential to assess metal  
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31 435 uptake from the food. A direct link has been demonstrated between AE and accumulated  
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34 436 metal concentration in mussels (Fisher et al., 1996), and a population of the clam, *Ruditapes*  
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36 437 *philippinarum*, from a metal-contaminated site exhibited a higher Cd AE than did clams  
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38  
39 438 originating from uncontaminated sites (Shi and Wang, 2004). In our study, the assimilation  
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41 439 efficiency for Cd from sediment differed significantly between the population from Río San  
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43  
44 440 Pedro (high) and the Blackwater (low), but it did not follow the rank order of sediment or  
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46 441 bioaccumulated Cd concentrations. Rainbow et al. (2009a) showed some limited inter-  
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48  
49 442 population differences in metal AE between populations of *N. diversicolor* from six UK  
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51 443 estuaries, but these differences were also not consistent with the local metal exposure history.  
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53 444 Similarly, no inter-population differences in Cd and Zn AE in mussel, *Perna viridis* collected  
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56 445 from sites differentially enriched with trace metals were recorded by Blackmore and Wang  
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58 446 (2003).  
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447 In our study, the efflux rate constants after dietary metal uptake showed no significant  
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2 448 differences between the populations, similarly to the results for Ag, Cd and Zn Ke observed in  
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4 449 the barnacle *Balanus amphitrite* (Rainbow et al., 2003) or in the Cd and Zn Ke of marine  
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6  
7 450 clams, *Ruditapes philippinarum* and *Macra veneriformis* (Shi and Wang, 2004). No  
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9  
10 451 differences between the two efflux rate constants of each metal after uptake from water or  
11  
12 452 ingested sediment ( $K_{ew}$  and  $K_{esed}$ ) suggest that the same physiological handling process is in  
13  
14 453 action irrespective of the route of metal uptake.

16  
17 454 Temperature generally enhances many physiological processes that determine the rates  
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19 455 of metal uptake, elimination or detoxification (Heugens et al., 2003). As increased  
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21  
22 456 temperature may affect both influx and efflux rates of metals, net bioaccumulation may or  
23  
24 457 may not increase (Luoma, 1983). Increase in temperature resulted in increased uptake rates of  
25  
26 458 dissolved Cd, Cr and Zn in the clam, *Corbicula fluminea*, and also their assimilation  
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28  
29 459 efficiency of Cd from sediment (Lee and Lee, 2005). Heugens et al. (2003) reported enhanced  
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31 460 Cd uptake rate at elevated temperature as well as some alterations in Cd elimination in  
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33  
34 461 *Daphnia magna*. Despite the numerous studies indicating higher metal uptake at higher  
35  
36 462 temperature due to the higher rate of metabolism, Wang and Fisher (1997) found a negative  
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39 463 relationship between Ag assimilation efficiency and temperature in *Mytilus edulis*. The  
40  
41 464 influence of the temperature on the kinetic parameters was not consistent in our study; Ag  
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44 465 uptake from solution and Zn efflux rate after uptake from solution showed significant positive  
45  
46 466 correlation with the temperature, but Cd assimilation from sediment was significantly lower at  
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48 467 18°C.

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51 468 We conclude that there was no consistent significant effect of the change in  
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53 469 temperature between 10°C and 18°C on the biodynamic accumulation parameters measured.  
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56 470 Thus we consider that it is valid to include British and Spanish populations of *N. diversicolor*  
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58 471 (and by extension populations of the worm from western Europe and north Africa) in any  
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472 attempt to produce a generalised biodynamic model of Ag, Cd and Zn bioaccumulation in *N.*  
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2 473 *diversicolor*.

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#### 6 7 475 4.2 Biodynamic modelling of trace metals in *Nereis diversicolor*

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12 477 We incorporated the population-specific biodynamic accumulation parameters of  
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14 478 *Nereis diversicolor* from the different estuaries determined here into a biodynamic model to  
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16  
17 479 predict the accumulated Cd, Ag and Zn concentrations in ragworms from each site (Table 5).  
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19 480 There was generally good agreement (Table 6, Figure 1a), although the model overpredicted  
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21  
22 481 in all cases the accumulated concentration of Cd, and underpredicted the Ag concentration of  
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24 482 the Blackwater worms. Predictions of Ag bioaccumulation for two Spanish sites and for Zn  
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26 483 bioaccumulation at all sites were excellent.

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29 484 The model also provides information about the contribution of the sediment or  
30  
31 485 solution as metal source to the overall metal accumulation (Wang and Rainbow, 2008). Our  
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33  
34 486 results are consistent with those previously recorded in the deposit feeding polychaetes  
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36 487 studied, in that more than 99% of Cd and Zn, and about 70% of Ag, are derived from  
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38  
39 488 sediment ingestion at low dissolved metal concentrations (*Arenicola marina* – Casado-  
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41 489 Martinez et al. (2009a) and *Nereis diversicolor* (Rainbow et al., 2009a).

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44 490 We have then gone on to conduct sensitivity analyses to check how the changes in  
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46 491 certain parameters -namely, the growth rate constant and dissolved metal concentration- affect  
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48  
49 492 the accumulated metal concentration. The growth rate constant was varied from 0.005 to 0.05  
50  
51 493 d<sup>-1</sup>, and the dissolved metal concentrations from 0.01 to 0.10 µg Cd l<sup>-1</sup>, 0.01 to 0.03 µg Ag l<sup>-1</sup>  
52  
53 494 and 0.03 to 5 µg Zn l<sup>-1</sup>.

54  
55  
56 495 Independently of the variation in growth rate constant and dissolved metal  
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58 496 concentration over these ranges, the worms always accumulated more than 90% of the total

497 Cd and Zn from sediment. The choice of a high or low  $C_w$  essentially had no effect on  
1  
2 498 predicted concentrations of Cd and Zn in the worms (compare Figs 1a and 1b at growth rate  
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4 499 constant  $0.02 \text{ d}^{-1}$ ). The effect of changes in the growth rate constant on predicted accumulated  
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6  
7 500 metal concentrations using the low  $C_w$  is shown in Figure 2. Any effect becomes more  
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9  
10 501 considerable only when growth rate constant is below  $0.02 \text{ d}^{-1}$ . The modelled Cd  
11  
12 502 bioaccumulation was overpredicted at all growth rate constants modelled, with the best fit  
13  
14 503 between predicted and measured concentrations using the maximum growth rate constant. In  
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16  
17 504 contrast, at higher growth rate constants the Zn bioaccumulation predicted by the model was  
18  
19 505 below the measured concentration (about 60%), while at lower growth rate constants the  
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21  
22 506 prediction was more similar to the field observation.

24 507 Ag showed a different pattern, as at high dissolved concentrations the contribution of  
25  
26 508 dissolved and sediment Ag was equal at all growth rate constants. For instance, using the  
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28  
29 509 maximum dissolved concentration, approximately 53 % of total Ag was accumulated from the  
30  
31 510 water (mean of the three populations), while this value decreased to 27 % at low  $C_w$ . This  
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33  
34 511 input of Ag from solution is much greater than in the case of Cd or Zn (Table 5). It has to be  
35  
36 512 mentioned that the atypically high seawater Ag concentrations used here for the sensitivity  
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38  
39 513 analysis were recorded in estuaries contaminated by mining activities in the 1990s and  
40  
41 514 nowadays are not common in the estuarine environment (Rainbow et al., 2009b). For Ag the  
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43  
44 515 predicted concentration at both low and high  $C_w$  accurately matched the observed  
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46 516 concentration in the worms, except in the case of the Blackwater population (Table 6, Figs.  
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48 517 1a, 1b).

#### 51 518 52 53 519 *4.3 Generalized model*

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521 The second question addressed is whether it is possible to choose standardised values  
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2 522 of the relevant parameters that can be applied in a generalised biodynamic model of Ag, Cd  
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4 523 and Zn accumulation by *N. diversicolor* and allow accurate prediction of bioaccumulated  
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6  
7 524 concentrations in the worm across a wide range of western European and even north African  
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9  
10 525 (Moroccan) estuaries of different degrees of contamination. To generate this model, we have  
11  
12 526 chosen approximately median values of the physiological parameters measured for each  
13  
14 527 population, together with a similar median value for the ingestion rate of sedimentary organic  
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16  
17 528 matter, as measured or calculated in this study and by Rainbow et al. (2009b). These have  
18  
19 529 been incorporated, together with the values of growth rate constant and dissolved metal  
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21  
22 530 concentrations used in the model detailed in Table 5, in the list of model parameters given in  
23  
24 531 Table 7.

26 532 The comparisons of the steady state concentrations predicted by this generalised  
27  
28  
29 533 model against measured concentrations from our studies and in the literature are given in  
30  
31  
32 534 Table 8. The predicted (y) and measured (x) concentrations have been entered into regression  
33  
34 535 models (Figures 3a-c) and the regression coefficients compared against unity which would  
35  
36 536 indicate perfect agreement.

38  
39 537 The regression model for the full data set for cadmium (Figure 3a) had a regression  
40  
41 538 coefficient of 1.763 (95% CL 0.650), significantly above 1, confirming overprediction of  
42  
43  
44 539 bioaccumulated concentrations by the model with the assumptions given in Table 7. In most  
45  
46 540 populations the concentrations of Cd predicted by modelling were approximately two-fold  
47  
48  
49 541 higher than the field measurements. The bioavailability of metals to *Nereis diversicolor* may  
50  
51 542 be affected by factors controlling the local partitioning of sediment-bound metals between  
52  
53 543 different sediment constituents (Luoma and Bryan, 1982). For instance, the enhanced metal  
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55  
56 544 concentration observed in the sediment did not result in raised Cd concentration in ragworms  
57  
58 545 from Boulogne harbour in the study of Berthet et al. (2003), as reflected here in the  
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546 underprediction by our model (Table 8). Thus atypical local factors affecting Cd  
1  
2 547 bioavailability in sediments will cause specific differences between predicted and observed  
3  
4 548 concentrations in the worms, over and above any general trend to overpredict or underpredict  
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6  
7 549 by the model. The reason for the general overprediction by the model of the accumulated Cd  
8  
9  
10 550 concentrations in *N. diversicolor* will presumably not lie in a wrong choice of growth rate  
11  
12 551 constant, for this would affect all predicted accumulated metal concentrations equally – not  
13  
14 552 the case here. A possible cause is the chosen assimilation efficiency for Cd from the sediment.  
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16  
17 553 It may be that the technique used here overestimates the typical assimilation efficiencies of  
18  
19 554 sediment cadmium in the field. A revised AE of 0.25 in the model would have given a very  
20  
21  
22 555 accurate prediction of the measured accumulated concentrations in the worm. Alternatively  
23  
24 556 (or additionally), the error may lie in the assumption that the Cd concentration of the organic  
25  
26 557 component of the sediment is modelled by the total metal concentration rather than a factor of  
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28  
29 558 it perhaps related to the percentage organic content of the sediment.  
30

31           The full data set for Ag is greatly affected by the large underprediction of the Ag  
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33  
34 560 concentration in the Seine worms (Table 8), almost certainly caused by an underestimation of  
35  
36 561 the dissolved Ag concentration in this contaminated estuary (Amiard et al., 2009). The  
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38  
39 562 regression coefficient for the full data set (0.234, 95% CL 0.156) comes closer to unity after  
40  
41 563 removal of this data point (0.662, 95% CL 0.138) (Figure 3b), but the model does still  
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43  
44 564 underpredict observed Ag bioaccumulation. Luoma and Bryan (1982) concluded that  
45  
46 565 extraction of sediment with HCl appeared to be the best technique to model the bioavailable  
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48  
49 566 fraction of many sedimentary metals. The Ag concentrations in most of the sediments here  
50  
51 567 were determined after stronger acid digestion (“total” metal) that probably did not reflect the  
52  
53  
54 568 biologically available quantity of metal, and HCl-extracted Ag concentrations in the  
55  
56 569 sediments may be more appropriate for use in this model. The model predictions when using  
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58 570 the nitric acid-extracted sediment concentrations was very close to the measured  
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571 concentrations for the worms from the Gannel, Tressilian and Bidasoa River, perhaps in  
572 reflection of local factors affecting local sediment Ag bioavailability. The physicochemical  
573 characteristics of any sediment will affect the metal bioavailability and consequent uptake by  
574 deposit feeders. Silver tends to form strong organo- or sulphide complexes that are of low  
575 bioavailability, but some organic complexes increase rather than decrease the bioavailability  
576 (Bryan and Langston, 1992). The presence of other trace metals also influences metal uptake;  
577 the Ag concentration in deposit feeding clam, *Scrobicularia plana* was inversely correlated  
578 with Cu concentration in sediment (Luoma and Bryan, 1982).

579         The very high Restronguet Creek data point (Table 8) dominates any regression of the  
580 zinc data, single handedly bringing about a very significant regression with a regression  
581 coefficient significantly greater than 1 (2.510, 95% CL 0.275). Elimination of this point  
582 eliminates any significance of the regression of the remaining data set (reg. coeff. 0.551, 95%  
583 CL 0.509). Furthermore the data seem to fall into two categories, perhaps partly  
584 discriminating between worms in which regulation of Zn body concentration has broken  
585 down or not (Rainbow et al. 2009b). Thus, we present two regression lines for zinc after  
586 exclusion of the Restronguet Creek point (Figure 3c). The regression coefficient for one of the  
587 lines is significantly raised above 1 (1.527, 95% CL 0.208) indicating the overprediction of  
588 accumulated Zn in populations that probably are better able to regulate their body Zn  
589 concentrations, while the coefficient of the other line fell below unity (0.726, 95% CL 0.560),  
590 with these latter worms containing higher Zn concentrations than predicted by the model.

591         In the absence of mean dry weight values of each population we used a mean (21.2  
592 mg) of mean dry weights of populations from UK estuaries for the generalised model. The  
593 ingestion rate is related to animal body weight; thus the sensitivity analysis was extended to a  
594 range of animal sizes (16.2-57.1 mg) as well. The IR is 0.175 g OM g<sup>-1</sup> d<sup>-1</sup> when using the  
595 average dry weight, whereas this value varies over the range of 0.134 to 0.186 g OM g<sup>-1</sup> d<sup>-1</sup> at

596 the chosen maximum and the minimum animal size, respectively. Considering that sediment  
597 is the main source of various metal accumulations, the use of a specific dry weight for the  
598 calculation of IR could improve the accuracy of the model prediction.

## 600 **5. Conclusion**

601  
602 Physiological metal bioaccumulation parameters in populations of ragworms from southern  
603 Spain and south England did not show any consistent inter-population variations relating to  
604 the local metal bioavailability or the effect of raised temperature. These findings allowed us to  
605 define metal-specific parameters of *N. diversicolor* and apply them in a generalized model  
606 with some success. According to the assumptions of this model, predicted steady state  
607 concentrations of Cd and Zn in *N. diversicolor* were overestimated, those of Ag  
608 underestimated, but still of the same order of, and comparable to, independent field  
609 measurements. We conclude that species-specific physiological metal bioaccumulation  
610 parameters are relatively constant over large geographical distances and climatic zones, and a  
611 single generalised biodynamic model does have the potential to predict accumulated Ag, Cd  
612 and Zn concentrations in this polychaete from a single sediment metal concentration.

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731 **Figure captions**

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2 732 Figure 1. Ag, Cd and Zn concentrations ( $\mu\text{g g}^{-1}$ ) measured ( $\pm 95\%$  CL) in *Nereis diversicolor*  
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4 733 from one UK and two Spanish estuaries compared to metal concentrations predicted from  
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7 734 independently derived biodynamic constants and sediment metal concentrations (see Table 5)  
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9 735 using (a) low or (b) high dissolved metal concentrations expected in metal contaminated  
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14 737  
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16 738 Figure 2. The effect of changes in growth rate constant from 0.005 to 0.05  $\text{d}^{-1}$  on the predicted  
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18 739 concentrations of Cd, Ag and Zn in polychaetes from one UK and two Spanish estuaries.  
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21 740 Other parameters are as in Table 5.  
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26 742 Figure 3. The predicted and measured concentrations of (a) Cd, (b) Ag and (c) Zn in *Nereis*  
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28 743 *diversicolor* from western European and Moroccan estuaries using the biodynamic parameters  
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30 744 presented in Table 8. In Figure 3c, open circles: populations considered able to regulate their  
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32 745 body Zn concentrations; filled circles: populations less able to regulate body Zn  
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35 746 concentrations.  
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Table 1. Collection details and concentrations of Cd, Ag and Zn ( $\mu\text{g g}^{-1} \pm \text{sd}$  dry weight) in oxic surface sediment and the polychaete worm *Nereis diversicolor*.

Site	Geographic coordinates	Dates of collection		Cd	Ag	Zn
Blackwater	051°44.08'N, 000°41.34'E,	13/09/07	Sediment	1.95 ± 0.26	0.46 ± 0.09	93 ± 17
			<i>N. diversicolor</i>	<0.2 - <0.8	1.38 ± 0.61	237 ± 37
Río San Pedro	036°32.77'N, 006°12.39'W	08/09/07	Sediment	0.60 ± 0.09	0.37 ± 0.01	101 ± 6
			<i>N. diversicolor</i>	0.22 ± 0.00	0.30 ± 0.00	152 ± 6
Puente Zuazo	036°28.35'N, 006°11.71'W	08/09/07	Sediment	0.56 ± 0.02	0.98 ± 0.28	121 ± 18
			<i>N. diversicolor</i>	0.21 ± 0.01	0.30 ± 0.00	160 ± 9
Trocadero	036°30.90'N, 006°14.36'W	08/09/07	Sediment	0.58 ± 0.07	0.45 ± 0.04	145 ± 8
			<i>N. diversicolor</i>	0.19 ± 0.02	0.26 ± 0.00	126 ± 5

Table 2. Ingestion rates of *Nereis diversicolor* from 3 Spanish and 1 UK estuaries following Cammen (1980). See text for details.

	Mean $\pm$ sd dry wt of worms  g	Organic matter consumption per worm mg OM d <sup>-1</sup>	Ingestion rate of organic matter  g OM g <sup>-1</sup> d <sup>-1</sup>	% organic matter in sediment	Ingestion rate of sediment  g g <sup>-1</sup> d <sup>-1</sup>
Blackwater	0.0172 $\pm$ 0.0084	3.15	0.183	12.1	1.51
Río San Pedro	0.0255 $\pm$ 0.0223	4.21	0.171	12.7	1.34
Puente Zuazo	0.0180 $\pm$ 0.0165	3.25	0.181	8.6	2.10
Trocadero	0.0571 $\pm$ 0.0239	7.66	0.134	11.1	1.21

Table 3. *Nereis diversicolor*: Uptake rate constants ( $K_u$ : mean, SE,  $l\ g^{-1}\ d^{-1}$ ) and efflux rate constants ( $K_{ew}$ : mean, SE,  $d^{-1}$ ) of different populations for Cd, Ag and Zn from solution at 18°C, and a comparison of constants between Blackwater populations at 18°C (Blackwater 18) and 10°C (Blackwater10, from Rainbow et al. 2009a). Constants sharing a common letter in any comparison are not significantly different ( $P>0.05$ ) by Tukey's *post hoc* test.

	Uptake $K_u$				$K_{ew}$			Efflux		% 'slow' pool	
	Mean	SE	n	Tukey's	Mean	SE	Tukey's	Half life (d)	Mean	SE	n
<b>Cadmium</b>											
Puente Zuazo	0.0410	0.0057	20	A	0.0184	0.0039	A	41.8	62.8		15
Río San Pedro	0.0243	0.0040	19	A, B	0.0277	0.0037	A	31.2	34.3		12
Blackwater	0.0117	0.0016	21	B, C	0.0175	0.0036	A	50.0			16
Trocadero	0.0100	0.0015	22	C	0.0163	0.0036	A	76.5			17
Blackwater 10	0.0134	0.0015	15	A	0.0184	0.0018	A				14
Blackwater 18	0.0117	0.0016	21	A	0.0175	0.0036	A				16
<b>Silver</b>											
Puente Zuazo	0.7775	0.3514	18	A	0.0519	0.0072	A	18.1	63.6		17
Blackwater	0.6717	0.4427	14	A	0.0553	0.0032	A	13.7			22
Río San Pedro	0.4971	0.1664	9	A	0.0683	0.0117	A	15.0			12
Blackwater 10	2.7856	0.6887	15	A	0.0436	0.0058	A				10
Blackwater 18	0.6717	0.4427	14	A	0.0553	0.0032	B				22
<b>Zinc</b>											
Blackwater	0.0769	0.0137	22	A	0.0422	0.0036	B	19.1	73.6	12.9	24
Río San Pedro	0.0557	0.0211	10	A, B	0.0469	0.0040	A, B	15.2	73.5		5
Puente Zuazo	0.1748	0.0416	20	B	0.0728	0.0065	A	11.6	59.1		20

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Blackwater 10	0.1021	0.0346	17	A	0.0359	0.0024	A	17
Blackwater 18	0.0769	0.0137	22	A	0.0422	0.0036	A	24

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Table 4. *Nereis diversicolor*: Assimilation efficiencies (AE: mean, SE) and efflux rate constants ( $K_{\text{esed}}$ : mean, SE,  $\text{d}^{-1}$ ) of different populations for Cd, Ag and Zn at 18°C, and a comparison of constants between Blackwater populations at 18°C (Blackwater 18) and 10°C (Blackwater10, from Rainbow et al. 2009a). Other details as for Table 3.

	Assimilation				$K_{\text{esed}}$	SE	Efflux		
	AE (%)	SE	n	Tukey's			Half time	n	Tukey's
<b>Cadmium</b>									
Río San Pedro	60.9	7.8	6	A	0.0229	0.0042	39.2	6	A
Puente Zuazo	47.1	14.2	4	A,B	0.0186	0.0079	33.5	4	A
Blackwater	20.9	5.9	4	B	0.0280	0.0080	31.1	4	A
Blackwater 10	50.7	5.1	7	A	0.0232	0.0029		7	A
Blackwater 18	20.9	5.9	4	B	0.0280	0.0080		4	A
<b>Silver</b>									
Río San Pedro	28.2	3.4	2	A	0.0917	0.0481	5.2	3	A
Puente Zuazo	26.6	2.6	2	A	0.1909	0.1168	5.8	2	A
Blackwater	13.4	0.4	2	A	0.0193		36.0	1	A
Blackwater 10	34.4	7.2	10	A	0.0835	0.0165		9	A
Blackwater 18	13.4	0.4	2	A	0.0193			1	A
<b>Zinc</b>									
Blackwater	67.8	9.2	6	A	0.0331	0.0033	21.8	6	A
Río San Pedro	54.5	12.7	5	A	0.0512	0.0099	18.9	5	A
Puente Zuazo	40.9	6.5	4	A	0.0467	0.0106	17.0	4	A
Blackwater 10	45.4	14.8	4	A	0.0059	0.0098		4	A
Blackwater 18	67.8	9.2	6	A	0.0331	0.0033		6	B

Table 5. Biodynamic modelling of accumulated Ag, Cd and Zn concentrations in *Nereis diversicolor* collected from 2 Spanish and 1 UK estuaries in 2007. (g: growth rate constant ( $0.02 \text{ d}^{-1}$ ); AE: assimilation efficiency; IR: ingestion rate; OM: organic matter).

	mean dry wt g	Accumulation from water				Accumulation from ingested sediment					Contribution to accumulation		Predicted steady state concn $\mu\text{g g}^{-1}$
		$K_u$ $\text{L g}^{-1} \text{d}^{-1}$	Concn $\mu\text{g L}^{-1}$	$K_{ew}$ $\text{d}^{-1}$	$K_{ew} + g$ $\text{d}^{-1}$	AE	IR of OM $\text{g OM/g/d}$	Concn in sediment $\mu\text{g g}^{-1}$	$K_{esed}$ $\text{d}^{-1}$	$K_{esed} + g$ $\text{d}^{-1}$	water %	sediment %	
<b>CADMIUM</b>													
Blackwater	0.0172	0.0117	0.01	0.0175	0.0375	0.209	0.183	1.95	0.0280	0.048	0.2	99.8	1.56
Río San Pedro	0.0255	0.0243	0.01	0.0277	0.0477	0.609	0.171	0.60	0.0229	0.0429	0.3	99.7	1.46
Puente Zuazo	0.0180	0.0410	0.01	0.0184	0.0384	0.471	0.181	0.56	0.0186	0.0386	0.9	99.1	1.25
<b>SILVER</b>													
Blackwater	0.0172	0.6717	0.01	0.0553	0.0753	0.134	0.183	0.46	0.0193	0.0393	23.7	76.3	0.38
Río San Pedro	0.0255	0.4971	0.01	0.0683	0.0883	0.282	0.171	0.37	0.0917	0.1117	26.1	73.9	0.22
Puente Zuazo	0.0180	0.7775	0.01	0.0519	0.0719	0.266	0.181	0.98	0.1909	0.2109	32.6	67.4	0.33
<b>ZINC</b>													
Blackwater	0.0172	0.0769	0.3	0.0422	0.0622	0.678	0.183	93	0.0331	0.0531	0.2	99.8	218
Río San Pedro	0.0255	0.0557	0.3	0.0469	0.0669	0.545	0.171	101	0.0512	0.0712	0.2	99.8	132
Puente Zuazo	0.0180	0.1748	0.3	0.0728	0.0928	0.409	0.181	121	0.0467	0.0667	0.4	99.6	135

Table 6. *Nereis diversicolor*: Comparisons of predicted (from Table 5) and measured concentrations of Cd, Ag and Zn ( $\mu\text{g g}^{-1} \pm 95\%$  CL dry weight) in worms from sampling sites.

	Cd		Ag		Zn	
	Predicted	Measured	Predicted	Measured	Predicted	Measured
<i>Nereis diversicolor</i>						
Blackwater	1.56	<0.2 - <0.8	0.38	$1.38 \pm 0.44$	218	$237 \pm 26$
Río San Pedro	1.46	$0.22 \pm 0.00$	0.22	$0.30 \pm 0.04$	132	$152 \pm 15$
Puente Zuazo	1.25	$0.21 \pm 0.03$	0.33	$0.30 \pm 0.00$	135	$160 \pm 22$



Table 7. Selected values of the accumulation biodynamic parameters used to generate model predictions of accumulated metal concentrations in *Nereis diversicolor*.

Parameter	Cd	Ag	Zn
$K_u$ ( $l\ g^{-1}d^{-1}$ )	0.015	3.0	0.07
$K_{ew}$ ( $d^{-1}$ )	0.025	0.05	0.04
AE	0.50	0.35	0.50
$K_{esed}$ ( $d^{-1}$ )	0.02	0.06	0.04
$g$ ( $d^{-1}$ )	0.02	0.02	0.02
IR ( $g\ OM\ g^{-1}d^{-1}$ )	0.175	0.175	0.175
$C_w$ ( $\mu g\ l^{-1}$ )	0.01	0.01	0.3

Table 8. *Nereis diversicolor*: Model-predicted metal concentrations using the chosen values of the parameters listed in Table 7, compared with measured concentrations in worms from western European and Moroccan estuaries ( $\mu\text{g g}^{-1}$  dry body weight).

	Cd		Ag		Zn	
	Predicted	Measured	Predicted	Measured	Predicted	Measured
Rio San Pedro	1.32	0.22	0.71	0.3	148	152
Puente Zuazo	1.23	0.21	1.18	0.3	177	121
Blackwater <sup>a</sup>	4.27	<0.2-<0.8	0.78	1.38	136	237
East Looe <sup>a</sup>	1.58	<1.6-<2.7	0.78	2.48	97	294
Gannel <sup>a</sup>	3.04	<0.8-<1.9	0.72	0.68	366	258
Restronguet Creek <sup>a</sup>	6.24	2.0	1.50	4.82	4769	1925
Tavy <sup>a</sup>	4.12	2.6	0.82	1.32	308	472
West Looe <sup>a</sup>	3.09	<1.1-<4.2	1.31	2.9	151	366
Bay of Somme <sup>b</sup>	0.11	0.05			153	105
Boulogne harbour <sup>b</sup>	0.13	0.06			129	88
Seine estuary <sup>c</sup>	1.10	1.6	1.50	16	123	334
Authie estuary <sup>c</sup>	0.53	0.1	1.04	1.3	60	212
Mylor Creek <sup>d</sup>	1.99	0.91	2.57	2.8	304	208
Cowlands Creek <sup>d</sup>	0.38	0.17	1.12	0.9	318	218
Truro River <sup>d</sup>	3.15	1.44	7.70	9.5	568	389
Tressilian River <sup>d</sup>	2.85	1.3	1.58	1.5	435	298
St Just Creek <sup>d</sup>	1.56	0.71	0.66	0.3	228	156
Hayle <sup>d</sup>	1.03	0.47	4.49	5.3	380	260
Tamar <sup>d</sup>	1.16	0.53	0.89	0.6	261	179
Avon <sup>d</sup>	0.31	0.14	0.51	0.1	288	197
Ría de Plencia <sup>e</sup>			0.96	0.30	177	181
Oualidia lagoon <sup>f</sup>			0.89	0.24	152	115
Khniiffis lagoon <sup>f</sup>	0.81	1.00	0.66	0.11	102	94
Bidasoa River <sup>g</sup> st.2			1.42	1.40		
Bidasoa River <sup>g</sup> st.4			0.96	1.30	320	186

<sup>a</sup> Rainbow et al. (2009a)

<sup>b</sup> Berthet et al. (2003)

<sup>c</sup> Amiard et al. (2009)

<sup>d</sup> Bryan and Gibbs (1983)

<sup>e</sup> Saiz-Salinas & Francés-Zubillaga (1997)

<sup>f</sup> Idardare et al. (2008)

<sup>g</sup> Saiz-Salinas et al. (1996)

Figure 1.

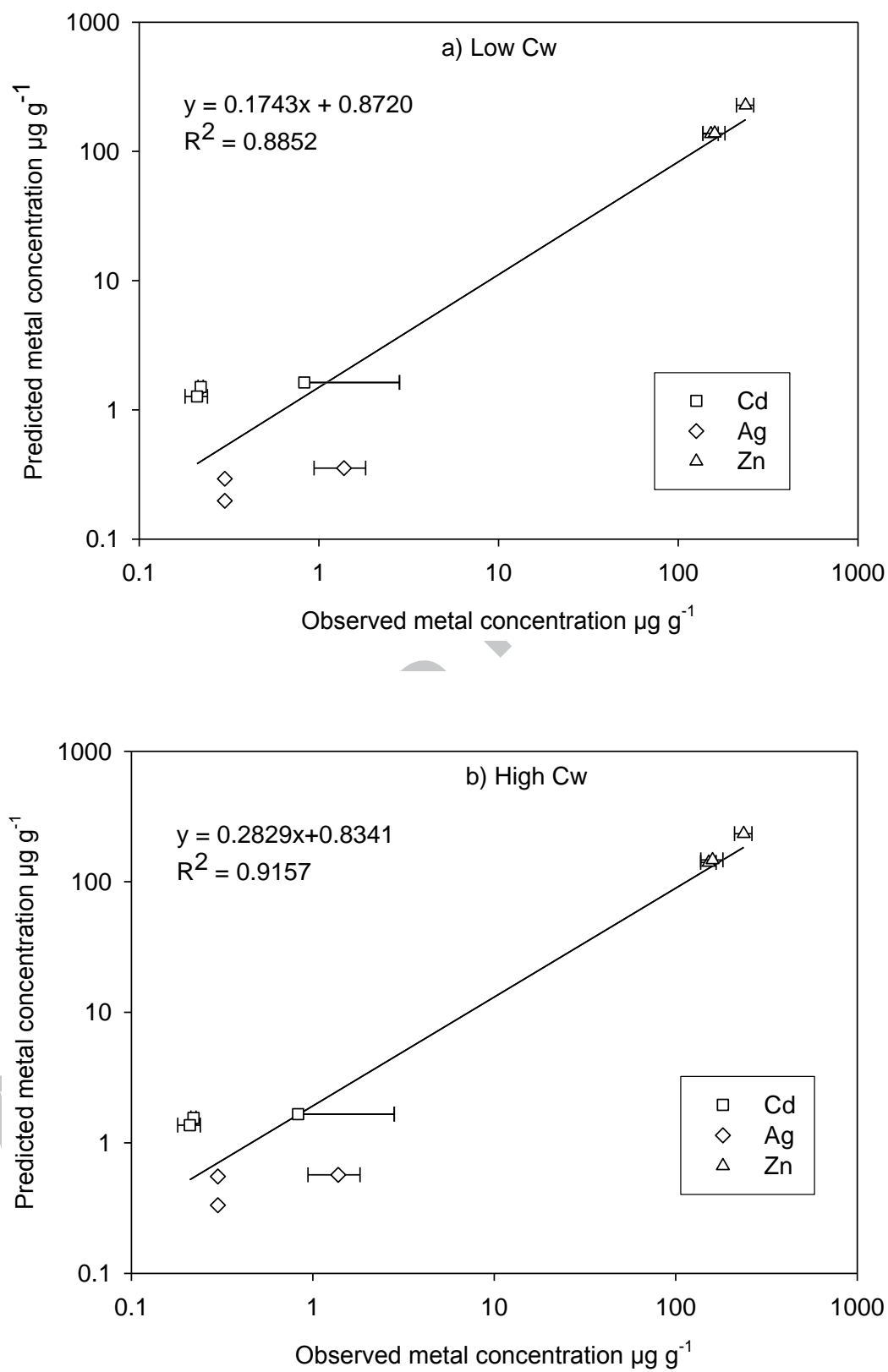


Figure 2.

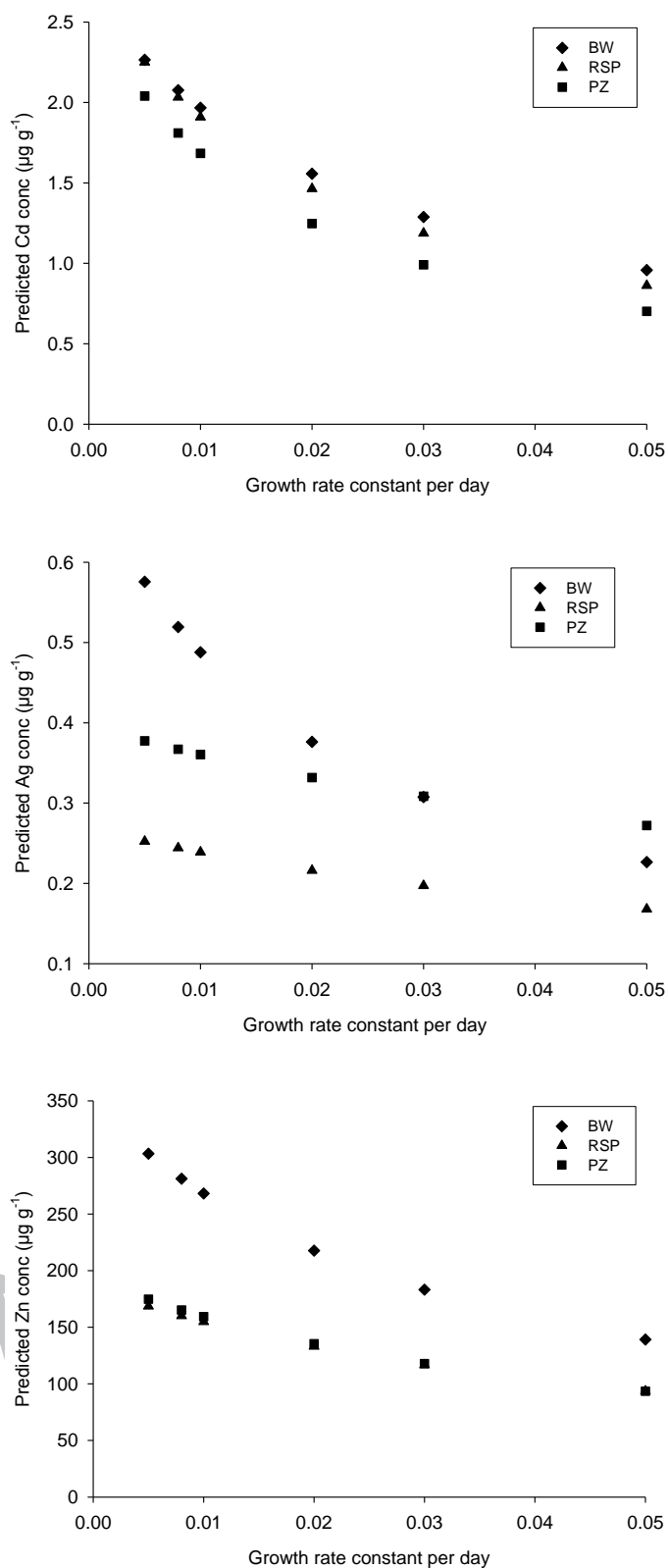


Figure 3.

