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Review

Sublethal effect modelling for environmental risk assessment of chemicals: Problem definition, model variants, application and challenges

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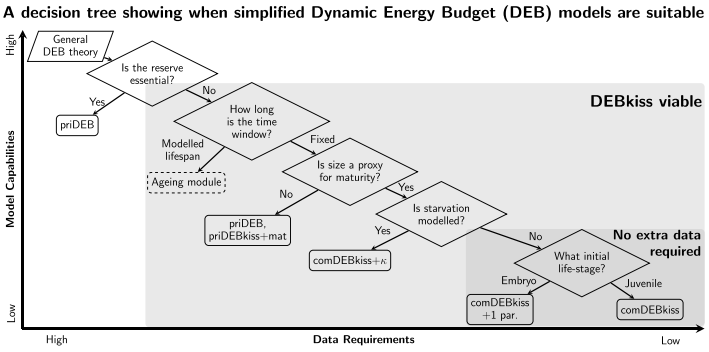
HIGHLIGHTS

- Comprehensive synthesis of many related models
- New, coherent and unifying nomenclature developed
- Establishes guidelines for when simplifications can be made
- Provides details and open problems for the use of these models in risk assessment

GRAPHICAL ABSTRACT



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ABSTRACT

Bioenergetic models, and specifically dynamic energy budget (DEB) theory, are gathering a great deal of interest as a tool to predict the effects of realistically variable exposure to toxicants over time on an individual animal. Here we use aquatic ecological risk assessment (ERA) as the context for a review of the different model variants within DEB and the closely related DEBkiss theory (incl. reserves, ageing, size & maturity, starvation). We propose a coherent and unifying naming scheme for all current major DEB variants, explore the implications of each model's underlying assumptions in terms of its capability and complexity and analyse differences between the models (endpoints, mathematical differences, physiological modes of action). The results imply a hierarchy of model complexity which could be used to guide the implementation of simplified model variants. We provide a decision tree to support matching the simplest suitable model to a given research or regulatory question. We detail which new insights can be gained by using DEB in toxicokinetic-toxicodynamic modelling, both generally and for the specific example of ERA, and highlight open questions. Specifically, we outline a moving time window approach to assess time-variable exposure concentrations and discuss how to account for cross-generational exposure. Where possible, we suggest valuable topics for experimental and theoretical research.

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Contents

1. Introduction

Man-made substances that enter the environment can pose a risk to exposed organisms. Prospective environmental risk assessments (ERA) are conducted for substance groups prior to their release into the environment (EFSA, 2010). The use of plant protection products can result in highly time-variable exposure to environmental organisms due to multiple applications (Carazo-Rojas et al., 2018), weather driven events (Spycher et al., 2018) (e.g. run-off) or the movement of animals through a landscape (Liu et al., 2013). The assessment of such time-variable exposures using the results of standard laboratory toxicity tests, usually conducted with constant exposure conditions, requires extrapolation. Mechanistic effect modelling is playing an ever-increasing role in extrapolating the effects of constant toxicant exposure to organisms (e.g. in standard laboratory toxicity tests) to effects expected under realistic, time-variable exposure in the environment (Galic et al., 2010; Thorbek et al., 2009). Within ERA the general unified threshold model of survival (GUTS) has been recognised as a suitable toxicokinetic-toxicodynamic (TKTD) model to extrapolate lethality in laboratory tests to predict the effects resulting from realistic, time-variable exposure profiles (EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2018). There is a clear need for models which can perform the same extrapolation for sublethal effects, i.e. models that translate a time-variable exposure concentration to predicted effects on growth, development, and reproduction over time (Ashauer et al., 2011). The general approach of sublethal effect modelling must differ from GUTS due to the increased complexity of the problem. Sensitivity to a substance may change as the organism grows and matures (Key et al., 1998) and the effects of the substance on growth and reproduction may in turn have an effect on the uptake, distribution and elimination of the substance (Bridges, 2000; Nichols et al., 2009). For these reasons it is essential to consider how exposure will affect organisms of different sizes and ages.

Put simply, organisms are systems that acquire energy and use it to grow and reproduce. Dynamic energy budget (DEB) theory offers a comprehensive set of rules for the assimilation, dissipation (including reproduction) and growth of a general organism which result in a system of ordinary differential equations (ODEs) (Kooijman, 2010). Using this as a basis it is possible to then model the uptake and elimination of a toxicant as well as the damage it causes to the organism by interfering with the allocation of energy to growth and reproduction (Fig. 1 "Organism"). A wide range of DEB-based models have been developed and tested to model these and other processes, each with TKTD additions in what are now commonly referred to as DEBtox models, although the name DEBtox was first coined for a specific piece of software (Kooijman and Bedaux, 1996). Therefore, to avoid any potential confusion we will refer to the approach and suite of potential model variants as DEB-TKTD.

In order for these models to be generally applicable the assumptions they make must be reasonable, at least within the context of the intended use. This is often difficult to assess. Further complications are introduced with variable food, environmental or exposure conditions which can induce starvation and are still not well accounted for within current models. The effects of such variability are complex and not always entirely negative (Jager et al., 2013a; Costantini et al., 2010). Furthermore, it is known that toxic exposure can have effects on future generations in two main ways, either via altered sensitivity to the substance (Guo et al., 2012; Kim et al., 2012; Rix and Cutler, 2018), or direct maternal transfer (Miller and Amrhein, 1995). Quantifying these effects may be crucial to identify the true effects of exposure yet is largely ignored in DEB models.

The number of different use cases and questions that have been addressed using DEB-TKTD has resulted in different assumptions and therefore different model variants. We review the prominent DEB-based model variants and explore the implications of each one's underlying assumptions in terms of its capability and complexity and propose a clear unifying naming scheme which encompasses all variants. The comparisons imply a hierarchy of model complexity which can be used to guide the identification of the simplest model variant that is fit for purpose both in terms of the species and stressor(s) of concern. The study also provides essential context to understand how different DEB-based models relate to each other.

While some have numerically compared different variants (Jager and Klok, 2010) an analytical examination of the differences between these models, both in the absence and presence of toxic stress, is currently lacking. We provide an analytical and numerical analysis detailing the impact of the most important assumptions on model output to investigate under which conditions they will impact the predicted effects of exposure.

Finally, we explore one potential method to implement DEB-TKTD in ERA, the moving time-window. While many open problems must still be solved in this area, we propose ideas which may be the first steps to a concrete and efficient implementation. For instance, the potential

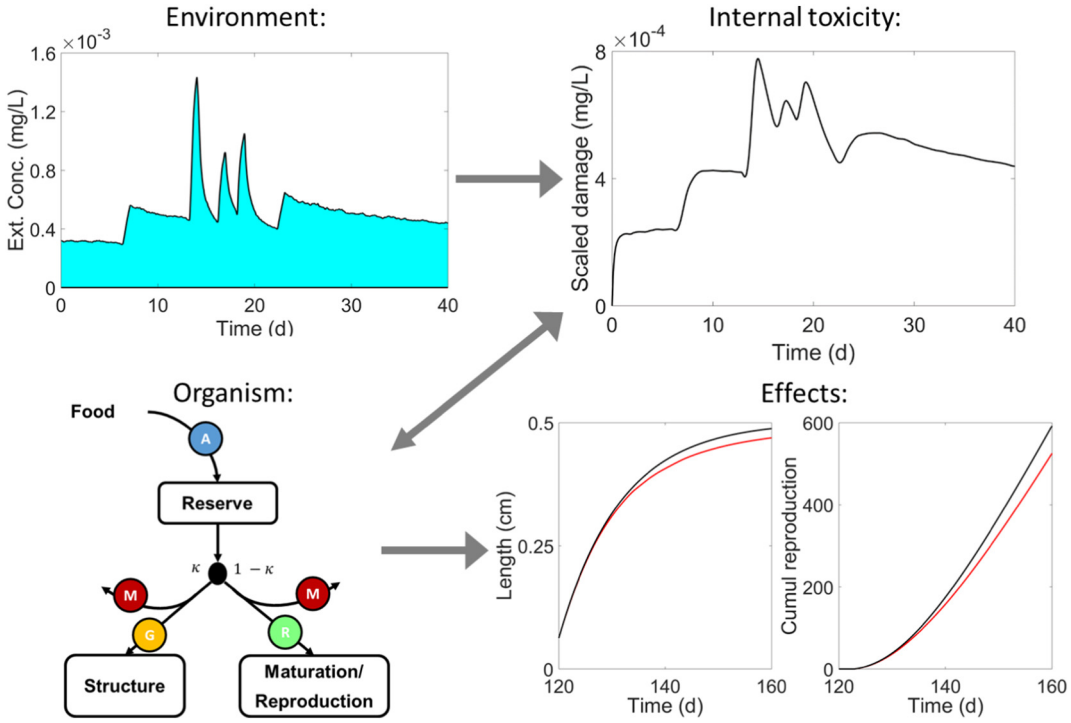

Fig. 1. Conceptual diagram of the framework of DEB-TKTD modelling for ERA to address the time-variable exposure problem. "Environment" contains an exposure profile for the toxicant of concern. "Internal toxicity" translates the exposure at any given time in the window to a level of physiological damage to the organism, based on the properties of the substance and the organism. "Organism" deals with the general DEB-TKTD processes, the arrows show the energy fluxes, and the circled letters denote the various physiological modes of action, that is, the processes which the stressor may affect (A = assimilation, M = maintenance, G = growth, R = reproduction). "Effects" shows a hypothetical example of growth and reproduction under control (black) and exposure (red) conditions. The fat bidirectional arrow between "Internal toxicity" and "Organism" indicates the feedback loop between them. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hazards of maternal transfer are discussed, and we derive a mathematical criteria to determine whether the age of the mother is a relevant factor.

Although many aspects of this paper are broadly applicable across different taxa and implementations of DEB theory, specific attention will be paid to the question of extrapolating results from standard chronic bioassays to realistic time-variable exposure for the purposes of ERA. Using DEB to predict the effects of toxic exposure is one of the most promising applications of the theory. Indeed, DEB-TKTD has already been used many times at the individual (Kooijman and Bedaux, 1996; Jager and Zimmer, 2012) and the population level (Martin et al., 2012; Martin et al., 2014) and could play an important role in bridging across even wider levels of biological organisation (Forbes and Galic, 2016). However, there remain a number of challenges which must be overcome and the current lack of direction is impeding progress. The goal of this paper is to discuss and, where possible, resolve as many of these challenges as possible.

2. General DEB theory

A full description of DEB theory and the derivation of the standard models have been presented many times before (see e.g. (Kooijman, 2010; Billoir et al., 2008; Jager et al., 2013b)). Here we provide a brief introduction to the core assumptions and energy fluxes illustrated in Fig. 1 (Organism). Readers with good knowledge of DEB theory may want to skip to Section 2.1.

The general model organism has three life-stages: embryo, juvenile and adult; and three state variables: structure (described by the cube of structural length L), reserve (E) and maturity (E_H: embryo, juveniles) or the reproductive buffer (E_R: adults). Energy in the reserve is mobilised and divided between the somatic (growth) and maturation (reproductive) branches, a constant fraction κ of mobilised energy goes towards growth, with the remaining $(1 - \kappa)$ going to maturation/reproduction. In each branch energy is first spent on maintenance, based on the current amount of structure (i.e. volume – L^3) or maturity. For endothermic organisms, one must also consider surface-area related somatic maintenance for temperature control, although these can be set to zero under most laboratory conditions. Once maintenance has been paid, remaining energy in the growth branch is spent to build new structure, and in the maturity branch on either maturation (embryos, juveniles) or reproduction (adults).

Life stage transitions occur once a threshold maturity value is reached. Embryos do not feed, instead they survive and develop solely from the initial reserve received from their mother. Feeding begins once embryos mature into juveniles. Once juveniles become adults (known as puberty) maturity no longer increases and the energy is used to build a reproductive buffer. How and when this buffer is emptied is species specific and not in the domain of DEB theory. However, for iteroparous batch-spawning organisms (which include standard aquatic test organisms) reproduction is often modelled as a continuous process (Jager and Zimmer, 2012; Jusup et al., 2017), hence no buffer is necessary.

The feeding rate is determined by the *functional response*, f, a Holling Type II response scaled between zero and one to represent the fraction of the maximum feeding rate the organism could achieve (Holling, 1959). The standard DEB theory assumes isomorphic growth, meaning the organism does not change shape as it grows and feeding scales with surface area. Many organisms do change shape, including many species of fish (Kooijman, 2014) and there are model extensions that account for this (see Kooijman, 2010 and the associated comments document). Although these are not explicitly considered in this paper, the same issues remain relevant for these extended models.

Arguably the highest profile modification to the standard DEB model is DEBkiss, which follows an almost identical set of rules to DEB theory but removes the reserve compartment (Jager et al., 2013b). Ingested food is immediately mobilised into either the somatic or maturation

branch. The central currency in DEBkiss is mass, not energy (although they obey the same general rules), this means that some state variables change, for example maturity is typically written as mass of assimilates spent on maturation, W_H. Embryos also behave differently in DEBkiss; as there is no reserve their growth is instead funded by an egg buffer which is assimilated as a food source (Jager et al., 2013b). Although it is not necessary, DEBkiss models typically also do away with the concept of maturity by assuming that there exists a constant ratio between (structural) size and maturity; as a result life stage shifts occur not only at a fixed maturity level but also a fixed size. The consequences and evidence in support of the simplification are discussed in Section 0.

Despite the listed differences, DEB and DEBkiss are generally very similar. For instance, von Bertalanffy growth emerges under constant conditions in both DEB and DEBkiss. Furthermore, many parameters in DEB have functionally equivalent counterparts in DEBkiss, although direct comparison of parameter values is unwise due to the different dimensions and slight nuances in certain processes. Table 1 lists the core parameters for both frameworks. Where possible, the differences between models are quantified and discussed in Section 4.3.

For both DEB and DEBkiss, moving from the model variables to real world measurements requires auxiliary (or conversion) parameters. Relevant examples for this paper are the dry weight density of structure d_V (similarly the densities of the reserve and reproductive buffer) and the shape coefficient δ_M, used to translate between physical length L_ω and the structural length L (which assumes a cubic shape). Other auxiliary parameters may be necessary depending on the type of data available (e.g. wet weight instead of dry weight).

The parameters of the core models of both DEB and DEBkiss are known as "primary parameters", which relate to individual biological processes or quantities but are generally abstract and difficult to measure from standard biological observations. The complexity of the models can be reduced by introducing "compound parameters" (Kooijman, 2010), but requires several simplifying assumptions, namely: a constant ratio between size and maturity, and a fixed energetic cost per egg. In the spirit of simplicity these models also assume that reproduction is a continuous process. Compound parameters group together primary (and sometimes auxiliary) parameters to reduce the total number of parameters. Compound parameters not only reduce the challenge of model calibration but are also generally more intuitive and often directly measurable. Knowledge of these relationships can also be used to help refine estimates for the primary parameters. Arguably the greatest benefit of using the compound parameter models is that their simplicity often means that it is feasible to fully calibrate the physiological part of the model from bioassay data (observations of growth and fecundity over time) alone (Jager and Zimmer, 2012; Jager, 2020).

2.1. Model variant naming convention

The primary and compound parameters inspire the naming of model variants within this paper. Models written in terms of primary parameters have the prefix "pri" (e.g. priDEB) and those written in terms of compound parameters the prefix "com". Full details of the naming scheme are given in Fig. 2. The relevant compound parameters are also listed and defined in Table 1. Many of these compound parameters appear in both comDEB and comDEBkiss, with equivalent purposes and similar (or possibly identical) values but different mathematical definitions in terms of primary parameters.

The history of DEB modelling briefly outlined above has led to several predominant variants which could be used as the physiological part of a DEB-TKTD model. These are: the standard DEB model, priDEB (Kooijman, 2010); the simplified standard model, comDEB (Jager and Zimmer, 2012); the reserve-less priDEBkiss (Jager et al., 2013b) model and its simplified variant, comDEBkiss (Jager, 2018). The equations and initial conditions of each model variant are stated in full in the supporting information (SI). These physiological models describe the

Table 1
Table showing all parameters involved in the physiological part of the DEB variants (V and S.A. are abbreviations of volume and surface area). Primary parameters are those used in the priDEB and priDEBkiss+mat variants. Parameters removed by priDEBkiss are shown in bold. The removal of maturity from priDEBkiss means that L_p appears in the model despite being listed as a compound parameter. Dimensions are given as ∕ for length, Ɛ for energy, t for time, m for mass (organism), m_a for mass of assimilates and a dash for dimensionless parameters. The von Bertalanffy growth rate in DEB is only true when the animal's reserve is at maximum capacity (see SI). N/a denotes parameters which are absent from DEBkiss models. Compound parameters for both DEB and DEBkiss models are also defined mathematically. The zero in L_{m0}^3 highlights that these values are the maximum size in the control. DEB notation follows the standard rules (Kooijman, 2010), i.e. a dot above the parameter denotes that it is a rate (dimension includes t^{-1}), quantities per structural surface area (dimension L^{-2}) have curly braces and per volume have square brackets (L^{-3}). Note that the shape coefficient δ_M (which translates between structural and physical size) can be incorporated into L_b, L_p, and L_m to directly model physical lengths and remove the explicit need for this parameter in compound parameter models. While many of these parameters share the same purpose care must be taken if values are compared between DEB and DEBkiss models due to the different underlying assumptions.

Primary parameter name	DEB notation	DEB dimension	DEBkiss notation	DEBkiss dimension
Maximum S.A. specific assimilation rate	$\{\dot{p}_{Am}\}$	$Ɛ∕^{-2}t^{-1}$	J_{Am}^a	$m_a∕^{-2}t^{-1}$
Energy conductance	$\dot{v}$	$∕t^{-1}$	N/a	–
Somatic fraction	κ	–	κ	–
V. specific costs for growth	$[E_G]$	$Ɛ∕^{-3}$	$\frac{d_V}{y_{VA}}$	$m_a∕^{-3}$
V. costs for somatic maintenance	$[\dot{p}_M]$	$Ɛ∕^{-3}t^{-1}$	J_M^v	$m_a∕^{-3}t^{-1}$
Maturity maintenance rate	$\dot{k}_J$	t^{-1}	J_J^h	t^{-1}
Egg production efficiency	κ_R	–	y_{BA}	–
Maturity threshold for birth	E_H^b	Ɛ	$\boldsymbol{W_H^b}$	$\boldsymbol{m_a}$
Maturity threshold for puberty	E_H^p	Ɛ	$\boldsymbol{W_H^p}$	$\boldsymbol{m_a}$
Energy cost per egg	E_0 (not constant in priDEB)	Ɛ	W_{B0}	m_a
Compound parameter name	**DEB notation**	**DEB definition**	**DEBkiss notation**	**DEBkiss definition**
Energy investment ratio	g	$\frac{[E_G]\,\dot{v}}{\kappa\,\{\dot{p}_{Am}\}}$	N/a	–
Length at birth	L_b	$\left(\frac{\kappa}{1-\kappa}\frac{E_H^b}{[E_G]}\right)^{\frac{1}{3}}$	L_b	$\left(\frac{\kappa}{1-\kappa}\frac{y_{VA}}{d_V}W_{B0}\right)^{\frac{1}{3}}$
Length at puberty	L_p	$\left(\frac{\kappa}{1-\kappa}\frac{E_H^p}{[E_G]}\right)^{\frac{1}{3}}$	L_p	$\left(\frac{\kappa}{1-\kappa}\frac{y_{VA}}{d_V}W_H^p\right)^{\frac{1}{3}}$
Maximum length	L_m	$\frac{\kappa\{\dot{p}_{Am}\}}{[\dot{p}_M]}$	L_m	$\frac{\kappa J_{Am}^a}{J_M^v}$
Somatic maintenance rate	$\dot{k}_M$	$\frac{[\dot{p}_M]}{[E_G]}$	N/a	–
Von Bertalanffy growth rate	$\dot{r}_B$	$\frac{[\dot{p}_M]g}{3[E_G](1+g)}$	r_B	$\frac{y_{VA}J_M^v}{3d_V}$
Maximum reproduction rate	$\dot{R}_m$	$\frac{\kappa_R}{E_0}\frac{1-\kappa}{\kappa}[\dot{p}_M]\left(L_{m0}^3-L_p^3\right)$	R_m	$\frac{y_{BA}}{W_{B0}}\frac{1-\kappa}{\kappa}J_M^v\left(L_{m0}^3-L_p^3\right)$

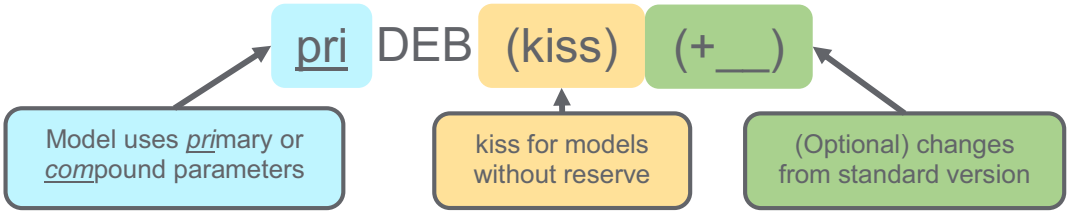

Fig. 2. Graphic explaining the proposed naming scheme for the DEB variants.

growth and reproduction of an organism which is not affected by significant toxicant related stress. The different assumptions have their own limitations and data requirements for calibration which will be discussed in Section 4.1. In order to model the effects of toxic exposure we must now add a TKTD module.

3. Toxicokinetics-toxicodynamics (TKTD)

TKTD models deal with the uptake, biotransformation and elimination of toxic substances and the physiological damage that they deal to the organism. Throughout this section we will assume aquatic exposure for the TKTD. Other forms of exposure may require a different structure, for example using ideas from body burden modelling for exposure through ingestion (Bednarska et al., 2013; Martin et al., 2019). However, the translation of the internal concentration (or damage) to effects on physiological parameters is universal.

Within DEB-TKTD models, toxicokinetics have typically been modelled as a scaled internal concentration (SIC), scaled to the same units as the external concentration of the substance, C_w (Kooijman, 2010; Jager and Zimmer, 2012; Jager et al., 2013b). However, substances vary in their TKTD properties, and in some cases the effects of exposure are still felt long after the substance itself has been eliminated.(Sadoul et al., 2019) To reflect these differences Jager (2020) proposed a generalised model equation for scaled damage, D

$$\frac{dD}{dt} = k_d(x_u\, C_w - x_e D) - (x_G + x_R)D, \tag{1}$$

where

$$[x_u, x_e, x_G, x_R] = [X_u, X_e, X_G, X_R]^{\circ}\left[\frac{L_m}{L}, \frac{L_m}{L}, \frac{3}{L}\frac{dL}{dt}, F_{BV}K_{RV}\frac{dR_c}{dt}\right] \tag{2}$$

$$x_u \mapsto \max(1, x_u)\; x_e \mapsto \max(1, x_e)$$

The circle denotes element-wise multiplication. The two terms in the first bracket of (1) describe the uptake and elimination of damage, the rates of which are mediated by the dominant rate constant, k_d. The x_i's represent feedbacks which can be "off" ($X_i = 0$) or "on" ($X_i = 1$) depending on the nature of the stressor(s). The processes in question are u (e): the rate of uptake (elimination) modulated by the surface area:volume ratio, G: damage diluted by growth and R: damage diluted by reproduction. Dilution by reproduction requires some additional parameters: F_{BV} is the mass of an egg as a fraction of the mass of the mother (set to a constant value for simplicity) and K_{RV} is the partition coefficient between egg material (composed of reserve for DEB organisms and mass of assimilates (energy) for DEBkiss) and structure. The classical SIC ODE (Jager and Zimmer, 2012; Zimmer et al., 2018) is a special case of Eq. (2), namely where the switches take the values $[X_u, X_e, X_G, X_R] = [1, 1, 1, 0]$. Importantly, the damage equation is compatible with any DEB model variant.

This physiological damage must be translated into effects on the organism's growth, reproduction and/or survival within the DEB model. This is done with the use of a non-dimensional linear-with-threshold stress equation:

$$s = b\max(0, D - c_0) \tag{3}$$

where c_0 is a threshold value below which the organism suffers no effects and b determines the sensitivity above the threshold.

For sublethal effects the stress value alters one or more of the primary parameters in the physiological part of the DEB(kiss) model. The parameters affected are determined by the physiological mode of action (pMoA) (Ashauer and Jager, 2018; Jager, 2019). In theory, any parameters could be affected by stress. However, for the vast majority of chemicals, the pMoA is generally one, or a combination of several, out of four pMoAs: Assimilation, Maintenance (both somatic and maturity), Growth and Reproduction (Ashauer and Jager, 2018). The schematic in Fig. 1 shows where these pMoAs act within the physiological model. The effects on primary parameters are given in Table 2, effects on compound parameters correspond to their definitions in terms of the primary parameters (Table 1). Identifying (or at least narrowing down) the dominant pMoA is possible through observation of the modelling and bioassay data (Jager, 2019).

Mortality is traditionally modelled according to the "stochastic death" approach.(Jager et al., 2011) Stress induced mortality generally occurs with different sensitivity and thus requires a second stress Eq. (3) with new c_0 and b values giving survival stress, h. The survival probability S is then determined by the ODE:

$$\frac{dS}{dt} = -(h + h_b)S \tag{4}$$

where h_b is some background mortality rate linked to accidental death and is not suitable to describe mortality through ageing, which requires an extension to the priDEB(kiss) model (Sec. 4.1.2). The alternative "individual tolerance" (Jager et al., 2011) approach may also be used, although it is less consistent with sublethal effects, both in terms of mathematical description and underlying rationale.

Table 2
List of the standard pMoAs and the corresponding effects on primary parameters. Under the growth pMoA stress is also applied to the flux to maturation in juveniles. This means that the ratio between structural volume and maturity is the same as under zero stress. In particular, this means that if size at puberty is constant, the growth pMoA does not alter it. There are multiple choices for how reproductive stress may be modelled which will typically produce similar results. The first of these is the most common choice.

Physiological mode of action	Effect on DEB parameters	Effect on DEBkiss parameters
Assimilation	$f \mapsto f(1-s)$	$f \mapsto f(1-s)$
Maintenance	$[\dot{p}_M] \mapsto [\dot{p}_M](1+s)$	$\dot{J}^v_M \mapsto \dot{J}^v_M(1+s)$
	$\dot{k}_J \mapsto \dot{k}_J(1+s)$	$\dot{J}^l_M \mapsto \dot{J}^l_M(1+s)$
Growth	$[E_G] \mapsto [E_G](1+s)$	$y_{VA} \mapsto y_{VA}/(1+s)$
	$\frac{dE_H}{dt} \mapsto \frac{dE_H}{dt}\frac{1}{1+s}$	$\frac{dW_H}{dt} \mapsto \frac{dW_H}{dt}\frac{1}{1+s}$
Reproduction – increased egg costs	$\kappa_R \mapsto \kappa_R/(1+s)$	$y_{BA} \mapsto y_{BA}/(1+s)$
Reproduction – decrease in yield	$\kappa_R \mapsto \kappa_R/(1-s)$	$y_{BA} \mapsto y_{BA}/(1-s)$
Reproduction – hazard to embryos during oogenesis	$\kappa_R \mapsto \kappa_R e^{-s}$	$y_{BA} \mapsto y_{BA}e^{-s}$

4. Model variants

4.1. Model trade-offs

Every model is a simplification of reality. The modeller must decide which elements are crucial to the behaviour of the system and which can be ignored without seriously affecting the ability of the model to answer a specific question. We define different DEB model variants by identifying the major elements which can either be included or ignored in a DEB model and order these in decreasing complexity, capabilities and data requirements (Fig. 3).

The question set (problem definition), the nature of the species, the requirements of the model, the available data and the assumed environmental conditions influence which elements are necessary. In many applications predictions will be made using constant environmental conditions identical to the laboratory toxicity experiments. Imposing fixed environmental conditions has implications for the required model complexity, which will be discussed where relevant. The answers to the questions in Fig. 3 give an indication of the minimum complexity required from the model and thus which variant minimizes data requirements while retaining all necessary capabilities. Reaching a model variant does not necessarily mean that the subsequent questions do not need to be answered, but rather that the answers will not impose greater demands on model complexity than what has already been determined.

All of these models have their roots in the general DEB theory. Using priDEB as the basis for a model offers the greatest flexibility and widest scope of any model variant. Within TKTD modelling it is theoretically possible to use parameter estimates for sensitivity to a substance and extrapolate to predict effects on a related, untested species. This has been done for models which predict mortality (Gergs et al., 2019) but to our knowledge has yet to be attempted for sublethal effects (Baas et al., 2018). More work must be done to investigate the feasibility and reliability of extrapolating toxic effects from one species to another before we can know how important the choice of model variant is. For instance, it is not sufficiently known whether a substance will act through the same pMoA in a different species (Ashauer and Jager, 2018), or how similarities in taxonomic or DEB properties may influence the sensitivity to a substance. However, there is some indication that phylogenetically close species show similar sensitivity patterns to a pesticide (Hammond et al., 2012), but these findings need more research to be generally applicable. Thus, at least in the short term, extrapolation from a tested species to a related, untested species is not a realistic goal for models used in regulatory risk assessment (EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2018). Furthermore, all current bioassays used for risk assessment maintain constant, (approximately) ideal conditions and thus in general we do not know how toxicity changes in a fluctuating environment. Thus predictive models are often limited to the same constant conditions as in the laboratory. The remainder of the subsection is dedicated to addressing each question in Fig. 3.

4.1.1. The reserve compartment

The reserve compartment forms a buffer between food density in the environment and the energy demand within the animal. The theory states that the reserve is more significant in larger species (Jusup et al., 2017) and thus it may be essential to model the reserve for animals of a certain size although this cannot be guaranteed. A reserve may also be beneficial if comparing results across species. Such comparisons benefit from using the most general theory in order to model the differences between organisms of similar size which differ significantly in their ratio of structure to reserve (Nisbet et al., 2000). These differences may be

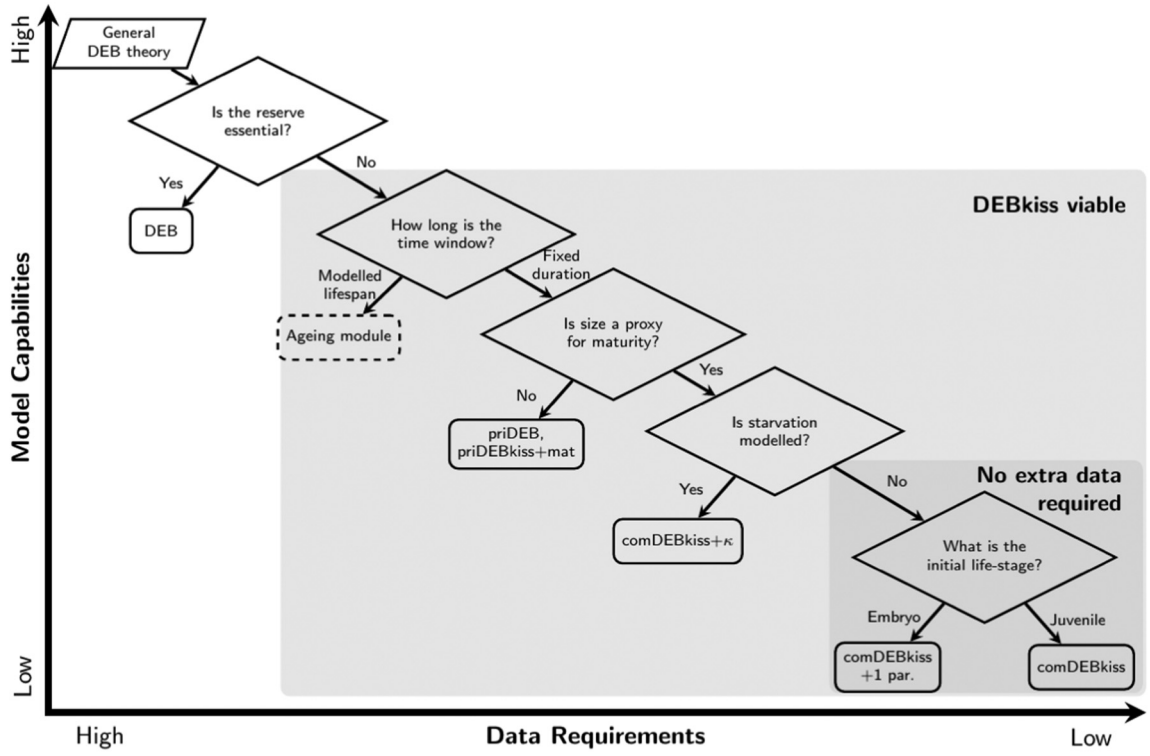

Fig. 3. A decision tree to identify the simplest applicable model based on necessary features. Endpoints of the decision tree do not necessarily mean that no more decisions need to be made, but that the chosen features have dictated the minimal complexity required (with the exception of "DEB" if the reserve is essential, subsequent questions in the flowchart must be answered to find the simplest possible DEB variant). The first shaded region denotes capabilities which can be incorporated by a DEBkiss model (as well as DEB). The smaller shaded region ("No extra data required") indicates the range where data gathered by standard (aquatic) bioassays are fully sufficient to calibrate the model variants. The model variants are: priDEB, priDEBkiss+mat, priDEBkiss, comDEBkiss+κ, comDEB and comDEBkiss. Ageing modules are only possible with priDEB and priDEBkiss(+mat). The dashed box around the ageing module is because it is misplaced with the respect to the axes, but not the shaded regions. Incorporating an ageing module will increase the model capabilities and data requirements beyond those of priDEB alone.

negligible for sufficiently similar species (e.g. of the same genus or family), allowing for extrapolation to other species using DEBkiss.

Removing the reserve compartment removes an ODE from the model and reduces the number of unknown parameters by at least one. In the simplest model, comDEBkiss, every parameter is a directly measurable quantity, the same cannot be said for comDEB. Furthermore, calibration of a DEB model will often lead naturally to a vanishingly small reserve compartment (Jager, 2019). On the other hand, without a reserve animals must immediately respond to an absence of food. While this seems unrealistic the same is true for a fully grown animal with a reserve, all mobilised energy in the somatic branch is required to pay somatic maintenance, thus any decrease in food availability still requires an immediate starvation response (see Section 4.1.4 for a discussion on starvation response). Questioning whether reserve is essential is thus natural and removing it is reasonable in many situations. Although for some organisms removing the reserve may be an over-simplification, there is a proven track record of successful calibration to many standard test species, namely aquatic invertebrates and small fish (Jager et al., 2013b; Sherborne and Galic, 2020), including similar reserve-less models which predate DEBkiss (Jager et al., 2013b; Sherborne and Galic, 2020; Kooijman and Metz, 1984).

Finally, in priDEB an embryo is produced with exactly the level of reserve so that the reserve density at birth is equal to the reserve density of the mother at the time of egg formation, known as maternal effect theory. All reduced models, and all DEBkiss variants instead impose a fixed energetic investment per egg. Evidence exists which supports each approach (see within Kooijman, 2010; Jager, 2018) but a fixed cost per egg allows for greater freedom to alter the investment in each egg in response to stress. A notable, relevant, and frequently observed phenomenon is the tendency of *Daphnia magna* to produce larger, stronger offspring in response to poor feeding conditions (Glazier, 1992; Guisande and Gliwicz, 1992). Barry (1996) tested endosulfan on *D. magna* and found a small (but not statistically significant) increase in mean egg mass at higher concentrations, despite the maternal *Daphnia* being smaller. These examples show the varying evidence on maternal effect theory and the benefits of allowing the energy invested in each egg to change. The best option may be to measure the mass of a subset of eggs in each treatment and use it as a fixed value for each treatment. This would also reduce the number of parameters to be fitted. Regardless, this is not the primary concern of removing the reserve. The decision should be based on the long-term behaviour of the organism. In either case, the subsequent questions are very similar.

4.1.2. Ageing

In order to predict chronic effects one must acknowledge the changing nature of the organism over time. The sensitivity of the organism may depend on its life-stage. For instance, a fully grown organism will not display any negative effects of exposure to a substance which affects only structural growth costs, whereas a growing organism under the same conditions could be severely affected.

Generally, as organisms age their behaviour changes, feeding and reproduction rates typically decrease (Álvarez et al., 2005; Dudycha, 2003). Ageing occurs as reactive oxygen species (ROS) are created during an animal's life. Since animals tend to live longer at lower food levels, within DEB theory the accrual of ageing stress is linked to assimilation or mobilisation of energy, requiring (at least) a priDEB(kiss) model base (Kooijman, 2010). Ageing is a form of damage which typically modulates survival and fecundity. Therefore, in order to make accurate predictions over the whole lifespan of an organism an ageing module should be incorporated. However, this imposes significant additional requirements on both the model and data. Not only would most bioassays need to be extended far beyond their current duration, but since ageing is a form of stress incorporating it raises the complicated issue of multiple stressors (Baas et al., 2010a). This would be especially difficult since, over a whole life, there is no way to study the effects of a toxicant independent of ageing. The alternative is to ignore ageing in the model and run it for a prescribed length of time (or until toxicant-induced mortality). In a risk-assessment context, this period will be the duration of the moving time-window (see Sec 5). In practice, modelling ageing is not only difficult but – for many applications in ERA – also unnecessary, meaning that ignoring ageing is the generally advisable approach.

4.1.3. Size and maturity

An organism reaches maturity when it crosses the maturity (puberty) threshold, by allocating energy to maturation. The "com" models and priDEBkiss all assume that (structural) size at puberty is fixed, i.e. that the threshold is a certain size that must be reached or exceeded for reproduction to occur. This assumption can also be used to parameterise priDEB(kiss) models when data is only available at a single food level (Kooijman, 2010). As it stands then, this assumption must be enforced if DEB models are to be parameterised only from bioassay data. A change in the size at puberty has been observed in response to changes in temperature (Dhillon and Fox, 2004), or the presence of parasites (Chadwick and Little, 2005), or predators (Coors et al., 2004). However, in order to be relevant to predictions made based on laboratory conditions it must change significantly in response to stress induced by a PPP.

A fixed size at puberty results from setting the somatic maintenance rate ($\dot{k}_M = [\dot{p}_M]/[E_G]$) equal to the maturity maintenance rate ($\dot{k}_J$). Otherwise, the same organism will mature at different sizes under different conditions. Ebert (1992) studied this expectation in *D. magna*. Egg production begins two instars before laying. Under reduced feeding *Daphnia* started spending energy on egg production several days and instars later, only after passing a size threshold. The *Daphnia* continued to grow during the development of the first brood, leading to variability in size at first reproduction (i.e. deposition of the first brood) between the groups fed at different levels. Dhillon and Fox (Dhillon and Fox, 2004) studied the temperature-size relationship for the size and age at puberty in Japanese medaka, another common test species. In some treatments fish were split between ad libitum food and an equilibrated ration designed to unify growth rates across temperatures. Fish fed ad libitum were only marginally larger at puberty. However, as puberty was measured as the time when eggs were first observed it is possible that the hypothesis made by Ebert also holds for medaka. If these size differences are significant then a time delay equal to the length of gestation can be incorporated into reproduction and the assumption of a fixed size at puberty retained.

Examples where size at puberty is altered by stress are typically characterised by an earlier onset of reproduction and reduced growth, this "fecundity compensation" is usually triggered by predators (Ebert, 1992) or parasites (Dhillon and Fox, 2004). Hall et al. (2007) used DEB to develop a model of parasitism which included a parasite driven influence on κ. This shift is suggestive of decreasing κ,providing more energy to reproduction. As such these examples do not provide evidence that contradict the assumption that somatic and maturity maintenance rates are equal. Modelling these examples requires a variant which can accept a dynamic κ and variable size at puberty, limiting the choice to priDEB or priDEBkiss+mat (Fig. 3). As these responses are not the result of exposure to a pesticide this pMoA is currently not necessary in ERA. A notable exception to this trend was found in nematodes, which the authors modelled as a descriptive decrease in L_p within a comDEB model (Álvarez et al., 2006). An investigation into whether using priDEB(kiss+mat) with the pMoAs identified in that study produces an effect on size at first reproduction similar to that observed in the data would be very valuable. Overall, our current knowledge suggests that the pMoAs considered in DEB-TKTD models are sufficient for PPPs (Ashauer and Jager, 2018).

One potential area of concern is applying a fixed size at puberty to organisms which are (almost) fully grown before they start to reproduce. Offspring are formed from energy remaining after maturity

maintenance has been paid. Under a fixed size at puberty the proxy for remaining energy is the difference between current size and size at puberty. If size at puberty is close to the maximum size fecundity will be extremely (and unrealistically) sensitive to any reduction in growth, regardless of the source and scale of stress. This will predominantly be a concern in determinate growers, including many species of birds and mammals rather than the fish and crustaceans which constitute the majority of aquatic test species.

It seems that a fixed size at puberty is a reasonable simplification in many cases. However, if this is found to be violated for a given species, or a PPP which alters κ is found then DEB variants which explicitly include maturity must be used, at least in those instances. The EPA guidelines for the mysid chronic toxicity test requires that organisms are measured when secondary sex characteristics are visible (EPA, 1996). This guarantees a data point where length is close to length at puberty. If similar guidelines were established for more species it would provide useful data to test this assumption and calibrate models.

4.1.4. Starvation

During reduced food availability growing organisms naturally slow their growth rate. This remains true until they hit the non-growth boundary, the point where the somatic branch is only receiving enough energy to pay somatic maintenance and growth ceases. This boundary point is slightly different for DEB and DEBkiss models. In the DEBkiss framework assimilated food is immediately mobilised, and thus the non-growth boundary is given by $f = \frac{L}{L_m}$, whereas for DEB models the reserve density $e = \frac{\dot{v}}{\{\dot{p}_{Am}\}} \frac{E}{L^3}$ determines the amount of energy mobilised, and the non-growth boundary is $e = \frac{L}{L_m}$. (These relations are identified by setting $\frac{dL}{dt} = 0$.) This gives the growing DEB model organism a buffer during times of food scarcity that the equivalent DEBkiss organism does not have. This is sometimes known as mild starvation (Kooijman, 2010). Mild starvation occurs when the reserve is being depleted but the reserve density is sufficient to cover somatic maintenance. Mathematically, this means that

$$f < \frac{L}{L_m} \leq e, \tag{5}$$

However, for $L = L_m$ optimal conditions are required just to remain at the non-growth boundary. Any decrease in the food level decreases the mobilisation rate and moves the organism below the non-growth boundary and into "severe" (or prolonged) starvation. Typically, models assume an "absolute" preference for maintenance to be paid from the reserve. However, this need not always be the case (Tolla et al., 2007).

Under severe starvation organisms must break the rules of the general DEB(kiss) framework. The theory in this area is still developing and is likely to be at least partially species or taxon specific. For instance, some organisms may deal with starvation by reducing or ceasing movement, shrinking (Huusko et al., 2011), or regressing to a juvenile state (Thomas and Ikeda, 1987). Starving organisms may still reproduce, using the reproduction buffer or the remaining flux into the reproduction branch. However, often a starvation response is modelled by ceasing growth and redirecting enough energy from the $1 - \kappa$ branch to pay for somatic maintenance (Kooijman, 2010; Jager, 2018; Pecquerie et al., 2009). This is the default response in comDEBkiss+κ. Real-world support for this comes from observations of oocyte resorption and decreasing gonad mass in starving organisms (Bell, 1971; Corriero et al., 2011). If all mobilised energy (i.e. from both the κ and $(1 - \kappa)$ branches) is still insufficient to pay somatic maintenance then typically the organism is assumed to either shrink (burn structure to pay maintenance costs) (Jager et al., 2013b), or die (Pecquerie et al., 2009). The priorities for the animal are thus: somatic maintenance, current structure, maturity maintenance, reproduction, growth of new structure. Larger values of κ mean that proportionally less energy is able to be redirected from the maturity branch, and thus that this adaptation provides less benefit to the organism. In other cases shrinking is the first and only response to severe starvation (Augustine et al., 2011), in which case maturity maintenance and reproduction are second and third in the list of priorities. An implication of both schemes is that maturity maintenance is, to some extent, optional. Consequences of this including rejuvenation (Thomas and Ikeda, 1987), or increased susceptibility to disease (Furlong and Groden, 2003) have been observed in experimental studies. These rankings are reasonable, but may not be true for all species. Indeed, different frameworks of early DEB models proposed different rankings, which were tested by Glazier and Calow (Glazier and Calow, 1992). They found evidence of different rankings in different strains of *D. magna*, and even differences across life-stages. An experiment by Bradley et al. (1991) suggested a priority order of maintenance, growth, reproduction. These responses can each be incorporated within a DEB (or DEB-like) model, but they highlight the difficulty of applying a single modelling approach to all organisms under extreme conditions.

When shrinking is the first and only response to starvation, an extra parameter for the efficiency of regaining energy from burning structure is all that is needed in priDEB or comDEB (Augustine et al., 2011), and this is true for DEBkiss as well. Until recently, the primary parameters were assumed to be necessary to correctly adjust the redirected fluxes under severe starvation. A notable exception to this is the recent work of Jager (2020) where comDEBkiss is extended to handle starvation with no more than two additional parameters needed (referred to as comDEBkiss+κ). This model is also detailed in the SI. While comDEB and comDEBkiss cannot model such responses to starvation without extension they can be used to identify the onset of severe starvation – i.e. the point where the organism crosses the non-growth boundary – this point is the same in the "com" and "pri" forms of the model.

Before starvation can be modelled in a standard manner there is a need for further investigation into the biological responses, particularly when caused by exposure to a PPP rather than a dedicated starvation experiment. Little is known about severe starvation in response to toxicant stress, largely because it is so unlikely in standard toxicity bioassays. Generally, chronic exposure studies begin with young, small animals who can modulate their growth to a size which is sustainable under continued constant exposure. Pulses of exposure later in life are more likely to induce severe starvation, but are rarely implemented in experiments.

Typically, organisms are maintained under constant environmental conditions and (approximately) ad libitum feeding conditions. In many cases these conditions will also be assumed in model predictions. Whether starvation can occur at all then depends on the pMoA of the substance. Stress to reproduction has no effect on the somatic branch and stress to growth only impacts the somatic branch once maintenance has been paid. That leaves assimilation and maintenance as the pMoAs which can induce starvation even under ad libitum food. How they induce starvation is crucially different. The DEB-TKTD schematic in Fig. 1 ("Organism") shows that the maintenance pMoA only directly affects the organism once the reserve has already been mobilised. This means that the scaled reserve density remains at equilibrium so long as feeding conditions do not change. Thus $f \equiv e$ and Eq. (5) shows that mild starvation cannot occur. Hence, under stress to maintenance DEB and DEBkiss organisms with identical growth curves will both enter severe starvation at the same time. By contrast, the assimilation pMoA disrupts the intake of energy since $f \mapsto f(1 - s)$ (see Table 2). This means that mild starvation can occur in DEB organisms. Consider stress to assimilation acting on an identically growing DEB and DEBkiss organism from initially optimal conditions. For the DEBkiss organism, the non-growth boundary becomes $f(1 - s) < L/L_m$, whereas for DEB it remains $e < L/L_m$. Since $e \geq f(1 - s)$ there will be a period of time where $f(1 - s) < L/L_m < e$, i.e. the DEBkiss organism will experience severe starvation whereas the DEB organism can still survive as usual thanks to its reserve. The DEB organism is therefore more resilient to assimilation stress than equivalent DEBkiss organisms. At the individual level results produced by assimilation and maintenance pMoAs are so similar that distinguishing between them generally requires feeding data. However,

they can have significantly different implications at the population level (Martin et al., 2014). Assimilation stress reduces the amount of food eaten by an individual, but that food is still available for others. As long as reproduction can still occur and population density is not a dominant factor, the population will shift to a higher number of smaller individuals. Under maintenance stress animals eat the same amount of food, but grow and reproduce less, so the negative effects to population biomass are much greater.

Perhaps more than any other question in Fig. 3 the extent to which these issues are of concern is dictated by what question is being asked and hence, what constitutes an unacceptable risk. The most conservative approach would be to register any time when the organism falls below the non-growth boundary as an unacceptable risk, and stop the model. This side-steps the complex issues of modelling the response to and recovery from starvation. However, if organisms were allowed to approach their ultimate size in the time-window then results may be biased towards the end of their life. A single instant where somatic maintenance could not be covered may be considered worse than a sustained period of decreased growth caused by a reduced energy supply. Ultimately, we need to look at the overall impact on the individual's fitness and whether exposure to a chemical significantly reduces its survival and reproductive potential, and, as a consequence, the persistence of its populations.

Hyperphagia is often observed once feeding conditions improve (Gurney and Nisbet, 2004; Metcalfe and Monaghan, 2001). This could be viewed as a compensatory response in growth as part of an organism's recovery, but the true picture is more complicated. Hyperphagia has been connected to negative effects later in life and could be an additional form of stress brought on by changing conditions (Metcalfe and Monaghan, 2001). Continuing during and after starvation in standard models for use in ERA requires a sound argument that the effects of hyperphagia are negligible and – depending on the starvation strategy – an accurate estimation for κ. The amount of shrinking that is possible before death cannot be answered for all species in general, but is dependent on other factors (Lika et al., 2014). The theory also dictates that, under starvation, the effect of dilution by growth is reversed to concentrate scaled damage, potentially leading to a feedback loop. This result also requires testing.

If the simplifications proposed by each of these questions so far have been accepted, it will generally be the case that the simplest model variants are applicable (exceptions discussed in Section 4.1.5). The major benefit of reaching this point is that the need for literature data in model calibration is minimal. Indeed, for comDEBkiss data on growth and reproduction will typically be sufficient, providing enough observations of growth and fecundity over time are taken. For comDEB, the compound parameter g remains difficult to fit unless control data are gathered over multiple feeding levels.

4.1.5. Initial stage

Initial conditions are necessary in any mathematical model. This means that we must define an initial state for the model organism. While often we will want to model the whole life-cycle of the organism, this is not always the case, for instance some organisms may only be vulnerable to some stressors during particular phases of their lifecycle (Zimmer et al., 2018). At the fullest extent this means that the model must begin from the embryo phase. For an embryo, the damage ODE (1) may need to change. If uptake or elimination is modified by the organism's surface area (i.e. $X_u = 1$ or $X_e = 1$ in (2)) all variants will require some estimate of the (approximately) constant surface area of the embryo.

Embryonic growth and development are less well understood within DEB theory than other life stages. Typically, (and especially for short-lived animals) the embryonic phase is short enough that any discrepancies are not viewed as damaging to the quality of the model as a whole. If this can also be assumed in TKTD modelling then the primary need to explicitly model effects on the embryonic phase is for differential sensitivity of lethal effects during this life stage. For instance, in some cases the embryo can show significantly reduced sensitivity (Knöbel et al., 2012). In such a case a threshold size and a new set of initial conditions are necessary to add to comDEB(kiss). This alone is challenging for the initial scaled reserve, $e_0 \gg 1$, but a relationship in terms of compound parameters can be found so that embryonic development in comDEB is approximately equivalent to that in priDEB. Given an (often arbitrarily small) initial size, L_0, and a (known or estimated) size at birth, L_b, $e_0 = \left(\frac{L_b}{L_0}\right)^3 (1+g)$ (see SI). Models in terms of primary parameters need no modification. In rare cases where sublethal effects on embryos must be explicitly modelled priDEB(kiss) will almost certainly be necessary; initial amounts of reserve or egg buffer and the rules for birth (onset of feeding) increase the complexity required. Sublethal effects on embryos are often difficult to quantify, for instance, abnormalities and stunted or aborted growth are commonly observed (Wiegand et al., 2001). Significant species-specific experimental work would need to be done in order to quantify the effect of – for example – a curved spine or shell on future survival, growth and fecundity.

4.1.6. Summary

Each of the assumptions in this section is a trade-off between capability and complexity. Each model variant makes its own compromises, summarised, along with the required number of physiological parameters, in Table 3. (Full details of these parameters are given in the SI.) At first glance, the reduction in the number of model parameters may seem small compared to the sacrificed capabilities. However, what matters more is the difficulty of calibrating those parameters. Kooijman et al. (2008) provide guidelines on which parameters can be estimated

Table 3
Table of potentially valuable capabilities of DEB-TKTD models and the required number of parameters for an ectothermic organism. For each variant the number of parameters are given in the "base model", where conditions are ideal and no stress is applied. Parameter counts include conversion parameters assuming observations are of length and fecundity over time, other data types may require different (and perhaps more) conversion parameters. The priDEB variant can be scaled to reduce the number of free parameters by one (see SI). Below, the potential capabilities are listed. Numbers in the cells denote how many additional parameters (on top of the base model) the variant requires to include the capability, "N/A" indicates that the model cannot incorporate the feature, and model extension names are given where relevant. The food density, f, and TKTD parameters are omitted as they are independent of the DEB variant. Details of all parameters are given in Table S2 of the supporting information.

Model features/capabilities	DEB		DEBkiss		
	priDEB	comDEB	priDEBkiss + mat	priDEBkiss	comDEBkiss
Base model	10	6	10	9	5
Modelled lifespan	2	N/A	2	2	N/A
Variable L_p	0	N/A	0	N/A	N/A
Starvation onset	0	0	0	0	0
Starvation – redirecting fluxes	0	N/A	0	0	1 **comDEBkiss+κ**
Starvation – shrinking	1	1	1	1	2 **comDEBkiss+κ**
Embryo phase	1	2	1	1	2
Influence of maternal exposure	0	0	0	0	0

in the priDEB model – and which must be set to fixed values – from different data sets. For example, to calibrate the maturity maintenance coefficient requires growth and reproduction data at multiple feeding levels. This data is not gathered during any standard bioassay and would require significant additional time and effort to gather even in bespoke experiments. Given that there are still many open questions when modelling sublethal effects, it was still not possible to recommend a single variant over the others. However, the conditions under which certain simplifications can be made have been explored; we have recast these simplifications as a series of questions to be asked. The answer to each question either imposes some necessary level of complexity or shows the avenue for further simplifications to be made. Ultimately, we hope this may be useful for regulators and non-specialists to determine the domain of applicability of a given model variant.

4.2. Model endpoints

In the context of ERA, model application will not just aim at accurately predicting an organism's growth and development in response to stress, but also to refine endpoints. Ashauer et al. (2011) produced a table of such endpoints. Table 4 contains the subset of those for which DEB-TKTD is suitable. Simplified variants lose the ability to explicitly model development and thus its suitability will be species-dependent (see Sec. 0).

4.3. Mathematical differences

Studies which discuss the differences between different DEB models are few and far between (Baas et al., 2018), and usually limited to comparing numerical fits (Jager and Klok, 2010). In this section we present analytical results which provide insight into the differences between DEB variants. The majority of these differences will be between all DEB and all DEBkiss model variants since, under constant conditions, all DEB variants are equivalent to each other, as are all DEBkiss variants.

Under ideal conditions all variants produce identical growth curves for feeding organisms providing that compound parameters in DEB and DEBkiss have the same value. Compound parameter definitions in Table 1 show how this constrains parameter values in the "pri" variants. In animals with a reserve, weak homeostasis dictates that reserve density does not change at constant food levels (Kooijman, 2010), this enforces a somewhat counter-intuitive finding that constant reduced food availability increases the von Bertalanffy growth rate in DEB organisms. This is not the case in DEBkiss organisms as there is no reserve. There is evidence to support DEB over DEBkiss in this regard (Lika and Kooijman, 2011), but whether it is true or not its impact on the quality of a model calibration or parameter values is rarely significant. The embryonic phase is slightly different between DEB and DEBkiss. DEBkiss embryos essentially feed on their egg buffer (yolk) at the maximum rate (i.e. $f = 1$) until it is depleted. In contrast, DEB embryos mobilise the energy in their reserve compartment. As embryos in DEB have a negligible amount of initial structure the reserve density tends to infinity at age zero. Such differences will have little impact due to the typically short duration of the embryonic phase in standard test organisms. If modelling begins from birth (i.e. the onset of feeding) then the growth of an organism beginning from the same length at birth will be identical under ad libitum feeding conditions whether it is modelled with a reserve or not.

There are more significant differences in how reproduction is modelled under ideal conditions. We can use the analytical solutions (derived in the SI) for reproduction to investigate how the difference between the two evolves over time. Assuming the same compound parameters for DEB and DEBkiss (and therefore identical growth curves from birth) we define R_c^s as cumulative reproduction in the DEB model and R_c^k in DEBkiss and $f = e = 1$

$$\frac{dR_c^s}{dR_c^k} = 1 - \frac{1}{1+g}\frac{L_m L^2 - L^3}{L_m L^2 - L_p^3}, \tag{6}$$

As organisms grow Eq. (6) approaches one as $L \to L_m$ and the rates of reproduction approach the same maximum rate $\dot{R}_m$. The differences in cumulative reproduction under constant conditions can be found using the analytical solutions in the SI. The energy investment ratio g is a crucial parameter in this context as it gives a sense of the significance of the reserve. Animals which do not have a reserve immediately dedicate all assimilated energy to growth and reproduction (in adults). For animals with a large reserve proportionally more energy is stored, meaning that under constant conditions at any given time less energy is available to produce offspring. As $g \to \infty$ the role of the reserve diminishes and cumulative reproduction in DEB and DEBkiss approach equality. This is shown over time for various levels of g in Fig. 4. For all but the extremely small values of g the difference is small compared to the total number of offspring.

4.4. Different responses to pMoAs

The previous subsection showed that under ad libitum feeding conditions and no toxicant stress, DEB and DEBkiss model organisms with equal compound parameters (Table 1) will have equivalent growth and similar fecundity except in extreme cases. It remains to see how similar the behaviour of DEB and DEBkiss is under stress, particularly the pMoAs defined in Table 2. Once again, we will assume that the environment and feeding conditions remain constant. Detailed mathematical calculations and results are provided in the SI, as well as a visual indicator of how each pMoA may alter the size at puberty in models with maturity (Fig. S1). The effects will depend on the ratio of somatic to maturity maintenance rates

$$k := \frac{\dot{k}_J}{\dot{k}_M} \tag{7}$$

in most cases where a fixed size at puberty ($k = 1$) is an unreasonable assumption maturity maintenance is much smaller than somatic maintenance, i.e. $k \ll 1$.

First we consider assimilation stress. As discussed in 4.1.4 the presence of a reserve can mean that the DEB organism follows qualitatively different behaviour than the DEBkiss organism. This, and the fact that reserve dynamics, structural growth and TKTD can all be interlinked limits theoretical analysis of the difference between DEB and DEBkiss.

Table 4
A table recording common endpoints in ERA which can be modelled using DEB-TKTD models (Ashauer et al., 2011). Symbols denote the capability of each model variant to predict the corresponding endpoint. The symbol ∝ denotes proportionality, i.e. the effect on the endpoint can only be modelled if it is consistent with some size (growth) effect.

Endpoint	Example Guidelines	priDEB	comDEB(+κ)	priDEBkiss+mat	priDEBkiss	comDEBkiss(+κ)
Survival	OECD 202–4, 207, 210, 212–4	✓	✓	✓	✓	✓
Growth	OECD 211	✓	✓	✓	✓	✓
Development	OECD 211, 218–9, 228, 231	✓	∝ size	✓	∝ size	∝ size
Reproduction	OECD 211, 220, 222, 226, 228, 232	✓	✓	✓	✓	✓
Larval development time	OECD 218, 219, 228, 233	✓	×	✓	∝ size	×

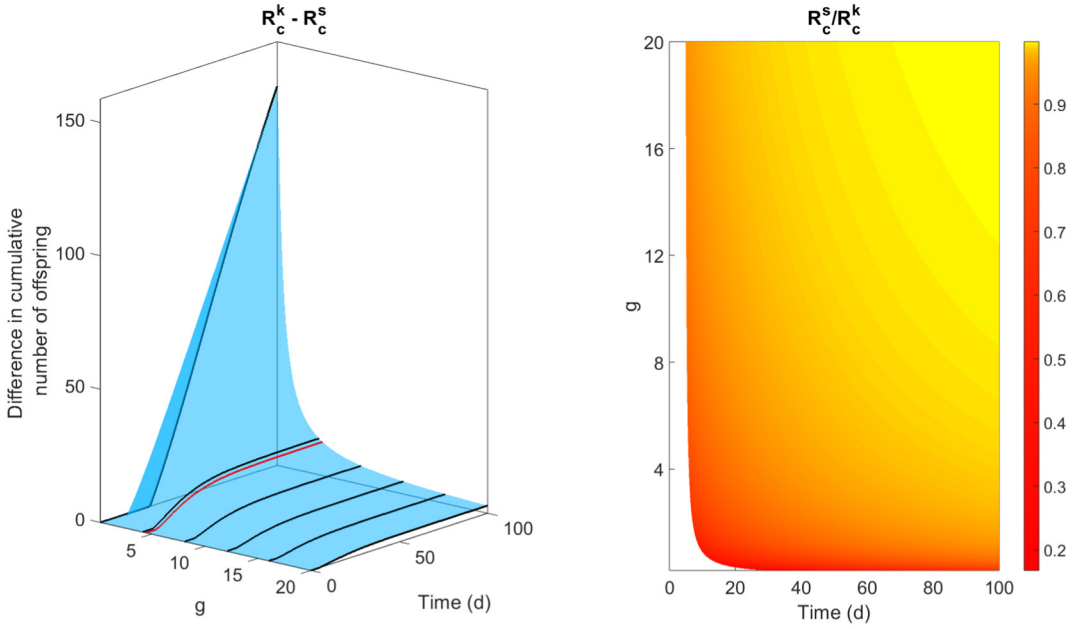

Fig. 4. Illustration of the difference between cumulative reproduction for DEB and DEBkiss models under ideal conditions. The parameter g gives an indication of the importance of the reserve, which vanishes as $g \to \infty$. The von Bertalanffy growth rate is recalculated for each g so that growth remains identical for DEB and DEBkiss. The differences between fecundity in DEBkiss (R_c^k) and DEB (R_c^s) thus only come from the difference in the form of the reproduction equation. This difference is expressed as a simple subtraction ($R_c^k - R_c^s$) in the left plot, and as the fraction R_c^s/R_c^k in the right plot. All other relevant parameters were taken from the Add-my-Pet database entry for Daphnia magna as of 30/08/2019. These are: $L_0 = 0.026\ cm, L_p = 0.069\ cm, L_m = 0.152, \dot{k}_M = 0.273 d^{-1}, \dot{R}_m = 15.544$. All length values are structural, not physical values. The red line on the left plot shows difference over time for the database value of D. magna, $g = 4.494$. In that case, after 100 days, the DEB model organism produced 1236 offspring, and the DEBkiss 1249. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

That said, perhaps unsurprisingly, in cases where the reserve is small ($g \to \infty$) and reserve dynamics are fast ($\dot{v} \to \infty$) the buffering effect disappears and the effects of assimilation stress to DEB and DEBkiss organisms become identical. Qualitatively at least, assimilation stress has the same effect on the size at puberty in priDEB and priDEBkiss+mat. For values of $k < 1$ increased stress decreases the size at puberty while the reverse is true for values above 1 (Fig. S1). The fact that both DEB (Kooijman, 2010) and DEBkiss (Jager et al., 2014) based models for assimilation stress can adequately describe a given data set suggests that the differences between the effects of assimilation stress for DEB and DEBkiss are generally negligible. The other pMoAs do not affect reserve dynamics, and thus permit further analysis.

Regardless of the model variant, stress to maintenance has the same effect. Counterintuitively, the von Bertalanffy growth rate increases by a factor $1 + s$ where s is as defined in Eq. (3), but this is countered by the larger effect of the reduction in the maximum size. All DEB-TKTD variants are equally affected by this pMoA and thus, if growth is equivalent before stress, it will remain equivalent under stress, at least until the onset of severe starvation. If size at puberty is fixed, then the differential effect on reproduction can be found from Eq. (6) (see SI). In cases where size at puberty is not fixed, the effect of maintenance stress on L_p is qualitatively identical to assimilation stress above.

Stress to growth initially appears to have different effects in DEB compared to DEBkiss (see SI). However, some analysis shows that stress to growth costs can produce identical effects on size. Indeed, for a DEB and DEBkiss model with identical growth curves in the absence of stress, stress s_s applied to the DEB organism is equivalent to stress $s_k = \frac{g}{1+g} s_s$ applied to the DEBkiss organism. This difference will be seen in the values of the tolerance parameter b (Eq. (3)). For instance, for these equivalently growing model organisms, if $g = 1$ for the DEB model organism, then if $b_k = \frac{1}{2} b_s$ stress will have equal effects on structural length if other TKTD parameters are equal. The change in the ratio of reproductive output under growth stress can then be found by setting $g \mapsto g(1 + s_s)$ in Eq. (6). Under this pMoA the stress is generally also applied to maturation (in models which have it). This is done so that under the special case where size at puberty is fixed (Sec. 0) it remains fixed under stress to growth. For priDEBkiss+mat animals, stress to growth modelled in this manner never changes size at puberty since both the growth and maturation ODEs are reduced by the same factor $(1/(1 + s))$. By contrast, for DEB animals L_p increases (decreases) for values of k greater (less) than one. However, in practice this change is minimal except for extremely low values of the energy investment ratio, g. Indeed, L_p is generally insensitive to stress on any pMoA (see Fig. S1), and in practical terms could be assumed constant. An exception to this could be organisms which reproduce in large numbers, even the modest increase in energy allocated to new offspring as a result of a smaller length at puberty could result in a great increase in numbers if the energetic costs per egg are small.

The reproduction pMoA is the simplest case as it does not affect the overall energy budget, has no consequences on growth or survival, and does not differ between DEB and DEBkiss models. Regardless of the form of stress chosen from Table 2 the effect is to multiply the rate of reproduction. As such all forms of reproductive stress will have the same *proportional* effect on reproduction regardless of which model variant is employed and the ratio Eq. (6) is unchanged.

The impact of stress on reproduction from any pMoA may be the most relevant change when it comes to assessing population health. Differences between DEB-TKTD variants come about from two different sources. Firstly, if growth of the DEB and DEBkiss organisms diverge then this will have a knock-on effect on the ratio of reproductive output Eq. (6). Secondly, the effects of stress may have a direct effect on the reproductive branch. The reserve means that, for both maintenance and growth pMoAs, reproduction for the DEB organism is less sensitive to stress than the DEBkiss organism. Precise details of how Eq. (6) changes when growth is held equal between DEB and DEBkiss are in the SI. In practical terms, calibrating both a DEB and DEBkiss model to the same data under either pMoA is unlikely to lead to identical growth curves, as each model will compromise to identify the best description of both growth and reproduction that it can.

Table 5
The features from Fig. 3 whose relevance is affected by the pMoA of the substance are listed. "Relevant" means that damage caused by the given pMoA can alter organismal growth and development from control conditions. Size and maturity are only relevant to Growth costs for DEB organisms since the growth pMoA does not change size at puberty for DEBkiss organisms, in almost all cases changes to L_p for the DEB organism will be negligible (see Fig. S1 of SI). Assimilation has an N/A for DEBkiss since maternal effect theory cannot be applied. However, it may be possible to apply stress to the size of the egg buffer to approximate it.

Model capability	pMoA assimilation	pMoA maintenance	pMoA growth	pMoA reproduction
Initial reserve per embryo	Potentially affected	Not affected	Not affected	Not affected
Size at puberty	Potentially affected	Potentially affected	Potentially affected (DEB only)	Not affected
Starvation	Potentially affected	Potentially affected	Not affected	Not affected
Maximum potential complexity required - DEB	priDEB	priDEB	priDEB	comDEB
Maximum potential complexity required - DEBkiss	N/A	priDEBkiss+mat	comDEBkiss	comDEBkiss

The pMoA of a substance will also impact on which of the model capabilities listed in Fig. 3 remain relevant. Consider a substance which only causes stress to reproduction in a laboratory exposure study. As all treatments experience the same feeding and environmental conditions one would expect growth and maturation of the test animals to be equivalent (within the range of biological variability). Thus the size at maturity will remain constant for all treatment levels, and the model fit and TKTD parameter estimates will also be identical regardless of whether the model uses primary or compound parameters. Of course, one should be mindful of simplifications made for such reasons if the results are ever translated to different conditions. A summary for all pMoAs and model capabilities is given in Table 5.

5. The moving time window and cross-generational exposure

Once a chosen DEB-TKTD variant is calibrated to data and, ideally, validated against independent data, it must be used to make predictions. Making predictions while mimicking the standardised toxicity test conditions is the clearest and simplest way to isolate the problem of time-variable exposure from other varying conditions and explore how time-variable exposure alone affects organism development. One can view this as a virtual toxicity test in which only the exposure profile differs from the experimental conditions. When the problem is phrased as such it is also easier to see where it applies in the regulatory risk assessment framework. However, as damage uptake and elimination and the effects of stress can change with the size and life-stage of the organism it is important to consider how an exposure will affect individuals of all sizes.

To deal with this, a moving time window approach is envisioned (EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2018). The moving time window is an interval of the predicted exposure profile where growth and reproduction are modelled from some starting point (e.g. birth or hatch) until some end condition is reached (either a certain duration, or until death). Within each window the "margin of safety" multiplier (Ashauer et al., 2013) will be found as the constant by which the exposure must be multiplied before unacceptable risks are predicted by the calibrated (and validated) DEB-TKTD model. In this context unacceptable risks could include a decrease in survival, growth and/or reproduction. The time window moves through the exposure profile by some (small) increment and reassesses the margin of safety multiplier. The minimum margin of safety found across all time windows can then inform the risk assessment in much the same way as the LP_x value is proposed for GUTS models (EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2018). That is, some threshold value will be set, if the multiplier value is greater than that threshold then that exposure profile will be deemed safe for the species in question.

The procedure is illustrated in Fig. 5. If the time window is longer than the exposure profile, for example when the test species has a long lifespan, the process inverts, and the exposure profile shifts along the age of the animal. The pre and post-exposure phases ensure that all parts of the exposure profile are fully considered (Fig. 5). Depending on which is considered most realistic or sufficiently conservative, these phases may have zero exposure, exposure from another part of the profile (e.g. wrap-around so that day −1 has the same exposure as the final day), or continue the observed trend (e.g. some exponential decay). To be comprehensive, these phases should be equal to the duration of the time window minus the incremental movement.

In general, only the exposure profile will change from the experimental setup, that is, we assume that feeding and temperature conditions remain constant and ideal – at exactly those conditions of the often standardised (e.g. OECD guidelines) laboratory toxicity test that generated the model calibration data. In the same spirit, it is tempting to suggest that the time-window should be exactly as long as the bioassay used to calibrate the model. However, consider a predicted exposure profile with two separate major pulses of exposure 25 days apart. Although a 21 day *D. magna* study may be sufficiently long to determine sensitivity to the substance this does not mean that the two exposures cannot both be relevant to a single *Daphnia*. Thus the duration of the window may need to be adaptive, especially if the endpoint of concern remains reduction in growth, reproduction or survival at a certain point in time.

The extent of reduction in chronic endpoints (e.g. growth, reproduction) which is acceptable is traditionally determined by statistical methods, for example using hypothesis tests to derive so called no effect concentrations (NOECs) or using regression models to find an effective concentration which has some x% effect on a given endpoint (ECx). The concept behind NOECs is fundamentally flawed (Laskowski, 1995; Kooijman, 1996; Crane and Newman, 2000; Landis and Chapman, 2011; Fox and Landis, 2016), and the use of EC values makes invalid assumptions (Jager, 2011; Baas et al., 2010b) that render both concepts unsuitable for assessment time-variable concentrations. Nevertheless, one could suggest that the traditionally accepted effect size is 10% (EC10) and therefore using a 10% reduction in growth or reproduction at the end of the moving time window is most consistent with the current regulatory ERA frameworks.

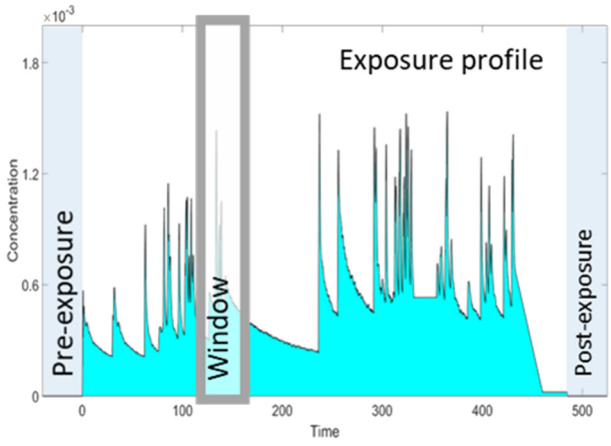

Fig. 5. An illustration of the moving time window progressing through a hypothetical exposure profile. The pre and post-exposure regions are padded areas of zero exposure to ensure that exposure early (late) in the profile can still be experienced by older and larger (younger and smaller) organisms.

Defining the acceptable effect size for growth and reproduction in environmentally relevant and realistic scenarios – as opposed to standardised laboratory bioassays – is also an open and species-specific problem. Within ERA, the typical protection goal is the population or ecosystem, not the individual. Thus an assessment of potential population level effects could inform risk assessment at the individual level. Using DEB-TKTD at the core of ERA offers a chance for coherent effect assessment and modelling across biological scales (Galic et al., 2010; Jager and Klok, 2010). In the future this could be developed further by also incorporating sub-individual models (Murphy et al., 2018).

Whatever criteria for measuring adverse effects are chosen, identifying the worst case time-window will require significant computational power. Happily, many of the potential time-windows can be excluded from the process prior to any modelling taking place. The simple procedure runs as follows: (i) construct the full list of possible time windows and accompanying exposures, (ii) let c_{maxmin} be the largest minimum concentration across all windows, (iii) any window whose maximum concentration is lower than c_{maxmin} will have lower concentration for any age within the window. As such, damage will be lower for any age and thus this cannot provide the worst case margin of safety. The window which has c_{maxmin} as its minimum is a leading candidate for the worst case exposure, but is not guaranteed. The benefits of this initial thinning of time windows can be seen in the SI (Fig. S2), and are maximised when the exposure profile contains one large, sustained pulse, with minimal exposure either side. This approach will require adaptation in cases where maternal transfer is significant.

Typically, chronic bioassays begin with embryos or young juveniles whose parents were acclimated to the conditions but not exposed to the toxicant. Thus models for calibration and validation can safely assume zero stress as an initial condition. However, in the real world embryos may be produced with some non-zero internal concentration of a potentially hazardous substance through maternal transfer. This is not just a theoretical concern, experiments with *D. magna* embryos have shown that maternal transfer can have serious adverse effects, even when transferred to clean water less than 24 h after birth (Palma et al., 2009; Kast-Hutcheson et al., 2001). The difficulty in assessing maternal transfer depends more on the TK equation being used than the choice of DEB model variant. Miller and Amrhein (Miller and Amrhein, 1995) showed a strong linear relationship between the concentrations of organochlorine pesticides and polychlorinated biphenyls in trout muscle and their eggs. If SIC is the driver of organism stress then the initial conditions for an embryo could readily be found from the SIC of the mother when the embryo was produced, provided estimates for the amount of the substance transferred from mother to offspring are accurate and reliable. However, this is not a universally applicable solution. The relationship between concentration in the mother and embryo may be non-linear (Fisk and Johnston, 1998), and in cases where scaled damage must be used the amount transferred is not so easy to quantify. Kast-Hutcheson et al. (2001) use a technique whereby they could compare effects on embryos developing in the mother versus those developing in culture media with an equivalent external concentration of the fungicide propiconazole. They found more severe effects in embryos which developed in the mother, suggesting that there is some biotransformation or adverse effect occurring within the mother that affects her offspring.

Environmental exposure sustained over multiple generations could alter the population's growth, fecundity and sensitivity to the substance. In some cases, this may strengthen the population as they adapt to the toxicant (Rix and Cutler, 2018). However, in other cases sensitivity increases over multiple generations (Guo et al., 2012; Kim et al., 2012), or there may be some trade-off, for example increased survival but reduced growth rates. These are changes to the (otherwise constant) DEB-TKTD parameters. These shifts are of more concern to short-lived species whose generations are also shorter. The current risk assessment framework does not consider these potential long-term effects and thus their potential should not necessarily be seen as an obstacle to the use of DEB-TKTD modelling. Rather, by understanding these changes as quantifiable changes to parameters, DEB-TKTD models offer the possibility of improving our understanding of them.

Moreover, experimental work can help quantify these changes. In most chronic studies embryos of the F_1 generation are maintained in the same conditions as their mothers. Under these conditions, one could begin by assuming zero initial damage and unchanged parameters, but the cause of any deviation of the data from the predictions could not be explained. An experiment could be designed to calibrate a DEB-TKTD model to the F_0 generation over its whole life. Embryos gathered from each treatment level would then be split randomly into two groups for the F_1 generation: one kept under the same concentration and the second transferred to clean media. Comparing observations for the survival, growth and development of the F_1 embryos exposed or in clean media can then be used to calibrate the initial damage level, keeping all parameter values from the F_0 generation. This quantifies a relationship for the amount of initial damage transferred from mother to embryo. Applying the same relationship to data from the embryos under continued exposure will give an indication of whether sensitivity has been altered between generations. The expectation is then to see emerging patterns across a variety of species and substances.

The desire for conservatism in regulatory environmental risk assessment schemes raises one further issue when attempting to quantify maternal transfer. Since most test species are iteroparous two embryos produced on the same day could be the offspring of two mothers of very different ages, sizes and thus levels of exposure and damage which will impact the initial condition and size of the embryo. These embryos will experience the same exposure throughout the remainder of their lives, but their growth and development may differ due to this initial difference.

We can estimate how long exposure to a toxicant remains relevant from the damage ODE (1). By considering only the elimination processes we can estimate the time taken to eliminate a given percentage of the damage caused. By setting this proportion to some value $(1-r)\approx 1$ we can estimate the time to (near) total recovery. Assuming that damage has never been above the NEC (i.e. the analytical solutions for $L(t)$ and $R_c(t)$ hold) the damage decreases over time according to:

$$D(t)=\begin{cases} D_0\left(\frac{L}{L_0}\right)^{-3X_G} e^{-k_d t} e^{-X_R F_{BV} K_{RV} R_c(t)} \text{ if } X_e = 0 \\ D_0\left(\frac{L}{L_0}\right)^{-3X_G-\frac{k_d}{f\dot{r}_B}} e^{-k_d t} e^{-X_R F_{BV} K_{RV} R_c(t)} \text{ if } X_e = 1 \end{cases} \quad (8)$$

where D_0 is the initial damage level and L_0 is the length at time (but not necessarily age) zero (see SI for the full derivation of the solution). Numerical solutions can replace Eq. (8) if growth or fecundity have been affected by stress. In the general case, numerical methods, such as the bisection method, must be used to find t corresponding to the damage level from Eq. (8). However, when all switches are off we have an analytical solution. The time to reach rD_0 for some small r is equivalent to the *time to equilibrium*, namely

$$t_r = -\frac{\ln(r)}{k_d}. \quad (9)$$

In this context, t_r can be thought of as the time until damage recovery or – more precisely – the time until only a fraction r of the initial damage remains. Note also that this result does not rely on the analytical solutions for $L(t)$ and $R(t)$ and thus holds for any value of D_0. Unless the organism is shrinking (in which case we assume it will also not be reproducing) this case is also the most conservative, that is, recovery will be slowest. There then exists an interval of potential ages which contains the worst-case maternal damage. For $r\approx 0$, Eq. (9) gives the upper bound of this interval. As a lower bound the mother must, of course, be old enough to reproduce, i.e. $t_a \geq t_p$ where t_a is the age of the mother and t_p is the age at puberty. This gives the interval

$$t_p \leq t_a \leq t_r \tag{10}$$

However, for particularly fast kinetics $t_r < t_p$ and the interval will collapse. Under these circumstances the maternal age is irrelevant to the scaled damage of the offspring since all mothers old enough to reproduce will have (approximately) the same scaled damage. In the most conservative case (9) gives a simple criterion for when maternal age plays a role in the damage of the offspring, namely

$$k_d > -\frac{\ln(r)}{t_p} \tag{11}$$

Determining the worst case maternal transfer – or at least an acceptable estimated level of damage – is not trivial. Simulating many different ages over the interval in Eq. (10) will add considerable computation time and in extreme cases risks pushing the question back one generation rather than solving it. In other words, we may need to answer the following question: what is the mother's worst case initial damage, and is it relevant for its offspring?

6. Discussion

The development of DEB theory has become fragmented over time, with different models designed with different focal species and applications in mind. While similar, these different variants all have nuanced differences in their assumptions, domains of applicability, strengths and weaknesses. As a result, it may be difficult and confusing for non-experts to assess which variant is best-suited for their problem, or to fully comprehend the implications of the implementation of a DEB based model. Using DEB to predict the lethal and sublethal effects of toxic exposure is one of the most promising applications of the theory. However, the current lack of direction is impeding progress.

In an attempt to unify research in this field, we have introduced the general theory behind DEB-TKTD modelling and the major model variants within DEB theory. The proposed naming structure provides a coherent scheme to facilitate communication of different DEB models, including any extensions or deviations from the standard form. The assumptions behind each of the major model variants have been reframed as a series of questions organised in (roughly) descending complexity. The result of this is a decision scheme which can provide modellers and non-modellers with a reference guide to make or review decisions on what level of complexity is required from a DEB-TKTD implementation.

The first comprehensive simplification of the standard DEB model was DEBkiss (Jager et al., 2013b). It has been shown that DEB and DEBkiss provide similar estimates for the growth and reproduction of an organism when calibrated to the same data (Jager and Klok, 2010). Our results in Section 4.3 provide analytical results on similarities and differences under constant conditions. These differences rapidly decrease as the role of the reserve becomes smaller ($g \to \infty$). The responses to the common pMoAs (Table 2) are always qualitatively similar and in some cases may be identical. Thus, both frameworks seem equally well suited to TKTD modelling for a single species at the individual level. Which framework is ultimately preferred will likely come down to the value of the simplicity gained by removing the reserve against the potential to expand to species or scenarios where the reserve may play an essential role. An additional benefit of comDEBkiss is that every parameter relates to a measurable quantity. It should therefore be possible to obtain reasonable parameter estimates directly from data prior to calibration. The reserve prevents the same being true for comDEB, as the parameter g cannot be measured directly and requires data at multiple feeding levels to estimate it reliably. If the reserve plays a significant role for the species one would expect to see systematic over-estimation of fecundity in a DEBkiss model based on measured (as opposed to calibrated) parameter values, since DEBkiss model organisms produce more offspring under ideal conditions (6).

To calibrate more complex models one must use ample empirical data for the given test species. In practice this would mean that each species (or strain) would have a set of reference values (or reference distributions in a Bayesian framework) for the physiological parameters. At least a subset of these would then need to be adjusted to better match the control data. Currently, the Add-my-pet database holds priDEB entries for more than 2000 species, with extensive data on many of the standard species used in ERA (Marques et al., 2018). Such data could be used to generate a database of reference parameter values for any DEB model variant, although the value of this (e.g. for cross species comparisons) may diminish if compound parameter variants are used. These data open the door to using primary parameter models in DEB-TKTD modelling without requiring vast quantities of data to be gathered in every experiment (Zimmer et al., 2018). However, using and reusing literature data also carries uncertainties and could lead to inconsistencies between the model and experimental data, since different species strains, laboratory conditions, handling stress and other factors can all introduce inter-experimental variability (Hickey et al., 2012). In many cases, using such external data will bring these additional hurdles without necessarily bringing any benefit.

The goal of TKTD modelling for ERA is not necessarily to characterise an entire species' life-cycle, but to most accurately describe and predict their response to toxic exposure. Under the simplest scenario, i.e. a substance whose (single) pMoA is reproductive, effects can be equally well-described by simplified models (either comDEB or comDEBkiss) without requiring external data. Although there are a great number of different model variants which may feasibly be applied in most cases, in practice the application is likely to encourage the use of the most general (priDEB) or the most concise (comDEBkiss) variant. The benefits of a model which requires zero external data speak for themselves. In scenarios where this is not possible, we believe that it will generally be simpler to maintain a database of parameters and literature data for a single model variant, in which case priDEB is the natural choice. This also reduces the number of variants (and the nuanced differences between them) with which non-modellers must be familiar.

Regardless of which model variant is used, a moving time-window is necessary to ensure that the risks of exposure to animals of all ages are considered for the whole predicted exposure profile (EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2018). However, to our knowledge the approach has not been comprehensively tested for sublethal effects. The hurdles to using this approach include how to interpret and determine the worst-case time-window and what metric should be used to measure the scale of effects. Furthermore, in some instances it may be necessary to consider transfer of damage from mother to offspring. There are two distinct challenges within this. Firstly, how does one transfer a measured or estimated amount of damage from the mother to the embryo? This is further complicated by the fact that damage to embryos often has more profound physiological effects (Kast-Hutcheson et al., 2001) which are not well expressed by DEB-TKTD models. Secondly, the worst-case transfer must be found. Considerable computational work must be added unless some logical or mathematical argument can be made to identify or approximate this value. However, such problems do not highlight flaws or shortcomings within DEB-TKTD models. Rather, they demonstrate the potential power of modelling to quantify such potential dangers to animals beyond what is feasible to assess in a laboratory.

DEB-TKTD models seem poised to become an important tool in ERA. However, there is currently no consensus as to which variant of the physiological model is best suited to the demands and data availability. We have illustrated how the many different DEB model variants fall into a hierarchy of complexities and capabilities. Under the conditions where they can be used simplified variants are equivalent to the more complex alternatives and avoid many of the challenges. For ERA, the greatest value may lie in supporting two approaches and two model

variants, each representing one end of the flowchart in Fig. 3. priDEB should be used in cases where the complexity is required and the existing validated physiological model is built on a wealth of high quality data, while comDEB(kiss) can be used when these data are not needed or (in many scenarios) when they do not exist. This flexibility maximises the opportunities to use DEB-TKTD models in ERA. The narrative of the decision making process should not frame priDEB as the "true model" and all simplifications as deviations from fact. Rather, DEB theory is itself a simplification of reality, the decisions made around simplifications or extensions should aim to strike the best balance between realism, complexity and the users' needs.

CRediT authorship contribution statement

Neil Sherborne: Conceptualization, Methodology, Software, Writing - original draft. **Nika Galic:** Conceptualization, Writing - review & editing. **Roman Ashauer:** Conceptualization, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.141027.

References

Álvarez, O.A., Jager, T., Kooijman, S.A.L.M., Kammenga, J.E., 2005. Responses to stress of Caenorhabditis elegans populations with different reproductive strategies. Funct. Ecol. 19, 656–664.

Álvarez, O.A., Jager, T., Redondo, E.M., Kammenga, J.E., 2006. Physiological modes of action of toxic chemicals in the nematode Acrobeloides nanus. Environ. Toxicol. Chem. 25, 3230–3237.

Ashauer, R., Jager, T., 2018. Physiological modes of action across species and toxicants: the key to predictive ecotoxicology. Environ. Sci. Process. Impacts 20, 48–57.

Ashauer, R., Agatz, A., Albert, C., Ducrot, V., Galic, N., Hendriks, J., Jager, T., Kretschmann, A., O'Connor, I., Rubach, M.N., et al., 2011. Toxicokinetic-toxicodynamic modeling of quantal and graded sublethal endpoints: a brief discussion of concepts. Environ. Toxicol. Chem. 30, 2519–2524.

Ashauer, R., Thorbek, P., Warinton, J.S., Wheeler, J.R., Maund, S., 2013. A method to predict and understand fish survival under dynamic chemical stress using standard ecotoxicity data. Environ. Toxicol. Chem. 32, 954–965.

Augustine, S., Litvak, M.K., Kooijman, S.A.L.M., 2011. Stochastic feeding of fish larvae and their metabolic handling of starvation. J. Sea Res. 66 (4), 411–418.

Baas, J., Jager, T., Kooijman, B., 2010a. A review of DEB theory in assessing toxic effects of mixtures. Sci. Total Environ. 408, 3740–3745.

Baas, J., Jager, T., Kooijman, S.A.L.M., 2010b. Understanding toxicity as processes in time. Sci. Total Environ. 408, 3735–3739.

Baas, J., Augustine, S., Marques, G.M., Dorne, J.-L., 2018. Dynamic energy budget models in ecological risk assessment: from principles to applications. Sci. Total Environ. 628, 249–260.

Barry, M.J., 1996. Effects of an Organochlorine pesticide on different levels of biological Organization in Daphnia. Ecotoxicol. Environ. Saf. 34, 239–251.

Bednarska, A.J., Edwards, P., Sibly, R., Thorbek, P., 2013. A toxicokinetic model for thiamethoxam in rats: implications for higher-tier risk assessment. Ecotoxicology 22, 548–557.

Bell, W.J., 1971. Starvation-induced oöcyte resorption and yolk protein salvage in Periplaneta americana. J. Insect Physiol. 17, 1099–1111.

Billoir, E., Delignette-Muller, M.L., Péry, A.R.R., Geffard, O., Charles, S., 2008. Statistical cautions when estimating DEBtox parameters. J. Theor. Biol. 254, 55–64.

Bradley, M.C., Perrin, N., Calow, P., 1991. Energy allocation in the cladoceran Daphnia magna Straus, under starvation and refeeding. Oecologia 86, 414–418.

Bridges, C., 2000. Long-term effects of pesticide exposure at various life stages of the southern leopard frog (Rana sphenocephala). Arch. Environ. Contam. Toxicol. 39 (1), 91–96.

Carazo-Rojas, E., Pérez-Rojas, G., Pérez-Villanueva, M., Chinchilla-Soto, C., Chin-Pampillo, J.S., Aguilar-Mora, P., Alpízar-Marín, M., Masís-Mora, M., Rodríguez-Rodríguez, C.E., Vryzas, Z.J.E.p., 2018. Pesticide monitoring and ecotoxicological risk assessment in surface water bodies and sediments of a tropical agro-ecosystem. Environ. Pollut. 241, 800–809.

Chadwick, W., Little, T.J., 2005. A parasite-mediated life-history shift in Daphnia magna. Proc. R. Soc. B 272, 505–509.

Coors, A., Hammers-Wirtz, M., Ratte, H.T., 2004. Adaptation to environmental stress in Daphnia magna simultaneously exposed to a xenobiotic. Chemosphere 56, 395–404.

Corriero, A., Zupa, R., Bello, G., Mylonas, C.C., Deflorio, M., Genovese, S., Basilone, G., Buscaino, G., Buffa, G., Pousis, C., et al., 2011. Evidence that severe acute stress and starvation induce rapid atresia of ovarian vitellogenic follicles in Atlantic bluefin tuna, Thunnus thynnus (L.)(Osteichthyes: Scombridae). J. Fish Dis. 34, 853–860.

Costantini, D., Metcalfe, N.B., Monaghan, P., 2010. Ecological processes in a hormetic framework. Ecol. Lett. 13 (11), 1435–1447.

Crane, M., Newman, M.C., 2000. What level of effect is a no observed effect? Environ. Toxicol. Chem. 19, 516–519.

Dhillon, R.S., Fox, M.G., 2004. Growth-independent effects of temperature on age and size at maturity in Japanese medaka (Oryzias latipes). Copeia 2004, 37–45.

Dudycha, J.L., 2003. A multi-environment comparison of senescence between sister species of Daphnia. Oecologia 135, 555–563.

Ebert, D., 1992. A food-independent maturation threshold and size at maturity in Daphnia magna. Limnol. Oceanogr. 37, 878–881.

EFSA, 2010. Panel on Plant Protection Products and their Residues (PPR), Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). EFSA J. 8 (10), 1821.

EFSA Panel on Plant Protection Products and their Residues (PPR), Ockleford, C.A.P., Berny, P., Brock, T., Duquesne, S., Grilli, S., Hernandez-Jerez, A.F., Bennekou, S.H., Klein, M., et al., 2018. Scientific opinion on the state of the art of toxicokinetic/toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms. EFSA J. 16, e05377.

EPA, 1996. Ecological Effects Test Guidelines OPPTS 850.1350 Mysid Chronic Toxicity Test.

Fisk, A.T., Johnston, T.A., 1998. Maternal transfer of organochlorines to eggs of walleye (Stizostedion vitreum) in Lake Manitoba and western Lake Superior. J. Great Lakes Res. 24 (4), 917–928.

Forbes, V.E., Galic, N., 2016. Next-generation ecological risk assessment: predicting risk from molecular initiation to ecosystem service delivery. Environ. Int. 91, 215–219.

Fox, D.R., Landis, W.G., 2016. Comment on ET&C perspectives, November 2015—a holistic view. Environ. Toxicol. Chem. 35, 1337–1339.

Furlong, M.J., Groden, E., 2003. Starvation induced stress and the susceptibility of the Colorado potato beetle, Leptinotarsa decemlineata, to infection by Beauveria bassiana. J. Invertebr. Pathol. 83, 127–138.

Galic, N., Hommen, U., Baveco, J.M., Van Den Brink, P., 2010. Potential application of population models in the European ecological risk assessment of chemicals II: review of models and their potential to address environmental protection aims. Integr. Environ. Assess. Manag. 6.

Gergs, A., Rakel, K.J., Liesy, D., Zenker, A., Classen, S., 2019. Mechanistic effect modeling approach for the extrapolation of species sensitivity. Environmental science & technology 53, 9818–9825.

Glazier, D.S., 1992. Effects of food, genotype, and maternal size and age on offspring investment in Daphnia magna. Ecology 73, 910–926.

Glazier, D.S., Calow, P., 1992. Energy allocation rules in Daphnia magna: clonal and age differences in the effects of food limitation. Oecologia 90, 540–549.

Guisande, C., Gliwicz, Z.M., 1992. Egg size and clutch size in two Daphnia species grown at different food levels. J. Plankton Res. 14, 997–1007.

Guo, R., Ren, X., Ren, H., 2012. Effects of dimethoate on rotifer Brachionus calyciflorus using multigeneration toxicity tests. J. Environ. Sci. Health B 47, 883–890.

Gurney, W.S.C., Nisbet, R.M., 2004. Resource allocation, hyperphagia and compensatory growth. Bull. Math. Biol. 66, 1731–1753.

Hall, S.R., Becker, C., Cáceres, C.E., 2007. Parasitic castration: a perspective from a model of dynamic energy budgets. Integr. Comp. Biol. 47 (2), 295–309.

Hammond, J.I., Jones, D.K., Stephens, P.R., Relyea, R.A., 2012. Phylogeny meets ecotoxicology: evolutionary patterns of sensitivity to a common insecticide. Evol. Appl. 5 (6), 593–606.

Hickey, G.L., Craig, P.S., Luttik, R., Zwart, D., 2012. On the quantification of intertest variability in ecotoxicity data with application to species sensitivity distributions. Environ. Toxicol. Chem. 31, 1903–1910.

Holling, C.S., 1959. Some characteristics of simple types of predation and parasitism. The Canadian Entomologist 91, 385–398.

Huusko, A., Mäki-Petäys, A., Stickler, M., Mykrä, H., 2011. Fish can shrink under harsh living conditions. Funct. Ecol. 25, 628–633.

Jager, T., 2011. Some good reasons to ban ECx and related concepts in ecotoxicology. Environ. Sci. Technol. 45, 8180–8181.

Jager, T., *DEBkiss: A simple framework for animal energy budgets*. Leanpub: https://leanpub.com/debkiss_book, version 2.0: 2018.

Jager, T., Making sense of chemical stress application of dynamic energy budget theory in ecotoxicology and stress ecology. Leanpub: https://leanpub.com/debtox_book, version 2.0: 2019.

Jager, T., 2020. Revisiting simplified DEBtox models for analysing ecotoxicity data. Ecol. Model. 416, 108904.
Jager, T., Klok, C., 2010. Extrapolating toxic effects on individuals to the population level: the role of dynamic energy budgets. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 365, 3531–3540.
Jager, T., Zimmer, E.I., 2012. Simplified dynamic energy budget model for analysing ecotoxicity data. Ecol. Model. 225, 74–81.
Jager, T., Albert, C., Preuss, T.G., Ashauer, R., 2011. General unified threshold model of survival - a toxicokinetic-toxicodynamic framework for ecotoxicology. Environ. Sci. Technol. 45, 2529–2540.
Jager, T., Barsi, A., Ducrot, V., 2013a. Hormesis on life-history traits: is there such thing as a free lunch? Ecotoxicology 22 (2), 263–270.
Jager, T., Martin, B.T., Zimmer, E.I., 2013b. DEBkiss or the quest for the simplest generic model of animal life history. J. Theor. Biol. 328, 9–18.
Jager, T., Gudmundsdóttir, E.M., Cedergreen, N., 2014. Dynamic modeling of sublethal mixture toxicity in the nematode Caenorhabditis elegans. Environ. Sci. Technol. 48 (12), 7026–7033.
Jusup, M., Sousa, T., Domingos, T., Labinac, V., Marn, N., Wang, Z., Klanjšček, T., 2017. Physics of metabolic organization. Phys Life Rev 20, 1–39.
Kast-Hutcheson, K., Rider, C.V., LeBlanc, G.A., 2001. The fungicide propiconazole interferes with embryonic development of the crustacean Daphnia magna. Environ. Toxicol. Chem. 20, 502–509.
Key, P.B., Fulton, M.H., Scott, G.I., Layman, S.L., Wirth, E.F., 1998. Lethal and sublethal effects of malathion on three life stages of the grass shrimp, Palaemonetes pugio. Aquat. Toxicol. 40 (4), 311–322.
Kim, H.Y., Lee, M.J., Yu, S.H., Kim, S.D., 2012. The individual and population effects of tetracycline on Daphnia magna in multigenerational exposure. Ecotoxicology 21, 993–1002.
Knöbel, M., Busser, F.J.M., Rico-Rico, Á., Kramer, N.I., Hermens, J.L.M., Hafner, C., Tanneberger, K., Schirmer, K., Scholz, S., 2012. Predicting adult fish acute lethality with the zebrafish embryo: relevance of test duration, endpoints, compound properties, and exposure concentration analysis. Environ. Sci. Technol. 46, 9690–9700.
Kooijman, S.A.L.M., 1996. An alternative for NOEC exists, but the standard model has to be abandoned first. Oikos 75, 310–316.
Kooijman, S.A.L.M., 2010. Dynamic Energy Budget Theory for Metabolic Organisation. Cambridge University Press.
Kooijman, S.A.L.M., 2014. Metabolic acceleration in animal ontogeny: an evolutionary perspective. J. Sea Res. 94, 128–137.
Kooijman, S.A.L.M., Bedaux, J.J.M., 1996. The Analysis of Aquatic Toxicity Data. VU University Press.
Kooijman, S.A.L.M., Metz, J.A.J., 1984. On the dynamics of chemically stressed populations: the deduction of population consequences from effects on individuals. Ecotoxicol. Environ. Saf. 8, 254–274.
Kooijman, S.A.L.M., Sousa, T., Pecquerie, L., Van der Meer, J., Jager, T., 2008. From food-dependent statistics to metabolic parameters, a practical guide to the use of dynamic energy budget theory. Biol. Rev. 83, 533–552.
Landis, W.G., Chapman, P.M., 2011. Well past time to stop using NOELs and LOELs. Integr. Environ. Assess. Manag. 7, vi–viii.
Laskowski, R., 1995. Some good reasons to ban the use of NOEC, LOEC and related concepts in ecotoxicology. Oikos 73, 140–144.
Lika, K., Kooijman, S.A.L.M., 2011. The comparative topology of energy allocation in budget models. J. Sea Res. 66, 381–391.
Lika, K., Augustine, S., Pecquerie, L., Kooijman, S.A.L.M., 2014. The bijection from data to parameter space with the standard DEB model quantifies the supply–demand spectrum. J. Theor. Biol. 354, 35–47.
Liu, C., Sibly, R.M., Grimm, V., Thorbek, P., 2013. Linking pesticide exposure and spatial dynamics: an individual-based model of wood mouse (Apodemus sylvaticus) populations in agricultural landscapes. Ecol. Model. 248, 92–102.
Marques, G.M., Augustine, S., Lika, K., Pecquerie, L., Domingos, T., Kooijman, S.A.L.M., 2018. The AmP project: comparing species on the basis of dynamic energy budget parameters. PLoS Comput. Biol. 14, e1006100.
Martin, B.T., Zimmer, E.I., Grimm, V., Jager, T., 2012. Dynamic energy budget theory meets individual-based modelling: a generic and accessible implementation. Methods Ecol. Evol. 3, 445–449.
Martin, B., Jager, T., Nisbet, R.M., Preuss, T.G., Grimm, V., 2014. Limitations of extrapolating toxic effects on reproduction to the population level. Ecol. Appl. 24, 1972–1983.
Martin, T., Thompson, H., Thorbek, P., Ashauer, R., 2019. Toxicokinetic—toxicodynamic modeling of the effects of pesticides on growth of Rattus norvegicus. Chem. Res. Toxicol. 32, 2281–2294.
Metcalfe, N.B., Monaghan, P., 2001. Compensation for a bad start: grow now, pay later? Trends Ecol. Evol. 16, 254–260.
Miller, M.A., Amrhein, J.F., 1995. Maternal transfer of organochlorine compounds in Lake Superior siscowet (Salvelinus namaycush siscowet) to their eggs. Bull. Environ. Contam. Toxicol. 55, 96–103.
Murphy, C.A., Nisbet, R.M., Antczak, P., Garcia-Reyero, N., Gergs, A., Lika, K., Mathews, T., Muller, E.B., Nacci, D., Peace, A., et al., 2018. Linking adverse outcome pathways to dynamic energy budgets: A conceptual model. A Systems Biology Approach to Advancing Adverse Outcome Pathways for Risk Assessment. Springer, pp. 281–302.
Nichols, J.W., Bonnell, M., Dimitrov, S.D., Escher, B.I., Han, X., Kramer, N.I., 2009. Bioaccumulation assessment using predictive approaches. Integr. Environ. Assess. Manag. 5 (4), 577–597.
Nisbet, R.M., Muller, E.B., Lika, K., Kooijman, S.A.L.M., 2000. From molecules to ecosystems through dynamic energy budget models. J. Anim. Ecol. 69, 913–926.
Palma, P., Palma, V.L., Fernandes, R.M., Bohn, A., Soares, A.M.V.M., Barbosa, I.R., 2009. Embryo-toxic effects of environmental concentrations of chlorpyrifos on the crustacean Daphnia magna. Ecotoxicol. Environ. Saf. 72, 1714–1718.
Pecquerie, L., Petitgas, P., Kooijman, S.A.L.M., 2009. Modeling fish growth and reproduction in the context of the Dynamic Energy Budget theory to predict environmental impact on anchovy spawning duration. J. Sea Res. 62, 93–105.
Rix, R.R., Cutler, G.C., 2018. Does multigenerational exposure to hormetic concentrations of imidacloprid precondition aphids for increased insecticide tolerance? Pest Manag. Sci. 74, 314–322.
Sadoul, B., Augustine, S., Zimmer, E., Bégout, M.-L., Vijayan, M.M., 2019. Prediction of long-term variation in offspring metabolism due to BPA in eggs in rainbow trout using the DEB model. J. Sea Res. 143, 222–230.
Sherborne, N., Galic, N., 2020. Modelling sublethal effects of chemicals: application of a simplified dynamic energy budget model to standard ecotoxicity data. Environ. Sci. Technol. 54 (12), 7420–7429.
Spycher, S., Mangold, S., Doppler, T., Junghans, M., Wittmer, I., Stamm, C., Singer, H., 2018. Pesticide risks in small streams—how to get as close as possible to the stress imposed on aquatic organisms. Environ. Sci. Technol. 52 (8), 4526–4535.
Thomas, P.G., Ikeda, T., 1987. Sexual regression, shrinkage, re-maturation and growth of spent female Euphausia superba in the laboratory. Mar. Biol. 95, 357–363.
Thorbek, P., Forbes, V., Heimbach, F., Hommen, U., Thulke, H.H., Van Den Brink, P., Wogram, J., Grimm, V. (Eds.), 2009. Ecological Models for Regulatory Risk Assessments of Pesticides: Developing a Strategy for the Future. Society for Environmental Toxicology and Chemistry and CRC Press.
Tolla, C., Kooijman, S.A.L.M., Poggiale, J.-C., 2007. A kinetic inhibition mechanism for maintenance. J. Theor. Biol. 244, 576–587.
Wiegand, C., Krause, E., Steinberg, C., Pflugmacher, S., 2001. Toxicokinetics of atrazine in embryos of the zebrafish (Danio rerio). Ecotoxicol. Environ. Saf. 49, 199–205.
Zimmer, E.I., Preuss, T.G., Norman, S., Minten, B., Ducrot, V., 2018. Modelling effects of time-variable exposure to the pyrethroid beta-cyfluthrin on rainbow trout early life stages. Environ. Sci. Eur. 30, 36.