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Energy-based modelling to assess effects of chemicals on *Caenorhabditis elegans*: a case study on uranium

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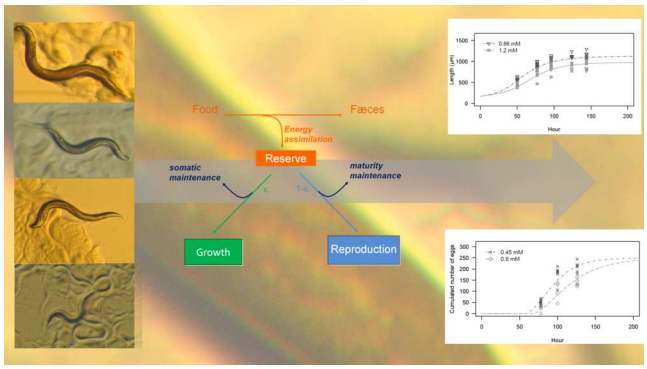
Abstract



The ubiquitous free-living nematode *Caenorhabditis elegans* is a powerful animal model for measuring the evolutionary effects of pollutants which is increasingly used in (eco)toxicological studies. Indeed, toxicity tests with this nematode can provide in a few days data on the whole life cycle. These data can be analysed with mathematical tools such as toxicokinetic-toxicodynamic modelling approaches. In this study, we assessed how a chronic exposure to a radioactive heavy metal (uranium) affects the life-cycle of *C. elegans* using a mechanistic model. In order to achieve this, we exposed individuals to a range of seven concentrations of uranium. Growth and reproduction were followed daily. These data were analysed with a model for nematodes based on the Dynamic Energy Budget theory, able to handle a wide range of plausible biological parameters values. Parameter estimations were performed using a Bayesian framework. Our results showed that uranium affects the assimilation of energy from food with a no-effect concentration (NEC) of 0.42 mM U which would be the threshold for effects on both growth and reproduction. The sensitivity analysis showed that the main contributors to the model output were parameters linked to the feeding processes and the actual exposure concentration. This confirms that the real exposure concentration should be measured accurately and that the feeding parameters should not be fixed, but need to be reestimated during the parameter estimation process.

Highlights

- We proposed an improved bioenergetic model to assess effects on nematodes
- The numerical behaviour of this model was evaluated
- The model proved to handle a wide range of plausible biological parameters values
- Uranium is likely to decrease the assimilation of energy by *C. elegans*

Keywords: *Caenorhabditis elegans* - Dynamic Energy Budget - Bayesian - Numerical behaviour - Uranium

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1 Introduction

The evaluation of the long term effects of pollutants on the environment is required for an effective protection of the ecosystems. According to Yeates and Bongers (1999), a useful bioindicator of ecosystemic stress should be the nematodes since they are abundant key decomposers in soil ecosystem. Within this phylum, a powerful animal model for measuring the effects of pollutant is the ubiquitous free-living nematode *Caenorhabditis elegans*. It measures 250 μm at hatching and up to 1.4 mm at adult stage. It has several advantages for ecotoxicological assessment such as (i) a short life cycle (*C. elegans* breeds in three days after hatching at 20°C), (ii) a short life span (21 days at 20°C), and (iii) a high fecundity (Byerly et al., 1976). *C. elegans* allows the assessment of life-history traits such as length, reproduction, or survival at various time steps during an experimentation. Therefore, this nematode is widely used to produce data for the assessment of chemical effects on the environment (Boyd et al., 2010; Dutilleul et al., 2013; Goussen et al., 2013; Smith et al., 2009; Swain et al., 2010).

The assessment of (eco)toxicological data through models based on the Dynamic Energy Budget (DEB) theory (Billoir et al., 2008b; Kooijman and Bedaux, 1996), known as DEBtox models is relevant to assess the effects of toxic compounds on organisms. Indeed, this method allows the simultaneous modelling of multiple endpoints (growth and reproduction) and the parameters estimated are independent of the exposure time (Billoir et al., 2008b; Jager et al., 2004). Such models can be used to assess physiological modifications (*e.g.* changes in growth rate, maximal length, reproduction rate, cumulated reproduction) (Augustine et al., 2012a; Billoir et al., 2008a; Massarin et al., 2011), or differential life strategy responses (Alda Álvarez et al., 2005) of an organism exposed to a pollutant. DEB modelling can also be adapted to other types of analysis. Thereby, individual based model (IBM) based on DEB modelling can permit to analyse population level data, especially multi-generation studies (Beaudouin et al., 2012; Martin et al., 2013).

Uranium, a pollutant of growing interest (OECD, International Atomic Energy Agency (IAEA), 2012), is a radioactive heavy metal that can be found naturally in the environment. It is both a chemical and a radiological toxic but its chemical toxicity is considered to be dominant (Sheppard et al., 2005; Zeman et al., 2008).

We recently produced multi-generation data for nematodes exposed to uranium (Goussen et al., 2013). These data will be analysed with a DEBtox model for nematodes. Prior to this, we had to propose a relevant and robust DEBtox modelling approach for nematodes. This is the aim of this paper.

Jager et al. (2005) presented a DEB model applied to the nematode. This model has been adapted to the assessment of ecotoxicological data (resulting in a so called DEBtox model) and used several times (Alda Álvarez et al., 2006, 2005; Swain et al., 2010). In the present study, we proposed an augmented version of the DEBtox equations for nematodes by Jager et al. (2005) based on biological characteristics that were not covered. We evaluated both nematode DEBtox models to analyse data on the effects of the uranium on the free living nematode *Caenorhabditis elegans*. We also assessed the output robustness and sensitivity to parameters.

2 Materials and Methods

Experimental data were extracted from (Goussen et al., 2013). Extended description is presented in supplementary information.

2.1 Model description

The Dynamic Energy Budget (DEB) theory (Kooijman, 2010) is based on a mathematical description of the uptake and use of energy within an organism. The general DEB framework generally predicts that individuals' growth follows a von Bertalanffy growth curve provided feeding is *ad libitum* or at constant density (Kooijman, 2010) and the growth is isomorphic (*i.e.* the organism shape do not change with growth). However as demonstrated by Byerly et al. (1976), *C. elegans* growth curve deviates from the expected von Bertalanffy curve. According to Knight et al. (2002), the *C. elegans* buccal cavity grows in a stepwise manner at each moult whereas the rest of the body growth is linear between the moults. Knight et al. (2002) assumed that this process induces a food limitation thus resulting in an initial growth slower than the later growth. Jager et al. (2005) dealt with this particularity assuming the *C. elegans* ingestion rate follows a size-dependent feeding limitation function and incorporated this in their nematode DEB model. These authors also assumed an immediate stop of the reproduction when the sperm runs out. Nevertheless, experimental data do not show an immediate stop of reproduction, but rather a more smooth transition (see for example Byerly et al. (1976) Fig. 6 and 8 and Muschiol et al. (2009) Fig. 2). We here propose two modifications of this set of equations in order to take into account some known biological specificities of the nematode. (i) The *C. elegans* male gamete limitation induces a progressive stop of the egg laying (Maupas, 1900; Ward and Carrel, 1979). (ii) We also modified the size-dependent feeding limitation function by adding a new parameter which allows the nematode to access food whatever its length. Indeed, the approach from Jager et al. (2005) assumed that a small nematode would never be able to access enough food to growth if food is just sub-optimal (see section results in this paper). With our approach, we assumed that this nematode will be able to access to a sufficient small quantity of food, such as small bacteria (bacteria is the main food source of *C. elegans* Abada et al., 2009), to initiate growth. The set of equations in our DEBtox model for nematodes is thus:

$$s_f(l) = \alpha \left[1 - \left(1 + \frac{l_f^3}{l^3} \right)^{-1} \right] \tag{1a}$$

$$\frac{dl}{dt} = r_B \left[(1 - s_f) f - l \right] \tag{1b}$$

$$\frac{dR}{dt} = \frac{R_M}{1 - l_p^3} \left[\frac{g + l}{g + (1 - s_f) f} (1 - s_f) f l^2 - l_p^3 \right] \frac{R_{Max} - R}{R_{Max}} \tag{1c}$$

with s_f the size-dependent ingestion limitation function, $(1 - \alpha)$ (-) the proportion of food available whatever the length, r_B (h^{-1}) the von Bertalanffy growth rate, f (-) the actual ingestion rate divided by the maximal ingestion rate for a body size, R (#) the cumulated reproduction, R_{Max} (#) the maximal cumulated reproduction, R_M (# h^{-1}) the maximum reproduction rate, and g (-) the investment ratio. L (μm) represents the body length at time t, L_0 (μm) the body length at birth (*i.e.* the start of feeding in the DEB framework), L_f (μm) the body length at which the ingestion rate is half the maximum ingestion rate, and L_p (μm) the body length at puberty. All these length data are scaled by the maximal length L_{inf} (μm) resulting in scaled parameters l, l_0, l_f, and l_p (-).

The internal concentration of the pollutant scaled by the ratio between the uptake rate and the elimination rate (which can be regarded as the bioconcentration factor; Péry et al., 2001), c_q, has been calculated as described by Kooijman and Bedaux (1996). These authors described a one-compartment toxicokinetic model with growth dilution, chemical exchanges depending on a surface area:volume ratio, and an elimination rate depending on food availability. This equation has been modified with the s_f function resulting in the following equation:

$$\frac{dc_q}{dt} = C \frac{k_e (1 - s_f) f}{l} - c_q \left[\frac{k_e (1 - s_f) f}{l} + \frac{d \ln l^3}{dt} \right] \tag{2}$$

where k_e (h^{-1}) is the elimination rate and C (mM) the real exposure concentration. The toxic stress function, s, was calculated as $s(c_q) = \max\left[0, b\left(c_q - NEC\right)\right]$ with b (mM^{-1}) the slope of the effect and NEC (mM) the no-effect concentration.

Combining Equations 1 with the DEBtox equations rederived from the original ones by Billoir et al. (2008b), we obtained the set of equations presented in Table 1.

According to a previous study, the uranium negatively affects both the growth and the reproduction of *C. elegans* at comparable exposure concentrations (Goussen et al., 2013). As a consequence, the modes of action that may apply to uranium on nematodes are the *growth*, *assimilation*, or *maintenance* models.

2.2 Estimation of the parameters

Parameters estimations were performed using the statistical computing software R 2.15 (R Core Team, 2012) and the JAGS 3.3.0 (Just Another Gibbs Sampler) software, which is a program for the statistical analysis of Bayesian hierarchical models by Markov Chain Monte Carlo (MCMC) (Plummer, 2003). The initial parameters' distributions were chosen according to the literature (Jager et al., 2005) (Table 2). We chose positive truncated normal distribution for biological parameters and we applied a coefficient of variation of 20% to these values. Parameters for which less information was available were distributed according to a non-informative uniform distribution. The different modes of action models were compared using the Deviance Information Criterion (DIC) which is suitable for Bayesian calibration comparison (Spiegelhalter et al., 2002). The DIC is a generalisation of the Akaike's Information Criterion (AIC). As for the AIC, the lower DIC points out the model that will make the best short-term predictions and a DIC difference of less than five indicates that the models show similar fits (Spiegelhalter et al., 2003). The DIC is a penalized deviance statistics as it includes penalty as a function of the effective number of parameters of the model (Spiegelhalter et al., 2002).

2.3 Sensitivity and uncertainty analysis

An uncertainty analysis was performed on the model parameters. Parameters' distributions were the same as for the estimation of the parameters apart for the use of triangle distributions instead of normal distributions. The triangle distribution permits a larger exploration of the space of parameters values, because extreme values have higher probability, resulting in a more robust model analysis. The experimental design was built using a Monte Carlo approach. The variances of the model output were estimated by Monte Carlo integrations. Two independent input sample matrices $(n \times p)$ were used to compute the Monte Carlo integration. The sample size (n) was of 30,000 and the number of parameters (p) is indicated in the Table S2 for each model.

We also performed a sensitivity analysis on the parameters using a variance-based Sobol method (scheme by Saltelli (2002)). The sensitivity analysis allows the identification of the main input contributors to the variation of the model outcome. Thus the sensitivity analysis indicates the parameters on which a particular emphasis must be paid. The Sobol's method is a global and model independent sensitivity analysis method that quantifies the amount of variance that each parameter contributes to the unconditional variance of the model output. The parameters' values, the distribution, and the experimental design were the same as for the uncertainty analysis. We then calculated the first order (Si; variance contribution of one parameter to the total model variance) and total order (STi; result of the main effect of one parameter and

Table 1: *C. elegans* DEBtox equations

Assimilation model:

$$\frac{dl}{dt} = r_B \frac{(1-s_f)\,f+g}{g+[(1-s_f)\,f\,(1-s\,(c_q))]}\,[(1-s_f)\,f\,(1-s\,(s_q))-l] \tag{3a}$$

$$\frac{dR}{dt} = \frac{R_M}{1-l_p^3}\left[\frac{g+l}{g+(1-s_f)\,f\,(1-s\,(c_q))}\,(1-s_f)\,f\,(1-s\,(c_q))\,l^2-l_p^3\right]\frac{R_{Max}-R}{R_{Max}} \tag{3b}$$

Maintenance model:

$$\frac{dl}{dt} = r_B\,[(1-s_f)\,f-(1+s\,(c_q))\,l] \tag{4a}$$

$$\frac{dR}{dt} = \frac{R_M}{1-l_p^3}\,(1+s\,(c_q))\left[(1-s_f)\,fl^2\frac{g\,(1+s\,(c_q))^{-1}+l}{g+(1-s_f)\,f}-l_p^3\right]\frac{R_{Max}-R}{R_{Max}} \tag{4b}$$

Growth model:

$$\frac{dl}{dt} = r_B\frac{(1-s_f)\,f+g}{(1-s_f)\,f+g\,(1+s\,(c_q))}\,[(1-s_f)\,f-l] \tag{5a}$$

$$\frac{dR}{dt} = \frac{R_M}{1-l_p^3}\left[(1-s_f)\,fl^2\frac{g\,(1+s\,(c_q))+l}{g\,(1+s\,(c_q))+(1-s_f)\,f}-l_p^3\right]\frac{R_{Max}-R}{R_{Max}} \tag{5b}$$

Hazard model:

$$\frac{dl}{dt} = r_B\,[(1-s_f)\,f-l] \tag{6a}$$

$$\frac{dR}{dt} = \frac{R_M}{1-l_p^3}\left[(1-s_f)\,fl^2\frac{g+l}{g+(1-s_f)\,f}-l_p^3\right]\exp^{-s(c_q)}\frac{R_{Max}-R}{R_{Max}} \tag{6b}$$

Costs model:

$$\frac{dl}{dt} = r_B\,[(1-s_f)\,f-l] \tag{7a}$$

$$\frac{dR}{dt} = \frac{R_M}{1-l_p^3}\left[(1-s_f)\,fl^2\frac{g+l}{g+(1-s_f)\,f}-l_p^3\right](1+s\,(c_q))^{-1}\frac{R_{Max}-R}{R_{Max}} \tag{7b}$$

of all its interactions with the other parameters) Sobol index. Calculations were performed thanks to the function "sobol2002" from the *sensitivity* R-package (Pujol et al., 2012) with the statistical computing software R 2.15 (R Core Team, 2012).

3 Results

3.1 Control data fit accuracy

Our model (Eq. 1) provided a relevant fit of both the growth and the reproduction data for the control conditions (Fig 1A and B). The parameters we obtained by fitting the control data set are presented in Table 2. Both our model and the model proposed by Jager et al. (2005) present a similar fit accuracy (ΔDIC < 5, see Table S2).

Uncertainty analysis performed on the model proposed by Jager et al. (2005) showed that this model does not allow growth of hatchlings at suboptimal food availabilities. Indeed, more than 50% of the simulated cases gave a non growing and therefore a non reproductive nematode (Fig. S1A and S2A). In contrast, our model allows the hatchlings nematodes to grow at much lower food levels (Eq. 1) (Fig. S1B and S2B). Model exploration also showed that the model we used is robust for the parameters estimations. Indeed, as shown by the Figures S1C and D, our model allows to test a larger range of values leading to a significant growth, while performing the parameters estimations, compared to the initial model proposed by Jager et al. (2005).

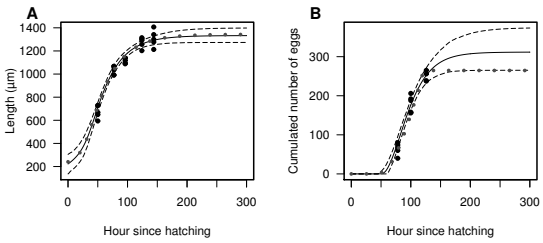

Figure 1: *C. elegans* length (**A**) and cumulated reproduction (**B**) in relation to age for the control conditions. Age is indicated as hour since hatching. Observed data are represented by *points*, the model fits (Eq. 1) are represented by the *regression line*. *Dashed lines* represent 95th percentiles of the posterior distribution. *Dotted lines* represent the model fitted with the equations of Jager et al. (2005).

3.2 Mode of action and sensitivity analysis

Table S2 presents the DIC comparison between the model by Jager et al. (2005) and our model for all the modes of action. The best mode of action is the *assimilation* model. Indeed, the DIC of the *assimilation* model is lower by over 32 points than all other modes of action DIC. In the same way as for the control model, the Jager et al. (2005) model and our model have a non distinguishable quality of fit (ΔDIC < 5) for the *assimilation* model.

Sensitivity analysis (Fig. 2 and S3) performed on our *assimilation* model showed that the main impact contributors to the growth model (Eq. 3a) output variations are parameters α (-) and the exposure concentration, followed by parameters b (mM^{-1}), f (-), L_f (μm), and NEC (mM). Although L_f (μm) has a non negligible *STi*, its *Si* remains negligible. Parameters R_{Max} (#), L_p (μm), R_M (# h^{-1}), and g (-) did not influence the growth model output. Regarding the reproduction model (Eq. 3b), parameters f (-) and the exposure concentration are the main contributors. These parameters are followed by parameters b (mM^{-1}), α (-), L_{inf} (μm), L_p (μm), and NEC (mM). Parameters L_0 (μm), R_M (# h^{-1}), R_{Max} (#), and g (-) did not influence the reproduction model output.

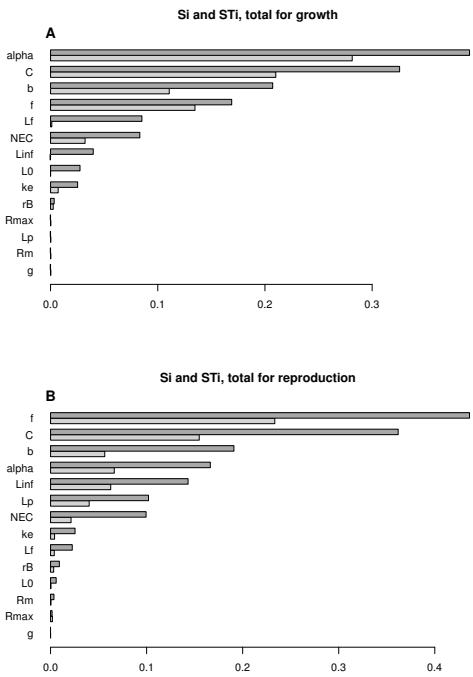

Figure 2: Sensitivity analysis for the *assimilation* model (Eq. 3). Mean of the sensitivity indices at the different times for the growth equation (A) and reproduction equation (B). Total order indices (*STi*) are presented in *dark grey* and first order indices (*Si*) in *light grey*. Parameters were ordered according to the total order Sobol sensitivity indices.

3.3 Parameter estimations

Parameter estimations for the *assimilation* mode of action (Eq. 3) are presented in Table 2 and Figure S4. The distributions of the parameters estimated with the control data only were close to the distributions of the parameters estimated with all the data. Indeed,

Table 2: Prior and posterior (mean±SD) distributions of the model parameters using the *assimilation* model (Eq. 3)

Parameter	**Prior**	**Control data set (mean±SD)**	**Full data set (mean±SD)**
α (-)	$U[0,1]$	0.88 ± 0.07	0.86 ± 0.06
f (-)	$U[0,1]$	0.97 ± 0.02	0.97 ± 0.03
L_0 (μm)	$\mathcal{N}(282,56)$ I $]0,+\infty]$	219 ± 43.53	165 ± 37.37
L_f (μm)	$\mathcal{N}(444,89)$ I $]0,+\infty]$	418 ± 50.96	364 ± 47.35
L_p (μm)	$\mathcal{N}(1020,204)$ I $]0,+\infty]$	764 ± 87.18	818 ± 57.64
L_{inf} (μm)	$\mathcal{N}(1540,308)$ I $]0,+\infty]$	1414 ± 46.32	1383 ± 41.14
r_B (h^{-1})	$\mathcal{N}(0.039, 7.8\ 10^{-3})$ I $]0,+\infty]$	0.034 ± 0.005	0.031 ± 0.003
g (-)	$U[0,20]$	10 ± 5.48	12 ± 4.88
R_M (# h^{-1})	$\mathcal{N}(12.67,2.53)$ I $]0,+\infty]$	15.8 ± 2.02	15.1 ± 1.90
R_{Max} (#)	$\mathcal{N}(265,53)$ I $]0,+\infty]$	312 ± 27.42	249 ± 22.13
k_e (h^{-1})	$U[0,1]$	—	0.16 ± 0.11
b (mM^{-1})	$U[0,5]$	—	0.31 ± 0.03
NEC (mM)	$U[0,2]$	—	0.42 ± 0.06

Prior and posterior (mean±SD) distributions of the model parameters using the *assimilation* model (Eq. 3) with the control data only or with all data (Full data set). The normal distribution of mean μ and standard deviation σ is noted $\mathcal{N}(\mu,\sigma)$, and the uniform distribution between a and b is noted $U[a,b]$. I $]c,d]$ denotes an interval censoring between c (excluded) and d

the means of the parameters estimates were within the confidence interval of each other. According to these estimations, the individuals were mostly fed *ad libitum* ($f = 0.97$). The estimated no-effect concentration (NEC (mM)) was of 0.42 ± 0.06 mM and the elimination rate k_e (h^{-1}) was of 0.16 ± 0.11 h^{-1} indicating that under continuous exposure, internal concentration steady state should be reached within one day. The slope of the effect (b (mM^{-1})) was estimated at 0.31 ± 0.03. The slope of the effect (b (mM^{-1})) and the no-effect concentration (NEC (mM)) were slightly correlated (correlation coefficient of 0.17). The newly introduced parameter α (-) was estimated at 0.88 ± 0.07 for the control data set and at 0.86 ± 0.06 for all the data.

As shown in Figure 3, uranium affects both growth (especially the individuals' maximum length) and reproduction (both the onset of the reproduction and the maximum cumulated reproduction).

4 Discussion

In this study, the effect of uranium on growth and reproduction of the nematode *C. elegans* has been assessed using a bioenergetic approach.

We have shown that our model presents a relevant fit for *C. elegans* growth and reproduction. The model we used presents a similar fit quality to the model proposed by Jager et al. (2005) (ΔDIC < 5). Nevertheless, our model has been shown to be more robust and to better account for the end of the reproductive period. Our model was more flexible, allowed a larger variation around the optimal values, and therefore, could handle a larger range of biological possibilities. Indeed, as shown in Figure S1C, the previous model did not allow growth for organisms slightly limited ($f \leq 0.9$) around the L_f (μm) optimal values presented by Jager et al. (2005) (≈ 444 μm). Nevertheless, as demonstrated by Palgunow et al. (2012), *C. elegans* can handle a large variation of feeding conditions. These authors submitted wild-type N2 nematodes to a so-called "developmental-Dietary Restriction" (dDR) experiment (organisms fed by 250 μL *E. coli*, OD_{600} ranging from 0.3 to 6, dDR starting from egg stage) and did not observed any arrest of growth (the authors observed a reduction of 74% of body length at adult stage for the most stringent dDR condition). According to Figure S1D, the new formulation allowed organisms growth for a larger range of dietary restriction. The new formulation of the reproduction is a descriptive model that also takes into account (i) the fact that sperm cells are limited since they are produced before oocytes during the early stage of development of *C. elegans* (mainly during the beginning of stage L4) (Hubbard and Greenstein, 2005; Lints and Hall, 2009) and (ii) the slow decrease of the laying at the end of the reproductive period (Byerly et al., 1976; Muschiol et al., 2009). A mechanistic understanding of this new formulation of the reproduction may be as follows: if it is assumed that an egg-laying involves an ejaculate of several male gamete, this may lead to a decrease of the density of sperm as the male gamete reserve decreases. Further experiments would be required to confirm this hypothesis. It should be necessary to be able to measure sperm density.

Our results show that uranium affects both the reproduction and the growth of *C. elegans*. Our modelling results suggest that the mode of action of uranium is an effect on the assimilation of energy from food. Indeed, the Deviance Information Criterion we obtained for the *assimilation* model was much better than for all other modes of action. This result is consistent with other experimental and modelling studies

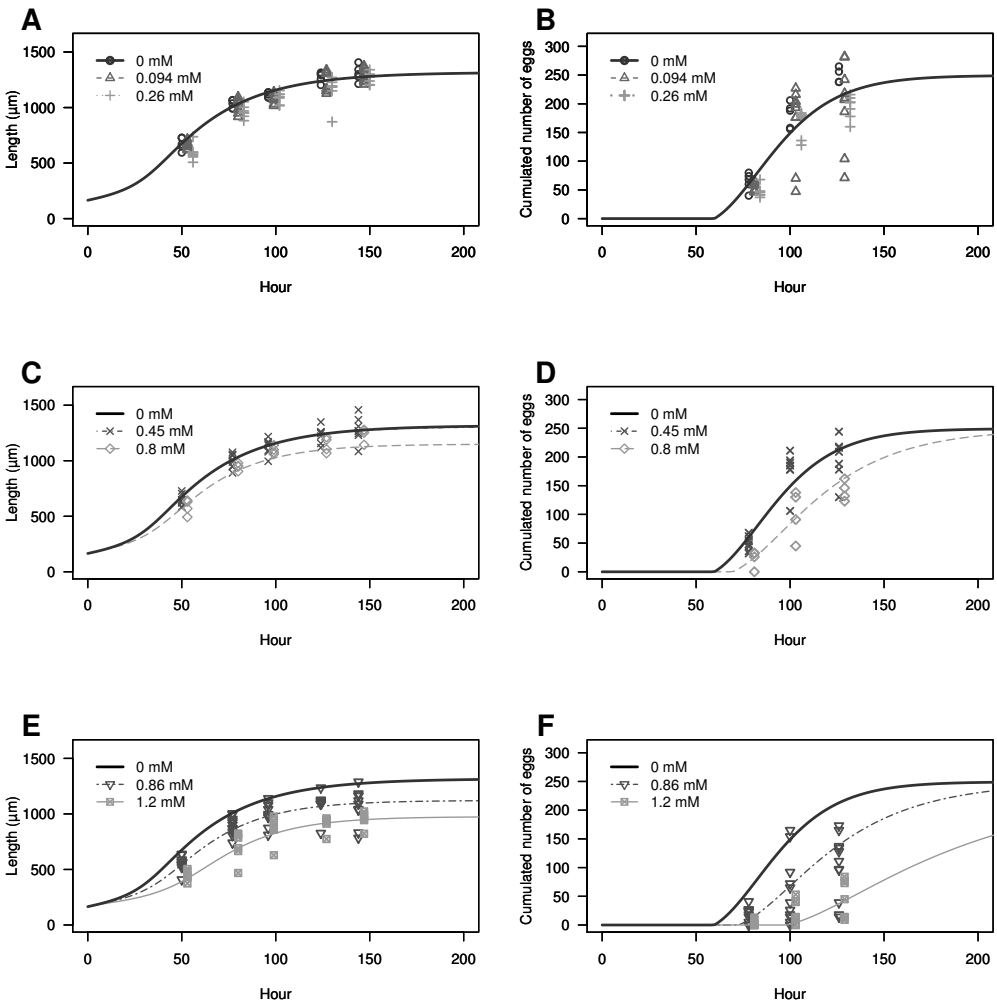

Figure 3: Growth and reproduction results for the *assimilation* model (Eq 3). Panels A and B represent the results for a concentration below the no-effect concentration (NEC (mM)) for respectively the growth and the reproduction. Panels C, D (two lowest), E, and F (two highest) represent the results for the concentrations over the non effect concentration (NEC (mM)) for the growth and the reproduction. Model fits are represented by regression *lines*. Data are represented by *symbols*. Symbols have been slightly shifted in time to facilitate graphics' reading.

which tackled the mode of action of uranium. Augustine et al. (2012a) showed that the mode of action of uranium on *Danio rerio* could either be *assimilation* or *maintenance*. A complementary study reveals that uranium induced an increase of damage in gut cell mitochondria, a loss of the gut wall architecture, the presence of large necrotic zones, and an overall decrease in gut bacteria (Augustine et al., 2012b). As these results reveal negative impacts on organs linked with the assimilation of food, they are in accordance with effects on the assimilation of energy from food. Similarly, DEBtox analysis of the uranium toxicity on *Daphnia magna* exposed during three generations showed that for the two first generations, the *assimilation* and the *maintenance* captured effects on growth and reproduction equally well, but for the last generation, the *assimilation* mode of action better captured the uranium effects (Massarin et al., 2011). The authors concluded in favour of the *assimilation* mode of action based on this last result and on histological evidences. These results were indeed supported by measurements demonstrating that uranium induced structural damages on intestinal epithelium (Massarin et al., 2011; Zeman, 2008), a significant decrease of the ingestion rate expressed per daphnid (Zeman et al., 2008), and a significant reduction in carbon ingestion and assimilation rates (Massarin et al., 2010). The same kind of histological damages to the digestive tract was also reported on the earthworm *Eisenia fetida* exposed to soil contaminated with uranium (Giovanetti et al., 2010).

Our conclusions relative to the mode of action are based on "goodness of fit" and on information from the literature. Using "goodness of fit" only would have required complementary experiments. Indeed, the outcome of the comparison between different modes of action may be biased by experimental variability. Another important point is that, in DEBtox modelling,

the level of effects is assumed to be linearly dependent on the internal concentration, once the NEC is exceeded. This is a strong assumption to account for the actual shape of the dose response with a minimum set of parameters to estimate. This may bias the comparison between different modes of action, if the actual dose response is far from linear. Thereby, previous studies assessing modes of action through DEBtox models proposed complementary measures to a standard test such as food limitation studies or histological observations (Massarin et al., 2011).

Even though we obtained a biologically pertinent model, it did not fit very well the maximum egg laying under stress conditions. A better fit may be obtained using a double effect (both direct and indirect effect) on the *C. elegans* reproduction (Table S1). Thereby a *hazard* additional effect (Eq. S1) or a *cost* additional effect (Eq. S2) on the male gametes could be considered in addition with the *assimilation* model (Eq. 3).

Applied to our data, Eq. S1 gave a DIC of 4,398 and Eq. S2 a DIC of 4,399. Although these DIC are lower than the DIC we obtained in Table S2, to conclude with certainty, and retain this model, further experimental analysis are required to assess and validate this double effect. It should be interesting to assess the evolution of the number of male gamete produced under uranium exposure through, for example, the use of DAPI staining and counting spermatid nuclei located within the gonad as described by Cutter (2004).

The sensitivity analysis we performed showed that the parameters related to food assimilation such as f (-) and α (-) greatly influence the model output. Indeed, according to the DEB framework, food assimilation is the main organism function for the acquisition of energy, and therefore, this function drives all other organism functions. Thereby we believe that the food distribution must be constant throughout the replicates and conditions in toxicity test experiments. Moreover, the food linked parameters should be re-estimated during the data analysis. The impacts of the variations of exposure concentration on the model output were also assessed thanks to the sensitivity analysis. As this parameter is one of the most influential parameters on the output variability of the model, we recommend the careful analysis of the actual concentration present in the medium.

5 Conclusion

Our study proposed a robust and consistent DEB model for the nematode. Our results supported the hypothesis that uranium affects the assimilation of energy from food in many species. We also confirmed the importance of accurately assessing the actual exposure concentration of contaminant and of food while analysing (toxicity) data through modelling. We highlight some points of attention when using modelling for assessing (eco)toxicological data such as the importance of supporting the modelling results by complementary studies. The model developed and evaluated in the present study appears suitable to be used in the analysis of multi-generational datasets such as the one we recently produced (Goussen et al., 2013) in order to assess the evolution of individuals exposed to long term stress.

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A Supplementary material

A.1 Test organism

The *C. elegans* population used in this study is the EEV-A_0 population created by Teotónio et al. (2012) from a mixture of 16 wild isolates. This androdioecious population was derived through a funnel cross strategy. Briefly, two-isolate hybrids were obtained by crossing, in a pair-wise fashion, each of the wild isolates. Then, four-isolate hybrids were obtained by inter crossing in a pair-wise fashion the two-isolate hybrids. Hybridizations continued until the 16-isolates hybrids were created. The population was then maintained over 140 generations and no significant loss of genetic diversity was observed by the authors after the recombination-selection equilibrium was mostly reached.

Prior to exposure, nematode populations were maintained at 20 °C, 80% RH in 9 cm Petri dishes filled with modified nematode growth medium (NGM; use of HEPES buffer instead of potassium phosphate buffer (Dutilleul et al., 2013; Goussen et al., 2013)) seeded with *Escherichia coli* strain OP50 (Brenner, 1974; Stiernagle, 2006). *E. coli* OP50 cultures were grown overnight in L-Broth rich medium at 37 °C. Then cultures ($OD_{600} = 3$) were washed twice with a 5 g L^{-1} NaCl solution in order to remove LB medium since it contains phosphate. Petri dishes were seeded with 1 mL of a 20:1 mixture and left overnight to allow the bacterial culture to dry. Petri dishes were then exposed to UV doses (Bio-Link Crosslinker, $\lambda = 254$ nm; intensity = 200 μwatt m^{-2}) for 15 minutes to stop bacterial growth and to avoid uncontrolled heterogeneity in food availability between Petri dishes. To ensure nematodes were fed *ad libitum* they were transferred into new dishes every three days. To do so, nematodes were washed off the Petri dishes with a M9-modified solution (use of HEPES buffer instead of potassium phosphate buffer). Nematodes picked up were pooled in a 15 mL falcon tube and the number of individuals in a tube was estimated based on three sample drops of 5 μL (Teotónio et al., 2012). The volume corresponding to 500 individuals was then transferred into new Petri dishes.

A.2 Experimental conditions

Experimental data were extracted from a previous study (Goussen et al., 2013). Briefly, individuals from the maintained population were exposed to a range of seven nominal concentrations of uranium: 0 (control), 0.1, 0.3, 0.5, 0.9, 1.1, and 1.2 mM U. For this purpose, 12-well tissue-plates were used. Plates were filled with modified NGM. The uranium stock solution was obtained by dilution of uranyl nitrate ($UO_2(NO_3)_2$,$6H_2O$, Sigma-Aldrich, France). Uranium solution was added to the modified NGM just before flowing the plates. To measure real exposure concentration, 100 μL of NGM samples were collected for each treatment and stored at 4 °C. The samples were digested with a combination of 1 mL HNO_3 and 1 mL H_2O_2 at 90 °C prior to measurement with ICP-AES (Optima 4300 DV, Perkin-Elmer, USA; detection limit 0.04 μM). Around 60 gravid hermaphrodites were randomly picked up from the maintained population dishes, placed on new plates, and allowed to lay for 90 minutes. Eggs were then pooled and randomly deposited in the 12-well tissue-plates. At least 12 wells were used for each treatment (one egg per well). Survival, growth, and egg laying were monitored individually for six days. Survival was measured by stimulating each worm with a platinum transfer pick. A nematode was scored as dead if no head or body movement was triggered by three repeated stimulations (Sutphin and Kaeberlein, 2009; Swain et al., 2004). Nematodes were photographed daily using a stereomicroscope (ZEISS SteREO Discovery V20, ×240 and ×160 magnification respectively for juveniles and adults) coupled with a computer-connected camera (Nikon D5000). Body length was measured using the ImageJ software (Rasband, 2012) and a micrometer scale measure. Egg laying was recorded by visual scoring. The few worms that were lost by crawling off the plates or desiccating on the sides of the plates were removed from the data.

A.3 Model description

According to the DEB theory, energy is taken up from food, assimilated, and stored into reserves. This energy is then dispatched between three main processes: (i) maintenance, (ii) growth, and (iii) reproduction. Models based on the DEB theory allowing the analyse of ecotoxicological data (*i.e.* DEBtox models) have been built by Kooijman and Bedaux (Kooijman and Bedaux, 1996a,b,c) and corrected by Billoir et al. (2008). DEBtox models describe the perturbation of energy management when an organism is exposed to a pollutant. This kind of model assumes that the effects appear when the internal concentration exceeds a threshold called the no-effect concentration (NEC). The DEBtox models describe five modes of action of chemicals on physiological processes. Two of these have a direct effect on the reproduction and the three others have indirect effects on the reproduction in conjunction with effects on growth. The two first are the *cost* model (overhead costs for the creation of eggs) and the *hazard* model (mortality during oogenesis), and the three others are the *assimilation* model (decrease of the assimilation of energy from food), the *maintenance* model (increase of maintenance costs), and the *growth* model (overhead costs for the creation of growth units).

A.4 Estimation of the parameters

For each effect model, we performed an adaptive phase of 10,000 iterations. We then performed another 90,000 iterations. These iterations were performed on three independent MCMC chains. Chains' convergences were assessed using the Gelman and Rubin (1992) convergence statistic modified by Brooks and Gelman (1998). For the few estimations for which

the convergence was not fully reached during this first run, we performed an adaptive phase of 15,000 iterations followed by 135,000 iterations on five independent MCMC chains. Model fit quality was checked by plotting the fits for both the growth and the reproduction data. This assessment was performed using the mean and confidence interval of the 1,000 calculations corresponding to the 1,000 last iterations of the estimation process.

A.5 Supplementary equations

Table S1: *C. elegans* DEBtox reproduction equations with two effects

Hazard additional effect:

$$\frac{dR}{dt} = \frac{R_M}{1-l_p^3}\left[\frac{g+l}{g+(1-s_f)\,f\,(1-s\,(c_q))}\,(1-s_f)\,f\,(1-s\,(c_q))\,l^2 - l_p^3\right]\frac{R_{Max}\exp^{-s(c_q)} - R}{R_{Max}\exp^{-s(c_q)}} \tag{S1}$$

Cost additional effect:

$$\frac{dR}{dt} = \frac{R_M}{1-l_p^3}\left[\frac{g+l}{g+(1-s_f)\,f\,(1-s\,(c_q))}\,(1-s_f)\,f\,(1-s\,(c_q))\,l^2 - l_p^3\right]\frac{R_{Max}\,(1+s\,(c_q))^{-1} - R}{R_{Max}\,(1+s\,(c_q))^{-1}} \tag{S2}$$

A.6 Supplementary Figures

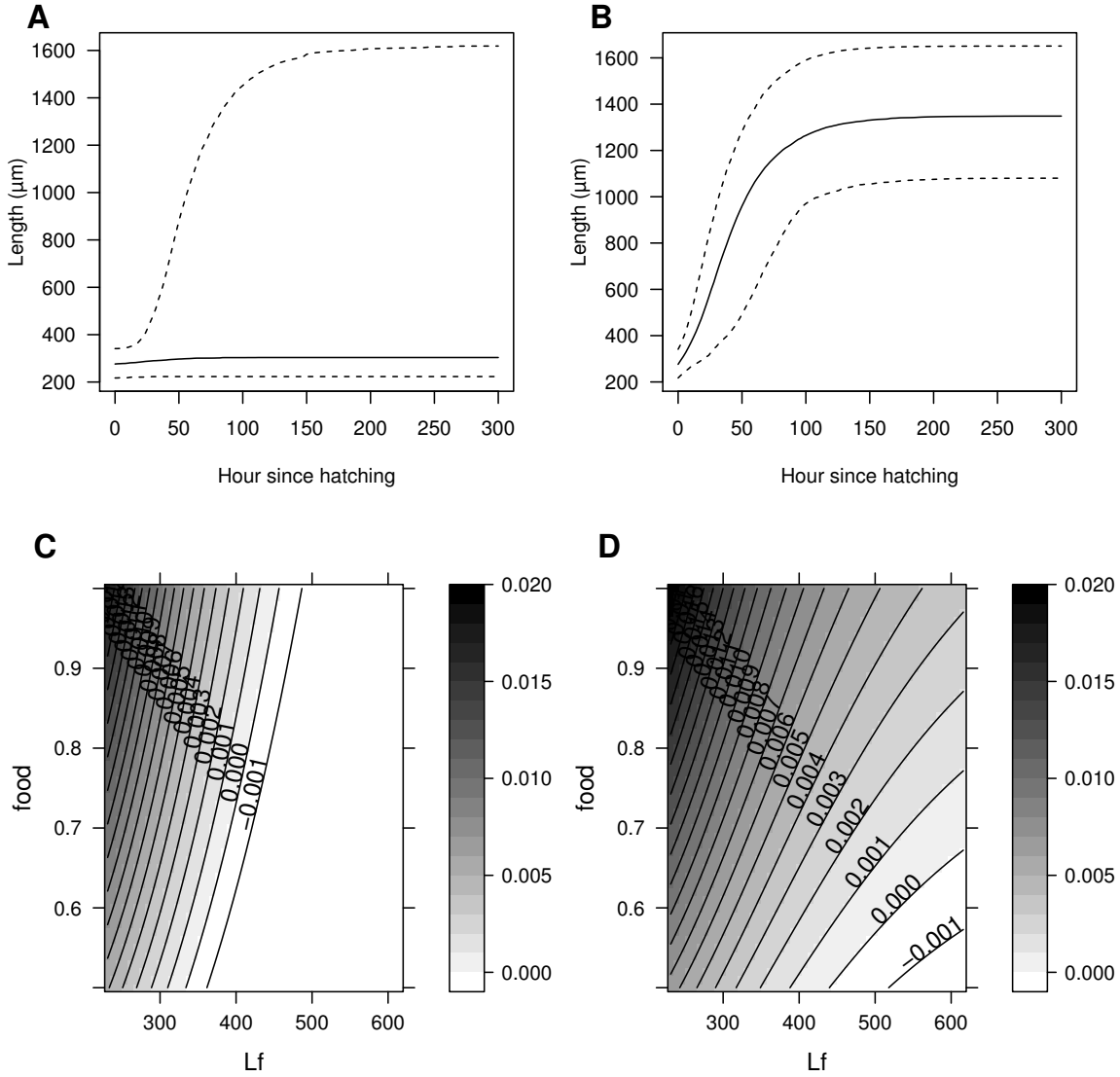

Figure S1: Uncertainty analysis and model exploration for growth. Panel A represents the uncertainty analysis of the model proposed by Jager et al. (2005) and panel B represents the uncertainty analysis of the model we used (Eq. 1). The *solid line* represents the median of the model output. *Dashed lines* represent the 95% quantiles of the model output. Panel C represents the scaled growth ($\frac{dl}{dt}$) at hatching length for the model proposed by Jager et al. (2005) and panel D represents the scaled growth ($\frac{dl}{dt}$) at hatching length for the model we used (Eq. 1). *Food* represents the resulting food $(1 - s_f)f$ and Lf is the body length at which the ingestion rate is half the maximum ingestion rate. The numbers on the contours in the plot present the $\frac{dl}{dt}$ variation. s_f includes the α (-) parameter in panel D but not in panel C

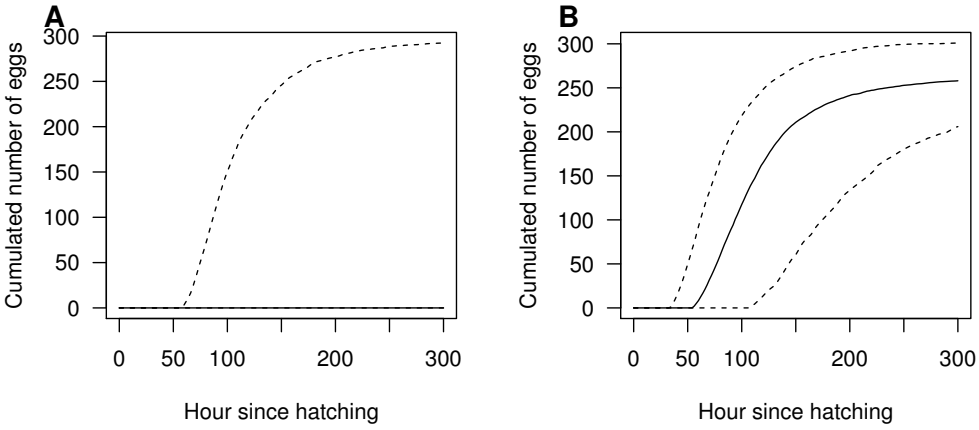

Figure S2: Uncertainty analysis for reproduction. Panel A represent the uncertainty analysis of the model proposed by Jager et al. (2005) and panel B represent the uncertainty analysis of the model we used (Eq. 1). The *solid line* represent the median of the model output. *Dashed lines* represent the 95% quantiles of the model output.

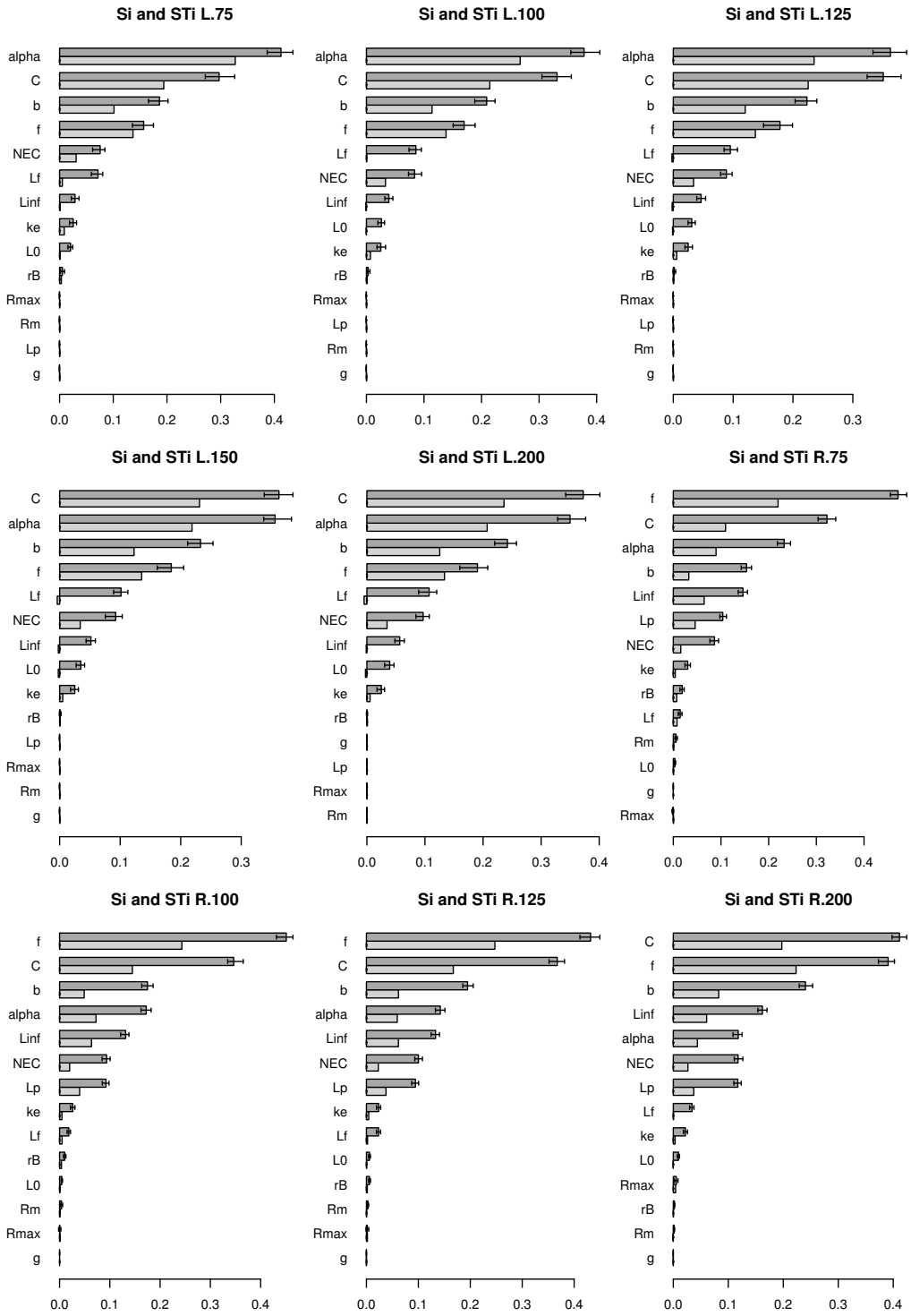

Figure S3: Sensitivity analysis for the *assimilation* model (Eq. 3). Sensitivity indices at the different times for the growth equation and reproduction equation (*e.g.* L.75 and R.75 represent respectively sensitivity indices at 75 hours for growthand reproduction equations). First order indices (Si) are presented in *light grey* and total order indices (STi) in *dark grey*. Parameters were ordered according to the total order Sobol sensitivity indices.

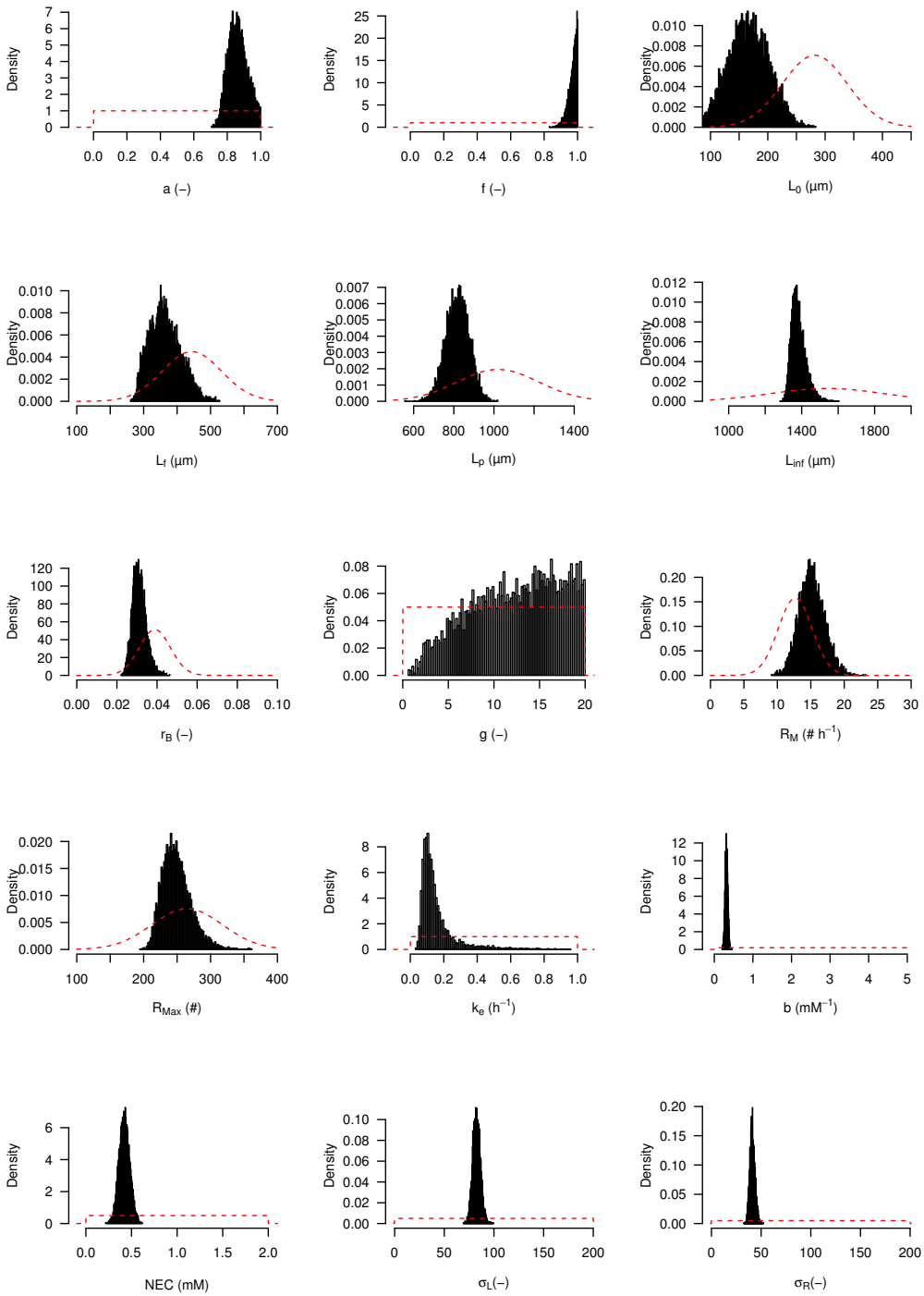

Figure S4: Prior and posterior distribution density for the *assimilation* model (Eq. 3). Prior distribution is presented with *dashed line* and posterior distribution is presented with *histograms*.

A.7 Supplementary Tables

Table S2: Comparison of the fit quality between models

	Mode of action	**Number of parameters**	**Log-likelihood**	**DIC**
Jager et al. (2005)	Control	9	-195.5	399
	Assimilation	12	-2,198.5	4,408
	Maintenance	12	-2,227.5	4,485
	Growth	12	-2,216.5	4,451
Present study	Control	10	-196.5	401
	Assimilation	13	-2,197.0	4,405
	Maintenance	13	-2,244.5	4,687
	Growth	13	-2,213.0	4,437

Comparison between the model from Jager et al. (2005) and our model for all the modes of action. The comparison is based on the log-likelihood and the Deviance Information Criterion (DIC)

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