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Review article

Trace metal bioaccumulation: Models, metabolic availability and toxicity

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Abstract

Aquatic invertebrates take up and accumulate trace metals whether essential or non-essential, all of which have the potential to cause toxic effects. Subsequent tissue and body concentrations of accumulated trace metals show enormous variability across metals and invertebrate taxa. Accumulated metal concentrations are interpreted in terms of different trace metal accumulation patterns, dividing accumulated metals into two components — metabolically available metal and stored detoxified metal. Examples of different accumulation patterns are described from crustaceans but have a general applicability to all aquatic invertebrates. Toxicity does not depend on total accumulated metal concentration but is related to a threshold concentration of internal metabolically available metal. Toxicity ensues when the rate of metal uptake from all sources exceeds the combined rates of detoxification and excretion (if present) of the metal concerned. The biodynamic model of trace metal bioaccumulation allows the prediction and explanation of widely differing accumulated trace metal concentrations in organisms, combining geochemical analyses of environmental metal concentrations with the measurement of key physiological parameters for a species from the site under consideration. The combination of the biodynamic model as a unified explanation of metal bioaccumulation with an understanding of the relationship between accumulation and toxicity sets the stage for a realistic understanding of the significance of trace metal concentrations in aquatic invertebrates.

Keywords: Trace metals; Accumulation; Detoxification; Metabolic availability; Biodynamic modelling; Crustaceans

Contents

Aquatic invertebrates take up and accumulate trace metals whether essential or not, all of which have the potential to cause toxic effects. Subsequent tissue and body concentrations of accumulated trace metals show enormous variability across metals and invertebrate taxa (Eisler, 1981; Rainbow, 1990, 1993; Phillips and Rainbow, 1994). This review will use crustacean examples (Rainbow, 1998) to explore general principles relating to the accumulation of trace metals by aquatic invertebrates (Rainbow, 2002). A distinction will be made between two different components of accumulated metals, specifically accumulated metal that has been detoxified and metal that is available metabolically to play an essential and in extreme circumstances a toxic role in biochemistry (Rainbow, 2002). This distinction has implications for any relationship between accumulated concentrations and metal toxicity, for it is rate processes (specifically the net difference between rates of metal uptake and those of metal excretion and detoxification combined), not total accumulated metal concentrations, that determine the onset of toxic effects in a metal-exposed animal (Rainbow, 2002; Marsden and Rainbow, 2004). Finally the review will highlight the application of the biodynamic model (Luoma and Rainbow, 2005) in predicting and explaining the widely different accumulated metal concentrations found in aquatic invertebrates.

Accumulated concentrations of trace metals in crustaceans vary widely between metals and between taxa (Rainbow, 1998), as illustrated for zinc, copper and cadmium in Table 1. Three taxa have been selected: barnacles (Cirripedia), and two malacostracan taxa — amphipods and caridean decapods. Barnacles have zinc concentrations an order of magnitude

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Table 1

A selection of body concentrations (μg g^{-1} dry weight) of three trace metals (Zn, Cu, Cd) in a systematic range of crustaceans from clean and metal-contaminated sites (after Rainbow, 1998; Rainbow and Wang, 2005)

Species	Location	Zinc	Copper	Cadmium	Reference
Cirripedia					
Capitulum mitella	Hung Hom, Hong Kong (contaminated)	19,890	545	10.0	Phillips and Rainbow, 1988
	Cape D'Aguilar, Hong Kong	2852	29.2	5.2	Phillips and Rainbow, 1988
Tetraclita squamosa	Hung Hom, Hong Kong (contaminated)	6963	94.9	2.8	Phillips and Rainbow, 1988
	Tung Chung, Hong Kong	2245	14.9	4.2	Phillips and Rainbow, 1988
Balanus amphitrite	Chai Wan Kok, Hong Kong (contaminated)	9353	3472	7.3	Phillips and Rainbow, 1988
	Lai Chi Chong, Hong Kong	2726	59.3	5.5	Phillips and Rainbow, 1988
Semibalanus balanoides	Dulas Bay, Wales (contaminated)	50,280	3750	–	Walker, 1977
	Menai Strait, Wales	19,230	170	–	Rainbow, 1987
	Southend, England	27,837	232	28	Rainbow et al., 1980
Malacostraca, Amphipoda					
Orchestia gammarellus	Restronguet Creek, England (contaminated)	392	139	9.8	Rainbow et al., 1989, 1999
	Dulas Bay, Wales (contaminated)	126	105	9.1	Rainbow et al., 1999
	Millport, Scotland	188	77.5	1.6	Rainbow et al., 1999
Talorchestia quoyana	St Kilda, Dunedin, New Zealand	481	31.9	17.2	Rainbow et al., 1993
	Sandfly Bay, Dunedin, New Zealand	133	15.6	8.9	Rainbow et al., 1993
Malacostraca, Eucarida					
Decapoda					
Pleocyemata, Caridea					
Palaemon elegans	Millport, Scotland	80.6	110	0.9	White and Rainbow, 1986
Pandalus montagui	Firth of Clyde, Scotland	57.5	57.4	–	Nugegoda and Rainbow, 1988a

above those of amphipods and carideans, even when the barnacles are from uncontaminated sites (Table 1). On the other hand, barnacles from uncontaminated sites have body copper concentrations usually below those of amphipods and carideans (Table 1), which as malacostracans have significant body contents of the copper-bearing respiratory protein haemocyanin absent from barnacles. Barnacles do, however, have the potential to increase their body concentrations of copper well above those of amphipods and carideans when at copper-contaminated sites such as Chai Wan Kok, Hong Kong (Phillips and Rainbow, 1988) or Dulas Bay, Wales (Walker, 1977) (Table 1). Differences between body cadmium concentrations in the three crustacean taxa are not so marked, although the caridean concentrations tend to be lowest (Table 1).

Any aquatic invertebrate will take up trace metals into the body from solution through permeable body surfaces and from the gut. Recently it has become increasingly appreciated that uptake of trace metals from the diet may be the major source of metals for many aquatic invertebrates (Wang, 2002), including barnacles (Wang et al., 1999a,b; Rainbow and Wang, 2001, 2005). Fig. 1 presents a schematic representation of the accumulation of a trace metal by a decapod crustacean (Rainbow, 1988). Metal passively adsorbed onto the exoskeleton of a crustacean (with the potential to be desorbed when dissolved conditions change) will contribute to the total body concentration of metal in the crustacean, but its proportional contribution is usually small (White and Rainbow, 1984b, 1986; Nugegoda and Rainbow, 1988b, 1989; Rainbow, 1988). Since, furthermore, such metal does not enter physiological pathways within the animal, it is not considered further here. When metal first enters the body of the crustacean after uptake either from solution through permeable ectodermal surfaces or across the endoderm of the gut, it will initially be metabolically available — that is it has the potential to bind to molecules in the receiving cell or elsewhere in the body after internal transport via the haemolymph (Fig. 1). In the case of an essential metal, it is available to bind to sites where it can play an essential role (e.g. zinc in the enzyme carbonic anhydrase or copper in haemocyanin) or, if present in excess (caused by entry at too high a rate), to sites where it may cause toxic effects. Such an excess of essential metal (and all non-essential metal) must be detoxified, i.e. bound tightly to a 'sacrificial' site from which escape is limited, probably in a storage organ beyond the site of uptake. The metal has now entered the second component of accumulated metal — the detoxified store (Fig. 1) which may be temporary or permanent. Trace metals taken up into the body may or may not be excreted, either from the metabolically available component or from a detoxified store (Fig. 1), dependent on the accumulation pattern

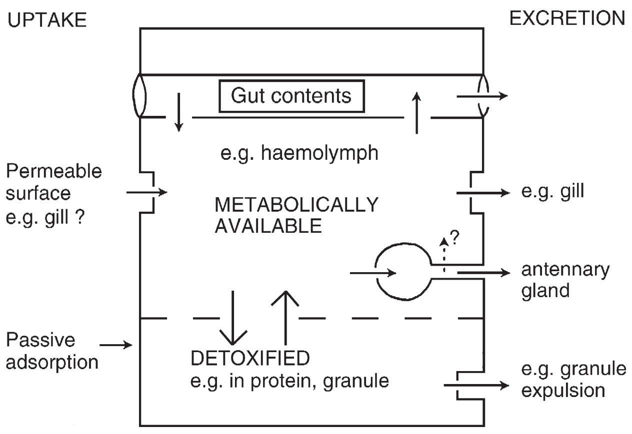

Fig. 1. A schematic representation of the body metal content of a decapod crustacean (from Rainbow, 1988).

of a particular crustacean for a particular metal (Rainbow, 1998, 2002).

It is possible to make estimates of the metabolic requirements of essential trace metals to try to gain an understanding of the size of the metabolically available component of accumulated metal (Fig. 1) in an aquatic invertebrate. White and Rainbow (1985) made theoretical calculations of enzyme requirements for copper and zinc, based on the number of copper and zinc-bearing enzymes and their contribution to the total concentration of enzymes in tissues. Making many assumptions such as the equal contribution of each enzyme to the total enzyme load, they estimated that metabolising tissue needs approximately 26.3 μg Cu g^{-1} and 34.5 μg Zn g^{-1} (Table 2) to fulfil enzyme requirements (White and Rainbow, 1985). In the same study, White and Rainbow (1985) made estimates the amount of copper needed by the respiratory protein haemocyanin in decapod crustaceans. Rainbow (1993) refined these theoretical calculations by making allowance for the different contributions of soft tissue and blood to total body weight in decapod crustaceans, as exemplified by the caridean *Pandalus montagui* in Table 3. In *P. montagui*, the concentration of copper needed by the haemocyanin present was estimated to be 22.9 μg Cu g^{-1}, giving a total theoretical body concentration of copper of 38.1 μg Cu g^{-1} (Table 3). The measured mean concentration of copper in *P. montagui* from the Firth of Clyde, Scotland is 57.4 μg Cu g^{-1} with a standard deviation of 18.9 (Nugegoda and Rainbow, 1988a) (Table 3), suggesting that the theoretical estimates may be approximately correct.

The second component of accumulated metal is that of detoxified metal (Fig. 1). Many trace metals are detoxified in the form of one of a variety of insoluble granules or deposits in invertebrate tissues (Hopkin, 1989; Marigomez et al., 2002). Hopkin (1989) described three types of intracellular granules: type A — consisting of concentric layers of calcium and magnesium phosphates which may contain trace metals such as manganese and zinc; type B — more heterogeneous in shape and always containing sulphur in association with metals that include copper and zinc; type C — often polyhedral with a crystalline form, mainly containing iron, probably derived from ferritin. In crustaceans, the most commonly reported metal-rich granules are type A and B granules (Al-Mohanna and Nott, 1987, 1989; Nassiri et al., 2000), while large ferritin crystals are characteristic of the ventral caecum cells of stegocephalid amphipods (Moore and Rainbow, 1984). Detoxification also occurs in the soluble phase. Certain trace metals (e.g. Zn, Cu, Cd, Ag, Hg) are associated with, and induce, metallothioneins, low molecular weight cytosolic proteins involved in the cellular regulation and detoxification of these metals (Roesijadi, 1993; Amiard et al., 2006). The presence of sulphur in the high proportion of cysteine residues in these proteins provides the high metal affinity of the molecule, sequestering metals in the cytoplasm and reducing their metabolic availability. It is the lysosomal breakdown of metallothioneins that probably gives rise to the sulphur-rich type B granules described above, as in the amphipod *Orchestia gammarellus* from copper-contaminated sites (Nassiri et al., 2000).

Table 2
Essential metal requirements in enzymes of metabolising soft tissue (after White and Rainbow, 1985; Rainbow, 1993)

	Number of metal-associated enzymes	Percentage of total number of enzymes	Average number of metal atoms per enzyme molecule	Estimated enzyme metal requirement in tissue (μg g^{-1} dry wt)
Copper	30	1.40%	2.95	26.3
Zinc	80	3.74%	1.41	34.5

Table 3
Estimates of the essential requirements for copper in the caridean decapod *Pandalus montagui* (after Rainbow, 1993, with data from Nugegoda and Rainbow, 1988a; Depledge, 1989)

Pandalus montagui	
% distribution dry weight	
Exoskeleton	40.0%
Blood	2.1%
Soft tissues	57.9%
Haemocyanin	
Blood Cu concentration	44 μg ml^{-1}
Blood volume in body	0.52 ml g^{-1} dry wt
Blood Cu concentration in body	22.9 μg g^{-1}
Enzyme requirement	
Metabolising soft tissue	26.3 μg g^{-1}
Whole body	15.2 μg g^{-1}
Total body Cu metabolic requirement	
Haemocyanin	22.9 μg g^{-1}
Enzymes	15.2 μg g^{-1}
Total	38.1 μg g^{-1}
Measured body Cu concentration	
Mean ± SD	57.4 ± 18.9 μg g^{-1}

The distinction of two components of accumulated metal has implications for the onset of toxic effects in an animal exposed to a high bioavailability of a trace metal. So long as the uptake rate of a trace metal (integrated across both dissolved and dietary sources) is less than the combined rates of detoxification and excretion, then accumulated metal will not build up in the metabolically available component and toxicity will not ensue (Rainbow, 2002; Marsden and Rainbow, 2004). If, on the other hand, the rate of uptake of the trace metal is faster than the rate of its excretion and detoxification combined, then the concentration of metabolically available metal will exceed a threshold and toxic metal will start binding at sites where it will interfere with normal metabolic functioning. As will be shown below, not all invertebrates excrete all trace metals, in which case it is the rate of detoxification alone that must match the rate of metal uptake to avoid toxic effects. Another corollary is that the concentration of detoxified metal is independent of the onset of any toxic effects, and thus may be extremely high as may be the total accumulated concentration without causing toxic effects (Rainbow, 2002). The onset of toxicity can occur at any total body concentration if the uptake rate changes such that it exceeds the combined rates of excretion and detoxification for sufficient time for the concentration of metabolically available metal to exceed a threshold.

As stated at the start of this review, different crustaceans have very different accumulated concentrations of trace metals (e.g.

Table 1). It is the accumulation pattern of a particular crustacean for a particular trace metal that determines the accumulated body concentration, and these accumulation patterns correspondingly vary within and between crustaceans and indeed other invertebrates (Rainbow, 1998, 2002).

The first accumulation pattern to be considered is that of the essential trace metal zinc in caridean decapods. The caridean *Palaemon elegans* is able to regulate its body concentration of zinc (to about 90 μg Zn g^{-1}) when exposed to a wide range of dissolved zinc bioavailabilities (White and Rainbow, 1982; Rainbow and White, 1989), an accumulation pattern for zinc also shown by *Palaemonetes varians* (*ca* 96 μg Zn g^{-1}) and *P. montagui* (*ca* 70 μg Zn g^{-1}) (Nugegoda and Rainbow, 1988a, 1989). Zinc is taken up by *P. elegans* in significant quantities (14% of total body Zn content per day at 100 μg Zn l^{-1} under defined physicochemical conditions at 20 °C), but the uptake rate is balanced by the excretion rate so that the body concentration remains unchanged (White and Rainbow, 1982, 1984a,b). Eventually at a high enough dissolved Zn concentration, the excretion rate fails to match the uptake rate, and there is a net increase in body zinc concentration to only about double the regulated body concentration with lethal toxic effect (White and Rainbow, 1982). The implication here is that much of the zinc remains in metabolically available form without detoxification, the concentration of metabolically available metal building up sufficiently to cause toxicity. It can also be concluded that in caridean decapods at least, the body concentrations of zinc are regulated to approximately those required to meet metabolic demand with relatively little stored in detoxified form (Rainbow, 1998, 2002).

The zinc accumulation pattern of barnacles occupies the other extreme of the range of zinc accumulation patterns (Rainbow, 1998, 2002). All zinc taken up from solution by barnacles is accumulated without excretion (Rainbow and White, 1989) and any excretion of zinc taken up from the diet is essentially insignificant (half life of 1346 days in *Elminius modestus* — Rainbow and Wang, 2001). Correspondingly accumulated body concentrations reach very high concentrations (e.g. 50,000 μg Zn g^{-1} or more — Table 1) (Rainbow, 1987, 1998) and most of this accumulated zinc inevitably is in detoxified form (Fig. 1). Thus zinc in barnacle bodies is bound as zinc pyrophosphate granules (Walker et al., 1975a,b; Pullen and Rainbow, 1991; Masala et al., 2004), a Type A granule in the categorisation of Hopkin (1989). Thus accumulation patterns for zinc in two taxa of crustaceans could not be more different — a) regulation to a constant body concentration with apparently little stored in detoxified form (caridean decapods), and b) stored in detoxified form without significant excretion (barnacles) resulting in some of the highest accumulated concentrations of any trace metal in any animal tissue (Eisler, 1981; Rainbow, 1987).

Accumulation patterns for copper also vary among crustaceans (Rainbow, 1998). The caridean *P. elegans* appears to regulate the body concentration of copper (*ca* 130 μg Cu g^{-1}) over a wide range of dissolved Cu exposures as it does for zinc (White and Rainbow, 1982; Rainbow and White, 1989), the lack of a suitable radiotracer for copper preventing confirmation that the rate of Cu excretion matches that of Cu uptake. It is likely that the regulated body copper concentrations of carideans approximate to their metabolic needs; indeed the measured body Cu concentration of *P. montagui* from the Firth of Clyde, Scotland (Nugegoda and Rainbow, 1988a) approximates to estimated theoretical requirements (Rainbow, 1993), as shown in Table 3. Therefore during regulation most accumulated copper will be in metabolically available form and excretion will be from this component (Rainbow, 2002). Once regulation has broken down at high dissolved copper bioavailabilities and the rate of Cu uptake exceeds the rate of Cu excretion, the accumulated concentration in *P. elegans* can reach higher levels than in the case of zinc (*ca* 600 μg Cu g^{-1}) (White and Rainbow, 1982). Under these circumstances, the hepatopancreas of *P. elegans* contains type B Cu-rich granules, probably representing residual bodies from the lysosomal breakdown of metallothionein binding copper (Rainbow, 1998). These granules have the potential to be excreted via the gut as hepatopancreatic epithelial cells complete their cell cycle (Fig. 1). The accumulation of copper by carideans may therefore show two excretory routes — excretion from the metabolically available component during copper regulation, and (much more rarely) excretion from an insoluble detoxified store (Fig. 1) during the net accumulation that follows regulation breakdown in high copper bioavailabilities (Rainbow, 2002).

As shown in Table 1, concentrations of copper in barnacles from uncontaminated sites are generally very low, even approximating to theoretical estimates of copper enzyme requirements (Table 3), barnacles lacking haemocyanin. Barnacles do, however, have the potential to accumulate high concentrations of copper (Table 1) and show strong net accumulation of copper from solution in the laboratory, as for zinc, with no suggestion of regulation (Rainbow and White, 1989). It is likely that, again as for zinc, all incoming copper is accumulated with the necessity for detoxification (Rainbow, 1998, 2002). Barnacles from Cu-contaminated sites such as Dulas Bay have many type B Cu-rich granules (Walker, 1977; Rainbow, 1987), probably again resulting from lysosomal breakdown of metallothionein binding copper.

The copper accumulation pattern of amphipods appears to be intermediate between the apparent copper regulation of carideans and the strong net accumulation of barnacles (Rainbow and White, 1989; Weeks and Rainbow, 1991; Rainbow, 1998, 2002). There is no evidence for regulation of copper body concentrations and amphipods exposed to a range of dissolved Cu exposures show net accumulation at all exposures (Rainbow and White, 1989; Weeks and Rainbow, 1991). Copper is accumulated in the cells of the ventral caeca (equivalent to the hepatopancreas of carideans), again in the form of type B Cu-rich granules (Icely and Nott, 1980; Nassiri et al., 2000), presumably derived from metallothionein. Copper detoxified in these granules will be excreted (Fig. 1) on completion of the cell cycle of the ventral caeca epithelial cells (Galay Burgos and Rainbow, 1998). This is not a regulation process for the body concentration of copper in the amphipods will reach a new steady state level as Cu bioavailabilities change, the availability of Cu being reflected in the number of granules in (and hence the Cu concentration of) the ventral caeca. As the Cu concentration increases the proportion of accumulated copper in the detoxified component increases, from a starting point whereby most copper in an amphipod from an uncontaminated site will probably be in metabolically available form (Tables 1 and 3). Amphipods have haemocyanin and their metabolic needs for copper might then be of the order of 38 μg Cu g^{-1} as calculated for the caridean *P. montagui* (Table 3). Indeed *Talorchestia quoyana* from St Kilda, New Zealand had 31.9 μg Cu g^{-1} (Table 1); the lower copper concentration in the same species from Sandfly Bay may reflect a temporary absence of haemocyanin in these amphipods, the haemocyanin content of talitrid amphipods being known to vary seasonally (Rainbow and Moore, 1990).

The accumulation patterns of amphipod crustaceans for other trace metals including zinc and cadmium are also those of net accumulation with detoxified storage in type B granules in the ventral caeca with the potential to be excreted with the faeces (Rainbow and White, 1989; Rainbow, 1998, 2002). The ferritin crystals that are characteristic of iron accumulation in stegocephalid amphipods are similarly lost into and from the alimentary tract (Moore and Rainbow, 1984).

No crustacean regulates the body concentration of the non-essential metal cadmium (Rainbow, 1998). In carideans (*P. elegans*), amphipods (*Echinogammarus pirloti*) and barnacles (*E. modestus*), all cadmium taken up from solution is accumulated without excretion over at least a 28-day period (Rainbow and White, 1989). The accumulated cadmium is necessarily detoxified, typically as metallothionein. The metallothionein will be broken down in lysosomes (Langston et al., 1998), but cadmium is rarely visualised in lysosome residual bodies (type B granules) unlike copper or zinc.

A knowledge of accumulation patterns is a prerequisite to understanding why aquatic invertebrates accumulate trace metals to such different body concentrations. A further stage in the understanding of the physiological processes controlling accumulated concentrations, specifically on a quantitative basis, is offered by the application of biodynamic modelling (Luoma and Rainbow, 2005).

The accumulated trace metal concentration (content per unit weight) of an aquatic invertebrate results from metal taken up from solution and metal taken up from food, less any metal that has been excreted. Depending upon the time period under consideration, it may be necessary to allow for the growth of the animal, growth dilution reducing the accumulated concentration.

The rate of metal uptake from solution (μg g^{-1} d^{-1}) can be expressed as $k_u C_w$, where C_w is the dissolved concentration of the metal and k_u is the dissolved metal uptake rate constant (μg g^{-1} d^{-1} per μg l^{-1}, or l g^{-1} d^{-1}). Over environmentally realistic dissolved concentrations, the rate of trace metal uptake from solution by an aquatic invertebrate is directly proportional to the dissolved concentration, and the uptake rate constant k_u is the slope of this direct relationship (Luoma and Rainbow, 2005). From a measured k_u for a given set of physicochemical variables of a medium, it is possible then to calculate an uptake rate at a particular dissolved concentration. The rate of metal uptake from the diet can be expressed as AE (IR) C_f where IR is the ingestion rate (g g^{-1} d^{-1}), C_f the concentration of the metal in the food (μg g^{-1}), and AE the assimilation efficiency of the metal from that food source. AE is measured in the laboratory using radiotracers (Wang and Fisher, 1999; Rainbow and Wang, 2001). Also measured in the laboratory with radiotracers is the efflux rate constant k_e (d^{-1}) (Rainbow and Wang, 2001). Growth rate constants (d^{-1}) allow for the effect of growth dilution. These

Table 4
Comparisons between observed concentrations (μg g^{-1}) and those predicted by the biodynamic model (after Luoma and Rainbow, 2005)

Species	Ecosystem	Metal	Predicted concentration			Observed concentration			Reference
			Min	Max	Median/mean	Min	Max	Median/mean	
Bivalves									
Mytilus edulis	San Francisco Bay	Cr	2.6	7.5	5.05			4.05	Wang et al., 1997
Mytilus edulis	San Francisco Bay	Cd	2.7	10.1	6.4	4.4	9.4	6.9	Wang et al., 1996
Mytilus edulis	San Francisco Bay	Se	1.0	5.6	3.3	2.5	6.7	4.6	Wang et al., 1996
Mytilus edulis	San Francisco Bay	Zn	54	265	160	54	130	92	Wang et al., 1996
Dreissena polymorpha	Hudson River	Ag			0.09			0.08	Roditi et al., 2000
Dreissena polymorpha	Hudson River	Cd			21.7			18.7	Roditi et al., 2000
Dreissena polymorpha	Hudson River	Cr			12.6			16.8	Roditi et al., 2000
Macoma balthica	San Francisco Bay	Ag	1.3	21.0	11.2			8.0	Griscom et al., 2002
Macoma balthica	San Francisco Bay	Cd	0.02	0.90	0.46			0.33	Griscom et al., 2002
Macoma balthica	San Francisco Bay	Co	0.03	2.7	1.5			2.4	Griscom et al., 2002
Macoma balthica	San Francisco Bay	Se	2.2	4.3	3.3			3.0	Luoma et al., 1992
Insect larvae									
Chaoborus punctipenis	Flavrian Lake, Quebec	Cd	0.7	3.0	1.8			1.3	Croteau et al., 2001
Chaoborus punctipenis	La Bruère Lake, Quebec	Cd	0.5	2.2	1.3			1.7	Croteau et al., 2001
Chaoborus punctipenis	Vaudray Lake, Quebec	Cd	1.1	4.4	2.7			2.9	Croteau et al., 2001
Barnacles									
Balanus amphitrite	Hong Kong	Zn	2610	11,560	7080	3100	11,000	6550	Rainbow et al., 2003
Elminius modestus	English Channel	Cd	7.6	11	9	15	27	21	Rainbow and Wang, 2001
Elminius modestus	English Channel	Zn	1500	4400	2950	2470	4730	3600	Rainbow and Wang, 2001

separate terms can then be combined into the biodynamic model to predict the accumulated concentration of a trace metal in an aquatic invertebrate.

$$C = [k_u C_w + \mathrm{AE}(\mathrm{IR})C_f] \ / \ [k_e + g]$$

The accumulation of zinc by barnacles can be used as an example of the application of the biodynamic model (Rainbow and Wang, 2005). Barnacles have high rates of uptake of zinc from solution in comparison to other crustaceans (Rainbow and White, 1989; Rainbow, 1998). Thus *Eliminius modestus* has a Zn uptake rate constant of 0.3 $\mathrm{l\,g^{-1}\,d^{-1}}$ at 10 °C (Rainbow and White, 1989, 1990), a Zn AE varying from 40% to 90% according to the nature of the food source and an efflux rate constant of 0.0022 $\mathrm{d^{-1}}$ (Rainbow and Wang, 2001). Using data from the literature for Zn concentrations in water and food (suspended material), ingestion rates and growth rates, Rainbow and Wang (2001) used the biodynamic model to predict an accumulated Zn concentration in *E. modestus* from Southend, UK of 1500 to 4400 μg Zn $\mathrm{g^{-1}}$ according to the growth rate constant chosen. The measured concentration for barnacles collected in summer 2000 was 3463 ± 1155 μg Zn $\mathrm{g^{-1}}$ (Rainbow and Wang, 2001), an excellent fit to the predicted concentration confirming the suitability of the biodynamic model. Furthermore the model predicted that more than 97% of both accumulated Zn and Cd in *E. modestus* is accumulated from dietary ingestion (Rainbow and Wang, 2001). Similarly Wang et al. (1999a,b) used biodynamic modelling to show that both Zn and Cd are overwhelmingly obtained from dietary ingestion rather than the dissolved phase in another barnacle *Balanus amphitrite* from Hong Kong coastal waters, again in spite of very high uptake rates from solution (Rainbow et al., 2003). Ultimately it is the high ingestion rate and high trace metal assimilation efficiencies of barnacles that cause dietary uptake to predominate (Rainbow and Wang, 2005), and the adoption of an accumulation strategy of storage detoxification with very limited excretion source that causes the high accumulated concentrations observed, with potential to increase further in circumstances of high metal bioavailability (Table 1).

Luoma and Rainbow (2005) illustrated the application of the biodynamic model to a very wide range of examples from the literature, considering concentration data that covered 7 orders of magnitude, for 7 metals and 14 species of animals from 3 phyla and 11 marine, estuarine and freshwater environments. The coefficient of determination (R^2) between predicted and independently measured accumulated concentrations from the field (e.g. Table 4) was 0.98, and most predictions agreed with observations within two-fold. Such good agreement indicates that the basic assumptions of the biodynamic model are tenable (Luoma and Rainbow, 2005).

Thus the biodynamic model of trace metal bioaccumulation allows the prediction and explanation of widely differing accumulated trace metal concentrations in aquatic invertebrates, combining geochemical analyses of environmental metal concentrations with the measurement of key physiological parameters for a species from the site under consideration. The combination of the biodynamic model as a unified explanation of metal bioaccumulation with an understanding of the relationship between accumulation and toxicity sets the stage for a realistic understanding of the significance of trace metal concentrations in aquatic invertebrates.

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