

Title	Shedding the load: moulting as a cause of variability in whole- body metal concentrations
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Publication date	2020-09-24
Original Citation	O'Callaghan, I. and Sullivan, T. (2020) 'Shedding the load: moulting as a cause of variability in whole-body metal concentrations', Journal of Crustacean Biology, 40(6), pp. 725-733. doi: 10.1093/ jcbiol/ruaa077
Type of publication	Article (peer-reviewed)
Link to publisher's version	https://academic.oup.com/jcb/article/40/6/725/5911240 - 10.1093/jcbiol/ruaa077
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Download date	2025-06-01 01:39:29
Item downloaded from	https://hdl.handle.net/10468/11828



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4	CONCENTRATIONS
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6	Shedding the load: moulting as a cause of variability in whole-body
7	metal concentrations
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18	(Received 24 July 2020; accepted 31 August 2020)
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21	ABSTRACT
22	Moulting is a biological process shared by aquatic macroinvertebrates, but while the exoskeleton
23	is believed to be a major sink of metal pollutants, the contribution of the moulting of the
24	crustacean exoskeleton to total accumulated metal concentrations is insufficiently considered.
25	We present a conceptual, qualitative model that illustrates the impact of moulting on the whole-
26	body burden of an unspecified metal analyte in a hypothetical moulting invertebrate. The model

27	demonstrates that moult stage is a contributor to the whole-body pollutant concentration, and that
28	this introduces a temporal component even in steady-state exposure conditions. The applicability
29	of this model is illustrated by comparison to published results of pre- and post-moult
30	accumulations. A solution for reducing this variability in the measurement of whole-body metal
31	concentrations is presented, and its potential application to both ex-situ and in-situ studies of
32	biomonitor species is discussed.
33	
34	Key Words: aquatic environment, bioaccumulation, body burden, crustaceans, ecdysis,
35	measurement errors, exoskeleton, macroinvertebrates, metal pollution
36	
37	INTRODUCTION
38	Biomonitoring is defined as the use of an organism, whether whole, in part, or communal, to
39	determine the quality of the environment, and it is commonly used as a method of detecting and
40	quantifying contaminants present in the environment (Markert, 2007). Biomonitoring is
41	generally employed for the quantification of environmental pollutants, wherein a measurable
42	parameter may be the abundance of an identified biomonitor species in a geographical area, or
43	under a defined set of conditions (Ketelaars & Frantzen, 1995; Bonada et al., 2006;).
44	A primary application of an aquatic biomonitor is the measurement of bioavailability and
45	uptake of trace metals in the environment, and hence an indication of environmental
46	concentrations (Johnson et al., 1993; Flessas et al., 2000). Trace metals may enter the aquatic
47	environment by means of natural processes, such as metals leaching into rivers following a forest
48	fire in an adjacent location, or through anthropogenic disturbance (Richardson et al., 2001). In
49	many instances, there is a higher ratio of metals entering the hydrosphere as a result of

anthropogenic activities than of metals from natural sources entering waterways (Callender,2003).

52 A distinction must be made between total and bioavailable pollutant concentrations in order to appreciate the advantage of biomonitoring over direct measurement of the pollutants. 53 Various physico-chemical processes can render a pollutant biologically inert. These can be 54 55 divided into environmental processes, that influence the "environmental availability," and internal biological processes within an organism, that influence the "toxicological 56 bioavailability" of the pollutant (Peijnenburg et al., 1997). While total pollutant concentrations 57 can be determined directly through chemical analysis, accounting for the concept of 58 bioavailability requires either the application of measured environmental parameters to a model 59 of the environmental processes (the *a priori* approach, often the application of partition 60 coefficients to measured total pollutant concentrations), or direct measurement of accumulated 61 concentrations within the organism of interest (the *a posteriori* approach). The classification of 62 63 pollutants according to their bioavailability and toxicity, such as the Priority Substances List of the Water Framework Directive and subsequent amendments (European Commission, 2000, 64 65 2008), is a similar approach to the former. Biomonitors exemplify the latter, as the measured 66 biological concentrations are the result of both the environmental availability and toxicological bioavailability processes, and, therefore, offer an insight into the results of these processes 67 68 without requiring knowledge of the processes themselves. This approach avoids the need to 69 simplify such processes, so long as the biomonitor is chosen such that the toxicological 70 bioavailability encountered is representative for the ecosystem under study.

The biomonitoring of bioavailable pollutant concentrations using biomonitor organisms
also presents another advantage. Metal pollutants are often concentrated within the organism of

interest, which can allow for the observation of concentrations of contaminants that would
otherwise be difficult to detect at background concentrations, especially at a high spatial or
temporal frequency that would otherwise be difficult and prohibitively expensive to carry out
using analytical methods (Bryan & Darracott, 1979). This concentration behaviour relaxes the
limits of detection and allows the quantification in the organism what would rarely be detected in
the water (Phillips, 1977).

There is still some debate around the extent to which crustaceans uptake and 79 bioaccumulate trace metals, and reproducibility of measured metal concentrations appears to be 80 the exception, rather than the norm (Depledge & Rainbow, 1990; O'Callaghan et al., 2019). This 81 is compounded by the uncertainty regarding the most likely sources, uptake pathways or sites of 82 bioaccumulation within the organism of interest (Fleming & Richards, 1982; Elangovan et al., 83 1999; Van Hattum et al., 1999; Robinson et al., 2003; Santoro et al., 2009). Several models have 84 been proposed for the processes of uptake, bioaccumulation, and excretion of various pollutants 85 86 in freshwater macroinvertebrates (Rainbow & Luoma, 2011; Awrahman et al., 2015), a task made more complicated by the varying behaviour of different metal analytes and potential 87 88 interactions between said metals (O'Callaghan et al., 2019). A survey of these models, however, 89 indicates that periodic moulting of the exoskeleton, a process common to many invertebrate 90 species (Lebrun *et al.*, 2011), may not have been adequately accounted for in regard to trace 91 metals loss from the whole organism upon renewal of the exoskeleton.

Failing to take into account exoskeletons could therefore be a substantial source of
variability in measurement of the bioavailability of trace metals or in biomonitoring programs.
Previous studies have shown that the moulted exoskeleton may contain sizable concentrations of
bioaccumulated pollutants, pointing to ecdysis as being a possible pathway through which

significant portions of the accumulated substance may be shed or lost from the organism
(Miramand *et al.*, 1981; Hall, 1982; Topcuoğlu *et al.*, 1987; Rauch & Morrison, 1999). Moulting
may ultimately influence survival of an organism, as periodic moulting may reduce or maintain
pollutant concentrations below critical concentrations for the organism (Bryan & Darracott,
100 1979; Bergey & Weis, 2007). Furthermore, in the context of quantifying the accumulated
concentrations, accuracy and applicability of existing pollutant uptake and release models could
be improved by consistently including moult stage as a variable.

We present a generalized description of the flow of an unspecified metal pollution in a 103 moulting aquatic macroinvertebrate, such as a crustacean. This conceptual description offers an 104 illustrative, non-specific picture of the potential impact of moulting on measured accumulations, 105 equivalent to a repeating, discontinuous depletion of total accumulated metal concentrations. We 106 demonstrate the applicability of this conceptual model by comparing to previously published pre-107 and post-moult measurements of overall body concentrations of crustaceans, and discuss the 108 109 relevance of this conceptual model to the study of accumulation in biomonitor species. We also present a possible technique for reducing this variability, and discuss the potential application of 110 this technique both in the field and the laboratory. 111

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113

### CONCEPTUALIZING THE IMPACT OF MOULTING

114 *Existing models* 

115 Common types of models used to describe the accumulation of metals in aquatic invertebrates
116 include bioconcentration, bioaccumulation, and accumulation factors (BCF/BAF/ACF), the
117 biotic ligand model (BLM), the free ion activity model (FIAM), and biodynamic models (Wang
118 & Tan, 2019).

BCF, BAF, and ACF factors provide an intuitive and relatively uncomplicated way of estimating accumulation rates, but rely on the assumption that equilibrium will be reached across the organism-environment interface (McGeer *et al.*, 2003; van den Brink *et al.*, 2019). Moulting consists of dynamic changes in the organism, which violates this key assumption, reducing the contribution of moulting to a static correction term rather than a time-varying process.

BLM, as well as the related FIAM and extensions thereof, are commonly applied to studies of the total accumulated concentration ionic metals in aquatic macroinvertebrates (Brown & Markich, 2000; Di Toro *et al.*, 2001; van den Brink *et al.*, 2019). Both models, however, focus on the interface between the environment and the proposed receptor site, and ignore the internal mechanisms of translocation, transformation, and excretion (Vijver *et al.*, 2004). The contribution of moulting is closely linked with the relative sequestration of metal pollutants in exoskeleton and soft tissue compartments, which relies on these internal mechanisms.

Biodynamic models, commonly referred to as physiologically-based pharmacokinetic 131 132 (PBPK), are better suited to modelling the dynamic potential contribution of moulting to the accumulated concentrations of contaminant (Ardestani et al., 2014; van den Brink et al., 2019). 133 This approach models the processes of uptake, accumulation, translocation, transformation, and 134 135 excretion across time, and does not rely on any steady-state assumptions. toxicokinetictoxicodynamic (TKTD) models, such as general unified threshold model of survival (GUTS) 136 137 (Jager et al., 2011; EFSA Panel on PPR et al., 2018), are an example of a biodynamic approach 138 applied to both contaminant accumulation and resultant biological effects. One of the most 139 comprehensive static metal-accumulation biodynamic models in the literature arguably shows a relatively good correlation with observed results across a large number of studies (Luoma & 140 Rainbow, 2005), and GUTS is considered sufficiently developed for use in risk assessment 141

applications (EFSA Panel on PPR *et al.*, 2018). These models, however, have not yet been
extended to include the contribution of moulting, a correction that would have to be separately
determined for each organism-analyte pair.

145

146 *Choice of approach* 

147 We take the approach of describing moulting using a non-specific model that is designed to capture the essence of the problem, while remaining broadly applicable to any moulting aquatic 148 invertebrates such as crustaceans, whose exoskeleton may act as a significant sink for 149 contaminants, and any transition metal, metalloid, or heavy metal species. Such approach should 150 be contrasted with the common approach deriving a quantitative, predictive model described 151 above. The aim of our model is instead to illustrate certain contributions of the moulting process 152 153 to measured concentrations that are common to all moulting aquatic invertebrates and metal analytes, without offering a prediction for the significance of these contributions in any one 154 155 scenario.

We limit the applicability of the presented model to the transition, metalloid and heavy 156 metals, as it has been observed that the accumulation of the alkali or alkaline earth metals in the 157 158 exoskeleton of an aquatic crustacean may differ from that of the aforementioned elements. The accumulation of calcium, in particular, has been extensively investigated throughout the various 159 160 moult stages, and has been found to undergo a series of storage and resorption processes. This is 161 said to be linked to the use of calcium in the release of the exoskeleton and hardening of the 162 newly developing cuticle (Greenaway, 1985). The chitinous nature of the exoskeleton, and, more specifically, the nitrogen groups therein, may play a role in the alternative behaviour of the 163 transition metals, metalloids, and heavy metals, as it has been noted that chitinous materials 164

show a poorer affinity towards the alkali and alkaline earth metals (Rae & Gibb, 2003). For this
reason, the assumptions made in the following section apply only to the accumulation of
transition metals, heavy metals, and the metalloids.

168

169 Assumptions

For the purpose of creating a concise and simplified conceptual model, we must introduce a
number of assumptions. These assumptions are chosen such that they adequately isolate the
impact of moulting on trace metals concentrations, while removing internal processes that are not
mediators of the moulting process.

174 1) In order to reduce the process to a flow network, we make the assumption that all 175 metal pathways are uni-directional from intake to depuration. This does not mean that there are 176 no bi-directional pathways, but rather that bi-directional flows can be replaced by a long-term 177 uni-directional approximation.

2) While the process of moulting may be complex and irregular, we assume that each moulting event happens similarly and that the properties of each moulted exoskeleton are largely identical, in that each sequential exoskeleton is capable of accumulating metal contaminants at a fixed rate, after consideration of the growth factor. This is a simplifying assumption, and the impact of moulting is qualitatively similar under non-uniform moulting behaviour.

3) The frequency of moulting is taken to be constant, for the purposes of illustration.Again, non-constant frequency of moulting would produce qualitatively similar results.

4) Contaminant intake occurs solely through the processes of respiration, ingestion, and
adsorption. The inclusion of these three pathways is intended to make the model as general as
possible, and the results still hold if uptake through either ingestion or respiration does not occur,

and/or if uptake through adsorption does not occur. Adsorption is defined as the uptake of metal
contaminants directly from the overlying and interstitial waters in direct contact with the surface
of the exoskeleton, and results in uptake of the contaminant directly into the pre-moult
exoskeleton; absorption through the exoskeleton and into the body is not directly considered for
the reasons explained in Assumption 1.

5) The only process of depuration included in the model is moulting. Gut contents are not
taken into account, and, therefore, excretion from the alimentary tract does not reduce
accumulated concentrations in the model; metal pollutants are taken to enter the system when
they are assimilated from the alimentary tract into the biological tissues.

6) This model only considers the movement of trace metals and assumes that no internal processes of biotransformation are taking place. This is true regardless when considering the elemental concentrations, but a more complex model would be required to account for change in speciation or complexation of metals due to biological processes.

7) In this conceptual system, we make the assumption that the rate of translocation
between the body and pre-moult exoskeleton is driven towards equilibrium by the presence of
open binding sites in the destination and high concentrations at the source. The flow of
translocation can therefore be approximated as proportional to the source concentrations. Other
models of translocation could be considered and would result in qualitatively similar results.

8) We assume that the described processes are not influenced by any biological damagethat may occur, and we do not account for the possibility of mortality as part of the model.

208

209 *The conceptual model* 

Based on the above assumptions, we present a simplified three-input, two-compartment model of 210 metal accumulation in a hypothetical moulting aquatic invertebrate (Fig. 1). The corresponding 211 212 rate diagram is shown in Figure 2. The model compartmentalizes metal concentrations accumulated within (and on) the pre-moult exoskeleton (Compartment E in Figure 2), which is 213 defined as the part of the body that is removed entirely during the moulting process, and 214 215 concentrations accumulated in the remainder of the body (Compartment B in Figure 2). For the purposes of simplifying the model, the body is inclusive of all non-moulting parts (gills, legs, 216 hepatopancreas, and other organs), but not the gut contents as explained in Assumption 5. 217 <Figs. 1 & 2> 218

Contaminants may enter Compartment B through ingestion or standard respiration, where 219 *i* denotes the concentration of contaminant present in the ingestate, *r* the concentration of 220 contaminant in the water overlying the gill regions, and  $k_i$  and  $k_r$  the rate constants of the 221 respective processes. Contaminants may enter Compartment E through surface adsorption 222 223 directly from overlying and interstitial waters in contact with the exoskeleton, where a denotes the concentration of contaminant present in these waters, and  $k_a$  the corresponding rate constant. 224 225 Contaminants may also flow from Compartment B to Compartment E through the process of 226 internal translocation or sequestration, where B is the concentration of contaminant in Compartment B, and  $k_t$  the rate constant of translocation. The features of this flow are 227 228 summarized in Assumption 7.

Moulting, or ecdysis, refers to the regular removal of the outer exoskeleton. As the exoskeleton is shed, a new exoskeleton develops beneath it (see Drach, 1967). The loss of the moulted exoskeleton cannot be modelled as a continuous flow of contaminants as it occurs suddenly and periodically, and, therefore, it is not included in the process diagram in Figure 2. Instead, the diagram describes the inter-moult movement of contaminants, and moulting is
implemented externally as a periodic discontinuous removal of the contaminants within
Compartment *E*. The moult period, or the rate at which an organism moults, will vary greatly
with species and other factors. It should be noted that occasional consumption of the organism's
own moulted exoskeleton has been observed in some macroinvertebrate species (Elangovan *et al.*, 1999), although there is consensus that metals bound within a chitinous exoskeleton are less
bioavailable than other forms of analyte (Khan *et al.*, 2010). The possibility of such an

240 occurrence is not considered in this model but would result in qualitatively similar results.

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# 242 Impact of moulting

The conceptual two-compartment model of Figure 1 can be converted into a causal diagram 243 describing the relationship between the variable of interest, namely the environmental 244 concentration of bioavailable metal, and the measured metal concentration. This diagram is 245 246 shown in Figure 3. The effect of environmental concentration on measured concentration occurs through the mediation variables of body and exoskeleton concentration. The hypothesis that the 247 measured whole-body concentration is an accurate estimator for the bioavailable environmental 248 249 concentration, given an acceptable measurement error, is, therefore, weakened by the direct 250 effect of moult stage on exoskeleton concentration.

251 
252 It can be directly observed from Figure 3 that the effect of the moult event on the overall
253 accumulated metal concentration depends greatly on the ratio of exoskeleton metal concentration
254 to body metal concentration. If a simplifying assumption is made that the moult stage of different

organisms is uncorrelated, then sampling more organisms should reduce, but not eliminate, theinfluence of moulting.

257

#### 258

# DERIVATION OF KEY EQUATIONS

259 Differential rate equations

260 The key aspects of the model are described by the following pair of differential rate equations,

which describe the change in inter-moult concentrations of metal contaminant in each

#### 262 compartment.

$$\frac{d[B]}{dt} = k_i[i] + k_r[r] - \frac{263}{k_t[B]}$$
(1)
264

$$\frac{d[E]}{dt} = k_t[B] + \frac{265}{k_a[a]}$$
(2)
266

267

# 268 *Growth factor*

The growth aspect is one of significant variability, as growth may indicate linear growth, lateral growth, or increasing thickness of the exoskeleton. Growth rate will vary considerably with species, as well as with the life stage of the organism. In order to account for the growth uncertainty, we use a growth factor, *G*. Equations 1 and 2 can, hence, be extended to account for growth by dividing each  $k_x$  term by *G*.

274

275 *Closed-form expressions* 

Equations 1 and 2 can be solved for instantaneous compartment concentrations, assuming all concentrations are 0 at time t = 0 and ignoring the effects of moulting. This produces the following closed-form expressions:

279 
$$[B](t) = (U/k_t)(1 - e^{-t/\tau})$$
 (3)

$$[E](t) = (U + k_a[a])t - U\tau(1 - e^{\frac{1}{2}\delta})$$
(4)

where the uptake rate is given by  $U = k_i [i] + k_r [r]$ , and the process time constant is  $\tau = G/k_t$ . Note that the concentrations in Compartment *B* can be modelled as a first-order underdamped system, where the concentration approaches an equilibrium value of  $U/k_t$ , while the concentration in Compartment *E* increases indefinitely as the loss due to moulting is not yet accounted for.

286

# 287 Steady-state equations

Steady-state is reached when  $t \gg \tau$  in all the above equations. This results in expressions for the final, steady-state accumulated concentration in Compartment *B* in an environmental equilibrium.

$$[B] = U/k_t \tag{5}$$

As moulting happens periodically, the steady-state equivalent in the case of the Compartment *E* has the appearance of a sawtooth pattern, rather than a fixed value. The period of the concentration in Compartment *E* is equal to the moulting period,  $T_M$ , while the peak concentration is given by:

296 
$$[E]_{MAX} = (U + k_a[a])T_M$$
 (6)

297 The variance of the corresponding error due to moulting is therefore given by:

$$\sigma^2 = \frac{([E]_{\text{MAX}})^2}{12}$$
(7)

300	
301	SIMULATION
302	Objectives
303	The conceptual model is designed to offer insights into the contribution of moulting to whole-
304	body concentrations in the general case. The following simulation is intended to provide an
305	example of how the model can describe the impact of moulting in an existing experimental
306	study. Its specificity to a particular organism and metal pollutant should not be taken to be a
307	statement about the limitations of the conceptual model, but rather an indication of how the
308	generalized model can be applied to a specific case. The implementation of the model employed
309	in the following sections is described in full in Supplementary material File S12.
310	
311	Simulation parameters
312	The simulation parameters shown in Table 1 were derived from studies of the uptake of
313	vanadium by the caridean shrimp Lysmata seticaudata (Risso, 1816) (Miramand et al., 1981).
314	The measurements extracted from Miramand et al. (1981) is available in Supplementary material
315	Table S1. Further details of how these parameters were derived are presented in Supplementary
316	material File S13 and compared with the cited measurements in Supplementary material Fig. S8.
317	<table 1=""></table>
318	

More explicit derivation of the above equations is presented in Supplementary material File S11.

319 *Simulation results* 

Figure 4 shows the bioaccumulated concentrations of metal contaminant in Compartment *B*,
when the environmental conditions are in equilibrium. The black dashed lines indicate the
steady-state values.

323 
324 As expected of a first-order system, the concentration in Compartment *B* reaches steady-

326 concentration reaches steady-state, there is no significant change in contaminant concentration
327 without a corresponding change in the environmental conditions.

state at a speed dictated by the rate of internal translocation of the contaminant. Once the internal

Figure 5 shows the bioaccumulated concentration of the contaminant in Compartment *E*. Moulting forces the pre-moult exoskeleton concentration to 0 every  $T_M$  days. This produces a periodic pattern with increasing amplitude, approaching a sawtooth pattern as Compartment *B* reaches steady-state.

332 < Fig. 5 >333 The total concentration of contaminant in the organism as a whole is shown in Figure 6. 334 The lower black dashed line shows the steady-state concentration for Compartment *B*, whereas 335 the upper black dashed line includes the peak concentration for Compartment *E*. Despite the 336 concentration in Compartment *B* reaching steady-state, the influence of moulting is still

significant. This results in a time variation in the overall concentration.

338
 339
 340
 DISCUSSION

341 *Contribution of moulting* 

Studies measuring the bioaccumulation of trace metals in freshwater macroinvertebrates
typically rely on the assumption that the accumulated concentrations of metals are relatively
time-invariant. Many models of pollutant uptake likewise rely on the steady-state assumption. In
both cases, individual measurements of total accumulated pollutant concentrations provide an
accurate quantification of the time-averaged accumulation flux.

347 The contribution of the conceptual model presented herein is to explain how periodic processes, exemplified by moulting, can produce fluctuations in the total accumulated whole-348 body concentrations. The L. seticaudata example demonstrates the significant effect this can 349 have on the measured whole-body concentration. Seeing as discontinuous loss occurs during 350 each moult, steady-state is not reached; the internal concentrations settle into a periodic 351 oscillation, with the total concentration varying between minimum and maximum values. The 352 error introduced by the continuous approximation is described in Supplementary material File 353 S14, and illustrated by Supplementary material Figs. S9, S10. From an experimental perspective, 354 355 this introduces a source of variability in the measurements, as the measured value depends not only on the mean total concentration, but also on the moult stage at the time at which the 356 357 measurement is taken.

358

#### 359 *Validity of simulated example*

Example parameters, listed in Table 1, were used for the purposes of demonstrating the effects of moulting on the measured whole-body concentration. This raises the question of whether the chosen parameters produce results that fairly represent realistic pollutant accumulations. This can only be answered through comparison with measured concentrations.

The model predicts that approximately 74% of the accumulated metal contaminant concentration 364 is lost during the process of moulting. Table 2 presents published measurements of analyte 365 concentrations accumulated within the exoskeleton of various species, expressed as a percentage 366 of whole-body accumulated concentrations. In cases where data extraction and/or post-367 processing was required to obtain the values given in Table 2, further details are given in 368 369 Supplementary material Tables S4–S7. It must be emphasized, when interpreting these figures, that most of the cited studies do not account for the effects of moulting on overall concentrations 370 we describe. Therefore, by assuming steady-state whole-body concentrations it would be 371 expected that the measured values presented herein represent approximately half of the 372 exoskeleton concentration at the time of moulting. Under these same assumptions, the 373 exoskeleton would contribute approximately 37% of the whole-body concentration using the 374 model parameters presented herein. 375

As is to be expected and considering the wide range of biological species, analytes, and environmental or experimental conditions, there is a broad variation in reported exoskeleton concentrations. Despite this variation, it is clear that such concentrations are a significant fraction of the whole-body accumulated concentration. Our model would, therefore, be correct in attributing a significant role to the contribution of moulting to whole-body pollutant concentrations.

382 
 383 If the results of the model are valid, the question then arises as to whether the
 384 mechanisms described in the model are also valid. Hall (1982) presented measurements of the
 385 accumulated concentrations of nickel in the cladoceran *Daphnia magna* Straus, 1820, presented

here in Supplementary material Tables S2, S3. Figure 7 shows the measured soft-tissue and

exoskeleton concentrations in individuals that have not moulted. It shows that the soft-tissue 387 concentrations rapidly reach steady-state, while the exoskeleton concentrations continue to 388 increase in the absence of moulting. A corresponding fit of the model is shown, with a value of P389 < 0.001 for both datasets. In-vivo measurements of whole-body nickel concentrations of one 390 individual over time are shown in Figure 8. A moulting event occurred between t = 20 h and t =391 392 49 h, depleting the whole-body concentration. Our model correctly describes the effects due to this moulting behaviour. These results indicate that our description of the processes that result in 393 depuration via moulting is likely valid. Further details of the derivation of these parameters is 394 given in Supplementary material File S15. 395 <Figs. 7 & 8> 396 397 398 *Reducing variability due to moulting* Figure 9 shows how accumulated concentrations can fluctuate through time. For most of the 399 400 moult period it is unclear at what point in the period the organism lies. In the context of crustaceans, however, it is usually relatively easy to identify if the organism is immediately at a 401 402 pre-moult or post-moult stage (Drach, 1967; Buchholz, 1982). The pre-moult stage is often, 403 depending on the species, associated with visual changes to the exoskeleton, such as changing colour or texture (Drach, 1967). The post-moult stage is, at the very least, signalled by the 404 405 appearance of a shed cuticle. Both these stages correspond to the maximum and minimum 406 exoskeleton concentrations, respectively. In the context of bioaccumulation studies, we therefore 407 propose that sampling could be undertaken synchronously with moulting (moult-synchronous 408 sampling) to ensure the robustness of the measurement by reducing the variability due to

exoskeleton concentration fluctuations. This would take the form of ensuring only specimens which are immediately pre-moult or post-moult are sampled.

411

410

<Fig. 9>

In the context of *ex-situ* studies, implementation of moult-synchronous sampling quite simply takes the form of delaying sampling until the desired moult stage has been reached for each organism. Implementation for *in-situ* studies, however, is less straightforward due to the requirement that samples be taken when the site is visited. In a case such as this, we propose acquiring the organisms as normal, but holding them in a suitable tank until the desired moult stage has been reached. This approach assumes that minimal depuration through other means occurs between acquisition and moulting.

An argument could be put forward that the variability due to moulting could be reduced by sampling multiple specimens. This argument relies on the assumption that the moult stage of each specimen is uncorrelated; however, moulting can be induced or accelerated by environmental stressors (Fowler *et al.*, 1971; Nugegoda & Rainbow, 1987), therefore, it could happen that specimens in similar conditions can moult together. For this reason, performing measurements on multiple specimens held in the same conditions may not be sufficient to overcome the effects of moulting on accumulated concentrations.

Even ignoring the possibility of correlated moult stage within the population under study, moult-synchronous sampling can offer a more efficient approach to the determination of mean total bioaccumulated concentrations. Both increased sampling size and moult-synchronous sampling aim to reduce the measurement error in the overall measured accumulated concentration due to moulting. Equation 7 quantifies the variance of the measurement error due to moulting. In the example of Figure 6, this corresponds to a mean error variance of  $\sigma^2 = 14$ 

432	ppb. If measurements were only made within the first post-moult day, this would reduce the
433	mean error variance of a single measurement to $\sigma^2 = 0.14$ ppb. In lay terms, the resulting increase
434	in statistical accuracy corresponds to that which would be obtained by increasing the number of
435	specimens by a factor of 100. Figure 9 shows the reduction of measurement error variance due to
436	moulting from restricting the sampling window. Moult-synchronous sampling is, therefore, a
437	more efficient means of reducing the measurement error due to pollutant loss during moulting
438	than simply increasing the sampling size.
439	
440	ACKNOWLEDGEMENTS
441	The authors wish to acknowledge the support of the Irish Research Council and the
442	Environmental Protection Agency (Ireland) under grant GOIPG/2018/3351. The authors would
443	also like to thank the two anonymous reviewers for their insightful comments, and Prof. Emer
444	Rogan of the School of BEES, University College Cork, for casting a critical, but kind eye over
445	the early development of this model.
446	
447	SUPPLEMENTARY MATERIAL
448	Supplementary material is available at Journal of Crustacean Biology online.
449	S1 Table. Accumulated vanadium concentration values derived from Miramand et al. (1981).
450	S2 Table. Accumulated nickel concentration values derived from Figure 6 of Hall (1982).
451	S3 Table. Accumulated nickel concentration values derived from Figure 2a of Hall (1982).
452	S4 Table. Accumulated concentration values derived from Bergey & Weis (2007).
453	S5 Table. Accumulated concentration values derived from Hennig (1984).
454	S6 Table. Accumulated concentration values derived from Keteles & Fleeger (2001).

456	S8 Figure. Accumulation data from Miramand et al. (1981) (CF actual), and fit to Equation 3
457	(CF fit), with $T_M = 21$ days and $G = 1$ .
458	S9 Figure. Simulated exoskeleton compartment pollutant concentration, with continuous
459	moulting approximation (given by Equation S14) overlaid in dashed black.
460	S10 Figure. Error in whole-body pollutant concentration when modelling moulting as a
461	continuous process.
462	S11 File. Expanded derivations of the model equations.
463	S12 File. Implementation of the model code.
464	S13 File. Derivation of model parameters from data presented in Miramand et al. (1981).
465	S14 File. Description of the continuous approximation assumption and resulting error.
466	S15 File. Derivation of model parameters from data presented in Hall (1982).
467	
468	REFERENCES
469	Ardestani, M.M., van Straalen, N.M. & van Gestel, C.A. 2014. Uptake and elimination
470	kinetics of metals in soil invertebrates: a review. Environmental Pollution, 193: 277–295.
471	Awrahman, Z.A., Rainbow, P.S., Smith, B.D., Khan, F.R., Bury, N.R. & Fialkowski, W.
472	2015. Bioaccumulation of arsenic and silver by the caddisfly larvae Hydropsyche siltalai
473	and <i>H. pellucidula</i> : A biodynamic modeling approach. <i>Aquatic Toxicology</i> , <b>161</b> : 196–
474	207.
475	Bergey, L.L. & Weis, J.S. 2007. Molting as a mechanism of depuration of metals in the
476	fiddler crab, Uca pugnax. Marine Environmental Research, 64: 556–562.

S7 Table. Accumulated vanadium concentration values derived from Reinecke et al. (2003).

477	Bertine, K.K. & Goldberg, E.D. 1972. Trace elements in clams, mussels, and shrimp.
478	Limnology and Oceanography, 17: 877–884.
479	Bonada, N., Prat, N., Resh, V.H. & Statzner, B. 2006. Developments in aquatic insect
480	biomonitoring: a comparative analysis of recent approaches. Annual Review of
481	Entomology, <b>51</b> : 495–523.
482	Brown, P.L. & Markich, S.J. 2000. Evaluation of the free ion activity model of metal-
483	organism interaction: extension of the conceptual model. Aquatic Toxicology, 51: 177-
484	194.
485	Bryan, G.W. & Darracott, A. 1979. Bioaccumulation of marine pollutants. Philosophical
486	Transactions of the Royal Society of London B: Biological Sciences, 286: 483–505.
487	Buchholz, F. 1982. Drach's molt staging system adapted for euphausiids. Marine Biology,
488	<b>66</b> : 301–305.
489	Callender, E. 2003. Heavy metals in the environment – historical trends. Treatise on
490	<i>Geochemistry</i> , <b>9</b> : 67–105.
491	Depledge, M.H. & Rainbow, P.S. 1990. Models of regulation and accumulation of trace
492	metals in marine invertebrates. Comparative Biochemistry and Physiology C, 97: 1–7.
493	Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R. & Santore, R.C. 2001.
494	Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environmental
495	Toxicology and Chemistry, <b>20</b> : 2383–2396.
496	Drach, P. 1967. Sur la méthode de détermination des stades d'intermue et son application
497	générale aux crustacés. Vie et Milieu, Série A: Biologie marine, 18: 595–610.
498	EFSA Panel on Plant Protection Products and their Residues (PPR), Ockleford, C.,
499	Adriaanse, P., Berny, P., Brock, T., Duquesne, S., Grilli, S., Hernandez-Jerez, A.F.,

500	Hougaard Bennekou, S., Klein, M., Kuhl, T., Laskowski, R., Machera, K., Pelkonen, O.,
501	Pieper, S., Smith, R.H., Stemmer, M., Sundh, I., Tiktak, A., Topping, C.J., Wolterink, G.,
502	Cedergreen, N., Charles, S., Focks, A., Reed, M., Arena, M., Ippolito, A., Byers, H. &
503	Teodorovic, I. 2018. Scientific Opinion on the state of the art of
504	Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of
505	pesticides for aquatic organisms. EFSA Journal, 16: e05377 [doi: 10.
506	2903/j.efsa.2018.5377].
507	Elangovan, R., Ballance, S., White, K.N., McCrohan, C.R. & Powell, J.J. 1999.
508	Accumulation of aluminium by the freshwater crustacean Asellus aquaticus in neutral
509	water. Environmental Pollution, 106: 257–263.
510	Eriksson, S.P. & Baden, S.P. 1998. Manganese in the haemolymph and tissues of the
511	Norway lobster, Nephrops norvegicus (L.), along the Swedish west coast, 1993–1995.
512	Hydrobiologia, <b>375/376</b> : 255–264.
513	
	European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the
514	European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field
514 515	European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. (OJ L 327, 22.12.2000, pp. 1–73). Office for Official Publications of the
514 515 516	European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. (OJ L 327, 22.12.2000, pp. 1–73). Office for Official Publications of the European Communities, Luxembourg.
514 515 516 517	<ul> <li>European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. (OJ L 327, 22.12.2000, pp. 1–73). Office for Official Publications of the European Communities, Luxembourg.</li> <li>European Commission. 2008. Directive 2008/105/EC of the European Parliament and of</li> </ul>
514 515 516 517 518	<ul> <li>European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. (OJ L 327, 22.12.2000, pp. 1–73). Office for Official Publications of the European Communities, Luxembourg.</li> <li>European Commission. 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of</li> </ul>
514 515 516 517 518 519	<ul> <li>European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. (OJ L 327, 22.12.2000, pp. 1–73). Office for Official Publications of the European Communities, Luxembourg.</li> <li>European Commission. 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC,</li> </ul>
514 515 516 517 518 519 520	<ul> <li>European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. (OJ L 327, 22.12.2000, pp. 1–73). Office for Official Publications of the European Communities, Luxembourg.</li> <li>European Commission. 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive</li> </ul>
514 515 516 517 518 519 520 521	<ul> <li>European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. (OJ L 327, 22.12.2000, pp. 1–73). Office for Official Publications of the European Communities, Luxembourg.</li> <li>European Commission. 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. (OJ L 348, 24.12.2008, p.</li> </ul>
514 515 516 517 518 519 520 521 522	<ul> <li>European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. (OJ L 327, 22.12.2000, pp. 1–73). Office for Official Publications of the European Communities, Luxembourg.</li> <li>European Commission. 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. (OJ L 348, 24.12.2008, p. 84). Office for Official Publications of the European Communities, Luxembourg.</li> </ul>

523	Fleming, T.P. & Richards, K.S. 1982. Uptake and surface adsorption of zinc by the
524	freshwater tubificid oligochaete Tubifex tubifex. Comparative Biochemistry and
525	<i>Physiology C</i> , <b>71</b> : 69–75.
526	Flessas, C., Couillard, Y., Pinel-Alloul, B., St-Cyr, L. & Campbell, P.G. 2000. Metal
527	concentrations in two freshwater gastropods (Mollusca) in the St. Lawrence River and
528	relationships with environmental contamination. Canadian Journal of Fisheries and
529	Aquatic Sciences, <b>57</b> : 126–137.
530	Fowler, S.W., Small, L.F. & Kečkeš, S. 1971. Effects of temperature and size on molting of
531	euphausiid crustaceans. Marine Biology, 11: 45-51.
532	Greenaway, P. 1985. Calcium balance and moulting in the crustacea. Biological Reviews,
533	<b>60</b> : 425–454.
534	Guary, J.C. & Fowler, S.W. 1990. Experimental study of the transfer of transuranium
535	nuclides in marine decapod crustaceans. Marine Ecology Progress Series, 60: 253–270.
536	Hall, T.M. 1982. Free ionic nickel accumulation and localization in the freshwater
537	zooplankter, Daphnia magna. Limnology and Oceanography, 27: 718–727.
538	Hennig, H.FK.O. 1984. Baseline surveys and metal binding proteins as metal pollution
539	indicators. Ph.D. thesis, University of Cape Town, Cape Town, South Africa.
540	Jager, T., Albert, A., Preuss, T.G. & Ashauer, R. 2011. General Unified Threshold Model of
541	Survival – a toxicokinetic-toxicodynamic framework for ecotoxicology.
542	Environmental Science & Technology, <b>45</b> : 2529–2540.
543	Johnson, R.K., Wiederholm, T. & Rosenberg, D.M. 1993. Freshwater biomonitoring using
544	individual organisms, populations, and species assemblages of benthic

545	macroinvertebrates. In: Freshwater biomonitoring and benthic macroinvertebrates (D.M.
546	Rosenberg & V.H. Resh, eds.), pp. 40–158. Chapman & Hall, London.
547	Ketelaars, H.A. & Frantzen, N.M. 1995. One decade of benthic macroinvertebrate
548	biomonitoring in the River Meuse. Netherland Journal of Aquatic Ecology, 29: 121–133.
549	Keteles, K.A. & Fleeger, J.W. 2001. The contribution of ecdysis to the fate of copper, zinc
550	and cadmium in grass shrimp, Palaemonetes pugio Holthius. Marine Pollution Bulletin,
551	<b>42</b> : 1397–1402.
552	Khan, F.R., Bury, N.R. & Hogstrand, C. 2010. Cadmium bound to metal rich granules and
553	exoskeleton from Gammarus pulex causes increased gut lipid peroxidation in zebrafish
554	following single dietary exposure. Aquatic Toxicology, 96: 124–129.
555	Lebrun, J.D., Perret, M., Uher, E., Tusseau-Vuillemin, M.H. & Gourlay-Francé, C. 2011.
556	Waterborne nickel bioaccumulation in Gammarus pulex: Comparison of mechanistic
557	models and influence of water cationic composition. Aquatic Toxicology, <b>104</b> : 161–167.
558	Luoma, S.N. & Rainbow, P.S. 2005. Why Is metal bioaccumulation so variable?
559	Biodynamics as a unifying concept. Environmental Science & Technology, 39: 1921-
560	1931.
561	Markert, B. 2007. Definitions and principles for bioindication and biomonitoring of trace
562	metals in the environment. Journal of Trace Elements in Medicine and Biology, 21: 77-
563	82.
564	McGeer, J.C., Brix, K.V., Skeaff, J.M., DeForest, D.K., Brigham, S.I., Adams, W.J. &
565	Green, A. 2003. Inverse relationship between bioconcentration factor and exposure
566	concentration for metals: implications for hazard assessment of metals in the aquatic
567	environment. Environmental Toxicology and Chemistry, 22: 1017–1037.

568	Miramand, P., Guary, J.C. & Fowler, S.W. 1981. Uptake, assimilation, and excretion of
569	vanadium in the shrimp, Lysmata seticaudata (Risso), and the crab, Carcinus maenas
570	(L.). Journal of Experimental Marine Biology and Ecology, 49: 267–287.
571	Nugegoda, D. & Rainbow, P.S. 1987. The effect of temperature on zinc regulation by the
572	decapod crustacean Palaemon elegans Rathke. Ophelia, 27: 17-30.
573	O'Callaghan, I., Harrison, S.S.C., Fitzpatrick, D. & Sullivan, T. 2019. The freshwater isopod
574	Asellus aquaticus as a model biomonitor of environmental pollution: A review.
575	<i>Chemosphere</i> , <b>235</b> : 498–509.
576	Peijnenburg, W.J.G.M., Posthuma, L.H.J.P., Eijsackers, H.J.P. & Allen, H.E. 1997. A
577	conceptual framework for implementation of bioavailability of metals for environmental
578	management purposes. Ecotoxicology and Environmental Safety, 37: 163–172.
579	Phillips, D.J. 1977. The use of biological indicator organisms to monitor trace metal
580	pollution in marine and estuarine environments – a review. <i>Environmental Pollution</i> , <b>13</b> :
581	281–317.
582	Rae, I.B. & Gibb, S.W. 2003. Removal of metals from aqueous solutions using natural
583	chitinous materials. Water Science & Technology, 47: 189–196.
584	Rainbow, P.S. & Luoma, S.N. 2011. Metal toxicity, uptake and bioaccumulation in aquatic
585	invertebrates – modelling zinc in crustaceans. Aquatic Toxicology, <b>105</b> : 455–465.
586	Rauch, S. & Morrison, G.M. 1999. Platinum uptake by the freshwater isopod Asellus
587	aquaticus in urban rivers. Science of the Total Environment, 235: 261–268.
588	Reinecke, A.J., Snyman, R.G. & Nel, J.A.J. 2003. Uptake and distribution of lead (Pb) and
589	cadmium (Cd) in the freshwater crab, Potamonautes perlatus (Crustacea) in the Eerste
590	River, South Africa. Water, Air, and Soil Pollution, 145: 395–408.

591	Richardson, G.M., Garrett, R., Mitchell, I., Mah-Poulson, M. & Hackbarth, T. 2001. Critical
592	review on natural global and regional emissions of six trace metals to the atmosphere.
593	Final Report. Prepared for the International Lead Zinc Research Organisation, the
594	International Copper Association, and the Nickel Producers Environmental Research
595	Association
596	[https://www.echa.europa.eu/documents/10162/13630/vrar_appendix_p2_en.pdf].
597	Robinson, K.A., Baird, D.J. & Wrona, F.J. 2003. Surface metal adsorption on zooplankton
598	carapaces: implications for exposure and effects in consumer organisms. Environmental
599	<i>Pollution</i> , <b>122</b> : 159–167.
600	Santoro, A., Blo, G., Mastrolitti, S. & Fagioli, F. 2009. Bioaccumulation of heavy metals by
601	aquatic macroinvertebrates along the Basento River in the South of Italy. Water, Air, and
602	<i>Soil Pollution</i> , <b>201</b> : 19–31.
603	Topcuoğlu, S., Birol, E. & Ünlü, M.Y. 1987. Factors affecting the accumulation and
604	elimination of silver (110mAg) in marine isopods. Marine Environmental Research, 21:
605	189–198.
606	van den Brink, N.W., Kokalj, A.J., Silva, P.V., Lahive, E., Norrfors, K., Baccaro, M.,
607	Khodaparast, Z., Loureiro, S., Drobne, D., Cornelis, G. and Lofts, S. 2019. Tools and
608	rules for modelling uptake and bioaccumulation of nanomaterials in invertebrate
609	organisms. Environmental Science: Nano, 6: 1985–2001.
610	Van Hattum, B., De Voogt, P., Van den Bosch, L., Van Straalen, N.M., Joosse, E.N.G. &
611	Govers, H. 1989. Bioaccumulation of cadmium by the freshwater isopod Asellus
612	aquaticus (L.) from aqueous and dietary sources. Environmental Pollution, 62: 129–151.

613	Vijver, M.G., Van Gestel, C.A., Lanno, R.P., Van Straalen, N.M. & Peijnenburg, W.J. 2004.
614	Internal metal sequestration and its ecotoxicological relevance: a review. Environmental
615	Science & Technology, <b>38</b> : 4705–4712.
616	Wang, WX. & Tan, QG. 2019. Applications of dynamic models in predicting the
617	bioaccumulation, transport and toxicity of trace metals in aquatic organisms.
618	Environmental Pollution, 252: 1561–1573.
619	
620	FIGURE CAPTIONS
621	Figure 1. Schematic representation of a general overview of the major pathways of trace metal
622	pollutant uptake (via ingestion, respiration and adsorption), translocation, and loss (via moulting)
623	in a moulting aquatic organism. The dashed arrow represents the elimination of accumulated
624	pollutants at the time of moulting, which is modelled as a repeating instantaneous event, while
625	the solid arrows represent the continuous pollutant flux.
626	Figure 2. Rate diagram of pollutant flux into and out of the body ( <i>B</i> ) and moulting exoskeleton
627	(E) compartments. Processes illustrated are respiration $(r)$ , ingestion $(i)$ , adsorption $(a)$ , and
628	internal translocation ( $t$ ); $r$ , $i$ , and $a$ represent the concentrations from which respiration,
629	ingestion and adsorption, respectively, occur, $B$ the body compartment concentration. The $k_x$
630	parameters represent the respective rate constants.
631	Figure 3. Causal diagram showing the connection between bioavailable environmental metal
632	concentrations and measured whole-body metal concentrations, showing the influence of moult
633	stage on the exoskeleton concentration mediator.

**Figure 4.** Simulation of theoretical concentration of a metal pollutant in Compartment *B* (the body of the organism, excluding the moulting exoskeleton) versus time, from application of the described parameters. The dashed black line represents the equilibrium value given by Equation 5 (see text). The lighter lines represent the effects of different values of  $k_t$  for the same equilibrium value.

**Figure 5.** Simulation of concentration of a metal pollutant in Compartment *E* (the moulting exoskeleton of the organism) versus time, from application of the described parameters. Unlike with the concentration in the body compartment, *B* (see Fig. 2), the concentration does not reach an equilibrium state, but oscillates between 0 (complete absence, due to moulting) and a maximum value. The equilibrium maximum value is given by Equation 6 (see text), and is denoted here by the dashed black line.

**Figure 6.** Simulation of overall concentration of a metal pollutant in the organism *versus* time. The overall concentration is a mass-weighted combination of the concentrations in the body and moulting exoskeleton compartments, *B* and *E*. This simulation represents the evolution of the actual measured whole-body concentration over time, where the variation in the concentration beyond day 200 is entirely due to contaminant loss through moulting. The equilibrium minimum and maximum are given by the dashed black lines, and are derived from Equations 5 and 6 (see text) after accounting for body mass.

Figure 7. Accumulated concentrations of nickel in the soft-tissue (*B*) and exoskeleton (*E*) of
multiple *Daphnia magna* individuals (from Hall, 1982). The corresponding fit of the model
qualitatively matches the behaviour seen, where *B* saturates, but *E* continues to accumulate
indefinitely in the absence of moulting. Hall (1982) also observed indefinite accumulation in the

656 filtering appendages, which contain parts of exoskeleton and soft tissue, so have not been657 included in this figure.

**Figure 8.** Accumulated concentrations, across time, of nickel in an individual specimen of

659 *Daphnia magna* (from Hall, 1982). A moulting event occurred between t = 20h and t = 49h. A fit

of the model, accounting for a moulting event just before t = 49 h, accurately describes the

observed behaviour, despite the relative simplicity of the model.

**Figure 9.** Proportional decrease in moult-induced measurement error with increasing accuracy of moult-synchronous sampling, where a "100%" sampling window is equivalent to ignoring moult stage when sampling. Using a sampling window of 10% of the moult period, for example, would reduce the variance by a factor of 100, for the same sample size.

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**Figure 2.** 



**Figure 3.** 





**Figure 4.** 





**Figure 5.** 















**Figure 8.** 



**Figure 9.** 

