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From food-dependent statistics to metabolic parameters, a practical guide to the use of dynamic energy budget theory

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ABSTRACT

The standard model of the dynamic energy budget theory for metabolic organisation has variables and parameters that can be quantified using indirect methods only. We present new methods (and software) to extract food-independent parameter values of the energy budget from food-dependent quantities that are easy to observe, and so facilitate the practical application of the theory to enhance predictability and extrapolation. A natural sequence of 10 steps is discussed to obtain some compound parameters first, then the primary parameters, then the composition parameters and finally the thermodynamic parameters; this sequence matches a sequence of required data of increasing complexity which is discussed in detail. Many applications do not require knowledge of all parameters, and we discuss methods to extrapolate parameters from one species to another. The conversion of mass, volume and energy measures of biomass is discussed; these conversions are not trivial because biomass can change in chemical composition in particular ways thanks to different forms of homeostasis. We solve problems like "What would be the ultimate reproduction rate and the von Bertalanffy growth rate at a specific food level, given that we have measured these statistics at abundant food?" and "What would be the maximum incubation time, given the parameters of the von Bertalanffy growth curve?". We propose a new non-destructive method for quantifying the chemical potential and entropy of living reserve and structure, that can potentially change our ideas on the thermodynamic properties of life. We illustrate the methods using data on daphnids and molluscs.

Key words: compound DEB parameters, mass-energy conversions, parameter identification, strong and weak homeostasis, surface-area-volume relationships, chemical composition, energy, entropy.

CONTENTS

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I. INTRODUCTION

Many biological quantities that are relatively easy to measure, such as body mass and respiration rate, have contributions from different processes, and are, therefore, not natural variables in explanatory models. The variables that such models have, such as reserve and structure, are typically not readily measurable, which calls for auxiliary theory for how to relate these variables to measurements. One of our own motivations originates in the analysis of toxicity data, where toxicants affect parameter values. Data from standardised bioassays to assess toxicity typically lack information about essential eco-physiological parameter values in the control, so this knowledge must be gained from other experiments. Our interest is in what type of data do we minimally need to have access to these parameter values. Knowledge of eco-physiological parameter values is also essential in the context of ecosystem modelling, and again in the analysis of the effects of toxicants at the ecosystem level (Brack *et al.*, 2005).

The dynamic energy budget (DEB) theory for metabolic organisation has all the essential components to deal with energy and mass balances, stoichiometric constraints on production and variable chemical composition of biomass (Kooijman, 2000, 2001). A problem in the application, however, is that many of the underlying processes are interlinked intimately, which makes it hard to study the various processes one by one. They need to be studied in coherence. An obvious question is "Why do we need difficult-to-quantify parameters of some model, and do not work with the easy-to-measure quantities only?". One application of the forward and backward translations of easy-to-measure quantities to (compound) DEB parameters that will be discussed is in solving problems like "What would be the ultimate reproduction rate and the von Bertalanffy growth rate at a specific food level, given that we have measured it at abundant food?". Knowledge of the compound DEB parameters are the key to the answer. This is a special case of a more general problem that is behind the words "quantities" *versus* "parameters". The difference is that quantities depend on experimental conditions, such as food availability, whereas parameters are constant (in principle). This has important consequences for extrapolation purposes and the understanding of biological phenomena.

This paper presents the required auxiliary theory for the standard model in DEB theory; this standard model deals with the metabolic organisation of an isomorph with a single reserve and a single structure that feeds on a single type of resource; see Nisbet *et al.*, (2000) for an introduction. Most animals fall into this category if we focus on simple nutritional situations. To make our contribution practical, the freely downloadable software package DEBtool can be used to perform all computations mentioned herein in a simple way. There is no need for the applied scientist to understand the technical details of this paper to use the software for the estimation of parameter values from data.

Sousa *et al.*, (2008) discuss the conceptual step from empirical patterns to formalised theory on metabolic organisation. The present paper provides the practical tools for this step using a minimum set of data. The aim is to discuss a natural sequence of steps in obtaining parameter values from data, starting with some compound parameters (i.e. functions of primary parameters), then the primary parameters themselves (which determine food uptake and changes in the state variables structure, reserve and maturity for a given trajectory of food density), then the composition parameters (of food, biomass and products), and finally the thermodynamic parameters. Many applications don't require knowledge on all parameters, so the sequence of steps can be followed partially only. For instance, if we want to predict an aspect of organisms that does not involve energy, we know *a priori* that there is no need to know any of the parameters that has energy in its dimension explicitly; it might be that ratios of energy parameters need to be known, but information on a ratio is weaker than that of the energy parameters themselves, and so requires less effort to obtain it from data. We make the relationship explicit between the strength of the data and the information that we can squeeze out of it.

We focus on the methodological and computational aspects, and only briefly reflect on statistical aspects. Our treatment is not meant to be exhaustive; auxiliary theory will develop further, just like the core theory. We provide some guidance in obtaining DEB parameters to supplement Kooijman (2000) and Van der Meer (2006a). A particularly nice application of the estimation of the parameter values of a species is to compare the values with those that can be expected on the basis of body size scaling relationships, that are implied by DEB theory (see Section IV.1); the differences represent specific evolutionary adaptations.

Energy is a useful and popular concept for comparative purposes, because using this concept seems to avoid the complexities inherent in the many chemical compounds organisms use. Although energy does facilitate comparison, we here demonstrate, however, that we cannot avoid the complexities inherent in chemical compounds and nutrition-related changes in the chemical composition of biomass. Although the quantification of thermodynamic parameters is demanding, it is still feasible, and we hope that this paper will motivate researchers to apply it to a selection of species.

First we discuss some basic problems that need to be solved and specify the standard DEB model being explicit on the model structure and the (primary) parameters that it contains. We don't show here how it follows from mechanistically inspired assumptions, or why this model is generic as well as biologically realistic. The latter topics are discussed in e.g. Kooijman (2000); Van der Meer (2006b) and (Sousa, Domingos & Kooijman, 2008). The following section presents the estimation of metabolic parameters. It is structured in a particular way, starting from a requirement for less-demanding measurements to more elaborate ones; the parameter estimates that are discussed in later sections make use of estimates obtained earlier. We give numerical examples for the application of our estimation procedure. We then consider parameters that quantify the (variable) chemical composition of biomass and the mass balance for the individual organism. The method is illustrated using data from the literature. We need this compositional information before we finally deal with the estimation of thermodynamic parameters. After looking forwards to more elaborate estimation procedures, the last section presents and discusses our conclusions.

II. PROBLEMS TO SOLVE

We first specify the problems for auxiliary theory that we need to solve in this paper in more detail.

To account for metabolic memory and nutrition-related changes in body mass composition, DEB theory partitions biomass into reserve and structure. Reserve is not a set of chemical compounds set apart for later use; reserve can have active metabolic functions. It is the dynamics that make the distinction between reserve and structure; being synthesised from food and used for metabolic purposes, reserve has an implied turnover, while structure is synthesised from reserve and requires (somatic) maintenance for turnover. There are no direct simple ways to quantify reserve and structure separately. They both contribute to body mass, for instance. The DEB theory is chemically (and biologically) implicit, meaning that it does not specify particular chemical compounds. Each particular compound, such as lipids, proteins and carbohydrates, can belong to reserve, to structure or to both, so how can we quantify these compartments?

To specify life-history events, DEB theory uses maturity, but there are also no direct methods to quantify maturity. Its formal status is information, it does not represent a mass or energy pool. DEB theory states that stage transitions (from embryo to juvenile, from juvenile to adult) occur if maturity reaches some threshold value, requiring a quantification of the maturity, but how can this be obtained from measurements?

One of the most characteristic elements of DEB theory is the κ-rule which states that a fixed fraction κ of the mobilised reserve is allocated to somatic maintenance plus growth (i.e. increase in the amount of structure), while the rest is allocated to maturity maintenance and maturation (in embryos and juveniles) or reproduction (in adults). A pertinent question is how to measure κ if we can't quantify reserve or maturity, and how to measure the maturity maintenance rate if we can't measure maturity? Only part of the reserve allocated to growth is actually fixed into structure, the rest (i.e. the overhead of growth) is converted into products that are excreted into the environment. How can we quantify the overhead costs? Most animals allocate to reproduction *via* a buffer that can contribute considerably to body mass. How should this buffer be quantified? In the DEB theoretical context it is even not obvious how to quantify somatic maintenance, because the basal respiration rate has contributions from other components (such as the overhead cost of growth).

These problems might seem to have no solutions at first sight, but the DEB theory is built on a number of solid principles that can be used here: strong and weak homeostasis. Strong homeostasis means that reserve and structure have a constant chemical composition, so any changes in the composition of the whole organism can be traced back to changes in the amount of reserve relative to that of structure. Weak homeostasis means that at constant food concentration, the body composition of the juvenile and the adult remains constant (possibly after an adaptation period) during growth and development. In combination with strong homeostasis this means that the reserve density, i.e. the ratio of the amounts of reserve and structure, remains constant, despite growth.

Strong homeostasis implies that reserve and structure can be quantified in terms of energy, volume as well as mass, see Table 1. Mass is quantified in C-moles (i.e. the number of C-atoms expressed as multiples of the Avogadro number; we use the dimension symbol #); mass is also quantified in gram (wet mass, dry mass, ash-free dry mass *etc.*). Although moles and grams are both units of mass, they are not equivalent. A (generalised) chemical compound whose mass is quantified in (C-)moles cannot change chemical composition, but a body whose mass is quantified in grams can (and actually does). Grams can only be converted easily to C-moles if the chemical composition is constant and known, but this rarely occurs in practice. There does not exist a

Table 1. The state variables of the standard dynamic energy budget (DEB) model, expressed in three different ways. The notation for energy in reserve $E_E = E$ and volume of structure $V_V = V$ is simplified. Energy is assessed by multiplying mass (in C-mole) by the chemical potential ($\bar{\mu}_E$ and $\bar{\mu}_V$). Maturity is quantified in invested reserve (in mass or energy). It does not represent a mass or energy pool, but information; it hardly makes sense to quantify it as volume, because there is no conservation law for volume. L stands for structural length

	Reserve E	Structure V	Maturity H
volume V		$V = L^3$	
mass M	M_E	$M_V = [M_V]V$	M_H
energy E	$E = \bar{\mu}_E M_E$	$E_V = \bar{\mu}_V M_V$	$E_H = \bar{\mu}_E M_H$

single most useful quantity for energetics. We need length because the feeding rate is linked to surface area. We need mass, however, to deal with mass conservation and energy to deal with energy conservation. DEB theory uses all of this.

With weak homeostasis we can assess the composition of both reserve and structure by comparing the body composition at different food densities, without being able to separate them physically. Weak homeostasis is only possible if strong homeostasis of the various compartments (here reserve and structure) applies. It fully specifies reserve dynamics in combination with strong homeostasis (Sousa *et al.*, 2008). The combination of presence (in juveniles and adults) and absence (in embryos) of weak homeostasis yields important information about metabolic organisation. We also show how starvation data can be used to replace (or supplement) embryo data (see Section IV.8).

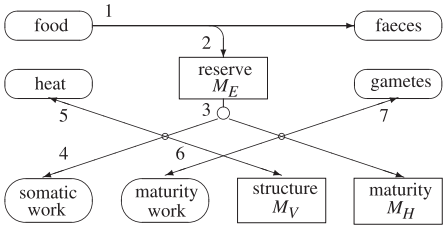
Parameter values are individual-specific in DEB theory, which is essential in evolutionary contexts (Kooijman *et al.*, 2003; Kooijman & Troost, 2007). We here treat parameter values as species-specific only, ignoring the relatively small differences within a species.

III. SPECIFICATION OF THE STANDARD DEB MODEL

This section summarises the standard DEB model. Fig. 1 presents the scheme of the standard DEB model and some notation. The state variables are listed in Table 1; the changes in the state variables are given in the Appendix.

Table 2 presents the primary parameters of the DEB model using time, length and mass only. This choice of primary parameters differs slightly from previous choices, e.g. Kooijman (2000). We here selected the specific searching rate $\{\dot{F}_m\}$ rather than the half-saturation coefficient $K = \{\dot{J}_{EAm}\}/y_{EX}\{\dot{F}_m\}$, because it is closer to the mechanism of the underlying feeding process. We use the maturity maintenance rate coefficient k_J rather than making an assumption about the maturity maintenance costs that causes that stage transitions also occur at fixed amounts of structure (so eliminating the need to think about maturation explicitly). This is because we want to allow for food-related differences in the amount of structure at stage transitions (birth, puberty). This also means that the maturity at birth M_H^b and at puberty M_H^p play explicit roles, rather than structural volume at birth V_b and at puberity V_p. The construct was avoided in the past, because these volumes are generally difficult to obtain numerically. This problem was, however, recently solved by Kooijman (2008). We here use the energy conductance $\dot{v}$ rather than the maximum reserve capacity $[M_{Em}] = \{\dot{J}_{EAm}\}/\dot{v}$ to have a better link with the mechanism of reserve mobilisation, see Kooijman & Troost (2007) for another recent gain in insight. We use the yield of reserve on food y_{EX} as a primary parameter rather than the maximum specific feeding rate $\{\dot{J}_{XAm}\} = \{\dot{J}_{EAm}\}/y_{EX}$ because this parameter is closer to the biochemical machinery, and therefore more conserved from an evolutionary perspective. Table 3 gives relationships between length, mass and energy and frequently occurring compound parameters on the basis of the chemical potential for reserve $\bar{\mu}_E$ (in J mol^{-1}) and the specific structural mass $[M_V]$ (in mol cm^{-3}). The parameter $[M_V]$ (which converts cubic centimetres into C-moles) has a similar status as a molecular mass (which converts moles into grams) or a specific density (which converts cubic centimetres into grams). Strong homeostasis makes these conversions simple.

Compound parameters (such as K, g, $\dot{k}_M$, L_m in Table 3) are (simple) functions of primary parameters. They typically have simple dimensions and are easier to extract from data than the primary parameters. Many applications only require knowledge of some compound parameters, so there is frequently no need to know all primary parameters. The somatic maintenance rate coefficient $\dot{k}_M = [\dot{J}_{EM}]y_{VE}[M_V]$ has the interpretation of the specific volume-linked somatic maintenance cost relative to that of a unit of structure.



Fluxes of food X and reserve E

1	$\dot{J}_{XA}$	feeding
2	$\dot{J}_{EA}$	assimilation
3	$\dot{J}_{EC}$	mobilisation
4	$\dot{J}_{EM}$	volume-linked somatic maintenance
5	$\dot{J}_{ET}$	surface-area-linked somatic maintenance
6	$\dot{J}_{EJ}$	maturity maintenance
7	$\dot{J}_{ER}$	reproduction

Fig. 1. The standard dynamic energy budget (DEB) model with fluxes (moles per time) and pools (moles) that illustrate the balance equations, equations (A1, A3, A4). Assimilation is zero during the embryo stage and becomes positive at the transition to the juvenile stage (birth) if food is available. Age is zero at the start of the embryo stage. Reproduction is zero during the juvenile stage and becomes positive at the transition to the adult stage (puberty), when further investment into maturation is ceased.

Table 2. The twelve primary parameters of the standard dynamic energy budget (DEB) model as represented in the length-mass-time frame. The cubed meters in the specific searching rate refer to the environment, all other meters to structure. Square brackets [ ] mean "per structural volume, curly braces { } mean "per structural surface area" and dots · mean "per time"

Symbol	Unit	Description	Process
$\{\dot{F}_m\}$	$m^3 d^{-1} m^{-2}$	surface-area-specific searching rate	feeding
$\{\dot{J}_{EAm}\}$	mol $d^{-1} m^{-2}$	surface-area-specific maximum assimilation rate	assimilation
y_{EX}	mol mol^{-1}	yield of reserve on food	digestion
y_{VE}	mol mol^{-1}	yield of structure on reserve	growth
$\dot{v}$	m d^{-1}	energy conductance	mobilisation
$\{\dot{J}_{ET}\}$	mol $d^{-1} m^{-2}$	surface-area-specific somatic maintenance	heating/osmosis
$[\dot{J}_{EM}]$	mol $d^{-1} m^{-3}$	volume-specific somatic maintenance	turnover/activity
k_J	d^{-1}	specific maturity maintenance	regulation/ immune defence
κ	-	allocation fraction	allocation
κ_R	-	reproduction efficiency	egg formation
M_H^b	mol	maturation at birth	life history
M_H^p	mol	maturation at puberty	life history

Likewise the maturity maintenance rate coefficient $\dot{k}_J$ is the maturity maintenance cost relative to that of a unit of maturity. But since we quantify maturity as the cumulative investment of reserve, the cost of a unit of maturity is one by definition.

The standard DEB model is specified mathematically in Eq. (A1 – A5) of the Appendix for the rate of change in the state variables. These equations can be considerably simplified under specific conditions, such as constant food density. These conditions can be created experimentally to

Table 3. Conversions and compound parameters. For descriptions of parameters and variables see Tables 1 and 2 and Fig. 1

Relationship	Unit	Description
$K = \frac{\{\dot{J}_{EAm}\}}{y_{EX}\{\dot{F}_m\}}$	mol m^{-3}	half-saturation constant
$\{\dot{J}_{XAm}\} = \{\dot{J}_{EAm}\}/y_{EX}$	mol $d^{-1} m^{-2}$	maximum specific ingestion rate
$M_{Vm} = L_m^3[M_V]$	mol	maximum structural mass
$[M_{Em}] = \{\dot{J}_{EAm}\}/\dot{v}$	mol m^{-3}	maximum reserve density
$m_H = M_H/M_V$	mol mol^{-1}	maturity density
$m_H^b = M_H^b/M_V$	mol mol^{-1}	maturity density at birth
$m_H^p = M_H^p/M_V$	mol mol^{-1}	maturity density at puberty
$m_E = M_E/M_V$	mol mol^{-1}	reserve density
$m_{Em} = [M_{Em}]/[M_V]$	mol mol^{-1}	maximum reserve density
$[E_m] = \{\dot{p}_{Am}\}/\dot{v}$	J m^{-3}	maximum reserve density
$L_m = \kappa\frac{\{\dot{J}_{EAm}\}}{[\dot{J}_{EM}]} = \kappa\frac{\{\dot{p}_{Am}\}}{[\dot{p}_M]} = \frac{\dot{v}}{\dot{k}_M g}$	m	maximum structural length
$L_T = \{\dot{p}_T\}/[\dot{p}_M]$	m	heating length
$U_E = M_E/\{\dot{J}_{EAm}\} = E/\{\dot{p}_{Am}\}$	d m^2	scaled reserve
$U_H = M_H/\{\dot{J}_{EAm}\} = E_H/\{\dot{p}_{Am}\}$	d m^2	scaled maturity
$\{\dot{p}_{Am}\} = \bar{\mu}_E\{\dot{J}_{EAm}\}$	J d^{-1} m^{-2}	maximum specific assimilation energy flux
$\{\dot{p}_T\} = \{\dot{J}_{ET}\}\bar{\mu}_E$	J d^{-1} m^{-3}	surface-area-specific maintenance energy flux
$[\dot{p}_M] = [\dot{J}_{EM}]\bar{\mu}_E = \dot{k}_M\bar{\mu}_{GV}[M_V]$	J d^{-1} m^{-3}	specific somatic maintenance energy flux
$[\dot{p}_J] = [\dot{J}_{EJ}]\bar{\mu}_E = \dot{k}_J E_H L^{-3}$	J d^{-1} m^{-3}	specific maturity maintenance energy flux
$\dot{k}_M = [\dot{p}_M]/[E_G] = j_{EM}\, y_{VE}$	d^{-1}	somatic maintenance rate coefficient
$\dot{J}_{EJ} = \dot{k}_J M_H$	mol d^{-1}	maturity maintenance mass flux
$j_{EM} = [\dot{J}_{EM}]/[M_V]$	mol mol^{-1} d^{-1}	specific somatic maintenance flux
$[E_G] = \bar{\mu}_E[M_V]/y_{VE}$	J m^{-3}	energy cost per structural volume
$\bar{\mu}_E = \{\dot{p}_{Am}\}/\{\dot{J}_{EAm}\}$	J mol^{-1}	chemical potential of reserve
$\bar{\mu}_{GV} = [E_G]/[M_V] = \bar{\mu}_E/y_{VE}$	J mol^{-1}	energy-mass coupler for growth
$g = \frac{[E_G]}{\kappa[E_m]} = \frac{\dot{v}[M_V]}{\kappa\{\dot{J}_{EAm}\}y_{VE}}$	–	energy investment ratio
$f = X/(K + X)$	–	scaled functional response
$e = \frac{m_E}{m_{Em}} = \frac{M_E\dot{v}}{L^3\{\dot{J}_{EAm}\}}$	–	scaled reserve density
$l = L/L_m$	–	scaled length

obtain parameter values that can be applied under more complex conditions. The parameter estimation steps focus on constant food densities, while varying food densities are discussed briefly in Section V. Consistency constraints apply to parameter values, which are discussed briefly below. Not all combinations of values make physical and physiological sense.

The scaled functional response, i.e. the feeding rate as a fraction of the maximum feeding rate of an individual of that size, $f = \frac{\dot{J}_{XA}}{\dot{J}_{XAm}} = \frac{\dot{J}_{EA}}{\dot{J}_{EAm}}$, is a function of food availability. Exactly how only becomes important when we want to make the step from compound to primary parameters and have to use masses for the first time. This is discussed in Section IV.6 and later.

If the scaled functional response remains constant, the scaled reserve density becomes equal to the scaled functional response, $e = f$, and the scaled ultimate length becomes equal to the scaled reserve density, $L_\infty/L_m = e$. Therefore, the food density that just covers maintenance cost increases with the structural length. If the somatic and maturity rate coefficients are equal, stage transitions not only occur at fixed maturity levels, but also at fixed amounts of structure. If this is the case, there is no need to deal explicitly with maturity as a state variable. The Appendix gives more details.

The volume-specific somatic maintenance flux $[\dot{J}_{EM}]$ and surface-area-specific somatic maintenance flux $\{\dot{J}_{ET}\}$ are assumed to be constant (at constant temperature); but $\{\dot{J}_{ET}\} = 0$ in the embryo stage ($M_H < M_H^b$) of most endotherms. Since $\{\dot{J}_{ET}\} = 0$ in all stages of most species, this paper does not discuss its estimation in detail. The somatic maintenance cost is a component of the somatic maintenance rate coefficient $\dot{k}_M$, which appears in the early steps. The maintenance cost itself is only discussed explicitly in Section IV.7, since it has mass in its dimension.

The change in structural mass of the Appendix can be converted to the change in structural length $L = (M_V/[M_V])^{1/3}$ as

$$\frac{d}{dt}L = \dot{r}_B(eL_m - L - L_T)$$

$$\text{with } \dot{r}_B = \frac{1}{3}\frac{\dot{k}_M g}{e+g} = \frac{1}{3}\frac{1}{\dot{k}_M^{-1} + eL_m/\dot{v}}. \qquad (1)$$

where the quantity $\dot{r}_B$ is known as the von Bertalanffy growth rate, see Fig. 3. Although it is constant at constant food density, it is not a parameter in the DEB context because its value depends on state variables (namely the scaled reserve density e, and so on the amounts of reserve M_E and structure M_V) and indirectly on food availability. The von Bertalanffy growth rate plays an important role in the early steps of the estimation of metabolic parameters; the literature reports values for many species, Kooijman (2000) provides them for 270 species. The heating length L_T is the length an organism remains smaller due to somatic maintenance costs that are linked to the surface area (heating in endotherms, osmosis in freshwater organisms). These costs don't affect the von Bertalanffy growth rate, only the ultimate length.

The reserve density at birth m_E^b equals that of the mother at the moment of egg formation; it represents maternal effect. This means, if the mother is living for some time at a constant food level, which corresponds with some functional response f, then the reserve density of the embryo at birth equals $m_E^b = fm_{Em}$. These initial conditions imply von Bertalanffy growth at constant food availability right after birth. Foetal development is a variant of egg development. The initial amount of reserve is given in the Appendix and the computational aspects are discussed in Kooijman (2008).

Maturity at birth M_H^b is a primary parameter. Length at birth L_b and age at birth a_b are functions of parameters and the nutritional state of the mother, *via* f. To avoid mass in the first estimation steps, we scale M_H^b with the specific maximum assimilation rate $\{\dot{J}_{EAm}\}$, which results in a rather abstract parameter $U_H^b = M_H^b/\{\dot{J}_{EAm}\}$ with dimension time times squared length. This 'trick' allows us to use statistics of the embryo stage in the early steps of the estimation of metabolic parameters; the way length at birth (or puberty) relates to food density provides valuable information about the maintenance ratio, i.e. the ratio of the maturity and the somatic maintenance rate coefficients.

We must have that length at birth L_b and puberty L_p are smaller than maximum length L_m, but not necessarily that $L_b < L_p$; aphids are an example of organisms for which $L_p < L_b$; they allocate to reproduction in the embryo stage. DEB theory has no problems with such cases and shows that we should think in terms of events in life histories (e.g. switching on of assimilation, and allocation to reproduction) rather than in terms of life stages.

DEB theory shows [see Eq. (A13)] that the age at birth should be less than $L_b/(L_\infty \dot{r}_B)$. If age at birth is close to this value we have a large value for $\dot{k}_M$ and a small value for g (see Fig. 2). A geometric interpretation of this constraint is given in Fig. 3. For humans, we have a birth mass of 3.5 kg, an adult mass of 70 kg, and a von Bertalanffy growth rate of 0.15 year^{-1}, so the gestation time should be less than $(3.5/70)^{1/3}/0.15 = 2.5$ year. A larger value would be inconsistent with DEB theory for any combination of parameter values. The relationship between foetal and egg development is given in Kooijman (2000) (see also Section VI). The fact that reserve contributes to mass is here not important, because weak homeostasis implies that reserve makes up a constant fraction of mass, as long as the baby and adult experience similar food conditions.

Age zero is defined as the start of development, an event that is not always easy to observe or to infer; sometimes the age at birth seems to be longer than the maximum possible value (e.g. in *Armadillo spp.*) because some species delay the onset of development. In other species with an age at birth exceeding the maximum value, the parameters of the embryo stage differ from that in later stages, this can be expected in species that exhibit metamorphosis, e.g. anchovy *Engraulis encrasicolus* (Pecquerie, 2008). The standard DEB model no longer applies in this case and more advanced DEB models should be used that take these details into account.

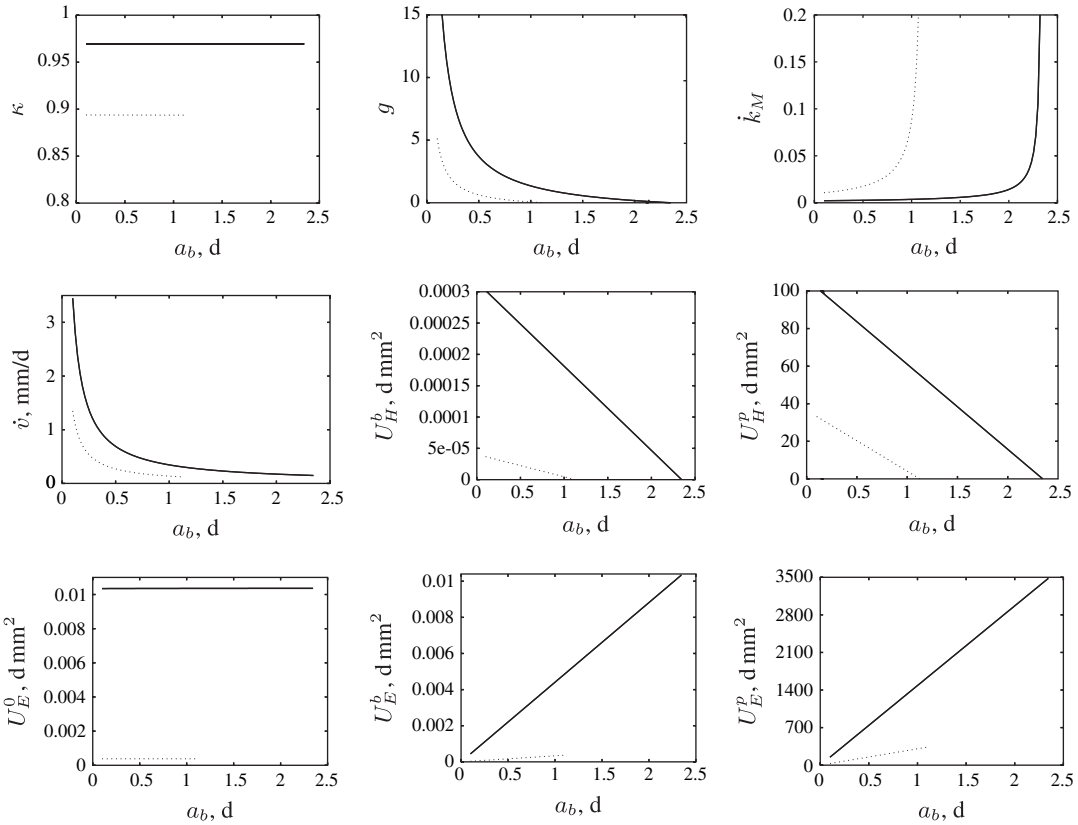


Fig. 2. Dynamic energy budget (DEB) parameters as function of age at birth, a_b, as obtained from DEBtool/animal/get_pars_r for *Haliotis tuberculata* (solid curves) and *Ruditapes philippinarum* (dotted curves). The easy-to-observe quantities for *Haliotis* are for $f = 1$: $L_b = 0.115$ mm, $L_p = 8$ mm, $L_\infty = 70$ mm, $\dot{r}_B = 0.0007$ d^{-1}, $\dot{R}_\infty = 5{\times}10^6$ year^{-1} (Fred Jean, personal communication) and for *Ruditapes* for $f = 0.5$: $L_b = 0.045$ mm, $L_p = 4.35$ mm, $L_\infty = 12.3$ mm, $\dot{r}_B = 0.00323$ d^{-1}, $\dot{R}_\infty = 7{\times}10^6$ year^{-1} (Jonathan Flye Sainte Marie, personal communication). All lengths are structural lengths and L_∞ stands for the measured ultimate length. The upper boundaries for the age at birth are the maximum possible ones for these data. The symbols are defined in Tables 2 – 3; superscrips 0, b and p indicate age zero, birth and puberty, respectively.

IV. ESTIMATION OF BUDGET PARAMETERS

The estimation of parameters can be structured naturally into 10 steps. In step 1 we avoid the estimation of parameter values and work with 'circumstantial evidence' using knowledge of the maximum length of a species only. The subsequent sequence of estimation steps starts with observations on growth and reproduction at a single food density (typically at abundant food, steps 2 and 3), and then at several food densities (steps 4 and 5), (Table 4). It is experimentally difficult to keep food constant at some low value. Alternatively one could work at abundant food (which does not need careful control to a constant density), but reduce its nutritional quality by mixing it with an indigestible compound, for example with silt for filtering bivalves, see e.g. Kooijman (2006). DEB theory was developed for dynamically changing food (and temperature) conditions, and if food densities are measured as functions of time, observations on growth and reproduction in dynamic environments yield the required information on all primary DEB parameters. This, however, involves advanced methods for parameter estimation that are briefly discussed in Section V. The 10 steps discussed in this section assume that food density is constant; it is also conceptually important to see what extra information on energetics can be gained from comparison of food densities.

Although steps 1 to 5 use values of the scaled functional response, this does not necessarily involve measurements on feeding, since the scaled functional response equals the scaled ultimate length, $f = L_\infty/L_m$, for instance. To obtain the maximum length, we need observations at abundant food. Steps 4 and 5 compare feeding, growth and reproduction at several food levels. For supply systems scaled functional responses might be chosen rather far apart from each other, which would contribute to the accuracy. Demand systems, however, would not survive such large differences.

Length measures are very popular in the literature, possibly because they can be obtained in a non-destructive way. Therefore, in steps 2 to 5 we discuss how particular compound parameters can be obtained from data using length and time only. Yet the detailed interpretation of length measures requires some discussion. We here assume that reserve contributes little to actual length (= the length

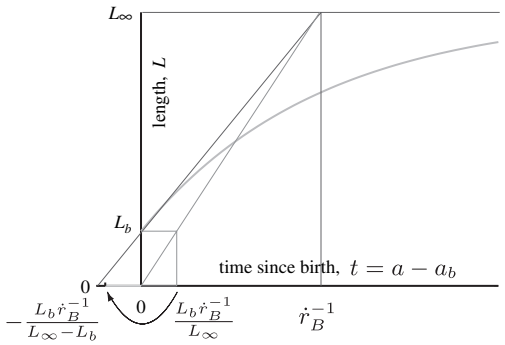


Fig. 3. The von Bertalanffy growth curve $\frac{d}{dt}L = \dot{r}_B(L_\infty - L)$, with the geometric interpretation of the von Bertalanffy growth rate $\dot{r}_B$, and the maximum possible age at birth $\frac{L_b \dot{r}_B^{-1}}{L_\infty}$ in the context of dynamic energy budget (DEB) theory. The tangent line at $t = 0$ intersects the asymptote (ultimate length L_∞) at the inverse von Bertlanffy growth rate, $\dot{r}_B^{-1}$; the line from the origin to this intersection point hits level length at birth L_b at time $\frac{L_b}{L_\infty \dot{r}_B}$, which is the maximum possible incubation time. a indicates ages, a_b age at birth.

that we can measure). So actual length is a proxy for structure. Structure as well as reserve contribute to the volume of the organism. Volumetric length is defined as the cubic root of the volume, so reserve contributes to volumetric length; since the specific density of organisms does not change dramatically, volume is typically proportional to wet mass. Structural length is the cubic root of structural volume. Actual length depends on shape and how the length measure is defined. The shape coefficient represents the ratio of the structural length and the actual length; we treat this as a parameter that needs to be quantified (see step 8). For comparison purposes it makes sense to use structural length, which is independent of shape, but involves a shape coefficient (see step 8). The assumption of isomorphy of the standard DEB model, i.e. a lack of change in shape during growth, is made for structural volume, not for volume.

We avoid the use of mass in the expressions until step 6, since the interpretation of mass is linked to chemical composition (that can change). We avoid the use of energy in the expressions until step 10, because free energies and entropy require knowledge of mass fluxes. We avoid the use of information to quantify maturity in all steps, and use the cumulated investment of mass of reserve in maturity instead.

In step 6 we deal with feeding rates, forcing us to include mass, as an introduction to step 7 where we obtain all primary parameters. Steps 8 – 10 consider mass, energy and entropy balances, the cornerstones of DEB theory, which we typically need in more advanced applications, which e.g. prepare for variations in composition of food and food selection.

All rate parameters depend on temperature in ways that are discussed in Kooijman (2000). The dependence of rates on temperature involves the measurement of Arrhenius temperatures (T_A) (Kooijman, 2000). Arrhenius temperatures are typically high ($T_A \simeq 12$ kK) for species that naturally experience small temperature changes (e.g. pelagic species), and much lower for species in e.g. the intertidal zone ($T_A \simeq 6$ kK). We here assume that environmental and body temperature are constant, and if the temperatures for two data sets differ, rates are corrected for this difference using $\dot{r}_B(T_2) = \dot{r}_B(T_1)\exp(T_A(T_1^{-1} - T_2^{-1}))$, for the von Bertalanffy growth rate $\dot{r}_B$, for instance. The Arrhenius temperature is specific-specific and this correction is only appropriate for temperature differences within a species-specific temperature range. We have to correct all rate parameters ($\{\dot{F}_m\}$, $\{\dot{J}_{EAm}\}$, $\dot{v}$, $\{\dot{J}_{ET}\}$, $[\dot{J}_{EM}]$, $\dot{k}_J$, see Table 2) for differences in temperature. See Kooijman (2000) for more advanced corrections for differences in temperature.

Table 4. Quantities that need to be measured in the different steps to estimate dynamic energy budget (DEB) parameters (see Section IV)

Symbol	Unit	Description	Steps one food level	several food levels
L_m	m	maximum length (at abundant food)	1	
L_∞	m	ultimate length (von Bertalanffy)	2,3	4,5
L_b	m	length at birth (first feeding)	2,3	4,5
$\dot{r}_B$	d^{-1}	von Bertalanffy growth rate	2,3	4,5
a_b	d	age at birth	2,3	4,5
L_p	m	length at puberty	3	5
$\dot{R}_\infty$	d^{-1}	reproduction rate at L_∞	3	5
$\dot{J}_{XA}$	mol d^{-1}	feeding rate		6
M_E^0	mol	mass of a freshly laid egg	7	
M_V^b	mol	mass of a neonate (first feeding)	7	
W_0	g	mass of a freshly laid egg	7	
W_b	g	mass of a neonate	7	
n_{*W}	#	elemental composition		8
X	mol m^3	food density		9
W	g	mass		9
L	m	length		9
$\dot{p}_{T+}$	W	dissipating heat		10

(1) Step 1: Maximum body length only

The first natural question is: how can we avoid estimating parameters? Parameter values are individual-specific. Within a particular taxon, primary parameters probably vary less than between taxa. This offers the possibility to estimate parameters for a particular species using parameters of another species and correcting for the difference in maximum structural length. The body-size-corrected mean values can be compared with parameter estimates from data to detect evolutionary adaptations. Such adaptations especially apply to life-history parameters (the specific maturities at birth and puberty, see Cardoso, van der Veer & Kooijman (2006), and much less to biochemical parameters, such as the yield coefficients y_{EX} and y_{VE} (Table 2), because all species use the same biochemical machinery. Part of the variability in egg size and in (relative) length at birth among species originates from variability of the maintenance ratio Kooijman (2008).

DEB theory implies simple rules for the co-variation of primary parameter values among species (Kooijman, 1986, 2000; Kooijman *et al.*, 2007). For the choice of primary parameters in Table 2, only three parameters vary systematically with maximum structural length L_m (see Table 3): the maximum specific assimilation rate $\{\dot{J}_{EAm}\}$, which is proportional to L_m, and the maturity at birth M_H^b and puberty M_H^p, which are proportional to L_m^3. Therefore, the mass-specific maturities $m_H^b = M_H^b/M_{Vm}$ and $m_H^p = M_H^p/M_{Vm}$ (with maximum structural mass M_{Vm} given in Table 3) are independent of maximum structural length. All other primary parameters do not vary systematically with maximum structural length.

The practical application of these rules is as follows: given $\{\dot{J}_{EAm}\}$, M_H^b and M_H^p for a reference species of maximum structural length L_{m1}, the parameters become $z\{\dot{J}_{EAm}\}$, $z^3 M_H^b$ and $z^3 M_H^p$ for zoom factor $z = L_{m2}/L_{m1}$, where L_{m2} is the maximum structural length of the species under consideration. It is essential to work with structural lengths here, unless the shapes of both species happen to be the same. All other parameters are the same for both species.

Compound parameters can depend on L_m, and many eco-physiological traits, such as respiration, can be written as compound parameters. Table 5 gives values that can be used to approximate the parameters discussed in the first five steps. This co-variation of values of some primary parameters explains why respiration (being the use of dioxygen or the production of carbon dioxide or heat) varies among species more or less allometrically with maximum body mass to the power somewhere between 2/3 and 1, and also explains why differences between taxa do exist. See Van der Meer (2006b) for a discussion. Notice that we here compared different species (so different parameter values), which is, within the context of DEB theory, very different from comparisons of different body sizes of a single individual at different points in its life cycle (the same parameter values, but different values of state variables). Respiration again depends more or less allometrically on body mass of an individual at different points in its life cycle, and the allometric coefficient is again somewhere between 2/3 and 1, but the explanation for this is very different: the allocation to growth and reproduction changes during the life cycle.

Table 5. Typical parameter values that occur in steps 1 to 5 for a species of maximum structural length L_m in cm at 20°C

$T_A = 12.5$ kK	Arrhenius temperature
$\dot{v} = 0.04$ cm d^{-1}	energy conductance
$g = 4/L_m$	energy investment ratio
$k_M = 0.015$ d^{-1}	somatic maintenance rate coefficient
$\dot{k}_J = 0.005$ d^{-1}	maturity maintenance rate coefficient
$\kappa = 0.8$	allocation fraction to soma
$\kappa_R = 0.95$	reproduction efficiency
$U_H^b = 4\ 10^{-8} L_m^3$ d cm^2	scaled maturity at birth
$U_H^p = 0.01 L_m^3$ d cm^2	scaled maturity at puberty

(2) Step 2: Growth at a single food density

Suppose that only information on growth at one food level is available, resulting in a scaled functional response f_1 (i.e. some value between 0 and 1). Food density does not need to be constant, as long as it is abundant ($f_1 \simeq 1$). We have no information about how length at birth depends on food level, so we are forced to assume that the somatic and maturity rate coefficients are equal, $\dot{k}_M = \dot{k}_J$, which implies that stage transitions occur at a fixed length as well. This assumption simplifies matters considerably; until puberty we have $U_H = L^3(1-\kappa)g/\dot{v}$, which means that maturity density, i.e. the maturity per amount of structure, remains constant (even at varying food density) removing the need to deal with maturity explicitly (until step 4, see Section IV.4).

The equations in the Appendix can then be used to make the following map

$$(L_b, L_\infty, a_b, \dot{r}_B \text{ at } f_1) \rightarrow (g, \dot{k}_M = \dot{k}_J, \dot{v}, U_E^0, U_E^b) \qquad (2)$$

where $U_E^0 = M_E^0/\{\dot{J}_{EAm}\}$ and $U_E^b = M_E^b/\{\dot{J}_{EAm}\}$ are the scaled reserves at age zero and at birth.

The inverse map can also be made

$$(g, \dot{k}_M = \dot{k}_J, \dot{v}, f_1) \rightarrow (L_b, L_\infty, a_b, \dot{r}_B, U_E^0, U_E^b). \qquad (3)$$

The functions ‘‘get_pars_g and ‘‘iget_pars_g in software package DEBtool perform the required computations for this map and its inverse. The function ‘‘elas_pars_g can be used to compute the elasticity coefficients to study how changes in the easy-to-measure quantities translate into changes in the DEB parameters.

If the energy conductance $\dot{v}$, having dimension length per time, is obtained using actual length measurements, the resulting value is in terms of these length measurements. Multiplication of $\dot{v}$ by the shape coefficient converts it to structural length per time; the values of the scaled initial reserve U_E^0 and the scaled reserve at birth U_E^b should then be multiplied by the squared shape coefficient. See also step 7 (Section IV.7).

(3) Step 3: Growth and reproduction at a single food density

Suppose now that we have information on both growth and reproduction at a single scaled function response f_1. We still have no information about how length at birth and at puberty depend on food level, so we are still forced to assume $\dot{k}_M = \dot{k}_J$. We infer the scaled threshold values for maturity at stage transitions from length at birth and puberty at abundant food.

We assume that the losses in the overhead of reproduction are small, $\kappa_R \simeq 0.95$, since reserve of the mother is transformed into reserve of the offspring with the same chemical composition, so little chemical work is involved. The compound parameters that can be obtained from easy-to-measure quantities at constant scaled functional response are:

$$(L_b, L_p, L_\infty, a_b, \dot{r}_B, \dot{r}_\infty \,\mathrm{at} f_1) \overset{\text{given } \kappa_R}{\rightarrow} (\kappa, g, \dot{k}_J = \dot{k}_M, \dot{v}, U_H^b, U_H^p, U_E^0, U_E^b, U_E^p) \quad (4)$$

where the superscripts 0, b and p refer to age zero, burth and puberty.

The equations in the appendix define this map. The inverse map can also be made

$$(\kappa, g, \dot{k}_J = \dot{k}_M, \dot{v}, U_H^b, U_H^p, f_1) \overset{\text{given } \kappa_R}{\rightarrow} (L_b, L_p, L_\infty, a_b, \dot{r}_B, \dot{R}_\infty, U_E^0, U_E^b, U_E^p). \quad (5)$$

The functions "get_pars_r" and "iget_pars_r" in software package DEBtool do the required computations for this map and its inverse. Table 6 gives a numerical example. The function "elas_pars_r" can be used to compute the elasticity coefficients to study how changes in the easy-to-measure quantities translate into changes in the DEB parameters.

Table 6. A numerical example for the (unique) conversion from easy-to-measure quantities to dynamic energy budget (DEB) parameters and quantities. The scaled reserve at age zero, U_E^0, and at birth, U_E^b, and puberty, U_E^p, are not parameters and depend on food density; their values follow from the conversion from easy-to-measure quantities to DEB parameters, as well as from the reversed conversion. The scaled functional response is assumed to be $f = 1$ and the reproduction efficiency $\kappa_R = 0.95$. The assumption of fixed amounts of structure at stage transition is made. For a description of symbols see Tables 1 – 3; superscripts 0, b and p indicate age zero, birth and puberty, respectively

Measured quantities	DEB parameters	DEB quantities
$L_b = 1.46$ mm	$\kappa = 0.6$	$U_E^0 = 3.95$ mm^{2}d
$L_p = 1.98$ mm	$g = 2$	$U_E^b = 1.25$ mm^{2}d
$L_\infty = 12.5$ mm	$\dot{k}_J = 0.1$ d^{-1}	$U_E^p = 3.13$ mm^{2}d
$a_b = 2.3$ d	$\dot{k}_M = 0.1$ d^{-1}	
$\dot{r}_B = 0.022$ d^{-1}	$\dot{v} = 2.5$ mm d^{-1}	
$\dot{R}_\infty = 15$ d^{-1}	$U_H^b = 1$ mm^{2}d	
	$U_H^p = 2.5$ mm^{2}d	

Note that the length at first allocation to reproduction, L_p, is smaller than the length at the first reproduction event because organisms start by depositing the allocated reserve in a buffer and convert this deposited reserve later into offspring. Various taxa use different environmental triggers for handling this buffer.

Fig. 2 shows how the DEB parameters and quantities depend on age at birth for *Haliotis tuberculata* and *Ruditapes philippinarum*. The conclusion is that this age needs to be known rather accurately, which is the reason why its use is avoided in the next estimation step.

(4) Step 4: Growth at several food densities

If we have more than one food density, information is available for how the food level affects the length at stage transition, and this determines the maturity maintenance rate coefficient $\dot{k}_J$ relative to the somatic maintenance rate coefficient $\dot{k}_M$. So from this step in the parameter estimation onwards, we no longer assume $\dot{k}_J = \dot{k}_M$, Within the context of DEB theory there is no reason to believe that stage transitions occur at fixed amounts of structure, although empirical evidence indicates that this is not too unrealistic. If $\dot{k}_J \neq \dot{k}_M$, maturity density varies, even at constant food density, which requires that maturity is explicitly included as a state variable.

From this step onwards we don't use the age at birth, because it is typically rather difficult to access. A resting stage can precede the development of the embryo (see Section VI). From Eq. (A8) it is clear that $\dot{r}_B^{-1}$ is linear in f, and the intercept and the slope can be used to obtain $\dot{k}_M$ and $\dot{v}$, see Fig. 4. Kooijman (2000, Fig. 3.14) shows that this perfectly matches data for *Daphnia magna*. Although the von Bertalanffy growth rate $\dot{r}_B$ at several food densities has information on the somatic maintenance rate coefficient $\dot{k}_M$, the information is weak only, and very weak if the smallest scaled function response is not that small.

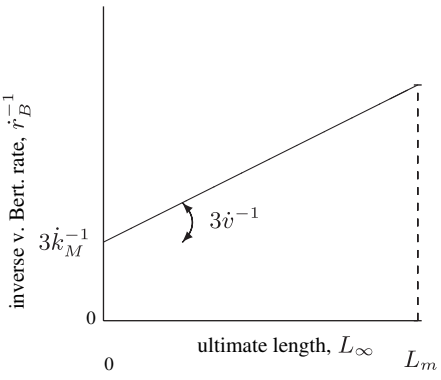


Fig. 4. The inverse von Bertalanffy growth rate $\dot{r}_B^{-1}$ as a function of ultimate length $L_\infty = L(\infty)$ results in a straight line with simple relationships with the somatic maintenance rate coefficient $\dot{k}_M$ and the energy conductance $\dot{v}$; see Eq. (A8). Since it is rarely possible to raise individuals at very low food levels, the lowest L_∞ is closer to maximum structural length L_m than to zero, which means that the information on $\dot{k}_M$ is only weak.

We can make the map

$$\begin{pmatrix} L_b, L_\infty, \dot{r}_B \text{ at } f_1 \\ \\ L_b, L_\infty, \dot{r}_B \text{ at } f_2 \end{pmatrix} \rightarrow \begin{pmatrix} & & U_E^0, U_E^b \text{ at } f_1 \\ g, \dot{k}_\jmath, \dot{k}_M, \dot{v}, U_H^b, & & \\ & & U_E^0, U_E^b \text{ at } f_2 \end{pmatrix}. \quad (6)$$

This map is over-determined, meaning that if (small) errors in the easy-to-observe quantities are present, the map is not exact and a minimisation of the weighted sum of squared deviations should lead to the best map. This also means that it is still possible to make the map even if some of the quantities are missing. Notice that this map only involves time and length, and neither mass in C-moles or grams, nor energy (Joules).

The inverse map can also be made

$$(g, \dot{k}_\jmath, \dot{k}_M, \dot{v}, U_H^b, f_1, f_2) \rightarrow \begin{pmatrix} L_b, L_\infty, \dot{r}_B, U_E^0, U_E^b \text{ at } f_1 \\ \\ L_b, L_\infty, \dot{r}_B, U_E^0, U_E^b \text{ at } f_2 \end{pmatrix}. \quad (7)$$

The map from easy-to-observe quantities on growth at several food densities and its inverse are computed by functions 'get_pars_h' and 'iget_pars_h' of software package DEBtool in toolbox 'animal' respectively. The functions 'get_pars_i' and 'iget_pars_i' do the same, assuming that $\dot{k}_\jmath = \dot{k}_M$. All routines can handle more than two food levels.

(5) Step 5: Growth and reproduction at several food densities

If information on both growth and reproduction is available for more than one food level, we can make the map

$$\begin{pmatrix} L_b, L_p, L_\infty, \dot{r}_B, \dot{R}_\infty \text{ at } f_1 \\ \\ L_b, L_p, L_\infty, \dot{r}_B, \dot{R}_\infty \text{ at } f_2 \end{pmatrix} \overset{\text{given } \kappa_R}{\rightarrow} \begin{pmatrix} & & U_E^0, U_E^b, U_E^p \text{ at } f_1 \\ \kappa, g, \dot{k}_\jmath, \dot{k}_M, \dot{v}, U_H^b, U_H^p, & & \\ & & U_E^0, U_E^b, U_E^p \text{ at } f_2 \end{pmatrix}. \quad (8)$$

Like the previous map, this map is also over-determined. Notice that this map only involves time, number and length, and neither mass in or grams, nor energy (Joules). Data involved in this step do not determine the fraction of reserve allocated to reproduction that is fixed in embryo's, κ_R.

The inverse map can also be made

$$(\kappa, g, \dot{k}_\jmath, \dot{k}_M, \dot{v}, U_H^b, U_H^p, f_1, f_2) \overset{\text{given } \kappa_R}{\rightarrow} \begin{pmatrix} L_b, L_p, L_\infty, \dot{r}_B, \dot{R}_\infty, U_E^0, U_E^b, U_E^p \text{ at } f_1 \\ \\ L_b, L_p, L_\infty, \dot{r}_B, \dot{R}_\infty, U_E^0, U_E^b, U_E^p \text{ at } f_2 \end{pmatrix}. \quad (9)$$

The function "get_pars_s" of software package DEBtool in toolbox "animal" can be used to make the conversion from easy-to-measure quantities to DEB parameters; the function "iget_pars_s" makes the inverse conversion and can be used to test the results. Both directions of conversion yield the scaled amounts of reserve at start, birth and puberty. The functions "get_pars_t" and "iget_pars_t" do the same, but assume that $\dot{k}_\jmath = \dot{k}_M$, which simplifies matters considerably; the function "get_pars_s" uses "get_pars_t" to obtain initial estimates and uses a variety of algorithms (genetic algorithms, followed by simplex and Newton-Raphson procedures) to make the map. These computations are rather complex.

DEB theory predicts that the reproduction rate varies considerably if food density varies around low levels. This is consistent with data, *cf.* Gurney & Nisbet (1998, Fig. 1.5b). So if this actually happened in the data, a small weight coefficient should be specified for such data; the DEBtool routines allow assignment of weight coefficients. Table 7 illustrates an application where the reproduction rate at low food levels is even ignored.

(6) Step 6: Food intake

Knowledge about food intake is especially relevant in population dynamics studies to quantify the loss rates of prey. DEB theory links food uptake

Table 7. A numerical example for the step from easy-to-measure quantities to dynamic energy budget (DEB) parameters and quantities for two food levels. The data are for female *Daphnia magna* at 20°C (Kooijman, 2000). Again we used $\kappa_R = 0.95$ and weight coefficients 1 for L_b, 1 for L_p, 0.5 for L_∞, 2 for $\dot{r}_B$, 0.1 for $\dot{R}_\infty$. This choice combines the accuracy of the measurements and the effect the numerical value should have on the result. Notice that data point $\dot{R}_\infty$ for $f = 0.7$ is missing; it turns out that 4.0 d^{-1} should be expected with these data. The scaled reserve at age zero and at birth and puberty are not parameters, nor is the age at birth. The reversed step can also be taken: from DEB parameters to easy-to-measure quantities and DEB quantities. This proves that the steps are unique. The values for the DEB quantities follow from the conversion steps in both directions. All symbols are defined in Tables 2 – 3; superscripts 0, b and p indicate age zero, birth and puberty, respectively

Measured quantities			DEB quantities	
$f = 1$	$f = 0.7$	DEB parameters	$f = 1$	$f = 0.7$
$L_b = 0.77$ mm	0.76 mm	$\kappa = 0.80$	$U_E^0 = 0.223$ mm^2d	0.183 mm^2d
$L_p = 2.5$ mm	2.3 mm	$g = 0.422$	$U_E^b = 0.140$ mm^2d	0.098 mm^2d
$L_\infty = 4.48$ mm	3.14 mm	$\dot{k}_\jmath = 1.70$ d^{-1}	$U_E^p = 4.25$ mm^2d	2.97 mm^2d
$\dot{r}_B = 0.158$ d^{-1}	0.216 d^{-1}	$\dot{k}_M = 1.71$ d^{-1}	$a_b = 0.80$ d	0.83 d
$\dot{R}_\infty = 14.7$ d^{-1}	–	$\dot{v} = 3.24$ mm d^{-1}		
		$U_H^b = 0.012$ mm^2d		
		$U_H^p = 0.366$ mm^2d		

$$\dot{\tilde{\jmath}}_{XA} = -f\{\dot{\tilde{\jmath}}_{XAm}\}L^2 \quad (10)$$

to surface area. The scaled functional response is the Holling type II, $f = \frac{X}{X+K}$, for food density X and half-saturation constant K. The maximum specific feeding rate $\{\dot{\tilde{\jmath}}_{XAm}\}$ can be obtained from measurements of the feeding rate at several food densities and fitting a hyperbola. The specific searching rate then follows from $\{\dot{F}_m\} = \{\dot{\tilde{\jmath}}_{XAm}\}/K$; this rate will generally depend on food and environmental details. We chose the specific searching rate $\{\dot{F}_m\}$ as a primary parameter, rather than the half-saturation constant K, because it is closer to the underlying feeding mechanism, and does not depend on the maximum size of a species (K and $\{\dot{\tilde{\jmath}}_{XAm}\}$ increase with the the maximum length of a species). We take the flux $\tilde{\jmath}_{XA}$ to be negative to indicate that food is disappearing ($\frac{d}{dt}X < 0$); we need this in Eq. (16) in estimation step 9 (Section IV.9), where positive fluxes indicate appearance.

We need information on the feeding process to make the step to the primary DEB parameters.

(7) Step 7: From compound to primary parameters

The mixture of primary and compound parameters mentioned above suffices for many applications already (e.g. to predict growth and reproduction in different situations), but other applications require more primary parameters explicitly. We need to supplement the measured quantities with other type of measurements (involving mass or energy) to make the step to the primary parameters, if necessary.

The missing information to obtain the full set of primary parameters for isomorphic ectotherms ($\{\dot{\tilde{\jmath}}_{ET}\} = 0$; see Table 2) can be extracted from the amount of carbon in a freshly laid egg M_E^0 and in a neonate $M_W^b = M_E^b + M_V^b$.

The map

$$(\{\dot{\tilde{\jmath}}_{XAm}\}, K, M_E^0, M_W^b; \kappa, g, \dot{k}_J, \dot{k}_M, \dot{v}, U_H^b, U_H^p) \overset{\text{given } \kappa_R}{\rightarrow} (\{\dot{\tilde{\jmath}}_{EAm}\}, \{\dot{F}_m\}, y_{EX}, y_{VE}, \dot{v}, [\dot{\tilde{\jmath}}_{EM}], \dot{k}_J, \kappa, M_H^b, M_H^p, [M_V]) \quad (11)$$

is made by function get_pars_u of software package DEBtool.

The logic behind this map is as follows. We first use the information in M_E^0 and obtain $\{\dot{\tilde{\jmath}}_{EAm}\} = M_E^0/U_E^0$, and then $y_{EX} = \{\dot{\tilde{\jmath}}_{EAm}\}/\{\dot{\tilde{\jmath}}_{XAm}\}$, $M_H^b = \{\dot{\tilde{\jmath}}_{EAm}\}/U_H^b$, $M_H^p = \{\dot{\tilde{\jmath}}_{EAm}\}/U_H^p$, $M_E^b = U_E^b\{\dot{\tilde{\jmath}}_{XAm}\}$. We then use the information in M_W^b, and obtain $M_V^b = M_W^b - M_E^b$, $[M_V] = M_V^b L_b^{-3}$, $y_{VE} = \dot{v}[M_V](\kappa\{\dot{\tilde{\jmath}}_{EAm}\}g)^{-1}$, $[\dot{\tilde{\jmath}}_{EM}] = \dot{k}_M[M_V]/y_{VE}$.

This completes the full set of primary parameters of the standard DEB model in the absence of a somatic maintenance cost that is linked to surface area (ectotherms). We now continue to determine conversion factors in preparation of determining composition parameters in the next step.

If the mass of a freshly laid egg W_0 and a neonate W_b are known, we can obtain the molecular masses of reserve and structure: $w_E = W_0/M_E^0$ and $w_V = (W_b - w_E M_E^b)/M_V^b$. On the assumption that the specific density of structure is $d_V = 1$ g cm^{-3} (i.e. that of water), the shape coefficient is $\delta_{\mathcal{M}} = d_V^{-1} W_V^b L_b^{-3}$. We can now convert actual lengths into structural lengths and correct the primary parameters that have length in their dimension: $\delta_{\mathcal{M}}\dot{v}$, $\delta_{\mathcal{M}}^{-2}\{\dot{\tilde{\jmath}}_{EAm}\}$, $\delta_{\mathcal{M}}^{-2}\{\dot{F}_m\}$, $\delta_{\mathcal{M}}^{-3}[\dot{\tilde{\jmath}}_{EM}]$. The parameter $[M_V]$ is not a primary one because it only converts one size-measure into another; it is best to convert it to $\delta_{\mathcal{M}}^{-3}[M_V]$ for comparative purposes.

At constant food density the mass of juveniles increases proportional to cubed length (in the standard model), and the proportionality constant relates to the (constant) reserve density. The masses in adults are typically above this mass-length curve, due to contributions of the buffer of reserve that is allocated to reproduction. The deviation can be used to quantify the size of this buffer, and to study the buffer handling rules for the transformation of the allocated reserve to offspring.

(8) Step 8: Composition parameters for biomass

The elemental composition of reserve and structure is required if predictions about fluxes of specific compounds (such as ammonia, carbon dioxide and dioxygen) are to be made. The chemical index of element * of reserve, n_{E*}, can be known from a freshly laid egg. If the chemical indices of a neonate, n_{*W}, are known as well, the chemical index of structure, i.e. the frequency of element * in structure, relative to carbon, is given by

$$n_{*V} = n_{*W} m_{\mathrm{w}}^b - -n_{*E} m_E^b \quad \text{for} * = H, O, N, \ldots \quad (12)$$

where $m_W^b = M_W^b/M_V^b$ and $m_E^b = M_E^b/M_V^b$.

This is just one of a series of related techniques to unravel the composition of reserve and structure using measurements of biomass. Suppose that we have the elemental frequencies of two individuals of the same length (so the same amount of structure) at two scaled functional responses. We can now use the knowlegde of DEB parameters of step 7 to partition total biomass $M_W = M_V + M_E$ into contributions from structure $M_V = M_W/(1 + m_E)$ with $m_E = M_E/M_V = f m_{Em} = f\frac{\{\dot{\tilde{\jmath}}_{EAm}\}}{\dot{v}[M_V]}$, see Table 3, and reserve $M_E = M_W - M_V$. Moreover, if an organism has actual length L and structural mass M_V, the shape coefficient is $\delta_{\mathcal{M}} = (M_V/[M_V])^{1/3}/L$.

We also have

$$M_W n_{*W} = M_V n_{*V} + M_E n_{*E} \quad (13)$$

so the chemical indices of reserve and structure of two individuals with the same amount of structure are

$$n_{*E} = \frac{M_{W1} n_{*W1} - M_{W2} n_{*W2}}{M_{W_1} - M_{W2}};$$

$$n_{*V} = m_{W1} n_{*W1} - (m_{W1} - 1) n_{*E} \quad (14)$$

for $m_W = M_W/M_V$. This technique to compute the chemical indices of reserve and structure can also be applied to compounds rather than chemical elements. The contribution of the reproduction buffer in the mass (and composition) of adults should be taken into account, but for juveniles we don't have these complications.

Knowledge about the chemical indices can be used to determine the molecular masses of reserve and structure, so to link grams and C-moles. A pertinent question is to include or exclude water in mass and volume measurements. If water replaces reserve in starving organisms (likely in aquatic arthropods and other taxa with exoskeletons), strong homeostasis can only apply when we exclude water. In many other cases the inclusion of water is more handy.

The decrease of compounds during starvation can be used to gain information on the composition of reserve and structure, using the following reasoning.

We first try to understand the decrease of a compound C in an organism during starvation, having measurements of how the amount M_C (in C-mol) changes in time t. At the start of the experiment, the organism has amounts of structure M_V and reserve M_E. Strong homeostasis prescribes that the densities of the compound in reserve M_{CE}/M_E and in structure M_{CV}/M_V remain constant. Suppose that reserve mobilisation during prolonged starvation deviates from the standard pattern and is just enough to cover the somatic maintenance cost, growth is absent, allocation to maturity mainteance and reproduction negligibly small. The amount of structure M_V remains constant, so if we focus on the mass of some compound M_C, e.g. protein, we have

$$\begin{aligned} M_C(t) &= M_{CV} + (M_{CE}/M_E)(t_0 - t) j_{EM} M_V \\ &= M_{C0} - t_{jCM} M_V \\ \text{with } j_{CM} &= (M_{CE}/M_E) j_{EM} \\ \text{and } M_{C0} &= M_{CV} + j_{CM} M_V t_0 \end{aligned} \tag{15}$$

where M_{CV} is the (constant) amount of compound C in structure, M_{CE}/M_E the constant density of the compound in reserve, j_{EM} the (constant) specific rate of use of reserve for somatic maintenance purposes and t_0 the moment at which all reserve is depleted. This shows that each compound can decrease linearly at its own rate, even under the strong homeostasis assumption.

It also shows that, if we only know how the compound changes in time, we have access to M_{C0} and j_{CM}, but not to the more informative M_{CV} and M_{CE} (i.e. information on the composition of structure and reserve).

We do have some relative information on the composition of reserve, if we know the time trajectories of several compounds: $j_{C_1M}/j_{C_2M} = M_{C_1E}/M_{C_2E}$. If we know when the reserve is depleted (namely at time t_0), we have access to the composition of structure M_{CV}/M_V, since $M_C(t_0) = M_{CV}$, but the individual will probably start to use structure to pay maintenance cost during prolonged starvation (causing deviations from a linear decrease). Moreover it is likely that the reserve buffer that is allocated to reproduction is used under extreme starvation. This makes it difficult to have access to t_0.

Suppose now that we have information for all compounds, that is $\sum_i M_{C_iV} = M_V$ and $\sum_i M_{C_iE} = M_E$. Although the actual number of chemical compounds is formidable, they can be grouped into a limited number of chemical categories (e.g. proteins, lipids *etc.*). We have $\sum_i j_{C_iM} = j_{EM}$, so $j_{C_iM}/\sum_j j_{C_jM} = M_{C_iE}/M_E$. We also have $\sum_i M_{C_i0} = M_V(1 + j_{EM} t_0)$, so $M_V = \sum_i M_{C_i0} - t_0 \sum_i j_{C_iM} M_V$, which we know if we have an estimate for t_0. We obviously must have that $t_0 < \sum_i M_{C_i0} / \sum_i j_{C_iM} M_V$. The composition of structure is then found from $M_{C_iV}/M_V = M_{C_i0}/M_V - t_0 j_{C_iM}$.

Fig. 5 and Table 8 give an example application. RNA might also contribute to biomass, but is neglected here. We treated the data as if it was referring to 100 g wet mass at time zero.

Chemical compounds can be used as proxies for reserve and structure. DNA probably belongs to the structure because differences in nutrition do not translate into differences in numbers of cells but in cell masses; the amount of DNA per cell remains constant. For this reason the amount of DNA can be used as a proxy for structure. Ribosomal RNA is primarily associated with reserve in yeast (Vrede *et al.*, 2004) and probably in many organisms; perhaps it is not associated with structure, which means that rRNA can be used as a proxy for reserve. Yolk lipoproteins can be used as proxy for the reserve buffer that is allocated to reproduction (Stibor, 2002).

(9) Step 9: Fluxes of compounds and mass balances

Knowledge of fluxes of compounds is essential to access quantifications of energy and entropy (see Section IV.10), since these more general measures need to be accessed *via* (dynamic) material balances at the individual level. We also need these balances to access the fraction of reserve allocated to reproduction that is fixed in embryos, κ_R. For these reasons we here summarise how these material fluxes can be obtained. We take mass fluxes as positive if the corresponding compounds appear in the environment, and negative if they disappear.

The fluxes of organic compounds (food X, structure V, reserve E, and faeces P) are given by

$$\dot{\boldsymbol{J}}_{\mathcal{O}}^T = (\dot{J}_{XA} \quad \dot{J}_V \quad \dot{J}_E + \kappa_R \dot{J}_{ER} \quad \dot{J}_{PA}). \tag{16}$$

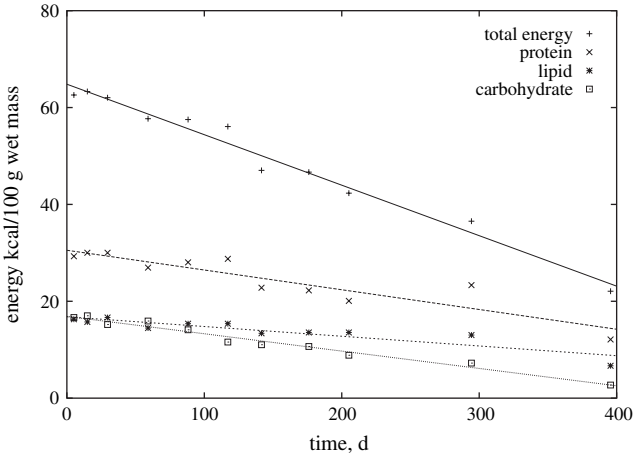


Fig. 5. The amount of energy in starving *Crassostrea gigas*. Data from Whyte, Englar & Carswell (1990). Parameters are presented in Table 8.

Table 8. The parameter estimates from Fig. 4, conversions, and their translation into composition information for three choices for the time at which the reserve is depleted; 1 cal = 4.184 J; caloric values are from ((Kooijman, 2000) p. 137). $\bar{\mu}_C$ is the specific chemical potential of (generalised) compound C, where C stands for total, protein, lipid or carbohydrate, M_{C0} is the initial mass of compound C, $\dot{J}_{CM}$ is the rate at which compound C is used for maintenance, M_{CE} is the mass of compound C in reserve, M_{CV} is the mass of compound C in structure. Other symbols are defined in Table 1

100 g wet mass	Total	Protein	Lipid	Carbohydrate
$\bar{\mu}_C M_{C0}$, kcal	64.81	30.54	16.80	16.87
$\bar{\mu}_C \dot{J}_{CM}$, kcal/d	0.1042	0.0408	0.0200	0.0358
$\bar{\mu}_C$, kJ/C-mol		401	616	516
M_{C0}, C-mol	0.570	0.319	0.114	0.137
$\dot{J}_{CM}$, mmol/d		0.426	0.136	0.290
M_{CE}/M_E, mol/mol		0.500	0.159	0.341
M_{CV}/M_V, mol/mol, $t_0 = 200$ d		0.546	0.191	0.263
M_{CV}/M_V, mol/mol, $t_0 = 400$ d		0.537	0.185	0.278
M_{CV}/M_V, mol/mol, $t_0 = 600$ d		0.531	0.181	0.288

Superscript T stands for transposition. Further, $\dot{J}_{XA}$ is given in Eq. (10), $\dot{J}_V = \frac{d}{dt}M_V$ is given in Eq. (A3), $\dot{J}_E = \frac{d}{dt}M_E$ is given in Eq. (A1), $\dot{J}_{ER}$ is given in Eq. (A5), see Appendix, and $\dot{J}_{PA} = -y_{PX}\dot{J}_{XA}$ where y_{PX} is the (constant) yield of faeces on food. At constant food density, where reserve density is constant, the change in reserve is proportional to the change in structure: $\frac{d}{dt}M_E = m_E\frac{d}{dt}M_V$.

Once the chemical indices of reserve and structure are known and the nitrogenous waste, e.g. ammonia or other products, identified, the mineral fluxes $\dot{\boldsymbol{J}}_{\mathcal{M}}$ can be obtained from the organic fluxes $\dot{\boldsymbol{J}}_{\mathcal{O}}$ using the mass balance equation

$$0 = \boldsymbol{n}_{\mathcal{M}}\dot{\boldsymbol{J}}_{\mathcal{M}} + \boldsymbol{n}_{\mathcal{O}}\dot{\boldsymbol{J}}_{\mathcal{O}}, \tag{17}$$

where $\dot{\boldsymbol{J}}_{\mathcal{M}}$ is the vector with the molar fluxes of the minerals (carbon dioxide $\dot{J}_{CO_2}$, water $\dot{J}_{H_2O}$, dioxygen $\dot{J}_{O_2}$, nitrogen waste $\dot{J}_{N_{waste}}$), and the matrices $\boldsymbol{n}_{\mathcal{M}}$ and $\boldsymbol{n}_{\mathcal{O}}$ represent the chemical indices of the mineral and organic compounds, respectively.

Given values for $\text{par} = (\{\dot{J}_{EAm}\}, \{\dot{F}_m\}, y_{EX}, y_{VE}, \dot{v}, [J_{EM}], \dot{k}_J, \kappa, \kappa_R, M_H^b, M_H^p, [M_V])$, the result of step 7, see Section IV.7) and for the chemical indices $\boldsymbol{n}_{\mathcal{M}}$, $\boldsymbol{n}_{\mathcal{O}}$ (the result of step 8, see Section IV.8), the map

$$(X, M_E, M_V, M_H; \boldsymbol{n}_{\mathcal{M}}, \boldsymbol{n}_{\mathcal{O}}; \text{par}) \to (\dot{\boldsymbol{J}}_{\mathcal{M}}, \dot{\boldsymbol{J}}_{\mathcal{O}}) \tag{18}$$

can be made with function 'flux' of software package DEBtool, where food density X, mass of reserve M_E and structure M_V, and mass of reserve invested in maturity M_H can be vectors rather than scalars. Step 8 showed how the masses of reserve M_E and structure M_V can be obtained from body masses. Maturity M_H can be obtained from the DEB parameters, and knowlegde of M_V and the food density X. So the present step does not provide new parameter estimates, but shows how organic and mineral fluxes can be obtained. This is an essential preparation for the final step, the estimation of thermodynamic parameters, and can also be used for checking consistency of parameter estimates if (some of) the fluxes are measured and compared with model predictions.

Knowledge of mineral fluxes can also be used to assess some compound parameters directly. If contributions from assimilation are excluded by pre-starving the subjects, the respiratory quotient, i.e. the ratio of carbon dioxide production to dioxygen consumption, varies in predictable ways with length, which can be used to extract parameter values. If it is independent of length, constraints on the composition of reserve relative to that of structure apply as evaluated in Kooijman (2000, p. 138). Similarly, if the ratio of nitrogen-waste production to dioxygen consumption is independent of length, another constraint on the composition of reserve and structure applies Kooijman (2000, p. 146). This also holds for the watering quotient, i.e. the ratio of water production to dioxygen consumption. If all three quotients are independent of length, the composition of reserve and structure must be identical in terms of elemental frequencies.

(10) Step 10: Thermodynamic parameters

Since they involve all aspects simultaneously, thermodynamic parameters are perhaps also most informative. Yet little is known about free energies and entropies, since biochemical methods cannot be applied to access entropies of living systems, and the first non-destructive method to quantify entropy of living organisms has been developed only very recently (Sousa *et al.*, 2006) and applied to microbial populations in a chemostat. The value of the entropy of living biomass so obtained did differ from Battley's empirical rule (Battley, 1997) and destructive methods. We expect that changes in entropy are especially important in transients from anaerobic and aerobic conditions and in large transients in pressure (deep ocean to surface). We consider statements such as "Life shows a tendency to maximise entropy production" as open claims that still have to be substantiated. We here provid the methodology to evaluate tendencies like these.

Strong homeostasis implies that the specific enthalpies, chemical potentials and entropies of reserve and structure are constant. The next subsections discuss how they can be obtained as discussed in (Sousa *et al.*, 2006) for microbial populations in a chemostat. To our knowledge, this method has not yet been applied to individual organisms. Our

methods can be applied under anaerobic as well as aerobic conditions if we replace dioxygen by the products that are formed. DEB rules for product formation are discussed in Kooijman (2000, p. 147). Under aerobic conditions, simplifications apply, which we briefly discuss.

(*a*) *Step 10a: Enthalpy and dissipating heat*

Given molar enthalpies for the minerals, $\bar{\boldsymbol{h}}_{\mathcal{M}}^T = (\bar{h}_{CO_2} \quad \bar{h}_{H_2O} \quad \bar{h}_{O_2} \quad \bar{h}_{N_{waste}})$ taken from the literature (Table 9), molar enthalpies of the organic compounds, $\bar{\boldsymbol{h}}_{\mathcal{O}}^T = (\bar{h}_X \quad \bar{h}_V \quad \bar{h}_E \quad \bar{h}_P)$ can be obtained from the energy balance equation

$$\begin{aligned} 0 &= \bar{\boldsymbol{h}}_{\mathcal{M}}^T \dot{\boldsymbol{J}}_{\mathcal{M}} + \boldsymbol{h}_{\mathcal{O}}^{-T} \dot{\boldsymbol{J}}_{\mathcal{O}} + \dot{p}_{T+} \\ &= (\bar{\boldsymbol{h}}_{\mathcal{O}} - \bar{\boldsymbol{h}}_{\mathcal{M}} \boldsymbol{h}_{\mathcal{M}}^{-1} n_{\mathcal{O}})^T \dot{\boldsymbol{J}}_{\mathcal{O}} + \dot{p}_{T+}, \end{aligned} \tag{19}$$

by measuring the net heat dissipated heat by the organism, $\dot{p}_{T+}$. This net dissipating heat can be negative if heat from the environment is required to keep the temperature of the individual constant. Generally measurements of dissipating heat at four different food levels are required to obtain the four enthalpies for the organic compounds; if the enthalpies of food X and faeces P are known then only measurements of dissipated heat at two different food densities are required.

The dissipated heat can be estimated for other food densities, knowing the enthalpies of organic compounds, using the method of indirect calorimetry that establishes a linear dependence between the mineral fluxes and the dissipated heat [see also Kooijman (2000, p. 155)].

The specific enthalpy of biomass equals $\bar{h}_W = \frac{m_E \bar{h}_E + \bar{h}_V}{m_E + 1}$.

(*b*) *Step 10b: Chemical potentials and entropy*

The specific chemical potential $\bar{\mu}$ of a compound converts a flux of this compound (in moles per time) into a flux of Gibbs energy, for instance the assimilation energy flux is $\dot{p}_A = \bar{\mu}_E \dot{J}_{EA}$. The chemical potentials $\bar{\mu}$ have to be computed simultaneously with the molar entropies $\bar{s}$. Work that is involved in changes in volumes is typically negligibly small at the surface of the earth, but in the deep ocean, this work has profound effects on energetics and biochemistry (Gibbs, 1997; Sébert, 1997). Neglecting this effect, the chemical potential and entropies of food $\bar{\mu}_X$ and $\bar{s}_X$, structure $\bar{\mu}_V$ and $\bar{s}_V$, reserve $\bar{\mu}_E$ and $\bar{s}_E$, and faeces $\bar{\mu}_P$ and $\bar{s}_P$ can be obtained with

Table 9. Formation enthalpies and absolute entropies of CO_2, H_2O and O_2 at 25°C taken from Dean (1979). Formation enthalpy and absolute entropy for NH_3 at 25°C taken from Atkins (1990)

Formula	State	Enthalpy (kcal/mol)	Entropy (cal/mol.K)
CO_2	gas	-94.05	51.07
H_2O	liquid	-68.32	16.71
O_2	gas	0	49.00
NH_3	dissolved	-19.20	26.63

$$\begin{aligned} 0 &= (\bar{\boldsymbol{\mu}}_{\mathcal{M}} + T\bar{\boldsymbol{s}}_{\mathcal{M}})^T / \dot{\boldsymbol{J}}_{\mathcal{M}} + (\bar{\boldsymbol{\mu}}_{\mathcal{O}} + T\bar{\boldsymbol{s}}_{\mathcal{O}})^T \dot{\boldsymbol{J}}_{\mathcal{O}} + \dot{p}_{T+} \\ &= ((\bar{\boldsymbol{h}}_{\mathcal{M}} - \bar{\boldsymbol{\mu}}_{\mathcal{M}} - T\bar{\boldsymbol{s}}_{\mathcal{M}}) \\ &\qquad \boldsymbol{n}_{\mathcal{M}}^{-1} \boldsymbol{n}_{\mathcal{O}} - \bar{\boldsymbol{h}}_{\mathcal{O}} + \bar{\boldsymbol{\mu}}_{\mathcal{O}} - T\bar{\boldsymbol{s}}_{\mathcal{O}})^T \dot{\boldsymbol{J}}_{\mathcal{O}}, \end{aligned} \tag{20}$$

by measuring the temperature T of the organisms and computing the organic and mineral flows at eight different food densities (or four different food densities if molar entropies and chemical potentials of food X and faeces P are known), where $\bar{\boldsymbol{\mu}}_{\mathcal{M}}$ and $\bar{\boldsymbol{s}}_{\mathcal{M}}$ collect the values of the molar chemical potentials and molar entropies for the four minerals, while $\bar{\boldsymbol{\mu}}_{\mathcal{O}}$ and $\bar{\boldsymbol{s}}_{\mathcal{O}}$ perform this function for the organic compounds, as before.

The rate of entropy production by the organism $\dot{\sigma}$ is a measure of the amount of dissipation that is occurring. It can be quantified for each food density if the temperature of the organism and the entropies of the organic compounds are known:

$$0 = \dot{\sigma} + \frac{\dot{p}_{T+}}{T} + \bar{\boldsymbol{s}}_{\mathcal{M}}^T \dot{\boldsymbol{J}}_{\mathcal{M}} + \bar{\boldsymbol{s}}_{\mathcal{O}}^T \dot{\boldsymbol{J}}_{\mathcal{O}}. \tag{21}$$

The chemical potentials of organic compounds are essential to obtain the energy parameters $\{\dot{p}_{Am}\}$, $[E_G]$, $\{\dot{p}_T\}$, $[\dot{p}_M]$ and $[\dot{p}_J]$, see Table 3.

The specific entropy of biomass equals $\bar{s}_W = \frac{m_E \bar{s}_E + \bar{s}_V}{m_E + 1}$

(*c*) *Step 10c: Aerobic conditions*

Formulae are simpler for aerobic conditions because for most important reactions in aerobic biological systems $T\Delta\bar{s}$ is very small compared to $\Delta\bar{h}$ and therefore the enthalpy of the reaction $\Delta\bar{h}_+$ is approximated using its Gibbs energy $\Delta\bar{\mu}_+$, since at constant temperature we have $\Delta\bar{\mu} = \Delta\bar{h} - T\Delta\bar{s} \simeq \Delta\bar{h}$ (Garby & Larsen, 1995).

The entropies of the organic compounds $\bar{\boldsymbol{s}}_{\mathcal{O}}$ can be obtained with

$$0 = \bar{\boldsymbol{s}}_{\mathcal{M}}^T \dot{\boldsymbol{J}}_{\mathcal{M}} + \bar{\boldsymbol{s}}_{\mathcal{O}}^T \dot{\boldsymbol{J}}_{\mathcal{O}}, \tag{22}$$

by computing the organic and mineral flows at four different food densities (or two different food densities if molar entropies of food X and faeces P are known) and constant temperature.

The specific chemical potentials of the organic compounds $\bar{\boldsymbol{\mu}}_{\mathcal{O}}$ can be computed with

$$0 = \dot{p}_{T+}^{\circ} + \bar{\boldsymbol{\mu}}_{\mathcal{O}}^T \dot{\boldsymbol{J}}_{\mathcal{O}} + \bar{\boldsymbol{\mu}}_{\mathcal{M}}^T \dot{\boldsymbol{J}}_{\mathcal{M}}, \tag{23}$$

where $\dot{p}_{T+}^{\circ}$ is the net heat release by all chemical reactions. If the temperature of the organism is constant, the net heat release $\dot{p}_{T+}^{\circ}$ is equal to the net heat dissipated by the organism $\dot{p}_{T+}$. The computation can be performed by measuring directly the dissipated heat $\dot{p}_{T+} \simeq \dot{p}_{T+}^{\circ}$, at four different food densities (or two different food densities if the chemical potentials of food X and faeces P are known), that is approximately equal to the total heat release by all chemical reactions $\dot{p}_{T+}^{\circ}$. Alternatively the dissipated heat can be obtained using indirect calorimetry.

The rate of entropy production by the organism $\dot{\sigma}$ can be quantified if the temperature of the organism is known: $\dot{\sigma} = -\frac{\dot{b}_{T+}}{T}$.

V. BEYOND THE TEN ESTIMATION STEPS

The sequence of estimation steps basically concerns a mixture of statistics and observations. We did this because sometimes this is the only information available, but in the first place to reveal the logical relationships between these quantities and DEB parameters. From a statistical point of view, it is better to use data directly (Van der Meer, 2006a), rather than *via* these statistics. This strategy also allows for a wider choice of types of data that can be used to obtain values for parameters. Data on embryo development, for instance, can be used to extract the energy conductance $\dot{v}$ and the somatic maintenance rate coefficient $\dot{k}_M$, see Kooijman (2000, p. 101). Growth and reproduction at varying (but measured) food densities can be used to extract parameter values. The basic idea is to use all available information simultaneously.

The regression routines of software package DEBtool can handle an arbitrary number of data sets simultaneously using algorithms, that vary from slow with a large domain of attraction (genetic algorithms, Nead-Melder method), to fast with a small domain of attraction (Newton-Raphson method). It is easy to change from fixing parameters at particular values to subjecting them to optimisation. The routines allow for continuation, i.e. the resulting parameters from one call can be used as a starting point for a next call. Since the possibility always exists that the resulting estimates correspond with a local minimum of the sum of weighted squared deviations, rather than with a global minimum, it is a good idea to try several values for initial estimates, and select the result with the smallest deviation.

A basic problem in estimating parameters from several data sets simultaneously is that it is less easy to figure out if the combined data do determine the parameters that are subjected to optimisation. A useful test is to check for non-singularity using the Newton-Raphson method (a warning appears for singularity); this test is not "waterproof", however. Moreover, a parameter might be determined by the combined data, but very imprecisely only. The standard deviations might indicate this (DEBtool has a function for the covariance matrix of parameter estimates, from which standard deviations are derived), but one should not conclude from a small standard deviation that the corresponding parameter is precisely determined by the data; a mistake that is easy to make. The simultaneous confidence interval with highly correlated parameters might be large. Moreover parameters might depend on each other in non-linear ways that are poorly quantified by the correlation matrix. Profile likelihood functions give a much more reliable idea about the real confidence of parameter values (DEBtool has functions for them), but the computation of these profile likelihood functions can be demanding.

It is always a good idea to finalise the estimation with the Newton Raphson method (because it is most accurate), and to check the results graphically (DEBtool has facilities for this); no formalised method can compete with the human eye.

Apart from optimising the goodness of fit we want to have physiological consistency. These different criteria frequently, but not always, coincide; a very unrealistic parameter value might give a slightly better fit than a realistic one. As long as the fit is not too bad, realism is a stronger criterion. Such an endpoint can be obtained using the concept of sloppy constraints, where "pseudo observations" are fitted for particular parameters, simultaneously with real observations. Choosing large weight coefficients in the regression procedure that minimises the weighted sum of squared deviations for the pseudo observations, the sloppy constraints become real constraints and the parameters are set to the "observed" values. By decreasing the weight coefficients, we can allow deviations from these values; if the weight coefficients equal zero, the "observation" is completely ignored. This procedure has relationships with Bayesian methods, but has a better biological foundation. See the first estimation step (SectionIV.1) for the logic of this procedure.

DEB theory can handle varying food conditions and temperatures, which are inherent to seasonal forcing. The implementation of the more advanced applications typically requires some data-set-specific coding and is beyond the scope of this paper.

VI. DISCUSSION

We made the balance explicit between research effort to collect data and yielded information captured in parameter values. This balance can improve the planning of research. Although the DEB variables and their interactions cannot be observed directly, indirectly they can. This shows that the testability of theories can be somewhere between "yes" and "no". Body masses and respiration rates are frequently used in energetics. Changes in body composition, however, mean that these quantities have complex interpretations. We here showed how to make use of these changes in composition; the comparison of growth and reproduction at different food levels is the key to metabolic organisation. For example, observed differences in amounts of structure at stage transitions under different food conditions can be used to quantify the maturity maintenance rate coefficient $\dot{k}_J$.

Parameter values capture important biological information about a species. It is not the purpose here to present new information on particular species, but a little more discussion might illustrate the general point. We found, for instance, that female *D. magna* allocates a fraction $1 - \kappa = 0.2$ (see Table 7) of the utilised reserve to maturity maintenance plus reproduction, while a fraction 0.46 would maximise the ultimate reproduction rate ($\dot{R}_\infty = 47.5\text{d}^{-1}$ rather than the observed 14.7 d^{-1}). We took into account that the energy investment ratio g depends on κ to arrive at this conclusion. This sheds a new light on the applicability of maximisation principles (Lika & Kooijman, 2003). Moreover, we arrive at an incubation time of less than a day, while the eggs are in the brood pouch during the intermoult period, which lasts 1.5 - 2 d at 20°C. This

suggests that the eggs are arrested in their development for half a day at abundant food. We also derived a maximum incubation time of $L_b/(L_\infty \dot{r}_B) = 0.77/(4.48\times0.158) = 1.09$ d (again at abundant food), which further supports the existence of a delay in development because this maximum does not depend on values of DEB parameters, only on the structure of DEB theory.

Apart from the information that is in the parameter values themselves, these values can be used to obtain more information. For example, they can be used to reconstruct food input from observations on growth (e.g. otolith data from fish (Pecquerie, 2008)) and/or reproduction (e.g. number of eggs in brood pouches in daphnids). See Kooijman (2000) for examples. Multiple regression of reconstructed food trajectories with measured quantities (amounts of chlorophyll, particulate organic matter, total organic matter) can then be used to assess the nutritional significance of these quantities.

The present paper only deals with the standard DEB model; the theory has been extended in many directions, including varying food quality (e.g. Muller *et al.*, (2001)) and preferences Kooijman (2000), social interactions (Kooijman & Troost, 2007), deviations from isomorphy Kooijman (2000), more reserve and more structure compartments (Leeuwen, Zonneveld & Kooijman, 2003). Practical application should teach to what extent simple DEB principles can be pushed into the extreme: models remain caricatures of reality, especially if we deal with such complex systems as living organisms. In practice, the principles should be applied less strictly and with some care. Reserve can be replaced by water during starvation, for instance, and the chemical composition of yolk might differ (in detail) from reserve in juveniles and adults. Small changes in shape during growth do occur, and length must be defined carefully to avoid such problems.

It is likely that a new class of techniques based on isotope analysis will soon be developed to supplement the methods that are described here. Some results from doubly labelled water are reported in Kooijman (2000). These techniques will come with new possibilities to extract parameter values in field situations, but they also have their own limitations that are inherent to the links between levels of organisation; these methods rely on pools of metabolites being well-mixed, for instance.

The package DEBtool is freely downloadable from http://www.bio.vu.nl/thb/deb/deblab/. Information about the DEB research program and its results can be found at http://www.bio.vu.nl/thb/deb/.

VII. CONCLUSIONS

(1) We present a recipe for how to obtain the twelve parameter values of the standard dynamic energy budget (DEB) model from observations that are (relatively) easy to make; the freely downloadable software package DEBtool offers computational support.

(2) The units of the parameters give information about the type of measurements that are required to arrive at parameter values.

(3) We showed that a natural sequence exists to convert quantities that are 'simple' to measure stepwise to compound and primary parameters of the standard DEB model, and then to composition and thermodynamic parameters.

(4) The sequence of estimation steps reflects an increase in information content that is captured in parameter values, but also an increase in experimental effort that needs to be invested to obtain the required data.

(5) Observations on growth and reproduction are required to obtain the fraction of mobilised reserve that is allocated to somatic maintenance plus growth.

(6) At constant food density, the standard DEB model for body length as a function of time since birth reduces to the von Bertalanffy growth curve. The inverse von Bertalanffy growth rate increases linearly with the ultimate length for different food levels; the slope relates to the energy conductance and the intercept to the somatic maintenance rate coefficient. Observations of growth at different food levels are required to obtain these two parameters.

(7) Observations on length at birth and at puberty are required for different food levels to obtain the maturity maintenance rate coefficient. If these observations are not available, this parameter should be set equal to the somatic maintenance rate coefficient, with the implication that these lengths do not depend on food level.

(8) Changes in chemical composition of biomass during starvation or during growth at different food levels can be used to obtain the chemical composition of reserve and structure.

(9) The age at birth should be less than the ratio of the relative length at birth and the von Bertalanffy growth rate, $a_b < L_b/(L_\infty \dot{r}_B)$, to be consistent with DEB theory. The measured age at birth typically includes a delay of the onset of development.

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IX. REFERENCES

Atkins, P. W. (1990). *Physical Chemistry*. Oxford: Oxford University Press.

Battley, E. (1997). Heat capacity measurements from 10 to 300 K and derived thermodynamic functions of lyophilized cells of *Saccharomyces cerevisae* including the absolute entropy and the entropy formation at 298.15 K. *Thermochimica Acta* **298**, 37–46.

Brack, W., Bakker, J., Deckere, E. D., Deerenberg, C., Gils, J. V., Hein, M., Jurajda, P., Kooijman, S., Lamoree, M., Lek, S.,

López de Alda, M.-J., Marcomini, A., Muñoz, I., Rattei, S., Segner, H., Thomas, K., Ohe, P. V. D., Westrich, B., Zwart, D. D., & Schmitt-Jansen, M. (2005). Models for assessing and forecasting the impact of environmental key pollutants on freshwater and marine ecosystems and biodiversity. *Environmental Science & Pollution Research* **12**, 252–256.

Cardoso, J. F. M. F., Veer, H. W. V. D., & Kooijman, S. A. L. M. (2006). Body size scaling relationships in bivalves: a comparison of field data with predictions by dynamic energy budgets (DEB theory). *Journal of Sea Research* **56**, 125–139.

Dean, J. (1979). *Lange's Handbook of Chemistry*. New York, NY: McGraw-Hill, 12th Ed.

Garby, L. & Larsen, P. (1995). *Bioenergetics – its thermodynamic foundations*. Cambridge University Press.

Gibbs, A. G. (1997). Biochemistry at depth. In: Randall, D. J. & Farrell, A. P., eds, *Deep-sea fishes*., chapter 6, pp. 239–278. San Diego: Academic Press.

Gurney, W. S. C. & Nisbet, R. M. (1998). *Ecological dynamics*. Oxford University Press.

Kooijman, S. A. L. M. (1986). Energy budgets can explain body size relations. *Journal of Theoretical Biology* **121**, 269–282.

Kooijman, S. A. L. M. (2000). *Dynamic Energy and Mass Budgets in Biological Systems*. Cambridge University Press.

Kooijman, S. A. L. M. (2001). Quantitative aspects of metabolic organization; a discussion of concepts. *Philosophical Transactions of the Royal Society B* **356**, 331–349.

Kooijman, S. A. L. M. (2006). Pseudo-faeces production in bivalves. *Journal of Sea Research* **56**, 103–106.

Kooijman, S. A. L. M. (2008). What the egg can tell about its hen: embryo development on the basis of dynamic energy budgets. *Journal of Mathematical Biology*, DOI 10.1007/s00285-008-0195-x, to appear.

Kooijman, S. A. L. M., Auger, P., Poggiale, J. C., & Kooi, B. W. (2003). Quantitative steps in symbiogenesis and the evolution of homeostasis. *Biological Reviews* **78**, 435–463.

Kooijman, S. A. L. M., Baas, J., Bontje, D., Broerse, M., Jager, T., Gestel, C. V., & Hattum, B. V. (2007). Scaling relationships based on partition coefficients and body sizes have similarities and interactions. *SAR and QSAR in Environmental Research* **18**, 315–330.

Kooijman, S. A. L. M. & Troost, T. A. (2007). Quantitative steps in the evolution of metabolic organisation as specified by the dynamic energy budget theory. *Biological Reviews* **82**, 1–30.

Leeuwen, I. M. M. V., Zonneveld, C., & Kooijman, S. A. L. M. (2003). The embedded tumor: host physiology is important for the interpretation of tumor growth. *British Journal of Cancer* **89**, 2254–2263.

Lika, K. & Kooijman, S. A. L. M. (2003). Life history implications of allocation to growth versus reproduction in dynamic energy budgets. *Bulletin of Mathematical Biology* **65**, 809–834.

Muller, E. B., Nisbet, R. M., Kooijman, S. A. L. M., Elser, J. J., & McCauley, E. (2001). Stoichiometric food quality and herbivore dynamics. *Ecology Letters* **4**, 519–529.

Nisbet, R. M., Muller, E. B., Lika, K., & Kooijman, S. A. L. M. (2000). From molecules to ecosystems through dynamic energy budget models. *Journal of Animal Ecology* **69**, 913–926.

Pecquerie, L. (2008). *Bioenergetic modelling of the growth, development and reproduction of a small pelagic fish: the Bay of Biscay anchovy*. Ph.D. thesis, Agrocampus Rennes & Vrije Universiteit, Amsterdam.

Sébert, P. (1997). Pressure effects on shallow-water fishes. In: Randall, D. J. & Farrell, A. P., eds, *Deep-sea fishes*., chapter 7, pp. 279–324. San Diego: Academic Press.

Sousa, T., Mota, R., Domingos, T., & Kooijman, S. A. L. M. (2006). The thermodynamics of organisms in the context of DEB theory. *Physical Review E* **74**(051901), 1–15.

Sousa, T., Domingos, T., & Kooijman, S. A. L. M. (2008). From empirical patterns to theory: A formal metabolic theory of life. *Philosophical Transactions of the Royal Society B* **363**, 2453–2464.

Stibor, H. (2002). The role of yolk protein dynamics and predator kairomones for the life history of *Daphnia magna*. *Ecology* **83**, 362–369.

Van der Meer, J. (2006a). An introduction to dynamic energy budget (DEB) models with secial emphasis on parameter estimation. *Journal of Sea Research* **56**, 85–102.

Van der Meer, J. (2006b). Metabolic theories in biology. *Trends in Ecology & Evolution* **21**, 136–140.

Vrede, T. J., Dobberfuhl, D., Kooijman, S. A. L. M., & Elser, J. J. (2004). Fundamental connections among organism C:N:P stoichiometry, macromolecular composition and growth. *Ecology* **85**, 1217–1229.

Whyte, J. N. C., Englar, J. R., & Carswell, B. L. (1990). Biochemical-composition and energy reserves in *Crassostrea gigas* exposed to different levels of nutrition. *Aquaculture* **90**, 157–172.

X. APPENDIX: CHANGES IN STATE VARIABLES OF THE STANDARD DEB MODEL

The changes in mass of reserve M_E and structure M_V, cumulative invested reserve mass in maturity M_H and the reproduction rate $\dot{R}$ are

$$\frac{d}{dt}M_E = \dot{J}_{EA} - \dot{J}_{EC} \text{ with } \dot{J}_{EA} = f\{\dot{J}_{EAm}\}L^2 \text{ and } f = 0 \text{ if } M_H < M_H^b, \tag{A1}$$

$$\dot{J}_{EC} = \{\dot{J}_{EAm}\}L^2\frac{ge}{g+e}\left(1 + \frac{L_T + L}{gL_m}\right) \text{ with } g = \frac{\dot{v}[M_V]}{\kappa\{\dot{J}_{EAm}\}y_{VE}}, \tag{A2}$$

$$\frac{d}{dt}M_V = (\kappa\dot{J}_{EC} - \dot{J}_{EM} - \dot{J}_{ET})y_{VE} \text{ with } \dot{J}_{EM} = [\dot{J}_{EM}]L^3 \text{ and } \dot{J}_{ET} = \{\dot{J}_{ET}\}L^2, \tag{A3}$$

$$\frac{d}{dt}M_H = (1-\kappa)\dot{J}_{EC} - \dot{J}_{E\jmath} \text{ with } \dot{J}_{E\jmath} = \dot{k}_\jmath M_H \text{ for } M_H < M_H^b, \text{ else } \frac{d}{dt}M_H = 0, \tag{A4}$$

$$\dot{R} = \kappa_R\dot{J}_{ER}/M_E^0 \text{ with } \dot{J}_{ER} = (1-\kappa)\dot{J}_{EC} - \dot{J}_{E\jmath} \text{ for } M_H = M_H^p, \text{ else } \dot{R} = 0, \tag{A5}$$

where the scaled reserve density $e = \frac{m_E}{m_{Em}} = \frac{M_E\dot{v}}{L^3\{\dot{J}_{EAm}\}}$ (dimensionless) and the reserve density $m_E = M_E/M_V = M_E(L^3[M_V])^{-1}$ (in mol mol^{-1}) represent ratios of masses of reserve and structure. Structural mass M_V relates to

structural length L as $M_V = [M_V]L^3$, where $[M_V]$ is a constant parameter. All fluxes are here taken to be non-negative.

Equations (A1, A3, A4) are basically just balance equations that follow from the scheme in Fig. 1, where κ is the fraction of mobilised reserve that is allocated to somatic maintenance plus growth, κ_R the fraction that is allocated to reproduction and actually fixed in embryo reserve and M_E^0 the initial amount of reserve of an embryo that is generated by a female. In the case of a male, the interpretation of M_E^0 is the mean amount of reserve invested in sperm for a successful fertilisation.

The flux of mobilised reserve $\dot{J}_{EC}$ has an ultimate value for ectotherms at constant food density (heating length $L_T = 0$, body length $L = L_\infty$, scaled reserve density $e = f$), given by

$$\dot{J}_{EC\infty} = \{\dot{J}_{EAm}\} f L_\infty^2. \tag{A6}$$

We need this in Eq. (A9). The rather complex expression for the reserve mobilisation rate corresponds with a much simpler one for the change in reserve density, and follows mathematically from the assumption of weak homeostasis (Kooijman, 2000); a mechanism is given in Kooijman & Troost (2007). Eq. (A2) presents the reserve flux, rather than the change in reserve density, because of its simpler links with empirical data (e.g. with respiration).

(1) Changes of scaled state variables

To remove the unit "mole" from the system (and so one degree of freedom), we work with scaled reserve $U_E = M_E/\{\dot{J}_{EAm}\}$ and scaled maturity $U_H = M_H/\{\dot{J}_{EAm}\}$. Notice that $\dim(U_E) = \dim(U_H) = tL^2$, where t stands for time and L for length.

In the absence of surface-related maintenance costs, $\{\dot{J}_{ET}\} = 0$, we obtain

$$\frac{d}{dt}L = \dot{r}_B(eL_m - L) \text{ with } e = \dot{v}\frac{U_E}{L^3} \text{ and} \tag{A7}$$

$$\dot{r}_B = \frac{\dot{k}_M g}{3(e+g)} = \frac{1/3}{L_\infty/\dot{v} + 1/\dot{k}_M}, \tag{A8}$$

$$\begin{aligned} &L(0) \simeq 0 \text{ and } L(a_b) = L_b \text{ and } L(\infty) = L_\infty, \\ &\dot{R} = ((1-\kappa)S_C - \dot{k}_J U_H^p)\kappa_R/U_E^0 \text{ for} \\ &U_H = U_H^p \text{ else } \dot{R} = 0 \\ &\text{with } S_C = \frac{\dot{J}_{EC}}{\{\dot{J}_{EAm}\}} = L^2\frac{ge}{g+e}\left(1 + \frac{L}{gL_m}\right) \text{and} \\ &U_E^0 = M_E^0/\{\dot{J}_{EAm}\} \text{ where } \dot{R} = \dot{R}_\infty \\ &\text{for } L = L_\infty, \text{ and } S_C = eL_\infty^2, \end{aligned} \tag{A9}$$

$$\begin{aligned} &\frac{d}{dt}U_E = fL^2 - S_C \text{ for } U_H > U_H^b \text{ else} \\ &\frac{d}{dt}U_E = -S_C \text{ with } U_E(0) = U_E^0 \text{ and } U_E(a_b) = fL_b^3/\dot{v}, \end{aligned} \tag{A10}$$

$$\begin{aligned} &\frac{d}{dt}U_H = (1-\kappa)S_C - \dot{k}_J U_H \text{ for } U_H < U_H^p \text{ else } \frac{d}{dt}U_H = 0 \\ &\text{with } U_H(0) = 0 \text{ and } U_H(a_b) = U_H^b \text{ and } U_H(a_p) = U_H^p. \end{aligned} \tag{A11}$$

These equations also apply at time-varying food density, but at constant food density we have $e = f$ in the juvenile and adult stage. Deviations can occur if parameters vary in time due to stress or change in temperature, for instance. We did not make the substitution of e by f directly to make clear that the use of reserve (that includes maintenance, growth and reproduction) does not depend on food directly, only *via* reserve. Hence, the history of food availability matters.

(2) Initial reserve

It turns out (Kooijman, 2008) that the scaled amount of reserve of an embryo at the start of its development equals

$$U_E^0 = \frac{M_E^0}{\{\dot{J}_{EAm}\}} = \dot{v}^{-1}\left(\frac{1}{L_b(g+f)^{1/3}} - \frac{B_{\frac{g}{g+f}}(\frac{4}{3},0)}{3g^{1/3}\dot{v}/\dot{k}_M}\right)^{-3} \tag{A12}$$

where $B_x(a,b) = \int_0^x y^{a-1}(1-y)^{b-1}dy$ is the incomplete beta function. Length and maturity at the start of the development of the embryo are taken to be negligibly small. So, the values of the state variables scaled maturity, scaled reserve and length develop from $(0, U_E^0, 0)$ at the start (age $a = 0$) to $(U_H^b, f L_b^3/\dot{v}, L_b)$ at birth (age $a = a_b$).

If $\dot{k}_J = \dot{k}_M$, length at birth $L_b = \left(\frac{U_H^b \dot{v}}{g(1-\kappa)}\right)^{1/3}$ is independent of food availability and can be observed directly. Otherwise, the (scaled) length at birth needs to be solved numerically; an efficient procedure is presented in Kooijman (2008).

(3) Age at birth

The age at birth is determined by e_b (equals e of the mother at egg laying), $\dot{k}_M$, g and scaled length at birth $l_b = L_b/L_m$ Kooijman (2008), and simplifies for small g and large $\dot{k}_M$, while $\dot{r}_B = \frac{\dot{k}_M g}{3(e_b+g)}$ remains fixed

$$\begin{aligned} a_b &= \frac{3}{\dot{k}_M}\int_0^{x_b}\frac{dx}{(1-x)x^{2/3}(3gx_b^{1/3}l_b^{-1} - B_{x_b}(\frac{4}{3},0) + B_x(\frac{4}{3},0))} \\ &\overset{g,\dot{k}_M^{-1}\text{small}}{\simeq} \frac{1}{3e_b\dot{r}_B}\int_0^{x_b}\frac{dx}{(1-x)x^{2/3}x_b^{1/3}(l_b^{-1} - (x_b^{4/3} - x^{4/3})/(4e_b))} \\ &\overset{g,\dot{k}_M^{-1}\text{verysmall}}{=} \frac{l_b}{3e_b\dot{r}_B}\int_0^{x_b}\frac{dx}{(1-x)x^{2/3}x_b^{1/3}} \\ &\overset{g,\dot{k}_M^{-1}\to 0}{=} \frac{l_b}{e_b\dot{r}_B} \end{aligned} \tag{A13}$$

where $x_b = \frac{g}{e_b + g}$. The significance of this result is in the fact that for fixed $\dot{r}_B$, L_b and $L\infty$, $g \to 0$ while $\dot{k}_M \to \infty$ if a_b is running from 0 to this upper boundary. See Fig. 3 for a graphical interpretation. So, if in practice a value for a_b is found that is larger than $\frac{l_b}{e_b \dot{r}_B} = \frac{L_b}{L_\infty \dot{r}_B}$ (the latter equality applies if the mother was in equilibrium with the same food level), this value is inconsistent with DEB theory, and if it is still used to estimate deb parameters, problems might be expected. Notice, however, that age zero is the time when development starts, which might be well after the appearance of the egg.

(4) Simple situations

In the absence of growth, i.e. Eq. (A3) equals zero, the catabolic flux reduces to $\dot{\mathcal{J}}_{EC} = M_E \dot{v}/L$. In the absence of surface-related maintenance costs, $\{\dot{\mathcal{J}}_{ET}\} = 0$, the catabolic flux just covers the somatic maintenance cost if $\kappa \dot{\mathcal{J}}_{EC} = [\dot{\mathcal{J}}_{EM}]L^3$. For scaled reserve density $e = M_E/M_{Em}$, this amounts to the reserve threshold $e = \frac{[\dot{\mathcal{J}}_{EM}]L}{\kappa\{\dot{\mathcal{J}}_{EAm}\}}$. At constant food density this reserve threshold translates to the food intake threshold, since $e = f$. Maturity can only exceed the threshold at birth if during the development of the embryo $(1 - \kappa)\dot{\mathcal{J}}_{EC} > \dot{k}_{\mathcal{J}} M_H^b$, so if $M_H^b < (1 - \kappa)\frac{M_E^b \dot{v}}{L_b \dot{k}_{\mathcal{J}}}$. Substitution of the previous threshold gives the constraints for viable eggs

$$f > \frac{[\dot{\mathcal{J}}_{EM}]L_b}{\kappa\{\dot{\mathcal{J}}_{EAm}\}} = l_b = \frac{L_b}{L_m} \text{ and } M_H^b < \frac{1 - \kappa}{\kappa} \frac{[\dot{\mathcal{J}}_{EM}]}{\dot{k}_{\mathcal{J}}} L_b^3 = \frac{1 - \kappa}{\kappa} \frac{j_{EM}}{\dot{k}_{\mathcal{J}}} M_V^b, \tag{A14}$$

where length at birth L_b is an implicit function of primary parameters that is determined by the relationships just discussed. If food is constant and $\dot{k}_M = \dot{k}_{\mathcal{J}}$, L_b and L_p are constant and there is no need to consider maturity explicitly.