Technical background document for:

Making Sense of Chemical Stress

Applications of Dynamic Energy Budget theory in ecotoxicology and stress ecology[1]

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[1]Version: Beta 1.2. This book is still in its beta test version. Make sure to check http://www.debtox.info/book.html for the latest version, as well as a link to the main book.

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Preface

About this book

This book is the technical document that belongs to the e-book "Making sense of chemical stress", which can be found on http://www.debtox.info/book.html. This document contains the equations for the DEB models discussed in the main book, including their derivation and alternative formulations for some modules. The first version of this book was in 2012, and I expected to update it regularly. However, it took me 2.5 years to come up with a small update. The main reason is that I started with the DEBkiss simplification (which removes the reserve compartment of standard DEB), which keeps me pretty busy. The e-book on DEBkiss can be found here: http://www.debtox.info/book_debkiss.html. However, I might return to standard DEB and DEBtox, and update this document again; check the website to see if there is a newer version.

This book does not provide a lot of background or rationale for the equations and the writing style is pretty sloppy, so there no point in reading it from cover to cover. It is best to use it as a reference manual (it also serves as a reference for me). The chapters in this book follow the chapters in the main text.

Disclaimer

This document *will* contain errors, which is inevitable given the sheer number of equations. If you spot one, please let me know so that I can include corrections in updates of this file. I do not accept liability or responsibility for any damage or costs incurred as a result of these errors.

Most of the presented 'model alternatives' are not well tested yet, but they can form the starting point for dedicated research.

Notation

In this book, I follow as strictly as possible the notation used in the DEB book of Kooijman [15] (see also http://www.bio.vu.nl/thb/research/bib/Kooy2010_n.pdf). This notation takes some getting used to, and deviates from common use of symbols in ecotoxicology and biology. This is caused by the fact that DEB theory covers so many fields, each with conflicting use of notation, that it is impossible to use a single unique notation that everybody is familiar with. Still, even this dedicated notation runs into consistency problems here and there (mainly with the subscripts). I follow the same notation to ensure that this book can be read next to the DEB book [15].

Some conventions:

- Parameters or variables indicated by the same leading symbol have the same dimensions. For example, L, L_m and L_w are all in body length (dimension of 'length of organism', L).

- A dot above a symbol specifies that this parameter is a rate, with a dimension that includes time^{-1}. This dot should *not* be read as the symbol representing a differential quotient (which will always be specified with a $\frac{d}{dt}$).

- Quantities that are expressed per unit of structural volume are put between square braces; quantities per unit of surface area are between curly braces. For example, $[\dot{p}_M] = \dot{p}_M / L^3$.

Common leading symbols are:

c	external or scaled internal concentration
e	scaled energy in reserve
E	energy in reserve
J	fluxes of compound
$\dot{k}$	specific rate constant
L	body length
M	mass (in moles)
$\dot{p}$	energy flux (power)
P	partition coefficient (volume based)
U	scaled reserve
V	body volume

Common indices are:

0	no effect, or the freshly laid egg
A	assimilation
b	birth (start of feeding)
C	mobilisation
d	dissolved (concentrations) or dry (weight)
E	reserve
H	maturity
J	maturity maintenance
m	maximum
M	somatic maintenance
p	puberty (start investment reproduction)
Q	toxicant
R	reproduction buffer
V	structure
w	physical (volume), wet (weight) or water
X	food

For the dimensions, I use # for countable numbers (e.g., moles of a compound or eggs), e for energy, t for time, l for length of environment (l^3 is an environmental volume), L for length of an organism, and m for mass (weight).

Many of the symbols that I use in this file are in the table below. This table is certainly not complete, but the equations in the rest of this document should be readable without referring to this table. In future versions of this file, I will try to get this table more complete.

Symbol	Description	Dimensions
a_b	age at birth (time after egg development started)	t
a_p	age at puberty (time after egg development started)	t
$\dot{b}_\dagger$	killing rate for toxicants	$l^3\#^{-1}t^{-1}$
c_0	scaled no-effect concentration for sub-lethal effects	$\#l^{-3}$
$c_{0\dagger}$	scaled no-effect concentration for lethal effects	$\#l^{-3}$
c_d	dissolved concentration of toxicant in the environment	$\#l^{-3}$
c_T	scaled tolerance concentration for sub-lethal effects	$\#l^{-3}$
c_V	scaled internal toxicant concentration in structure	$\#l^{-3}$
d_{Vd}	dry weight density of structure	mL^{-3}
d_{Vw}	wet weight density of structure	mL^{-3}
e	scaled energy density in reserve: E/E_m	$-$
e_0	initial scaled energy density in an egg: E_0/E_m	$-$
e_R	scaled energy density in reproduction buffer: E_R/E_m	$-$
E	energy in the reserve	e
E_H	accumulated energy investment in maturation	e
E_H^b	maturation threshold for birth (start of feeding)	e
E_H^p	maturation threshold for puberty (start investment reproduction)	e
E_m	maximum energy in the reserve	e
E_R	energy in the reproduction buffer	e
E_0	initial energy in the egg	e
$[E_G]$	volume-specific energy costs per unit of structe	eL^{-3}
$[E_m]$	maximum reserve density	eL^{-3}
f	scaled functional response	$-$
$\{\dot{F}_m\}$	maximum surface-area-specific searching rate	$l^3L^{-2}t^{-1}$

Symbol	Description	Dimensions
g	energy investment ratio	$-$
$\dot{h}$	hazard rate (instantaneous probability to die)	t^{-1}
$\dot{h}_0$	hazard rate for 'accidents' in control (background mortality)	t^{-1}
$\dot{h}_D$	hazard rate due to damage from ageing	t^{-1}
$\dot{h}_Q$	hazard rate due to toxicant exposure	t^{-1}
$\ddot{h}_a$	Weibull ageing acceleration	t^{-2}
$\dot{J}_{XA}$	ingestion rate of food	$\#t^{-1}$
$\{\dot{J}_{XAm}\}$	maximum surface-area-specific ingestion rate	$\#t^{-1}L^{-2}$
k	maintenance ratio $\dot{k}_J/\dot{k}_M$	$-$
$\dot{k}_e$	elimination (or dominant) rate constant	t^{-1}
$\dot{k}_e^{\mathrm{ref}}$	reference elimination rate constant	t^{-1}
$\dot{k}_J$	maturity maintenance rate coefficient	t^{-1}
$\dot{k}_M$	somatic maintenance rate coefficient	t^{-1}
L	structural length of organism	L
L_m	maximum structural length of organism	L
L_{m0}	maximum structural length of organism in control	L
L_w	physical length of organism (L/δ_M)	L
M_Q	total amount of chemical in organism (in moles)	$\#$
ΔM_Q	change in amount of chemical at spawning (in moles)	$\#$
$[M_Q]$	internal concentration in organism (in moles)	$\#L^{-3}$
M_Q^0	initial amount of chemical in an egg (in moles)	$\#$
$[M_Q^0]$	initial internal concentration in an egg (in moles)	$\#L^{-3}$
M_{QR}	amount of chemical in reproduction buffer (in moles)	$\#$
M_{QV}	amount of chemical in structure (in moles)	$\#$
$[M_V]$	number of C-atoms per unit structural volume	$\#L^{-3}$
$\dot{p_A}$	energy flux (power) of assimilation	et^{-1}
$\{\dot{p}_{Am}\}$	maximum surface-area-specific assimilation rate	$et^{-1}L^{-2}$
$\dot{p_C}$	energy flux (power) of mobilisation	et^{-1}
$\dot{p_J}$	energy flux (power) of maturity maintenance	et^{-1}
$\dot{p_M}$	energy flux (power) of somatic maintenance	et^{-1}
$[\dot{p_M}]$	volume-specific costs for somatic maintenance	$et^{-1}L^{-2}$
P_{EV}	partition coefficient between reserve and structure	L^3L^{-3}
P_{ow}	partition coefficient between octanol and water	l^3l^{-3}
P_{Vd}	partition coefficient between structure and water	l^3L^{-3}
P_{Wd}	partition coefficient between total body and water	l^3L^{-3}
$\ddot{q}$	scaled level of damage inducing compounds	t^{-2}
$\dot{r}$	specific volumetric growth rate	t^{-1}
$\dot{R}$	reproduction rate	$\#t^{-1}$
ΔR	number of eggs in a spawning event	$\#$
s_G	Gompertz stress coefficient	$-$
S	survival probability	$-$
S_C	scaled mobilisation from the reserves	L^2
t_p	handling time for a food item	t
t_R	time for a spawning event	t
u_H^{bp}	ratio of maturity thresholds for birth and puberty	$-$
U_E	scaled reserve level ($E/\{\dot{p}_{Am}\}$)	tL^2

Symbol	Description	Dimensions
U_E^0	initial scaled reserve in egg	tL^2
U_H	scaled energy investment in maturition	tL^2
U_H^b	scaled maturity threshold for birth	tL^2
U_H^p	scaled maturity threshold for puberty	tL^2
U_R	scaled reproduction buffer	tL^2
$[U_{Rm}]$	volume-specific maximum scaled reproduction buffer	tL^{-1}
$\dot{v}$	energy conductance	Lt^{-1}
V	structural body volume of organism (L^3)	L^3
V_w	physical body volume of organism (L^3)	L^3
X	food density in the environment	$\#l^{-3}$
δ_M	shape coefficient	$-$
κ	allocation fraction to growth and maintenance	$-$
κ_R	fraction of allocated energy fixed in eggs	$-$
μ_{AX}	energy-mass coupler for assimilation from food	$e\#^{-1}$
ω_d	contribution of reserve to dry weight	$-$
ω_V	contribution of reserve to physical volume	$-$
ω_w	contribution of reserve to wet weight	$-$

Chapter 1

Practical energy budget models

In this chapter, I will present the sets of equations for DEB models that are practical to use in ecotoxicology. Here, they are presented without their derivation and without alternative sub-models (which will be provided in the next chapters). In this way, it is immediately clear what the basic models are without having to wade through pages of derivation.

1.1 The scaled standard model

The basic state variables

The change in scaled reserve is given by ($t = 0$ indicates the start of embryonic development):

$$\frac{d}{dt}U_E = \begin{cases} -S_C & \text{if } U_H < U_H^b \\ fL^2 - S_C & \text{otherwise} \end{cases} \quad \text{with } U_E(0) = U_E^0 \tag{1.1}$$

The scaled reserve density is between 0 and 1 and is defined as:

$$e = \dot{v}\frac{U_E}{L^3} \tag{1.2}$$

The scaled mobilisation power is calculated as:

$$S_C = L^2\frac{ge}{g+e}\left(1 + \frac{\dot{k}_M}{\dot{v}}L\right) \tag{1.3}$$

The equation for change in structural length:

$$\frac{d}{dt}L = \frac{1}{3L^2}\left(\frac{\dot{v}}{g}S_C - \dot{k}_M L^3\right) \quad \text{with } L(0) \approx 0 \tag{1.4}$$

The change in scaled maturity is given by:

$$\frac{d}{dt}U_H = \begin{cases} \kappa_H((1-\kappa)S_C - \dot{k}_J U_H) & \text{if } U_H < U_H^p \\ 0 & \text{otherwise} \end{cases} \quad \text{with } U_H(0) = 0 \tag{1.5}$$

Note that $U_H(a_b) = U_H^b$ and $U_H(a_p) = U_H^p$. The energy flux into the reproductive buffer is given by:

$$\frac{d}{dt}U_R = \begin{cases} 0 & \text{if } U_H < U_H^p \\ (1-\kappa)S_C - \dot{k}_J U_H^p & \text{otherwise} \end{cases} \quad \text{with } U_R(0) = 0 \tag{1.6}$$

Two compound parameters can be used to facilitate parametrisation: the ratio of the two rate constants for maintenance (with a default value of 1), and the ratio of the maturity at birth and at puberty:

$$k = \frac{\dot{k}_J}{\dot{k}_M} \quad \text{and} \quad u_H^{bp} = \frac{U_H^b}{U_H^p} \tag{1.7}$$

Reproductive energy to eggs

Ignoring the buffer, the mean reproduction rate is given by:

$$\dot{R} = \frac{\kappa_R}{U_E^0} \frac{d}{dt} U_R \tag{1.8}$$

If we do consider the buffer, than at spawning events, the buffer is converted into a batch of eggs (ΔR) as follows:

$$\Delta R = \text{floor}\left(\frac{\kappa_R U_R}{U_E^0}\right) \quad \text{if } U_R = [U_{Rm}]L^3 \text{ or } t = t_R \text{ or } ... \tag{1.9}$$

The 'floor' function means to round off to the nearest integer below the value in brackets. This is needed as only whole eggs can be produced. The conditions for spawning is highly species specific; here I suggest the possibility for a threshold on the size-dependent filling of the reproduction buffer $[U_{Rm}]L^3$ or a specific time t_R

If the remaining energy is kept in the buffer, U_R is reset to:

$$U_R = U_R - \frac{U_E^0 \Delta R}{\kappa_R} \tag{1.10}$$

The scaled initial reserves in the egg U_E^0 is not a free parameter in the standard DEB model. The initial reserves must be such that the embryo will hatch at the same reserve density as its mother had at egg formation. We can simulate the embryonic development with different values of U_E^0 until we observe the correct reserve density at hatching, or use dedicated procedure (see [14]).

Alternative rule for egg costs and hatching

As an alternative, we can take the scaled initial reserves in the egg U_E^0 as a free parameter (which removes U_H^b as a parameter), and assume hatching occurs when the reserve density reaches a threshold (e.g., $e = 1$). This is less calculation intensive than the standard rule, and has the advantage that it allows more freedom for the mother to adapt investment per offspring, or size of offspring, in response to environmental conditions or toxicant stress.

Starvation response

Starvation occurs when the somatic maintenance costs cannot be paid anymore:

$$e < \frac{L}{L_m} \quad \text{where} \quad L_m = \frac{\dot{v}}{\dot{k}_M g} \tag{1.11}$$

Some possible scenatios:

Option 1: Do nothing It is possible to let the system of equations of the standard model run with no modification, even under starvation. This implies that structural body size will shrink, and for juveniles the maturity level, and for adults the reproduction buffer. The unrealistic part of this strategy is that it assumes that the energy used to make a unit of structure, maturation or buffer is completely returned when it is being used to pay maintenance costs (this can be corrected by adding a parameter).

Option 2: Use the reproduction buffer Another option is to fix body size to the point where starvation started, and take the energy that is short on the somatic maintenance requirements from the reproduction buffer (or the flow of energy into maturation or reproduction). The mobilisation under constant structural size is:

$$S_C = eL^2 \tag{1.12}$$

The ODE for the reproduction buffer is changed to:

$$\frac{d}{dt}U_R = (1-\kappa)S_C - \dot{k}_J U_H^p - \kappa L^2\left(\frac{L}{L_m} - e\right) \tag{1.13}$$

This can continue until the reproduction buffer is exhausted and $\frac{d}{dt}U_R$ becomes negative.

Option 3: Only mobilise for somatic maintenance The last option is to only mobilise enough energy from the reserves to fulfil the maintenance needs. This implies that:

$$\frac{d}{dt}L = 0, \frac{d}{dt}U_H = 0, \frac{d}{dt}U_R = 0 \tag{1.14}$$

The mobilisation from the reserves is recalculated to the minimal flux to pay somatic maintenance:

$$S_C = \frac{\dot{k}_M \kappa g}{\dot{v}} L^3 = \kappa \frac{L^3}{L_m} \tag{1.15}$$

This recalculated flux might be less or more than what can be maximally mobilised under normal conditions, which is eL^2. This scenario can continue until the reserves are fully depleted. However, you might also decide that no more reserves can be mobilised than the maximum under normal conditions, so to include the condition $S_C \leq eL^2$.

1.2 Simplified standard model

The reserve dynamics is (note that $t = 0$ now indicates the start of the experiment):

$$\frac{d}{dt}e = (f-e)\frac{\dot{v}}{L} \quad \text{with } e(0) = 1 \tag{1.16}$$

For structural length, the equation is:

$$\frac{d}{dt}L = \frac{\dot{k}_M g}{3(e+g)}\left(e\frac{\dot{v}}{\dot{k}_M g} - L\right) \quad \text{with } L(0) = L_0 \tag{1.17}$$

We can use the same relationships for the link with physical size measures as for the scaled standard model (see Section 1.4). However, we would need to add an additional parameter

for the contribution of the buffer to volume or weight, as the relationship between offspring number and their energy or mass content is lost in the simplification.

For the reproduction rate we use:

$$\dot{R} = \begin{cases} 0 & \text{if } L < L_p \\ \frac{\dot{R}_{m0}}{L_{m0}^3 - L_p^3} \left(\left(\frac{\dot{v}}{\dot{k}_M} L^2 + L^3 \right) \frac{e}{e+g} - L_p^3 \right) & \text{otherwise} \end{cases} \tag{1.18}$$

It is possible to simulate a reproduction buffer by collecting $\dot{R}$, and emptying it (in discrete offspring) during spawning events.

Instead of $\dot{k}_M$ and $\dot{v}$, we can use the more intuitive maximum length (in the control at maximum food) and Von Bertalanffy growth rate constant (in the control at maximum food) as our parameters. Therefore, if g_0 is given:

$$\dot{k}_{M0} = \dot{r}_{B0} \frac{3(1+g_0)}{g_0} \quad \text{and} \quad \dot{v} = L_{m0} \dot{k}_{M0} g_0 \tag{1.19}$$

1.3 Mortality

In a DEB context, mortality is treated as a chance process. Chance processes in time are modelled using the hazard rate, $\dot{h}$. If we know the hazard rate as function of time, we can calculate the survival probability over time as:

$$S(t) = \exp\left(- \int_0^t \dot{h}(\tau)\, d\tau \right) \tag{1.20}$$

The hazard rate can be increased due to the action of toxicants (as treated in the section on toxicodynamics below), but also due to old-age effects (discussed in Section 4.2, and not repeated here, as I do not know which model alternative is most appropriate).

1.4 Structural to physical size

As a first approach, it is possible to use actual length, cubic root of body weight or volume, or square root of body surface, as a proxy for structural length. This ignores the contributions of reserve and reproduction buffer to the length measure, which might be acceptable for actual body length, but generally not for weight, volume or surface area. Furthermore, parameters with length in their dimension are sensitive to how this length is determined. If the aim is to compare different species, it also makes sense to distinguish between structural length and actual length.

An actual (well-chosen) length measure can be related to structural length by the shape-correction coefficient:

$$L = \delta_M L_w \tag{1.21}$$

The physical volume is a weighted sum of structure, reserve and reproduction buffer:

$$V_w = L^3 + \omega_V (e + e_R) L^3 \quad \text{with} \quad e_R = \dot{v} \frac{U_R}{L^3} \tag{1.22}$$

The wet and dry weight of an organism are related to structural volume in a similar way, accounting for the density of the wet or dry structure (d_V and d_{Vd}):

$$W_w = d_V L^3 (1 + \omega_w(e + e_R)) \quad (1.23)$$
$$W_d = d_{Vd} L^3 (1 + \omega_d(e + e_R)) \quad (1.24)$$

1.5 Toxicokinetics

Here, I only present the models for scaled internal concentrations.

Basic model

The basic equation for the scaled internal concentration of a toxicant in a growing animal is:

$$\frac{d}{dt} c_V = \dot{k}_e^{\text{ref}} \frac{L_{m0}}{L} (c_d - c_V) - c_V \frac{3}{L} \frac{d}{dt} L \quad (1.25)$$

Where $\dot{k}_e^{\text{ref}}$ is the elimination rate in a fully-grown adult. For non-growing organisms, this equation simplifies to:

$$\frac{d}{dt} c_V = \dot{k}_e (c_d - c_V) \quad (1.26)$$

Extended model

In the scaled non-simplified DEB model, we can take into account changes in composition and the effects of the reproduction buffer. For the scaled internal concentration, we scale with the bioconcentration factor for structure P_{Vd}. The whole-body partition coefficient P_{Wd} can change over time. I introduce a new symbol for the scaled amount of toxicant in the body[1] as $m_Q = M_Q / P_{Vd}$:

$$\frac{d}{dt} m_Q = \dot{k}_e \left(c_d L^3 (1 + P_{EV}(e + e_R)\omega_V) - m_Q \right) \quad (1.27)$$
$$m_{QV} = \frac{m_Q}{P_{EV}(e + e_R)\omega_V + 1} \quad (1.28)$$
$$c_V = \frac{m_{QV}}{L^3} \quad (1.29)$$

When the elimination rate is determined by the structural surface area:

$$\dot{k}_e = \dot{k}_e^{\text{ref}} \frac{L_{m0}}{L} \frac{1 + \omega_V}{1 + P_{EV}(e + e_R)\omega_V} \quad (1.30)$$

When the area for exchange is determined by the physical volume, instead of the structural volume:

$$\dot{k}_e = \dot{k}_e^{\text{ref}} \frac{L_{m0}}{L} \left(\frac{1 + \omega_V}{1 + P_{EV}(e + e_R)\omega_V} \right)^{1/3} \quad (1.31)$$

When the reproduction buffer is emptied, the scaled internal mass of toxicant changes by:

[1] I use the symbol m different from the DEB book, where it is used as moles of compound relative to C-moles of structure.

$$\Delta m_Q = -\frac{e_0 \Delta R}{e + e_R}(m_Q - m_{QV}) \tag{1.32}$$

$$e_0 = \dot{v}\frac{U_E^0}{L^3} \tag{1.33}$$

For constant reproduction rate:

$$\frac{d}{dt}m_Q = \dot{k}_e \left(c_d L^3 \left(1 + P_{EV}\, e\, \omega_V\right) - m_Q\right) - \dot{R}\frac{e_0}{e}(m_Q - m_{QV}) \tag{1.34}$$

1.6 Toxicodynamics

For the effects of toxicants, I introduce a stress function as function of the dose metric. If the dose metric is the scaled internal concentration:

$$s = \frac{1}{c_T}\max\left(0, c_V - c_0\right) \equiv \frac{1}{c_T}(c_V - c_0)_+ \tag{1.35}$$

This stress factor is applied to one or more primary parameters. How this works out on compound parameters is specified in Table 4.1 and 4.2.

The scaled internal concentration affects the hazard rate as:

$$\dot{h}_Q = \dot{b}_\dagger \max\left(0, c_V - c_{0\dagger}\right) \equiv \dot{b}_\dagger \left(c_V - c_{0\dagger}\right)_+ \tag{1.36}$$

The hazard rate due to toxicant exposure ($\dot{h}_Q$) can simply be added to other hazard rates, assuming that they are independent effects. Some deaths are accidental, and can be included by a (low) constant background hazard rate ($\dot{h}_0$). For short laboratory experiments, this is usually sufficient. The total hazard is thus:

$$\dot{h} = \dot{h}_Q + \dot{h}_0 + \dot{h}_D \tag{1.37}$$

Chapter 2

The model for the standard animal

2.1 The standard model in powers

We start with a global description of DEB in terms of 'powers' (energy fluxes, in et^{-1}) as indicated in Figure 2.1; in Section 2.1.1 and 2.1.3, these powers will be further specified. In the standard DEB animal model, there are two types of biomass: structure (which requires maintenance) and reserve (which fuels metabolic processes). Structure ans reserve have a constant composition (strong homeostasis) and at constant food density (and when reserves are in steady state with the food level) the ratio between reserves and structure is constant from birth to death (weak homeostasis). Both structure and reserve are state variables. DEB identifies three life-cycle stages: embryo, juvenile and adult. The switch from embryo to juvenile is marked by the initiation of feeding, the switch from juvenile to adult marks the end of maturation and the start of investment in reproduction. The switching is determined by the 'complexity' of the organism, which is captured by the DEB state variable 'maturity' (the cumulated investment of reserves in development). There are thus maturity thresholds for birth E_H^b and puberty E_H^p. Note that $t = 0$ indicates the start of embryonic development, unless specified otherwise.

The feeding power for juveniles and adults $\dot{p}_X$ is turned into an assimilation flux $\dot{p}_A$ (for embryos, $\dot{p}_X = 0$, by definition) with a constant conversion efficiency (κ_X):

$$\dot{p}_A = \begin{cases} 0 & \text{if } E_H < E_H^b \\ \kappa_X \dot{p}_X & \text{otherwise} \end{cases} \tag{2.1}$$

The change in absolute amount of reserves (E) is given by the difference between the powers for assimilation ($\dot{p}_A$) and mobilisation ($\dot{p}_C$):

$$\frac{d}{dt}E = \begin{cases} -\dot{p}_C & \text{if } E_H < E_H^b \\ \dot{p}_A - \dot{p}_C & \text{otherwise} \end{cases} \quad \text{with } E(0) = E_0 \tag{2.2}$$

Structural body volume L^3 is calculated from the allocated mobilisation flux to the soma, after withdrawing the maintenance power $\dot{p}_M$. The energy flux is converted into structural volume using the energetic costs for structure $[E_G]$:

$$\frac{d}{dt}L^3 = \frac{1}{[E_G]}\left(\kappa \dot{p}_C - \dot{p}_M\right) \quad \text{with } L(0) \approx 0 \tag{2.3}$$

Maturation (as cumulated reserve investment into maturity, for embryos and juveniles) is given in the same manner, as the difference between the allocated mobilisation power, after withdrawing maturity maintenance $\dot{p}_J$:

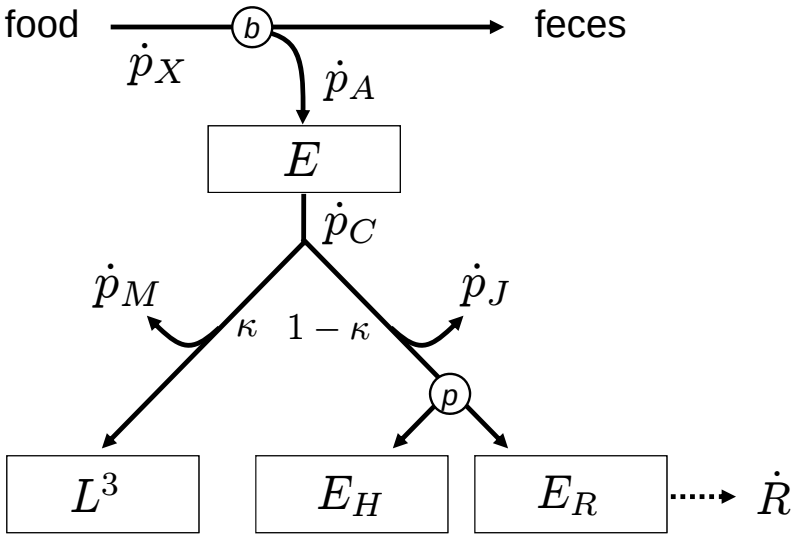

Figure 2.1: Schematic diagram of the energy flows in a standard DEB animal, with the associated parameter symbols. The nodes b and p denote switches at birth (start of feeding) and puberty (start of reproductive investment). The mobilisation flux is split according to a constant fraction κ.

$$\frac{d}{dt}E_H = \begin{cases} \kappa_H\left((1-\kappa)\dot{p}_C - \dot{p}_J\right) & \text{if } E_H < E_H^p \\ 0 & \text{otherwise} \end{cases} \quad \text{with } E_H(0) = 0 \tag{2.4}$$

In addition to DEB theory, we include here κ_H as the fraction of the energy flux available for maturity that is fixed in maturation. Normally, we do not have to bother with this parameter, and set it to 1, as we define maturity in terms of the reserves used to build it up. However, we need this parameter when we add toxicants, to include an effect on the costs for maturation.

The investment in maturation stops when the invested reserves hit the threshold for puberty E_H^p. At that point (thus for adults only), maturity maintenance is frozen at the value at puberty $\dot{p}_J^p$, and the energy flux is channelled into the reproduction buffer E_R:

$$\frac{d}{dt}E_R = \begin{cases} 0 & \text{if } E_H < E_H^p \\ (1-\kappa)\dot{p}_C - \dot{p}_J^p & \text{otherwise} \end{cases} \quad \text{with } E_R(0) = 0 \tag{2.5}$$

The mean reproduction rate can be calculated using the costs for a single egg (E_0), and assumes that a fraction κ_R of the allocated flux is fixed into eggs (the remainder is lost as overheads):

$$\dot{R} = \frac{\kappa_R}{E_0}\frac{d}{dt}E_R \tag{2.6}$$

The egg costs need to be found in such a way that they lead to hatching of an animal with the same reserve density $[E]$ as its mother had at the moment of egg formation (a maternal effect). The egg starts with a very small amount of structure, no maturity, no reproduction buffer, and an amount E_0 of reserves. It hatches when the maturity hits the birth threshold E_H^b. Note that in the simplified DEB model, we will assume constant egg costs, and do not deal with the embryo stage.

2.1.1 Specification of the power for assimilation

Instead of the feeding power $\dot{p}_X$, it is generally easier to work with the feeding rate $\dot{J}_{XA}$ in C-moles per time (these two are related by the chemical potential of food $\bar{\mu}_X$). The feeding rate is taken proportional to the structural surface area. Therefore, the surface-specific feeding rate $\{\dot{J}_{XAm}\}$ is constant. The feeding rate is thus given by:

$$\dot{J}_{XA} = \frac{\dot{p}_X}{\bar{\mu}_X} = f\{\dot{J}_{XAm}\}L^2 \tag{2.7}$$

The scaled functional response f is specified further as a hyperbolic relationship in food density (also known as the Holling type II functional response):

$$f = \frac{X}{X + \frac{\{\dot{J}_{XAm}\}}{\{\dot{F}_m\}}} \tag{2.8}$$

where the ratio $\{\dot{J}_{XAm}\}/\{\dot{F}_m\}$ is the half-saturation coefficient for the feeding rate. This f now varies between 0 (no food) and 1 (*ad libitum* food density; when X goes to infinity). For many ecotoxicological applications, we do not want to think about food densities, and we usually have tests performed at a (more or less) constant (usually high) food density. For that purpose, we can work with f as a model parameter, and ignore all of the feeding details.

The feeding rates $\dot{J}_{XA}$ and $\{\dot{J}_{XAm}\}$, as well as the searching rate $\{\dot{F}_m\}$ and the food density X, are specified in C-moles. When food is expressed in another unit (e.g., grammes of prey, prey items), a conversion factor is required. However, it can be easily seen from the equations above that in the calculation of f the conversion falls out.

The assimilation flux (for juveniles and adults) scales with the structural surface area and depends on the available food. The surface-area specific maximum assimilation power $\{\dot{p}_{Am}\}$ is a species-specific constant. The actual assimilation power $\dot{p}_A$ is than given by:

$$\dot{p}_A = fL^2\{\dot{p}_{Am}\} \tag{2.9}$$

The assimilation flux is logically related to the feeding rate, through a mass-energy coupler (converts the C-moles of $\{\dot{J}_{XAm}\}$ into energy):

$$\{\dot{p}_{Am}\} = \mu_{AX}\{\dot{J}_{XAm}\} \tag{2.10}$$

2.1.2 Specification of the powers for maintenance

The somatic maintenance power is proportional to the structural volume. The volume-specific somatic maintenance power is thus a species-specific constant $[\dot{p}_M]$:

$$\dot{p}_M = [\dot{p}_M]L^3 \tag{2.11}$$

The maturity maintenance power is proportional to the cumulated investment into maturation:

$$\dot{p}_J = \dot{k}_J E_H \tag{2.12}$$

2.1.3 Specification of the mobilisation power

For the mobilisation power, we have to do a bit more work. We start with the differential equation for the reserve, Eq. 2.2, which we rewrite to an equation for the reserve density $[E] = E/L^3$:

$$\frac{d}{dt}[E] = [\dot{p}_A] - [\dot{p}_C] - [E]\dot{r} \tag{2.13}$$

The last term stands for the dilution of the reserves by growth, where:

$$\dot{r} = \frac{1}{L^3}\frac{d}{dt}L^3 \tag{2.14}$$

The weak homeostasis assumption of DEB theory [15] demands that $[E]$ does not change at constant food density, and thus does not depend on body size. However, we know that $[\dot{p}_A]$ scales with L^{-1}, because $\dot{p}_A$ scales with L^2 (see Eq. 2.9). For $\frac{d}{dt}[E]$ to stay zero when $[\dot{p}_A]$ scales with L^{-1}, the last two terms in Eq. 2.13 ($-[\dot{p}_C] - [E]\dot{r}$) should together be some function that scales with L^{-1}. We can write this in Eq. 2.13 by introducing some function H of $[E]$, where the other parameters of this function (θ) cannot depend on L:

$$\frac{d}{dt}[E] = [\dot{p}_A] - \frac{1}{L}H([E];\theta) \tag{2.15}$$

Following an argument about partitionability of the reserves, Kooijman [15] argues that the only possible function for H is a simple one: $H = \dot{v}[E]$. This implies that the volume-specific mobilisation power ($[\dot{p}_C]$) should be found by combining the last terms in Eq. 2.13 and 2.15:

$$-[\dot{p}_C] - [E]\dot{r} = -\frac{\dot{v}}{L}[E] \tag{2.16}$$

and thus:

$$[\dot{p}_C] = [E]\left(\frac{\dot{v}}{L} - \dot{r}\right) \tag{2.17}$$

$$\dot{p}_C = E\left(\frac{\dot{v}}{L} - \dot{r}\right) \tag{2.18}$$

Next, we can fill in $\dot{r}$, using Eq. 2.14 and the equation for growth Eq. 2.3, using the maintenance power of Eq. 2.11:

$$\dot{p}_C = E\left(\frac{\dot{v}}{L} - \frac{1}{L^3[E_G]}\left(\kappa\dot{p}_C - [\dot{p}_M]L^3\right)\right) \tag{2.19}$$

$$= E\frac{\dot{v}}{L} - E\frac{\kappa\dot{p}_C}{L^3[E_G]} + E\frac{[\dot{p}_M]}{[E_G]} \tag{2.20}$$

Next, we can move all terms including $\dot{p}_C$ to the left side of the equation:

$$\dot{p}_C + E\frac{\kappa\dot{p}_C}{L^3[E_G]} = E\frac{\dot{v}}{L} + E\frac{[\dot{p}_M]}{[E_G]} \tag{2.21}$$

$$\dot{p}_C\left(1 + \frac{[E]\kappa}{[E_G]}\right) = E\left(\frac{\dot{v}}{L} + \frac{[\dot{p}_M]}{[E_G]}\right) \tag{2.22}$$

Which leads to:

$$\dot{p}_C = E\frac{\dot{v}/L + \frac{[\dot{p}_M]}{[E_G]}}{1 + \frac{[E]\kappa}{[E_G]}} \tag{2.23}$$

$$= E\frac{[E_G]\dot{v}/L + [\dot{p}_M]}{[E_G] + [E]\kappa} \tag{2.24}$$

This is the equation that is also derived in the DEB book, Page 39, Eq. 2.12 (when multiplied with structural volume, L^3). We can further use Equation 2.23, introducing the somatic maintenance rate coefficient $\dot{k}_M = [\dot{p}_M]/[E_G]$, the scaled reserve densiy $e = [E]/[E_m]$ (where $[E_m]$ is the maximum reserve density, which is taken as a constant), and the energy investment ratio $g = [E_G]/(\kappa[E_m])$:

$$\dot{p}_C = E\frac{\dot{v}/L + \dot{k}_M}{1 + \kappa\frac{[E]}{[E_G]}} \tag{2.25}$$

$$= e[E_m]L^3\frac{\dot{v}/L + \dot{k}_M}{1 + e\kappa\frac{[E_m]}{[E_G]}} \tag{2.26}$$

$$= [E_m]L^3\left(\frac{\dot{v}}{L} + \dot{k}_M\right)\frac{eg}{e+g} \tag{2.27}$$

2.2 The scaled standard model

Several formulations of DEB models are available that are useful for different purposes. In the previous section, I presented a formulation in terms of energy fluxes. This is not the most practical one to work with in an ecotoxicological setting because of the dimension 'energy' that we will generally not have access to.

I will use the DEB3 version with scaled reserves and maturity [19], which is phrased only in terms of the dimensions length and time. Reserves and maturity are scaled with the maximum assimilation rate per unit of surface area, which removes the unit 'mole' from the system (and one parameter). All stage transitions are initiated by maturity levels; $U_H = U_H^b$ for birth (start of feeding) and $U_H = U_H^p$ for puberty (start of investment in reproduction, and stop investment in maturity). Note that the age $a = 0$ marks the start of the development of the embryo in the egg; age $a = a_b$ is the age of birth, and $a = a_p$ is the age at puberty.

In this formulation, two compound parameters will be used to remove the dimension 'energy' from the complete parameter set: the somatic maintenance rate coefficient $\dot{k}_M$, and the energy investment ration g, defined as:

$$\dot{k}_M = \frac{[\dot{p}_M]}{[E_G]} \quad \text{and} \quad g = \frac{[E_G]}{\kappa[E_m]} \tag{2.28}$$

2.2.1 Reserve and mobilisation

The change in scaled reserve is given by:

$$\frac{d}{dt}U_E = \begin{cases} -S_C & \text{if } U_H < U_H^b \\ fL^2 - S_C & \text{otherwise} \end{cases} \quad \text{with } U_E(0) = U_E^0 \tag{2.29}$$

It turns out to be handy to work with the scaled reserve density:

$$e = \dot{v}\frac{U_E}{L^3} \tag{2.30}$$

Under constant conditions, and the absence of toxic stress on feeding or assimilation, $e = f$. The scaled mobilisation power is then calculated as:

$$\begin{aligned} S_C &= \frac{\dot{p}_C}{\{\dot{p}_{Am}\}} = \frac{\dot{p}_C}{\dot{v}[E_m]} && (2.31)\\ &= \frac{L^3}{\dot{v}}\left(\frac{\dot{v}}{L} + \dot{k}_M\right)\frac{eg}{e+g} && (2.32)\\ &= L^2\frac{ge}{g+e}\left(1 + \frac{\dot{k}_M}{\dot{v}}L\right) && (2.33) \end{aligned}$$

2.2.2 Derivation of the growth equation

Growth can be seen as in DEB context in terms of energy as follows:

$$\frac{d}{dt}L^3 = \frac{1}{[E_G]}\left(\kappa\dot{p}_C - [\dot{p}_M]L^3\right) \tag{2.34}$$

Here, $\kappa\dot{p}_C$ is the energy flux allocated to growth and somatic maintenance. The energy flux needed for maintenance is deducted, and the remainder is divided by the energetic costs for growth. I decided to work with scaled reserves to remove the dimension of energy. The scaled mobilisation flux was S_C, which relates to $\dot{p}_C$ as:

$$\dot{p}_C = S_C\{\dot{p}_{Am}\} \tag{2.35}$$

Using this relationship, the growth equation becomes:

$$\frac{d}{dt}L^3 = \frac{\kappa\{\dot{p}_{Am}\}}{[E_G]}S_C - \frac{[\dot{p}_M]}{[E_G]}L^3 \tag{2.36}$$

There are two expressions for maximum length, that we both will use:

$$L_m = \frac{\kappa\{\dot{p}_{Am}\}}{[\dot{p}_M]} = \frac{\dot{v}}{\dot{k}_M g} \tag{2.37}$$

Because $\dot{k}_M = [\dot{p}_M]/[E_G]$, we can use this equation to rewrite Eq. 2.36 to:

$$\frac{d}{dt}L^3 = \frac{\dot{v}}{g}S_C - \dot{k}_M L^3 \tag{2.38}$$

To change this from volumetric to length growth, we need to take care that $\frac{d}{dt}L^3 = 3L^2\frac{d}{dt}L$, which leads to this equation:

$$\frac{d}{dt}L = \frac{1}{3L^2}\left(\frac{\dot{v}}{g}S_C - \dot{k}_M L^3\right) \quad \text{with } L(0) \approx 0 \tag{2.39}$$

This equation is different from the one given in [19], although it is mathematically equivalent. I prefer this form as it reveals the close links with the maturation and reproduction process; growth is the net result of the mobilised reserved that are allocated to growth and somatic maintenance (κ is included in g), minus the maintenance needs.

Filling in the equation for S_C, and 'some manipulation' gives the well-known Von Bertalanffy equation:

$$\frac{d}{dt}L = \dot{r}_B\,(eL_m - L) \quad \text{with } L(0) \approx 0 \tag{2.40}$$

where:

$$\dot{r}_B = \frac{\dot{k}_M g}{3(e+g)} \quad \text{and} \quad L_m = \frac{\dot{v}}{\dot{k}_M g} \tag{2.41}$$

2.2.3 Maturation and reproduction

The change in scaled maturity is given by:

$$\frac{d}{dt}U_H = \begin{cases} \kappa_H((1-\kappa)S_C - \dot{k}_J U_H) & \text{if } U_H < U_H^p \\ 0 & \text{otherwise} \end{cases} \quad \text{with } U_H(0) = 0 \tag{2.42}$$

Note that $U_H(a_b) = U_H^b$ and $U_H(a_p) = U_H^p$. The energy flux into the reproductive buffer is given by:

$$\frac{d}{dt}U_R = \begin{cases} 0 & \text{if } U_H < U_H^p \\ (1-\kappa)S_C - \dot{k}_J U_H^p & \text{otherwise} \end{cases} \quad \text{with } U_R(0) = 0 \tag{2.43}$$

At spawning events, the reproduction buffer will be converted to eggs. Ignoring the buffer, the mean reproduction rate is given by:[1]

$$\dot{R} = \frac{\kappa_R}{U_E^0}\frac{d}{dt}U_R \tag{2.44}$$

What triggers emptying of the reproduction buffer? Perhaps, filling of the buffer to a critical level depending on structural size ($U_R = [U_{Rm}]L^3$), some time interval (t_R), external conditions (e.g., temperature, rainfall, food conditions). At such an event, the buffer is converted into a batch of eggs (ΔR) as follows:[2]

$$\Delta R = \text{floor}\left(\frac{\kappa_R U_R}{U_E^0}\right) \quad \text{if } U_R = [U_{Rm}]L^3 \text{ or } t = t_R \text{ or } \ldots \tag{2.45}$$

The 'floor' function (rounding to lower integer) is needed as only whole eggs can be produced. What happens to the remaining energy in the buffer, which was insufficient for the production of one egg? It may either be lost, or kept in the buffer for the next reproductive event. If it is kept in the buffer, U_R is reset:

$$U_R = U_R - \frac{U_E^0 \Delta R}{\kappa_R} \tag{2.46}$$

This assumes that the overhead costs for reproduction $(1-\kappa_R)$ are only paid for the eggs that were produced, not for the entire buffer at the moment of spawning. This sounds realistic, but the numerical influence of this choice will anyway be small, especially when a large number of eggs are produced per batch.

[1] In this case, we do not need to follow U_R as a state variable. However, we can use $\dot{R}$ as a state; its integrated counterpart is the cumulative reproductive output.

[2] When we work with a buffer, we do not need to use an additional state variable in our calculations. We can simply re-use U_H. For an adult, we can continue to build up U_H, but consider that $U_R = U_H - U_H^p$.

One problem is left open: the determination of the scaled initial reserves in the egg, U_E^0. However, following the standard assumptions of the DEB model, this quantity does not require additional parameter. The initial reserves must be such that the embryo will hatch at the same reserve density as its mother had at egg formation. The most intuitive way to solve it would be to simply simulate the embryonic development with different values of U_E^0 until we observe the correct reserve density at hatching. However, more efficient procedures are available (see [14]).

When $\dot{k}_J$ equals $\dot{k}_M$ we have the situation where stage transitions take place at the same structural length for different food levels. This situation is of special interest as a constant size at puberty appears to be common in many of the invertebrates that are used as standard test organisms in ecotoxicology. For this reason, I introduce the ratio of the two rate constants as a new parameter (with a default value of 1). Similarly, I would like to introduce a parameter for the ratio of the maturity at birth and at puberty:

$$k = \frac{\dot{k}_J}{\dot{k}_M} \quad \text{and} \quad u_H^{bp} = \frac{U_H^b}{U_H^p} \tag{2.47}$$

The reason for introducing this new parameter is that the absolute value of U_H is difficult to interpret because of the non-intuitive dimensions. Furthermore, all U are scaled using the maximum surface-specific assimilation rate. This rate depends on details of food quality and might therefore differ between experiments. However, the ratio of maturity at birth and puberty should be constant in a species under a much broader range of environmental conditions.

This leaves the following complete set of parameters: g, $\dot{v}$, $\dot{k}_M$, k, κ, κ_R, κ_R, U_H^p, u_H^{bp}. For the assimilation part, we can either treat f as a model parameter or use the feeding module to calculate f using $\{\dot{J}_{XAm}\}$, $\{\dot{F}_m\}$ and X.

2.2.4 Initial conditions at the start of an experiment

We can start our simulations at $t = 0$ at the moment of a freshly laid egg. However, life-cycle toxicity tests are generally started with juveniles of which the age is (approximately) known. In that case, it may be convenient to take $t = 0$ at the moment of hatching (especially as the embryonic development might include a diapause). At hatching, maturity is known ($U_H = U_H^b$). Reserve density can be taken equal to that of the mother at egg production. When the mothers come from a well-fed culture, it is appropriate to assume $e = 1$ for the hatchling. The exact value usually has little impact as the reserves of the (small) hatchling will rapidly reach a steady state with its food density in the test. The length at birth (L_b) will have to derived from the initial reserves in the egg U_E^0, and the parameter values.

It is also possible to start with $t = 0$ later in life. Let's take the situation where length at the start of the experiment is known. We need to obtain the value of the state variables U_E and U_H at this point. The value for the reserves can be derived from rewriting Equation 2.30 (providing we can estimate e, for example,if we can assume $e = 1$). The value for the maturity is more problematic. We can, of course, simulate the development from the moment of hatching (or from the freshly produced egg).

DEBtool offers more efficient methods to derive L_b and U_H at $L > L_b$.

2.3 Starvation responses

Problems on starvation occur when the somatic maintenance costs cannot be paid anymore. This corresponds to the point where the the scaled reserve density is insufficient to maintain

the actual structural size:

$$e < \frac{L}{L_m} \quad \text{where} \quad L_m = \frac{\dot{v}}{\dot{k}_M g} \tag{2.48}$$

At this point, there is a whole range of options that the organism could follow. These strategies will be highly specific, but here I will work out a few simple options. The first, and simplest option, is to do nothing. Just allow $\frac{d}{dt}L$ to become negative, and thus allow shrinking of structure. The underlying assumption is that structure is used for maintenance, and that burning a unit of structure yields exactly the amount of energy as was needed to build this unit of structure (which hardly seems realistic, but may suffice as an initial guess). If the animal is a juvenile, $\frac{d}{dt}U_H$ can become negative too, when maturity maintenance cannot be paid anymore. Maturity is information, so there is nothing to burn here. However, we could interpret this as a degradation of maturity when maturity maintenance cannot be paid anymore. The degradation of maturity will than exactly match what is needed to pay maturity maintenance (which also seems unrealistic). For mature animals, $\frac{d}{dt}U_R$ can become negative so that the reproduction buffer is used to pay maturity maintenance.

Another option is to keep the complete metabolic organisation intact, but to maintain current levels of structure and maturity from the reproduction buffer (conform [23]). Because there is no change in structural volume anymore, the equation for the mobilisation of reserves is extremely reduced.

2.3.1 Mobilisation under no-growth conditions

Let's start from the equation for the volume-specific mobilisation power:

$$[\dot{p}_C] = [E]\left(\frac{\dot{v}}{L} - \dot{r}\right) \tag{2.49}$$

where $\dot{r}$ is the volumetric growth rate. Under the simple starvation strategies that I present, structural growth completely ceases. Therefore $\dot{r} = 0$ and L is constant. The equation for $[\dot{p}_C]$ is therefore easily translated into an equation for the absolute power under no-growth conditions:

$$\dot{p}_C = E\frac{\dot{v}}{L} \tag{2.50}$$

Because $S_C = \dot{p}_C/\{\dot{p}_{Am}\}$ and $U_E = E/\{\dot{p}_{Am}\}$, we can rewrite this to the scaled mobilisation flux:

$$S_C = U_E\frac{\dot{v}}{L} \tag{2.51}$$

And because:

$$e = \dot{v}\frac{U_E}{L^3} \tag{2.52}$$

we end up with the simple result:

$$S_C = eL^2 \tag{2.53}$$

2.3.2 Pay maintenance costs from the reproduction buffer

One option is to keep the complete metabolic organisation intact, but to maintain current levels of structure and maturity from the reproduction buffer (conform [23]). Because there is no change in structural volume anymore, the equation for the mobilisation of reserves is extremely reduced:

$$S_C = eL^2 \tag{2.54}$$

This simple result was obtained in the previous section for a constant structural volume. Under starvation, this mobilisation is insufficient to maintain structural volume. First, we need to calculate how large the allocated mobilisation rate to soma κS_C needs to be, to just match the needs for maintenance. This can be done from Eq. 2.39, by setting growth to zero. First, I will write Eq. 2.39 slightly differently to show explicitly the mobilisation flux to somatic maintenance and growth κS_C (κ is otherwise hidden in the compound parameter g):

$$\frac{d}{dt}L = \frac{1}{3L^2}\left(\frac{\dot{v}}{\kappa g}\kappa S_C - \dot{k}_M L^3\right) \tag{2.55}$$

Solving for zero growth yields:

$$\kappa S_C = \frac{\dot{k}_M \kappa g}{\dot{v}} L^3 \tag{2.56}$$

This is the amount that is needed for somatic maintenance alone. An alternative (and perhaps more logical) way to derive this is to explicitly derive the scaled somatic maintenance flux S_M:

$$S_M = \frac{[\dot{p}_M]L^3}{\{\dot{p}_{Am}\}} \tag{2.57}$$

$$L_m^{-1} = \frac{[\dot{p}_M]}{\kappa\{\dot{p}_{Am}\}} = \frac{\dot{k}_M g}{\dot{v}} \Rightarrow \frac{[\dot{p}_M]}{\{\dot{p}_{Am}\}} = \kappa\frac{\dot{k}_M g}{\dot{v}} \tag{2.58}$$

$$S_M = \kappa\frac{\dot{k}_M g}{\dot{v}} L^3 \tag{2.59}$$

The difference between the actual mobilisation flux S_C for zero growth and the required flux for somatic maintenance S_M is than subtracted from the flow into the reproduction buffer, changing the ODE for the buffer to:

$$\frac{d}{dt}U_R = (1-\kappa)S_C - \dot{k}_J U_H^p - \left(\frac{\dot{k}_M \kappa g}{\dot{v}} L^3 - \kappa e L^2\right) \tag{2.60}$$

$$= (1-\kappa)S_C - \dot{k}_J U_H^p - \kappa L^2\left(\frac{\dot{k}_M g}{\dot{v}} L - e\right) \tag{2.61}$$

$$= (1-\kappa)S_C - \dot{k}_J U_H^p - \kappa L^2\left(\frac{L}{L_m} - e\right) \tag{2.62}$$

This can continue until the reproduction buffer is exhausted and $\frac{d}{dt}U_R$ becomes negative. At that point, there may still be reserves left ($e > 0$), but the mobilisation is insufficient to yield a flux large enough to cover the maintenance costs. When the reproduction buffer is exhausted,

this scenario is almost equivalent to a change in κ to match somatic maintenance needs (a change in κ would also affects the value of g). A modification of this strategy could be that the organism stops emptying the reproduction buffer, or also reduces maturity maintenance (which should have consequences somewhere, e.g., in the hazard rate).

For this scenario, one would have to think carefully about the availability of the energy in the reproduction buffer; energy which has already been allocated to eggs. To what extent can this energy be used again to fuel maintenance? Experiments with *Daphnia* suggest that reserves early in egg formation can be used to increase survival under starvation [26]. For juveniles, we might take the required energy for somatic maintenance out of the maturation flux.

2.3.3 Minimise mobilisation from the reserve

The last option that I would like to discuss here is to only mobilise enough energy from the reserves to fulfil the maintenance needs. This implies that:

$$\frac{d}{dt}L = 0, \frac{d}{dt}U_H = 0, \frac{d}{dt}U_R = 0 \tag{2.63}$$

I also assume that maturity maintenance costs are not paid anymore. It is not entirely certain what happens to an organism when maturity maintenance is not paid anymore, but an increase in the hazard rate and a decrease in the level of maturity ('rejuvenation') seem plausible. In this scenario, the mobilisation from the reserves is recalculated to the minimal flux to pay somatic maintenance:

$$S_C = \frac{\dot{k}_M \kappa g}{\dot{v}} L^3 = \kappa \frac{L^3}{L_m} \tag{2.64}$$

This recalculated flux than has to be withdrawn from the scaled reserves U_E (this might be less or more than what can be maximally mobilised, which is eL^2). This scenario can continue until the reserves are fully depleted. However, you might also decide that no more reserves can be mobilised than the maximum under normal conditions, so to include the condition $S_C \leq eL^2$.

When the food density increases again, the starvation scenario stops as soon as the energy reserves U_E are sufficient to support a mobilisation flux S_C that can lead to positive growth again. The DEB book [15] has worked out more options, that allow for more realistic shrinking of structure and rejuvenation.

2.4 The simplified standard model

The simplified system that we derive here follows from two additional assumptions:

1. There is a always a constant ratio between structure and maturity for juveniles and embryos, also under (toxicant) stress. The start of investment into reproduction (puberty) therefore occurs at a fixed structural length, and we do not need to follow maturity as a state variable.

2. Egg costs are constant, unless there is a direct toxicant effect on them. This assumption overrides the 'maternal effect' of DEB theory (see [15]).

In contrast to the original DEBtox equations [16], we include the scaled reserve (e) as a dynamic state variable. Thus, we do not have to assume that the reserve density is always in steady state.

Assumption 1 limits the parameters that are allowed to change under stress. For example, if we increase the costs for structure, this implies a change in the length at puberty, unless the costs for maturation are affected by the same factor. Similarly, a change in κ will affect the length at puberty. To allow changing any arbitrary DEB parameter, one would have to use a full DEB model (see [12]). The use of scaled reserves implies that we cannot incorporate an effect on the energy conductance $\dot{v}$ (as a change in $\dot{v}$ would affect $[E_m]$ that is used to scale the reserves, see next section).

2.4.1 Reserve dynamics

From the derivation in Section 2.1.3 (Eq. 2.15 and 2.9), it follows that (see also the DEB book [15] Page 39, Eq. 2.10-2.11):

$$\frac{d}{dt}[E] = [\dot{p}_A] - [E]\frac{\dot{v}}{L} = \frac{\{\dot{p}_{Am}\}f - [E]\dot{v}}{L} \tag{2.65}$$

The maximum reserve density is achieved ($[E] = [E_m]$) when $f = 1$, and thus $\frac{d}{dt}[E] = 0$ when $[E_m] = \{\dot{p}_{Am}\}/\dot{v}$. The scaled reserve density was defined as $e = [E]/[E_m]$, and because $[E_m]$ is taken as constant, we can rewrite the reserve equation to:

$$\frac{d}{dt}e = (f - e)\frac{\dot{v}}{L} \tag{2.66}$$

When the dynamics of the reserves are fast, or when f is constant, we can take $f = e$, and effectively ignore the reserve altogether.

2.4.2 Maturity is a constant fraction of structure

In this section, we will remove maturity as a state variable, using assumption 1 above. The change in structural volume L^3 and in the investment in maturity E_H are given by the following two equations:

$$\frac{d}{dt}L^3 = \frac{1}{[E_G]}\left(\kappa\dot{p}_C - [\dot{p}_M]L^3\right) \tag{2.67}$$

$$\frac{d}{dt}E_H = \kappa_H\left((1-\kappa)\dot{p}_C - \dot{k}_J E_H\right) \tag{2.68}$$

When maturity is always a constant fraction of structural volume, we can work with length instead of maturity; investment in reproduction will always start at a constant structural size. Both structure and maturity start at embryonic development approximately at zero. Thus we demand that:

$$\frac{E_H}{L^3} = \frac{\frac{d}{dt}E_H}{\frac{d}{dt}L^3} = \frac{E_H^p}{L_p^3} = [E_H] = \text{constant} \tag{2.69}$$

and thus (note that $\dot{k}_M = [\dot{p}_M]/[E_G]$):

$$\frac{d}{dt}E_H = [E_H]\frac{d}{dt}L^3 \tag{2.70}$$

$$\kappa_H\left((1-\kappa)\dot{p}_C - \dot{k}_J E_H\right) = \frac{[E_H]}{[E_G]}\left(\kappa\dot{p}_C - [\dot{p}_M]L^3\right) \tag{2.71}$$

$$\kappa_H(1-\kappa)\dot{p}_C - \kappa_H\dot{k}_J E_H = \frac{\kappa[E_H]}{[E_G]}\dot{p}_C - [E_H]\frac{[\dot{p}_M]}{[E_G]}L^3 \tag{2.72}$$

$$\kappa_H(1-\kappa)\dot{p}_C - \kappa_H\dot{k}_J[E_H]L^3 = \frac{\kappa[E_H]}{[E_G]}\dot{p}_C - [E_H]\dot{k}_M L^3 \tag{2.73}$$

$$\kappa_H\frac{1-\kappa}{[E_H]}\dot{p}_C - \kappa_H\dot{k}_J L^3 = \frac{\kappa}{[E_G]}\dot{p}_C - \dot{k}_M L^3 \tag{2.74}$$

For $\dot{p}_C$, we can include Eq. 2.27:

$$\kappa_H\frac{(1-\kappa)[E_m]}{[E_H]}L^3\left(\frac{\dot{v}}{L}+\dot{k}_M\right)\frac{eg}{e+g} - \kappa_H\dot{k}_J L^3 = \frac{\kappa[E_m]}{[E_G]}L^3\left(\frac{\dot{v}}{L}+\dot{k}_M\right)\frac{eg}{e+g} - \dot{k}_M L^3 \tag{2.75}$$

$$\kappa_H\frac{(1-\kappa)[E_m]}{[E_H]}\left(\frac{\dot{v}}{L}+\dot{k}_M\right)\frac{eg}{e+g} - \kappa_H\dot{k}_J = \frac{\kappa[E_m]}{[E_G]}\left(\frac{\dot{v}}{L}+\dot{k}_M\right)\frac{eg}{e+g} - \dot{k}_M \tag{2.76}$$

$$\kappa_H\frac{1-\kappa}{[E_H]}H(L) - \kappa_H\dot{k}_J = \frac{\kappa}{[E_G]}H(L) - \dot{k}_M \tag{2.77}$$

We thus have only a single function H of L, times two constants, and minus two constants. This equality can only hold when $\kappa_H\dot{k}_J = \dot{k}_M$, and when the factors before $H(L)$ are the same. This latter requirement translates into:

$$\kappa_H\frac{1-\kappa}{[E_H]} = \frac{\kappa}{[E_G]} \tag{2.78}$$

$$\kappa_H\frac{1-\kappa}{\kappa}[E_G] = [E_H] \tag{2.79}$$

We can use this result to obtain an expression that links E_H^p to L_p, because we would like to use the latter as a model parameter (and remove the first):

$$\kappa_H\frac{1-\kappa}{\kappa}[E_G] = \frac{E_H^p}{L_p^3} \tag{2.80}$$

$$E_H^p = \kappa_H\frac{1-\kappa}{\kappa}[E_G]L_p^3 \tag{2.81}$$

Thus, we now have a constraint for using a constant length at puberty ($\kappa_H\dot{k}_J = \dot{k}_M$) and an expression for how the length at puberty (L_p^3) relates to the invested reserves into maturation (E_H^p). Because we assume that E_H^p is constant, this implies that an increase in $[E_G]$ needs to be accompanied by a decrease of κ_H by the same factor (note that $\dot{k}_M$ includes $[E_G]$, so the equality $\kappa_H\dot{k}_J = \dot{k}_M$ still holds). Furthermore, a change in somatic maintenance costs $\dot{k}_M$ should be accompanied by the same change in maturity maintenance costs $\dot{k}_J$.

2.4.3 Rewriting the reproduction rate

In this section, we will rewrite the reproduction equation in such a way that many of the model parameters are combined into a new compound parameter, the maximum reproduction rate $\dot{R}_m$. Starting from the energy-based equation for reproduction, we can use the results derived in the previous section ($\kappa_H \dot{k}_J = \dot{k}_M$ and the expression for E_H^p in Eq. 2.81) to rewrite the equation:

$$\begin{aligned}
\dot{R} &= \frac{\kappa_R}{E_0}\left((1-\kappa)\dot{p}_C - \dot{k}_J E_H^p\right) && (2.82)\\
&= \frac{\kappa_R}{E_0}\left((1-\kappa)\dot{p}_C - \frac{\dot{k}_M}{\kappa_H}\kappa_H\frac{1-\kappa}{\kappa}[E_G]L_p^3\right) && (2.83)\\
&= \frac{\kappa_R}{E_0}\frac{1-\kappa}{\kappa}[E_G]\left(\frac{\kappa}{[E_G]}\dot{p}_C - \dot{k}_M L_p^3\right) && (2.84)
\end{aligned}$$

Next, we need expressions for the mobilisation flux, which was already derived in Section 2.1.3 (Eq. 2.27), as well as the maximum mobilisation flux (without stress, with $L = L_m$ and $e = 1$). We need this maximum flux because we will write the reproduction equation in terms of the maximum reproduction rate later on, to remove a number of parameters. For that purpose, we introduce the maximum length in the controle L_{m0}, and parameters in the control $\dot{k}_{M0}$ and g_0 (remember that we do not consider stress on $\dot{v}$ or κ as they violate the simplifying assumptions). This is necessary to correctly introduce stress factors due to the toxicant at a later stage. The maximum mobilisation power is:

$$\dot{p}_{Cm} = [E_m]L_{m0}^3\left(\frac{\dot{v}}{L_{m0}} + \dot{k}_{M0}\right)\frac{g_0}{1+g_0} \qquad (2.85)$$

Note that $L_{m0} = \dot{v}/(\dot{k}_{M0}g_0)$ is the maximum length in the control, and thus:

$$\begin{aligned}
\dot{p}_{Cm} &= [E_m]L_{m0}^3\left(\dot{k}_{M0}g_0 + \dot{k}_{M0}\right)\frac{g_0}{1+g_0} && (2.86)\\
&= [E_m]L_{m0}^3\dot{k}_{M0}\left(1+g_0\right)\frac{g_0}{1+g_0} && (2.87)\\
&= [E_m]L_{m0}^3\dot{k}_{M0}g_0 && (2.88)\\
&= [E_m]\dot{v}L_{m0}^2 && (2.89)
\end{aligned}$$

The maximum reproduction rate (in the control, at maximum size, and $e = 1$) is now given by introducing the expression for $\dot{p}_{Cm}$ into Eq. 2.84, and using control parameters only (κ_{R0}, $[E_{G0}]$, $\dot{k}_{M0}$) (note that $g = [E_G]/(\kappa[E_m])$):

$$\begin{aligned}
\dot{R}_{m0} &= \frac{\kappa_{R0}}{E_0}\frac{1-\kappa}{\kappa}[E_{G0}]\left(\frac{\kappa}{[E_{G0}]}[E_m]\dot{v}L_{m0}^2 - \dot{k}_{M0}L_p^3\right) && (2.90)\\
&= \frac{\kappa_{R0}}{E_0}\frac{1-\kappa}{\kappa}[E_{G0}]\left(\frac{\dot{v}}{g_0}L_{m0}^2 - \dot{k}_{M0}L_p^3\right) && (2.91)\\
&= \frac{\kappa_{R0}}{E_0}\frac{1-\kappa}{\kappa}[E_{G0}]\dot{k}_{M0}\left(L_{m0}^3 - L_p^3\right) && (2.92)
\end{aligned}$$

The reproduction rate of Eq. 2.84 can be filled in with the regular mobilisation flux too:

$$\dot{R} = \frac{\kappa_R}{E_0}\frac{1-\kappa}{\kappa}[E_G]\left(\frac{\kappa}{[E_G]}[E_m]L^3\left(\frac{\dot{v}}{L}+\dot{k}_M\right)\frac{eg}{e+g}-\dot{k}_M L_p^3\right) \tag{2.93}$$

$$= \frac{\kappa_R}{E_0}\frac{1-\kappa}{\kappa}[E_G]\left(L^3\left(\frac{\dot{v}}{L}+\dot{k}_M\right)\frac{e}{e+g}-\dot{k}_M L_p^3\right) \tag{2.94}$$

$$= \frac{\kappa_R}{E_0}\frac{1-\kappa}{\kappa}[E_G]\dot{k}_M\left(\left(\frac{\dot{v}}{\dot{k}_M}L^2+L^3\right)\frac{e}{e+g}-L_p^3\right) \tag{2.95}$$

We can remove a number of parameters from this equation by calculating the ratio $\dot{R}/\dot{R}_{m0}$, and use that to form an expression for $\dot{R}$ that includes $\dot{R}_{m0}$ as parameter:

$$\frac{\dot{R}}{\dot{R}_{m0}} = \frac{\kappa_R}{\kappa_{R0}}\frac{[E_G]}{[E_{G0}]}\frac{\dot{k}_M}{\dot{k}_{M0}}\frac{\left(\frac{\dot{v}}{\dot{k}_M}L^2+L^3\right)\frac{e}{e+g}-L_p^3}{L_{m0}^3-L_p^3} \tag{2.96}$$

$$\dot{R} = \frac{\dot{R}_{m0}}{L_{m0}^3-L_p^3}\frac{\kappa_R}{\kappa_{R0}}\frac{[E_G]}{[E_{G0}]}\frac{\dot{k}_M}{\dot{k}_{M0}}\left(\left(\frac{\dot{v}}{\dot{k}_M}L^2+L^3\right)\frac{e}{e+g}-L_p^3\right) \tag{2.97}$$

$$= \frac{\dot{R}_{m0}}{L_{m0}^3-L_p^3}\frac{\kappa_R}{\kappa_{R0}}\frac{[\dot{p}_M]}{[\dot{p}_{M0}]}\left(\left(\frac{\dot{v}}{\dot{k}_M}L^2+L^3\right)\frac{e}{e+g}-L_p^3\right) \tag{2.98}$$

The last step makes use of the fact that $\dot{k}_M = [\dot{p}_M]/[E_G]$. The stress-to-control ratios κ_R/κ_{Ro} and $[\dot{p}_M]/[\dot{p}_{M0}]$ are of course only relevant when there is exposure to a toxicant, otherwise they are one and can be removed. These two model parameters only appear as a ratio. The advantage is that a stress on κ_R or $[\dot{p}_M]$ does not require the absolute value of these parameters. The stress factor s can simply be applied on the parameter $\dot{R}_m$.

2.4.4 Rewriting the growth equation

Starting with the growth equation in terms of energetics (making use of $\dot{k}_M = [\dot{p}_M]/[E_G]$):

$$\frac{d}{dt}L^3 = \frac{1}{[E_G]}\left(\kappa\dot{p}_C - [\dot{p}_M]L^3\right) \tag{2.99}$$

$$3L^2\frac{d}{dt}L = \frac{1}{[E_G]}\left(\kappa\dot{p}_C - [\dot{p}_M]L^3\right) \tag{2.100}$$

$$\frac{d}{dt}L = \frac{1}{3}\left(\frac{\kappa\dot{p}_C}{[E_G]L^2} - \dot{k}_M L\right) \tag{2.101}$$

Next, we can include Eq. 2.27 for the mobilisation rate (making use of $g = [E_G]/(\kappa[E_m])$):

$$\begin{aligned}
\frac{d}{dt}L &= \frac{1}{3}\left(\frac{\kappa[E_m]}{[E_G]}L\left(\frac{\dot{v}}{L}+\dot{k}_M\right)\frac{eg}{e+g}-\dot{k}_M L\right) && (2.102)\\
&= \frac{1}{3}\left(\left(\dot{v}+\dot{k}_M L\right)\frac{e}{e+g}-\dot{k}_M L\right) && (2.103)\\
&= \frac{1}{3}\left(\dot{v}\frac{e}{e+g}+\dot{k}_M L\frac{e}{e+g}-\dot{k}_M L\right) && (2.104)\\
&= \frac{1}{3}\left(\dot{v}\frac{e}{e+g}+\dot{k}_M L\left(\frac{e}{e+g}-1\right)\right) && (2.105)\\
&= \frac{1}{3}\left(\dot{v}\frac{e}{e+g}+\dot{k}_M L\frac{e-(e+g)}{e+g}\right) && (2.106)\\
&= \frac{1}{3(e+g)}\left(\dot{v}e-\dot{k}_M Lg\right) && (2.107)\\
&= \frac{\dot{k}_M g}{3(e+g)}\left(e\frac{\dot{v}}{\dot{k}_M g}-L\right) && (2.108)
\end{aligned}$$

This is the Von Bertalanffy result of DEB theory, as long as the parameters are constant.

2.5 Link to physical measures of body size

The amount of structure of an organism cannot be directly observed. So how do we relate structural length L to measurements of 'real' body size L_w? In an organism that grows strictly isomorphically, any length measure is proportional to the structural length. However, the proportionality constant depends on how the length is taken. The proportionality is the shape-correction coefficient:

$$L = \delta_M L_w \tag{2.109}$$

The shape coefficient is specific for each length measure. It is difficult to obtain though, as the real structural length cannot be directly obtained from measurements. As an approximation, one could use (assuming that reserves do not contribute appreciably to the total volume):

$$\delta_M \approx \frac{V_w^{1/3}}{L_w} \tag{2.110}$$

The physical volume or the weight is a weighted sum of structure, reserve and reproduction buffer (and possibly gut contents). The physical volume is given by:

$$V_w = L^3 + \omega_V(e+e_R)L^3 \quad \text{with} \quad e_R = \dot{v}\frac{U_R}{L^3} \tag{2.111}$$

where ω_V is a dimensionless parameter for the contribution of the reserves (including the reserves in the reproduction buffer) to the total volume.

In the absence of an appreciable reproduction buffer, and when e is constant, the physical volume is proportional to structural volume L^3. The proportionality constant than only matters when you want to compare parameter values between species, or when comparing life history at different food levels.

The wet and dry weight of an organism is related to structural volume in a similar way, accounting for the density of the wet or dry structure (d_V and d_{Vd}):

$$W_w = d_V L^3 (1 + \omega_w(e + e_R)) \quad (2.112)$$
$$W_d = d_{Vd} L^3 (1 + \omega_d(e + e_R)) \quad (2.113)$$

One way to estimate ω values is to follow volume or weight under mild starvation. Under starvation, the organism will initially only burn reserves, but under prolonged starvation, it will also burn structure and/or the contents of the reproduction buffer. Another option is to follow the volume or weights of individuals on egg production (for organisms that sport a substantial reproduction buffer). Alternatively (and probably most practically), we can estimate ω values from the egg size. The egg is completely made up of reserve, and in the standard DEB animal, there is only one type of reserve (although the water content of the egg may differ from that of the juvenile and adult).

For example, the volume of a single egg V_w^0 is given by:

$$V_w^0 = \omega_V e_R^0 L^3 \quad \text{with} \quad e_R^0 = \dot{v} \frac{U_E^0}{L^3} \quad (2.114)$$

Where e_R^0 is the scaled reproduction buffer density corresponding to a single egg. And thus:

$$\omega_V = \frac{V_w^0}{\dot{v} U_E^0} \quad (2.115)$$

As a last option, we can use the add-my-pet defaults for unknown parameters. Following the DEB book (Section 3.2), the ω_V is given by:

$$\omega_V = [E_m] \frac{w_E}{d_E \mu_E} \quad (2.116)$$
$$\omega_w = [E_m] \frac{w_E}{d_V \mu_E} \quad (2.117)$$
$$\omega_d = [E_m] \frac{w_{Ed}}{d_{Vd} \mu_E} \quad (2.118)$$

For three of these parameters, we can use the defaults as given in the add my pet approach. For μ_E (the chemical potential of reserve), a default value of $550 \cdot 10^3$ J/mol is taken. Further, we can take $d_E = d_V = 1$ g/cm^3, and $d_{Vd} = 0.1$ g/cm^3. The values for w_E and w_{Ed} (molecular weights of wet and dry reserve) can be estimated from the general elemental composition of reserve (assuming that wet reserve contains a factor of 10 more H and O relative to C):

$$w_{Ed} = 1 \cdot 12 + 2 \cdot 1 + 0.75 \cdot 16 + 0.2 \cdot 14 = 28.8 \text{ g/mol} \quad (2.119)$$
$$w_E = 1 \cdot 12 + 20 \cdot 1 + 7.5 \cdot 16 + 0.2 \cdot 14 = 154.8 \text{ g/mol} \quad (2.120)$$

We can use these defaults to provide a default for the values for the ω's (and replacing $[E_m] = \{\dot{p}_{Am}\}/\dot{v}$):

$$\omega_V = [E_m] \frac{154.8}{1 \cdot 550 \cdot 10^3} = \frac{\{\dot{p}_{Am}\}}{\dot{v}} 2.8 \cdot 10^{-4} \quad (2.121)$$
$$\omega_w = [E_m] \frac{154.8}{1 \cdot 550 \cdot 10^3} = \frac{\{\dot{p}_{Am}\}}{\dot{v}} 2.8 \cdot 10^{-4} \quad (2.122)$$
$$\omega_d = [E_m] \frac{28.8}{0.1 \cdot 550 \cdot 10^3} = \frac{\{\dot{p}_{Am}\}}{\dot{v}} 5.2 \cdot 10^{-4} \quad (2.123)$$

Using defaults of $\dot{v} = 0.02$ cm/d and $\{\dot{p}_{Am}\} = 22.5\, z$ J/cm^2/d, we see that the value for ω_V and ω_w is expected to be around $0.32\, z$, and ω_{Vd} around $0.59\, z$.

2.6 Mortality in DEB models

In a DEB context, mortality is treated as a chance process. Chance processes in time are modelled using the hazard rate. The hazard rate is the instantaneous probability to die, or more precisely:

$$\dot{h}(t) = \lim_{\Delta t \to 0} \frac{S(t) - S(t + \Delta t)}{\Delta t S(t)} = -\frac{1}{S}\frac{d}{dt}S \tag{2.124}$$

Thus, the hazard rate times a very small time increment gives the probability to die in that interval, given that you are alive at the start of that interval. In experiments with a short duration, the hazard rate can usually be taken constant. However, for longer durations, we will probably see an increase in the hazard rate with the age of the organisms (senescence). If we do not feed the organisms in the test, we will also see an increase in the hazard rate due to starvation.

If we know the hazard rate as function of time, we can calculate the survival probability over time as:

$$S(t) = \exp\left(-\int_0^t \dot{h}(\tau)\, d\tau\right) \tag{2.125}$$

Ageing or senescence is treated as an effect of a toxicant; in this case, produced by the organism itself, as a byproduct of metabolism.

2.6.1 Tolerance distributions

We can define the spread of any distribution that is symmetrical on log-scale by a spread factor F_s. I will define F_s as the factor between the median α and the 2.5-th and 97.5-th percentile. The range between α/F_s and $\alpha \times F_s$ thus covers 95% of the distribution. For the lognormal distribution, we can link F_s to the standard deviation of the associated normal distribution after log transformation. After log transformation, we can find the lower 2.5th percentile at:

$$0.025 = F(-1.96\sigma_{\log} + \log\alpha) \tag{2.126}$$

where F is the cumulative normal distribution, $\sigma_{\log}$ is the normal standard deviation after log transformation, and $\log\alpha$ the mean of that distribution (which equals the median of the log-normal distribution).

$$\frac{\alpha}{F_s} = 10^{-1.96\sigma_{\log} + \log\alpha} \tag{2.127}$$

$$\frac{\alpha}{F_s} = \alpha 10^{-1.96\sigma_{\log}} \tag{2.128}$$

$$\frac{1}{F_s} = 10^{-1.96\sigma_{\log}} \tag{2.129}$$

$$-\log F_s = -1.96\sigma_{\log} \tag{2.130}$$

$$\frac{\log F_s}{1.96} = \sigma_{\log} \tag{2.131}$$

The fraction survival thus follows from the cumulative density function of the normal distribution:

$$S(x) = 1 - F\left(\log x; \log \alpha, \sigma_{\log}\right) \tag{2.132}$$

For the log-logistic distribution, we can also define the spread F_s in the same way. The fraction of surviving individuals is the reciprocal of the CDF at a value of the dose metric x:

$$S(x) = \frac{1}{1 + \left(\frac{x}{\alpha}\right)^{\beta}} \tag{2.133}$$

where α is the median of the tolerance distribution, and β the shape parameter. We define F_s in such a way that at $x = \alpha \times F_s$ the survival $S = 0.025$. Thus:

$$\begin{aligned}
\frac{1}{1 + \left(\frac{x}{\alpha}\right)^{\beta}} &= 0.025 && (2.134)\\
\left(\frac{x}{\alpha}\right)^{\beta} &= \frac{1}{0.025} - 1 && (2.135)\\
x &= \alpha \left(40 - 1\right)^{1/\beta} && (2.136)\\
F_s = \frac{x}{\alpha} \Rightarrow F_s &= 39^{1/\beta} && (2.137)\\
\beta &= \frac{\ln 39}{\ln F_s} && (2.138)
\end{aligned}$$

Or, as Wikipedia puts it more generally, the p-th quantile can be calculated from the inverse cumulative distribution:

$$\begin{aligned}
F^{-1}(p; \alpha, \beta) &= \alpha \left(\frac{p}{1-p}\right)^{1/\beta} && (2.139)\\
F_s &= \left(\frac{0.975}{1 - 0.975}\right)^{1/\beta} && (2.140)
\end{aligned}$$

2.7 Changes in temperature

We can assume that all metabolic rate constants are affected by temperature by the same factor. The elimination rate for chemicals is a special case, as it is not a metabolic rate constant (unless the elimination is dominated by biotransformation). Here, I give the expression to calculate the maintenance rate coefficient $\dot{k}_M$ at temperature T from its reference value $\dot{k}_M^{\text{ref}}$ at the reference temperature T_{ref}:

$$\dot{k}_M(T) = \dot{k}_M^{\text{ref}} \exp\left(\frac{T_A}{T_{\text{ref}}} - \frac{T_A}{T}\right) \tag{2.141}$$

where T_A is known as the Arrhenius temperature. Note that all T are here as absolute temperature in Kelvin.

Temperature also affects the maximum surface-specific assimilation rate $\{\dot{p}_{Am}\}$. This parameter is used to scale all of the reserves. Changing the scaling over time is not really advisable. We can scale with $\{\dot{p}_{Am}\}$ at a reference temperature though. Introduce a temperature correction factor for all rates:

$$F_T = \exp\left(\frac{T_A}{T_{\text{ref}}} - \frac{T_A}{T}\right) \tag{2.142}$$

Now rewrite the reserve dynamics with this factor:

$$\frac{d}{dt}E = fL^2F_T\{\dot{p}^{\mathrm{ref}}_{Am}\} - \dot{p}_C \tag{2.143}$$

$$\dot{p}_C = [E_m]L^3\left(\frac{\dot{v}}{L} + \dot{k}_M\right)\frac{eg}{e+g} \tag{2.144}$$

We now scale all energy-containing states with the reference assimilation $\{\dot{p}^{\mathrm{ref}}_{Am}\}$ to obtain U_E and S_C (note that $\{\dot{p}^{\mathrm{ref}}_{Am}\} = \dot{v}^{\mathrm{ref}}[E_m]$):

$$\frac{d}{dt}U_E = fL^2F_T - S_C \tag{2.145}$$

$$S_C = \frac{L^3}{\dot{v}^{\mathrm{ref}}}\left(\frac{\dot{v}}{L} + \dot{k}_M\right)\frac{eg}{e+g} \tag{2.146}$$

$$S_C = \frac{L^3}{\dot{v}^{\mathrm{ref}}}\left(\frac{\dot{v}^{\mathrm{ref}}F_T}{L} + \dot{k}^{\mathrm{ref}}_M F_T\right)\frac{eg}{e+g} \tag{2.147}$$

$$S_C = L^2F_T\left(1 + \frac{\dot{k}^{\mathrm{ref}}_M}{\dot{v}^{\mathrm{ref}}}L\right)\frac{eg}{e+g} \tag{2.148}$$

For the growth equation:

$$\frac{d}{dt}L^3 = \frac{1}{[E_G]}\left(\kappa\dot{p}_C - \dot{p}_M\right) \quad \text{with } L(0) \approx 0 \tag{2.149}$$

$$= \frac{\kappa\{\dot{p}^{\mathrm{ref}}_{Am}\}}{[E_G]}S_C - \frac{\dot{p}_M}{[E_G]} \tag{2.150}$$

$$= \frac{\dot{v}^{\mathrm{ref}}}{g}S_C - F_T\dot{k}^{\mathrm{ref}}_M L^3 \tag{2.151}$$

This derivation makes use of the relationship given in Equation 2.37. The final body size equation is thus:

$$\frac{d}{dt}L = \frac{1}{3L^2}\left(\frac{\dot{v}^{\mathrm{ref}}}{g}S_C - F_T\dot{k}^{\mathrm{ref}}_M L^3\right) \tag{2.152}$$

For maturation and reproduction, only application of the correction factor F_T on $\dot{k}^{\mathrm{ref}}_J$ is needed.

2.8 Deviating growth curves

2.8.1 Slow initial growth

One possibility to include an initial food limitation for juveniles was presented by [6]. The scaled functional response was taken as a hyperbolic function in structural volume:

$$f(L) = f\left(1 + \frac{L_f^3}{L^3}\right)^{-1} \tag{2.153}$$

There is nothing much mechanistic about this function, but it worked well for nematodes.

2.8.2 Acceleration through a V1-morphic stage

For a number of species, the feeding stages grow substantially faster than the embryo. In other words, the parameter set for the juvenile/adult yields predictions for the embryonic stage that are too short. One way to solve this issue is to assume an acceleration through a V1 stage between birth and 'metamorphosis' (at maturity level U_H^j) [18, 1]. V1 morphy implies that $\dot{v}$ and $\{\dot{p}_{Am}\}$ need to be multiplied by a factor δ, which depends on body size:

$$U_H < U_H^b \quad \rightarrow \quad \delta = 1 \tag{2.154}$$

$$U_H^b < U_H < U_H^j \quad \rightarrow \quad \delta = \frac{L}{L_b} \tag{2.155}$$

$$U_H > U_H^j \quad \rightarrow \quad \delta = \frac{L_j}{L_b} \tag{2.156}$$

For the unscaled model, we can simply apply this factor on $\dot{v}$ and $\{\dot{p}_{Am}\}$. The only thing to take care of is that L_j is not known before we start the model run. We thus have to remember its value when the organism reaches that point in its development.

For the scaled model, we first rewrite the reserve dynamics with this factor:

$$\frac{d}{dt}E = fL^2\delta\{\dot{p}_{Am}\} - \dot{p}_C \tag{2.157}$$

$$\dot{p}_C = [E_m]L^3\left(\frac{\delta\dot{v}}{L} + \dot{k}_M\right)\frac{eg}{e+g} \tag{2.158}$$

Now we can scale with $\{\dot{p}_{Am}\}$, which is now the specific assimilation of the embryo:

$$\frac{d}{dt}U_E = fL^2\delta - S_C \tag{2.159}$$

$$S_C = \frac{L^3}{\dot{v}}\left(\frac{\delta\dot{v}}{L} + \dot{k}_M\right)\frac{eg}{e+g} \tag{2.160}$$

$$S_C = L^2\left(\delta + \frac{\dot{k}_M}{\dot{v}}L\right)\frac{eg}{e+g} \tag{2.161}$$

The growth, maturity and reproduction do not directly depend on $\dot{v}$ and $\{\dot{p}_{Am}\}$ (only through S_C). The $\dot{v}$ that appears in the growth equation is the one for the embryo (because S_C was scaled to the embryonic specific assimilation). This is quite comparable to the derivation for temperature effects in the previous section, but without the effects on maintenance. Note that the final size is now δL_m.

2.9 Alternative rules for hatching

In the standard DEB model, E_H^p is a model parameter, and the egg costs (E_0) have to be found through iteration to ensure hatching of the embryo with the same reserve density as its mother (the maternal effect). Instead of hatching at a fixed maturity level, we can also assume hatching when the embryo reaches $e = 1$, and use E_0 as a model parameter. This allows for more freedom for the mother to play with investment per egg (as is often observed). E_H^b is than a model output, and not a parameter.

In *Daphnia*, hatchling size increases with the size of the mother, and increases with a decrease in food level (opposite to the DEB prediction). Furthermore, exposure to a particular compound leads to the production of more but smaller offspring, which also cannot be explained in the standard framework. It seems more intuitive to see this as a change in the investment per egg than as a change in maturity at birth. When hatchling size increases with size of the mother, we can be assume a linear increase with length as a first approach. When we know egg costs at one specific size (U_E^{0*} at L^*), we can calculate them at any other size with a proportionality α:

$$U_E^0 = \alpha(L - L^*) + U_E^{0*} \tag{2.162}$$

Chapter 3

Toxicokinetics

3.1 Deriving the one-compartment model from diffusion

First, consider a box filled with water, floating in a well-mixed swimming pool with water. At a certain time point, an amount of chemical is dropped in the pool; the outside walls of the box allow the chemical to pass in both directions. The transport of chemical between the swimming pool and the box takes place through molecular diffusion (the box is just like the pool considered to be well mixed). According to Fick's first law, diffusion of a chemical in a single solvent is proportional to the surface area for exchange and the concentration difference between two regions ΔC. The surface area for diffusive exchange is proportional to the volumetric length of the box squared (the proportionality is determined by a shape correction δ_Q), assuming it does not change in shape. The mass flux is thus given by (M_Q is the moles of toxicant in the box):

$$\frac{d}{dt}M_Q = \dot{v}_Q(\delta_Q L)^2\Delta C \tag{3.1}$$

The proportionality constant $\dot{v}_Q$ is the ratio of the diffusion coefficient and the distance over which transport takes place. This constant is a conductance (dimensions of a length per time) and is known in environmental chemistry as a mass-transfer coefficient or 'piston velocity'. The chemical conductance depends on the chemical (mainly on molecular weight, when considering transport in a single solvent), on the nature of the interface, and on the temperature, but does not depend on the size of the box. We will discuss the nature of this conductance in Section 3.2.

We can change this mass flux into a concentration change by dividing both sides of Eq. 3.1 by the volume of the box V. However, the volume may not be constant; the box may grow or shrink in a particular manner (however, let's assume it does not change in shape, so all growth and shrinking is isomorphic). Using the product rule for the derivative:

$$\frac{d}{dt}[M_Q] = \frac{d}{dt}M_Q V^{-1} = V^{-1}\frac{d}{dt}M_Q + M_Q\frac{d}{dt}V^{-1} \tag{3.2}$$

and the chain rule for the last term:

$$\begin{aligned}\frac{d}{dt}[M_Q] &= V^{-1}\frac{d}{dt}M_Q - M_Q\frac{1}{V^2}\frac{d}{dt}V &(3.3)\\ &= \frac{1}{V}\frac{d}{dt}M_Q - [M_Q]\frac{1}{V}\frac{d}{dt}V &(3.4)\end{aligned}$$

This equation, we can use to rewrite Eq. 3.1, noting that $1/V \frac{d}{dt}V = \frac{d}{dt}lnV$:

$$\frac{d}{dt}[M_Q] = \dot{v}_Q \frac{(\delta_Q L)^2}{V}\Delta C - [M_Q]\frac{d}{dt}lnV \tag{3.5}$$

The last term accounts for the effects of a change in volume on the concentration. The parameters in front of the concentration gradient ΔC together form the 'elimination rate constant' $\dot{k}_e$, which is the concentration-specific elimination rate from the box. Therefore, $\dot{k}_e$ is given by:

$$\dot{k}_e = \dot{v}_Q \frac{(\delta_Q L)^2}{V} = \frac{\dot{v}_Q \delta_Q^2}{L} \tag{3.6}$$

When the box changes in size, the surface:volume ratio changes, which implies that $\dot{k}_e$ is not a constant anymore but is proportional to surface area/volume, and therefore scales with L^{-1}. Even though $\dot{k}_e$ is a function of the size of the box, we can still work with an elimination rate constant as a parameter, but we would have to define a reference situation. One possible reference is the situation where $L = L_{m0}$:

$$\dot{k}_e^{\text{ref}} = \frac{\dot{v}_Q \delta_Q^2}{L_{m0}} \tag{3.7}$$

In this way, $\dot{k}_e^{\text{ref}}$ has the interpretation of the elimination rate of an organism at the maximum size reached under control conditions, L_{m0} (smaller individuals will have a larger elimination rate). Thus, the elimination rate constant can be written as a modification of the reference rate constant:

$$\dot{k}_e = \dot{k}_e^{\text{ref}} \frac{L_{m0}}{L} \tag{3.8}$$

We still need to think about ΔC. In ecotoxicology, we are not dealing with a chemical in a single solvent but with the transfer from the environment to an organism. This can be considered as a situation with two solvents (still, a rather heroic simplification). We cannot directly compare the concentration in the environment to the concentration in the organism, but we can use the difference between the actual and the final concentration as the driving force:

$$\Delta C = [M_Q]_\infty - [M_Q] = P_{Vd} c_d - [M_Q] \tag{3.9}$$

With that, the full equation for the body density of toxicant becomes:

$$\frac{d}{dt}[M_Q] = \dot{k}_e^{\text{ref}} \frac{L_{m0}}{L} \left(P_{Vd} c_d - [M_Q]\right) - [M_Q]\frac{d}{dt}lnV \tag{3.10}$$

$$= \dot{k}_e^{\text{ref}} \frac{L_{m0}}{L} \left(P_{Vd} c_d - [M_Q]\right) - [M_Q]\frac{3}{L}\frac{d}{dt}L \tag{3.11}$$

Here, it is written in terms of a single rate constant (the 'elimination' rate constant, $\dot{k}_e$) and a partition coefficient (the bioconcentration factor, P_{Vd}). This formulation is completely equivalent to the one with two rate constants (for uptake and elimination) that is also commonly used. I prefer this formulation for its link with diffusion: the flux into the organism is determined by the concentration gradient (corrected for the differences in affinity using the partition coefficient).

3.1.1 The scaled internal concentration

We cannot estimate P_{Vd} from effects data alone. When measured internal concentrations are not available, we have to resort to scaling: both sides of Eq. 3.1 can be divided by P_{Vd}. The scaled version of the internal concentration ($c_V = [M_Q]/P_{Vd}$) has the dimensions of an external concentration (in equilibrium, $c_{V\infty} = c_d$, for constant c_d):

$$\frac{d}{dt}c_V = \dot{k}_e^{\text{ref}}\frac{L_{m0}}{L}(c_d - c_V) - c_V\frac{3}{L}\frac{d}{dt}L \tag{3.12}$$

For organisms that do not change in size appreciably, this equation can be simplified by removing the size influence (there is no need for $\dot{k}_e^{\text{ref}}$ anymore):

$$\frac{d}{dt}c_V = \dot{k}_e(c_d - c_V) \tag{3.13}$$

3.2 The nature of the chemical conductance

Elimination rates depend on the chemical; they tend to decrease with increasing hydrophobicity. The only chemical-specific component in Eq. 3.6 is the conductance $\dot{v}_Q$. This conductance will undoubtedly depend on details of the interface between the organism and its environment. It is safe to assume that chemicals need to be dissolved in a water phase before they can cross biological membranes. Therefore, if we consider uptake as a diffusion process, we have to consider the transport from the water phase, across a lipid membrane, into the organism.

In environmental chemistry, transport from one phase to another (e.g., water to air) is generally described by the 'two-film model' (see e.g., [24]). This model considers that the bulk of the medium is well mixed, but that there is a stagnant boundary layer that 'sticks' to each side of the interface. Transport through these boundary layers has to rely on molecular diffusion, which is slow. The total resistance for diffusion is given by the sum of the chemical's resistances in each phase (the resistance of the interface itself is usually ignored). Strictly speaking, the resulting model is not a 'one-compartment model' anymore as the concentration is not homogeneous within the phase. In the stagnant boundary layer, there is a concentration gradient between the bulk concentration and the concentration at the interface. The mathematics of this model are rather complex (see [17]), but usually, a somewhat crude approximation is made. When we assume that the flux across the interface is steady, the concentration profiles at each side remain constant, and the model simplifies extremely. Of course, a constant flux is unlikely to persist for long in a system of limited capacity such as a living organism, nevertheless, this assumption seems to provide an adequate approximation.

In steady flux, we obtain a one-compartment model for the organism again, when we can ignore the volume of the unstirred boundaries. The elimination rate than depends on the total conductance $\dot{v}_Q$, which is made up of the conductances of the chemical in water $\dot{v}_{Qw}$ and membrane lipids $\dot{v}_{QV}$, and on the partition coefficient:

$$\dot{v}_Q = \left(\frac{1}{\dot{v}_{QV}} + \frac{P_{Vd}}{\dot{v}_{Qw}}\right)^{-1} \tag{3.14}$$

Note that I assume that the membrane side is part of the structure. Therefore I indicate the conductance and the partition coefficients with a subscript for structure (V). The partition coefficient is used to translate the water conductance (dimension lt^{-1}) to the organism perspective that is needed for the total conductance (dimension Lt^{-1}). Also note that this model is exactly skew symmetric. That is, we can change the perspective from organism to water and

obtain the same relations. The relationship between the elimination rate from the organism to the water phase and the organism-water partition coefficient is the same as the relationship between the elimination rate from water to the organism is with the water-organism partition coefficient.

The two conductances do not depend very much on chemical properties (mainly on molecular weight). Therefore, at low values of the bioconcentration factor, the total conductance (and therefore the elimination rate) hardly depends on P_{Vd}. At high values of the bioconcentration factor, the elimination rate decreases proportional to P_{Vd}.

Kooijman [15] promotes a square-root relationship between the elimination rate and the octanol-water partition coefficient (P_{ow}). I don't endorse this relationship for several reasons. First, and least important, the elimination rate should be related to P_{Vd} and not P_{ow}. Even though these two are correlated, organisms do not contain octanol. My other reason is that the square-root relationship follows from the very strict one-compartment assumption that both phases (organism and the water phase in the environment) are well mixed, up to the interface. In my opinion, this assumption is less defensible than the assumption of 'steady flux' across two stagnant boundary layers. In artificial membrane systems, the existence of these stagnant boundaries (and their effect on diffusive resistance) have been experimentally confirmed [3]. Furthermore, the predictions of the two-film steady-flux model for the rate constants in SPME-coated fibers are extremely accurate [17]. However, in organisms, the observations contain too much scatter to distinguish between these two alternatives. A final piece of evidence is the observation that dissolved organic carbon can enhance the uptake of PAHs in organisms and fibers, when the water-side film is rate limiting [25]. This strongly suggests that such a film exists and that it can be rate limiting.

Even though it would be nice to predict elimination rates for untested compounds, the above discussion is of limited relevance for toxic effects, in my opinion. These elimination rates are at best relevant for whole-body residues, and these concentrations are probably not directly relevant for toxic effects (see main text).

3.3 Considering reserve and structure

Changes in composition due to varying food levels and/or reproductive behaviour has logical effect on toxicokinetics in DEB animals. In the following, I work out these effects on toxicokinetics, under the assumption that the internal redistribution between structure, reserves and reproduction buffer is fast (relative to the exchange with the environment). First, reserve and reproduction buffer are assumed to have the same composition, an assumption that is later on loosened.

3.3.1 Same composition of reserves and structure

In this section, we complicate things a little further by acknowledging the fact that reserves and reproduction buffer add to the total physical volume. First, we assume that reserves and reproduction buffer behave exactly the same as structure for toxicokinetics (they thus have the same composition). Instead of the structural volume V, we therefore have to consider the (dynamics of) the physical volume V_w. First, we assume that the exchange with the environment occurs through structure only; and thus that the surface area for exchange is proportional to L^2. think for example of fish, where the main surface area for exchange is the gills, or situations where the main route of uptake is across the gut wall.

The physical volume is made up from contributions of structure, reserve and reproduction buffer:

$$V_w = V + (e + e_R)\omega_V V \quad \text{where} \quad V = L^3 \tag{3.15}$$

The scaled buffer e_R is defined as:

$$e_R = \dot{v}\frac{U_R}{L^3} \tag{3.16}$$

The elimination rate constant depends on the ratio of the surface area for exchange and the volume of interest. The volume is now the physical volume, and not the structural volume. Therefore, $\dot{k}_e$ is given by:

$$\dot{k}_e = \dot{v}_Q \frac{(\delta_Q L)^2}{V_w} = \dot{v}_Q \frac{\delta_Q^2 L^2}{V + (e + e_R)\omega_V V} = \frac{\dot{v}_Q \delta_Q^2}{L}\frac{1}{1 + (e + e_R)\omega_V} \tag{3.17}$$

Interestingly, this equation shows that the elimination rate not only depends on structural length but also on the reserve density and the filling of the reproduction buffer. More reserves implies a lower elimination rate. This is not surprising as reserves add to the total volume, but not to the surface area for exchange (at least, that is what I have assumed).

We can still work with an elimination rate constant as a parameter, but we would have to define a reference situation. One possible reference is the situation where $L = L_{m0}$, $e = 1$ and $e_R = 0$:

$$\dot{k}_e^{\text{ref}} = \frac{\dot{v}_Q \delta_Q^2}{L_{m0}}\frac{1}{1 + \omega_V} \tag{3.18}$$

Thus, the elimination rate constant can be written as a modification of the reference rate constant:

$$\dot{k}_e = \dot{k}_e^{\text{ref}}\frac{L_{m0}}{L}\frac{1 + \omega_V}{1 + (e + e_R)\omega_V} \tag{3.19}$$

When we only deal with situation under ad libitum food and no effects on assimilation ($e = 1$) and when we can ignore the contribution of the reproduction buffer, we obtain:

$$\dot{k}_e = \dot{k}_e^{\text{ref}}\frac{L_{m0}}{L} \tag{3.20}$$

We can also assume that the area for exchange is determined by the physical volume, instead of the structural volume. Think for example of the situation where the outer surface area of an organism is the exchange area (e.g., an earthworm that does not feed on soil). We obtain:

$$\dot{k}_e = \frac{\dot{v}_Q \delta_Q^2}{V_w^{1/3}} = \frac{\dot{v}_Q \delta_Q^2}{(V + (e + e_R)\omega_V V)^{1/3}} = \frac{\dot{v}_Q \delta_Q^2}{L\,(1 + (e + e_R)\omega_V)^{1/3}} \tag{3.21}$$

Similarly, we can introduce a reference rate constant at $L = L_{m0}$, $e = 1$ and $e_R = 0$, which leads to:

$$\dot{k}_e = \dot{k}_e^{\text{ref}}\frac{L_{m0}}{L}\left(\frac{1 + \omega_V}{1 + (e + e_R)\omega_V}\right)^{1/3} \tag{3.22}$$

Again, when we only deal with situation under ad libitum food and without effects on assimilation ($e = 1$) and when we can ignore the contribution of the reproduction buffer, we obtain the simple Eq. 3.6. However, it is unlikely that changes due to reserve and reproduction buffer lead to an isomorphic change in size. To account properly for this option thus would

require considering these shape changes (which might not be trivial). Again, when we only deal with situation under ad libitum food and without effects on assimilation ($e = 1$) and when we can ignore the contribution of the reproduction buffer, we obtain the simple $\dot{k}_e$ as in Eq. 3.20.

Because the physical volume V_w can change in time rather suddenly (when the reproduction buffer is emptied), it is perhaps easier to keep the mass flux equation for M_Q, and not go to concentrations with an ODE:

$$\frac{d}{dt}M_Q = \dot{k}_e V_w \left(P_{Vd} c_d - \frac{M_Q}{V_w} \right) \tag{3.23}$$

$$= \dot{k}_e V \left(P_{Vd} c_d \left(1 + (e + e_R)\omega_V\right) - \frac{M_Q}{V} \right) \tag{3.24}$$

The total mass of chemical in the body is made up of parts in each compartment, structure, reserve and reproduction buffer. However, in this situation, the concentration is the same in each compartment, M_Q/V_w. What happens during a spawning event? Probably the main part of the reproduction buffer is turned into a batch of eggs. Some reserves, not enough to turn into a single egg, might stay in the buffer or may be lost. Let's assume it stays in the buffer, and that from the total reproduction buffer U_R, an amount $U_E^0 \Delta R$ is used to make eggs and leaves the body. The remainder, with the associated compounds, is assumed to stay in the mother. The production of eggs leads to a decrease in physical volume, from resetting of the reproduction buffer. Further, there is a decrease in the total amount of chemical in the body. Of the chemical in the reproduction buffer, some leaves in the eggs, and some stays (the fraction that was not enough to build eggs, and the chemicals associated with the fraction of the buffer that is lost as overhead). What leaves is the fraction associated with the eggs, which is the concentration in the reproduction buffer (equal to the whole body) times the total volume of eggs produced:

$$\Delta M_Q = \frac{M_Q}{V_w} e_0 \omega_V V \Delta R \tag{3.25}$$

where e_0 is the scaled energy content of a single egg:

$$e_0 = \frac{E_0}{E_m} = \dot{v} \frac{U_E^0}{L^3} \tag{3.26}$$

Interestingly, these assumptions lead to slight increase in the mother's whole-body concentration, because part of the buffer is burnt off as overhead in the production of eggs (or: $e_0 \Delta R < e_R$).

Apparently, for the concentration it does not matter much that there are spawning events. The only things that matters is that the presence of reserves and reproduction buffer influence the elimination rate constant, and that the change in structure and reserves, and the build-up of the reproduction buffer, influences growth dilution.

3.3.2 Different composition of reserves and structure

In this section, I present the situation were the reserves and reproduction buffer have a different composition than structure, and thereby a different affinity for the chemical. The total mass of chemical in the body is made up of parts in each compartment, structure, reserve and reproduction buffer:

$$M_Q = M_{QV} + M_{QE} + M_{QR} \tag{3.27}$$

Let's assume that the internal redistribution between these compartments is fast, relative to the exchange with the outside environment. Further, assume that the reproduction buffer has the exact same properties as the reserves. This implies that we can treat the system as a one-compartment model, although the total partition coefficient is now a weighted sum[1] of the partition coefficients for structure P_{Vd} and reserve P_{Ed}:

$$P_{Wd} = \frac{M_Q}{c_d V_w} = \frac{P_{Vd}V + P_{Ed}(e+e_R)\omega_V V}{V_w} \tag{3.28}$$

Next, I introduce the partition coefficient P_{EV} as the ratio between the concentrations in reserve (including the reproduction buffer) and structure:

$$P_{EV} = \frac{M_{QE} + M_{QR}}{(e+e_R)\omega_V V}\frac{V}{M_{QV}} = \frac{P_{Ed}}{P_{Vd}} \tag{3.29}$$

Now, I can rewrite Eq. 3.28; after some manipulation:

$$P_{Wd} = P_{Vd}\frac{1 + P_{EV}(e+e_R)\omega_V}{1 + (e+e_R)\omega_V} \tag{3.30}$$

This equation shows that when $P_{EV} = 1$, there is no effect on the total partition coefficient, $P_{Wd} = P_{Vd}$.

The total chemical mass in the organism changes according to a one-compartment model, with time-varying parameters:

$$\frac{d}{dt}M_Q = V_w \dot{k}_e \left(P_{Wd}c_d - \frac{M_Q}{V_w}\right) \tag{3.31}$$

The elimination rate $\dot{k}_e$ also needs to be modified when reserve and structure have a different composition. Now, we cannot simply add both volumes; we need to convert the reserve and reproduction buffer to an equivalent volume of structure using P_{EV}. When exchange is assumed to related to a structural surface, we can modify Eq. 3.19:

$$\dot{k}_e = \dot{k}_e^{\mathrm{ref}}\frac{L_{m0}}{L}\frac{1+\omega_V}{1 + P_{EV}(e+e_R)\omega_V} \tag{3.32}$$

If exchange to occurs through the total surface, we can modify Eq. 3.22:

$$\dot{k}_e = \dot{k}_e^{\mathrm{ref}}\frac{L_{m0}}{L}\left(\frac{1+\omega_V}{1 + P_{EV}(e+e_R)\omega_V}\right)^{1/3} \tag{3.33}$$

In Section 3.2, I explained that elimination rates depend on the bioconcentration factor, and here we have a situation that the whole-body partitioning P_{Wd} can change in time. Should this also affect the elimination rate? Apart from the changes in elimination rate brought about by changes in representative volume (as implemented above), I do not think we need additional changes. The elimination rate depends on the properties of the interface, and thus (probably) on membrane lipids. I think it is safe to assume that the relevant partition coefficient depends on a structural component, which does not change with a change in reserves or reproduction buffer. Therefore, I think it is safe to assume that $\dot{k}_e^{\mathrm{ref}}$ depends on P_{Vd}, which remains constant in time (because of strong homeostasis, see also Section 3.2).

[1] I weigh with the volumes as the P_* are also on volume basis. This is contrary to common practice of expressing bioconcentration per (wet, dry or lipid) weight of organism.

It is probably easiest to follow the total mass of chemical, and not change the ODE to concentrations. Especially because the total volume might change due to growth, starvation (decrease of reserve), and reproduction (decrease of reproduction buffer). Furthermore, reproduction also may lead to a decrease in the total amount of chemical in the body due to transfer to the eggs. Keeping things on mass basis facilitates the mass balance. Any time we need the total concentration in the organism, we can calculate M_Q/V_w. This concentration is on a volume basis, so a translation to weights would require the total density (which is a weighted sum of the densities of structure and reserve).

We can fill in Eq. 3.30 for P_{Wd}:

$$\frac{d}{dt}M_Q = V_w\dot{k}_e\left(P_{Vd}\frac{1+P_{EV}(e+e_R)\omega_V}{1+(e+e_R)\omega_V}c_d - \frac{M_Q}{V_w}\right) \tag{3.34}$$

$$= V\dot{k}_e\left(P_{Vd}\left(1+P_{EV}(e+e_R)\omega_V\right)c_d - \frac{M_Q}{V}\right) \tag{3.35}$$

$$= \dot{k}_e\left(P_{Vd}c_dV\left(1+P_{EV}(e+e_R)\omega_V\right) - M_Q\right) \tag{3.36}$$

The toxic effects are most likely related to the concentration in the structure and not to the total body concentration. Further, the concentration in the eggs will likely be the same as the concentration in the reproduction buffer. Therefore, we also need access to M_{QV} and M_{QR}. From Eq. 3.27 it follows that $M_{QE}+M_{QR} = M_Q - M_{QV}$, which we can introduce in Eq. 3.29:

$$P_{EV} = \frac{M_Q - M_{QV}}{(e+e_R)\omega_V V}\frac{V}{M_{QV}} \tag{3.37}$$

which we can manipulate until:

$$P_{EV}(e+e_R)\omega_V = \frac{M_Q}{M_{QV}} - 1 \tag{3.38}$$

and thus:

$$M_{QV} = \frac{M_Q}{P_{EV}(e+e_R)\omega_V + 1} \quad \text{and} \quad M_{QR} = \frac{e_R}{e+e_R}(M_Q - M_{QV}) \tag{3.39}$$

An interesting consequence is that if the organism is starved, the concentration in the structure will increase, at least when $\dot{k}_e$ is small compared to the decrease rate of e. This is known in migrating birds to cause problems: during migration, toxicants stored in the reserves are mobilised, which can kill the bird. This example support the idea that toxic effects are most likely related to the concentration in structure and not in the whole body. The concentration in structure can at any moment be calculated as $M_{QV}/V = [M_{QV}]$, and related to metabolic processes.

What happens at spawning? We can use the scaled reserve density of Eq. 3.26 again. The total amount in the body is reduced by the amount in the reproduction buffer that is used for egg production (assuming that the remainder of the reserves, together with the associated toxicants, stays in the reproduction buffer). I take the total amount of chemical in the reserves and the buffer, and multiply it by the fraction of the (scaled) reserves that is actually transferred to eggs. This is the change in the total amount of chemical in the body:

$$\Delta M_Q = -\frac{e_0 \Delta R}{e_R} M_{QR} \tag{3.40}$$

$$= -\frac{e_0 \Delta R}{e + e_R}(M_Q - M_{QV}) \tag{3.41}$$

$$e_0 = \dot{v}\frac{U_E^0}{L^3} \tag{3.42}$$

where e_0 is the scaled reserves in a single egg (scaled to the maximum reserve density of the mother). The reproduction efficiency κ_R is already included through the calculation of ΔR. I assume that chemicals are transported only with the part of the reproduction buffer that actually makes it into the eggs. The remainder of the buffer (insufficient to make a whole egg) stays in the buffer, and the fraction $1 - \kappa_R$ is lost as overheads. I assume that the chemical load associated associated with these fractions of the buffer also stays in the mother.

Even though technically, the amount is not taken from M_Q but from M_{QR} only, this does not matter as I assumed rapid redistribution between the compartments. Furthermore, I would only like to follow the change in M_Q, to make life simple. Because Eq. 3.36 does not contain V_w anymore, I do not have to worry about the change in physical volume, brought about by emptying the buffer (which is taken care of by e_R). The production of eggs leads to a decrease in physical volume. The new physical volume follows from the structural volume again, and from e and e_R (Eq. 2.111), where the latter is reset after spawning (see Eq. 2.46).

3.3.3 Scaled version

For the standard TK model, we used a scaling with P_{Vd} to obtain a scaled internal concentration. Here, we should not scale with P_{Wd} as this partition coefficient can change over time. However, we can still scale M_Q with P_{Vd}. We can take the ODE for M_Q of Eq. 3.36, and divide both sides with P_{Vd}. I introduce a new symbol for the scaled amount[2] as $m_Q = M_Q/P_{Vd} = c_W V_w$:

$$\frac{d}{dt} m_Q = V\dot{k}_e \left((1 + P_{EV}(e + e_R)\omega_V)c_d - \frac{m_Q}{V} \right) \tag{3.43}$$

$$= \dot{k}_e \left(c_d V (1 + P_{EV}(e + e_R)\omega_V) - m_Q \right) \tag{3.44}$$

We can calculate the scaled amount m_Q without knowing the value of P_{Vd}. We can also simply calculate scaled amounts in structure m_{QV} and reserve m_{QR} as above. For the link with effects, we can calculate a scaled internal concentration as:

$$c_V = \frac{m_{QV}}{V} = \frac{M_{QV}}{P_{Vd} V} \tag{3.45}$$

The interpretation of this c_V is similar to that of the standard TK model. It is the scaled concentration in structure and not in the whole body. However, the interpretation that $c_{V\infty} = c_d$ is lost; the value of $c_{V\infty}$ now also depends on P_{EV}, ω_V and e, e_R (the relationship may thus vary in time).

[2] I use the symbol m different from the DEB book, where it is used as moles of compound relative to C-moles of structure.

3.3.4 Constant reproduction

This is a variation of the previous model, accounting for continuous reproduction. Again, for the elimination rate, either 3.19 or 3.22 can be used, with $e_R = 0$. The total mass of chemical is given by an additional term to Eq. 3.36:

$$\frac{d}{dt}M_Q = \dot{k}_e \left(c_d V P_{Vd} \left(1 + P_{EV}\, e\, \omega_V\right) - M_Q\right) - \dot{R}\frac{e_0}{e}(M_Q - M_{QV}) \tag{3.46}$$

The last term is the flux of toxicant with reproduction. This equation can again be scaled with P_{Vd}:

$$\frac{d}{dt}m_Q = \dot{k}_e \left(c_d V \left(1 + P_{EV}\, e\, \omega_V\right) - m_Q\right) - \dot{R}\frac{e_0}{e}(m_Q - m_{QV}) \tag{3.47}$$

3.3.5 Toxicokinetics in eggs

At this moment, there appears to be little known about TK inside the egg. The difference between the egg and the juvenile/adult lies in the surface area for exchange (a rather constant egg wall vs. a size-dependent exchange area such as gills or gut surface). It seems likely that the elimination rate during the egg phase is considerably lower than for the juvenile/adult, but how much lower is impossible to say without experimental data. For the chemical kinetics, we can basically use the same equations for the egg as for the juvenile/adult under time-varying composition. In the egg situation, we really need to account for this change in composition as the ratio of structure to reserve drastically increases during embryonic development. Of course, the embryo is not reproducing, so we do not need to account for e_R and buffer dynamics.

The initial amount of chemical in a single egg is:

$$M_Q^0 = \frac{\Delta M_Q}{\Delta R} \tag{3.48}$$

The initial total concentration in the eggs is than obtained from the change in chemical load and the total weight of the eggs[3] (note that V is the structural volume of the mother):

$$[M_Q^0] = \frac{\Delta M_Q}{e_0 \omega_V V \Delta R} = \frac{M_Q - M_{QV}}{(e + e_R)\omega_V V} \tag{3.49}$$

Or, in other words, equal to the concentration in the reserves and the reproduction buffer just before spawning. This also implies that the initial concentration in the structure of the embryo equals the concentration in the structure of the mother just before spawning (as the same P_{EV} applies).

3.4 Uptake from food

In case that the kinetics are dominated by uptake through the gut, and if feeding rates vary, it might make sense to include the scaled functional response as follows:

$$\frac{d}{dt}c_V = f\dot{k}_e^{\mathrm{ref}}\frac{L_{m0}}{L}\left(c_d - c_V\right) - c_V\dot{r} \tag{3.50}$$

Note that the inclusion of f in this manner does not lead to different ultimate body residues $c_{V\infty}$; it only affects the rate for chemical exchange with the environment. This is a very

[3] In the DEB book, the symbol $[M_Q^0]$ is used for the NEC on unscaled internal concentration.

simplistic solution to a rather complex problem, but it does not require additional parameters. Probably a more realistic model for uptake from food is provided in [8].

3.5 Saturating kinetics

In some cases, the uptake flux of a toxicant may not increase proportionally to the external concentration as the concentration increases. Such saturating uptake may occur when uptake is through an active process, but also when the dissolved concentration (often unknowingly) approaches the water solubility. We can accommodate this behaviour as:

$$\frac{d}{dt}c_V = k_e \left(\frac{c_{dK}}{c_d + c_{dK}} c_d - c_V \right) \tag{3.51}$$

where c_{dK} is the external dissolved concentration at which the uptake flux is half of the flux if not saturation would occur.

One problem that occurs in this way is that the actual bioconcentraition factor $[M_{QV}]/c_d$ is not constant anymore, but becomes a function of c_d. This does not invalidate the scaling used for c_V, because that is scaled with a constant P_{Vd}, which should now be interpreted as the maximum bioconcentration factor (thus at very low values of c_d). The NEC c_0 is defined against c_V (and can be called c_{V0}), and normally can be interpreted as the external concentration that does not lead to exceedance of the internal threshold ($c_{d0} = c_{V0}$). However, this interpretation is not valid anymore in the saturation case. We can still calculate the external threshold c_{d0} though, by starting from the steady-state situation where c_V is constant, evaluated exactly at the NEC:

$$\begin{aligned}
k_e \frac{c_{dK}}{c_{d0} + c_{dK}} c_{d0} &= k_e c_{V0} && (3.52)\\
c_{dK} c_{d0} &= c_{V0} c_{d0} + c_{V0} c_{dK} && (3.53)\\
c_{dK} c_{d0} - c_{V0} c_{d0} &= c_{V0} c_{dK} && (3.54)\\
c_{d0} &= \frac{c_{V0} c_{dK}}{c_{dK} - c_{V0}} && (3.55)\\
c_{d0} &= \left(\frac{1}{c_{V0}} - \frac{1}{c_{dK}} \right)^{-1} && (3.56)
\end{aligned}$$

From this equation, it can also easily be seen that:

$$c_{V0} = \left(\frac{1}{c_{d0}} + \frac{1}{c_{dK}} \right)^{-1} \tag{3.57}$$

In a similar fashion, we can model a saturating biotransformation rate as an additional elimination mechanism:

$$\frac{d}{dt}c_V = k_e \left(c_d - c_V \right) - \dot{k}_m \frac{c_{VK}}{c_V + c_{VK}} c_V \tag{3.58}$$

where $\dot{k}_m$ is the maximum rate constant for metabolic elimination, and c_{VK} is the half-saturation concentration in structure.

Chapter 4

Toxicodynamics

4.1 Effects of toxicants on metabolic parameters

For the effects of toxicants, I introduce a stress function as function of the dose metric. If the dose metric is the scaled internal concentration:

$$s = \frac{1}{c_T} \max\left(0, c_V - c_0\right) \equiv \frac{1}{c_T}(c_V - c_0)_+ \tag{4.1}$$

In this definition, s is a dimensionless indicator of the degree of stress on a DEB parameter. Below the no-effect concentration c_0, there is no effect. When the scaled internal concentration exceeds the NEC, the stress function increases in a linear fashion. Note that c_V is a scaled internal concentration, and therefore has the dimensions of an external concentration. This implies that c_0 and c_T also have the dimension of an external concentration. The NEC can thus be interpreted as the external concentration that does not lead to exceedance of the internal threshold, even after prolonged exposure.

Instead of the scaled internal concentration c_V we can use any dose metric that we like, including the actual internal concentration M_{QV}, or the damage density $[m_D]$. Choosing a different dose metric also implies that the threshold and tolerance obtain a different interpretation and different dimensions (e.g., using damage density as metric means that the threshold and tolerance are also damage densities).

Subsequently, s can be applied to one or more primary DEB parameters. Because the DEB formulations I presented earlier includes two compound parameters (g and $\dot{k}_M$) we have to be a bit more careful with these ones. The most obvious effect mechanisms are shown in Table 4.1.

4.1.1 Effects on the acquisition of energy

In Table 4.1, I provide several options for effects on the acquisition of energy for the reserves from food. I think that this covers all of the possible effects on the acquisition, apart perhaps from an effect on the incorporation of the assimilation flux into the reserves μ_{EA}, which would be hard to distinguish from an effect on the assimilation efficiency μ_{AX}.

Note that I did not show an option for a toxicant to decrease $\{\dot{p}_{Am}\}$. This is because this parameter follows from $\{\dot{J}_{XAm}\}$ (see Eq. 2.10). This latter is considered as primary parameter here. However, a change in $\{\dot{J}_{XAm}\}$ or μ_{AX} leads to a change in $\{\dot{p}_{Am}\}$, which leads to problems in the scaled DEB model, as all reserve amounts and fluxes are scaled with this parameter. Scaling with a parameter that itself changes in time is asking for problems. Fortunately, we can see from Eq. 2.10 and 2.7 that we can account for all effects on the

Mode of action	Target parameter	DEB parameters under stress
Effects on energy acquisition		
Decrease assimilation efficiency	μ_{AX}	$\mu_{AX}(1-s)_+$
Decrease in maximum feeding rate	$\{\dot{J}_{XAm}\}$	$\{\dot{J}_{XAm}\}(1-s)_+$
Increase in food handling time	t_p	$\{\dot{J}_{XAm}\}\frac{1}{1+s}$
Decrease in food searching or filtering rate	$\{\dot{F}_m\}$	$\{\dot{F}_m\}(1-s)_+$
Effects on energy use		
Increase in somatic maintenance	$[\dot{p}_M]$	$\dot{k}_M(1+s)$
Increase in maturity maintenance	$\dot{k}_J$	$\dot{k}_J(1+s)$
Increase in overhead costs for structure	$[E_G]$	$g(1+s), \dot{k}_M/(1+s)$
Increase in overhead costs for maturation	$\frac{d}{dt}U_H$	$\kappa_H/(1+s)$
Increase in overhead costs for making an egg	κ_R	$\kappa_R/(1+s)$
Hazard during oogenesis	κ_R	$\kappa_R \exp(-s)$
Increase in allocation to soma	κ	$\kappa(1+s)_a, g/(1+s)_a$
Decrease in allocation to soma	κ	$\kappa(1-s)_+, g/(1-s)_+$
Increase in mobilisation from reserve	$\dot{v}$	$\dot{v}(1+s), g(1+s)$
Decrease in mobilisation from reserve	$\dot{v}$	$\dot{v}(1-s)_+, g(1-s)_+$
Increase in Weibull ageing	$\dot{k}_W$	$\dot{k}_W(1+s)$
Increase in Gompertz ageing	$\dot{k}_G$	$\dot{k}_G(1+s)$

Table 4.1: Possible effect mechanisms for the scaled DEB model as presented in Section 2.2. Combinations of these mechanisms is possible. The $(1+s)_a$ means that an upper boundary for s has to be used, such that $\kappa \leq 1$

acquisition of energy by including them in the scaled functional response f. However, if we need to build a mass balance for food or nutrients, we have to be careful with the various options. For example, an effect on assimilation efficiency does not change the removal of food from the environment, but leads to an increased nutrient content of the faeces. In contrast, an increase in food handling time leads to a decreased removal of food items.

An effect on assimilation efficiency would be the simplest case, as it would act as a simple decrease of f (this was the only feeding-related option in the original DEBtox equations of [16]):

$$f \to f(1-s)_+ \tag{4.2}$$

For the other options, we have to dive a bit deeper in the feeding module (Section 2.1.1). It is possible to translate the effects on $\{\dot{J}_{XAm}\}$ and $\{\dot{F}_m\}$ to an effect of f. For a decrease in maximum surface-specific feeding rate only:

$$f \to f\left(\frac{f}{(1-s)_+} + 1 - f\right)^{-1} \tag{4.3}$$

Note that for $f = 1$, this equation results in Eq. 4.2 again. For an increase in food handling time:

$$f \to f\,(1+sf)^{-1} \tag{4.4}$$

For a decrease in searching or filtering rate:

$$f \to f\left(f + \frac{1-f}{(1-s)_+}\right)^{-1} \tag{4.5}$$

In this equation, there is no effect on f at maximum food $f = 1$. This makes sense as the organism does not have to search when there is food ad libitum. An effect on the maximum feeding rate and the maximum searching rate, by the same factor, would again lead to the basic effect on f as in Eq. 4.2.

Thus, all effects on the feeding and assimilation process can be lumped into an effect on f. In practice, it will generally be impossible to distinguish between these options without access to actual feeding rates and/or effects at different food densities. However, the choice of the mechanism of action can have consequences for the extrapolation to untested food densities, and for the food dynamics in population models.

4.1.2 Derivation of effects on feeding

Effects on feeding can be thought of as occurring through (at least) four pathways: increase in handling time, decrease in maximum ingestion rate, decrease in searching rate, or a decrease in assimilation efficiency from the gut to the reserves. In most ecotoxicological situations, we do not want to include details of the feeding process, because this requires details about the feeding biology of the organism, which is generally not observed. Furthermore, this would require two extra parameters and access to actual food densities. To simulate the consequences of an effect through each pathway, I would like to include all mechanisms of action as if it were an effect on f alone.

The feeding rate (in moles per time) is given by:

$$\dot{J}_{XA} = f\{\dot{J}_{XAm}\}L^2 \tag{4.6}$$

Which can be rewritten using the definition of f:

$$\dot{J}_{XA} = \frac{1}{1 + \frac{\{\dot{J}_{XAm}\}}{X\{\dot{F}_m\}}}\{\dot{J}_{XAm}\}L^2 \tag{4.7}$$

Assuming a stress on the maximum surface-specific ingestion rate as primary parameter, we obtain for the feeding rate under stress:

$$\dot{J}_{XAs} = \frac{1}{1 + \frac{\{\dot{J}_{XAm}\}(1-s)}{X\{\dot{F}_m\}}}\{\dot{J}_{XAm}\}(1-s)L^2 \tag{4.8}$$

The ratio between the feeding rate under stress and without stress is the factor that needs to be applied on f. For notational ease, I will use J for $\{\dot{J}_{XAm}\}$ and F for $\{\dot{F}_m\}$ This ratio is (after a little manipulation):

$$\frac{\dot{J}_{XAs}}{\dot{J}_{XA}} = \frac{1 + \frac{J}{XF}}{1 + \frac{J(1-s)}{XF}}(1-s) \tag{4.9}$$

No we can use the definition of f as:

$$f = \frac{1}{1 + \frac{J}{XF}} \Rightarrow \frac{J}{XF} = \frac{1-f}{f} \tag{4.10}$$

and after some more manipulations:

$$\frac{\dot{J}_{XAs}}{\dot{J}_{XA}} = \frac{1}{\frac{f}{1-s} + 1 - f} \tag{4.11}$$

Note that f is here the value under non-stressed conditions. For ad libitum conditions, $f = 1$, which implies that the stress function simplifies to $1 - s$. However, for lower food densities in the environment, the stress on the feeding rate is somewhat less than that on $\{\dot{J}_{XAm}\}$, because the saturation coefficient also decreases. When we have no interest in actual feeding levels, we can accommodate this stress on the maximum surface-specific ingestion rate through an effect on f as follows:

$$f_s = f\frac{1}{\frac{f}{1-s} + 1 - f} \tag{4.12}$$

If we want to include an effect on the handling time per food item, this implies that (the handling time is the inverse of the maximum ingestion rate):

$$\{\dot{J}_{XAms}\} = \{\dot{J}_{XAm}\}\frac{1}{1+s} \tag{4.13}$$

Through a similar derivation, this effect can be translated into an effect on f as:

$$f_s = f\frac{1}{1+sf} \tag{4.14}$$

An effect on the maximum surface-specific searching rate $\{\dot{F}_m\}$ leads to the following effect on f:

$$f_s = f\frac{1}{f + \frac{1-f}{1-s}} \tag{4.15}$$

Interestingly, when the food level is ad libitum, there is no effect of the stressor. This makes sense: when you are swimming in food, you do not have to search for it. However, f reaches 1 asymptotically only, and therefore never truly is 1. Thus, the analysis of the effects becomes very sensitive to the actual level of f in the test.

The last option is that a stressor reduces the uptake of food from the gut, or the assimilation into the reserves (these two options cannot be distinguished without energetic analysis of the faeces). This leads to the 'standard' DEBtox relationship:

$$f_s = f(1-s) \tag{4.16}$$

4.1.3 Effects on use of reserve

In Table 4.1, I provide several options for effects on the use of energy from the reserves. I think that this covers the most of the possible effects on the use of reserves. This list is far greater than the original four mechanisms for the use of energy in DEBtox.

A peculiar effect is perhaps on the costs for maturation. Maturation is quantified by the amount of reserves invested in it, so there is no concept of overhead costs in most of the DEB publications. However, if a toxicant can change this overhead, there needs to be a parameter. Therefore, I suggest to introduce the parameter κ_H, which is added in Eq. 2.42 (in the control, $\kappa_H = 1$).

An effect on mobilisation $\dot{v}$ does not seem particularly likely, and was not considered in the original DEBtox formulation. However, in principle it is possible that this parameter is

affected by toxicants. A decrease in $\dot{v}$ leads to an increase in maximum reserve density $[E_m]$, as long as $\{\dot{p}_{Am}\}$ remains constant. Therefore, g will increase (see Eq. 2.28). A decrease in $\dot{v}$ and g by the same factor means that maximum structural body size is not affected. However, because $[E_m]$ is increased, the ultimate physical volume will be increased. Even though there is no effect on ultimate structural size, there is an effect on the growth rate, comparable to an increase in the growth costs. This effect is caused by a less intuitive change in mobilisation flux S_C, because the effect of the growth dilution factor for reserve works out slightly different (see Eq. 2.33). An increase in $\dot{v}$ will lead to a short-lived increase in S_C, which is followed by a decrease in $[E_m]$.

4.1.4 Effects on mortality

Effects on mortality are modelled somewhat independently from effects on the energy budget. In principle, the same scaled internal concentrations should be used, but survival has its own threshold for effects and its own proportionality:

$$\dot{h}_Q = \dot{b}_\dagger \max(0, c_V - c_{0\dagger}) \equiv \dot{b}_\dagger (c_V - c_{0\dagger})_+ \tag{4.17}$$

Similarly as explained for sub-lethal effects, we can use any dose metric for survival, which has consequences for the interpretation and dimensions of the NEC and the killing rate.

The hazard rate due to toxicant exposure ($\dot{h}_Q$) can simply be added to other hazard rates, assuming that they are independent effects. Some deaths are accidental, and can be included by a (low) constant background hazard rate ($\dot{h}_0$). For short laboratory experiments, this is usually sufficient. For full life-cycle situation, we probably want to use an ageing module of Section 4.2, which yields $\dot{h}_D$. The total hazard is thus:

$$\dot{h} = \dot{h}_Q + \dot{h}_0 + \dot{h}_D \tag{4.18}$$

For one particular data analysis [4], I decided to use a Weibull function for $\dot{h}_0$ to accommodate the increase in background hazard as the organisms died from starvation in the experiment. This is pragmatic if the interest is in effects of a toxicant only, but starvation does much more than increase the hazard rate (and might interact with the toxicity parameters). It would be more scientific (but also much more complicated) to deal with such starvation in an energy budget context.

4.1.5 Modes of action for the DEBtox simplification

For the simplified model, we are severely limited in the number of mechanisms that we can invoke (Table 4.2). The reason is that we have to insure that the length at puberty remains constant. In Table 4.2, I only showed one mechanism for the acquisition of energy. Even though all of the mechanisms in Table 4.1 can be used, they can hardly be distinguished in the standard tests that the simplified model was meant for.

4.1.6 Mixture toxicity

The general DEB approach for mixtures was presented in [12]. In the 'same target' model, we can sum op the (scaled) internal concentration (or damage levels), with a weight factor ($\mathcal{W}$). When using scaled internal concentrations, this weight factor can represent differences in the bioconcentration factor of the various components in the mixture. Further, these factors may represent differences in efficiency with which the components interact with the target site.

Mode of action	Target parameter	DEB parameters under stress
Effects on energy acquisition		
Decrease in assimilation from food	$\{\dot{p}_{Am}\}$	$f(1-s)_+$
Effects on energy use		
Increase in maintenance costs	$[\dot{p}_M]$, $\dot{k}_J$	$\dot{k}_M(1+s)$, $\dot{R}_m(1+s)$
Increase in costs for structure and maturation	$[E_G]$, $\frac{d}{dt}U_H$	$g(1+s), \dot{k}_M/(1+s)$
Increase in overhead costs for making an egg	κ_R	$\dot{R}_m/(1+s)$
Hazard during oogenesis	κ_R	$\dot{R}_m \exp(-s)$

Table 4.2: Possible physiological modes of action for the simplified DEBtox model as presented in Section 2.4

When departing from scaled body residues, the total concentration of the components a, b, etc. is:

$$c_V^{a+} = c_V^a + \mathcal{W}_b\, c_V^b + \mathcal{W}_c\, c_V^c + \ldots \tag{4.19}$$

In this case, the total concentration c_V^{a+} is referenced to chemical a. In other words, the other components are weighted as if they were diluted or concentrated forms of a. The total stress factor and the total hazard coefficient are than calculated as:

$$s = \frac{1}{c_T^a}(c_V^{a+} - c_0^a)_+ \tag{4.20}$$

$$h_Q = \dot{b}_\dagger^a(c_V^{a+} - c_{0\dagger}^a)_+ \tag{4.21}$$

In the 'different target' model, we calculate a stress factor for each component individually as s_a, s_b etc. and apply them all to a target DEB parameter. If two compounds affect the same parameter, the stress factors are multiplied as follows for the example of an effect on $\dot{k}_M$:

$$\dot{k}_M = \dot{k}_{M0}(1+s_a)(1+s_b)\ldots \tag{4.22}$$

For effects on the hazard rate, we can sum the independent hazard rates for the individual components:

$$h_Q = h_Q^a + h_Q^b + \ldots \tag{4.23}$$

For mixture that are made up of a combination of chemicals that effect the same and different targets, we can first make a (weighted) sum of the components that affect the same target, and than combine the summed concentrations in the different target model.

4.2 Ageing or senescence

4.2.1 Derivation DEB book

The standard ageing module in DEB consists of a system of two ODEs. Starting from an equation for damage-inducing (M_Q, changed genes, affected mitochondria) and damage compounds (M_D, 'wrong proteins'), this system can be rewritten to equations for 'acceleration' $\ddot{q}$ and 'hazard' $\dot{h}_D$. In this section, I will work out the derivation in detail, starting with the equations from [15]:

$$\frac{d}{dt}M_Q = \eta_{QC}\dot{p}_C + \frac{1}{E_{sG}}\dot{p}_C M_Q \tag{4.24}$$

$$\frac{d}{dt}M_D = \dot{k}_W y_{DQ} M_Q \tag{4.25}$$

The parameter E_{sG} is a constant with the dimensions of energy. To remove the energy dimension we can scale with a constant property of the organism that also has an energy dimension. Thus we can scale with the maximum absolute reserve level in the control:

$$\frac{1}{E_{sG}} = \frac{s_G}{[E_{m0}]L_{m0}^3} \tag{4.26}$$

We can now translate the amount of damage-inducing compounds and damage compounds to densities, note that $m_* = M_*/M_V$:

$$\frac{d}{dt}m_Q = \eta_{QC}\frac{\dot{p}_C}{M_V} + \frac{s_G}{[E_{m0}]L_{m0}^3}\dot{p}_C m_Q - \dot{r}m_Q \tag{4.27}$$

$$\frac{d}{dt}m_D = \dot{k}_W y_{DQ} m_Q - \dot{r}m_D \tag{4.28}$$

The hazard rate is proportional to the damage density m_D. The reference density m_D^{ref} is introduced to allow the interpretation of the proportionality $\dot{h}_a$ as a hazard rate (although this is not essential for the final equations).

$$\dot{h} = \frac{\dot{h}_a}{m_D^{\text{ref}}} m_D \tag{4.29}$$

We can now free m_D from this equation, and use it to rewrite Eq. 4.28:

$$\frac{m_D^{\text{ref}}}{\dot{h}_a}\frac{d}{dt}\dot{h} = \dot{k}_W y_{DQ} m_Q - \dot{r}\frac{\dot{h} m_D^{\text{ref}}}{\dot{h}_a} \tag{4.30}$$

$$\frac{d}{dt}\dot{h} = \frac{\dot{h}_a}{m_D^{\text{ref}}}\dot{k}_W y_{DQ} m_Q - \dot{r}\dot{h} \tag{4.31}$$

The entire part before m_Q is constant. To simplify the notation, we can introduce a new state variable:

$$\ddot{q} = \frac{\dot{h}_a}{m_D^{\text{ref}}}\dot{k}_W y_{DQ} m_Q \tag{4.32}$$

This simplifies the equation for the hazard rate to:

$$\frac{d}{dt}\dot{h} = \ddot{q} - \dot{r}\dot{h} \tag{4.33}$$

Next, we also have to rewrite the ODE for m_Q to a corresponding change in $\ddot{q}$:

$$\frac{m_D^{\text{ref}}}{\dot{h}_a \dot{k}_W y_{DQ}}\frac{d}{dt}\ddot{q} = \eta_{QC}\frac{\dot{p}_C}{M_V} + \frac{s_G}{[E_{m0}]L_{m0}^3}\dot{p}_C\ddot{q}\frac{m_D^{\text{ref}}}{\dot{h}_a \dot{k}_W y_{DQ}} - \dot{r}\ddot{q}\frac{m_D^{\text{ref}}}{\dot{h}_a \dot{k}_W y_{DQ}} \tag{4.34}$$

$$\frac{d}{dt}\ddot{q} = \frac{\dot{h}_a \dot{k}_W y_{DQ}}{m_D^{\text{ref}}}\eta_{QC}\frac{\dot{p}_C}{M_V} + \frac{s_G}{[E_{m0}]L_{m0}^3}\dot{p}_C\ddot{q} - \dot{r}\ddot{q} \tag{4.35}$$

Next step is to include the equation for the mobilisation $\dot{p}_C$:

$$\dot{p}_C = E\left(\frac{\dot{v}}{L} - \dot{r}\right) = e[E_m]L^3\left(\frac{\dot{v}}{L} - \dot{r}\right) \tag{4.36}$$

which gives (in the second equation, note that $M_V = [M_V]L^3$):

$$\frac{d}{dt}\ddot{q} = \left(\frac{\dot{h}_a \dot{k}_W y_{DQ}}{m_D^{\mathrm{ref}}}\frac{\eta_{QC}}{M_V} + \frac{s_G}{[E_{m0}]L_{m0}^3}\ddot{q}\right) e[E_m]L^3\left(\frac{\dot{v}}{L} - \dot{r}\right) - \dot{r}\ddot{q} \tag{4.37}$$

$$= \left(\frac{\dot{h}_a \dot{k}_W y_{DQ}}{m_D^{\mathrm{ref}}}\frac{\eta_{QC}}{[M_V]}[E_m] + s_G\frac{[E_m]L^3}{[E_{m0}]L_{m0}^3}\ddot{q}\right) e\left(\frac{\dot{v}}{L} - \dot{r}\right) - \dot{r}\ddot{q} \tag{4.38}$$

As long as $[E_m]$ is not affected by a toxicant, the first term between brackets is constant (here, $\ddot{h}_a$ is used as a new compound parameter), and $[E_m] = [E_{m0}]$. Therefore, we can simplify the equation to:

$$\frac{d}{dt}\ddot{q} = \left(\ddot{h}_a + s_G\frac{L^3}{L_{m0}^3}\ddot{q}\right) e\left(\frac{\dot{v}}{L} - \dot{r}\right) - \dot{r}\ddot{q} \tag{4.39}$$

In which cases is $[E_m]$ not constant? When either $\dot{p}_{Am}$ or $\dot{v}$ change. In the scaled DEB model, I include all changes in $\dot{p}_{Am}$ as a change in f. Therefore, $[E_m]$ will also be constant in that way. However, a change in $\dot{v}$ is more tricky. A decrease in $\dot{v}$ implies an increase in $[E_m]$. This means that the ageing constants $\ddot{h}_a$ and s_G will also increase by the same factor, if we are to use Eq. 4.39.

4.2.2 Derivation for scaled model

The original derivation of the ageing model in the DEB book has a few problems in a chemical-stress context. Firstly, it depends on the constancy of $[E_m]$ (which changes when $\dot{v}$ changes), and it depends on the equation for the mobilisation flux $\dot{p}_C$ (which changes under some of the starvation regimes). Therefore, I have derived a new formulation, which is mathematically equivalent, but includes the scaled mobilisation explicitly as used in the scaled DEB model. I start again, without specifying the proportionality constants (I just call them a_1-a_3 to simplify notation):

$$\frac{d}{dt}M_Q = a_1\dot{p}_C + a_2\dot{p}_C M_Q \tag{4.40}$$

$$\frac{d}{dt}M_D = a_3 M_Q \tag{4.41}$$

Next, I can change the equation for M_Q and M_D to amounts per structural volume by dividing by L^3:

$$\frac{d}{dt}[M_Q] = \frac{a_1}{L^3}\dot{p}_C + a_2\dot{p}_C[M_Q] - \dot{r}[M_Q] \tag{4.42}$$

$$\frac{d}{dt}[M_D] = a_3[M_Q] - \dot{r}[M_D] \tag{4.43}$$

I assume that the hazard rate is proportional to the amount of damage per structural volume (instead of per C-mole of structure as in the original):

$$\dot{h}_D = \dot{b}_D[M_D] \tag{4.44}$$

The set of equations now has four parameters: a_1, a_2, a_3, $\dot{b}_D$. However, these parameters can not be independently estimated. I do not see an easy scaling for this set of equations, as was done for the internal concentration. Therefore, I follow the same route as in the original DEB3 derivation and rewrite the equations for $[M_Q]$ and $[M_D]$ using:

$$[M_D] = \frac{\dot{h}_D}{\dot{b}_D} \tag{4.45}$$

Note that I use a subscript D for 'damage' for the hazard rate. In the DEB book, this hazard rate has no subscript. Because I have to deal with multiple causes of death (due to chemicals in the environment, and accidental deaths in the laboratory) I feel it is better to make this one of all possible hazard rates. The symbol $\dot{h}$ without subscript is than reserved for the overall hazard rate.

Rewriting the equation for $[M_D]$ to:

$$\frac{d}{dt}\dot{h}_D = a_3\dot{b}_D[M_Q] - \dot{r}\dot{h}_D \tag{4.46}$$

Next we can call the first term in this equation $\ddot{q}$, so that:

$$\ddot{q} = a_3\dot{b}_D[M_Q] \quad \text{and thus} \quad [M_Q] = \frac{\ddot{q}}{a_3\dot{b}_D} \tag{4.47}$$

which we can use to rewrite the equation for $[M_Q]$:

$$\frac{d}{dt}\ddot{q} = a_1a_3\dot{b}_D\frac{\dot{p}_C}{L^3} + a_2\dot{p}_C\ddot{q} - \dot{r}\ddot{q} \tag{4.48}$$

The scaled mobilisation flux S_C equals $\dot{p}_C/\{\dot{p}_{Am}\}$, and thus we can rewrite the equation to:

$$\frac{d}{dt}\ddot{q} = \left(\{\dot{p}_{Am}\}a_1a_3\dot{b}_D\frac{1}{L^3} + \{\dot{p}_{Am}\}a_2\ddot{q}\right)S_C - \dot{r}\ddot{q} \tag{4.49}$$

The string of variables $\{\dot{p}_{Am}\}a_1a_3\dot{b}_D$ is a constant[1] with the dimensions L/t^3; the combination $\{\dot{p}_{Am}\}a_2$ has dimensions $1/(L^2t)$. These are not such practical dimensions; I would prefer rate constants with dimensions $1/t$. To achieve that, I can introduce a length measure that is constant into the equation: the maximum length under control conditions. Furthermore, I add a power of 3 to the first rate constant to provide the dimension of cubed time. This leads to:

$$\frac{d}{dt}\ddot{q} = \left(\dot{k}_W^3\frac{L_{m0}}{L^3} + \dot{k}_G\frac{\ddot{q}}{L_{m0}^2}\right)S_C - \dot{r}\ddot{q} \tag{4.50}$$

$$\frac{d}{dt}\dot{h}_D = \ddot{q} - \dot{r}\dot{h}_D \tag{4.51}$$

Now we have two rate constants as parameters. Note that I use the symbol $\dot{k}_W$ here for a different parameter than in the original derivation. The reason is that I like the subscript W for 'Weibull', and G for 'Gompertz'.

[1] I can do this because I take all of the effects on $\{\dot{p}_{Am}\}$ as effects on the scaled functional response f to preserve the scaling in the scaled DEB version.

4.2.3 Relationship between the sets of ageing parameters

The two formulations are mathematically identical, at least in the absence of stress on $\dot{v}$. Therefore, we can transform the old parameters in the new ones. For the two expressions for $\frac{d}{dt}\ddot{q}$ to be identical the following must hold:

$$\left(\dot{k}_W^3 \frac{L_{m0}}{L^3} + \dot{k}_G \frac{\ddot{q}}{L_{m0}^2}\right) S_C = \left(\ddot{h}_a + s_G \frac{L^3}{L_{m0}^3}\ddot{q}\right) e \left(\frac{\dot{v}}{L} - \dot{r}\right) \tag{4.52}$$

Using Eq. 4.36, and as $\dot{p}_C = S_C\{\dot{p}_{Am}\}$, we can rewrite this equation to:

$$\left(\dot{k}_W^3 \frac{L_{m0}}{L^3} + \dot{k}_G \frac{\ddot{q}}{L_{m0}^2}\right) S_C = \left(\ddot{h}_a + s_G \frac{L^3}{L_{m0}^3}\ddot{q}\right) S_C \frac{\{\dot{p}_{Am}\}}{[E_m]L^3} \tag{4.53}$$

$$\left(\dot{k}_W^3 \frac{L_{m0}}{L^3} + \dot{k}_G \frac{\ddot{q}}{L_{m0}^2}\right) S_C = \left(\ddot{h}_a \frac{L_{m0}}{L^3} + s_G \frac{\ddot{q}}{L_{m0}^2}\right) S_C \frac{\{\dot{p}_{Am}\}}{[E_m]L_{mo}} \tag{4.54}$$

Clearly, the important difference between the parameter sets lies in one parameter combination:

$$\frac{\{\dot{p}_{Am}\}}{[E_m]L_{mo}} = \frac{\dot{v}}{L_{mo}} \tag{4.55}$$

Maximum length in the control is given by:

$$L_{mo} = \frac{\dot{v}_0}{\dot{k}_{M0} g_0} \tag{4.56}$$

and the factor is therefore given by:

$$\dot{k}_W^3 = \ddot{h}_a \frac{\dot{v}}{\dot{v}_0} \dot{k}_{M0} g_0 \tag{4.57}$$

$$\dot{k}_G = s_G \frac{\dot{v}}{\dot{v}_0} \dot{k}_{M0} g_0 \tag{4.58}$$

Without stress on $\dot{v}$, this a straightforward transformation. However, when $\dot{v}$ is affected, this leads to a an increase in $[E_m]$, and thereby an increase in $\ddot{h}_a$ and s_G. The factor $\dot{v}/\dot{v}_0$ compensates for this effect; the new parameters $\dot{k}_W$ and $\dot{k}_G$ remain constant under a change in $\dot{v}$.

4.2.4 Alternative ageing model

The DEB3 ageing module as presented in the previous sections has some problems for me. Mainly, for invertebrates such as nematodes and springtails (and probably many others) the shape of the survival curves is such that the ageing model does not predict increased longevity at food limitation. This is unacceptable behaviour, as food limitation seems quite consistently to increase longevity in animals.

An alternative I would like to explore further departs from the same situation as the standard model, but links the hazard rate to the damage-inducing compounds directly (without additional damage compounds). Furthermore, I take the absolute amount of damage-inducing compounds, and not their density. This is partly for pragmatic reasons: dividing by the body volume kills much of the life-extending effect of food limitation due to the counteracting effect of growth dilution. However, it might not be such a stretch to take the absolute amount

of damage, as it appears that damaged mitochondria are not diluted by growth, but are replicated [20]. The starting equations are now:

$$\frac{d}{dt}M_Q = a_1\dot{p}_C + a_2\dot{p}_C M_Q \tag{4.59}$$
$$\dot{h}_D = a_3 M_Q \tag{4.60}$$

We can rewrite these two equations into a single ODE for $\dot{h}_D$ by using $M_Q = h_D/a_3$:

$$\frac{1}{a_3}\frac{d}{dt}\dot{h}_D = a_1\dot{p}_C + a_2\dot{p}_C\frac{\dot{h}_D}{a_3} \tag{4.61}$$
$$\frac{d}{dt}\dot{h}_D = \dot{p}_C\left(a_1a_3 + a_2\dot{h}_D\right) \tag{4.62}$$

As $\dot{p}_C = \{\dot{p}_{Am}\}S_C$, we obtain:

$$\frac{d}{dt}\dot{h}_D = \{\dot{p}_{Am}\}S_C\left(a_1a_3 + a_2\dot{h}_D\right) \tag{4.63}$$
$$= S_C\left(\{\dot{p}_{Am}\}a_1a_3 + \{\dot{p}_{Am}\}a_2\dot{h}_D\right) \tag{4.64}$$

The terms $\{\dot{p}_{Am}\}a_1a_3$ and $\{\dot{p}_{Am}\}a_2$ are constants, but they have a bit unpleasant dimensions ($1/(t^2L^2)$ and $1/(tL^2)$). We can, however, turn them into two rate constants with dimensions $1/t$ by using another constant; the maximum length in the control (L_{m0}):

$$\dot{k}_W = \sqrt{\{\dot{p}_{Am}\}a_1a_3L_{m0}^2} \tag{4.65}$$
$$\dot{k}_G = \{\dot{p}_{Am}\}a_2L_{m0}^2 \tag{4.66}$$

And the ageing equation is now written as:

$$\frac{d}{dt}\dot{h}_D = \frac{S_C}{L_{m0}^2}\left(\dot{k}_W^2 + \dot{k}_G\dot{h}_D\right) \tag{4.67}$$

4.2.5 Slight variation

One of the things that I don't like about the ageing models in this form is that the hazard rate ($\dot{h}_D$) is the only resulting state variable. To include an ageing effect on reproduction, we would have to include a stress relative to the hazard rate, which is awkward. Fortunately, it is straightforward to rearrange the same simple model.

$$\frac{d}{dt}M_Q = a_1\dot{p}_C + a_2\dot{p}_C M_Q \tag{4.68}$$

Next, we can scale M_Q using a_1/a_2, which implies $m_Q = M_Q a_2/a_1$. This leads to the equation for scaled (dimensionless) damage:

$$\frac{d}{dt}m_Q = a_2\dot{p}_C + a_2\dot{p}_C m_Q \tag{4.69}$$
$$= a_2\dot{p}_C(1 + m_Q) \tag{4.70}$$
$$= a_2\{\dot{p}_{Am}\}S_C(1 + m_Q) \tag{4.71}$$

We can introduce $\dot{k}_G = a_2\{\dot{p}_{Am}\}L_{m0}^2$:

$$\frac{d}{dt}m_Q = \dot{k}_G \frac{S_C}{L_{m0}^2}(1 + m_Q) \tag{4.72}$$

Scaled damage can be related to the hazard rate by:

$$\dot{h}_D = \dot{k}_W m_Q \tag{4.73}$$

Note that $\dot{k}_W$ is not exactly the same as in the previous derivation (in fact this $\dot{k}_W$ equals the previous one squared and divided by $\dot{k}_G$)! Advantage of this parameterisation is that it is now easy to include a threshold for scaled damage, and to include effects in reproduction.

4.2.6 Ageing effects on reproduction

Ageing also affects reproduction rates. One option is to decrease the efficiency for the production of eggs from the reproduction buffer (a decrease of κ_R) [7]. However, it is generally observed that old animals feed less. We could depart from the starvation strategies outlined earlier.

4.2.7 Ageing related to respiration

In the previous sections, ageing was related to the mobilisation of reserves (through $\dot{p}_C$ or S_C). However, part of these mobilised reserves are used as building materials for structure and offspring. It is possible that these do not lead to production of ROS and the associated damage. The full DEB model provides direct access to the individual fluxes that lead to respiration: somatic and maturity maintenance, maturity, and the overheads of growth and reproduction (ignoring respiration related to assimilation):

$$\dot{p}_D = \begin{cases} \dot{p}_M + (1-\kappa)\dot{p}_C + (1-\kappa_G)\dot{p}_G & \text{if } E_H < E_H^p \\ \dot{p}_M + \dot{p}_J^p + (1-\kappa_R)\dot{p}_R + (1-\kappa_G)\dot{p}_G & \text{otherwise} \end{cases} \tag{4.74}$$

For the scaled model, we have to do a bit more work to calculate the scaled total dissipation. The scaled somatic maintenance power is given by (note that L_m is *not* in the control!):

$$S_M = \frac{[\dot{p}_M]}{\{\dot{p}_{Am}\}}L^3 = \kappa\frac{L^3}{L_m} \tag{4.75}$$

This recalculation makes use of the definitions of L_m, $\dot{v}$, g and $\dot{k}_m$. The scaled growth power is thus:

$$S_G = \kappa S_C - \kappa\frac{L^3}{L_m} \tag{4.76}$$

The scaled maturity plus maturity maintenance power in juveniles:

$$S_J + S_H = (1-\kappa)S_C \tag{4.77}$$

Maturity maintenance in adults:

$$S_J = \dot{k}_J U_H^p \tag{4.78}$$

And the scaled power for reproduction:

$$S_R = (1-\kappa)S_C - \dot{k}_J U_H^p \tag{4.79}$$

This leaves as unknown parameter the fraction of growth energy fixed in new structure:

$$\kappa_G = \mu_V \frac{[M_V]}{[E_G]} \tag{4.80}$$

Using the default add_my_pet values for these parameters yields a κ_G of 0.73.

At this moment, I have not compared all these different ageing models so more work is needed here. An interesting result of this model would be that because relative respiration decreases after puberty, so does the rate of ageing (although this can be partly counteracted by the ageing acceleration).

4.3 Damage models and receptor kinetics

4.3.1 Damage kinetics

In the papers that use it, the concept of damage is kept quite vague. Here, I assume that damage is diluted by growth (just as assumed for damage due to ROS in the ageing module), and that actual internal concentration $[M_Q]$ is available:

$$\frac{d}{dt}[M_D] = \dot{k}_D y_{DQ}[M_Q] - \dot{k}_r[M_D] - \dot{r}[M_D] \tag{4.81}$$

where $\dot{k}_D$ is the rate constant for damage 'accural', y_{DQ} the yield of damage on internal chemical concentration, and $\dot{k}_r$ is the damage repair rate. Note that the dilution factor enters in the same way as for internal concentrations (with $\dot{r}$ as the specific growth rate). This equation has at least two parameters, which cannot both be identified from the data (as damage cannot be measured). This situation is completely equivalent to the problem that we cannot identify two TK rate constant from toxicity data alone. The solution is the same: scale with the partition coefficient. Next, I can introduce a partition coefficient between damage density and internal concentration:

$$P_{DQ} = \frac{\dot{k}_D y_{DQ}}{\dot{k}_r} \tag{4.82}$$

Now we can divide both sides of the equation for $[M_D]$ with P_{DQ} to obtain a scaled damage density ($[m_D] = [M_D]/P_{DQ}$ with the dimensions of an internal concentration):

$$\frac{d}{dt}[m_D] = \dot{k}_r \left([M_Q] - [m_D]\right) - \dot{r}[m_D] \tag{4.83}$$

See also the explanation in [5].

4.3.2 Receptor kinetics

The receptor model as presented by [10] follows from the assumption that the internal concentration knocks out functional receptors (N_f), and makes them non-functional (N_n). Here, I assume that non-functional receptors are diluted by growth, and thus that the total number of receptors per unit of structural volume is constant (which is by no means certain). Furthermore, I assume that the actual internal concentration $[M_Q]$ is available. It is possible to substitute the scaled concentration c_V, but it will be difficult to estimate all parameters from toxicity data alone. The equation for the number of non-functional receptors is:

$$\frac{d}{dt}N_n = \dot{b}_{fn}[M_Q]N_f - \dot{k}_r N_n \tag{4.84}$$

where $\dot{b}_{fn}$ is the receptor 'knock-out' rate and $\dot{k}_r$ is the repair rate. The total amount of receptors is limited, and here I assume that they are a fixed fraction of the structural body volume:

$$N_+ = N_f + N_n = [N_+]V \tag{4.85}$$

We can use this relation to rewrite the equation:

$$\frac{d}{dt}N_n = \dot{b}_{fn}[M_Q]\left([N_+]V - N_n\right) - \dot{k}_r N_n \tag{4.86}$$

Next, we can rewrite this equation to the fraction of non-functional receptors $n_n = N_n/N_+$:

$$\frac{d}{dt}n_n = \dot{b}_{fn}[M_Q](1 - n_n) - \dot{k}_r n_n - \dot{r}n_n \tag{4.87}$$

The dilution factor $\dot{r}$ enters in the same manner as for the internal concentration.

If n_n remains much small than 1, this equation reduces to the scaled damage model (if we scale the receptor equation by a partition coefficient equal to $\dot{b}_{fn}/\dot{k}_r$).

Chapter 5

Fitting models to data

5.1 Initial values for the energy-budget parameters

Fitting more than two or three parameters to a data set requires good starting values. Most of the DEB model parameters are rather abstract, and cannot be directly observed. Furthermore, there are plenty of parameter combinations that do not produce a working life cycle. Therefore, it is good to start with parameter values that are already somewhat close, and work from there.

It is possible to start with the body-size-specific parameter values as offered in the DEB book in 5.1. They might work for your species, but care must be taken. Especially, $\dot{k}_M$ seems very low to me, and perhaps better to start with $k = 1$. Table 5.3 gives some representative values for several invertebrates that I have worked with. I do not claim that these are appropriate or accurate; they might serve as a starting point for your analyses.

5.2 Likelihood functions

5.2.1 Continuous endpoints (body size and reproduction)

Here, we will go into a bit more detail about normal likelihood functions, and especially on treating the standard deviation of the error distribution as a nuisance parameter, and working with the means of replicate observations. See also the supporting information of [13].

Observations are represented as Y_{ijr}, where i is the time point of the observation (from 1 to k), j the concentration (or the treatment, from 1 to m), and r the replicates (the number of individual organisms on which there are observations at that time and concentration, from 1 to n). The number of observation times may depend on the treatment (and thus k_j), and the number of replicates may depend on both time and treatment (and thus n_{ij}). The observed value is compared to the predicted value $\hat{Y}_{ij}$ from the model, which depends on the parameter set θ. Our reference situation is the log-likelihood function where the deviations from the model predictions $\hat{Y}$ are normal, independent and homoscedastic:

$$\ell(\theta, \sigma^2|Y) = -\frac{N}{2}\ln(2\pi\sigma^2) - \frac{1}{2\sigma^2}\sum_{j=1}^{m}\sum_{i=1}^{k_j}\sum_{r=1}^{n_{ij}}(Y_{ijr} - \hat{Y}_{ij}(\theta))^2 \tag{5.1}$$

where N is the total number of data points:

Parameter	Symbol	Initial value
Specific searching rate	$\{\dot{F}_m\}$	$6.5\,\mathrm{L\,cm^{-2}\,d^{-1}}$
Maximum specific ingestion rate (C-moles)	$\{\dot{J}_{XAm}\}$	$51{\cdot}10^{-6}\,z\,\mathrm{mol\,cm^{-2}\,d^{-1}}$
Maximum specific ingestion rate (volume)	$\{\dot{J}_{XAm}\}/[M_V]$	$0.013\,z\,\mathrm{cm^3\,cm^{-2}\,d^{-1}}$
Fraction of mobilisation to soma	κ	0.8 [-]
Reproduction efficiency	κ_R	0.95 [-]
Energy conductance	$\dot{v}$	$0.02\,\mathrm{cm\,d^{-1}}$
Energy investment ratio	g	$3.1\,z^{-1}$
Somatic maintenance rate coefficient	$\dot{k}_M$	$0.0064\,\mathrm{d^{-1}}$
Maintenance ratio	k	0.31 [-] or 1 [-]
Scaled maturity at puberty	U_H^p	$7.4\,z^2\,\mathrm{cm^2\,d}$
Maturity ratio	u_H^{bp}	0.0017 [-]
Weibull ageing rate	$\dot{k}_W$	$0.0027\ z^{2/3}\,\mathrm{d^{-1}}$
Gompertz ageing rate	$\dot{k}_G$	$2\ {\cdot}10^{-4}\,z\,\mathrm{d^{-1}}$

Table 5.1: Initial values for parameters at 20 °C. The zoom factor is defined as $L_m = zL_m^{\mathrm{ref}}$ and $L_m^{\mathrm{ref}} = 1$ cm (note that this is structural length). All values recalculated from DEB book ... Ageing parameters recalculated from "add my pet". The conversion of feeding rate from C-moles to volume was made on the assumption that the number of C-moles per volume is the same for the food as for the feeding animal ($[M_V] = 4.1$ mmol/cm^3)...

Parameter	Symbol	Initial value
Contribution of reserves to volume	ω_V	$\frac{\{\dot{p}_{Am}\}}{\dot{v}}\ 2.8 \cdot 10^{-4} = 0.32\,z$ [-]
Contribution of reserves to wet weight	ω_w	$\frac{\{\dot{p}_{Am}\}}{\dot{v}}\ 2.8 \cdot 10^{-4} = 0.32\,z$ [-]
Contribution of reserves to dry weight	ω_d	$\frac{\{\dot{p}_{Am}\}}{\dot{v}}\ 5.2 \cdot 10^{-4} = 0.59\,z$ [-]
Density of structure (wet weight)	d_V	$1\ \mathrm{g\,cm^{-3}}$
Density of structure (dry weight)	d_{Vd}	$0.1\ \mathrm{g\,cm^{-3}}$

Table 5.2: Default values for the translation of structure and reserve to actual volume or weight. Derived from the 'add my pet' project; the zoom factor is the same as in Table 5.1.

Parameter	*D. magna*	*D. octaedra*	*C. teleta*
κ	0.80	0.92	0.82
$\dot{v}$	$3.2\ \mathrm{mm\,d^{-1}}$	$1.3\ \mathrm{mg^{1/3}\,d^{-1}}$	$1.68\ \mathrm{mm\,d^{-1}}$
g	0.42	1	2.4
$\dot{k}_M$	$1.7\ \mathrm{d^{-1}}$	$0.18\ \mathrm{d^{-1}}$	$0.25\ \mathrm{d^{-1}}$
k	1	1	1
U_H^p	$0.37\ \mathrm{mm^2\,d}$	$8.1\ \mathrm{mg^{2/3}\,d}$	$1.39\ \mathrm{mm^2\,d}$
u_H^{bp}	0.033	0.016	0.00087

Table 5.3: Initial parameters values for some invertebrates. *Daphnia magna* parameters at 20 °C from [19]; length measure is probably from eye to base of spine. *Dendrobaena octaedra* parameters at 15 °C from [9]; length measure is cubic root of wet weight. *Capitella capitata* parameters at 20 °C from [11]; length measure is cubic root of estimated volume.

$$N = \sum_{j=1}^{m} \sum_{i=1}^{k_j} n_{ij} \tag{5.2}$$

5.2.2 Transformation

Using untransformed data and model represents the situation where we can assume independent normal distributions for the residuals, with a constant variation. Alternatively we can transform the model and data using square-root or log-transformation:

$$\text{SSQ}(\theta; Y) = \sum_{j=1}^{m} \sum_{i=1}^{k_j} \sum_{r=1}^{n_{ij}} \left(\hat{Y}_{ij}^{1/2}(\theta) - Y_{ijr}^{1/2} \right)^2 \tag{5.3}$$

$$\text{SSQ}(\theta; Y) = \sum_{j=1}^{m} \sum_{i=1}^{k_j} \sum_{r=1}^{n_{ij}} \left(\ln(\hat{Y}_{ij}(\theta)) - \ln(Y_{ijr}) \right)^2 \tag{5.4}$$

The log-tranformation implies log-normal distributions for the residuals with a variation that increases with the predicted value for the endpoint. The square root transformation assumes a squared normal distribution with a variation that also increases with the predicted value, but less extreme than the log-transformation. At this moment, I prefer the square-root version.

5.2.3 Standard deviation known

If the standard deviation of the scatter is known, the first term in Eq. 5.1 becomes trivial (it does not depend on the parameters, so it is a constant). The log-likelihood function thus reduces to:

$$\ell(\theta | Y, \sigma^2) = -\frac{1}{2\sigma^2} \sum_{j=1}^{m} \sum_{i=1}^{k_j} \sum_{r=1}^{n_{ij}} (Y_{ijr} - \hat{Y}_{ij}(\theta))^2 + C \tag{5.5}$$

To include heteroscedasticity in the analysis, we can also take different error variances for each time and concentration:

$$\ell(\theta | Y, \sigma^2) = -\sum_{j=1}^{m} \sum_{i=1}^{k_j} \frac{1}{2\sigma_{ij}^2} \sum_{r=1}^{n_{ij}} (Y_{ijr} - \hat{Y}_{ij}(\theta))^2 + C \tag{5.6}$$

We can easily work with means ($\bar{Y}_{ij}$)instead of individual data points (Y_{ijr}), as long as the correct error variance is used. Note that:

$$\sigma_{\bar{Y}_{ij}}^2 = \frac{\sigma^2}{n_{ij}} \tag{5.7}$$

And thus if σ^2 is known and constant:

$$\ell(\theta | Y, \sigma^2) = -\frac{1}{2\sigma^2} \text{wSSQ}_n(\theta; Y) + C \tag{5.8}$$

$$\text{wSSQ}_n(\theta; Y) = \sum_{j=1}^{m} \sum_{i=1}^{k_j} n_{ij} (\bar{Y}_{ij} - \hat{Y}_{ij}(\theta))^2 \tag{5.9}$$

The SSQ is thus replaced by the weighted sum-of-squares; each residual is weighted with the number of replicates for each mean. If the variance of the error is known, no information is lost when we work with the means instead of the individual replicates.

5.2.4 Standard deviation as a nuisance parameter

Usually, we do not know the value of the residual variation σ^2, and we are not interested in, so we can replace it with its maximum-likelihood estimate. The maximum-likelihood estimate for the variance σ^2 at each θ is the SSQ divided by the number of observations N:

$$\hat{\sigma}^2(\theta, Y) = \frac{1}{N}\sum_{j=1}^{m}\sum_{i=1}^{k_j}\sum_{r=1}^{n_{ij}}(Y_{ijr} - \hat{Y}_{ij}(\theta))^2 \tag{5.10}$$

In fact, replacing σ^2 with its maximum likelihood estimate leads to a profile likelihood where σ^2 is 'profiled out' as a nuisance parameter:

$$\ell(\theta|Y) = \max_{\sigma^2} \ell(\theta, \sigma^2|Y) \tag{5.11}$$

This profile likelihood can be treated like a normal likelihood in all respects [22]. The log-likelihood simplifies to:

$$\ell(\theta|Y) = -\frac{N}{2}\ln\left(\sum_{j=1}^{m}\sum_{i=1}^{k_j}\sum_{r=1}^{n_{ij}}(Y_{ijr} - \hat{Y}_{ij}(\theta))^2\right) + C \tag{5.12}$$

All constant terms (that do not depend on the parameters) are absorbed in C, including the factor $\ln 2\pi/N$, and can subsequently be ignored.

In practice, we might have to work with the mean observed values at each timepoint and concentration $\bar{Y}_{ij}$. This requires some consideration because the simplification rests on the estimation of σ^2 from the data (see Eq. 5.10), and the number of replicates on which the mean is based might differ between the observation times or the tested concentrations. We can make use of the relationship between the standard error of the mean (the expected deviation between the average of a number of replicates and the true mean) and the standard deviation of a number of replicates (the expected deviation between individual replicate and the true mean). Consider a set of replicate observations for one timepoint i and one treatment j:

$$\sigma^2_{\bar{Y}_{ij}} = \frac{\sigma^2}{n_{ij}} \tag{5.13}$$

$$\mathcal{E}\left((\bar{Y}_{ij} - \hat{Y}_{ij}(\theta))^2\right) = \frac{1}{n_{ij}}\mathcal{E}\left((Y_{ijr} - \hat{Y}_{ij}(\theta))^2\right) \tag{5.14}$$

We can estimate the expected squared deviations $(Y_{ijr} - \hat{Y}_{ij})^2$ with the standard deviation:

$$\mathcal{E}\left((Y_{ijr} - \hat{Y}_{ij}(\theta))^2\right) \approx \frac{1}{n_{ij}}\left(\sum_{r=1}^{n_{ij}}(Y_{ijr} - \hat{Y}_{ij}(\theta))^2\right) \tag{5.15}$$

Combining Eq. 5.14 and 5.15, we can therefore estimate the sum-of-squares over the replicate observations by the following:

$$\sum_{r=1}^{n_{ij}}(Y_{ijr} - \hat{Y}_{ij}(\theta))^2 \approx n_{ij}^2(\bar{Y}_{ij} - \hat{Y}_{ij}(\theta))^2 \tag{5.16}$$

Even though these expressions are not necessarily equal, their expectations are the same. We can thus estimate the error variance from the data using the n^2-weighted sum-of-squares (see Eq. 5.10):

$$\hat{\sigma}^2(\theta, Y) = \frac{1}{N}\text{wSSQ}_{n^2}(\theta; Y) \tag{5.17}$$

$$\text{wSSQ}_{n^2}(\theta; Y) = \sum_{j=1}^{m}\sum_{i=1}^{k_j} n_{ij}^2(\bar{Y}_{ij} - \hat{Y}_{ij}(\theta))^2 \tag{5.18}$$

Now, we have to go back to the full log-likelihood of Eq. 5.1, and fill in Eq. 5.17 and 5.9:

$$\ell(\theta, |Y) = -\frac{N_Y}{2}\ln\left(\frac{2\pi}{N}\text{wSSQ}_{n^2}(\theta; Y)\right) - \frac{N}{2\text{wSSQ}_{n^2}(\theta; Y)}\text{wSSQ}_n(\theta; Y) \tag{5.19}$$

Where N_Y is the total number of means (whereas N is the total number of observations on individuals):

$$N_Y = \sum_{j=1}^{m}\sum_{i=1}^{k_j} 1 \tag{5.20}$$

We can further simplify the log-likelihood function by including all constant terms (that do not depend on the parameters) into a constant C:

$$\ell(\theta|Y) = -\frac{N_Y}{2}\ln(\text{wSSQ}_{n^2}(\theta; Y)) - \frac{N\text{wSSQ}_n(\theta; Y)}{2\,\text{wSSQ}_{n^2}(\theta; Y)} + C \tag{5.21}$$

In contrast to the likelihood with fixed error variance, in this case, information is lost when using the means instead of the replicate observations. The reason is that the individual data points allow for a better estimation of the variance. Note that when the number of replicates per mean (n_{ij}) is constant, the second term of the equation is constant and can be absorbed into the constant C. In other words, if the means result from the same number of observations, we can treat them as individual observations.

5.2.5 Multinomial distribution for mortality

For survival, the likelihood function follows from the multinomial distribution. The log-likelihood of the parameter set θ, given the data set Y, is given by (see e.g., [2, 5]).

$$\ell(\theta|Y) = \sum_{j=1}^{m}\sum_{i=1}^{k}(Y_{ij} - Y_{i+1j})\ln(S_{ij}(\theta) - S_{i+1j}(\theta)) + C \tag{5.22}$$

In this equation, Y_{ij} is the number of surviving organisms at observation time i at concentration j. Similarly, S_{ij} is the survival probability at that point. Note that the subscript $i+1$ in this equation means that we have to deal with the situation where $i = k+1$. This is an additional interval which catches the organisms that survive until the end of the experiment (they are expected to die in the interval from the end of the experiment to infinity). Therefore we can specify:

$$Y_{k+1j} = 0 \quad \text{and} S_{k+1j} = 0 \tag{5.23}$$

For this likelihood function, the fact the follow the same group of animals over time is not a problem because we only use one observation for each individual (in which interval it dies). Precondition is that the death of one individual in a container does not affect the death probability of the others.

In some cases, we can have the situation that organisms are removed from the survival test for destructive analysis (e.g., measurement of body residues), or that have escaped from the test container. We can still use the observations from these organisms for our likelihood, as they still contain information (they were alive up to the point where they were removed for analysis or lost). We can still use Eq. 5.22 for the individuals that have actually died, but add to it another log-likelihood function for the ones that were removed or lost (Z):

$$\ell(\theta|Z) = \sum_{j=1}^{m}\sum_{i=1}^{k} Z_{ij}\ln S_{ij}(\theta) + C \tag{5.24}$$

5.2.6 SSQs for reproduction

It is possible to compare the number of offspring produced by an individual mother in an interval between $t-1$ and t, Y_{ijr}, to the integrated reproduction rate over that interval:

$$\mathrm{SSQ}(\theta;Y) = \sum_{j=1}^{m}\sum_{i=2}^{k_j}\sum_{r=1}^{n_{ij}}\left(\int_{t_{i-1}}^{t_i} R_j(\tau,\theta)d\tau - Y_{ijr}\right)^2 \tag{5.25}$$

Often, we have observations for the number of offspring produced by a group of mothers in an interval, Y_{ij}. We can derive a weighted SSQ by calculating an average reproduction per female, using the average number of mothers alive in that interval, and again compare it to the integrated reproduction rate over that interval. We also have to weigh the residuals with the average number of mother over the interval:

$$\mathrm{wSSQ}_n(\theta;Y) = \sum_{j=1}^{m}\sum_{i=2}^{k_j}\left(\frac{n_{ij}+n_{i-1j}}{2}\right)\left(\int_{t_{i-1}}^{t_i} R_j(\tau,\theta)d\tau - \frac{2\,Y_{ij}}{n_{ij}+n_{i-1j}}\right)^2 \tag{5.26}$$

The n^2-weighted SSQ can be derived by scaling with the average number of mothers squared.

5.3 Optimisation and confidence intervals

5.3.1 Likelihood optimisation and confidence intervals

In many cases, we have data for more than one endpoint, or more data sets for the same endpoint. These data sets should not be treated in isolation as they contain information about the same parameters. The likelihood framework allows to combined the individual likelihoods. The overall log likelihood for the parameter set θ, based on all available data Y_+, for all individual data sets s, is the sum of the individual log likelihoods for each data set:

$$\ell(\theta|Y_+) = \sum_s \ell(\theta|Y_s) \tag{5.27}$$

Thereby, I assume that each data set can be treated as an independent source of information on the parameter value. The log-likelihood function can be maximised to find the best-fitting parameter set $\hat{\theta}$:

$$\ell(\hat{\theta}|Y_+) = \max_{\theta} \ell(\theta|Y_+) \tag{5.28}$$

To compare two fits with a different set of parameters, we can use the likelihood-ratio principle. The two models we compare should be nested; that is, one model is a reduced version of the other (reduced by fixing one or more parameters to a certain value). The ratio of two likelihoods (and thus the difference in two log likelihoods) from nested models can simply be recalculated into a criterion that follows (for 'large' numbers of observation) a chi-square distribution with as degrees of freedom the number of parameters in which the two models differ.

One application of this principle is to see whether the addition of a certain parameter significantly improves the model fit. For example, whether adding a damage model improves the fit. The standard model is a reduced version of the model with damage; the standard model can be obtained by taking the damage repair rate infinitely large.

Another application of the likelihood ratio principle is in the construction of confidence intervals (see [21, 22]. We can define the confidence interval of a parameter as all of the values that will not be rejected in a likelihood-ratio test. This is called the profile likelihood. Lets take the example of making a profile likelihood for a parameter η. We now consider the parameter set θ, and a reduced parameter set that does not include η, which I will call θ_1. The profile log-likelihood of the parameter η is:

$$\ell_p(\eta|Y_+) = \max_{\theta_1} \ell(\theta|Y_+) \tag{5.29}$$

Plotting this profile log-likelihood provides insight into the effect of the parameter η on the likelihood. We can see how the fit deteriorates when the parameter η is forced to a value different from the best one.

We can use this information to construct a confidence interval, using the likelihood-ratio principle. Minus 2 times the log of the ratio of two likelihoods follows approximately a chi-square distribution with v degrees of freedom. The degrees of freedom is the difference in the number of parameters that were estimated (so the difference between θ_1 and θ), in our case one (when η is a single parameter). The confidence level of a value of η follows from:

$$-2\left(\ell_p(\eta|Y_+) - \ell(\hat{\theta}|Y_+)\right) \sim \chi^2_{v,1-\alpha} \tag{5.30}$$

Using chi-square assumes 'large' numbers of observations. The production of a profile is rather computation intensive as a new optimisation has to be performed for every value on the x-axis, for every parameter that requires a confidence interval.

5.3.2 Joint confidence regions for more parameters

The technique of profiling the likelihood function, as outlined in the previous section, also works for more than one parameter. Just make sure that the degrees of freedom v is the same as the difference in number of free parameters between the two sets θ_1 and θ. Comparing the confidence interval for a single parameter a to the confidence region for two parameters (a and b) of the same model, however, produces a (for me, at least) counter-intuitive result. The critical value of the χ^2 distribution increases with increasing degrees of freedom; going from one to two degrees, the 95% threshold increases from 3.84 to 5.99. Therefore, the joint confidence region of a and b includes values of a that are *outside* the single-parameter confidence interval for parameter a. For me, this result was paradoxical: clearly, the interval and the region are both correctly calculated, so how can they give contrasting results?

Some internet searching provided me with the right direction.[1] Both the interval and the joint region are correct, and I will try to explain how this can be understood.

Consider a model with two parameters, a and b. Using profile likelihoods, I derive 95% confidence intervals for each of them. In a two-dimensional plot, these single-parameter intervals would (rather naively, perhaps) indicate a rectangular joint region of confidence (Fig. 5.1).

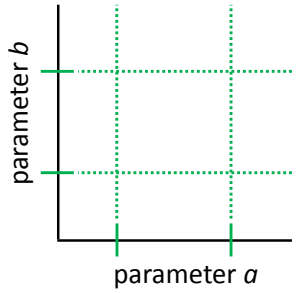

Figure 5.1: Two single-parameter 95% confidence intervals would indicate a rectangular joint region of confidence.

In practice, the joint confidence region of the two parameters will not be rectangular (although it would likely be possible to construct a model and a data set with these proprties). For example, it might be shaped as an ellipse. Imagine an ellipse that snugly fits the rectangular boundaries (Fig. 5.2). Now, the edges of the joint confidence region (the ellipse) coincide with both single parameter intervals.

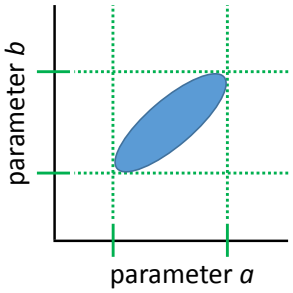

Figure 5.2: Fitting an ellipsoid joint confidence region into the rectangular joint region indicated by two single-parameter 95% confidence intervals.

The thing is now the white spaces within the rectangle. If the ellipse captures the x% joint confidence region of a and b, the rectangle will contain more than x%. Since our single-parameter intervals specify 95% intervals, the joint interval will capture less than that. In other words: the joint 95% confidence should extend beyond the boundaries of the rectangle (Fig. 5.3).

The edges of the ellipsoid are thus wider than the single-parameter intervals (shown in Fig. 5.4). The reason is that the white spaces within the rectangle are excluded from the joint interval. Values of parameter a smaller than its lower single-parameter confidence interval are now possible, but *only* given certain values of parameter b.

[1] Especially, the web pages of Prof. Ranjan Maitra of the Iowa State University set me in the right direction. In his lecture series http://www.public.iastate.edu/~maitra/stat501/lectures.html, with the lecture on "Inference for Means – Confidence Regions".

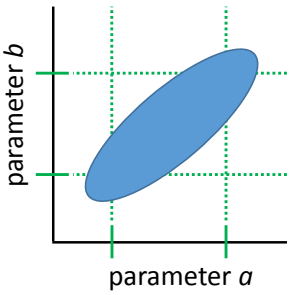

Figure 5.3: The 95% joint confidence region in relation to the rectangular joint region indicated by two single-parameter 95% confidence intervals.

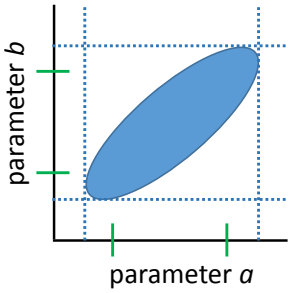

Figure 5.4: The edges of the 95% joint confidence region extend beyond the two single-parameter 95% confidence intervals.

In summary, the single-parameter interval and the joint region tell a different part of the same story. If we are interested in the uncertainty in parameter a, we should look at the single-parameter interval for parameter a. If we are interested in the joint uncertainty in a and b, we should focus on the joint confidence region. Parameter sets from within the joint region can be used to generate model predictions with associated uncertainty bounds.[2]

5.3.3 Asymptotic standard errors

To calculate standard errors for the parameter estimates, it is popular to assume that the maximum likelihood estimate for the parameters $\hat{\theta}$ follows a multivariate normal distribution. This assumption holds asymptotically for 'large' data sets and converges more rapidly to this asymptote for 'well-behaved' models. The profile likelihood is a more robust way to explore the confidence intervals for the parameters, but is more calculation intensive.

The Fisher information matrix $\boldsymbol{I}$ is the square matrix with the second-order partial derivatives of the log-likelihood to the parameters. These derivatives are evaluated in the maximum likelihood value of the parameters ($\theta = \hat{\theta}$).

[2]This is very similar to using a Bayesian sample from the posterior to make model predictions with uncertainty bounds. The big difference is, however, that for a Bayesian sample from the posterior, one would probably like to look at quantiles from the model predictions (e.g., the $2.5 - 97.5\%$ quantiles to produce 95% credible bounds on model curves). The values from the joint confidence region as discussed here are, however, *already* cut off at a certain confidence level (e.g., 95%). Therefore, it makes no sense to take quantiles on the model predictions again.

$$\boldsymbol{I}_{ij}(\hat{\theta}) = -\frac{\delta^2}{\delta\theta_i \delta\theta_j} \ell(\theta|Y_+)\Big|_{\theta=\hat{\theta}} \tag{5.31}$$

$$\approx -\frac{\ell(\hat{\theta}+\epsilon_i+\epsilon_j) - \ell(\hat{\theta}+\epsilon_i-\epsilon_j) - \ell(\hat{\theta}-\epsilon_i+\epsilon_j) + \ell(\hat{\theta}-\epsilon_i-\epsilon_j)}{4\epsilon_i\epsilon_j} \tag{5.32}$$

The covariance matrix is the inverse of the information matrix:

$$\mathbf{Cov} = \boldsymbol{I}^{-1} \tag{5.33}$$

The standard errors of the parameters can be derived from the diagonal variances:

$$\sigma_i = \mathbf{Cov}_{ii}^{1/2} \tag{5.34}$$

The matrix with correlation coefficients between the parameters can be obtained by normalising each element of the covariance matrix with the product of the standard errors of the parameters:

$$\mathbf{Cor}_{ij} = \frac{\mathbf{Cov}_{ij}}{\sigma_i \sigma_j} \tag{5.35}$$

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