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3 Running head: O'CALLAGHAN & SULLIVAN: MOULTING AND METAL

4 CONCENTRATIONS

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6 **Shedding the load: moulting as a cause of variability in whole-body**  
7 **metal concentrations**

8

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20

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

50 anthropogenic activities than of metals from natural sources entering waterways (Callender,  
51 2003).

52         A distinction must be made between total and bioavailable pollutant concentrations in  
53 order to appreciate the advantage of biomonitoring over direct measurement of the pollutants.  
54 Various physico-chemical processes can render a pollutant biologically inert. These can be  
55 divided into environmental processes, that influence the “environmental availability,” and  
56 internal biological processes within an organism, that influence the “toxicological  
57 bioavailability” of the pollutant (Peijnenburg *et al.*, 1997). While total pollutant concentrations  
58 can be determined directly through chemical analysis, accounting for the concept of  
59 bioavailability requires either the application of measured environmental parameters to a model  
60 of the environmental processes (the *a priori* approach, often the application of partition  
61 coefficients to measured total pollutant concentrations), or direct measurement of accumulated  
62 concentrations within the organism of interest (the *a posteriori* approach). The classification of  
63 pollutants according to their bioavailability and toxicity, such as the Priority Substances List of  
64 the Water Framework Directive and subsequent amendments (European Commission, 2000,  
65 2008), is a similar approach to the former. Biomonitoring exemplify the latter, as the measured  
66 biological concentrations are the result of both the environmental availability and toxicological  
67 bioavailability processes, and, therefore, offer an insight into the results of these processes  
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.

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

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

79         There is still some debate around the extent to which crustaceans uptake and  
80 bioaccumulate trace metals, and reproducibility of measured metal concentrations appears to be  
81 the exception, rather than the norm (Depledge & Rainbow, 1990; O'Callaghan *et al.*, 2019). This  
82 is compounded by the uncertainty regarding the most likely sources, uptake pathways or sites of  
83 bioaccumulation within the organism of interest (Fleming & Richards, 1982; Elangovan *et al.*,  
84 1999; Van Hattum *et al.*, 1999; Robinson *et al.*, 2003; Santoro *et al.*, 2009). Several models have  
85 been proposed for the processes of uptake, bioaccumulation, and excretion of various pollutants  
86 in freshwater macroinvertebrates (Rainbow & Luoma, 2011; Awrahman *et al.*, 2015), a task  
87 made more complicated by the varying behaviour of different metal analytes and potential  
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.

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

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

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

112

## 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).

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

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

131 Biodynamic models, commonly referred to as physiologically-based pharmacokinetic  
132 (PBPK), are better suited to modelling the dynamic potential contribution of moulting to the  
133 accumulated concentrations of contaminant (Ardestani *et al.*, 2014; van den Brink *et al.*, 2019).  
134 This approach models the processes of uptake, accumulation, translocation, transformation, and  
135 excretion across time, and does not rely on any steady-state assumptions. toxicokinetic-  
136 toxicodynamic (TKTD) models, such as general unified threshold model of survival (GUTS)  
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  
140 relatively good correlation with observed results across a large number of studies (Luoma &  
141 Rainbow, 2005), and GUTS is considered sufficiently developed for use in risk assessment

142 applications (EFSA Panel on PPR *et al.*, 2018). These models, however, have not yet been  
143 extended to include the contribution of moulting, a correction that would have to be separately  
144 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  
148 capture the essence of the problem, while remaining broadly applicable to any moulting aquatic  
149 invertebrates such as crustaceans, whose exoskeleton may act as a significant sink for  
150 contaminants, and any transition metal, metalloid, or heavy metal species. Such approach should  
151 be contrasted with the common approach deriving a quantitative, predictive model described  
152 above. The aim of our model is instead to illustrate certain contributions of the moulting process  
153 to measured concentrations that are common to all moulting aquatic invertebrates and metal  
154 analytes, without offering a prediction for the significance of these contributions in any one  
155 scenario.

156 We limit the applicability of the presented model to the transition, metalloid and heavy  
157 metals, as it has been observed that the accumulation of the alkali or alkaline earth metals in the  
158 exoskeleton of an aquatic crustacean may differ from that of the aforementioned elements. The  
159 accumulation of calcium, in particular, has been extensively investigated throughout the various  
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  
163 specifically, the nitrogen groups therein, may play a role in the alternative behaviour of the  
164 transition metals, metalloids, and heavy metals, as it has been noted that chitinous materials



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

168

### 169 *Assumptions*

170 For the purpose of creating a concise and simplified conceptual model, we must introduce a  
171 number of assumptions. These assumptions are chosen such that they adequately isolate the  
172 impact of moulting on trace metals concentrations, while removing internal processes that are not  
173 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.

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

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

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

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

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

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

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

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

208

209 *The conceptual model*

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

218 *<Figs. 1 & 2>*

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

229 Moulting, or ecdysis, refers to the regular removal of the outer exoskeleton. As the  
230 exoskeleton is shed, a new exoskeleton develops beneath it (see Drach, 1967). The loss of the  
231 moulted exoskeleton cannot be modelled as a continuous flow of contaminants as it occurs  
232 suddenly and periodically, and, therefore, it is not included in the process diagram in Figure 2.

233 Instead, the diagram describes the inter-moult movement of contaminants, and moulting is  
234 implemented externally as a periodic discontinuous removal of the contaminants within  
235 Compartment *E*. The moult period, or the rate at which an organism moults, will vary greatly  
236 with species and other factors. It should be noted that occasional consumption of the organism's  
237 own moulted exoskeleton has been observed in some macroinvertebrate species (Elangovan *et*  
238 *al.*, 1999), although there is consensus that metals bound within a chitinous exoskeleton are less  
239 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.

241

#### 242 *Impact of moulting*

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

251

<Fig. 3>

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

255 organisms is uncorrelated, then sampling more organisms should reduce, but not eliminate, the  
 256 influence 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,  
 261 which describe the change in inter-moult concentrations of metal contaminant in each  
 262 compartment.

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

264

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

266

267

### 268 *Growth factor*

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

274

### 275 *Closed-form expressions*

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

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

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

281 where the uptake rate is given by  $U = k_i [i] + k_r [r]$ , and the process time constant is  $\tau = G/k_t$ .

282 Note that the concentrations in Compartment  $B$  can be modelled as a first-order  
 283 underdamped system, where the concentration approaches an equilibrium value of  $U/k_t$ , while  
 284 the concentration in Compartment  $E$  increases indefinitely as the loss due to moulting is not yet  
 285 accounted for.

286

### 287 *Steady-state equations*

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

$$291 \quad [B] = U/k_t \quad (5)$$

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

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

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

$$298 \quad \sigma^2 = \frac{([E]_{\text{MAX}})^2}{12} \quad (7)$$

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

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>

318

319 *Simulation results*

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

323 *<Fig. 4>*

324 As expected of a first-order system, the concentration in Compartment *B* reaches steady-  
325 state at a speed dictated by the rate of internal translocation of the contaminant. Once the internal  
326 concentration reaches steady-state, there is no significant change in contaminant concentration  
327 without a corresponding change in the environmental conditions.

328 Figure 5 shows the bioaccumulated concentration of the contaminant in Compartment *E*.  
329 Moulting forces the pre-moult exoskeleton concentration to 0 every  $T_M$  days. This produces a  
330 periodic pattern with increasing amplitude, approaching a sawtooth pattern as Compartment *B*  
331 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  
337 significant. This results in a time variation in the overall concentration.

338 *<Fig. 6>*

339

## 340 DISCUSSION

341 *Contribution of moulting*



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

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

358

### 359 *Validity of simulated example*

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

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

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

382 *<Table 2>*

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

387 exoskeleton concentrations in individuals that have not moulted. It shows that the soft-tissue  
388 concentrations rapidly reach steady-state, while the exoskeleton concentrations continue to  
389 increase in the absence of moulting. A corresponding fit of the model is shown, with a value of  $P$   
390  $< 0.001$  for both datasets. *In-vivo* measurements of whole-body nickel concentrations of one  
391 individual over time are shown in Figure 8. A moulting event occurred between  $t = 20$  h and  $t =$   
392 49 h, depleting the whole-body concentration. Our model correctly describes the effects due to  
393 this moulting behaviour. These results indicate that our description of the processes that result in  
394 depuration via moulting is likely valid. Further details of the derivation of these parameters is  
395 given in Supplementary material File S15.

396 *<Figs. 7 & 8>*

397

#### 398 *Reducing variability due to moulting*

399 Figure 9 shows how accumulated concentrations can fluctuate through time. For most of the  
400 moult period it is unclear at what point in the period the organism lies. In the context of  
401 crustaceans, however, it is usually relatively easy to identify if the organism is immediately at a  
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  
404 colour or texture (Drach, 1967). The post-moult stage is, at the very least, signalled by the  
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

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

411 *<Fig. 9>*

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

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

426 Even ignoring the possibility of correlated moult stage within the population under study,  
427 moult-synchronous sampling can offer a more efficient approach to the determination of mean  
428 total bioaccumulated concentrations. Both increased sampling size and moult-synchronous  
429 sampling aim to reduce the measurement error in the overall measured accumulated  
430 concentration due to moulting. Equation 7 quantifies the variance of the measurement error due  
431 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. Moulst-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

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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).

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

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## 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 *H. pellucidula*: A biodynamic modeling approach. *Aquatic Toxicology*, **161**: 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.

- 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*, **51**: 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 **66**: 301–305.
- 489 Callender, E. 2003. Heavy metals in the environment – historical trends. *Treatise on*  
490 *Geochemistry*, **9**: 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*, **20**: 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*, **375/376**: 255–264.
- 513 European Commission. 2000. *Directive 2000/60/EC of the European Parliament and of the*  
514 *Council of 23 October 2000 establishing a framework for Community action in the field*  
515 *of water policy*. (OJ L 327, 22.12.2000, pp. 1–73). Office for Official Publications of the  
516 European Communities, Luxembourg.
- 517 European Commission. 2008. *Directive 2008/105/EC of the European Parliament and of*  
518 *the Council of 16 December 2008 on environmental quality standards in the field of*  
519 *water policy, amending and subsequently repealing Council Directives 82/176/EEC,*  
520 *83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive*  
521 *2000/60/EC of the European Parliament and of the Council*. (OJ L 348, 24.12.2008, p.  
522 84). Office for Official Publications of the European Communities, Luxembourg.



- 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 *Physiology C*, **71**: 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*, **57**: 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 **60**: 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.F.-K.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*, **45**: 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 **42**: 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*, **104**: 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, *Lysemata 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 *Chemosphere*, **235**: 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. *Environmental Pollution*, **13**:  
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*, **105**: 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](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 *Pollution*, **122**: 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 *Soil Pollution*, **201**: 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*, **38**: 4705–4712.

616 Wang, W.-X. & Tan, Q.-G. 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 ( $B$ ) 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.

634 **Figure 4.** Simulation of theoretical concentration of a metal pollutant in Compartment *B* (the  
635 body of the organism, excluding the moulting exoskeleton) versus time, from application of the  
636 described parameters. The dashed black line represents the equilibrium value given by Equation  
637 5 (see text). The lighter lines represent the effects of different values of  $k_t$  for the same  
638 equilibrium value.

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

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

652 **Figure 7.** Accumulated concentrations of nickel in the soft-tissue (*B*) and exoskeleton (*E*) of  
653 multiple *Daphnia magna* individuals (from Hall, 1982). The corresponding fit of the model  
654 qualitatively matches the behaviour seen, where *B* saturates, but *E* continues to accumulate  
655 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 been  
657 included in this figure.

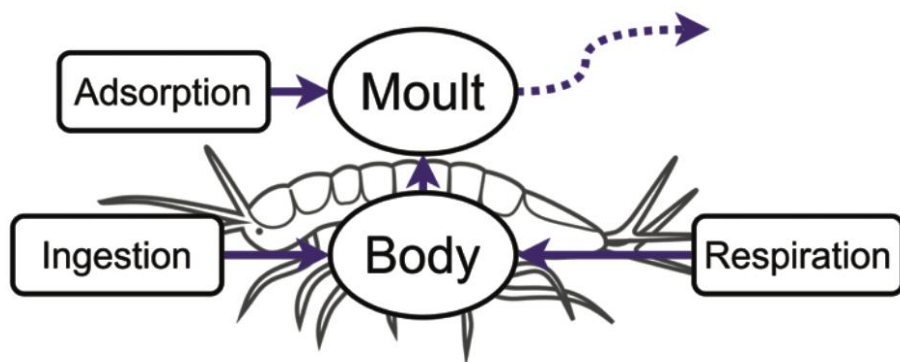
658 **Figure 8.** Accumulated concentrations, across time, of nickel in an individual specimen of  
659 *Daphnia magna* (from Hall, 1982). A moulting event occurred between  $t = 20\text{h}$  and  $t = 49\text{h}$ . A fit  
660 of the model, accounting for a moulting event just before  $t = 49\text{ h}$ , accurately describes the  
661 observed behaviour, despite the relative simplicity of the model.

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

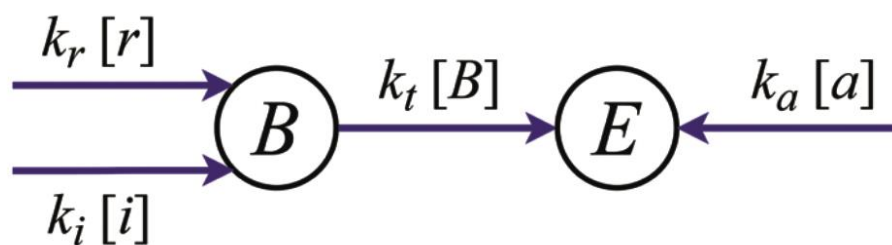
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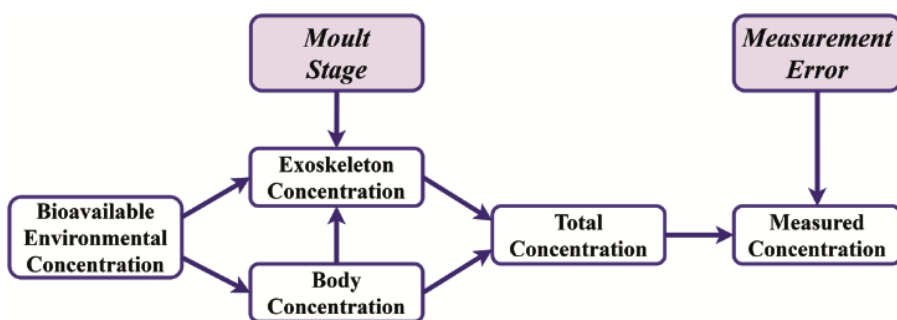
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669 **Figure 1.**

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671 **Figure 2.**

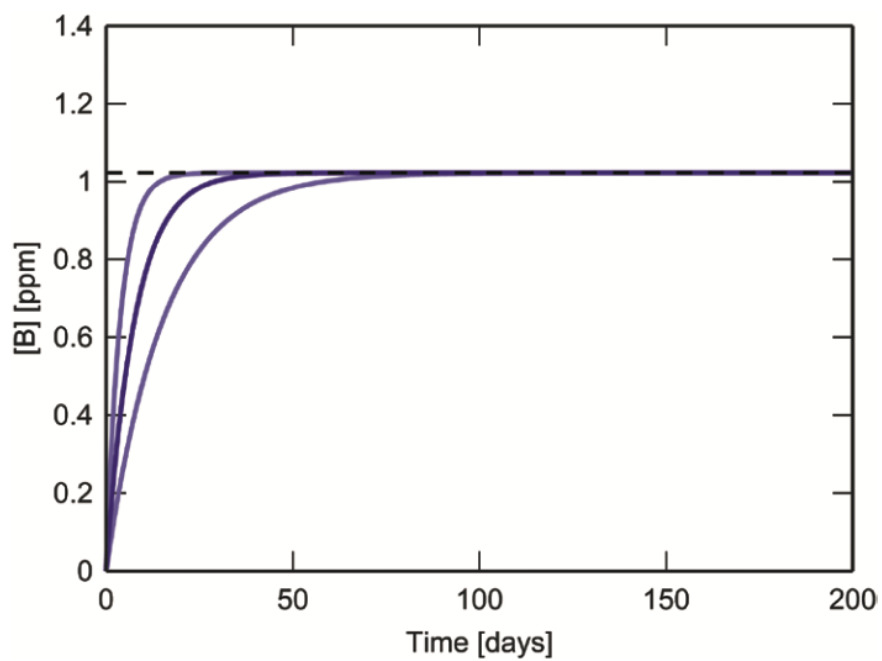
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673 **Figure 3.**

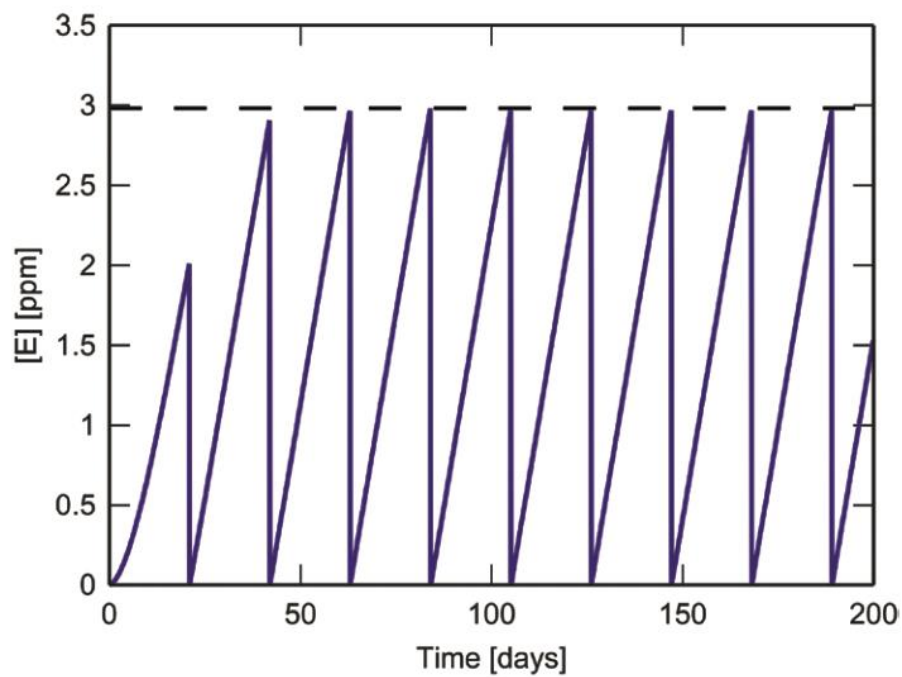
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675 **Figure 4.**



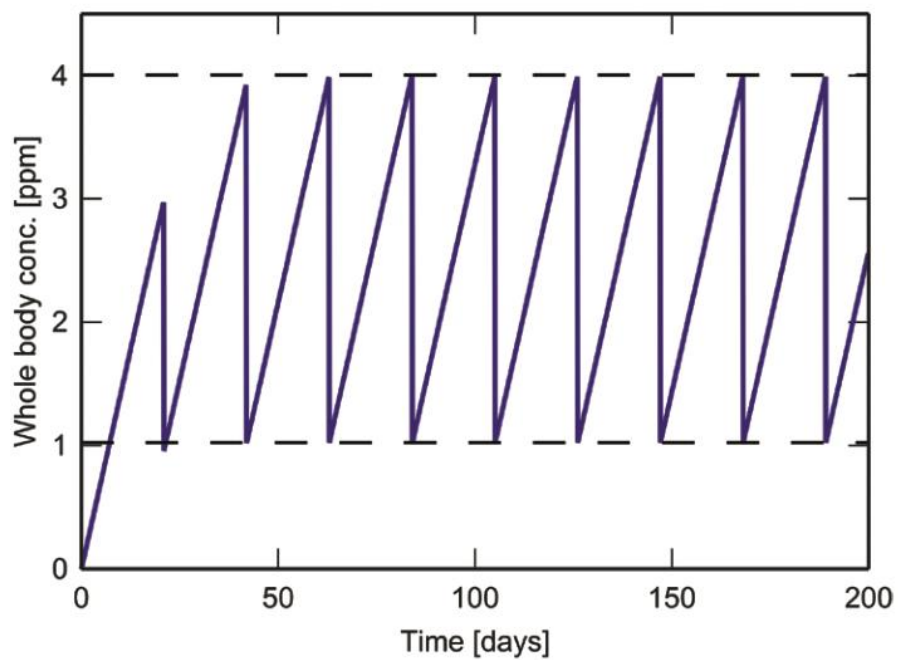


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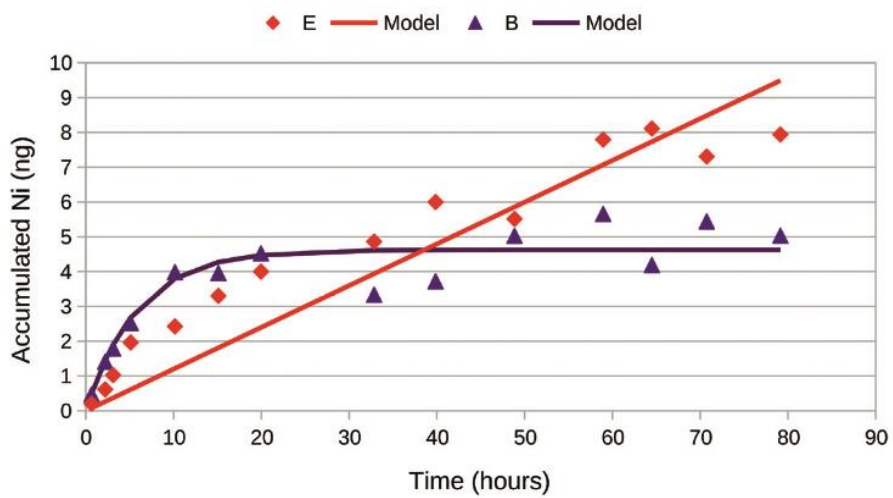
677 **Figure 5.**

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679 **Figure 6.**

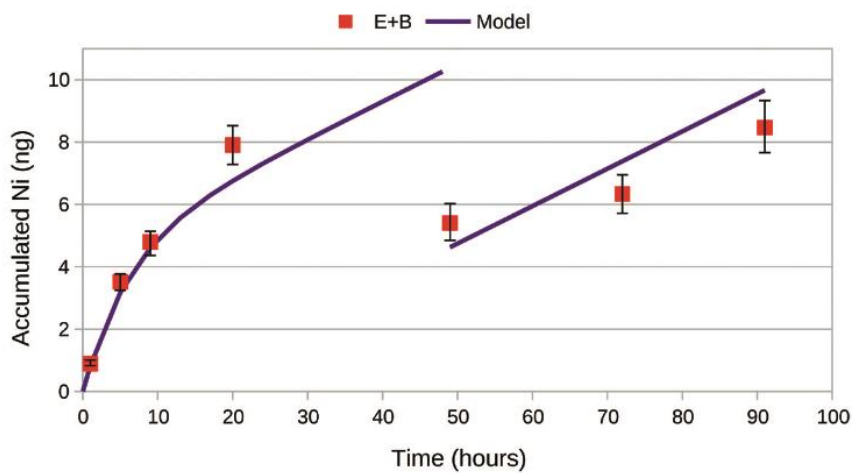


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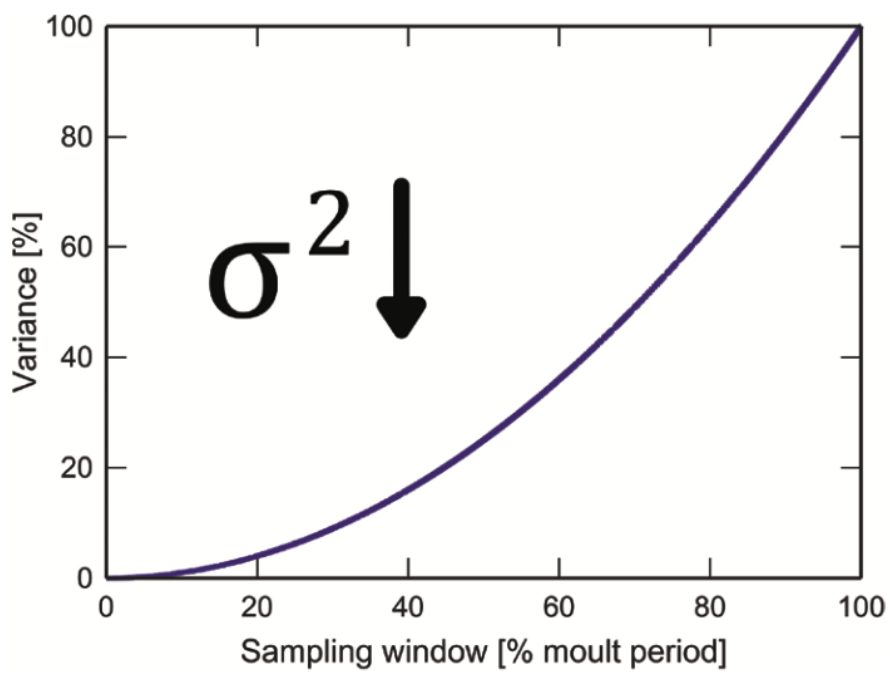
681 **Figure 7.**

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683 **Figure 8.**



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685 **Figure 9.**

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