Simplified Dynamic Energy Budget model for analysing ecotoxicity data*

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Abstract

Models based on Dynamic Energy Budget (DEB) theory offer important advantages in the interpretation of toxicant effects on life-history traits. In contrast to descriptive approaches, they make use of all of the data (all time points, and all endpoints) in one framework, and yield time-independent parameters. In 1996, a suite of simplified DEB models for the analysis of standard toxicity tests was presented under the name 'DEBtox'. Unfortunately, the original equations contained a few errors and inconsistencies. In this paper, we revisit DEBtox, presenting a new and consistent set of simplified DEB equations. The full derivation is presented in the supplementary material to facilitate critical examination of our work. The simplification is appropriate for situations where body size at the start of investment in reproduction remains constant, as well as the egg costs (and thus hatchling size). These conditions are probably met in many ecotoxicological tests, allowing this framework to be used, at least as a first approach. Additionally, we present a statistical framework for fitting the model to experimental data sets, and to calculate intervals on parameter estimates, model curves and model predictions. As an illustration, we provide a case study for the effects

 The paper was published as: Jager T, Zimmer EI. 2012. Simplified Dynamic Energy Budget model for analysing ecotoxicity data. Ecological Modelling 225:74-81. http://dx.doi.org/10.1016/j.ecolmodel.2011.11.012.

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of fluoranthene on *Daphnia magna*, although the framework is by no means limited to this species or toxicant.

Keywords:
Dynamic Energy Budgets, DEBtox, dose-response analysis, TKTD modelling, parameter estimation

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1. Introduction

Models based on Dynamic Energy Budget (DEB) theory (Kooijman, 2010; Nisbet et al., 2000) offer important advantages in the analysis and interpretation of toxicants effects on life-history traits such as growth, reproduction and survival. In contrast to descriptive approaches, a DEB-based analysis can make use of all of the data (all time points, and all endpoints) in one framework, and yields time-independent parameters that can be compared between chemicals (Jager et al., 2004; Billoir et al., 2008b). The theory offers a link between effects on various endpoints, which is essential for extrapolation to the population level (Jager and Klok, 2010; Klanjscek et al., 2006), and to interpret effects at the level of gene expression (Swain et al., 2010). Furthermore, the DEB concept is easily extended to deal with effects resulting from time-varying exposure (Pieters et al., 2006) and mixtures of toxicants (Jager et al., 2010). The underlying principle is that toxicants, once taken up in the body, influence the acquisition and/or use of energy by the organism. Toxicants may for example decrease the feeding rate, or increase maintenance costs. The idea of focussing on the energy budget comes quite natural if we approach the problem from the other side: if there is a decrease in growth or reproduction of an individual, there is obviously less energy devoted to these processes. So, where did that energy go to? Was it never assimilated from food in the first place, or was there an additional energy drain somewhere in the organism?

The principle of interpreting toxic effects based on energy budgets was first formulated in the seminal paper of Kooijman and Metz (1984). More than a decade later, this approach was streamlined into a simple model that could be used to analyse results from standard toxicity tests (Kooijman and Bedaux, 1996b,a). This approach was called 'DEBtox', and was also implemented into freely-available software with the same name. These models were simple enough to make use of the results from toxicity tests conducted according to standard test protocols. The simplifications necessary to derive

these models do limit the possibilities for data analysis in potentially serious ways (Jager et al., 2010). A full-scale DEB model can provide an entirely consistent analysis of toxic effects, but requires more parameters to be estimated from the data, and often additional information (such as egg size and hatching time) (Jager and Klok, 2010). Furthermore, there are quite a number of situations were the full model does not provide much of an added benefit, given the available experimental data (Jager and Klok, 2010). For these reasons, we consider that there is a need for simplified DEB models in ecotoxicology, although a new derivation is required, as the original model equations of DEBtox contained a few errors and inconsistencies. The major errors were spotted, and corrected, by Billoir et al. (2008b), but a number of issues remained.

In this paper, we present a set of simplified DEB equations, derived from the equations in Kooijman (2010). This derivation solves the issues with the original set of equations; the full derivation is presented as supplementary material. Furthermore, we discuss the underlying assumptions and associated limitations of this approach, and provide a pragmatic statistical framework to analyse experimental data. We illustrate the possibilities of this framework by analysing a simple partial life-cycle study for the water flea *Daphnia magna*.

2. Theory

2.1. Background of the simplified model for animals

The standard DEB animal is an animal that feeds on one kind of food, and does not change its shape over its life cycle (an isomorph). Its biomass consists of two components, each with constant composition: structure (which requires maintenance) and reserve (which can fuel metabolic processes). The energy flows are schematically presented in Figure 1. Food is taken up by the organism, and part of the energy is assimilated into the reserve. The reserve is mobilised and split into two fluxes: a fraction κ to the soma, and the rest to maturation and reproduction. Somatic maintenance costs have to be satisfied first, and the rest of the flux to the soma can be used for growth. Similarly, maturity maintenance costs have to be satisfied first and the remainder is used for maturation (in embryos and juveniles) or reproduction (in adults). The investment in reproduction is collected in a reproduction buffer, which is converted into eggs at spawning. None of the state variables

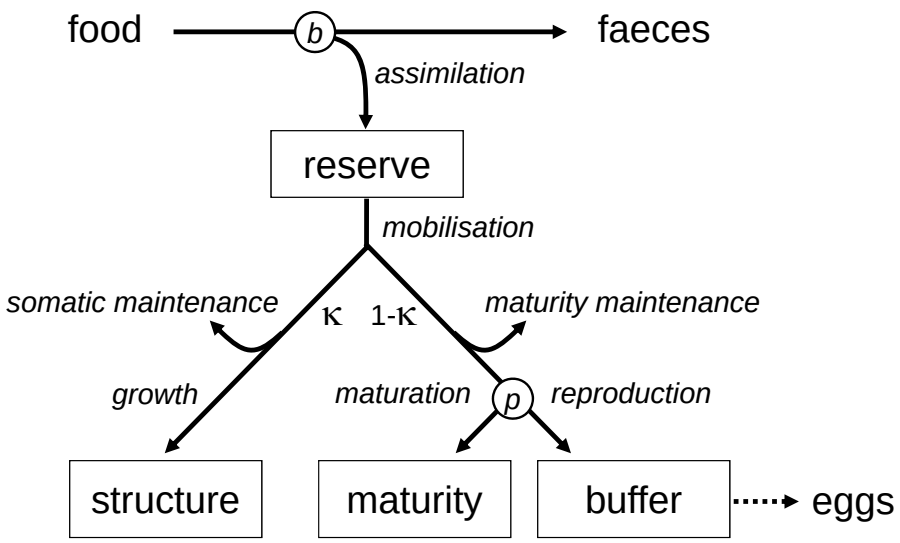

Figure 1: Schematic diagram of the energy flows in a standard DEB animal. The nodes b and p denote switches at birth (start of feeding) and puberty (start of reproductive investment). The mobilisation flux is split according to a constant fraction κ.

can be measured directly; auxiliary theory is needed to link model properties to observable quantities. For example, the physical size of an organism contains contributions from reserve, structure, and possibly the reproduction buffer. More information about this model can be found in Kooijman (2010) and Kooijman et al. (2008), and the equations provided in the supplementary material.

The equations for DEBtox (Kooijman and Bedaux, 1996b) have been derived from the full standard model by a re-parametrisation (to remove the dimension of 'energy' from the model system), and by using three additional assumptions. The first assumption is that maturity is always a constant proportion of structure (for embryos and juveniles). Therefore, instead of a maturity threshold for birth (the start of feeding) and puberty (the start of investment into reproduction), we can take thresholds for structural length. Therefore, we do not have to follow maturity as a state variable. This assumption does not only have to hold for different food levels, but also under toxicant stress (see discussion in Jager et al., 2010). The second assumption is that the energetic costs for an egg are constant under all circumstances. This contrasts the assumption for 'maternal effects' in DEB theory, where egg costs depend on the state variables of the mother (feeding status, and

possibly toxicant body burden). The third assumption is that the reserve is always in a steady state with the food level. This is realistic when we only consider situations with constant food levels, or when the changes in food availability are slow relative to the dynamics of the reserve. Other assumptions that are usually made when working with DEBtox models (although not absolutely required) is that there is no reproduction buffer (offspring are produced as a continuous flux), and that the measured size of the organisms is proportional to the structural size in the DEB model.

In the original DEBtox equations (Kooijman and Bedaux, 1996b), the reproduction equations for effects on assimilation or maintenance were incorrect, probably owing to the complexities of working consistently within a scaled-length framework. Billoir et al. (2008b) corrected these errors, but other issues remained. In the original equations, the Von Bertalanffy growth rate is not a constant but a function of food availability (this is mentioned in Kooijman and Bedaux, 1996b). To use this rate as a model parameter is quite impractical, because its value will depend on the food level, and thus also changes when effects on assimilation are considered. A less conspicuous problem with the original formulation is in its approach for considering an effect on growth costs. The simplified approach assumes that length at puberty is always constant, also under toxicant stress. For this to hold, the ratio of maturity to structural size must remain constant. However, this ratio changes when the costs for growth are affected, which implies that the length at puberty will shift. A simple way to repair this problem is to assume that the toxicant also affects the costs for maturation with the same factor as the costs for growth. This ensures that the length at puberty remains constant, although the applicability of this assumption remains to be tested. A final problem is that body size in the equations is represented in scaled length (scaled to the maximum size in the control), which makes a consistent application of toxicant stress cumbersome and difficult to check. To solve these issues, we derived a new set of simplified DEB equations. Additionally, we included the reserve compartment as a dynamic state. This does not cost additional parameters, and leads to a more consistent approach when food levels vary or when there is a rapid toxicant effect on assimilation.

2.2. Re-derived simplified DEB equations

An extensive derivation of the new set of DEBtox equations is provided in the supplementary material. The state variables for the organism are

Symbol	interpretation	dimensions
$\dot{b}_\dagger$	scaled killing rate	$L^3\#^{-1}t^{-1}$
c_0	scaled internal threshold concentration	$\#L^{-3}$
$c_{0\dagger}$	scaled internal threshold for survival	$\#L^{-3}$
c_d	dissolved external concentration of toxicant	$\#L^{-3}$
c_T	scaled internal tolerance concentration	$\#L^{-3}$
c_V	scaled internal concentration of toxicant	$\#L^{-3}$
e	scaled reserve density (0-1)	$[-]$
f	scaled functional response (0-1), in control f_0	$[-]$
g	energy investment ratio, in control g_0	$[-]$
$\dot{h}_0$	background hazard rate for survival	t^{-1}
$\dot{k}_e$	elimination or 'dominant' rate constant (at $L = L_m$)	t^{-1}
$\dot{k}_M$	somatic maintenance rate coefficient, in control $\dot{k}_{M0}$	t^{-1}
L	structural body length	l
L_0	structural body length at start of experiment	l
L_p	structural body length at puberty	l
L_m	maximum structural body length, in control at $f = 1$	l
$\dot{r}_B$	Von Bertalanffy growth rate, in control at $f = 1$	t^{-1}
$\dot{R}$	reproduction rate	$\#t^{-1}$
$\dot{R}_m$	max. reproduction rate (at $f = 1, L = L_m$), in control $\dot{R}_{m0}$	$\#t^{-1}$
s	stress factor (0 in control)	$[-]$
$\dot{v}$	energy conductance	lt^{-1}

Table 1: DEB model parameters used in this paper with their symbols, interpretation and dimensions (# for numbers, L for length of environment, l for length of organism, t for time).

structural body length (L), scaled reserve density (e, scaled with the maximum reserve density in the control, and so between 0 and 1), and the scaled internal concentration of the toxicant (c_V, scaled with the bioconcentration factor). A list of variables and parameters is given in Table 1. We strictly follow the notation as laid down by Kooijman (2010), including the convention to use a dot above the symbol to indicate that a parameter is a rate with a dimension that includes 'per time' (and thus not a derivative).

The differential equation for the scaled reserve dynamics is ($t = 0$ indicates the start of the experiment):

$$\frac{d}{dt}e = (f - e)\frac{\dot{v}}{L} \quad \text{with } e(0) = 1 \tag{1}$$

Here, f is the scaled functional response (1 indicates ad libitum food availability, 0 implies no food at all), and $\dot{v}$ is the energy conductance, which controls the rate at which reserves are mobilised. We assume that the animals that are used to start the experiment are from an ad-libitum fed culture, and hence $e(0) = 1$. Including reserve dynamics does not require any additional parameters, but it does require an additional state variable, and thus calculation time. If this is an issue, it can be assumed that $e = f$, which is acceptable when f is constant or changes slowly, relative to the reserve dynamics.

For structural length, the resulting differential equation is:

$$\frac{d}{dt}L = \frac{\dot{k}_M g}{3(e+g)}\left(e\frac{\dot{v}}{\dot{k}_M g} - L\right) \quad \text{with } L(0) = L_0 \tag{2}$$

Here, $\dot{k}_M$ is the rate coefficient for somatic maintenance (ratio of the volume-specific maintenance costs and the cost for structure), and g the energy investment ratio (the ratio between the energetic costs for structure and the maximum potentially available energy for the soma). This equation reduces to the Von Bertalanffy growth curve when the parameters are constant. Some deviations from this growth pattern may in fact be experimental artefacts, and can for example be included as a size-dependent food limitation (Jager and Klok, 2010).

In DEB theory, L stands for structural length, which does not equal physical body length. For isomorphs however, appropriate length measures will be proportional to the structural length, and the proportionality can be absorbed in the value of $\dot{v}$. An appropriate size measure is one that is

little affected by changes in reserve or the build up of a reproduction buffer. Examples are the distance from the eye to the base of the spine in *Daphnia*, and shell length in snails. The cubic root of body weight or volume can also be treated as proportional to structural length, although these measures will be more sensitive to changes in reserve and the build up of a buffer. Of course, the contribution of buffer and reserve to the size measurements can be included, at the cost of extra parameters.

For the reproduction rate $\dot{R}$ we end up with the following equation:

$$\dot{R} = \begin{cases} 0 & \text{if } L < L_p \\ \frac{\dot{R}_m}{L_m^3 - L_p^3} \left(\left(\frac{\dot{v}}{\dot{k}_M} L^2 + L^3 \right) \frac{e}{e+g} - L_p^3 \right) & \text{otherwise} \end{cases} \tag{3}$$

Where $\dot{R}_m$ is the maximum reproduction rate (in the control, at maximum food and maximum size), L_m is the maximum body length (in the control, at maximum food), and L_p is the length at puberty (i.e., at the start of reproductive investment). This equation yields a continuous reproduction rate whereas in reality animals produce discrete offspring. We can add a reproduction buffer to collect the reproduction flow, and allow only discrete offspring. However, we can also work with the continuous rate and compare the integrated reproduction to the observed offspring produced in an interval (see Section 3.4).

Instead of the rather abstract $\dot{k}_M$ and $\dot{v}$, we can use the more intuitive maximum length L_m and Von Bertalanffy growth rate constant $\dot{r}_B$ (both in the control at maximum food) as our parameters. These relate to the DEB parameters in the control, which are indicated with an additional subscript 0 ($\dot{k}_{M0}$ and g_0):

$$\dot{r}_B = \frac{\dot{k}_{M0} g_0}{3(1+g_0)} \quad \text{and} \quad L_m = \frac{\dot{v}}{\dot{k}_{M0} g_0} \tag{4}$$

Note that we do not consider effects on $\dot{v}$ because that would affect the scaling of reserve density e; $\dot{v}$ therefore does not need a subscript. If g_0 is given, we can derive $\dot{k}_{M0}$ and $\dot{v}$ as:

$$\dot{k}_{M0} = \dot{r}_B \frac{3(1+g_0)}{g_0} \quad \text{and} \quad \dot{v} = L_m \dot{k}_{M0} g_0 \tag{5}$$

The parameters $\dot{k}_{M0}$ and $\dot{v}$ are thus calculated from L_m, $\dot{r}_B$ and g_0. This brings the list of input parameters for the simplified DEB model to: L_0, L_p, L_m, $\dot{r}_B$, $\dot{R}_{m0}$, g_0, f_0.

2.3. Effects on toxicants on the energy budget

Toxicants need to be taken up into the body before they can exert their effects. However, body residues are not routinely determined in toxicity tests, and it is by no means certain that the concentration in the whole body is truly representative for toxicity. Therefore, the internal concentration will play the role of a hidden variable and its kinetics will be deduced from the development of the toxic effects on the observed endpoints over time (see Jager et al., 2011). The scaled internal concentration (scaled with the bioconcentration factor) c_V has the dimensions of an external concentration; in steady state, the scaled internal concentration will equal the external concentration c_d. The scaling concept, and the derivation of Eq. 6, is explained in more detail in the supplementary material.

Because we are dealing with growing organisms, the change in body size needs to be accounted for in the uptake model. An increase in size leads to dilution of the internal concentration, but also to a decrease in the surface:volume ratio (we assume isomorphy). The exchange of the toxicant with the environment is across a surface area, so this factor needs to be included as well. The model for the scaled internal concentrations as a function of body length L than becomes:

$$\frac{d}{dt}c_V = \dot{k}_e \frac{L_m}{L}\left(c_d - c_V\right) - c_V \frac{3}{L}\frac{d}{dt}L \tag{6}$$

Here, $\dot{k}_e$ is the elimination rate of an organism at the maximum size L_m; smaller individuals will have a larger elimination rate. The last term in the equation accounts for dilution of the internal concentration by growth. The single toxicokinetic parameter $\dot{k}_e$ has to be estimated from the effects data. Survival data generally provide sufficient information to fit this parameter (Jager et al., 2011), but its identifiability from sub-lethal data is often poor (Billoir et al., 2008b). Equation 6 is the simplest toxicokinetics model that accounts for a change in body size in a consistent manner, but this equation can be replaced by more elaborate models if needed.

The defining principle of the DEBtox approach is that some internal concentration affects the value of one or more parameters in a DEB model. For the relationship between the scaled internal concentration and the stress on a model parameter, we assume:

$$s = \frac{1}{c_T}\max\left(0, c_V - c_0\right) \tag{7}$$

Mode of action	DEB parameters under stress
Assimilation from food	$f = f_0 \max(0, 1 - s)$
Somatic and maturity maintenance	$\dot{k}_M = \dot{k}_{M0}(1 + s)$, $\dot{R}_m = \dot{R}_{m0}(1 + s)$
Costs for structure and maturation	$g = g_0(1 + s), \dot{k}_M = \dot{k}_{M0}(1 + s)^{-1}$
Overhead costs for making an egg	$\dot{R}_m = \dot{R}_{m0}(1 + s)^{-1}$
Hazard during oogenesis	$\dot{R}_m = \dot{R}_{m0} \exp(-s)$

Table 2: Possible physiological modes of action for the simplified DEBtox model (combinations of these 5 are also possible). Parameters that are not mentioned in the table for a specific mode of action are set to their value in the control (e.g., $f = f_0$, for all but the first mode).

In this definition, s is a dimensionless indicator of the degree of stress on a model parameter. Below the no-effect concentration c_0, there are no effects. When the scaled internal concentration exceeds c_0 (the no-effect concentration), the stress function increases in a linear fashion. As c_V has the dimensions of an external concentration, so c_0 and c_T also have this dimension. The c_0 can thus be interpreted as the external concentration that does not lead to exceedance of the internal threshold, even after prolonged exposure. To use the simplified DEB model with toxicants, we thus add three parameters to our list: k_e, c_0 and c_T.

The choice for this particular relationship is partly for simplicity (it requires only two parameters), but the use of a threshold has a more fundamental logic. We cannot expose an organism to a single toxicant in isolation; all chemicals are toxic, and there will always be a multitude of unidentified chemicals in the test medium and inside the organism. Invoking the threshold concept, we can assume that all these unknown chemicals are below their respective thresholds, and ignore their effects.

For effects on mortality, we could use the same scaled internal concentration c_V to link to a mortality mechanism such as stochastic death (see Jager et al., 2011, for details). In that way, effects on mortality will have their own toxicity parameters, but are linked to the sub-lethal effects because they share the same internal concentrations.

Which model parameter will be affected by a toxicant? An effect on each DEB parameter has specific consequences for the life-history traits, what we can call a physiological mode of action (Alda Álvarez et al., 2006). Table 2 lists the physiological modes of action (i.e., the DEB parameter affected by toxic stress) that we can invoke for the simplified model (see suppl. mat.).

The actual model parameters are calculated from their counterparts in the controls (with subscript 0) depending on the mode of action. We are limited in the number of modes of action that we can apply, because we have to insure that the length at puberty remains constant (the main assumption for the simplification). For this reason, we have to increase somatic and maturity maintenance costs by the same factor, and we have to increase the costs for maturation when we increase costs for growth. Changing these processes independently, or analysing effects through other parameters such as κ, violates the assumptions behind the simplification and thus leads to inconsistencies.

The set of equations presented here is essentially equivalent to those presented by Billoir et al. (2008b) for the case where reserve dynamics can be ignored ($e = f$), under ad libitum food availability ($f = 1$), and working in a scaled-length framework. Alternative simplified DEB models for toxicity analysis have been published by Klok and De Roos (1996) and Muller et al. (2010). Both approaches do not consider body residues, reserves and maturity as (explicit) state variables and are therefore further reduced than the model presented here (which includes toxicokinetics and reserve dynamics). The work of Klok and De Roos was based on the original model of Kooijman and Metz (a discussion of the differences can be found in Jager and Klok, 2010). The approach Muller and co-authors is closer to the model presented here, although a different strategy is followed for the incorporation of toxic effects.

2.4. Limitations of the simplified model

The simplified model as presented in the previous section has limitations that need to be considered. The simplification rests heavily on the constancy of the length at puberty and the egg costs. Whenever there are indications in the experimental data that this condition is not satisfied, a full-scale DEB model for toxicants (Jager et al., 2010) is appropriate. Adding an ad hoc parameter to decrease L_p as a function of toxicant stress (as done in Alda Álvarez et al., 2006) is not generally recommendable and may lead to bias in the interpretation of the effects.

In this simplified model, we do not deal with the embryonic phase, and also not with ageing. DEB theory (Kooijman, 2010) deals with these aspects, but it remains to be investigated how these concepts are best translated to the simplified model, and how toxicants can affect them. Also, we do not explicitly consider starvation, which occurs when somatic maintenance costs

cannot be paid from the mobilised reserves. Starvation implies a deviation from the standard rules, which is probably species specific, and rapidly requires a full-scale model.

3. Applying the theory in practice

3.1. Data needs

It is difficult to generalise the data needs for this simplified model. Weaker data sets can still be analysed by making use of defaults, prior information, or educated guesses. The weakness of a data set will be reflected in the confidence intervals of the parameters. In general, the most appropriate data would be (partial) life-cycle studies were body size, reproduction and survival are followed from juvenile to fully-grown adult, with regular observations over time. Such data provide the best opportunity to estimate all model parameters, and allow for ecologically relevant predictions (see Section 4). Data for body size alone over time can also be used effectively, reducing the model even further (see also Kooijman and Bedaux, 1996a). The use of reproduction data without observation on body size is not generally recommendable. Even though defaults may be used (see Kooijman and Bedaux, 1996b), it may be difficult to select an appropriate mode of action. Effects on survival alone can be analysed, as long as effects on body size can be ignored. In that case, the simplified model reduces to the DEBtox version of the general survival framework (GUTS, see Jager et al., 2011).

3.2. Selecting a mode of action

Energy fluxes in DEB are model abstractions and cannot be directly measured; we cannot measure maintenance fluxes or reproduction overheads. These processes do, however, have consequences for measurable properties such as body size and offspring production. Thus, we can infer the affected process from the time patterns of effects on the endpoints. Each physiological mode of action has specific consequences for the patterns of growth and reproduction over the life cycle. Effects on assimilation and maintenance lead to similar patterns with smaller ultimate body size, and delayed reproduction. These two modes are often difficult to distinguish without additional observations (e.g., oxygen use). Increasing the costs for structure leads to slower growth, but no effect on the ultimate body size, and also a delay in

reproduction. The last two modes (costs for eggs and hazard during oogenesis) lead to similar effects on reproduction only: no delay in the start of reproduction, but only a decrease in reproduction rate.

In some cases, the data may strongly suggest one particular mode of action, whereas in other cases several can provide an adequate explanation. The estimation of an effect threshold (c_0) does not seem to be very sensitive to the choice of action mode (Kooijman and Bedaux, 1996b), but there can be differences when extrapolating to untested conditions (e.g., food, temperature), or for unobserved endpoints (e.g., feeding rates, oxygen use). If the choice for an appropriate action mode matters, the only solution would be to set up additional experiments, using model simulations to show where alternative explanations will yield different predictions.

3.3. Statistical approach to fit the model

Fitting realistic models to realistic data sets is often a statistical minefield. When we follow the same group of organisms in a test over time, the resulting data will not be independent. The model predicts reproduction as a continuous rate (e.g., number of eggs per day), whereas we observe discrete number of offspring produced by one or more females in a time interval. Growth and reproduction are graded endpoints (we measure the degree of response in every individual), whereas survival is a quantal endpoint (we count the number of surviving individuals over time; each individual is either dead or alive). Clearly, these endpoints are not directly comparable, yet they share information about the same underlying parameters, as all endpoints are linked. As an additional complication, most of the deviation between model and data is not caused by random measurement errors. This is a popular simplifying assumption in statistics, but in reality these deviations will mainly result from biological variation and, obviously, because the model is incorrect.

Given the complexity of this issue and the general quantity and quality of the available data, we have to settle for a pragmatic approach. A likelihood framework is our first choice, as it is powerful, general, and allows us to combine the fits of all endpoints (quantal and graded) into a single value to be optimised (Jager et al., 2004). For survival data, the multinomial likelihood follows naturally (Bedaux and Kooijman, 1994; Jager et al., 2011, see suppl. mat.). For graded endpoints like body size and reproduction, the selection of an appropriate scatter structure is more troublesome. Here, we will stick to the common assumption in regression analysis of independent normal distributions for the error. Even though this assumption is almost

always violated, more appropriate alternatives will often be too complex for analysing basic toxicity data. Nevertheless, in our opinion, this is an area that needs further consideration in the future.

Under the assumption of independent normal distributions with constant variance σ^2, the log-likelihood ℓ of the parameter set θ, given the data Y is:

$$\ell(\theta, \sigma^2|Y) = -\frac{N}{2}\ln(2\pi\sigma^2) - \frac{1}{2\sigma^2}\mathrm{SSQ}(\theta; Y) \tag{8}$$

where N is the total number of data points, and SSQ the sum of the squared residuals (worked out in the next section). There are two obvious ways to simplify the likelihood equation. The first would be to select a value for σ^2, e.g., estimate it from the data set (see Billoir et al., 2008a). When σ^2 is constant, the first term of Eq. 8 does not depend on the parameters and can be ignored (we only need to know the likelihood up to a proportionality):

$$\ell(\theta|Y, \sigma^2) = -\frac{1}{2\sigma^2}\mathrm{SSQ}(\theta; Y) + C \tag{9}$$

Heteroscedasticity can be accommodated by an appropriate choice for σ^2 for different parts of the data set. Alternatively, we could also apply a transformation for model and data in the calculation of the SSQ. For example, log-transformation would be equivalent to taking a skewed error distribution, with a variance that increases with the value of the endpoint.

In the second simplification, we use the method of profile likelihoods to remove the error variance; i.e., replace this parameter by its maximum likelihood estimate (Pawitan, 2001). The estimate for σ^2 depends on θ, and is the SSQ divided by the number of observations N. Replacing this estimate in the likelihood function leads to the following simple result:

$$\ell(\theta|Y) = -\frac{N}{2}\ln\,\mathrm{SSQ}(\theta; Y) + C \tag{10}$$

Here, the error variance is taken homoscedastic, although appropriate transformation of model and data in the SSQ can be used. Furthermore, it is possible to use this equation on parts of the data set separately, and add the resulting log-likelihood functions, which allows a different variance for each part.

Both simplifications will yield the same best-fitting set of parameters, when applied on a single data set. However, when combining different data sets into the fit (e.g., body size and reproduction data), results can differ.

Furthermore, both methods can yield different confidence intervals. The profile likelihood is generally preferable unless we have a good estimate for σ^2 (Pawitan, 2001).

3.4. Deriving sums-of-squares

Replicated observations are represented as Y_{ijr}, where i is the time point of the observation (from 1 to k), j the exposure concentration (from 1 to m), and r the replicate individuals (1 to n). The number of observation times may depend on the treatment (and thus k_j), and the number of replicates may depend on both time and treatment (and thus n_{ij}). The total number of observations N is thus:

$$N = \sum_{j=1}^{m} \sum_{i=1}^{k_j} n_{ij} \tag{11}$$

The sum-of-squares (SSQ) is calculated as:

$$\mathrm{SSQ}(\theta; Y) = \sum_{j=1}^{m} \sum_{i=1}^{k_j} \sum_{r=1}^{n_{ij}} \left(\hat{Y}_{ij}(\theta) - Y_{ijr} \right)^2 \tag{12}$$

If needed, we can modify the scatter distribution by transforming the model predictions and the data, e.g., using log-transformation.

For reproduction, we need to compare the continuous reproduction from the model to the discrete observations in the data. In previous analyses (Kooijman and Bedaux, 1996b), both model and data were recalculated to cumulatives over time, and the (weighted) SSQ determined. This cumulation procedure, however, induces even more dependence in the data set. In our opinion, a better approach is to compare the number of offspring produced by an individual mother in an interval between $t-1$ and t, Y_{ijr}, to the integrated reproduction rate over that interval:

$$\mathrm{SSQ}(\theta; Y) = \sum_{j=1}^{m} \sum_{i=2}^{k_j} \sum_{r=1}^{n_{ij}} \left(\int_{t_{i-1}}^{t_i} R_j(\tau, \theta) d\tau - Y_{ijr} \right)^2 \tag{13}$$

But what do we do with the offspring produced when the mother dies in the interval? We could throw away this observation, or divide the model prediction by two (assuming that the mother died half-way in the interval).

Often, we do not have the data for the individuals, but only the mean of a number of replicates. For the first simplified likelihood (Eq. 9), we can use means if we apply the correct error variance for that observation (note that the variance of a mean is the variance of the replicates σ^2 divided by the number of replicates, n_{ij}). When the variance of the replicates is known, the means carry the same information for the likelihood function as the individual measurements, and we can simply exchange the SSQ in Eq. 9 with a weighted SSQ (see suppl. mat.):

$$\mathrm{wSSQ}_n(\theta; Y) = \sum_{j=1}^{m} \sum_{i=1}^{k_j} n_{ij} (\bar{Y}_{ij} - \hat{Y}_{ij}(\theta))^2 \tag{14}$$

For the second likelihood simplification (Eq. 10), the situation is somewhat more complex. The variance is optimised based on the data and the model parameters, but information is lost when taking the means. In this case, the log-likelihood function requires not only the weighted SSQ of Eq. 14, but also an SSQ weighted with n^2 (see suppl. mat.):

$$\ell(\theta|Y) = -\frac{N_Y}{2} \ln(\mathrm{wSSQ}_{n^2}(\theta; Y)) - \frac{N \, \mathrm{wSSQ}_n(\theta; Y)}{2 \, \mathrm{wSSQ}_{n^2}(\theta; Y)} + C \tag{15}$$

$$\mathrm{wSSQ}_{n^2}(\theta; Y) = \sum_{j=1}^{m} \sum_{i=1}^{k_j} n_{ij}^2 (\bar{Y}_{ij} - \hat{Y}_{ij}(\theta))^2 \tag{16}$$

where N_Y is the number of means, whereas N is the total number of individual data points on which these means were based (see Eq. 11). Note that when n_{ij} is the same for all means, the second term of the likelihood function is constant and can be absorbed in C. In that situation, means can be treated like individual data points.

For reproduction tests, it is common that the adults are kept in groups. In that case, we have to use the mean of the offspring produced. Problems occur when one or more of the mothers die between observations. A pragmatic solution would be to use the average number of adults to calculate the mean reproduction, and to use as n_{ij} to weigh the SSQs analogous to Eq. 14 and 16.

3.5. Optimisation and confidence intervals

If multiple data sets share common parameters, we need to combine the likelihoods for each data set into one overall likelihood. If the data sets are

independent, we can take the product of the individual likelihoods, and thus the sum of the log-likelihoods. Even though the assumption of independence is also usually violated, we consider it a pragmatic simplification. This overall log-likelihood can be maximised, and used to calculate confidence intervals using profile likelihoods (see e.g., Pawitan, 2001) or applied in a Bayesian framework (see e.g., Billoir et al., 2008a). In the absence of strong prior information, a Bayesian calculation will generally produce results similar to a likelihood approach. However, with the Bayesian framework, it is more straightforward to calculate simultaneous credible intervals for multiple parameters, and to construct intervals around model predictions (e.g., Ashauer et al., 2010). Because the assumptions regarding the scatter structure are generally violated, the confidence intervals have to be regarded as approximate.

In many cases, the available data sets will be insufficient to accurately identify all model parameters. In the original DEBtox approach (Kooijman and Bedaux, 1996b), several parameter were fixed to default values for *Daphnia magna*. This allows working with results from standard toxicity tests, which usually do not include determination of body size. Billoir et al. (2008a) advocate a Bayesian approach, using informative priors for such parameters.

The simplified set of equations and the fitting procedure have been implemented in Matlab R2010a. The complete series of scripts and functions can be downloaded from `http://www.debtox.info/`. The log-likelihood function is maximised using a Nelder-Mead Simplex routine (fminsearch). For Bayesian calculations, we implemented the slice sampler (slicesample) as provided in the Matlab Statistics Toolbox to yield a random sample from the posterior distribution. Slice sampling is a Markov chain technique that is efficient and easy to implement for routine application (Neal, 2003). This random sample is subsequently used to calculate credible intervals for the parameter estimates (as 2.5-97.5 percentiles of the sample from the posterior distribution), and for model curves (as 2.5-97.5 percentiles of the model values for each sample at each time point, see Ashauer et al., 2010).

4. Case study

To illustrate the model behaviour and statistical procedure, we take a data set for *Daphnia magna* exposed to fluoranthene. Observations on body size, offspring production, and survival are available over 21 days. This data set is part of the mixture study published by Jager et al. (2010), who per-

formed an analysis with a full DEB model and interpret the results in more detail. The observations on body size were made on a separate group of animals, from which five animals were destructively sampled at each time point, for each concentration. Because sampling was destructive, the assumption of an error following independent normal distributions is defensible, and the scatter structure does not reveal appreciable heteroscedasticity. For the observations on survival and offspring production, a different group of animals was followed, starting at $t = 0$ with 10 animals in each treatment. Because survival and reproduction over time were determined on the same animals, independence is compromised. Another problem is that the animals do not always produce a brood in each observation interval, which implies the regular occurrence of zeros in the data. Instead of trying to model this scatter structure in detail, we decided to move it closer to normality by working with the means for each time point and concentration. Observations for the two controls were combined, and several parameters were fixed. The food availability was assumed to be ad libitum ($f = 1$), initial size was fixed to the mean observed size (because its error is likely much smaller than that of the other observations), and the energy investment ratio g was fixed to the value provided by Kooijman et al. (2008), as this parameters could not be identified from this data set.

A more subtle issue is that reproduction in the model is the transformation of the buffer into eggs. For *Daphnia*, however, we count the release of neonates from the brood pouch, which occurs several days later. We compensate for this by shifting the observations in the comparison with the model prediction by 2.5 days, which is a reasonable estimate for instar duration (Nogueira et al., 2004).

In Figure 2, the maximum-likelihood fit of the model to the data is presented. The selected mode of action was an increase in the costs for producing offspring. A hazard during oogenesis provides a worse, but still reasonable fit. The other modes of action in Table 2 are unlikely as these are associated with strong effects on body size, which were not observed. This does not proof that the reproduction costs are indeed increased by fluoranthene, but rather that this is the simplest explanation within the DEB framework that is consistent with the observed effect patterns. More detailed studies in *D. magna* (Barata and Baird, 2000) showed that fluoranthene affects both the production of eggs as well as egg mortality during incubation in the brood pouch. However, that study is not directly comparable as it showed substantial effects on body size in contrast to our data set. Such differences in effect

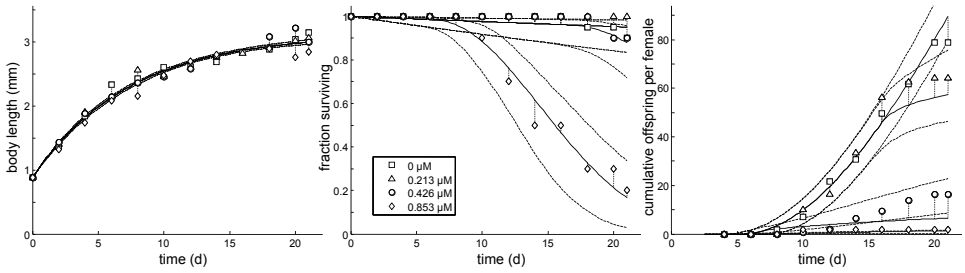

Figure 2: Fit of the simplified DEB model to data for *Daphnia magna*, exposed to fluoranthene. Symbols are means for the concentration and time point; dashed lines are 95% credible intervals on the model curves.

Parameter	value	2.5-97.5 percentiles	unit
f	1	n.e.	[-]
g	0.422	n.e.	[-]
L_0	0.88	n.e.	mm
L_p	1.67	1.39-2.03	mm
L_m	3.13	3.08-3.19	mm
$\dot{r}_B$	0.136	0.126-0.144	d^{-1}
$\dot{R}_m$	11.5	11.7-13.0	$\#\mathrm{d}^{-1}$
$\dot{h}_0$	2.42	0.821-8.61	$10^{-3}\mathrm{d}^{-1}$
$\dot{k}_e$	0.0247	0.00742-0.0567	d^{-1}
$c_{0\dagger}$	0.102	0.0285-0.238	μM
$\dot{b}_\dagger$	1.69	1.70-2.90	$\mu\mathrm{M}^{-1}\mathrm{d}^{-1}$
c_0	0.0418	0.0136-0.0798	μM
c_T	1.69	0.205-13.5	nM

Table 3: Results of the fit of the simplified DEB model on the dataset for *D. magna* exposed to fluoranthene. Maximum likelihood estimates with approximate credible intervals (n.e. means not estimated).

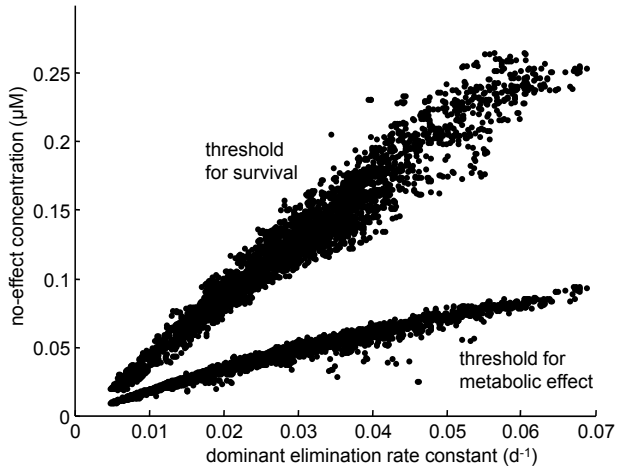

Figure 3: Sample from the posterior distribution, shown as no-effect concentration for survival ($c_{0\dagger}$) and metabolic effects (c_0) versus the dominant elimination rate constant ($\dot{k}_e$).

patterns may relate to genotypic differences between clones of this species, or to differences in the experimental conditions.

The parameter estimates associated with the fit in Figure 2 are given in Table 3. For the credible intervals, an MCMC sample from the posterior distribution was taken (5000 samples, a burn in with 1000 samples, and taking every second sample), applying uniform (presumably uninformative) prior distributions. This sample was used to calculate intervals for the parameter estimates (Table 3), and to produce credible intervals on the model curves (Fig. 2). Interestingly, the credible intervals for both no-effect concentrations (Table 3, $c_{0\dagger}$ for survival and c_0 for sub-lethal effects) overlap. However, plotting the samples from the posterior for these no-effect concentrations versus the dominant elimination rate constant $\dot{k}_e$ (Figure 3) clearly shows how distinct both parameter estimates actually are. Both thresholds are strongly correlated to this rate constant.

The sample from parameter space can also be used to produce intervals on model predictions. As an example, we provide an estimation of the intrinsic rate of population increase in Figure 4, as calculated from the parameters in Table 3 (see Jager et al., 2004; Kooijman and Bedaux, 1996b, integrating over 42 days; twice the duration of the study). This rate integrates the responses of all endpoints and their associated uncertainty into an ecologically meaningful statistic. Here, we plotted the population growth rate normalised

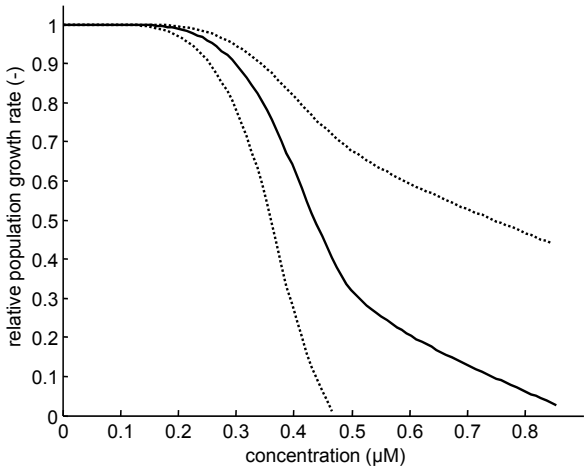

Figure 4: Population growth rate (intrinsic rate of increase), calculated from the model parameters of Table 3, with 95% credible intervals for the model curve. Population growth rate is normalised to the value in the control for each sample from the posterior.

to the rate in the control, which is probably most relevant. Figure 4 clearly shows that despite the substantial confidence interval on the no-effect concentrations in Table 3), the model predicts very little effect of the compound on the population, up to a concentration of about 0.2 μM. At higher concentrations, the predictions rapidly become very uncertain, reflecting the limited information in the data set.

5. Conclusions

In this paper, we present a new and consistent set of simplified DEB equations that can be used to interpret ecotoxicity test results. The full derivation of these equations is presented in the supplementary material to facilitate critical examination of our work. The simplification is appropriate for situations where length at puberty and egg costs (and thus hatchling size) remain constant. These conditions are probably met in many ecotoxicological tests, making this framework a useful tool, at least as a first approach. As an example, the analysis by Jager and Klok (2010) showed that the conclusions drawn from a simple DEBtox calculation can be quite comparable to those of a full-blown DEB model. The statistical framework that we present enables fitting the model to experimental data sets, and allows calculation of intervals on parameter estimates, model curves and model predictions, as

illustrated with the case study. This statistical framework is certainly not a perfect fit to the problem; in general, the available data will violate the underlying assumptions for the scatter structure. However, we believe this is a pragmatic solution to keep the analysis simple, although further study is certainly needed.

The case study shows how this framework is applied to a real data set; analysing all data simultaneously. Even though we used the popular test species *D. magna* for our illustration, the model is by no means limited to this species. Simplified DEB models have been successfully used to analyse toxicity data for a range of species, including springtails (Jager et al., 2004), nematodes (Alda Álvarez et al., 2006), earthworms (Jager and Klok, 2010), and bivalves (Muller et al., 2010). We hope that the presentation of this modelling framework, together with the detailed derivation and the available Matlab code, increases the acceptance of such dynamic modelling approaches in the field of ecotoxicology and ecotoxicological risk assessment.

6. Acknowledgements

This research has been supported by the European Union under the 7th Framework Programme (project acronym CREAM, contract number PITN-GA-2009-238148, http://cream-itn.eu/). We like to thank Elise Billoir and Pierre-Albin Biron for their comments on the derivation in the supplementary materials.

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