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DEBtox theory and matrix population models  
as helpful tools in understanding the  
interaction between toxic cyanobacteria and  
zooplankton

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**Abstract**

1 Bioassays were performed to find out how field samples of the toxic cyanobacteria  
2 *Microcystis aeruginosa* affect *Moina micrura*, a cladoceran found in the tropical  
3 Jacarepagua Lagoon (Rio de Janeiro, Brazil). The DEBtox approach has been pro-  
4 posed for use in analysing chronic toxicity tests as an alternative to calculating  
5 the usual safety parameters (NOEC, ECx). DEBtox theory deals with the energy  
6 balance between physiological processes (assimilation, maintenance, growth and re-  
7 production), and it can be used to investigate and compare various hypotheses  
8 concerning the mechanism of action of a toxicant. Even though the DEBtox frame-  
9 work was designed for standard toxicity bioassays carried out with standard species  
10 (fish, daphnids), we applied the growth and reproduction models to *M.micrura*, by  
11 adapting the data available using a weight-length allometric relationship. Our mod-  
12 elling approach appeared to be very relevant at the individual level, and confirmed  
13 previous conclusions about the toxic mechanism. In our study we also wanted to  
14 assess the toxic effects at the population level, which is a more relevant endpoint in  
15 risk assessment. We therefore incorporated both lethal and sublethal toxic effects  
16 in a matrix population model used to calculate the finite rate of population change  
17 as a continuous function of the exposure concentration. Alongside this calculation,  
18 we constructed a confidence band to predict the critical exposure concentration for  
19 population health. Finally, we discuss our findings with regard to the prospects for  
20 further refining the analysis of ecotoxicological data.

21 *Key words:* allometry, DEBtox models, matrix population model, Bayesian  
22 inference, *Moina micrura*, *Microcystis aeruginosa*

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## 23 1 Introduction

24 One of the main objectives in ecotoxicology is to provide an estimation of  
25 safety parameters, e.g. No Observed Effect Concentration (NOEC) and x%  
26 Effect Concentration (ECx), that can be derived from standardized toxic-  
27 ity tests (OECD, 1998; ISO, 2000). These approaches have been criticized for  
28 many reasons: 1) NOEC and ECx usually focus on a single endpoint (mortality,  
29 growth or reproduction); 2) the standard tests involve a fixed exposure time,  
30 irrespective of the properties of the chemical being tested; 3) they generally  
31 use only a few standard species, which may not be relevant to the ecosystem  
32 being investigated; 4) NOEC and ECx are based on purely descriptive regres-  
33 sion models, and are unrelated to the physiological processes of the organisms  
34 that are being tested, and to the toxicokinetics of the compound being tested  
35 (Chapman, 1996; Kooijman & Bedaux, 1996; Péry *et al.*, 2002; Jager *et al.*,  
36 2004, 2006). Biology-based models, such as DEBtox (Dynamic Energy Budget  
37 Theory applied to toxicity data) (Kooijman & Bedaux, 1996), have been pro-  
38 posed to overcome these shortcomings: 1) this modelling approach is based on  
39 the concept of No Effect Concentration (NEC), which is common to several bi-  
40 ological processes and consequently to several sublethal endpoints (e.g. growth  
41 and reproduction); 2) DEBtox models take into account the toxicokinetics of  
42 the chemical being tested; 3) they were originally developed for a standard  
43 panel of species, but they can be adapted to others; 4) DEBtox theory is a  
44 mechanistic modelling approach, based on several assumptions about how the  
45 test compound disrupts the energy balance between the various physiological  
46 processes.

47 In addition, ecotoxicology is now attempting to assess the impact of chemical

48 compounds not only on individuals, but also at higher organizational levels.  
49 For example, the population level is particularly relevant when different life  
50 stages may display different susceptibilities to toxic compounds (Emlen &  
51 Springman, 2007). In this case, the finite rate of population increase  $\lambda$  is  
52 considered to be a robust endpoint (Forbes & Calow, 1998). Various methods  
53 can be used to relate the effects of a compound on individuals to its impact  
54 on the finite rate of population increase. The one we chose was the matrix  
55 population model approach, which has already been successfully combined  
56 with DEBtox models (Lopes *et al.*, 2005; Billoir *et al.*, 2007).

57 Cyanobacteria are some of the most ancient organisms on Earth ( $\simeq$  4 billion  
58 years), and the first to develop the ability to fix atmospheric carbon and re-  
59 lease oxygen through photosynthesis. Another distinguishing characteristic of  
60 cyanobacteria is the fact that they produce secondary metabolites with toxic  
61 properties, known as cyanotoxins (Carmichael, 1992). Cyanotoxins have been  
62 responsible worldwide for deaths of wild and domestic animals, and also for  
63 some human fatalities (Ressom *et al.*, 1994; Jochimsen *et al.*, 1998; Carmichael  
64 *et al.*, 2001). The most common cyanotoxins are the hepatotoxic peptides,  
65 which are produced by some genera of freshwater bloom-forming cyanobac-  
66 teria such as *Microcystis*, *Anabaena* and *Oscillatoria*. In spite of the effects  
67 observed in non-target organisms, such as mammals, one of the most gener-  
68 ally accepted hypotheses to explain the evolutionary role of these toxins is  
69 that they are produced to protect cyanobacteria against their main predators,  
70 including the herbivorous zooplankton, such as *Daphnia*. According to this hy-  
71 pothesis, cyanotoxins act as chemical defenses against zooplankton predators,  
72 by decreasing their fitness (Lampert, 1981, 1987). The interaction between  
73 cyanobacteria and zooplankton has been widely used as a model system to

74 study the evolutionary role of cyanotoxins. Also, since cyanotoxins also pose  
75 some risk to human health, the ecotoxicology of the cyanobacteria-zooplankton  
76 system is used to study the mechanisms of action of these toxins in aquatic  
77 organisms, and can be used as the basis for guidelines for environmental health  
78 and water quality as well.

79 In this study, we used ecotoxicological data from the study of Ferrão-Filho &  
80 Azevedo (2003), which reported the effects of naturally-occurring cyanobac-  
81 teria from a hypereutrophic coastal lagoon in Brazil, including data from life-  
82 table and growth experiments with *Moina micrura* Kurs, a tropical freshwa-  
83 ter cladoceran. In contrast to the big temperate cladocerans, such as *Daphnia*  
84 (2.0-4.0 mm body size), *M. micrura* is much smaller (0.8-1.0 mm body size),  
85 reaches maturity after only 2-3 days, and has a shorter life-cycle (no more  
86 than 20 days).

87 Our main goal was to study the interaction between *Microcystis* and *M. mi-*  
88 *crura* by applying the DEBtox modelling framework to experimental data, in  
89 order to validate some hypotheses, in particular concerning the mechanism of  
90 action of the toxicant. Our second goal was to improve the ecotoxicological  
91 relevance of the analysis. To do this, both lethal and sublethal effect models  
92 were included in matrix population models, allowing us to extrapolate the im-  
93 pact of toxic effects observed in individuals to the population level. Moreover,  
94 a confidence band was added to the population endpoint to predict critical  
95 exposure concentrations for population health.

## 96 2 Models and Methods

### 97 2.1 Data

98 The data were obtained from life-table and growth experiments (Experiments  
99 2 and 5, respectively, described in Table I in the study of Ferrão-Filho &  
100 Azevedo (2003)), carried out with a sample of a naturally occurring bloom  
101 of cyanobacteria from Jacarepaguá Lagoon, in Rio de Janeiro (Brazil). The  
102 sample consisted of particulate material (seston) collected with a plankton net  
103 ( $25\ \mu\text{m}$ ). This sample was centrifuged, and the supernatant containing large  
104 colonies was discarded, while the settled material was used in the experiments.  
105 This material consisted mostly of single cells and small colonies of *Microcystis*  
106 ( $5\text{-}20\ \mu\text{m}$ ), and a small proportion of algae and detritus. Different concen-  
107 trations of seston, expressed as organic carbon ( $0.25$  to  $1.5\ \text{mg C L}^{-1}$ ), were  
108 used in the experiments. The microcystin (toxin) concentration in this sample  
109 was  $3.1\ \text{mg g}^{-1}$  of dry weight, and nominal microcystin concentrations in the  
110 seston treatments ranged from  $1.6$  to  $9.4\ \mu\text{g L}^{-1}$ . The controls ( $0.0\ \text{mg C L}^{-1}$   
111 of seston) consisted of animals fed solely with nutritious green algae at the  
112 concentration of  $1.0\ \text{mg C L}^{-1}$ . To avoid interference from nutrition factors,  
113 as a result of a poor food supply, the same amount of green algae was mixed  
114 with all the seston treatments.

115 The life-table experiment was performed with 20 replicate animals (females)  
116 per treatment, and lasted 16 days. Survivorship and neonates produced per  
117 female, as the cumulative number of offspring, were recorded daily. The growth  
118 experiment was performed with three replicate bottles containing 50 newborns  
119 ( $< 24\ \text{h}$ ), and lasted 6 days, with samplings for weighting the animals on days



120 2, 4 and 6.

## 121 2.2 DEBtox modelling

122 At the individual level, we used DEBtox modelling (Kooijman & Bedaux,  
 123 1996) to describe the effects of the contaminant on life history traits measured  
 124 during the experiments (e.g. growth, reproduction). The DEBtox framework  
 125 is based on the DEB theory, which assumes that energy is derived from food,  
 126 and is assimilated to constitute reserves. These reserves are shared between  
 127 three main processes: maintenance, growth, and reproduction. DEBtox models  
 128 deal with sublethal effects by assuming that the contaminant affects the en-  
 129 ergy balance, and consequently affects growth and reproduction. The DEBtox  
 130 theory also comprises a contamination kinetics model and a survival model  
 131 for the lethal effects of the toxicant. These different parts are presented below.  
 132 All DEBtox variables and parameters are summarized in Table 1.

### 133 2.2.1 Toxicological aspects

134 Lethal and sublethal effects of a contaminant are modelled using the following  
 135 stress function:

$$136 \quad s(c_q(t)) = c_*^{-1}(c_q(t) - NEC_*)_+ \quad (1)$$

137 where  $(c_q(t) - NEC_*)_+ = \max(0, c_q(t) - NEC_*)$ . Indeed, in accordance with  
 138 the DEBtox theory (Kooijman & Bedaux, 1996), the contaminant is assumed  
 139 to produce an effect when the concentration inside the organisms ( $c_q(t)$ ) ex-  
 140 ceeds a concentration called the No Effect Concentration ( $NEC_*$ ,  $*$  =  $L$  for  
 141 the lethal effects,  $*$  =  $S$  for the sublethal effects). Moreover, the toxic effect is

142 assumed to be proportional to the excess above the  $NEC_*$ .  $c_*$  is the so-called  
 143 tolerance concentration (Kooijman & Bedaux, 1996), which can be seen as a  
 144 kind of toxicity rate reciprocal ( $*$  =  $L$  for the lethal effects,  $*$  =  $H$ ,  $R$ ,  $A$ ,  $G$ ,  
 145 or  $M$  for the sublethal effects, depending on the mechanism of action being  
 146 considered (see *Sublethal effects*)).  $c_q(t)$  corresponds to the scaled concentra-  
 147 tion of the toxic compound inside the organism at time  $t$ , determined by  
 148 the following one-compartment kinetic model:

$$149 \quad \frac{dc_q(t)}{dt} = \frac{ck_e}{l(t)} - c_q(t) \left( \frac{\dot{k}_e}{l(t)} + \frac{d \ln(l(t)^3)}{dt} \right) \quad (2)$$

150 where  $\dot{k}_e$  is the elimination rate,  $c$  the exposure concentration and  $l(t)$  the  
 151 scaled body length at time  $t$ . The initial condition for the contamination ki-  
 152 netics equation was set at  $c_q(t=0) = 0$ , as exposure started at the beginning  
 153 of the experiment. For a more detailed explanation of this kinetics, see Kooi-  
 154 jman & Bedaux (1996).

### 155 2.2.2 Lethal effects

156 As far as the lethal effects were concerned, we used a classical modelling with  
 157 a tolerance function  $q(t, c)$  expressed as the exponential of minus a cumulative  
 158 hazard function. Thus, the probability  $q(t, c)$  of surviving until time  $t$  with an  
 159 exposure concentration of  $c$  can be written as follows:

$$160 \quad q(t, c) = \exp\left(-\int_0^t \dot{h}(\tau, c) d\tau\right) \quad (3)$$

161 where  $\dot{h}(\tau, c)$  is the hazard rate at time  $\tau$  and exposure concentration  $c$ .  $\dot{h}(\tau, c)$   
 162 is written as follows:

$$163 \quad \dot{h}(\tau, c) = \begin{cases} \dot{m} + s(c_q(\tau)) & \text{if } c > NEC_L \text{ and } \tau > t_0 \\ \dot{m} & \text{else} \end{cases}$$

164 where  $t_0$  is the time at which  $c_q(\tau)$  exceeds  $NEC_L$ :  $t_0 = -\dot{k}_e^{-1} \ln(1 - \frac{NEC_L}{c})$   
 165 being defined only if  $c > NEC_L$ .

### 166 2.2.3 Sublethal effects

167 Five possible mechanisms of action were proposed to deal with sublethal ef-  
 168 fects of the contaminant on the various processes considered in the DEBtox  
 169 framework (assimilation, maintenance, growth, reproduction): an increase in  
 170 maintenance costs (*Maintenance*), an increase in growth costs (*Growth*), a  
 171 decrease in assimilation (*Assimilation*), an increase in egg production costs  
 172 (*Costs*), or a surmortality during oogenesis (*Hazard*) (Kooijman & Bedaux,  
 173 1996). These five assumptions led to different equations for modelling growth  
 174 and reproduction, the endpoints potentially measured in toxicity tests involv-  
 175 ing zooplankton. Contaminant kinetics, individual growth and reproduction  
 176 are interrelated, so the corresponding equations had to be considered simulta-  
 177 neously. All the equations are summarized in Table 2 (Billoir *et al.*, in press),  
 178 and their variables and parameters in Table 1.

## 179 2.2.4 DEBtox parameter estimation

180 In this study, the investment ratio  $g$  was fixed at  $g = 1$ , the reference value for  
181 control organisms at optimal temperature (Kooijman & Bedaux, 1996; Kooi-  
182 jman *et al.*, 2003), and the ingestion rate as a fraction of its maximum value,  
183  $f$ , was fixed at  $f = 1$ , as the organisms were fed *ad libitum*. We collected data  
184 for 16 days, but we only used the data from day 0 to 10 to fit the models,  
185 because after 10 days, senescence effects appeared, which were not taken into  
186 account in the models. For the estimation of survival DEBtox parameters ( $\dot{k}_e$ ,  
187  $NEC_L$ ,  $\dot{k}_t$  and  $m$ ), we performed nonlinear regression of equation (3) on the  
188 survival data, based on a least squares criterion, implying data independence,  
189 homoscedasticity and normal error assumptions. To minimize the residual sum  
190 of squares, we used the *optim()* function implemented in the R language (R  
191 Development Core Team, 2007). For the estimation of the growth and repro-  
192 duction DEBtox parameters ( $\dot{k}_e$ ,  $NEC_S$ ,  $c_*$ ,  $L_m$ ,  $\gamma$ ,  $\dot{R}_m$  and  $l_p$ ), Billoir *et al.*  
193 (in press) have shown that nonlinear regression is sometimes inadequate, so we  
194 used Bayesian inference with WinBugs (Lunn *et al.*, 2000) and WBDiff (Lunn,  
195 2004) softwares, as proposed by Billoir *et al.* (resubmitted). From the arbitrary  
196 *prior* probability distribution for each parameter, Bayesian inference provides  
197 estimates as samples of *posterior* distributions given the data. Moreover, this  
198 estimation method made it easy to fit growth and reproduction data simulta-  
199 neously. In this study, we used slightly informative *prior* distributions which  
200 are summarized in Table 3. After checking the convergence of the estimation  
201 process, we considered the *posterior* distribution means as estimates.  $\dot{k}_e$  was  
202 involved in both the lethal and sublethal models, and consequently it was es-  
203 timated in two different ways, which could yield different values. Thereafter,  
204 though this is questionable (see Discussion), we kept both these estimates,

205 and used each for the models for which it had been estimated.

### 206 2.3 Allometric relationship

207 As only dry body weight data were available, rather than the body length  
208 data required to fit DEBtox models, these two quantities were related using  
209 an allometric relationship. The typical form for such a relationship is as follows  
210 (Jerison, 1973):

$$211 \quad y = ax^b \quad \text{or} \quad \log(y) = \log(a) + b \log(x) \quad (13)$$

212 where  $y$  and  $x$  are biological quantities,  $a$  and  $b$  are both regression parameters.  
213 In our case,  $y$  corresponded to the body length, and  $x$  corresponded to the  
214 dry body weight. To estimate the  $a$  and  $b$  parameters, we performed a linear  
215 regression in the log-log representation with data collected between day 2 and  
216 6 in five independent growth experiments performed on *M. micrura* fed with  
217 different kinds of food (data from Ferrão-Filho *et al.* (2005)). As neither body  
218 length nor dry body weight was a controlled variable, we used an orthogonal  
219 regression method. Once the allometric relationship had been established, we  
220 used it to infer body lengths from dry body weights, once again for weight  
221 data collected between day 2 and 6 in the toxicity experiments with seston.

### 222 2.4 Matrix population model

#### 223 2.4.1 Principles

224 To extrapolate the effects of contaminants from the individual to the popu-  
225 lation level we used matrix population models, which were first proposed by

226 (Leslie, 1945, 1948), and which have already been coupled to DEBtox models  
 227 (Lopes *et al.*, 2005; Billoir *et al.*, 2007). These discrete time models deal with  
 228 populations subdivided into classes based on age or body length. Individuals  
 229 pass from one class to the next at each time step, the number depending on  
 230 their survival rates. The number of age-class 1 offspring produced by adults  
 231 depended on their fecundity rates. Only females were taken into consideration  
 232 in our study. Let  $\mathbf{N}(t)$  represent the population at time  $t$  (the components  
 233 of the vector are the size of each class). If  $\mathbf{L}$  denotes the Leslie matrix, the  
 234 population dynamics is modelled using the following matrix equation:

$$235 \quad \mathbf{N}(t + 1) = \mathbf{L}\mathbf{N}(t)$$

236 Within this matrix modelling framework, the dominant eigenvalue of  $\mathbf{L}$ , de-  
 237 noted by  $\lambda$ , corresponds to the finite rate of population increase (Caswell,  
 238 2001; Skalski *et al.*, 2007). If  $\lambda > 1$ , the population increases. The finite rate  
 239 of population increase,  $\lambda$ , is related to another common index at the popula-  
 240 tion level, the intrinsic rate of population increase,  $r$ , with  $\lambda = \exp(r)$ .

#### 241 2.4.2 Application

242 In our case, an age-specific population structure was more appropriate, be-  
 243 cause we had far more information about reproduction and survival as a func-  
 244 tion of age than about body length, which was deduced from weight measure-  
 245 ments using the allometric relationship. The effect models of DEBtox theory  
 246 provided survival (Equation (3)) and reproduction (Equations (8 to 12)) as  
 247 functions of time,  $t$ , and of the toxicant exposure concentration,  $c$ . This also  
 248 made it possible to calculate the vital rates as continuous functions of  $t$  and  $c$ .  
 249 We decided to use a pre-breeding census (Caswell, 2001), meaning that data

250 were collected just before the birth pulse. Let  $P_i$  be the probability of sur-  
 251 viving from one age class to the next and  $F_i$  be the fecundity rate, *i.e.* the  
 252 number of offspring reaching age class 1 per female of age class  $i$ . Hence, we  
 253 got:

$$254 \quad P_i(c) = \frac{q(i+1, c)}{q(i, c)} \quad (14) \quad \text{and} \quad F_i(c) = \int_i^{i+1} P_1(c) \dot{R}(t, c) dt \quad (15)$$

255 where  $q(i, c)$  is the probability of surviving until age class  $i$  for a toxicant  
 256 exposure concentration  $c$  (Equation (3)), and  $R(t, c)$  is the reproduction rate  
 257 at time  $t$  and exposure concentration  $c$  (Equations (8 to 12)).

258 We used a matrix model with 10 age classes and a time step of 1 day (0-1 day,  
 259 1-2 days, ..., 9-10 days), because we used data over 10 days to fit individual  
 260 models. *M. micrura* can live and reproduce for more than 10 days, so we added  
 261 a term  $G_{10}$  to the diagonal of the matrix, allowing the organisms to loop in  
 262 the last age class. However, in the experiments under consideration, almost all  
 263 of the organisms had died after 16 days, including the controls. Consequently,  
 264 we used  $G_{10} = 0.5P_9$ , ensuring a survival probability of less than 1 % after 16  
 265 days.

266 Assuming that reproduction can occur from age class 2, the matrix is written  
 267 as follows:

$$268 \quad \mathbf{L} = \begin{pmatrix} 0 & F_2 & F_3 & \cdots & F_9 & F_{10} \\ P_1 & 0 & 0 & \cdots & 0 & 0 \\ 0 & P_2 & 0 & \cdots & 0 & 0 \\ 0 & 0 & P_3 & \cdots & 0 & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ 0 & 0 & 0 & \cdots & P_9 & G_{10} \end{pmatrix}$$

269 where all the matrix coefficients depend on the exposure concentration. To  
 270 evaluate the effects of cyanobacteria on zooplankton at the population level,  
 271 the finite rate of population increase,  $\lambda$ , was plotted as a function of the  
 272 exposure concentration.

### 273 2.4.3 Confidence interval for $\lambda$

274 In order to evaluate a confidence interval for the finite rate of population  
 275 increase,  $\lambda$ , we proposed a method based on bootstrapping. For both lethal  
 276 and sublethal parameters, we drew 10000 DEBtox parameter sets: in their 95  
 277 % Beale joint confidence region (Beale, 1960) for the lethal ones ( $\dot{k}_e$ ,  $NEC_L$ ,  $\dot{k}_t$   
 278 and  $m$ ) which were estimated by nonlinear regression, and in the sample of the  
 279 joint posterior distribution for the sublethal ones ( $\dot{k}_e$ ,  $NEC_S$ ,  $c_*$ ,  $L_m$ ,  $\gamma$ ,  $\dot{R}_m$   
 280 and  $l_p$ ) which was provided by the Bayesian inference. From each parameter  
 281 set, we next deduced the vital rates of the matrix population model and the



282 corresponding  $\lambda$ , thus we obtained 10000  $\lambda$  values. Finally, we considered the  
283  $2.5^{th}$  and  $97.5^{th}$  quantiles of this sample as the limits of a 95% confidence  
284 interval for  $\lambda$ , the finite rate of population increase.

### 285 **3 Results**

#### 286 *3.1 Allometric relationship*

287 All the data we used to calculate the allometric relationship are plotted in  
288 Figure 1 (A). They were consistent with underlying hypotheses of the model,  
289 we did not identify any outlier or marginal value. The orthogonal linear regres-  
290 sion gave good results, with distances to the regression line close to normality  
291 (Figure 1 (B)). We obtained the following allometric relationship (Jerison,  
292 1973):

$$293 \quad y = \exp(6.42)x^{0.144} \quad \text{or} \quad \log(y) = 6.42 + 0.144 \log(x)$$

294  $x$  being the dry weight and  $y$  being the body length.

#### 295 *3.2 Lethal effects*

296 The survival data were fitted by a nonlinear regression based on the least  
297 squares criterion. Results are presented in Figure 2. Although the theoretical  
298 curves did not all fit the data perfectly (Figure 2), we considered that the fits  
299 were satisfactory for our purposes. We were not attempting to describe the  
300 survival data accurately, but just wanted to extrapolate the results from the  
301 individual level to the population level.

## 302 3.3 Sublethal effects

303 Simultaneous fitting performed by Bayesian inference on growth and repro-  
304 duction data with the five assumptions about the mechanism of action of the  
305 toxicant are shown in Figure 3. For reasons of clarity, data replicates are not  
306 shown, and we only plotted the replicate mean for each time and each expo-  
307 sure concentration. Figure 3 allowed us to visualize the quality of fit, and to  
308 compare the different assumptions about the seston mechanism of action. The  
309 two best models seemed to be the *Assimilation* and the *Maintenance* models  
310 (Figure 3 (A) and (C)). With these two assumptions of either decreased as-  
311 similation or increased maintenance costs, the DEBtox models fitted both the  
312 reproduction and growth data well, except for reproduction at  $1.0 \text{ mg C L}^{-1}$ .  
313 However, we had only few replicates of the data at this concentration, because  
314 of high mortality. Consequently, the reproduction data at  $1.0 \text{ mg C L}^{-1}$  were  
315 not as reliable as the data measured at lower exposure concentrations.

316 As far as the other assumptions about mechanism of action of the seston were  
317 concerned (Figure 3 (B), (D) and (E)), they clearly disagreed with the growth  
318 data. The *Costs* and *Hazard* models considered no effect of the contaminant  
319 on the growth process (Figure 3 (D2) and (E2)), whatever the exposure con-  
320 centration. The assumption of increased growth costs (*Growth* model) (Figure  
321 3 (B2)) did not match the body length data. Moreover, this mechanism of  
322 action is marked by a delay in the onset of reproduction as soon as the No  
323 Effect Concentration (*NEC*) is exceeded. This feature is not obvious from the  
324 reproduction data (Figure 3 (B1)), as a delay in the onset of reproduction  
325 was only noticed for the two highest concentrations of seston ( $1.0$  and  $1.5 \text{ mg}$   
326  $\text{C L}^{-1}$ , which were less reliable), and although a clear effect of seston was

327 already observed at lower concentrations (0.25 and 0.5 mg C L<sup>-1</sup>), this was  
328 not accompanied by any delay in the onset of reproduction.

### 329 3.4 Population level

330 As a population endpoint, the finite rate of population increase  $\lambda$  is repre-  
331 sented as a function of seston exposure concentration in Figure 4 (A), with  
332 the five possible assumptions concerning the toxicological mechanism of ac-  
333 tion. We have also included in the graph the results reported by Ferrão-Filho  
334 & Azevedo (2003): in their study, they calculated the intrinsic population  
335 rate of increase  $r$ , and we were able to compare this to our results by using  
336  $\lambda = \exp(r)$ . For exposure concentrations between 0 and 0.5 mg C L<sup>-1</sup>, our re-  
337 sults seemed to be consistent with those obtained by Ferrão-Filho & Azevedo  
338 (2003), though we obtained slightly lower values in general. From about 0.7  
339 mg C L<sup>-1</sup>, with the *Maintenance*, *Growth* and *Assimilation* models, we ob-  
340 served a drastic drop in population growth due to effects on reproduction.  
341 Indeed, using these three assumptions to explain the mechanism of action of  
342 the seston, the DEBtox models predicted no reproduction at all for exposure  
343 concentrations from 1.0 mg C L<sup>-1</sup> (Figure 3 (A1, B1 and C1)). Consequently,  
344 the choice of the mechanism of action strongly influenced the threshold con-  
345 centration from which the population was predicted to go extinct ( $\lambda = 1$ ), as  
346 this threshold concentration ranged from 0.75 to 1.2 mg C L<sup>-1</sup>, depending on  
347 the mechanism of action considered. Ferrão-Filho & Azevedo (2003) obtained  
348  $\lambda \approx 1.05$  for the exposure concentration of 1.0 mg C L<sup>-1</sup>. This prediction was  
349 much more optimistic than our results ( $\lambda \approx 0.25$ ) using the two best models  
350 of sublethal effects at the individual level (the *Assimilation* and *Maintenance*

351 models).

352 We also calculated a confidence interval for  $\lambda$ , and in Figure 4 (B) we show  
353 the results obtained with the *Assimilation model* which appeared to be one of  
354 the two best models for describing sublethal effects. Calculating a confidence  
355 interval for  $\lambda$  as a continuous function of exposure concentration led to a  
356 confidence band that could also be read horizontally. This enabled us to deduce  
357 a prediction interval for the threshold concentration leading to population  
358 extinction ( $\lambda = 1$ ): 0.68-0.82 mg C L<sup>-1</sup> with the *Assimilation model*. We  
359 obtained similar results with the *Maintenance model*.

#### 360 4 Discussion

361 The best fits for our data were obtained with the *Assimilation* and *Mainte-*  
362 *nance* models, which suggests that these indirect effects (a decrease in food  
363 assimilation and an increase in maintenance costs) are the most probable  
364 mechanisms of action of cyanobacteria on *M. micrura*. This was consistent  
365 with the results of Ferrão-Filho & Azevedo (2003), which showed that ses-  
366 ton containing toxic *Microcystis* reduced the filtering rate (and therefore the  
367 food assimilation) and fitness (measured as the intrinsic rate of population  
368 increase,  $r$ ) in this cladoceran species. Moreover, microcystins are known to  
369 inhibit the activity of protein phosphatase 1 and 2A in *Daphnia* (DeMott &  
370 Dhawale, 1995), which leads ultimately to the disruption of the cell cytoskele-  
371 ton (Carmichael, 1992). Recent research has also shown that microcystins  
372 can cause gut damage in *Daphnia* by disrupting contact between gut cells  
373 (Rohrlack *et al.*, 2005). This biochemical-cellular mechanism of action of the  
374 microcystins is therefore completely compatible with the decrease in assimi-

375 lation predicted by DEBtox models used to analyse the data of *M. micrura*  
376 exposed to toxic *Microcystis* containing microcystins.

377 An important assumption of DEB theory is that food is shared between four  
378 main metabolic processes: assimilation, somatic maintenance, growth and re-  
379 production. As cyanobacteria form part of the zooplankton diet, they are also  
380 a source of energy, and so toxins (i.e. microcystins) can be assimilated di-  
381 rectly with food from the gut. Our findings show that the DEBtox theory  
382 can provide an excellent framework for studying the effects of cyanobacteria  
383 on zooplankton, since the exposure of zooplankton to cyanotoxins is directly  
384 coupled to the assimilation of food in the gut, and can therefore interfere with  
385 the energy balance.

386 As far as DEBtox parameter estimation was concerned, we used a set of meth-  
387 ods that had already been proposed: nonlinear regression for survival models  
388 (Kooijman & Bedaux, 1996; Billoir *et al.*, 2007), and Bayesian inference for  
389 the growth and reproduction models (Billoir *et al.*, resubmitted). However,  
390 when growth, reproduction and survival data are all available, it would be  
391 more logical to estimate all the parameters simultaneously especially because  
392 the kinetic parameter  $\dot{k}_e$  is involved in both the sublethal and lethal models.  
393 Jager *et al.* (2004) performed a simultaneous estimation of this type, but they  
394 ran into some statistical issues, such as very large confidence intervals, and  
395 the need to fix  $\dot{k}_e$ . We are currently trying to extend the Bayesian approach  
396 by integrating a survival module into the Bayesian model. We hope to report  
397 the results of this work in the near future.

398 At the population level, our results and those of Ferrão-Filho & Azevedo (2003)  
399 differ somewhat. Using the data collected for the exposure concentration of

400 1.0 mg C L<sup>-1</sup>, they obtained an intrinsic rate of population increase  $r = 0.042$   
401 (equivalent to  $\lambda = \exp(r) = 1.043$ ). In contrast, our results for the *Assimila-*  
402 *tion* and *Maintenance* models were much more pessimistic ( $\lambda \ll 1$ ), indicating  
403 that population health was threatened using the same exposure concentration.  
404 However, we obtained fewer data for this exposure concentration than for the  
405 lower concentrations, as a result of high mortality. Using data collected solely  
406 at one exposure concentration did not seem to be as reliable as that obtained  
407 by our methodology. Indeed, with the DEBtox modelling approach, all the  
408 exposure concentrations tested were considered simultaneously, as the models  
409 were continuous in exposure concentration. Moreover, the Bayesian inference  
410 took into account the amount of data available for each exposure concentra-  
411 tion tested. Combining both approaches made it possible to achieve the best  
412 inference of the finite rate of population increase,  $\lambda$ , as a continuous function  
413 of the exposure concentration.

414 The use of native species, such as *M. micrura*, and naturally occurring sam-  
415 ples of cyanobacteria are important issues in ecotoxicology, as this leads to  
416 better extrapolation of results from laboratory to the field. Although this  
417 is not a standard test species, like *D. magna*, the biology of *M. micrura* is  
418 relatively well known, and has been shown to be very sensitive to cyanobacte-  
419 ria, especially to toxic *Microcystis* (Ferrão-Filho *et al.*, 2000; Nandini, 2000).  
420 However, although the application of DEBtox and matrix population models  
421 to natural samples gave more realistic results than when we used laboratory  
422 samples (i.e. cultures), caution was still called for when extrapolating from  
423 these results to field situations. The threshold concentrations calculated from  
424 laboratory toxicity tests could sometimes be underprotective, because other  
425 stresses (predation, density-dependence, *etc...*) were not taken into account.

426 In contrast, our results seemed to be overprotective. The predicted thresh-  
427 old concentration range of 0.68-0.82 mg C L<sup>-1</sup>, above which the population  
428 would become extinct was much lower than the values commonly found in the  
429 Jacarepaguá Lagoon (2.7-38.1 mg C L<sup>-1</sup>; (Ferrão-Filho *et al.*, 2002)). Also, in  
430 spite of some reported outbreaks of *M. micrura* populations in this lagoon,  
431 it is important to note that this species coexists with high concentrations of  
432 *Microcystis* and microcystins (Ferrão-Filho *et al.*, 2002). This was attributed  
433 mainly to the predominance of *Microcystis* colonies of large size (> 20  $\mu$ m)  
434 that cannot be eaten by most cladocerans (Ferrão-Filho & Azevedo, 2003).

435 Even though the DEBtox framework has been applied to standard toxicity  
436 bioassays involving *D. magna*, a much larger cladoceran, our results showed  
437 that this model can also be applied to other zooplankton species, such as *M.*  
438 *micrura*, which is a much smaller cladoceran with a shorter life cycle. Once  
439 again, the combination of DEBtox and matrix population models appeared  
440 very useful when extrapolating from the individual to the population level. All  
441 lethal and sublethal effects were integrated into the finite rate of population  
442 increase, which is much more ecologically relevant than any statistically-based  
443 parameter (such as NOEC and ECx) derived from a single endpoint (such as  
444 mortality, growth or reproduction). Moreover, our methodology made it possi-  
445 ble to calculate the finite rate of population increase as a continuous function  
446 of time. Thanks to matrix population modelling, sensitivity analysis could  
447 also be used to gain demographic information, as in Billoir *et al.* (2007). Here,  
448 we used a fresh approach: parameter uncertainty was taken into account and  
449 integrated into a confidence band, which enabled us to provide a prediction in-  
450 terval for the threshold concentration leading to population extinction (when  
451  $\lambda$  becomes < 1). In conclusion, our results showed the potential value of us-

452 ing nested models to predict the threshold concentrations of cyanobacteria  
453 required for zooplankton population extinction to occur. These could be used  
454 as parameters in guidelines for protecting both aquatic life and environmental  
455 health.

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Symbol	Status	Dimension	Interpretation
$t$	covariable	T	time from the experiment beginning, and also organism age
$c$	covariable	C	exposure concentration
$c_q$	variable	C	internal concentration
$s$	variable	-	stress function
$q$	variable	-	survival probability
$\dot{h}$	variable	$T^{-1}$	hazard rate
$l$	variable	-	scaled body length
$\dot{R}$	variable	$\#T^{-1}$	reproduction rate
$NEC_*$	parameter	C	No Effect Concentration for lethal ( $* = L$ ), sublethal effects ( $* = S$ )
$c_*$	parameter	$CT^{-1}$ or C	tolerance concentration, $* = L, H, R, A, G,$ or $M$
$\dot{k}_e$	parameter	$T^{-1}$	elimination rate
$\dot{m}$	parameter	$T^{-1}$	control mortality rate
$l_b$	parameter	-	scaled body length at birth
$\dot{\gamma}$	parameter	$T^{-1}$	von Bertalanffy growth rate
$l_p$	parameter	-	scaled body length at puberty
$\dot{R}_M$	parameter	$\#T^{-1}$	maximum reproduction rate
$f$	parameter	-	ingestion rate as a fraction of its maximum
$g$	parameter	-	investment ratio

Table 1

Quantities considered in the DEBtox framework, their status (variable, covariable, or parameter), their dimension and their biological interpretation. T, C and # denote the dimensions of time, concentration and number respectively. Dots denote rates.

Mode of action	Growth	Reproduction
Growth	$\frac{dl}{dt} = \dot{\gamma} \frac{f+g}{f+g(1+s(c_q(t)))} (f - l(t))$ (4)	$\dot{R}(l(t)) = \frac{\dot{R}_M}{1-l_p^3} [fl(t)^2 (\frac{g(1+s(c_q(t)))+l}{g(1+s(c_q(t)))+f}) - l_p^3]$ (8)
Maintenance	$\frac{dl}{dt} = \dot{\gamma} (f - l(1 + s(c_q(t))))$ (5)	$\dot{R}(l(t)) = \frac{\dot{R}_M}{1-l_p^3} (1 + s(c_q(t))) [fl(t)^2 (\frac{g(1+s(c_q(t)))^{-1}+l(t)}{g+f}) - l_p^3]$ (9)
Assimilation	$\frac{dl}{dt} = \dot{\gamma} \frac{f+g}{f(1-s(c_q(t)))+g} (f(1 - s(c_q(t))) - l(t))$ (6)	$\dot{R}(l(t)) = \frac{\dot{R}_M}{1-l_p^3} [f(1 - s(c_q(t)))l(t)^2 (\frac{g+l(t)}{g+f(1-s(c_q(t)))}) - l_p^3]$ (10)
Hazard	$\frac{dl}{dt} = \dot{\gamma} (f - l(t))$ (7)	$\dot{R}(l(t)) = \frac{\dot{R}_M}{1-l_p^3} [fl(t)^2 (\frac{g+l(t)}{g+f}) - l_p^3] e^{-s(c_q(t))}$ (11)
Costs	$\frac{dl}{dt} = \dot{\gamma} (f - l(t))$ (7)	$\dot{R}(l(t)) = \frac{\dot{R}_M}{1-l_p^3} [fl(t)^2 (\frac{g+l(t)}{g+f}) - l_p^3] (1 + s(c_q(t)))^{-1}$ (12)

Table 2

DEBtox growth and reproduction equations for each assumption about the toxicant mode of action.

Parameter (unit)	Distribution
$L_m$ ( $\mu\text{m}$ )	$N(1000, 100) I(500, +\infty)$
$\dot{\gamma}$ (-)	$U(0, 2)$
$l_p$ (-)	$N(0.75, 0.1) I(0.5, 1)$
$\dot{R}_m$ ( $\#\text{d}^{-1}$ )	$N(5, 2) I(0, +\infty)$
$k_e$ ( $\text{d}^{-1}$ )	$U(0.0001, 10)$
NEC ( $\text{mg C L}^{-1}$ )	$U(0, 1.5)$
$c_*$ * = H, R, G, or M ( $\text{mg C L}^{-1}$ )	$U(0, 20)$
$c_A$ ( $\text{mg C L}^{-1}$ )	$U(2, 20)$

Table 3

Prior distributions used for the DEBtox sublethal parameter estimation which was performed by Bayesian inference.  $N(m, sd)I(a, b)$  denotes a normal distribution with mean  $m$  and standard deviation  $sd$ , where  $I(a, b)$  represents interval censoring (see Spiegelhalter et al., 2003, p. 12 for details) (Spiegelhalter *et al.*, 2003).  $U(c, d)$  denotes a uniform distribution between  $c$  and  $d$ .

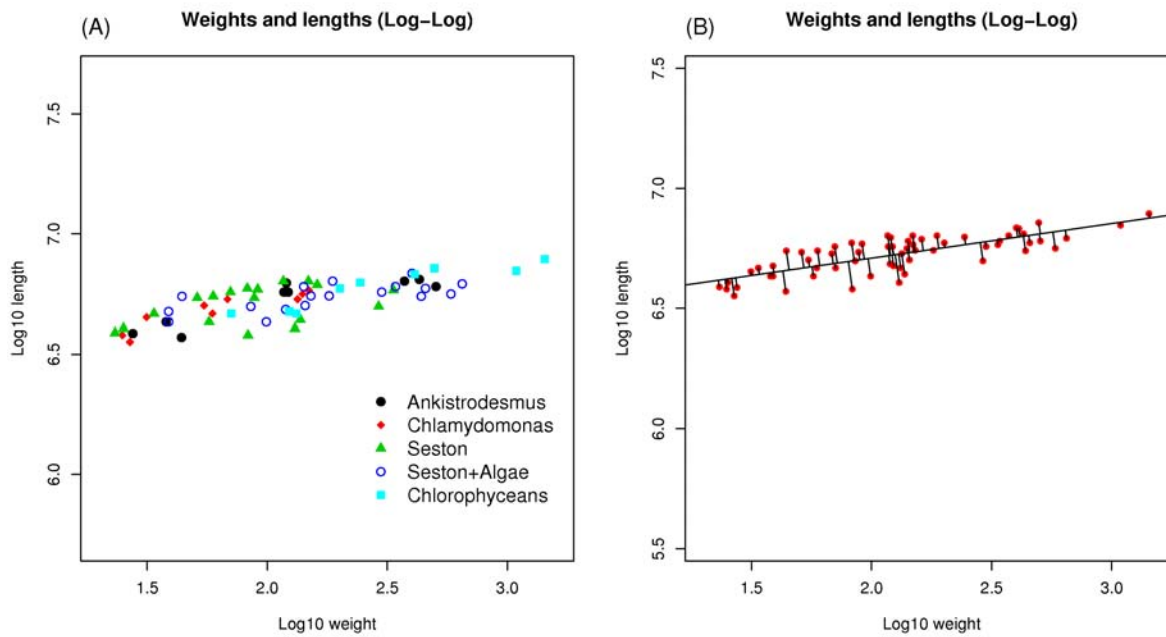


Fig. 1. (A) Log-Log representation of the data used to establish the length/dry weight allometric relationship. Data were collected during experiments involving various kinds of food (data from Ferrão-Filho *et al.* (2005)). (B) Orthogonal linear regression fit on these data.

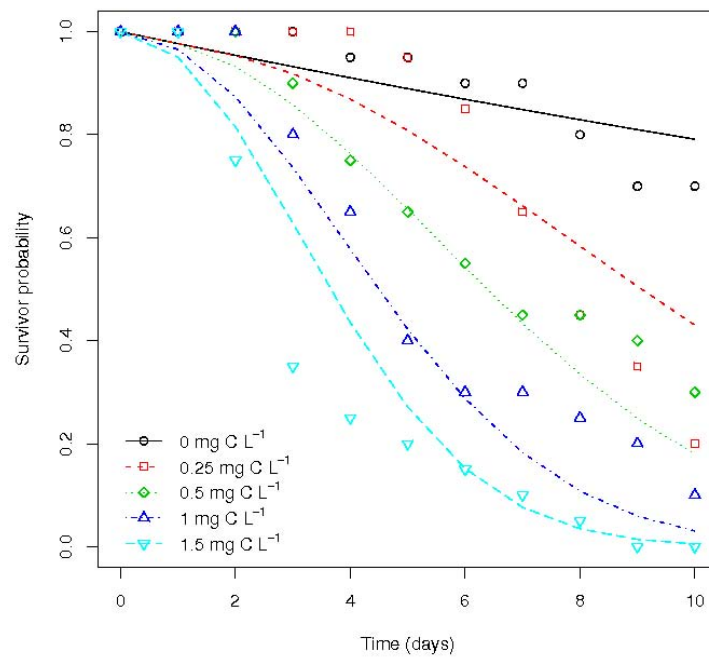


Fig. 2. Fitting performed by nonlinear regression of survival data: time profile of survivor probability for various seston exposure concentrations (0, 0.25, 0.5, 1.0, 1.5 mg C L<sup>-1</sup>)



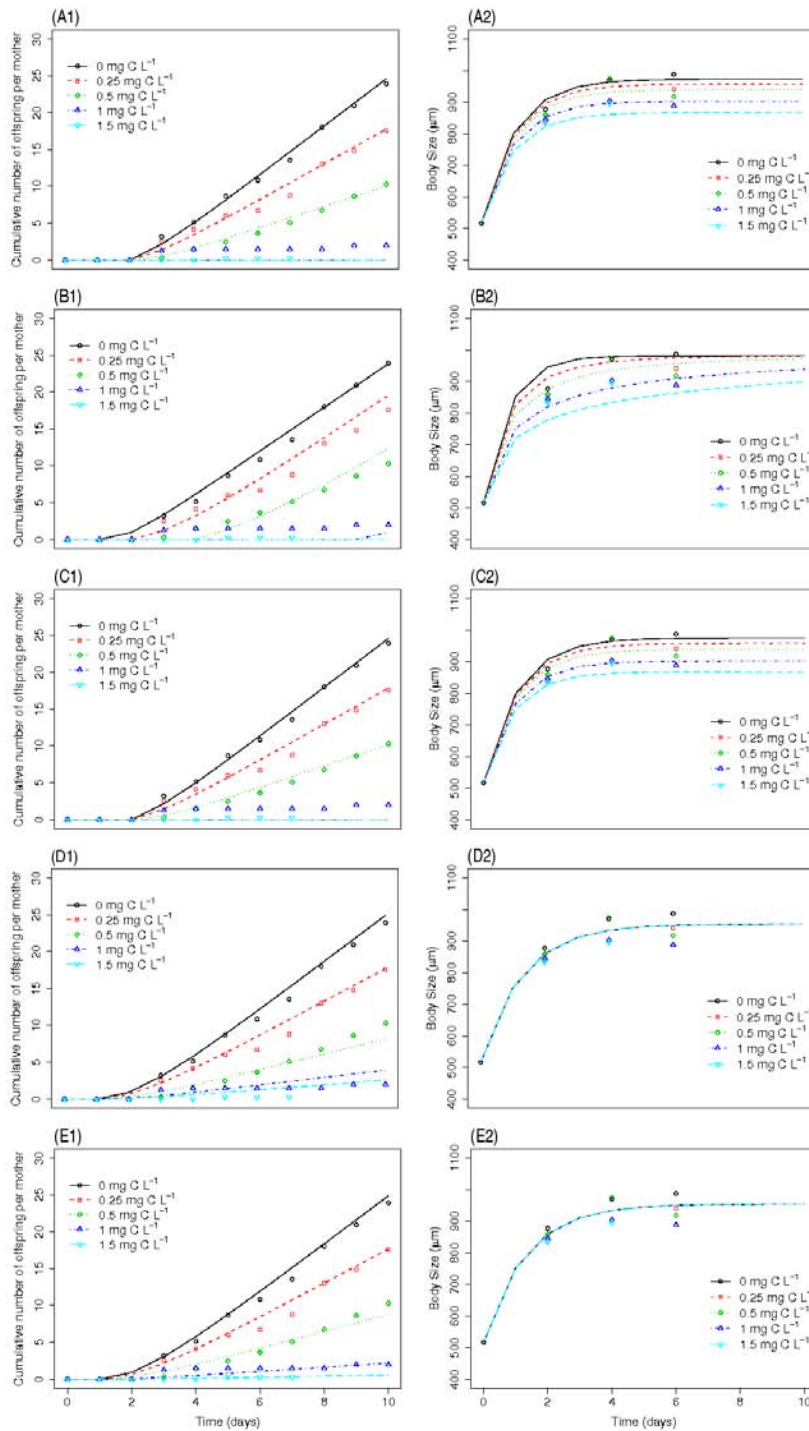


Fig. 3. Simultaneous fitting performed by Bayesian inference of (1) reproduction and (2) growth data under the assumption of (A) a decrease in *Assimilation* (B) an increase in the *Growth* costs (C) an increase in the *Maintenance* costs (D) an increase in egg production *Costs*, and (E) excess mortality during oogenesis (*Hazard*): time profiles of the cumulative number of offspring and body length for various seston exposure concentrations (0, 0.25, 0.5, 1.0, 1.5 mg C L<sup>-1</sup>)

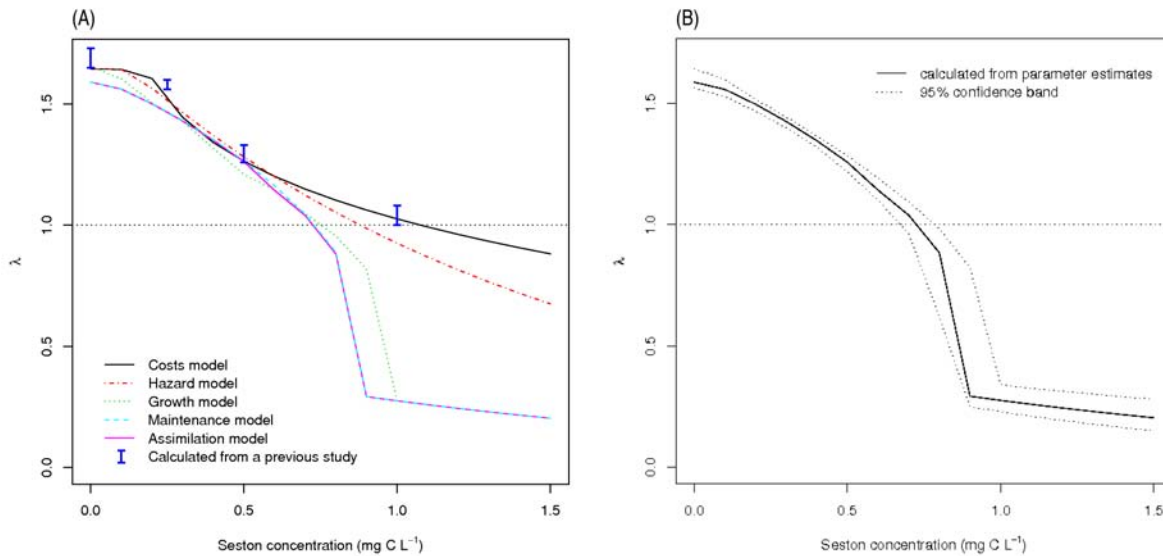


Fig. 4. (A) Evolution of the finite rate of population increase  $\lambda$  of *M. micrura* as a function of the seston exposure concentration ( $\text{mg C L}^{-1}$ ). Comparison of the five assumptions about the toxicological mechanism of action: *Hazard*, *Costs*, *Maintenance*, *Growth* and *Assimilation*. The results from Ferrão-Filho & Azevedo (2003) (means  $\pm$  SE) are also shown on this graph. (B) Change in the finite rate of population increase  $\lambda$  of *M. micrura* and its confidence interval as a function of seston exposure concentration ( $\text{mg C L}^{-1}$ ) when we assumed that a decrease in *Assimilation* was the toxicological mechanism of action.