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Biodynamic modelling and the prediction of accumulated trace metal concentrations in the polychaete *Arenicola marina*

M. Carmen Casado-Martinez [a,*], Brian D. Smith [a], T. Angel DelValls [b], Samuel N. Luoma [c], Philip S. Rainbow [a]

[a] Department of Zoology, The Natural History Museum, Cromwell Rd, London SW7 5BD, UK
[b] Unesco UNITWIN Wicop Chair, Department of Physical-Chemistry, University of Cadiz, Poligono Industrial Rio San Pedro s/n, C.P. 11510 Puerto Real (Cadiz), Spain
[c] John Muir Institute of the Environment, University of California at Davis, Davis, CA 95616, USA

Biodynamic modelling predicts accumulated field concentrations of Ag, Cd and Zn in the deposit-feeding polychaete Arenicola marina.

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ABSTRACT

The use of biodynamic models to understand metal uptake directly from sediments by deposit-feeding organisms still represents a special challenge. In this study, accumulated concentrations of Cd, Zn and Ag predicted by biodynamic modelling in the lugworm *Arenicola marina* have been compared to measured concentrations in field populations in several UK estuaries. The biodynamic model predicted accumulated field Cd concentrations remarkably accurately, and predicted bioaccumulated Ag concentrations were in the range of those measured in lugworms collected from the field. For Zn the model showed less but still good comparability, accurately predicting Zn bioaccumulation in *A. marina* at high sediment concentrations but underestimating accumulated Zn in the worms from sites with low and intermediate levels of Zn sediment contamination. Therefore, it appears that the physiological parameters experimentally derived for *A. marina* are applicable to the conditions encountered in these environments and that the assumptions made in the model are plausible.

1. Introduction

Biodynamics is becoming the preferred approach among the scientific community to study metal accumulation in aquatic organisms (e.g. Chong and Wang, 2001; Griscom et al., 2002; King et al., 2005; Luoma and Rainbow, 2005; Xie et al., 2008). The development of the key parameters of metal uptake and efflux from dietary and dissolved pathways for a determined species allows the construction of mechanistic models that describe the relative importance of each of these exposure pathways. Since these parameters are measured under relatively controlled conditions, they are readily comparable between studies and species (e.g. Wang and Fisher, 1999). Predictions of bioaccumulation from the model also agree well with independent observations from the field. Luoma and Rainbow (2005) showed strong agreement between observations and predictions across diverse data sets, validating the use of biodynamic models for 7 metals and 14 species of freshwater and marine organisms, including bivalves, crustaceans, insects and fish.

Biodynamic models thus represent a definite upgrade from previous models used to characterise metal exposure and effects in risk assessment, for which water was considered to be the main source of metals to aquatic invertebrates (see discussion by Luoma and Rainbow, 2008). Integration of biodynamic models into environmental risk assessment methods would also benefit from additional field measurements and additional physiological data for species that have not been studied to date.

The use of biodynamic models to understand metal uptake directly from sediments by deposit-feeding organisms represents a special challenge. Feeding and digestion strategies differ among deposit-feeding species (Levinton, 2001), ingestion rates are challenging to determine (Cammen, 1980), and simple concepts are lacking for addressing metal bioavailability from sediments (Griscom et al., 2002). Polychaetes comprise an important proportion of the total biomass of deposit-feeding aquatic benthic invertebrates and are key species of the infaunal benthic community in coastal and estuarine sediments. Biodynamic models have been developed for a few species of annelids (Selck et al., 1998; Wang et al., 1999; Rainbow et al., 2009). We recently described the relative importance of the pathways of trace metal uptake in the lugworm *Arenicola marina* (Casado-Martinez et al., 2009), showing that dietary uptake is the prevalent exposure route for the metal

* Corresponding author. Tel.: +44 (0) 20 7942 5565; fax: +44 (0) 20 7942 6126.
E-mail address: c.casado-martinez@nhm.ac.uk (M.C. Casado-Martinez).

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contaminants studied. In the present paper, we validate this model by comparing biodynamic model predictions with independently measured bioaccumulation data from the field. The results are further discussed in relation to the physiological constraints for this deposit-feeding polychaete and the geochemical properties and characteristics found in sediments from different metal-contaminated UK estuaries.

2. Materials and methods

2.1. Field data

For collection of lugworms we selected different UK estuaries characterised by a well reported history of trace metal contamination and bioavailability in their sediments (e.g. Bryan et al., 1985; Rainbow et al., 1999, 2002): the Gannel and Lynher in Cornwall in southwest England, Poole Harbour in the south coast of England, Dulas Bay in Anglesey, north Wales, and the outer Thames in southeast England (Table 1). *A. marina* were collected by digging in the littoral zone and transported back to the laboratory in cool boxes in sediment from the collection site. Once in the laboratory the worms were kept for 48 h in artificial sea water (TM Tropic Marin, Tropicarium Buchshlag, Dreieich, Germany) at a salinity of 33 at 10 °C to empty the gut. Following this depuration period, lugworms were frozen after drying off excess water with tissue, before drying to constant weight at 60 °C and digestion in concentrated nitric acid (Aristar grade, Merck) at 100 °C. Each digest was made up to a known volume with double distilled water for trace metal analysis.

A sample of the oxic surface sediment from which the lugworms were collected was taken for characterisation of physicochemical properties. Sediment samples were sieved through a 125 μm stainless steel mesh with distilled water, and the two separated fractions dried to constant weight at 60 °C to provide an estimate of the proportion of fines in the sample. The fine fraction was ground to a fine powder with mortar and pestle. Replicates (0.5 g) of the fine fraction were digested for metal analysis by refluxing in 10 ml of concentrated HNO_3, before evaporation to dryness and reconstitution in 0.6 N HCl for metal analysis. This extraction is relatively strong, but it has the disadvantage of not providing a complete dissolution of the sediment (Hornberger et al., 1999). Further 0.5 g replicates of the fine sediment samples were extracted in 10 ml of 0.6 N HCl for 2 h at room temperature to provide an estimate of relatively easily extracted metal, as a model for potentially bioavailable metals. Organic matter content was estimated as weight loss after ignition at 550 °C for 30 min.

Analysis of lugworm and sediment digests for Ag, Cd, Fe, Mn and Zn was carried out on a Vista-Pro CCD simultaneous inductively coupled plasma-optical emission spectrometer (ICP-OES), with additional analyses for Cd and Fe by flame atomic absorption spectrometry (Varian SpectrAA 220 FS spectrophotometer with background correction as appropriate) if concentrations were out of concentration range for ICP-OES. Comparative samples of mussel tissue (ERM CE278, Institute of Reference Materials and Measurements, Geel, Belgium) and blanks were processed and analysed simultaneously, and agreement is considered satisfactory (Table 2). All metal concentration data are expressed in terms of dry weight.

The individual size of the organisms varied significantly between collection sites (see Table 1). Data sets were examined for any effect of body size on accumulated metal concentrations in the lugworms. Size effects were observed for Cd (Fig. 1) and Ag, for which concentrations decreased with increasing dry body weight of the organism, but not for Zn. Thus, the concentrations of the former two metals in lugworms were expressed as the concentration in a standard-sized organism of 0.3 g dry weight as estimated from the best-fit least squares regression of the log–log plot of metal concentration against individual dry weight for each population. Concentrations of Zn in the lugworms are expressed as means.

For Zn, correlation analyses of the relationships between bioaccumulated and sediment concentrations were performed after logarithmic transformation of the individual concentration data. In the case of Cd, differences in the bioaccumulated concentrations in the worms between sites were potentially caused by differences in the size of the worms from the different sites. To use single estimated Cd concentrations in a standard-sized worm in any correlation analysis would erroneously give equal weight to data from each site even if these estimations were derived from regressions using different numbers of worms. Therefore, to account for differences in sample size (number of organisms) between collection sites, an artificial data set was created from modelled normal distributions derived from the mean and standard error for each estimated Cd concentration in the standard-sized worm; *n* data points were then randomly sampled from each of these modelled normal distributions where *n* is the number of worms sampled in each population, and these data points were logged and regressed against logged sediment concentration at the relevant site to give a regression coefficient. This process was repeated 100 000 times, and the mean regression coefficient with 95% CL calculated. Correlations were considered significant if the value 0 was not included in the confidence interval. These calculations were done using the MATHEMATICA program. There were insufficient data for Ag to perform such correlations. Subsequent correlation analyses, carried out as above, also considered possible relationships between bioaccumulated metals and other sediment characteristics. We have quoted those worthy of mention when appropriate.

All statistical analyses were performed on log-transformed data except percentages, which were arcsine-transformed, using the STATISTICA program, with a significance level of 0.05.

Table 1
Collection details of *Arenicola marina* from UK estuaries.

Site	Co-ordinates	Collection date	Individual dry weight range
Dulas Bay	053°22.30N 04°16.73W	11 June 2008	0.12–0.58
Gannel estuary	050°20.50N 05°05.93W	23 May 2008	0.085–0.18
Lynher estuary	050°20.50N 04°15.96W	23 May 2008	0.32–1.13
Poole Harbour	050°41.35N 01°56.42W	9 May 2008	0.15–0.54
Thames estuary	051°31.92N 00°37.62E	11 May 2008	0.054–0.23

Table 2
Measured and certified concentrations (μg g^{-1} dry weight ± 95% CL) of trace metals from mussel tissue standard reference material (ERM CE278).

	Measured	Certified
As	5.79 ± 0.03	6.07 ± 0.13
Cd	0.28 ± 0.01	0.35 ± 0.01
Cr	0.81 ± 0.01	0.78 ± 0.06
Cu	7.99 ± 0.17	9.45 ± 0.13
Mn	6.44 ± 0.14	7.69 ± 0.23
Pb	2.22 ± 0.03	2.00 ± 0.04
Zn	71.0 ± 1.73	83.1 ± 1.7

2.2. Modelling trace metal accumulation in A. marina

Biodynamic models estimate steady state concentrations that result from a balance of uptake rate from diet, uptake rate from solution, and loss rates of metal accumulated from either source, incorporating a growth rate constant where appropriate (Luoma and Rainbow, 2005). Accumulation from solution is defined by an uptake rate constant (k_u, in units μg g^{-1} d^{-1} per μg l^{-1} or l g^{-1} d^{-1}), the concentration in water (C_W in μg l^{-1}) and a rate constant of loss (k_{ew} in d^{-1}) of metal taken up from solution. Accumulation from food is defined by the concentration in food (C_F in μg g^{-1}), the ingestion rate (IR in g g^{-1} d^{-1}), the assimilation efficiency (AE) and the loss rate constant of metals assimilated from food (k_{ef} in d^{-1}). The final equation used to calculate a steady state bioaccumulated concentration (C_{SS}) in a determined organism after considering dilution due to growth (growth rate constant g in d^{-1}) is

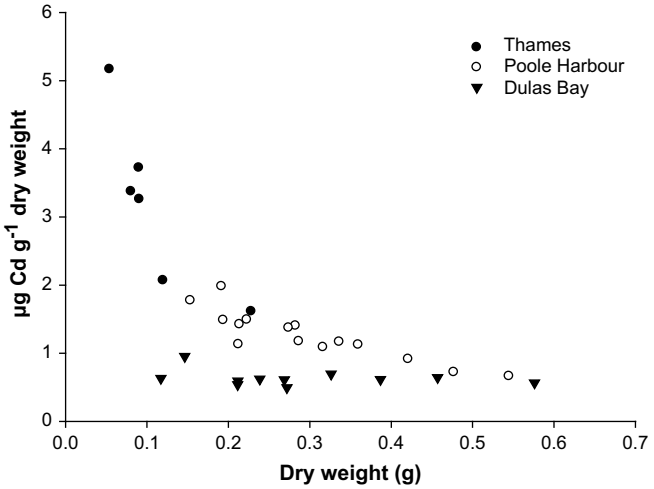

Fig. 1. Relationship between size and bioaccumulated concentration of Cd in field collected lugworms.

$$C_{SS} = [(k_u \times C_W)/k_{ew} + g] + \left[(AE \times IR \times C_F)/\left(k_{ef} + g\right)\right]$$

Table 3 presents the range of values for k_u, k_{ew}, AE and k_{ef} used to generate the model predictions. These parameters were calculated using standard laboratory experiments for Ag, Cd and Zn for *A. marina* for worms collected both from the Thames site and from another site in Northumberland, England (Casado-Martinez et al., 2009). The growth rate constant has been obtained from the available literature (Taghon, 1988; WO/2003/007701) and IR was estimated following the empirical model developed by Cammen (1980) for aquatic sediment-ingesting deposit-feeders according to the formula

$$IR = 0.435W^{0.771}OM^{-0.920}$$

where OM is the fraction of organic material in the ingested sediment and W is the dry body weight of the organism (in mg). The calculated IR was expressed as grams of OM ingested per grams of organism per day, considering that lugworms digest the organic matter component of the sediment fine fraction (<125 μm) for assimilation. Correspondingly, the metal concentration of the ingested food that is appropriate for the model would be the concentration of metal in this organic component. Metal in oxidised sediment is distributed between organic and inorganic components, particularly iron and manganese oxides (Luoma and Rainbow, 2008; Simpson and Batley, 2007). It is not valid to assume that all metal in the sediment is associated with organic matter, nor is it valid to assume that no metal is associated with sedimentary organic matter. The default assumption made here then is that the different sedimentary components have approximately similar affinities for the metals, and thus the same metal concentrations (content per unit weight of that component). This concentration is necessarily the same as metal concentration of the total sediment (<125 μm). Our estimate of the metal concentration of the organic component of the sediment (C_F) is therefore the metal sediment concentration measured in the fine fraction at each site. As for dissolved concentrations of the metals (C_W), we used the range of concentrations measured in UK estuaries for Cd and Zn (Luoma and Rainbow, 2008). In the absence of a broad database of concentrations of Ag in solution in UK estuaries, we used the range 0.006–0.03 μg Ag l^{-1} (Smith and Flegal, 1993; Ranville and Flegal, 2005).

3. Results

3.1. Field measurements

The concentrations of Zn, Cd and Ag in *A. marina* and sediments from the different estuaries are presented in Tables 4 and 5. Cd and Ag data are also quoted as the estimated concentrations for a standard-sized organism of 0.3 g dry weight for comparison purposes.

3.1.1. Zn

Zn concentrations in individual specimens of *A. marina* from the different collection sites ranged from 43.3 to 141 μg g^{-1} (Table 4). Lugworms from Dulas Bay showed the highest accumulated mean (±SE) concentration (89.6 ± 4.2 μg g^{-1}), significantly raised above that in Poole Harbour (55.6 ± 1.9 μg g^{-1}). After Dulas Bay there was a sequence of mean accumulated Zn concentrations from the Gannel (76.2 ± 2.5 μg g^{-1}) to the Lynher (62.5 ± 2.67 μg g^{-1}). The individual accumulated Zn concentrations in lugworms from Poole Harbour and the Thames varied more widely (Table 4), and the mean accumulated concentration for the Thames population (70.4 ± 0.52 μg g^{-1}) was higher, but not significantly so, than that for Poole Harbour.

Table 4
Concentrations (μg g^{-1} dry body weight) of Ag, Cd and Zn measured in field collected lugworms. Summary data for Zn are expressed as means with 95% confidence limits; those for Cd and Ag as the concentration in a 0.3 g dry weight worm with 95% CL as estimated from a power regression of accumulated concentration against dry weight. See text for further details.

Site		Ag	Cd	Zn
Dulas Bay ($n = 10$)	Max	0.30	0.95	141
	Min	0.05	0.49	68.4
	Mean			89.6
	95% CL			85.2–94.0
	Estimated	0.10	0.62	–
	95% CL	0.09–0.12	0.57–0.66	
Gannel ($n = 4$)	Max	0.25	2.05	81.9
	Min	<0.10	1.29	70.1
	Mean			76.2
	95% CL			77.9–81.2
	Estimated	–	0.97	
	95% CL		0.23–4.10	
Lynher ($n = 4$)	Max	0.87	1.27	69.8
	Min	0.81	0.60	57.2
	Mean			62.5
	95% CL			60.7–64.3
	Estimated	0.09	0.28	–
	95% CL	0.08–0.09	0.84–1.95	
Poole ($n = 15$)	Max	<0.052	1.98	90.4
	Min	<0.018	0.66	43.3
	Mean			55.6
	95% CL			53.5–57.7
	Estimated	–	1.15	–
	95% CL		1.11–1.20	
Thames ($n = 6$)	Max	0.94	5.17	105
	Min	0.15	1.61	55.9
	Mean			70.4
	95% CL			65.5–75.3
	Estimated	0.14	1.18	–
	95% CL	0.08–0.26	0.96–1.47	

The increased Zn accumulated concentrations in the Dulas Bay lugworms (Table 4) were associated with a mean HNO_3–Zn sediment concentration (612 μg g^{-1}) significantly greater than that of the other sites (Table 5). Simple linear regression analysis of log-transformed data showed that Zn concentrations in lugworms were significantly correlated with local sediment concentrations (both after HNO_3 and HCl extraction, $R^2 = 0.40$ and 0.42 respectively; $p < 0.05$; $n = 39$); almost certainly as a result of the high Dulas Bay concentrations. The accumulated Zn concentrations in the worms from the other sites, however, did not reflect the HNO_3–Zn concentrations in local sediments, with a sequence of mean HNO_3–Zn concentrations from the Lynher to Gannel, Poole, with the lowest mean concentration from the Thames (Table 5). Nor were the relatively higher bioaccumulated concentrations of Zn in lugworms from the Thames and the Gannel (Table 4) correlated with either concentrations of

Table 3
Ranges and/or mean values of the bioaccumulation parameters used to generate biodynamic model predictions of accumulated metal concentrations in *Arenicola marina* (from Casado-Martinez et al., 2009).

Parameter	Ag		Cd		Zn	
	Range	Mean	Range	Mean	Range	Mean
g (d^{-1})		0.02		0.02		0.02
k_u (l g^{-1} d^{-1})		1.2144		0.0120		0.0260
k_{ew} (d^{-1})		0.033		0.003		0.037
IR (g OM g^{-1} d^{-1})	0.078–0.120		0.078–0.120		0.078–0.120	
AE (%)	1–15	6.75	1–70	23.5	2–22	10.5
k_{ef} (d^{-1})	0.044–0.069	0.056	0.020–0.025	0.023	0.042–0.056	0.049
C_W (μg l^{-1})	0.006–0.03	0.01	0.01–0.1	0.035	0.35–5	2

Table 5
Sediment properties (percentage of sediment with grain size <125 μm and percentage organic matter (OM)) and concentrations of Cd, Ag and Zn in the fine fraction of the sediments after near total (HNO_3) and partial extraction (HCl) (μg g^{-1} dry weight, mean ± SE).

Site	% Age <125 μm	% Age OM	Ag		Cd		Zn	
			HNO_3	HCl	HNO_3	HCl	HNO_3	HCl
Dulas Bay	6.48	10.9	<0.040	<0.039 to <0.042	<0.14	<0.14	612 ± 7.36	347 ± 0.71
Gannel estuary	3.41	11.6	0.19 ± 0.040	<0.10	2.27 ± 0.02	2.74 ± 0.01	157 ± 4.27	96.7 ± 0.45
Lynher estuary	39.1	10.7	0.57 ± 0.016	0.13 ± 0.001	1.82 ± 0.04	1.87 ± 0.03	372 ± 4.60	198 ± 0.46
Poole Harbour	52.3	14.5	<0.098–0.12	<0.096 to <0.10	1.91 ± 0.30	2.25 ± 0.02	135 ± 8.58	74.4 ± 0.41
Thames estuary	57.8	3.03	0.15 ± 0.005	<0.099 to <0.10	1.52 ± 0.01	1.96 ± 0.04	50.7 ± 0.24	29.2 ± 0.05

HCl–Zn or raised proportions of Zn (57.6 and 61.2%, in the Thames and Gannel, respectively) in the sediments extractable with HCl in comparison with Poole (55.7%).

Zn accumulated concentrations showed a significant negative correlation with the proportion of fines in the local sediment ($R^2 = 0.62$; $p < 0.05$), and HNO_3 and HCl–Fe concentrations in the sediments ($R^2 = 0.33$ and 0.66, respectively; $p < 0.05$). On the contrary, a significant positive correlation was observed between Zn bioaccumulated concentrations and HNO_3 and HCl–Mn concentrations ($R^2 = 0.63$ and 0.67, respectively; $p < 0.05$).

3.1.2. Cd

Where detectable, Cd concentrations in individual *A. marina* ranged from 0.49 to 5.17 μg g^{-1} (Table 4). A clear size effect was observed in the bioaccumulated concentrations of Cd in lugworms from the Thames and Poole Harbour, for which concentrations decreased with increasing individual lugworm size, whereas Cd concentrations in lugworms from Dulas Bay, with Cd sediment concentrations below the detection limit, were maintained below 1 μg g^{-1} independently of size (Fig. 1).

Lugworms from the Thames showed a raised bioaccumulation of Cd after allowing for size effects, significantly different (ANCOVA) from the other populations. There was a decreasing sequence of estimated accumulated Cd concentrations in 0.3 g worms from the Thames to the Lynher population (Table 4).

HNO_3–Cd sediment concentrations varied over a lower range than Zn, with the highest concentration at the Gannel and the lowest in the Thames; sediment Cd concentrations at Dulas Bay were below the detection limit for the analytical technique (Table 5). Surprisingly the extraction of sediments with HCl produced concentrations above those after HNO_3 extraction with significant increases of 30% at the Thames and 20% at the Gannel ($p < 0.05$), and insignificant increases of 17% at Poole and 2% at the Lynher. It is possible that uncorrected interferences occurred in the HNO_3 digests, perhaps stemming from the high concentrations of major elements released by the strong decomposition of sediments by the concentrated acid.

The statistical analysis performed to allow for the different sample size at the different collection sites showed no statistical significant correlation after allowing for size effects between bioaccumulated Cd concentrations and concentrations in local sediments.

3.1.3. Ag

The concentrations of Ag in field collected lugworms ranged between concentrations below the detection limit (0.01 μg g^{-1}) and 0.94 μg g^{-1} dry weight (Table 4). Ag concentrations were always below the analytical detection limit in lugworms from Poole Harbour, whereas Ag concentrations in lugworms from the Thames varied over the whole range of measured concentrations, partly arising from size effects. After allowing for size effects, the estimated concentrations for a 0.3 g worm decreased from the Thames (0.14 μg g^{-1} dry weight) to the Lynher (0.09 μg g^{-1}dry weight); the difference between these two populations was statistically significant.

Dulas and Poole showed sediment concentrations of HNO_3–Ag below the detection limit, Thames and Gannel presented similar concentrations, and the highest concentrations were found at the Lynher (Table 5). Concentrations of HCl–Ag were only above the detection limit at the Lynher. No further statistical analyses were performed on Ag data due to the small database available.

3.2. Model predictions

The concentrations of accumulated metals predicted by the biodynamic model are presented in Table 6. Model predictions were calculated using single values of k_u and k_{ew} of 1.21 l g^{-1} d^{-1} and 0.033 d^{-1} for Ag, 0.012 l g^{-1} d^{-1} and 0.003 d^{-1} for Cd and 0.026 l g^{-1} d^{-1} and 0.037 d^{-1} for Zn. The range of values of k_{ef} was 0.044–0.069 d^{-1} for Ag, 0.020–0.025 d^{-1} for Cd and 0.042–0.056 d^{-1} for Zn, which is the range of values of the means of the two populations used in the laboratory experiments. Given the greatest sensitivity of metal predictions to variations in AEs (Casado-Martinez et al., 2009), we considered a range of values for this parameter of 1–15% for Ag, 1–70% for Cd and 2–22% for Zn, which correspond to the whole range of values found for individuals of *A. marina*. A mean AE of 6.75% for Ag, 23.5% for Cd and 10.5% for Zn was also used, corresponding to the mean of the means calculated individually for two different populations (Table 3).

We include an average predicted concentration, developed using the mean values of the parameters presented in Table 3 and the mean HNO_3- or HCl-extracted concentrations in sediments at each site. We include the minimum and maximum predicted bioaccumulated concentrations, which are calculated using a combination of different physiological parameters and concentrations in water. The minimum predicted bioaccumulated concentrations are generated using the minimum assimilation efficiency and minimum concentration in water and maximum efflux rate constants. Conversely, the maximum predicted concentrations are generated by using maximum assimilation efficiency and maximum concentration in water and minimum efflux rate constants.

The predicted concentrations are directly compared (Table 6) to measured concentrations and not with the estimated concentrations for a standard-sized organism, as the model allowed for size effects by using site-specific and worm-specific IRs.

3.2.1. Zn

The maximum and minimum possible Zn bioaccumulation predicted by the model covered about one order of magnitude. This is the maximum variability resulting from the use of the maximum and minimum values for physiological parameters and a broad range of dissolved concentrations. This range of maximum variability of predicted concentrations captured the order of magnitude of bioaccumulated concentrations found in the worms from the field, especially at sites with medium and high sediment concentrations of Zn (Fig. 2). Mean Zn concentrations in lugworms from

Table 6
Model-predicted metal concentrations in *Arenicola marina* using the ranges and mean values of the parameters as presented in Table 3 and sediment concentrations after extractions with HNO_3 and HCl compared with measured concentrations in lugworms from UK estuaries (μg g^{-1} dry body weight; see text for calculations).

		Ag		Cd		Zn	
		Predicted	Measured	Predicted	Measured	Predicted	Measured
Dulas Bay	HNO_3-extractable C_F					89.1 (14.1–218)	89.6 (68.4–141)
	HCl-extractable C_F					50.0 (9.2–125)	
Gannel estuary	HNO_3-extractable C_F	0.25 (0.12–1.20)	0.21 (0.17–0.25)	1.51 (0.07–4.29)	1.60 (1.29–2.05)	28.2 (4.5–69.2)	76.2 (70.1–81.9)
	HCl-extractable C_F			1.82 (0.08–5.17)		17.7 (3.2–43.5)	
Lynher estuary	HNO_3-extractable C_F	0.27 (0.12–1.26)	0.84 (0.81–0.87)	0.80 (0.037–2.27)	0.85 (0.60–1.27)	43.1 (6.8–106)	62.5 (57.2–69.8)
	HCl-extractable C_F	0.24 (0.05–1.17)		0.82 (0.04–2.33)		23.4 (4.2–57.3)	
Poole Harbour	HNO_3-extractable C_F			1.06 (0.05–3.03)	1.26 (0.66–1.98)	20.5 (3.3–50.2)	55.6 (43.3–90.4)
	HCl-extractable C_F			1.25 (0.06–3.55)		11.7 (2.1–28.7)	
Thames estuary	HNO_3-extractable C_F	0.24 (0.12–1.19)	0.59 (0.15–0.94)	0.95 (0.043–2.70)	3.20 (1.61–5.17)	9.1 (1.5–22.4)	70.4 (55.9–105)
	HCl-extractable C_F			1.22 (0.05–3.47)		5.7 (1.0–13.9)	

Dulas Bay and the Lynher estuary were very accurately predicted by the biodynamic model (Table 6) using mean values of the physiological parameters measured on Thames and Northumberland lugworms by Casado-Martinez et al. (2009), and the Zn sediment concentrations measured after HNO_3 extraction. For the other populations from estuaries with lower Zn sediment concentrations, average predicted concentrations were below the range of concentrations measured in field collected lugworms, but still of the same order of magnitude. In general, model predictions in the high range were more similar to the measured accumulated concentrations. The greatest differences between predictions and measured concentrations were observed for the Thames population, with predictions and field measurements within a factor of 8 for average values and within a factor of 2 for the highest forecasts.

3.2.2. Cd

Average Cd bioaccumulated concentrations were very accurately predicted (Table 6) by the model using mean values of the parameters of Casado-Martinez et al. (2009) and HNO_3-extracted sediment concentrations. Again, the greatest differences were shown for the Thames population, in which case only the predicted concentrations in the high range fell in the range of the measured concentrations. The average predictions were closer to the mean Cd accumulated concentrations when the concentration of Cd in sediment used in the model was that measured after weak extraction (Table 6).

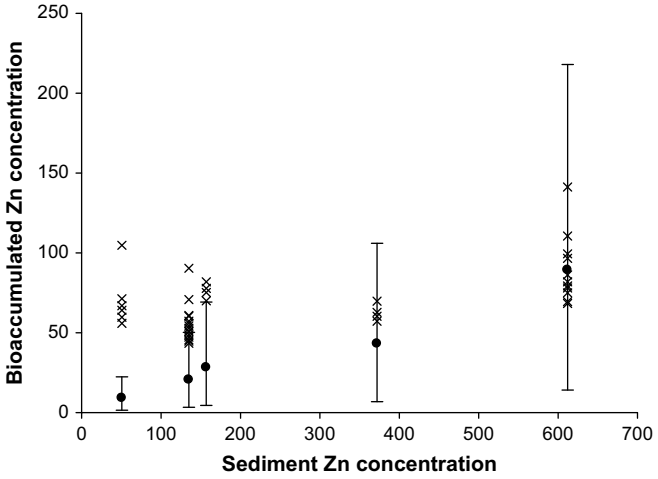

Fig. 2. Bioaccumulated Zn concentrations measured in field collected lugworms (×), and average and maximum ranges of concentrations predicted by the model plotted against sediment Zn concentrations. All values expressed as μg g^{-1} dry weight.

3.2.3. Ag

Average predicted bioaccumulated Ag concentrations were very similar at all sites, between 0.25 μg g^{-1} dry weight at the Thames and 0.27 μg g^{-1} dry weight at the Lynher. The model predictions when using the mean parameters of Casado-Martinez et al. (2009) and HNO_3-extracted sediment concentrations were very close to the mean measured concentration for the Gannel population (Table 6). The average predicted concentration was in the low range of the measured concentrations for the population in the Thames, although the predicted range fitted exactly the range of measured concentrations in field collected organisms. For the population at the Lynher, where the highest sediment and lugworm concentrations were measured, the model predictions were accurate when the extreme values of uptake and efflux rate constants were used.

4. Discussion

In this study Cd, Ag and Zn concentrations in lugworms predicted by biodynamic modelling were compared to independently measured concentrations in field populations from selected UK estuaries with different degrees of metal contamination. By definition models make assumptions, such as appropriate growth rate constants, ingestion rates, water and food concentrations chosen for incorporation into the model, and any goodness of fit between predicted and observed concentrations is itself a test of the validity of the assumptions made. Table 6 shows what is in effect excellent agreement between Ag, Cd and Zn accumulated concentrations in field collected lugworms and those predicted by the model, showing that the model is robust and the assumptions made potentially supportable. The factors and processes that may compromise the explanatory power of the metal include metal-specific chemistry, concentration, geochemical influences on bioavailability, exposure route, and species-specific physiological attributes (Luoma and Rainbow, 2005).

4.1. Metal concentrations and essentiality

The metal concentrations in sediments from the different collection sites varied over one order of magnitude, from baseline levels measured in relatively pristine estuaries to concentrations found at sites with moderate levels of contamination (Bryan and Langston, 1992). The highest sediment concentrations were measured for Zn (50–612 μg g^{-1}), although this range of sediment contamination was not reflected in the range of accumulated concentrations in the lugworms (43–141 μg g^{-1}). An increase in the mean Zn concentration accumulated in lugworms from Dulas Bay was observed, but this increase can be considered small for a difference in sediment concentrations of one order of magnitude. The model, using mean parameters and a concentration in water of

2 μg l^{-1}, predicts that the mean Zn bioaccumulated concentration in *A. marina* should change from 9 to 89 μg g^{-1} as sediment concentrations range from 50 to 612 μg g^{-1} (Table 6). Such a small change in predicted concentrations reflects the low k_u and the low AE of Zn from sediment for *A. marina*. Because of the individual to individual variability in Zn bioaccumulation observed in animals from the field, it is not surprising that such a small change in bioaccumulation would be difficult to detect without massive sampling sizes (a very high *n* is necessary to increase the power of the observed data).

In addition, the lugworms may hold a regulated body concentration of Zn to meet Zn needs. Zn concentrations as low as 9 μg g^{-1} are rarely if ever seen in aquatic organisms; thus the processes considered in the model would not account well for such a regulatory process. The model is well equipped to deal with increases beyond such a basal level, however. Other polychaetes such as *Nereis diversicolor* are able to regulate body concentrations of zinc to relatively constant body concentrations over a wide range of environmental Zn bioavailabilities and consequent uptake rates, until uptake rates are so great that regulation breaks down and net accumulation of Zn occurs (Bryan and Hummerstone, 1973; Bryan et al., 1980; Amiard et al., 1987; Rainbow et al., 2009). Our results suggest, as previously observed by Turner et al. (2008), that lugworms may be doing the same. The increase in bioaccumulation in response to the observed increase in sediment concentrations would, under such circumstances of regulation, be even smaller and more difficult to detect than stated above.

4.2. Physiological parameters of metal uptake and efflux

Ranges of values of the physiological parameters used in the biodynamic model were estimated according to now well-established approaches (Casado-Martinez et al., 2009). Uptake rate constants (k_u) were determined using short-term exposure to estimate unidirectional metal influx rates, and efflux rate constants (k_e) were those for the slow component of loss for metal accumulated from water and food separately. Assimilation efficiency was determined from the proportion of labelled metal remaining after defaecation of an ingested bolus of labelled sediment. Our results add to the body of recent literature on the validity of these empirical parameters for biodynamic modelling (Luoma and Rainbow, 2005).

Rainbow et al. (2009) showed that there is limited interpopulation variation in the biodynamic parameters controlling bioaccumulation of trace metals between populations of the polychaete *N. diversicolor* from metal-rich estuaries in SW England. In the present study, the range of values for the parameters of metal uptake was not experimentally derived specifically for each population. The parameters of metal uptake from solution were developed from a single population from Northumberland, and those for food were developed using two populations, that from Northumberland and that from the Thames. The results obtained here suggest that the range of values of these parameters derived experimentally for surrogate populations account for the major processes governing metal accumulation in lugworms, and that their use may be extended to other populations. They provide quantitative values for these constants that can be used in other studies of *A. marina*. On the other hand, population-specific values may have helped close the small gaps typically seen between predicted and observed bioaccumulation.

A sensitivity analysis has previously suggested that the strongest determining parameter for metal uptake from food by *A. marina* is AE (Casado-Martinez et al., 2009). In this study we used the range of values of AE determined in the laboratory from the proportion of labelled metal remaining in the body after defaecation of unassimilated metals from a single bolus of radiolabelled sediment previously ingested. AE varied over one order of magnitude between individuals, which is the range of variability found for metal release in gut juices extracted from *A. marina* (Mayer et al., 1996, 2001). AE can vary with the nature of the food (Wang and Fisher, 1999), but other biological and geochemical factors can determine different digestive release and uptake of metals from food in *A. marina* (Cheng et al., 2000), possibly leading to changes in AE. Intraspecific differences were accounted for by using AEs measured for two different populations, one from Northumberland and the one for the Thames, and both of them were fed sediments from the Thames. The mean of the means for both populations was used to develop average predictions, and seems representative of metal assimilation from sediment ingestion in different environmental conditions and populations.

4.3. Exposure routes of metals to lugworms

Our earlier conclusions on the pathways of metal uptake in the lugworm *A. marina* showed that more than 90% of the Zn and Cd and more than 70% of the Ag in lugworms is accumulated from sediment ingestion at environmental realistic concentrations (Casado-Martinez et al., 2009). This is in agreement with the positive relationships between bioaccumulated concentrations in lugworms and total sediment concentrations for Zn. Accumulated Cd concentrations were not significantly correlated with Cd concentrations in sediments, however. This is at least partly because the sediment Cd concentration differences among sites were small. A larger range would allow more statistical power in testing such a relationship. But model-predicted Cd uptake from sediment ingestion alone was sufficient to account for more than 90% of the total Cd accumulated by lugworms from the Gannel and Lynher and more than 80% for Poole and the Thames (for this last population only when maximum assimilation efficiency from sediment is considered, which offers the best agreement between predictions and measured concentrations). The model predicts, for mean conditions, that average Cd concentrations should only change from 0.82 to 1.82 μg g^{-1}dry weight in *A. marina* over this range of sediment concentrations (Table 6), very similar to what was observed in nature.

If we perform this same analysis for Ag, the proportion of metal accumulated from sediment ingestion accounts for 3–10% of the total average concentration in field collected organisms, a percentage that is not increased by adjusting the AE to the maximum values found by Casado-Martinez et al. (2009). In the range of sediment concentrations in this study, only an increase of the dissolved concentrations increases the concentrations predicted by the model to the range of concentrations measured in field collected lugworms, compatible with the high k_u for this metal. Furthermore, the model is accurate using low dissolved concentrations with low sediment concentrations (i.e. Gannel) and high dissolved concentrations with high sediments concentrations (i.e. Lynher). This may indicate that solution may have a higher contribution to the total Ag accumulated by lugworms than previously expected, and that it is probable that lugworms are exposed to relatively high dissolved concentrations in the water passing through their burrows. Consequently, it may be wise to test site-specific dissolved concentrations to improve the performance of the model for Ag.

4.4. Organism feeding and sediment properties

According to the relative importance of the pathways of Cd and Zn uptake, the strongest determinants for the model are those related to the uptake of metals from sediment ingestion. Uptake rate from diet is determined by feeding rate, AE and metal concentration in food. In our model, feeding rate was assumed to

refer to the amount of organic matter ingested as calculated by the model of Cammen (1980). This model was based on observations of the inverse correlation between organic matter content and the ingestion of total dry material, dependent on organism size. Our field measurements showed that metal concentrations of Cd and Ag are determined by body size, and this factor can be effectively accounted for in biodynamic models by calculating IR for a specific size of organism. In our study, IR calculated for the average dry weight of lugworms from each population resulted in model predictions readily comparable with bioaccumulated concentrations.

By expressing IR in terms of OM and the sediment concentrations as those measured after extraction of the fine fraction, we are assuming that lugworms feed preferentially on the OM present in fine sediments, that approximately the metals bound to this fraction are available for uptake, and that metals are distributed with similar affinity between the organic and inorganic sedimentary components binding the metals. In oxidised estuarine sediments Cd, Zn and Ag are associated preferentially with Fe oxides, Mn oxides and organic materials in different proportions (Luoma and Bryan, 1981), but it is still difficult to determine the exact distribution of metals among phases (Luoma and Rainbow, 2008). We assume that it is a valid assumption to consider that metals are distributed across sediment metal-binding components rather than distributed exclusively in organic material. The latter assumption would imply that the same quantity of metal measured in the fine sediment is concentrated in a small proportion of material (only OM), increasing considerably the metal concentration available to lugworms. The result would be greatly increased predicted metal concentrations out of line with the measured concentrations. Our results suggest that our assumptions are tenable.

Different studies have tried to understand the factors that determine the bioavailability of sediment-bound metals to sediment-ingesting deposit-feeders such as *A. marina*. We performed statistical analyses on the field measurements to check whether the partitioning of metal among different binding substrates in sediments is affecting metal bioavailability and uptake by lugworms, specifically considering organic matter and both HNO_3 and HCl extractable phases of Fe and Mn, but the limited number of estuaries, the narrow sediment concentration ranges for these estuaries and the small responses predicted by the model prevented the establishment of definite relationships, and none of the normalizations used to express the sediment concentrations showed a significantly better correlation than raw data (data not shown). A broad database of metal accumulated concentrations in lugworms for a larger number of estuaries may bring out specific influences of such differences in metal form in the sediments (e.g. Luoma and Bryan, 1981). But it is interesting that under these same conditions, the biodynamic model did capture the appropriate magnitude of bioaccumulation and critical aspects of the variability among the estuaries, without considering the form of metal in the sediment.

Among all the sites considered in this study, the largest differences between model predictions and measured concentrations were observed at the Thames site. Field measurements showed a higher bioavailability of metals for this population than for other populations living at sites with significantly higher metal concentrations in local sediments. This seems partly explained by a higher capacity of lugworms to assimilate metals from ingested sediment, as the model accuracy improves when using AEs in the high range. Indeed, lugworms from the Thames population showed a higher assimilation efficiency of metals than lugworms from Northumberland when fed the same sediment, in this case the local sediment from the Thames (Casado-Martinez et al., 2009). The population at the Thames site feeds on sediments with a considerably lower quality (lower OM content), although we took into account this factor in the calculation of site-specific IR. Whether this higher efficiency in assimilating metals from sediments is the result of long-term adaptation of lugworm populations to environments with low OM content is not known (Cammen, 1980), as it is not known if these site-specific differences in metal availability can be associated with a different distribution of metals among the different organic and inorganic phases of the sediment, leading to different exposure availabilities to the lugworms.

Another possibility is the size effect. We took into consideration this effect for comparative purposes by expressing the bioaccumulated concentrations in a standard size organism, while the model accounts for this factor through the calculation of IRs for a specific weight of organism. As can be observed in Fig. 1, the size of the lugworms collected from the Thames is in the low range for all populations, and it is possible that both the model to calculate IR and the correction to a standard-sized worm are not capable of capturing the variability in this extreme part of the curve, where bioaccumulated concentrations increase sharply for a small range of body weight. This would explain why the model prediction using average values of the parameters are close to the bioaccumulated concentration of Cd for a standard-sized organism but below the average bioaccumulated concentrations of Cd in small field collected organisms.

5. Conclusions

A biodynamic model has been used to predict accumulated concentrations of Ag, Cd and Zn in populations of the polychaete *A. marina* in metal-contaminated UK estuaries, showing a relatively robust agreement between predicted and measured bioaccumulated concentrations. Maximum variability of modelled concentrations can be as much as one order of magnitude, but captures the data range of the field observations. Mean coefficients are relatively robust in predicting field concentrations; therefore the parameters experimentally derived for *A. marina* are applicable to the conditions encountered in these environments. The model predicts responses expected from changes in sediment concentrations seen here for Cd and Zn, where sediment is the dominant source of metal. In both cases, responses in lugworms from the field would be difficult to detect because Zn bioaccumulation would vary over only a small range, and the range of Cd concentrations in sediments was small. Ag predictions were in the range of the measured accumulated concentrations, although the model suggests that solution may have a higher contribution to the total Ag accumulated by lugworms than previously expected. In this case, the accuracy of predicting may be diminished according to the wide ranges for the physiological parameters of metal uptake and the variability of metal partitioning in sediments.

With the increasing evidence that exposure and effects can vary with diet, efforts are being made to develop and test multipathway models not only for predicting bioaccumulation (Luoma and Rainbow, 2005) but also toxicity (Simpson and Batley, 2007). Multipathway models can be used to develop exposure–effects models for benthic organisms, but both organism physiology and sediment properties are strikingly important to predicting toxic effects in sediments (Simpson, 2005; Simpson and King, 2005). The lugworm *A. marina* lives in sediments with relatively large differences in OM and particle size, and consequently metal-binding properties. Because lugworms receive most of their metal uptake from dietary sources (particle ingestion), the influence of sediment type and metal partitioning may be only an issue for some metals such as Ag, for which a larger uptake from the dissolved phase may be expected. Nevertheless, sediment properties may still compromise the accuracy of the model for predicting bioaccumulation

(and possibly effects) due to its determinant role in the feeding behaviour of this species.

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