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Biodynamic modelling and the prediction of Ag, Cd and Zn accumulation from solution and sediment by the polychaete *Nereis diversicolor*

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ABSTRACT: Biodynamic modelling has been used to predict bioaccumulated concentrations of Ag, Cd and Zn in the deposit-feeding polychaete *Nereis diversicolor* from 5 metal-contaminated estuaries in SW England and a relatively non-contaminated estuary in SE England. The modelling employed previously measured physiological parameters of bioaccumulation — uptake rate constant, assimilation efficiency (AE) and efflux rate constants after uptake from water and sediment ingestion — and measured sediment metal concentrations specific for each population. AEs were considered to relate to metals in the organic component of the ingested sediment and ingestion rates were therefore expressed in these terms, with the further assumption that the total sediment metal concentration is a proxy for the metal concentration in the sediment organic component. A range of growth rate constants was extracted from the literature, as were concentration ranges of dissolved Ag, Cd and Zn in contaminated coastal waters. The model showed that >99% Cd and >98% Zn accumulated by *N. diversicolor* is derived from sediment ingestion; more bioaccumulated Ag is derived from solution, the percentage contribution of the dissolved source increasing from 46 to 80% with an increase in Ag dissolved concentration from low to high values for coastal waters. Bioaccumulated metal concentrations predicted from the model generally showed excellent agreement with independently measured concentrations in field-collected worms, supporting the assumptions made in the model.

KEY WORDS: Bioavailability · Biodynamic modelling · Sediments · Uptake · Efflux

INTRODUCTION

Sediments, especially estuarine sediments, are major sinks for contaminants such as toxic metals in coastal waters (Luoma & Rainbow 2008). Since sediments are ingested by deposit-feeding invertebrates, there is potential for sediment-associated trace metals to be taken up by these animals with possible toxic consequences in highly contaminated circumstances.

Various approaches (Luoma & Rainbow 2008) have been taken to assess the bioavailability of sediment-associated metals to deposit feeders (and indeed to suspension feeders if sediments are resuspended in the water column; Luoma 1989). These range from statistical modelling of metal extraction from oxic sediments with weak acids (Luoma & Bryan 1982) to experimental studies that take metal–sulphide interactions into account (Ankley et al. 1993, 1996), and mechanistic studies of metal releases by digestive juices from the alimentary tracts of deposit-feeding invertebrates (Mayer et al. 1996, 2001). More recently, interest has turned to the use of biodynamic modelling as a powerful tool to predict bioaccumulation and discriminate the relative importance of different routes of metal uptake by aquatic animals (Luoma et al. 1992, Wang et al. 1996, Wang & Fisher 1999). In general, predictions of bioaccumulation from these models agree well with observations of bioaccumulated metals when compared across metals and a variety of aquatic habitats in animals with different feeding strategies (Luoma & Rainbow 2005). Biodynamic modelling has also been applied to deposit-feeding organisms, including poly-

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chaetes (Selck et al. 1998, Wang et al. 1998, 1999, Casado-Martinez et al. 2009a,b), sipunculids (Wang et al. 2002, Yan & Wang 2002), bivalve molluscs (Lee et al. 1998, Griscom et al. 2000, 2002, King et al. 2005, Simpson & King 2005) and crustaceans (King et al. 2005, Simpson & King 2005). When applied to deposit feeders, these models show the importance of ingested sediments as (often) the predominant source of metals with potentially direct toxic effects to the ingestor but also potentially indirect toxic effects to predators higher up the local food chain (Rainbow et al. 2004, 2006).

Most applications of biodynamic studies to date have considered relatively broad applications, assuming that physiological parameters are species-specific and relatively constant. Fewer studies have addressed population differences and their implications for bioaccumulation, partly because such specific studies probably require determination of population-specific physiological parameters (e.g. Rainbow et al. 2009).

Here we apply biodynamic modelling to compare bioaccumulation and the relative importance of water and sediment as sources of the trace metals Ag, Cd and Zn among 6 populations of the widespread infaunal estuarine polychaete *Nereis diversicolor*. *N. diversicolor* can feed using a variety of mechanisms (Harley 1950), but not least as a deposit feeder ingesting local surface oxic sediments, underlying its use as an excellent biomonitor of the bioavailability of many trace metals from the sediment (Bryan et al. 1980, 1985, Luoma & Rainbow 2008). We use population-specific data on the biodynamic parameters controlling the uptake and subsequent accumulation of these 3 metals from solution and ingested sediment for populations of *N. diversicolor* from 6 UK estuaries with different degrees of metal contamination of the sediments (Rainbow et al. 2009). We predict the accumulated steady state concentrations in the worms from biodynamic modelling, compare these predictions with independently measured accumulated concentrations in the worms and ask whether population-specific differences in physiology are more important than environmental concentrations in determining bioaccumulation differences among the estuaries.

Biodynamic modelling

In a biodynamic model (Wang et al. 1996, Wang & Fisher 1999), the steady state accumulated metal concentration (C_{SS}, µg g^{-1}) in an animal can be described by:

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

where K_u is the metal uptake rate constant from solution (l g^{-1} d^{-1}), C_w the metal concentration in solution (µg l^{-1}), AE the assimilation efficiency of the metal from ingested food with metal concentration C_f (µg g^{-1}) (in this case sediment with metal concentration C_{sed}), IR the ingestion rate (g g^{-1} d^{-1}) and g the growth rate constant (d^{-1}). K_e is the efflux rate constant (d^{-1}) of metal taken up, K_{ew} for metal taken up from solution and K_{ef} for metal taken up from food (K_{esed} in this case where the food is the ingested sediment). The equation is essentially in 2 parts: (1) additive, describing firstly uptake of metal from solution and its subsequent efflux; and (2) uptake from ingested food and its subsequent efflux, allowing partitioning of the total accumulated concentration into metal derived from each route. Any effect of growth, as reflected in the growth rate constant, affects metal taken up from either source.

MATERIALS AND METHODS

Collection. *Nereis diversicolor* were collected from intertidal mudflats (upper 20 cm sediment depth) in the upper estuaries of the East Looe (050° 22.38′ N, 004° 27.74′ W), Gannel (050° 24.32′ N, 005° 05.17′ W), Restronguet Creek (050° 12.36′ N, 005° 05.41′ W), Tavy (050° 27.12′ N, 004° 10.17′ W) and West Looe (050° 21.82′ N, 004° 28.86′ W) in southwest England, and the Blackwater estuary (051° 44.08′ N, 000° 41.34′ E) in southeast England in April 2007, with further subsidiary collections for biodynamic modelling experiments in July and October 2007 (Rainbow et al. 2009). Restronguet Creek receives discharge from the Carnon River, draining a catchment with a long history of mining, and correspondingly contains extraordinarily high levels of As, Cu, Fe, Mn and Zn (Bryan et al. 1980, Bryan & Gibbs 1983). The Gannel estuary has been shown to have high bioavailabilities of Pb and Zn, Tavy of Cu, East Looe of Ag, Cu and Pb and West Looe of Cu and Pb (Bryan et al. 1980). The Blackwater estuary in Essex, SE England, was used as a control site with expectedly no atypically raised trace metal bioavailabilities. Table 1 presents comparative data on the total metal concentrations in the sediments of these estuaries.

Table 1. Total concentrations (mean ± SD, n = 3, µg g^{-1} dry weight) of Ag, Cd and Zn in oxic surface sediment from 6 UK estuaries, April 2007

Estuary	Ag	Cd	Zn
Blackwater	0.46 ± 0.09	1.95 ± 0.26	93 ± 17
East Looe	0.46 ± 0.23	0.72 ± 0.24	66 ± 15
Gannel	0.38 ± 0.04	1.39 ± 0.15	251 ± 20
Restronguet Creek	1.40 ± 0.47	2.85 ± 0.28	3270 ± 260
Tavy	0.51 ± 0.10	1.88 ± 0.08	211 ± 18
West Looe	1.15 ± 0.11	1.41 ± 0.14	103 ± 4

Worms were transported back to the laboratory in coolboxes in sediment from the collection site, and were kept in sediment from the site of origin covered by artificial seawater (Tropic Marin, Tropicarium Buchshlag) at a salinity of 16 at 10°C. Samples of associated oxic surface sediments were also collected at the same time in April 2007 for metal and organic matter analysis.

Metal analyses. Ten worms collected from each site in April 2007 were allowed to depurate their gut contents in artificial seawater at a salinity of 16 at 10°C in the absence of sediment for 2 d before being frozen, dried to constant weight at 60°C and acid-digested in concentrated nitric acid (Aristar grade, Merck) at 100°C. Each digest was made up to a known volume with double distilled water and analysed for Ag, Cd and Zn on a Vista-Pro CCD Simultaneous inductively coupled plasma-optical emission spectrometer (ICP-OES). Comparative analyses of the Standard Reference Material Tort-2 (lobster hepatopancreas, NRC) for Cd and Zn (certified Ag concentrations not available) gave measured mean concentrations within 5% of certified values, and agreement was considered satisfactory.

Total sediments were dried to constant weight at 60°C and acid-digested in concentrated nitric acid (Aristar grade, Merck) at 100°C before analysis of digests on a Vista-Pro CCD Simultaneous ICP-OES.

All metal concentrations are quoted in terms of dry weight.

Organic matter. Further samples of oxic surface sediments were wet-filtered with distilled water through a 125 µm stainless steel sieve, and separated fractions dried to constant weight at 60°C. The fine fraction was ground to a fine powder with a mortar and pestle. The organic matter content of this fine fraction was estimated by weight loss after heating at 550°C for 30 min.

Biodynamic modelling parameter measurement. Worms from each estuary were used in radiolabelled experiments to measure biodynamic parameters associated with the accumulation of Ag, Cd and Zn — methods and results from these experiments are presented in Rainbow et al. (2009). In order to ensure discrete measurement of dietary uptake by the worms of metals from sediment, a common sediment from the Blackwater estuary collection site had been radiolabelled with each metal separately for at least 1 mo at 4°C, with subsequent confirmation that there was no significant loss of any radiolabelled metal to overlying medium; radiolabelled sediment was subsequently packaged in gelatin for ingestion by the worms with no leakage of radiolabelled metal into the medium surrounding the worms (Rainbow et al. 2009). In the present study we used the coefficients developed in that study (Rainbow et al. 2009) in the biodynamic models.

RESULTS

Our objective was to apply biodynamic modelling to address the relative importance of physiology and metal concentrations in water or sediment in determining bioaccumulation of Ag, Cd and Zn in *Nereis diversicolor.* We compared metal bioaccumulation in *N. diversicolor* across the 6 estuaries using the metal concentrations in sediments determined for the present study. As is often the case in this type of modelling, ingestion rates, growth constants and dissolved concentrations were determined from the literature (e.g. Griscom et al. 2002). An important goal in this study is to discuss some of the choices that must be made in choosing such constants, and to conduct sensitivity analyses to investigate the effect of variation in the values chosen.

Estimated parameters

Ingestion rate and metal concentration in food

Estuarine particulate material is a complex mixture of organic matter and inorganic phases derived from the rocks weathered in the local catchment. Organic matter can originate and particles can be formed internally within the estuary or transported to the estuary by the river draining the catchment. Although deposit feeders usually ingest both organic and inorganic components of these particles, their assimilation processes are focused on the organic matter component in order to maximize the nutritional value of the ingested sediments (e.g. Cammen 1979). Some studies have assumed that organic content has a negative effect on bioavailability of metals from sediments (e.g. Di Toro et al. 2005). However, that assumption contradicts a body of data that show that metals such as Ag, Cd, Zn and Cr are assimilated more efficiently from both suspended sediments and bed sediments with higher natural organic content (especially living material) than from sedimentary material with less organic material (e.g. Lee et al. 1998), and, in general, more efficiently from organic ligands than from inorganic ligands (Harvey & Luoma 1985, Decho & Luoma 1994). There will necessarily be metals associated with inorganic components of oxidised sediment, not least manganese and iron oxide components (Luoma & Rainbow 2008). But the literature supports the concept that metal associated with organic material is typically of greater bioavailability than that associated with inorganic ligands (Lee et al. 1998). Thus, it is at least defensible to argue that it is the metal associated with the organic component of the sediment on which assimilation processes are focused, as empirically determined by measures of metal assimilation efficiency. It is therefore justifiable to assume for the sake of modelling that most of the metal expected to be bioavailable after ingestion is metal associated with the organic component of the sediment (Casado-Martinez et al. 2009a,b).

Under the conditions of the above assumption, the ingestion rate most relevant to the modelling being undertaken here is the rate of ingestion of organic matter (OM) in the sediment, expressed in $g_{OM}\ g_{organism}^{-1}\ d^{-1}$, rather than the ingestion rate of total sediment. Indeed it is reassuring for this argument that deposit-feeding invertebrates in general appear to maintain a rate of ingestion of organic matter which is independent of the organic content of the ingested sediment (Cammen 1979). Cammen (1979) showed that data for many deposit feeders of different taxa, including polychaetes, oligochaetes, bivalve molluscs, gastropod molluscs and amphipod and brachyuran crustaceans, fitted the relationship $C = 0.381W^{0.742}$, where C is the organic matter (mg) ingested per organism per day and W the body dry weight (mg) of the organism. Data for polychaetes including *Nereis succinea*, a close relative of *N. diversicolor*, fell particularly close to this generalised model (Cammen 1979). Therefore, the most defensible generalized ingestion rate for *N. diversicolor* appears to be one that allows a constant organic matter ingestion rate (Casado-Martinez et al. 2009b), even though that rate could vary substantially relative to the total mass of sediment ingested. This is in contrast to previous studies that have derived ingestion rates on the basis of $g_{sediment}\ g_{organism}^{-1}\ d^{-1}$ (Wang et al. 1998, 1999, Griscom et al. 2002).

As noted above, ingestion rate is size-dependent in nereid polychaetes. We therefore used the size model of Cammen (1979) to adjust the ingestion rate of organic matter for the worms in each population (Table 2). To characterize the mean size for each population, we used the mean dry weight of the worms used in the estimation of the biodynamic model parameters for which dry weight data were available (Rainbow et al. 2009). Table 2 therefore shows the calculated ingestion rate of organic matter in each population of *Nereis diversicolor* adjusted for their dry weight differences. Also quoted are ingestion rates in terms of total sediment, based on the measured organic matter content of the local sediment, for easier comparison with quoted literature values. These estimated ingestion rates range from 0.96 to 2.72 g g^{-1} d^{-1} (Table 2), in comparison with quoted ingestion rates of 2.2 g g^{-1} d^{-1} for 0.0024 g *N. succinea* at 10°C (Cammen 1980) and 3.5 g g^{-1} d^{-1} for 0.0058 g *N. succinea* at 15°C (Cammen 1979). Wang et al. (1998, 1999) used the latter figure of 3.5 g g^{-1} d^{-1} of Cammen (1979) in their biodynamic modelling studies of trace metal accumulation by *N. succinea* at 12°C. The ingestion rates reported in Table 2 are therefore in good agreement with those used by other authors, but take into account local sedimentary conditions and population differences that could affect ingestion.

Table 2. *Nereis diversicolor*. Ingestion rates from 6 UK estuaries following Cammen (1979). OM: organic matter; IR: ingestion rate

Estuary	Mean dry weight of worms (g)	OM consumption per worm (mg d^{-1})	IR of OM (g g^{-1} d^{-1})	% OM in sediment	IR of sediment (g g^{-1} d^{-1})
Blackwater	0.0188	3.36	0.179	12.1	1.48
East Looe	0.0247	4.11	0.167	8.1	2.06
Gannel	0.0174	3.17	0.182	6.7	2.72
Restronguet Creek	0.0209	3.64	0.174	10.5	1.66
Tavy	0.0158	2.95	0.187	11.2	1.67
West Looe	0.0289	4.62	0.160	16.6	0.96

We need also to assess the relevant measure of metal concentration in the ingested sediment. Arithmetically, if we are going to use an ingestion rate on the basis of $g_{OM}\ g_{organism}^{-1}\ d^{-1}$ in the determination of uptake rates from food, then the concentration of metal in the sediment must be in similar units. The arguments above also make the point that the most relevant measure is the metal concentration in the organic component of the sediment, as that is the component digested for assimilation.

There is no empirical method to directly separate metal associated with the organic components of sediment from that associated with inorganic ligands. One way to obtain the correct units would be to divide the mass of metal in a gram of sediment by the mass of organic matter. But this implicitly assumes that all metal is associated with the organic matter. As stated above, Ag, Zn and Cd in the oxidised sediment are distributed between organic and inorganic components (Luoma & Rainbow 2008). Thus 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. A default assumption is that the different sedimentary components have a generally similar affinity for the metals, and thus the same metal concentration (content per unit weight of that component); i.e. the metal is distributed evenly among ligands in the sediment. Although unlikely to be exactly correct (e.g. Luoma & Bryan 1982), this is probably a more valid generalization than the alternatives. Under such conditions, the metal concentration per unit mass of organic material is necessarily the same as the metal concentration per unit mass of total sediment. Our estimate of the metal concentration of the organic component of the sediment is therefore the total metal sediment concentration (Casado-Martinez et al. 2009b).

Growth rate

The biodynamic model requires an estimate of the growth rate constant of the worms. Gillet et al. (2008) have reported on the growth of 2 populations of *Nereis diversicolor* living on the shore of 2 estuaries in France: the metal-contaminated Seine estuary and a control estuary, the Authie. Gillet et al. (2008) followed the growth of different cohorts of the worms over extended periods between 2002 and 2004, expressed in terms of the combined length of the prostomium, peristomium and chaetigerous segment 1. These lengths (L, mm) can be transformed to total dry weights (W, mg) of the worms using the quoted equation for the Seine worms ($W = 0.4697e^{1.7209L}$) and a corrected equation ($W = 0.3218e^{2.5482L}$, P. Gillet pers. comm.) for the Authie worms. Thus growth curves expressed in terms of the above length can be expressed in terms of dry weight for different cohorts of the 2 worm populations. A growth rate constant of 0.006 d^{-1} can be calculated for a cohort of the Seine population increasing in size from about 4 to 12 mg between February and September 2002, with comparable growth rate constants of 0.008 and 0.010 d^{-1} for Authie cohorts increasing from 4 to 40 mg and 44 to 290 mg, respectively, over the same spring and summer (from Gillet et al. 2008). Growth rate constants for *N. diversicolor* in the literature refer to more juvenile worms under conditions of added food and are not surprisingly higher. Batista et al. (2003) reported growth rate constants of about 0.08 d^{-1} for juvenile worms fed with fish food pellets, and quoted growth rate constants of 0.04 to 0.07 d^{-1} for the same species reared under similar conditions with added fish food (Fidalgo e Costa 1999, Fidalgo e Costa et al. 2000). Nielsen et al. (1995) found a growth rate constant of 0.07 d^{-1} for *N. diversicolor* fed with shrimp meat, and one of 0.03 d^{-1} when the worm was filter feeding on a suspension of phytoplanktonic diatoms.

In the light of the size of the polychaetes used to evaluate metal bioaccumulation in each estuary (16 to 29 mg dry weight, Table 2), the absence of artificially added food and the similarity of our collection sites to those of Gillet et al. (2008), we have used a growth rate constant of 0.02 d^{-1} for *Nereis diversicolor* in the models described here and investigated the effects of change in this constant over the range 0.005 to 0.05 d^{-1}.

Dissolved concentrations

Although resident in burrows in the sediment, *Nereis diversicolor* strongly irrigates its burrow with a current of oxygenated water from the overlying water column (Banta et al. 1999, Kristensen 2001), as can easily be seen from the rust brown (iron oxide-rich) outlines of this worm's burrows in otherwise black iron sulphide-rich sediments. Any water bathing the worm is therefore water from the water column and not pore water in chemical equilibrium with the sediment particles. We have therefore resisted the approach of other authors (e.g. Wang et al. 1998, 1999) who have supposed that it is sediment pore water that is bathing the polychaete and calculated the metal concentrations therein by using the relationship between K_d (the partition coefficient) and the sediment metal concentration.

Dissolved metal concentrations are not available for the water column in the estuaries sampled (Luoma & Rainbow 2008). We have therefore fallen back on ranges of dissolved concentrations reported in the literature, with a subsequent sensitivity analysis of the effects of our chosen ranges on the results of the models employed.

The dissolved concentration ranges were chosen to extend from low concentrations typical of uncontaminated coastal waters to concentrations recognised as atypically high and indicative of significant anthropogenic contamination in estuaries. The ranges were 0.006 to 0.03 µg Ag l^{-1} (Smith & Flegal 1993, Ranville & Flegal 2005), 0.01 to 0.10 µg Cd l^{-1} and 0.3 to 5 µg Zn l^{-1} (Luoma & Rainbow 2008, their Table 5.5).

Biodynamic model

Table 3 illustrates one example of the biodynamic model applied to the accumulation of Ag, Cd and Zn by *Nereis diversicolor* from each of the 6 estuaries sampled. The physiological parameters of accumulation are those provided by Rainbow et al. (2009), with the absent data for Ag K_{ew} for the Gannel population and Ag K_{esed} for the East Looe population being replaced by the mean value for the remaining 5 populations. The growth rate constant in this model is 0.02 d^{-1}, the metal concentration in the food is the total metal concentration in the sediment and the dissolved concentrations of the metals are the bottom of the chosen range in each case (see above).

Contribution of sediment

Table 3 clearly indicates the predominance of sediment metal as the source of Cd and Zn to all the populations of *Nereis diversicolor*, in this case at low dis-

Table 3. *Nereis diversicolor*. Biodynamic modelling of accumulated Ag, Cd and Zn concentrations in *N. diversicolor* collected from 6 UK estuaries in 2007. In this model, growth rate (g) is entered as 0.02 d^{-1}. K_u: metal uptake rate constant from solution; K_{ew}: efflux rate constant of metal taken up from solution; AE: assimilation efficiency; IR: ingestion rate; OM: organic matter organic matter; K_{esed}: efflux rate constant of metal taken up from ingested sediment as food

Estuary	Mean dry weight (g)	Accumulation from water: K_u (l g^{-1} d^{-1})	Conc. (µg l^{-1})	K_{ew} (d^{-1})	$K_{ew}+g$ (d^{-1})	Accumulation from ingested sediment: AE	IR of OM (g g^{-1} d^{-1})	Conc. in sediment (µg g^{-1})	K_{esed} (d^{-1})	$K_{esed}+g$ (d^{-1})	Contr. to accumulation: Water (%)	Sediment (%)	Predicted steady state conc. (µg g^{-1})
Ag													
Blackwater	0.0188	2.7856	0.006	0.0436	0.0636	0.344	0.179	0.46	0.0835	0.1035	49.0	51.0	0.54
East Looe	0.0247	3.4559	0.006	0.0518	0.0718	0.293	0.167	0.46	0.0611	0.0811	51.0	49.0	0.57
Gannel	0.0174	1.7796	0.006	0.0340	0.0540	0.245	0.182	0.38	0.0628	0.0828	51.1	48.9	0.40
Restronguet Creek	0.0209	7.0624	0.006	0.0172	0.0372	0.341	0.174	1.40	0.0619	0.0819	52.9	47.1	2.15
Tavy	0.0158	7.3353	0.006	0.0250	0.0450	0.772	0.187	0.51	0.0440	0.0640	46.0	54.0	2.13
West Looe	0.0289	4.4229	0.006	0.0322	0.0522	0.528	0.16	1.15	0.0532	0.0732	27.7	72.3	1.84
Cd													
Blackwater	0.0188	0.0134	0.01	0.0184	0.0384	0.507	0.179	1.95	0.0232	0.0432	0.1	99.9	4.10
East Looe	0.0247	0.0157	0.01	0.0217	0.0417	0.710	0.167	0.72	0.0261	0.0461	0.2	99.8	1.86
Gannel	0.0174	0.0115	0.01	0.0336	0.0536	0.728	0.182	1.39	0.0177	0.0377	0.0	100.0	4.89
Restronguet Creek	0.0209	0.0087	0.01	0.0312	0.0512	0.660	0.174	2.85	0.0246	0.0446	0.0	100.0	7.34
Tavy	0.0158	0.0267	0.01	0.0311	0.0511	0.500	0.187	1.88	0.0163	0.0363	0.1	99.9	4.85
West Looe	0.0289	0.0149	0.01	0.0243	0.0443	0.614	0.16	1.41	0.0115	0.0315	0.1	99.9	4.40
Zn													
Blackwater	0.0188	0.1021	0.3	0.0359	0.0559	0.454	0.179	93	0.0059	0.0259	0.2	99.8	292
East Looe	0.0247	0.0173	0.3	0.0389	0.0589	0.461	0.167	66	0.0000	0.0200	0.0	100.0	254
Gannel	0.0174	0.0859	0.3	0.0386	0.0586	0.300	0.182	251	0.0170	0.0370	0.1	99.9	371
Restronguet Creek	0.0209	0.0660	0.3	0.0393	0.0593	0.653	0.174	3270	0.0441	0.0641	0.0	100.0	5797
Tavy	0.0158	0.0752	0.3	0.0235	0.0435	0.386	0.187	211	0.0712	0.0912	0.3	99.7	168
West Looe	0.0289	0.0336	0.3	0.0321	0.0521	0.588	0.16	103	0.0292	0.0492	0.1	99.9	197

solved concentrations of each metal. This predominance holds even if the dissolved concentrations of Cd and Zn are set at the top of the range chosen (Table 4). Much more of the silver accumulated is delivered from solution, matching that from the sediment at low dissolved concentrations but increasing to 80% at the highest dissolved concentration modelled (Tables 3 & 4).

Prediction

Biodynamic modelling can be used to predict the accumulated steady state concentrations in the worms from these 6 estuaries with different degrees of sediment metal contamination. The predictions were compared to independently measured accumulated concentrations in the worms sampled in April 2007, using the model run in Table 3 with low dissolved concentrations, and a further model employing high dissolved concentrations (Table 5). To gain further insight into local variability of accumulated metal concentrations in the worms, additional comparisons were made (Table 5) against accumulated concentrations in worms sampled in September 2003 from the same locations and analysed in identical fashion to the 2007 worms (S. N. Luoma et al. unpubl. data). More Cd accumulated concentrations were also available in the 2003 data set, many of the 2007 digests containing Cd concentrations beneath the detection limit.

As shown in previous studies (Luoma & Rainbow 2005), the model accurately predicted the difference in bioaccumulated concentrations among metals. The differences between Zn bioaccumulation and that of Cd or Ag were largely driven by differences in the concentrations in the sediments. Within metals, the model captured the variability in the observed data especially at the extremes of these narrow data ranges (Fig. 1). For Ag and Cd together, where sediments and bioaccumulated metal

Table 4. Effect of high and low dissolved Ag, Cd and Zn concentrations on the percent contributions of water and ingested sediments towards the accumulation of Ag, Cd and Zn by *Nereis diversicolor* from 6 UK estuaries. Other parameters are as in Table 3

Estuary	Water	Sediment	Water	Sediment
Ag	**Low (0.006 μg l^{-1})**		**High (0.03 μg l^{-1})**	
Blackwater	49.0	51.0	82.8	17.2
East Looe	51.0	49.0	83.9	16.1
Gannel	49.1	50.9	82.9	17.1
Restronguet Creek	52.9	47.1	84.9	15.1
Tavy	46.0	54.0	81.0	19.0
West Looe	27.7	72.3	65.7	34.3
Mean	45.9	54.1	80.2	19.8
SD	9.2	9.2	7.2	7.2
Cd	**Low (0.01 μg l^{-1})**		**High (0.1 μg l^{-1})**	
Blackwater	0.1	99.9	0.8	99.2
East Looe	0.2	99.8	2.0	98.0
Gannel	0.0	100.0	0.4	99.6
Restronguet Creek	0.0	100.0	0.2	99.8
Tavy	0.1	99.9	1.1	98.9
West Looe	0.1	99.9	0.8	99.2
Mean	0.1	99.9	0.9	99.1
SD	0.1	0.1	0.6	0.6
Zn	**Low (0.3 μg l^{-1})**		**High (5 μg l^{-1})**	
Blackwater	0.2	99.8	3.0	97.0
East Looe	0.0	100.0	0.6	99.4
Gannel	0.1	99.9	1.9	98.1
Restronguet Creek	0.0	100.0	0.1	99.9
Tavy	0.3	99.7	4.9	95.1
West Looe	0.1	99.9	1.6	98.4
Mean	0.1	99.9	1.8	98.2
SD	0.1	0.1	1.9	1.9

concentrations are in the same order of magnitude, the model predicted that differences among locations would be driven by metal concentrations in sediments both at high and low dissolved concentrations of Ag and Cd (Fig. 2). The model has already made allowance for physiological differences between populations that may affect bioaccumulation. Thus the 6-fold difference in sedimentary metal concentrations among sites is much more important in explaining bioaccumulation than the small differences in physiology among populations from the different sites. The coefficient of variation around

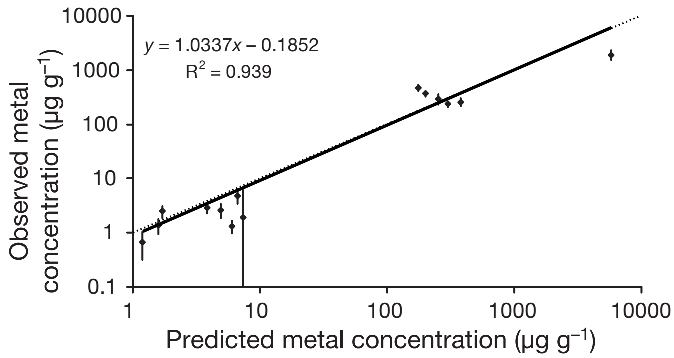

Fig. 1. Ag, Cd and Zn concentrations (μg g^{-1}, ±5% CL) observed in *Nereis diversicolor* collected from 6 UK estuaries compared to metal concentrations predicted (Table 5) from independently derived biodynamic constants, sediment metal concentrations and the higher end of dissolved metal concentrations expected in contaminated estuaries. The best fit regression line (solid) for all 3 metals combined is almost confluent with the 1:1 line (dashed)

Table 5. Comparisons of Ag, Cd and Zn mean concentrations (μg g^{-1} dry weight with 95% CL, or range of detection limits for some Cd concentrations [denoted with 'a']) measured in whole *Nereis diversicolor* collected from 6 UK estuaries in April 2007 and September 2003, against those steady state concentrations (C_{SS}) predicted from biodynamic modelling using parameters measured on the 2007 worms. Predicted concentrations assume either low (as in Table 3) or high dissolved metal concentrations (C_w)[a]

Estuary	2007 Mean	2007 95% CL	2007 n	2003 Mean	2003 95% CL	2003 n	Predicted C_{SS} Low C_w	Predicted C_{SS} High C_w
Ag								
Blackwater	1.38	0.44	10	1.62	0.43	5	0.54	1.59
East Looe	2.48	0.62	10	2.05	1.73	3	0.57	1.72
Gannel	0.68	0.36	6	1.51	1.24	5	0.40	1.19
Restronguet Creek	4.82	1.44	10	2.15	1.08	5	2.15	6.71
Tavy	1.32	0.34	9	0.82	1.06	5	2.13	6.04
West Looe	2.90	0.61	10	0.46	0.22	5	1.84	3.87
Cd								
Blackwater	<0.83–<1.98[a]		9	0.36	0.05	5	4.10	4.13
East Looe	<1.64–<2.65[a]		7	<0.29–<0.62[a]		3	1.86	1.89
Gannel	<0.73–<1.89[a]		9	0.33	0.09	2	4.89	4.91
Restronguet Creek	1.95	4.18	2	0.63	0.10	5	7.34	7.36
Tavy	2.62	0.75	5	0.42	0.13	5	4.85	4.89
West Looe	<1.08–<4.17[a]		10	<0.13–<0.60[a]		5	4.40	4.43
Zn								
Blackwater	237	26.1	10	176	63.0	5	292	301
East Looe	294	64.0	9	139	61.9	3	254	254
Gannel	258	43.0	10	208	56.1	5	371	378
Restronguet Creek	1925	405	10	189	22.5	5	5797	5802
Tavy	472	64.1	10	153	14.6	5	168	176
West Looe	366	46.0	10	130	30.3	5	197	200

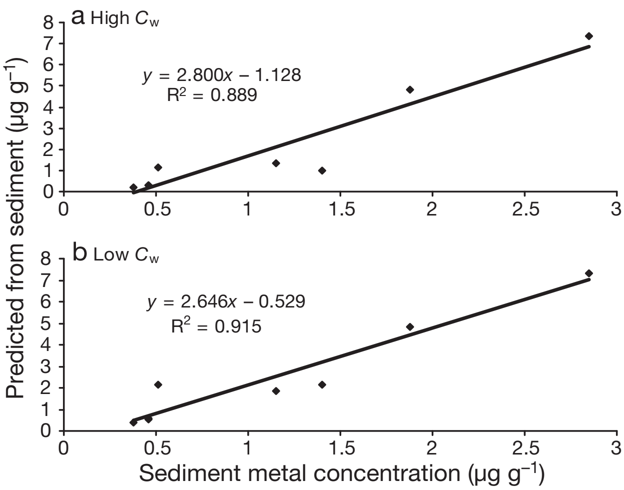

Fig. 2. Relationships between the predicted concentrations of both Ag and Cd together (Table 5) accumulated by *Nereis diversicolor* from ingested sediment and sediment metal concentrations when ambient dissolved metal concentrations (C_W) are (a) high or (b) low in the range of dissolved concentrations expected in contaminated estuaries. Solid lines are best fit regression lines

the mean metal concentration found in worms at each field site was consistently ~30% for Cd and Ag, except for Cd at Restronguet Creek. All predictions from the high water concentrations were within 2× the 95% confidence limits of the field data from 2007. The variability between years in the field data (Table 5) is indicative of the order of variability that can occur as estuarine and biological processes fluctuate from year to year. All model predictions for Ag and most for Zn fell within that range of variability. In general, the model appeared to overpredict Cd bioaccumulation, and it overpredicted both Cd and Zn bioaccumulation at the most contaminated site, Restronguet Creek. The most likely explanation for the latter is less bioavailability to the worms in the field than predicted from the measurements of AE in the laboratory. Restronguet Creek sediments are especially rich in sulphides which could have a negative effect on bioavailability. Given the predominance of the ingested sediment as a source of accumulated metal in the cases of both Cd and Zn, it is not surprising that the choice of dissolved concentrations at either end of the range made little difference to the predicted steady state concentrations. The modelling also suggested that dissolved Ag concentrations in the estuaries were intermediate between the 2 scenarios we chose, a not unreasonable conclusion.

Effect of growth rate

The models used to derive the data in Tables 3–5 have assumed a growth rate constant of 0.02 d^{-1} in every case. It is therefore logical to carry out a sensitivity analysis to see the effect of changes in growth rate on accumulated concentrations. These effects are shown in Fig. 3, using low dissolved concentrations for each metal as in the example in Table 3. As can be seen, there is relatively little effect of different growth rate constants, particularly above growth rate constants of 0.01 d^{-1}.

DISCUSSION

As generally concluded by Luoma & Rainbow (2005), biodynamic modelling has proved to be a powerful tool to interpret and predict the bioaccumulation of Ag, Cd

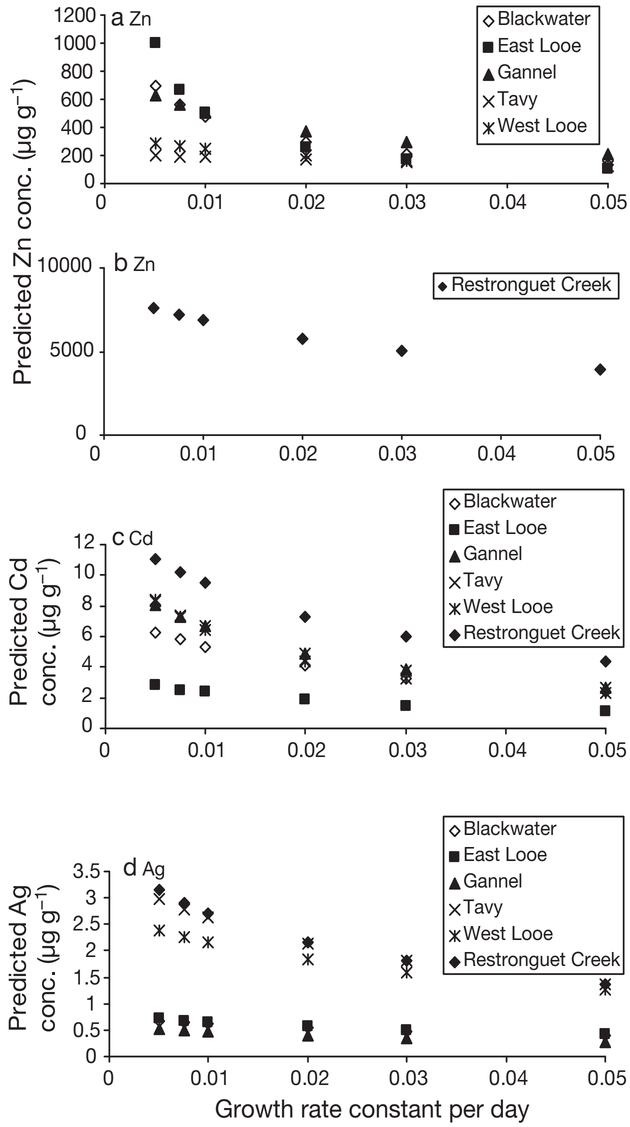

Fig. 3. Effect of changes in growth rate constant from 0.005 to 0.05 d^{-1} on the predicted concentrations of (a,b) Zn, (c) Cd and (d) Ag in *Nereis diversicolor* from 6 UK estuaries. Other parameters of the model are as in Table 3

and Zn from solution and ingested sediment by the infaunal polychaete *Nereis diversicolor*. Modelling metal bioaccumulation from sediments is a challenge because of the complexity of metal distributions within the sediment matrix and the diversity of strategies that different species employ to obtain nutrition from sediments. The methodologies we used to determine site-specific ingestion rates, metal concentrations and growth rates were important to the successful deployment of the model. 'Calibrating' the model with mechanistically justifiable assumptions adds credibility to the information on the relative importance of solution and sediment as sources of these trace metals to *N. diversicolor* and helped explain differences in bioaccumulated concentrations of the metals in 6 estuaries with different degrees of contamination.

A clear conclusion is that more than 99% of Cd and more than 98% of Zn accumulated by *Nereis diversicolor* are derived from the sediment over a range of sediment and dissolved metal concentrations. More bioaccumulated Ag is derived from solution, the mean percentage contribution of the dissolved source of Ag increasing from 46 to 80% with an increase in dissolved Ag concentration from low to high values for coastal waters. Many of the estuaries of SW England have been contaminated by mine wastes. Remediation has reduced dissolved metal inputs to these systems, but sedimentary contamination remains (P. S. Rainbow et al. unpubl. data). While that strategy could reduce Ag exposure of *N. diversicolor*, it will have little effect on Cd and Zn exposure.

These conclusions for *Nereis diversicolor* agree well with equivalent data published for other polychaetes. Wang et al. (1998, 1999) concluded that more than 98% of bioaccumulated Cd, Co, Se and Zn in *N. succinea* is derived from sediment ingestion, together with >70% of bioaccumulated inorganic Hg, while between 5 and 35% of bioaccumulated Ag originated in dissolved form. Selck et al. (1998) similarly concluded that 95% of bioaccumulated Cd in *Capitella* sp. was sediment-derived. Casado-Martinez et al. (2009a) also found that >98% of Cd and Zn in *Arenicola marina* was derived from sediment ingestion, while there was significant input (up to 30%) from solution in the case of Ag. Perhaps the most important factor in these differences is the unusually high Ag uptake rate from solution compared to other metals.

Steady state bioaccumulated concentrations of all 3 metals predicted by biodynamic modelling with tenable assumptions agreed very well with measured concentrations. The exceptions were in the cases of zinc and cadmium in the Restronguet Creek population of *Nereis diversicolor.* The modelling predicted greatly raised bioaccumulated concentrations but not to the degree reflected in the measured 2007 bioaccumulated concentrations. For Zn, this is probably not surprising given the atypical bioaccumulation of Zn in this population (Rainbow et al. 2009). *N. diversicolor* typically regulates the accumulated body concentration of Zn to an approximately constant level (Bryan & Hummerstone 1973, Bryan et al. 1980, Amiard et al. 1987), regulation being brought about by a matching of efflux rate to total uptake rate, until such a point that uptake rate exceeds the efflux rate achievable and net accumulation ensues with associated detoxification of the extra accumulated Zn (Rainbow 2002, Rainbow et al. 2009). The major detoxified stores of Zn in this population of *N. diversicolor* are spherocrystals in the gut wall (Mouneyrac et al. 2003). Any Zn regulation has clearly broken down in the case of the Restronguet Creek population in 2007 (although not in 2003), and the accumulation pattern of Zn under these circumstances differed greatly from that of the other populations. The physiological parameters used in the biodynamic model in the present study may therefore not be quite appropriate to this atypical accumulation pattern.

In general, it is remarkable that quantification of physiological uptake and loss processes for a species can be combined with crude measures of metal concentrations in water and sediment and result in predictions of bioaccumulation within an order of that independently observed in nature. On the other hand, it is important not to overstate what such a model can do. In most cases, physiological processes that are generically quantified might be expected to vary with environmental conditions. At least for *Nereis diversicolor* in these estuaries, we show that physiological variation is less important than variability in sediment metal concentrations, with perhaps the exception of variability in the AEs of Cd and Zn at a very contaminated site. Considerations important to improving conditions might include better characterization of environmental concentrations of metals and determination of AEs specific to the materials ingested by the organisms at each specific site.

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