

Summary of concepts of Dynamic Energy Budget theory

for metabolic organisation



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Contents

9	Basic concepts 9											1	1																										
9										•	. .									•			ns.	sten	sy	nic	ıan	lyn	s d	as	als	ua	idu	ivi	nd	-	1.1		
11	•		•			•					· •		• •		•	•				•		•••	• •		ife	o li	y to	key	is l	is i	asi	sta	eos	me	Ю		1.2		
12	•	•••	•	•		•	•	•	•	•	•••		• •	•	•	•	•	•		•		tes	c ra	bolio	etal	me	ts :	fec	aff	re	tur	rat	er	npe	Гer	'	1.3		
15	-															y	зgy	er	en	ıd	ı ar	ıgtł	ler	me,	tir	in	el i	ode	mo	3 r	ΞB	ЭE	D	rd	la	an	Sta	2	-
15																																r	ng	edir	Fee		2.1		
18																														m.	tioi	lati	nila	sim	Ass		2.2		
18	•																								•			\mathbf{ics}	ımi	na	dyr	e d	ve	serv	Res		2.3		
19																							oma	o sc	n t	tio	oca	allc	or a	for	le f	rul	ι-r	eκ	Гh	'	2.4		
20												ι.	$^{\rm th}$	w	\mathbf{ro}	g	nd	aı	tion	lat	imi'	i ass	s of	iead	erł	ove	les	lud	\mathbf{xcl}	і ех	on	tio	oat	sip	Dis		2.5		
21	. '																							ture	uct	str	of s	se	eas	cre	ind	1: i	$^{\mathrm{th}}$	owt	Gre		2.6		
22	. '																		ve.	erv	$res\epsilon$	ped	rapı	of wr	n c	tior	cret	exc	1: e	ion	ctic	luc	odı	pro	Rej		2.7		
22			•										•	•	•	e	ime	ti	and	ιS i	ıgth	, ler	ers	umb	: n	n I:	tioı	nat	tin	est	er e	ete	ne	ran	Pai		2.8		
25	í																					\mathbf{sm}	ooli	etał	me	d I	an	ds	ınd	ou	DO	mī	or	. c(gv.	\mathbf{er}	Ene	3	-
25																											pv	rot	enti	l e	nd	ar	gv	erg	En		3.1		
25																								on.	siti	\mathbf{pos}) mr		nd	ar	ISS	nas	m	dv	Зо		3.2		
25																						ns .	nisr	orga	n c	ls ii	ınd	oou	mp	\cos	of c	of	\mathbf{es}	isse	Cla		3.3		
26																					ıme	volt	nd	ss a	ma	y, 1	erg	ene	ofe	s o	ons	sio	ers	nve	Coi		3.4		
26																						s	ion	quat	ı eo	ion	act	rea	al	nica	em	che	ocl	crc	Мa		3.5		
26																		on	nati	ior	acti	d fr	g an	fling	nuf	esh	3: r	ics	am^{i}	/na	dy	es d	pes	top	so		3.6		
27																	ces	ux	n fl	l o	ased	ıs bi	, tion	rmat	sfor	ans	tra	ed	iate	edi	me	e-n	me	zvr	En		3.7		
28	•									•	•						•	•		•			• •		•					n.	sm	olis	bo	tał	Me		3.8		
29	•																									le	del	no	m		Œ	D	Δ	iati	ari	iv	Un	1	,
20	4																							me	tio	ndi	col	no	, m		r fe	ng	oir	and	Th		ОШ. Л 1	-	
20	•	• •	·	•	• •	•	•	•	•	•	•	•••	• •	•	•	•	•	·	• •	•	• •	•••	•••	. 611	.010	.iui	COI	18 28	ane	 zha	5 IC rel	ng	gii oir	ane	Ch		4.1 1/2		
31	•	• •	•	•	• •	•	•	•	•	•	•	•••	• •	• •	•	•	•	•	•••	•	els.	mod	₹Β 1	DF	ate	ari	niv	ло f пп	upe Lof	ots	s or nect	me sne	as	ss	√n Ma		4.3		
31		•••	•	•		•	•	•	•	•	•	•••	• •	•	•	•	•	•	• •	•	.010		101		auc			. ui	01) 1	on	atic	ra:	snii	Res		4.4		
31	•	•••	•	•	• •	•	·	•	•	•	•	•••	• •	• •	•	•	•	•	• •	•	• •	•••	• •		•	•	• •	°e	anc	ı. ala	ha	n l	ന്ന	roc	Nit	-	4.5		
32	•	• •	·	•	• •	•	•	•	•	•	•	•••	• •	•	•	•	•	·	• •	•	• •	•••	•••		•	•	• •	λ		nc	ba Isi	hal	gu r ł	ter	Wa	-	4.0 4.6		
32	•	•••	•	•	• •	•	·	•	•	•	•	•••	• •	• •	•	•	•	•	del	mr	εR ι	I DI	lard	tand	o g	the	in	cs.	mie	nar	lvn	bai bai	ne.	tor	SO	-	$\frac{1.0}{4.7}$		
32	•	•••	·	•	• •	•	•	•	•	•	•	•••	• •	• •	•	•	•	•	s		lan	v he	ero	e en	fre	h h	nn ean	nv	tro	nai.	су 11 Г — Ф	ruy nv	 alm	the	.50 7.m		 1 8		
33	•	•••	•	•	•••	•	•	•	•	•	•	•••	• •	• •	•	•	•	·		00		, 00	- 8.	0.011		iu I	an	РУ	10]	,110.	, 0	ey, :ts	act act	ndu Julu	\sum_{r}		49		
33		•••	•	•		•	•	•	•	•						v	201	$\frac{1}{trc}$	en	nd	v a	nerg	s, ei	mass	I: 1	n T	tio	nat	tin	est	er e	ete	ne	ran	2 ₈₁	0	4.10		
$2 \\ 3 \\ 3 \\ 3 \\ 3 \\ 3 \\ 3 \\ 3 \\ 3 \\ 3 \\ $		· · · · · · · · · · · · · · · · · · ·	· · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	· · · · · · · ·	· · · ·	· · · · ·	· · · · · · · · · · · · · · · · · · ·	- - - - - - - - - - - -	· · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	- · · · · · · · · · · · · · · · · · · ·	• • • • • • • • • • • • • • • • • • •			 	ma	lels EB 1 	mod l DI y ba nerg	EB 1 lard ergy s, ei	ns DE tand e en	tio ate e st fre I: 1	ndi aria the nd f	con niv in an tion	ng es i un ce cs py nat	din ape of anc ce mic troj	eec ha ts ala nco nar ent	g fe g sl ect on ba dan lyn g g er (ng spe atic 2n bal bal 2 dy py, ets ete:	gir gir as rat gen r b pe alp uct ne	ang ang ss : spin rog ter top tha odu ran	Ch Ma Res Nit Wa So En Pro Pau	0	$\begin{array}{c} 4.1 \\ 4.2 \\ 4.3 \\ 4.4 \\ 4.5 \\ 4.6 \\ 4.7 \\ 4.8 \\ 4.9 \\ 4.10 \end{array}$		

	4.11 Trajectory reconstruction	33
5	Multivariate DEB models	35
	5.1 Several substrates	35
	5.2 Several reserves	36
	5.3 Several structural masses	36
0		
6	Effects of compounds on budgets	39
	6.1 Ageing: Effects of ROS	39
	6.2 Toxins and toxicants	41
	6.3 One-compartment kinetics is the standard	41
	6.4 Energetics affects toxicokinetics	42
	6.5 Toxicants affect energetics	42
7	Extensions of DEB models	45
	7.1 Handshaking protocols for SUs	45
	7.2 Feeding	45
	7.3 Digestion in guts	47
	7.4 Division	47
	7.5 Cell wall and membrane synthesis	47
	7.6 Organelle-cytosol interactions and dual functions of compounds	47
	7.7 Mother-foetus system	48
	7.8 Extra life stages	48
	7.9 Changing parameter values	48
8	Covariation of parameter values	51
-	8.1 Intra-specific parameter variations	52
	8.2 Inter-specific parameter variations	52
	8.3 Quantitative structure-activity relationships	55
	8.4 Interactions between QSARs and body size scaling relationships	56
9	Living together	57
	9.1 Trophic interactions	57
	9.2 Population dynamics	57
	9.3 Food chains and webs	58
	9.4 Canonical community	59
10	Evolution	61
10	10.1 Before the first cells	61
	10.2 Early substrates and taxa	61
	10.3 Evolution of individual as dynamic system	62
	10.4 Merging of individuals in steps	63
	10.5 Multicellularity and body size	64
	10.6 Control over local conditions	64
	10.7 Control over global conditions	65
	10.8 Effects of climate on life	65

11	11 Evaluation												
	11.1 Empirical models that are special cases of DEB theory	67											
	11.2 A weird world at small scales	70											
	11.3 Static Energy Budgets	70											
	11.4 Net production models	70											

This document summarizes the concepts of the DEBbook and its comments-document (as available in the DEBlab), excluding all mathematical formulations, biological examples, empirical evidence or literature references. The focus of this document is on concepts only, including explanations for why we need them, to reveal their interrelationships. Like the comments-document, this document is regularly updated to boost its effectiveness.

An energy budget is the specification of the uptake of energy from the environment by an organism (feeding and digestion) and of the use of this energy for the various purposes: maintenance, development, growth and reproduction. A static energy budget represents a kind of snapshot of these fluxes for an individual in a given state, while a dynamic energy budget follows the changes of these fluxes during the life cycle of an organism in an environment that can change in temperature and resource availability. An analysis of the energy budget does not imply that energy would be limiting. In many situations it is useful to follow the energy aspects of systems that are nitrogen limited, for instance.

Organisms typically grow during their life cycle, and food uptake and maintenance are coupled to the size of the organism. Ultimate size of organisms (i.e. the ceasing of growth) is controlled by the balance between uptake of energy and drain in the form of maintenance and reproduction. Just after the start of maturation, during the embryo stage, organisms typically don't take up food. Food uptake is initiated at a moment called birth, i.e. the onset of the juvenile stage. Maturation, a form of metabolic learning, ceases at puberty, i.e. the onset of the adult stage, after which energy (metabolite) allocation to reproduction starts. Although not yet very detailed, this natural sequence already structures the underlying processes profoundly.

A theory is a set of coherent and consistent assumptions that, ideally, uniquely specifies models. Models are mathematical expressions that relate (typically abstract) quantities. They typically also include case-specific elements that are necessary to apply the theory in a particular situation, which is why one theory can imply many models. A theory can, for instance, specify the way organisms interact with their environment, but when this is applied to bacteria in chemostats or in batch cultures, different models result. Working with theories and models require a full separation of the imaginary and the real world, where auxiliary theory links quantities that can be measured in the real world to variables that play a role in the imaginary world. It is the task of core theory to structure the imaginary world, which we should fully understand, and we use this to understand particular aspects of the real world. See the back cover for the role of models in scientific research.

The Dynamic Energy Budget (DEB) theory is a formal theory for the processes of uptake and use of substrates by organisms. With field-biology as my hobby, and having visited remote habitats on all continents, I am aware of the huge biodiversity that exists. Rather than emphasising the differences between organisms, I asked myself the question what they all have in common in a rather abstract perspective. It is much more than I thought 30 years ago, when I started focused research on this topic.

The setup of DEB theory is axiomatic, where a small set of simple assumptions fully determines the quantitative behaviour of the individual as a dynamic system, using variables that make logical sense, aiming at understanding, rather than describing. The empirical testing and application of this theory require auxiliary theory that specifies how variables that can be measured relate to the variables used in the specification of the dynamic system. Basic to the theory is the coherence between levels of organisation, using the life cycle of an individual as primary focus, from which sub- and supra-organismic levels are considered. Space and time scales are tightly coupled methodologically. Since many species are unicellular, the step to biochemical systems is not always big. Populations are considered as sets of interacting individuals, ecosystems as sets of interacting populations. While walking up- and down the time-space-scale, some processes loose their importance, others gain.

The primary motivation in my research on the theory is to answer the question: how can we deal with the local coherence of levels of metabolic organisation, while avoiding the massive complexity of models with many variables and parameters. For me, models are tools, not aims; they should help to acquire insight. I have never seen any complex model that provided that insight in biology. The challenge is then to find an alternative strategy, working at several levels of organisation simultaneously. To do this in a consistent way is far for easy, however.

DEB theory is a simple theory about complex phenomena. This makes that the question of being 'right' or 'wrong' is easy to answer: it is bound to be 'wrong'. A more interesting question is: can it be useful? It is for you to judge.

Acknowledgements

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1 Basic concepts

Concepts that we need to set-up the standard DEB model are introduced first, while many complexities will be discussed later. I explain why individuals are the primary target for DEB theory and discuss the various types of homeostasis that will be used. The standard DEB model is the simplest in the family of DEB models, and deals with individuals that are composed of a single reserve and structure, living in a dynamic environment in terms of temperature and a single food source. It applies to most animals, since animals eat other organisms that don't vary that much in chemical composition.

1.1 Individuals as dynamic systems

DEB theory exploits the conservation of energy, mass and time as intensively as possible, and at the level of the individual it is most easy to check what goes in and out. This is much more difficult at the sub- and supra-organismic levels. Moreover the individual is the survival machine of life and the target for evolutionary change; the evolutionary perspective is very important for biologically implicit theories.

By selecting the individual as primary focus, we implicitly selected a size and a time scale, namely that of the life span of an individual. Ranging from bacteria to blue whales, the range of space-time scales is still considerable (see Chapter 8), but from a biochemical perspective, this choice already sets important priorities. It explains, for instance, why ATP cannot play a key-role in DEB theory: its life span is much too short, compared to the life cycle of a cell. It is never a good idea to have very fast as well as very slow variables in a single model.

Yet the level of the individual is not always clearly defined; how many individuals of grass has a meadow of a grass species that forms stolons that actively transport metabolites? Moreover we will sometimes feel the need to work with super-individuals, such as a school of fish, a forest or a mussel bank, and with sub-organismal structures, such as organs and organelles. The challenge here is to relate the behaviour of sub- and super-individuals to that of individuals, while respecting the theory. This calls for sandwich-modelling, where several levels of organisation are involved simultaneously.

I present 6 compelling arguments to partition biomass into two compartments: reserve

and structure. the significance of some of the arguments will only become clear later on.

- 1 To include metabolic memory.
- 2 To smooth out fluctuations in resource availability to make sure that no essential type of resource is temporarily absent.
- 3 To allow that the chemical composition of the individual depends on the growth rate.
- 4 To understand why mass fluxes are linear sums of three basic energy fluxes: assimilation, dissipation and growth.
- 5 To explain observed patterns in respiration and in body size scaling relationships.
- 6 To understand how the cell decides on the use of a particular (organic) substrate, as building block or as source of energy.

The reason for being that detailed is because this complicates the theory and its application quite a bit, so there is a need for a careful cost-benefit analysis in composing the theory. Reserve does NOT mean 'set apart for later use'; compounds in the reserve can (and do) have active metabolic functions. The difference between reserve and structure is in their dynamics. The turnover of reserve is paid from overheads in assimilation and mobilisation and the turnover time of reserve depends on food availability and size. The turnover of structure is paid from somatic maintenance and the turnover time of structure is independent of food availability or size. Structure needs (somatic) maintenance, reserve does not. A substantial part of maintenance relates to the turnover of structure, so compounds in both reserve and structure have a limited life span; later I will discuss 'waste-to-hurry', where species increase somatic maintenance to boost growth and reproduction. The overhead of assimilation can be found in the yield of reserve on food, that of mobilisation in all end-points of mobilised reserve: somatic and maturity maintenance, growth, maturation or reproduction.

Metabolic learning during ontogeny is quantified by the state of maturity, or more specifically, by the cumulated investment of reserve in maturity. Maturity does not represent mass, energy or entropy; it has the formal status of information. Metabolic switches occur when maturity reaches a threshold, e.g. at cell division, at the initiation of feeding (e.g. the transition from embryo to juvenile, called birth), at the redirection of investment in maturity to that in reproduction (e.g. the transition from juvenile to adult, called puberty), at the start and ending of metabolic acceleration (in some species).

If maturity is homogeneously distributed in structure and part of the structure is removed, part of maturity is removed as well. You can think of unicellulars that propagate by division, or the daughter cells of a cleaving egg cell of a multicellular organism separate, or a body part is removed (e.g. of plants due to grazing or of a sea anemone that is propagating vegetatively). Each structure should have a maturity. Most organisms can be represented with a single structure, but plants need at least two: root and shoot, each with its own assimilation activity. The number of reserves should match the number of essential resources (= substrates) that are acquired independently and are possibly limiting metabolism, e.g. phosphate, nitrate, light.

1.2 Homeostasis is key to life

The number of different chemical compounds in any organism can be safely assumed to be infinitely large. Two modelling strategies for individuals are possible: (1) select a few important compounds and hope that the rest will hardly matter, or (2) delineate a few pools of mixtures of compounds, called generalised compounds, that don't change in composition. DEB theory follows the second strategy using the argument of homeostasis: the ability of organisms to run their metabolism independent of the (fluctuating) environment. This ability is less than perfect; we need 5 homeostasis concepts to capture what organisms do. All species have strong and weak homeostasis, to some extend, but structural, thermal and acquisition homeostasis are sported by a decreasing number of species.

- 1 Strong homeostasis is the strict constancy of the chemical composition of pools. This implies stoichiometric constraints on the synthesis of generalised compounds. By delineating more and more pools, strong homeostasis becomes less restrictive. The water-content of some pools sometimes seems to escape strong homeostasis.
- 2 Weak homeostasis is the constancy of the chemical composition of the individual as a whole as long as substrate availability in the environment remains constant, *even when growth continues.* This implies constraints on the dynamics of the pools. Notice that, if substrate availability varies, the relative pool size varies, and so does the chemical composition of the individual. Weak homeostasis in fact implies strong homeostasis.
- 3 Structural homeostasis is the constancy of the shape of the individual during growth. This implies that surface area is proportional to volume to the power 2/3; a condition referred to as isomorphy, or, V_3^2 -morphy (later I will introduce other morphs). The significance of shape in the DEB context originates from the assumptions that transport of mass (substrate) to the individual will be linked to surface area, and maintenance to (structural) volume. Surface area-volume ratios also play a key role in transport in the environment; transport dominates ecosystem functioning. Core DEB theory makes no assumptions on the relationship between surface area and volume, but particular models do.

When reserve can be considered as blobs in a matrix of structure, at subcellular level, isomorphy implies that the surface area of the interface between reserve and structure scales with the ratio of the mass of reserve and structural length. We need this observation in setting up a mechanism for reserve dynamics.

Auxiliary theory treats body length as a proxy for structural length. To remove effects of shape in inter-species comparisons, volumetric structural length is introduced: the cubic root of structural volume. The ratio of volumetric structural length and body length, called the shape coefficient, is treated as a fixed parameter. The standard model assumes perfect structural homeostasis. Particular animal taxa, in particular those with larval stages, deviate temporarily from isomorphy.

Supply	Demand
eat what is available	eat what is needed
high half saturation coefficient	low half saturation coefficient
can handle large range of intake	can handle small range of intake
reserve density varies wildly	reserve density varies little
large range of ultimate sizes	small range of ultimate sizes
survives some shrinking well	survives shrinking badly
physiological birth control	behavioural birth control
low peak metabolic rate	high peak metabolic rate
open circulatory system	closed circulatory system
iso- & centro-lecithal eggs	a- & telo-lecithal eggs
rather passive, simple behaviour	rather active, complex behaviour
little parental care	advanced parental care
sensors less developed	sensors well developed
typically ectothermic	typically endothermic
evolutionary original	evolved from supply systems
has demand components	has supply components
(maintenance)	(some food must be available)

- 4 Thermal homeostasis is the constancy of the body temperature. Endotherms oxidise compounds for heating; most mammals and birds do it 'perfectly', tunas and insects much less so. Homeotherms don't do this, but make use spatial differences in temperature to reduce variations in body temperature. Ectotherms (by far the majority of species) have a body temperature (almost) equal to the environmental temperature.
- 5 Acquisition homeostasis is the constancy of the feeding rate, independent of food availability. This is, to some extent, sported by animals near the demand-end of the supply-demand spectrum at which organisms can be ranked. Most organisms are near the supply-end, see Table 1.1. Demand systems evolved from supply systems, and developed several adaptations for this while preserving many other properties of supply systems; see Section 7.2. DEB theory assumes that maturity and somatic maintenance are independent of substrate availability (reserve in this case), but growth and reproduction are. So it has demand as well as supply elements.

Temperature affects metabolic rates 1.3

When the log of any metabolic rate is plotted against the inverse absolute temperature, a straight line results in a species-specific tolerance range of temperatures; the slope is called the Arrhenius temperature. This Arrhenius relationship can be understood from fundamental principles under very simple very idealised conditions, remote from the situation in living organisms. I treat this relationship empirically only and observe that all rates in the

standard DEB model that affect conversion efficiencies should depend on the temperature in the same way to avoid that conversion efficiencies become temperature-dependent. The latter is unlikely, because the same biochemical machinery is used for the conversion of substrates into products; both generalised compounds, so they have constant composition. Since the specific searching rate does not affect any conversion efficiency (being ratios of rates), its Arrhenius temperature and tolerance range might differ from other rates.

Enzymes that control rates are chemically modified to operate at a particular temperature. This involves an adaptation period and complicates the interpretation of fast responses to temperature changes. If temperature fluctuates wildly most of the time, as in the intertidal zone, compared to the deep ocean, organisms are forced to use 'general purpose' enzyme configurations and refrain from continuous adaptation. This implies a low Arrhenius temperature for them.

Outside the temperature tolerance range, rates are typically lower than expected on the basis of the Arrhenius relationship. At the high-temperature end, the rates are typically a lot less and the individual dies. At the low-temperature end, the individual typically manages to send itself into a state of torpor. This situation typically occurs during the bleak season, where substrate availability is low. This deviating behaviour can be captured by delineating temperature-dependent transitions of enzymes from an active state and two inactive states (relating low and high temperatures); these transitions again follow the Arrhenius relationship.

Since substrate uptake affects substrate availability, and the Arrhenius temperature is species-specific, temperature can have complex effects. Ultimate size (i.e. a state) relates to the ratio of two rates: uptake (food) and drain (maintenance), are affected by temperature; food uptake can affect food availability.

If more than one reserve is present, the corresponding assimilation rates might differ in the way they depend on temperature. So these systems are more flexible than the singlereserve systems. Photon capture hardly depends on temperature, for instance, which implies that carbohydrate content typically increases for decreasing temperature. This explains why phytoplankton becomes more nutritious for zooplankton towards the poles. $\mathbf{2}$

Standard DEB model in time, length and energy

The standard DEB model is the simplest non-degenerated model implied by DEB theory. It assumes a single substrate (of constant chemical composition), a single reserve, a single structure and isomorphy. This situation applies (approximately) to most animals, but they evolved from multiple reserve systems (see Chapter 10); the reduction of the number of reserves increases the degree of homeostasis. This is why individuals in this chapter are called animals, for convenience, although its applicability is not confined to animals. The logical links between substrate, reserve, structure and maturity are given in Figure 2.1; this chapter explains why and how the assumptions in Table 2.1 quantify all fluxes in this figure uniquely and how they change during the life cycle of the individual. Table 2.2 gives an overview of the primary parameters of the standard DEB model; κ_X^P (or the equivalent y_{PX}) could be added to quantify faeces production and the fluxes of dioxygen and carbon dioxide in association with assimilation.

2.1 Feeding

In terms of food availability small animals typically live in a three-dimensional world, big ones in a two-dimensional world, others in the twilight-zone between these worlds. This implies a minimum spatial structure for ecosystems, with complex interaction between the players of the game. At the planetary level, life is confined to a kind of membrane that wraps the Earth, which receives its mass and energy from both of its surfaces. This con-



Figure 2.1: Energy fluxes in the standard DEB model. The rounded boxes indicate sources or sinks. The symbols stand for: X food intake; P defecation; A assimilation; C mobilisation; S somatic maintenance; J maturity maintenance; G growth; R reproduction.

Table 2.1: The assumptions that specify the standard DEB model quantitatively.

- 1 The amounts of reserve, structure and maturity are the state variables of the individual; reserve and structure have a constant composition (strong homeostasis) and maturity represents information.
- 2 Substrate (food) uptake is initiated (birth) and allocation to maturity is redirected to reproduction (puberty) if maturity reaches certain threshold values.
- 3 Food is converted into reserve and reserve is mobilised at a rate that depends on the state variables only to fuel all other metabolic processes.
- 4 The embryonic stage has initially a negligibly small amount of structure and maturity (but a substantial amount of reserve). The reserve density at birth equals that of the mother at egg formation (maternal effect). Foetuses develop in the same way as embryos in eggs, but at a rate unrestricted by reserve availability.
- 5 The feeding rate is proportional to the surface area of the individual and the food-handling time is independent of food density.
- 6 The reserve density at constant food density does not depend on the amount of structure (weak homeostasis).
- 7 Somatic maintenance is proportional to structural volume, but some components (osmosis in aquatic organisms, heating in endotherms) are proportional to structural surface area.
- 8 Maturity maintenance is proportional to the level of maturity
- 9 A fixed fraction of mobilised reserves is allocated to somatic maintenance plus growth, the rest to maturity maintenance plus maturation or reproduction (the κ -rule).
- 10 The individual does not change in shape during growth (isomorphism). This assumption applies to the standard DEB model only.

strains its impact from a geochemical and climatological perspective. Later I will explain that big-bodied species live on a large space-time scale that small-bodied ones, but scale only matters if space-time is inhomogeneous. It always is in practice, but not always in models.

Food intake, i.e. the disappearance of food from the environment, is proportional to the surface area of the individual. The argument rests on the more general principle that transport in volumes is across surface areas. Food uptake, i.e. the passing of food-derived metabolites across the gut wall, is taken to be proportional to food intake. This assumption constrains the activity of enzymes and gut flora in the digestion process. Feeding activity in general is taken to be a fixed proportion of somatic maintenance, independent of the feeding rate. Feeding rate-dependent costs are taken from food, again as a fixed proportion.

How feeding rate depends on food availability follows from a classification of behaviour

Table 2.2: The 12 primary parameters of the standard DEB model in a time-length-energy and a time-length-(dry)mass frame and typical values among species at 20 °C with maximum length $L_m = zL_m^{\text{ref}}$ for a dimensionless zoom factor z and $L_m^{\text{ref}} = 1 \text{ cm}$. The two frames relate to each other via $\overline{\mu}_E = 550 \text{ kJ mol}^{-1}$ and $[M_V] = 4 \text{ mmol cm}^{-3}$. The typical value for the Arrhenius temperature $T_A = 8 \text{ kK}$. See the text for a discussion of the values.

A = 0 km. See the text for a discussion of the values.											
specific searching rate	$\{\dot{F}_m\}$	$6.5 \mathrm{l cm^{-2} d^{-1}}$	$\{\dot{F}_m\}$	$6.5 \mathrm{lcm^{-2}d^{-1}}$							
assimilation efficiency	κ_X	0.8	y_{EX}	$0.8\mathrm{mol}\mathrm{mol}^{-1}$							
max spec. assimilation rate	$\{\dot{p}_{Am}\}$	$22.5 z \mathrm{J cm^{-2} d^{-1}}$	$\{\dot{J}_{EAm}\}$	$0.041 z \mathrm{mmol} \mathrm{cm}^{-2} \mathrm{d}^{-1}$							
energy conductance	\dot{v}	$0.02{ m cm}{ m d}^{-1}$	\dot{v}	$0.02{ m cm}{ m d}^{-1}$							
allocation fraction to soma	κ	0.8	κ	0.8							
reproduction efficiency	κ_R	0.95	κ_R	0.95							
volume-spec. som. maint. cost	$[\dot{p}_M]$	$18{ m Jcm^{-3}d^{-1}}$	$[\dot{J}_{EM}]$	$0.033{ m mmolcm^{-3}d^{-1}}$							
surface-spec. som. maint. cost	$\{\dot{p}_T\}$	$0{ m Jcm^{-2}d^{-1}}$	$\{\dot{J}_{ET}\}$	$0\mathrm{mol}\mathrm{cm}^{-2}\mathrm{d}^{-1}$							
maturity maint. rate coeff.	\dot{k}_J	$0.002{\rm d}^{-1}$	\dot{k}_J	$0.002{\rm d}^{-1}$							
specific cost for structure	$[E_G]$	$2800\mathrm{Jcm^{-3}}$	y_{VE}	$0.8\mathrm{mol}\mathrm{mol}^{-1}$							
maturity at birth	E_H^b	$275z^3\mathrm{mJ}$	M_H^b	$500 z^3 \mathrm{nmol}$							
maturity at puberty	$E_H^{\overline{p}}$	$166 z^3 \mathrm{J}$	$M_{H}^{\overline{p}}$	$0.3 z^3 \mathrm{mmol}$							

in just two categories that mutually exclude each other (sequential processing): food searching and food handling. The mean food searching time is inversely proportional to the meeting frequency between food items and consumer, which itself is proportional to the food density on the basis of the mass action law. The searching times naturally follow a timeinhomogeneous Poisson process, but the simplest formulation takes them deterministically. The mean food handling time is taken to be independent of the food density. The standard model takes the size of food particles constant, and the handling time proportional to the size of the food particle. Generally, the size of food particles follows some distribution, which makes handling times stochastic. These components result in the Holling type II functional response, where the half saturation constant is the ratio of the maximum food intake rate and the maximum searching rate. Searching rate is at maximum in absence of food and is defined as the volume (or surface area) of environment that is searched for food per unit of time. These components fully quantify the scaled functional response: food intake as fraction of its maximum for an individual of that size, as function of food density.

This simple set-up for the feeding process can be, and will be, generalised in many ways. The food handling time can, for instance be considered proportional to the mass of the food item, if food items vary in size. Later we will consider food preferences, if food items vary in quality, and consider classifications of behaviour in more than two categories, and allow traits to occur simultaneously. For such extensions, we first need to set-up the dynamics of Synthesizing Units (SUs); we just saw its simplest mode of operation.

2.2 Assimilation

Assimilation is defined as the inflow of reserve from food-derived metabolites. In the simple situation of constant food quality, it is assumed to be proportional to food uptake, so also to food intake. The ratio of the chemical potentials of food that has been ingested, and reserve that enters the reserve pool is called assimilation efficiency, which is taken to be a constant, independent of the feeding rate. Since animal food consists of other organisms, having reserve(s) and structure(s), the constancy of food quality is really constraining here.

Later we will allow for variations in food quality, and discuss stoichiometric constraints on digestion efficiency on the basis of SU-dynamics.

Notice that feeding and assimilation applies to juveniles and adults, not to embryos.

2.3 Reserve dynamics

The use (or mobilisation) of reserve, which fuels all metabolism, is taken to be dependent on the amounts of reserve and structure only, not on food availability; this fits the homeostasis philosophy. You will not find any assumption in the list in Table 2.4 that directly refers to reserve dynamics; this is because how the mobilisation of reserve depends on the amounts of reserve and structure follows from the weak homeostasis assumption; the derivation is rather technical, however. Dilution by growth, the phenomenon that reserve density, i.e. the ratio of the amounts of reserve and structure, decreases if the amount of structure increases, contributes to the complexity of the derivation.

The derivation uses the concept of partitionability, which is implied by weak homeostasis, i.e. we should be able to partition the reserve without affecting the overall dynamics, while each partition follows the same dynamics. It is very similar to the opposite: mergeability, i.e. we should be able to merge reserves with identical dynamics without affecting the overall dynamics. Contrary to mergeability, partitionability not only leads directly to the reserve dynamics, but also implies that the fraction of mobilised reserve that is allocated to the soma, i.e. somatic maintenance plus growth, should be independent of the amount of reserve. Partitionability and, especially, mergeability, are key concepts in the evolution of DEB systems.

Although the derivation is technical, the resulting reserve dynamics is very simple: the change in reserve density is proportional to the reserve density; the proportionality factor involves the ratio of the energy conductance and structural length. The energy conductance is a parameter with dimension length per time, where length actually stands for the ratio of volume and surface area. Its dimension thus links directly to the assumption of isomorphy. The maximum reserve capacity is the ratio of the surface area-specific assimilation rate and the energy conductance. So a high energy conductance results in a low maximum reserve capacity, so a short period for which the individual can do without food. The result that the change in reserve density is inversely proportional to length explains why young (small) individuals need to feed more frequently than the old (large) ones.

The maximum reserve turnover time equals the ratio of the maximum (structural) length and the energy conductance. The maximum structural length equals the ratio of source (a fraction of specific assimilation) and sink (specific somatic maintenance). Likewise, the maximum reserve density equals the ratio of source (specific assimilation) and sink (energy conductance).

A possible mechanism behind the reserve dynamics is that the mobilisation rate is proportional to the surface area of the interface between reserve and structure. The mobilised reserve flux is sent to the SUs for growth; they belong to the structure. Part of the received reserve flux is rejected and sent back to the reserve, following the rules of SU-dynamics. The constant relative abundance of SUs for growth in structure is such that the ratio of the rejected reserve and the synthesized structure equals the existing reserve density; the existence of such a constant relative abundance follows from SU-dynamics. This mechanism allows a large mobilisation combined with a large rejection. This 'needless' mobilisation can subsequently be killed with a simple self-inhibition of monomerisation, where reserve is supposed to be present as polymers, but mobilised as monomers. This killing of 'needless' mobilisation is especially important for starting embryos, which have an amount of reserve, but hardly any structure, with the implication that the surface area of the interface between reserve and structure is infinitely large.

The turnover time of reserve of juveniles and adults at constant food increases with the feeding rate and the structural length. Part of the reserve consists of proteins that catalise metabolic transformations (enzyme). These enzymes are thermally rather unstable and active at formation, but sooner or later lose their activity. The fraction of enzymes that are in the active state, therefore, decreases during growth and may lead to a loss of metabolic performance, compared to the small (young) individual. Such systematic changes are linked to ageing in the literature, but don't need to be associated to ageing in a DEB context.

2.4 The κ -rule for allocation to soma

Although weak homeostasis allows that the fraction of mobilised reserve that is allocated to the soma, called κ , depends on the amount of structure, the simplest rule that turns out to be realistic is that this fraction is constant during the life cycle of the individual. The rest of the mobilised reserve is allocated to maturity maintenance plus maturation (in embryos and juveniles) or reproduction (in adults). A consequence of the κ -rule is that growth competes directly with somatic maintenance, but only indirectly with reproduction; this uncouples body size control from reproductive output. Given the rules that will follow, a constant κ leads to von Bertalanffy growth at constant food density; this curve fits many data very well. Changes in κ translate to particular changes in growth curves, that can sometimes be induced by parasites or toxicants. Even more convincing for κ being constant is the implied body size scaling relationships, see Chapter 8. In fully-grown individuals, κ equals the ratio of somatic maintenance and assimilation, so a fraction of a rate equals a ratio of rates for them. The κ -rule have nice static and dynamic generalisations, which allow more flexibility in the growth of body parts (tissues, organs, tumours) in relation to there function (Chapter 5).

2.5 Dissipation excludes overheads of assimilation and growth

Dissipation is, chemically, the set processes where reserves are used, without direct links with growth; the resulting products are typically dissipated into the environment. Reproduction is primarily an export of reserve (with minor overheads), so not a use of reserve in the chemical sense. Since growth and assimilation have overheads, what dissipates from the individual into the environment is more than the dissipation flux. Strong homeostasis implies that the dissipation flux has a constant chemical composition, but what dissipates into the environment can vary in composition. This is why 4 fluxes are collected in the dissipation category:

- 1 Somatic maintenance, a flux taken to be mainly proportional to the amount of structure, but heating (confined to endothermic species) and osmosis (mostly confined to freshwater species) are somatic maintenance costs that are linked to (structural) surface area. Somatic maintenance is supposed to include the turnover of structure and activity (behaviour).
- 2 Maturity maintenance, a flux taken to be proportional to the level of maturity; the proportionality constant is called the maturity maintenance rate coefficient.

The existence of this rather esoteric flux is illustrated by

1) a thought-experiment, where we expose two individuals to two tiny differing constant food levels; one food level is just below the level at which puberty can be reached, the other is just above. If maturity maintenance would not exist, one individual will not reproduce, and the other at a substantial rate. Such a big difference in response to a tiny difference in environmental conditions has never been observed.

2) the observation that the total cumulative energy investment in development at any given size of the individual depends on food density, which is counter-intuitive; this can be removed by allowing for maturity maintenance.

Maturity maintenance is supposed to include maintenance of regulation and defence (e.g. immune) systems.

3 Maturation, i.e. the increase in the level of maturity. Two observations motivate the ideas on maturation:

1) Age at birth or puberty varies a lot with food density. Volume at birth or puberty varies a little with food density. This means that stage transitions cannot be linked to age.

2) Some species continue growing after puberty; other species, such as birds, do not. This means that stage transitions cannot be linked to size.

Metabolic switching is linked to maturity, which allows for some nutrition-dependent scatter of amounts of structure at birth and at puberty.

4 Reproduction overhead. Since starting embryos are assumed to consist of reserve, and hardly have any structure or maturity, reproduction is the process of conversion of reserve of the mother to that of the offspring. This involves little chemical work, mostly that of wrapping reserve packages into eggs. The ratio of the reserves fixed into offspring and allocated to reproduction is called the reproduction efficiency, which is assumed to be close to 1.

The reason why dissipation excludes assimilation and growth overheads is stoichiometric constraints on conversions The conversion from food to reserve, or from reserve to structure, has not only energy aspects, but also stoichiometric ones. The efficiency in terms of energy, does not need to equal that of nitrogen, for instance, if the compositions of reserve and structure differ. This introduces an uneasy aspect of the labels: dissipation is not 'all that dissipates'. The necessity of this discrepancy directly follows from energy as well as mass conservation.

It sometimes turns out to be handy to express the specific somatic maintenance as fraction of the specific costs for structure: the somatic maintenance rate coefficient, which has dimension 'per time'. Since maturity, unlike structure, has no mass or energy, maturity maintenance is directly expressed in terms of a similar fraction: the maturity maintenance rate coefficient, which thus also has dimension 'per time'. If the ratio of the latter and the first rate coefficients equals one, maturity density, i.e. the ratio of maturity and structure, remains constant till puberty and metabolic switching occurs when the amount of structure hits threshold values. But if this maintenance ratio is less than one, which is typically the case, maturity density increases till puberty, and then decreases.

2.6 Growth: increase of structure

A fraction κ of the mobilised reserve, minus the somatic maintenance flux, is allocated to growth; the conversion of reserve into structure has a constant efficiency, because of strong homeostasis. While maintenance is demand-driven, growth is supply-driven; even supply systems have demand components, which makes that ultimate length equals κ times the ratio of the surface area-specific assimilation rate and the volume-specific maintenance rate. If surface area-linked maintenance costs are present, the heating length should be subtracted from the result, i.e. the ratio of the surface area-linked and the volume-linked maintenance costs. At constant temperature, or constant ionic strength, the heating length is a constant. In the resulting expression for growth, a compound parameter shows up that comes back repeatedly, the energy investment ratio: the ratio of the specific costs for structure and κ times the maximum reserve density.

If food density is constant, the result is that post-natal growth follows the von Bertalanffy growth curve. By comparing growth at different food densities, the von Bertalanffy growth rate relates to the ultimate length in a very special way: the inverse von Bertalanffy growth rate is linear in the ultimate length; the slope relates to the energy conductance, the intercept to the somatic maintenance rate coefficient, i.e. the ratio of the volume-specific somatic maintenance cost and the volume-specific cost for structure. The von Bertalanffy growth rate is independent of the heating length. DEB theory assumes a maternal effect: the reserve density at birth equals that of the mother at egg formation. This indirectly specifies the costs of an egg, which can be computed using a shooting method: choose an initial amount of reserve, let the embryo evolve till maturity hits the threshold at birth, evaluate the reserve density at birth and adjust the initial amount of reserve. Since food levels generally vary, the costs of an egg generally varies and, in a population of many individuals, this evaluation method for the initial amount of reserve becomes computationally intensive. To solve this problem, I developed an alternative scheme, which is, however, rather technical, but efficient. An important side-result of this scheme is that age at birth must be smaller than the ratio of the relative length at birth (the ratio of the birth- and ultimate-lengths) and the von Bertalanffy growth rate. In practice many incubation times seems to be longer due to diapauses; age zero is when development starts.

Foetal development is a variation on egg development, where the foetus receives reserves from the mother during development and can use the draining system of the mother to get rid of its nitrogen waste. If this occurs fast enough (so reserve density can assumed to be large), the length of the foetus will be proportional to its age; a well-known empirical finding. Egg development deviates from this pattern because reserve density decreases during development, which slows it down.

2.7 Reproduction: excretion of wrapped reserve

A fraction $1 - \kappa$ of the mobilised reserve, minus the maturity maintenance flux, is allocated to reproduction in adults; the conversion of reserve of the mother into that of offspring has a constant efficiency. Since allocation to reproduction per time increment is incrementally small, and the initial amount of reserve of an embryo is not, we need a reproduction buffer to accumulate the invested reserve, and buffer handling rules.

Buffer handling rules are rather species specific. One example is: make an egg as soon as enough reserve is accumulated. This rule does not involve any new parameters. Many aquatic species use temperature to trigger spawning. This rule implies a (diurnal or yearly) temperature cycle.

Some metabolic switches in adults are linked to the reproduction buffer density, such as spawning events in multiple-spawners, or pupation in holo-metabolic insects.

Quite a few species sport a post-reproductive stage; DEB theory treats this as an effect of ageing, which will be discussed in Chapter 6.

2.8 Parameter estimation I: numbers, lengths and time

Half of the 12 primary parameters of the standard DEB model (see Table 8.1) contain energy in their dimension. The estimation of these parameters from data requires that energy is measured, somehow. Powerful auxiliary theory shows, however, that it is possible to estimate energy-independent ratios of energy parameters from data that has no energy in its dimension. This is why so much attention is given to compound parameters with simple dimensions; they can be estimated from simple data, while energy parameters cannot. This reasoning can also be reversed: we don't need knowledge about energy parameters when we want to predict quantities that have no energy in their dimension. Knowledge about a ratio is weaker than that about the numerator and the denominator separately. A good strategy is to collect weak knowledge before stronger knowledge. The key dimensions of the standard DEB model, time, length and energy, and easily be replaced by time, length and mass. We cannot avoid length, because of the assumption that assimilation is linked to surface area.

The problem that the state variables of the standard DEB model cannot be measured (and tested) directly is solved by considering several measurable quantities simultaneously, from which the values of the state variables can be deduced. The Add_my_Pet collection of data and DEB parameters of over 1000 animal species demonstrates that this is no handicap in practice, and powerful software is freely available.

3

Energy, compounds and metabolism

Before we are ready to deal with mass and energy aspects of univariate DEB models, we need to introduce several concepts first.

3.1 Energy and entropy

DEB theory assumes that the mass-specific chemical potentials and entropies of all pools (food, reserve, structure) are constant. Their values will be obtained from an input-output analysis of living individuals at different food levels.

3.2 Body mass and composition

The quantification of mass in terms of grams or C-moles is basically different, because changes in chemical composition prohibits the use of C-moles. Weights, and to a minor extent also physical volumes, have contributions from structure and reserve and sometimes also from the reproduction buffer. We can use changes in chemical composition of biomass to infer the composition of reserve and structure, thanks to the weak homeostasis assumption. The book only follows the 4 most abundant elements (carbon, hydrogen, oxygen and nitrogen), but this restriction is not basic to DEB theory (because it is chemically implicit). An extension to more chemical elements involves extra element-conservation rules and extra minerals that must be excreted, containing the new elements.

3.3 Classes of compounds in organisms

Compounds that are followed are classified into 2 categories: mineral and organic, just for convenience. The number of mineral compounds equals that of the elements (carbon dioxide, water, dioxygen, nitrogen-waste), which is convenient because this makes that all mineral fluxes follow from the conservation law of the elements, without involving new modelling aspects. If we want to delineate more minerals, we should also add assumptions that specify the excretion of those minerals. The organic compounds also have 4 members: food, faeces (as an example of a product), reserve and structure. This is implied by the strong homeostasis assumption. Again all this is not basic to DEB theory, because it is chemically implicit. Applications and testing of the theory require these evaluations, which also serve to introduce the notation.

3.4 Conversions of energy, mass and volume

Due to the strong homeostasis assumption, the conversion of energy, mass and volume of quantities is in principle simple. The use of these measures is to a large extend equivalent. It is possible, and frequently useful, to follow energy fluxes in nitrogen-limited systems, for instance. Table 3.3 is convenient for conversions and includes several compound parameters that frequently pop-up.

3.5 Macrochemical reaction equations

Macrochemical reaction equations (not to be confused with mathematical equations) make explicit which compounds can be considered to be substrates and which can be considered to be products in a (typically complex) chemical transformation. The chemical indices should be known and constant, generally. This means that biomass can only be a compound if the composition of reserve and structure are the same. The yield coefficients are subjected to conservation laws for elements (stoichiometric constraints) and energy. The fact that these coefficients can vary in complex ways, contrary to typical chemical reaction equations, limits the usefulness of macrochemical reaction equations. The variation can be restricted, however, by splitting up these equations in several microchemical reaction equations. DEB theory can frequently be used to quantify the variation in yield coefficient, which boosts the usefulness of these equations. Yield coefficients represent ratios of fluxes of compounds, the compound in the denominator is the reference compound, which defines 'the' reaction rate. In many cases in biology it is more useful to think of the chemical transformation as a set of coupled fluxes of compounds. Each compound appears (positive flux) or disappears (negative flux) at a compound-specific rate; these fluxes are subsequently constrained by conservation laws and ideas on links between them, such as DEB theory.

3.6 Isotopes dynamics: reshuffling and fractionation

Now that the technology to measure (stable) isotopes has made a leap forward, isotope data is rapidly accumulating in the literature. We need, however, sound models, to arrive at useful conclusions from these data. DEB theory is, in principle, ideal for this because it already follows all compounds to, in and from organisms. Since (isotopes of) elements are locked in compounds, and compounds transform to other compounds using several pathways simultaneously, the notation becomes rather demanding.

Although the various isotopes of an element generally behave identical from a chemical perspective, they differ in mass, so affect the velocity of their molecules, and they affect the

binding strength with other atoms within their molecule. Because of their weaker binding, molecules with light isotopes are more likely to be selected for catabolic functions than for anabolic ones. Isotope dynamics has two aspects: reshuffling and fractionation.

In a simple chemical reaction, the fate of each atom in the set of substrates is typically uniquely determined. In metabolism, which involves metabolic networks, the fate is no longer uniquely determined, and we have to deal with a stochastic mapping of atoms in substrates to that in products of the chemical elements of interest. All these probabilities become model parameters that should be known or estimated from data. If the metabolic network is really complex, reshuffling can be completely random: we take all atoms of a particular element from all substrates simultaneously, and place them randomly in the products with the correct frequency per compound. The reshuffling probabilities can be obtained from the chemical indices and yield coefficients, so they lose their role as parameters.

Molecules with various isotopes in one or more of their atoms are subjected to selection, not atoms directly. Fractionation can be from (large) pools or from fluxes; the difference is that fractionation from fluxes is converted to that of pools by multiplication by a time increment, which gives an incrementally small pool. Fractionation from (large) pools involves the binomial distribution, but that from fluxes involves Fisher's non-central hypergeometric distribution. Fractionation in carbon fixation (by C3 plants) is from a pool (of atmospheric carbon dioxide). Fractionation in the mobilised reserve, for instance, must be from a flux for consistency reasons; the isotope frequency in the reserve must equal that in the mobilised flux, because the mobilisation rate is supposed to be independent of the isotope frequency. Moreover, reserve is a generalised compound, consisting of chemical compounds that have a large variety of molecular masses and binding strengths. If this huge variation did not affect the relative rate of use of these compounds, then why should tiny variations caused by isotopes?

3.7 Enzyme-mediated transformations based on fluxes

The chemically implicit theory on enzyme kinetics is very useful for particular technical applications, but less so in cellular biology. The first reason is that it works with the concept concentration, which implies a well-mixedness at the molecular level and homogeneous space, and the second one is that by working with enzymes as chemical species, its application in metabolic transformations rapidly becomes too complex. DEB theory works with Synthesizing Units (SUs) dynamics; SUs are generalised enzymes that basically follow the rules of enzyme kinetics, with two important modifications. The first one is that SU-dynamics works with fluxes, not with concentrations. In homogeneous space, fluxes of arriving substrates to the SUs can be considered proportional to concentrations on the basis of convection-diffusion arguments; section 11.2 discusses why these arguments are typically problematic. This modification represents an extension of enzyme kinetics, because it gives room for transport modelling. The second one is that SU-dynamics ignores back-transformations, using the argument that transport of substrates to the SUs, and removal of products from the SUs is typically under cellular control. This modification

represents a restriction, and a substantial simplification, of enzyme kinetics.

Since SU-dynamics has a less strict link with molecular phenomena, it can be used for modelling behaviour, where the individual is considered as an SU. The simplest application in modelling the feeding process was already discussed.

Substrates can be classified as substitutable or complementary, and the processing of substrates as sequential or parallel; this gives 4 basic classes. Mixtures of these classes can be made by (weighted) sums of changes in the various binding fractions of SUs, where time scale separation arguments are used to derive pseudo-steady state kinetics. Further extensions, e.g. to cope with preference, inhibition and co-metabolism, are possible by letting the binding probabilities of new substrates depend on what is already bound to the SUs.

3.8 Metabolism

While animals are typically biotrophs, so the uptake of all the required substrates is more or less coupled, other organisms frequently take their various substrates independently from the environment. This requires a reserve per substrate that is taken up independently (see Chapter 5). The sources of energy and carbon can differ, even in bacteria that live of organic compounds. In many situations mixtures of various possibilities occur, even in plants, for instance.

The central metabolism, which deals with the energy housekeeping, consists of 4 biochemical modules. These modules are briefly discussed for two reasons. First as part of the strategy to model cellular performance not directly in terms of molecules, but in terms of (syntrophycally) interacting modules, inserting extra levels of organisation (see e.g. Section 7.6). Second because its evolution backbones the general structure of DEB theory, as will be discussed in Chapter 10. The modules were repeatedly recombined in ways that emphasise partitionability and mergeability as being basic to evolution. 4

Univariate DEB models

Univariate DEB models are an extension of the standard DEB model by removing the constraint of isomorphy. This chapter not only explores this extension, but also considers energy and mass aspects that were skipped in the presentation of the standard DEB model for didactic reasons, using the previous chapter. These considerations provide useful tests against experimental data. We start with considering consequences of changes in food density in more detail.

4.1 Changing feeding conditions

Feeding is typically in meals, where the scaled functional response jumps back and forth from 0 to 1. These fluctuations are typically somewhat smoothed out by the digestive system (see Section 7.3), but otherwise they induce a scatter structure of size data that is realistic. When individuals experience transitions to higher or lower food levels in their life, we observe predictable responses of growth; this supports the mechanism behind what sets the ultimate size. Growth continues (for some time) during starvation, which supports the loose coupling between growth and feeding. Existing alternatives to DEB theory have problems in capturing this feature (see Chapter 11).

When starvation continues, and the mobilised reserve flux is no longer sufficient to cover somatic maintenance costs, a variety of responses might occur. It turns out that the diurnal cycle can sometimes affect the response by affecting whether or not reproduction continuous during starvation and by affecting κ . Changes in κ induce a set of coupled responses, which further support the organisational structure of metabolism in DEB theory. Most of somatic maintenance relates to the turnover of structure. While the mobilisation of structure continues, its re-synthesis during continued starvation is not complete, which causes shrinking, and so a reduction of maintenance costs. If shrinking exceeds a given fraction of the maximum structure that has been reached in the past, death occurs; this fraction is higher for demand-systems than for supply systems. If mobilisation is insufficient to pay maturity maintenance, rejuvenation occurs: the decrease of the level of maturity. Maturity in adults has to be restored before allocation to reproduction can resume. Rejuvenation increases the hazard rate proportional to the difference of the maximum maturity level the individual has reached in the past and the actual level.

4.2 Changing shapes

Changes in shape are captured with the shape correction function: the ratio of the actual surface area (that is involved in substrate uptake) and that of an isomorph, where the two are set equal (in surface area and shape) for a reference size. This gives a dimensionless function of structural volume as it changes during the life cycle.

Apart from isomorphs ($V_{\overline{3}}^2$ -morphs), two special cases repeatedly pop-up in applications of DEB theory:

- V0-morphs, where surface area is proportional to structural volume to the power 0, so it remains constant. Biofilms, and organisms that increase their structure at the expense of their vacuoles are examples.
- V1-morphs, where surface area is proportional to structural volume to the power 1. Growing filaments and sheets are examples.

Many other cases can be seen as static or dynamic mixtures of these three basic types; rods are static mixtures, plants naturally evolve from V1-, via iso-, to V0-morphs during their life cycle and crusts from V1- to V0-morphs such that their diameter grows linearly in time at constant substrate. For me, Figure 4.11 is very convincing, where the substantial differences in the shapes of 4 growth curves can fully be attributed to the relative importance of the contributions of V0- and V1-morphic components in static mixtures of both. Then food intake of isomorphs is experimentally controlled and constant (independent of size), growth curves result that are very similar to that of V0-morphs; the (small) differences are due to the behaviour of the energy conductance. For isomorphs it is a constant, but for other morphs it becomes a function of structural volume (so of structural length).

V1-morphs have the unique feature that the significance of the levels of the individual and the population completely merge; a population of many small V1-morphs behaves identical to that of a few big V1-morphs with equal total structure and reserve. V1-morphs also have no size-control as an individual (if they would not reset their size by division); they continue to growth exponentially as long as substrate density remains constant. This argument can also be reversed: if we want to understand population characteristics (such as the maximum specific growth rate) in terms of properties of individuals (such as size at division), we cannot consider them as V1-morphs. Otherwise, the population dynamics of V1-morphs is so much simpler than that of other morphs, that it remains attractive to make this simplification for other purposes. This can, for dividing organisms, be defended mathematically as being a good approximation in quite a few situations.

The literature in microbiology speculates why the yield of biomass on substrate in not only low at low specific growth rates (all agree that this is because of maintenance), but also at high population growth rates. The explanation offered by DEB theory is because reserve (that corresponds to the limiting substrate) increases with the specific growth rate, which causes a quality shift; the goodness of fit with data is striking, if we treat these micro-organisms as V1-morphs. At zero maintenance, the reserve dynamics of V1-morphs is identical to the Droop model for cell quota. At very high reserve turnover rate, the growth-dynamics of V1-morphs is identical to that of the Marr-Pirt model, but the implied product formation is not. The Marr-Pirt model pays maintenance from structure, and this limit lets maintenance pay from assimilation. To mimic this (unrealistic) aspect the limit must be taken in the extended DEB model that accounts for the turnover of structure

4.3 Mass aspects of univariate DEB models

DEB theory specifies the fluxes of organic compounds (food, structure, reserve, faeces), from which follows the fluxes of mineral compounds (carbon dioxide, water, dioxygen, nitrogenwaste) uniquely on the basis of conservation of chemical elements. We have to evaluate them all simultaneously, however, not just dioxygen, for instance. It turns out that all mass and energy that go in and out an individual that follow the rules of univariate DEB models are weighted sums of 3 basic fluxes: assimilation, dissipation and growth. Moreover, the 3 basic fluxes turn out to be cubic polynomials in length, the coefficients depending on reserve density and primary DEB and composition parameters.

I demonstrate how data on different constant substrate levels, or data during starvation, can be used to access the composition of reserve and structure, and give arguments why particular compounds can be treated as proxies for reserve and structure.

4.4 Respiration

The literature on animal physiology treats the respiration quotient (RQ), i.e. the ratio of carbon dioxide production and dioxygen consumption, as constant, independent of size and nutritional condition. Contributions from assimilation are typically excluded, experimentally. I explore the implications of this assumption in a DEB context, and conclude that the RQ can only be constant if the elemental composition of reserve and structure relate to each other in a very special way, which includes to situation that they are identical. This is why microbial physiologists never make this assumption. The fact that animal the elemental composition of reserve and structure don't differ too much in animals makes that dioxygen consumption is approximately proportional to the flux of mobilised reserve; a result that will be used in the module for ageing (Chapter 6).

The literature on animal physiology speculates on the reason why dioxygen consumption sensitively depends on assimilation. DEB theory explains this on the basis of elemental conservation.

4.5 Nitrogen balance

Like the RQ, an urination (UQ) or a watering (WQ) quotient can be defined as ratios nitrogen-waste or water production and dioxygen consumption, with the remarkable implication if RQ, UQ as well as WQ are all constant, the elemental composition of reserve and structure must be identical for the 4 most abundant elements.

Ammonia excretion can be linked to assimilation, maintenance and growth; a view very different from that in static energy budgets (Chapter 11). These results are important for interactions between organisms, where other organisms use excreted nitrogen-waste.

4.6 Water balance

The production of metabolically derived water is specified by DEB theory. If water losses by transpiration are quantified as well, a model for drinking results on the basis of strong homeostasis. The water content of biomass might actually vary somewhat, but typically within rather narrow bounds for most animals.

Plants evaporate water via shoots, which is compensated by uptake from the soil via roots. This water flux carries a nutrient flux, that plants need for growth (and maintenance). It turns out that the ratio of the (functional) surface areas of roots and shoots appear in the half saturation constant for nutrient-uptake. This coupling between water and nutrient uptake makes that the half saturation constant for nutrient uptake changes dynamically.

4.7 Isotope dynamics in the standard DEB model

Like other mass fluxes, the fluxes of isotopes have contributions from assimilation, dissipation and growth. The rules for reshuffling and fractionation can be applied to each of these fluxes, by separating them in anabolic and catabolic aspects. This is to make explicit the role of each compound as substrate or product. Carbon dioxide that originates from food, reserve or structure can have different isotope signatures. Assimilation has a catabolic aspect because substrate is used to 'pay' its conversion to reserve. Dissipation has an anabolic aspect because the turnover of structure implies catabolic as well as anabolic aspects; structure appears both as substrate and as product in this transformation. Growth has a catabolic aspect because reserve is used to 'pay' its conversion to structure.

Applications of this theory still have to be developed; the analysis of the isotope signature in persistent products such as wood of plants, otoliths of fish and shells of molluscs is promising, because DEB theory fully specifies both their production as well as their isotope signature. The hope is that these applications allow the reconstruction of environmental quality trajectories from observations on isotope signatures.

4.8 Enthalpy, entropy and free energy balances

Like mass fluxes, dissipating heat has contributions from assimilation, dissipation and growth. This makes that dissipating heat can also be written as weighted sums of dioxygen consumption, carbon dioxide and nitrogen water production. DEB theory, therefore, explains the empirically justified method of indirect calorimetry; a firm support for DEB theory, that will stand as a rock. By treating the specific energy potentials and entropies of (organic) compounds as unknown parameters, their values can be estimated from data at a variety of (constant) food levels. The entropy production is by far the best quantifier for metabolic activity that can be applied to all organisms (including the ones that produce dioxygen).

In addition to the heat production as specified by DEB theory, the modelling of heat fluxes to and from the individual can further detail the thermal balance, which we need for endotherms outside their thermo-neutral zone. Heat production can understandably be proportional to the chemical potential of substrate when micro-organisms are fed with a variety of substrates.

4.9 Products

Like other mass fluxes, product production has contributions from assimilation, dissipation and growth. The production occurs in the overheads of these fluxes. I show with an example, pyruvate in fermenting yeast, that the contribution from growth might be negative (representing a consumption). This gives a very particular relationship between the production of that product and the specific growth rate, which is very well-captured by the theory. The well-known Leudeking-Piret model links product formation to maintenance and growth in micro-organisms. This model cannot capture the observed pattern, because reserve is essential to uncouple assimilation from maintenance and growth, so to create a three- rather than a two-dimensional basis for product formation; without reserve, assimilation equals maintenance plus growth. Fermentation occurs in the absence of dioxygen, and the rate at which products are formed follows from DEB theory.

As long as substrates are strictly non-limiting, such as dioxygen in aerobic situations, they can considered to be products that have negative production.

4.10 Parameter estimation II: mass, energy and entropy

By extending the data that were required to estimate essential compound parameters of the standard DEB model by data on composition and dissipating heat, I show how all primary DEB parameters and composition parameters can be obtained from data. A natural sequence exists: entropies can only be obtained if energy parameters are known; chemical potentials can only be obtained if mass parameters are known; mass parameters can typically only be obtained if other DEB parameters are known.

4.11 Trajectory reconstruction

DEB theory is simple enough to allow inverting the reasoning and reconstruct environmental quality trajectories (temperature, food availability) from observations on individuals. I work it out for observations on body weight, on length-dependent reproduction and on

otolith opacity as examples. These reconstructions assume that particular DEB parameters are know for the species under consideration. These reconstructions might, for instance, help to understand with what 'eyes' organisms look to their local environment and learn about food preference and the nutritional value of the various resources.

 $\mathbf{5}$

Multivariate DEB models

The univariate food, reserve and structure can be replaced by multivariate alternatives. This chapter discusses how this can be done in a way that is consistent with univariate DEB models.

5.1 Several substrates

The rules for SUs can be used to model food preferences. In the case of very strong preferences, an appreciated food type can replace a depreciated one that is already bounded to the SU. Biotrophy implies the coupled consumption of both reserve and structure of the prey by the consumer, which typically serve as substitutable substrates, or sometimes as mixed substitutable-complementary substrates.

The reserved situation also occurs; glucose can be taken up by two independently operating carriers in yeast. Low-affinity-high-capacity carriers operate aerobically are well as anaerobically and are linked to product formation, while high-affinity-low-capacity carriers operate only aerobically and are not linked to product formation. The transition from the functional dominance of one type of carrier to another as a function of the specific growth rate can be really sharp, such that it resembles a metabolic switch (as suggested in the literature), while in reality there is no switch.

Silt can compete with detritus and algae for access to the filtering machinery of bivalves, while its digestion efficiency is nil; the rules are again given by SU-dynamics. The overall effect is that the half saturation coefficient for algae is proportional to the silt concentration; this implies a model for pseudo-faeces production by bivalves.

In oxygenic photosynthesis, the binding of photons by photosystem I and II, and the binding of carbon dioxide to synthesise carbohydrates again follows from SU-dynamics. This also applies to the binding of dioxygen by Rubisco, which leads to the oxidation of carbohydrate in a process called photorespiration. This reversed reaction implies the existence of a compensation point, i.e. a ratio of carbon dioxide and dioxygen concentrations at which the net synthesis of carbohydrates is nil. This odd situation is generally thought to be an evolutionary relict; dioxygen was virtually absent when oxygenic photosynthesis evolved. Even the inhibition process of photons at high arrival rates can be modelled this way.

Calcification can be modelled by treating carbon dioxide and bicarbonate as substitutable substrates, with photons as a supplementary substrate, for the synthesis of lipids as reserve. Calcium uptake is coupled to that of bicarbonate; excreted calcium carbonate is formed as a product in this assimilation process and carbon dioxide is used for metabolism. In this way calcifiers can use bicarbonate as carbon source; 98 % of all inorganic carbon in the sea is in this form.

5.2 Several reserves

Chapter 10 explains why there should be a reserve for each set of substrates that is taken up independently. The discussion on several reserves is restricted to (dividing) V1-morphs for convenience. In V1-morphs maturity and somatic maintenance can be added and maturation and growth can be added, which makes the value of κ irrelevant, and the explicit evaluation of maturity can be avoided. The reserve turnover rate is constant for V1-morphs (independent of length), and the turnover rates of the various reserves seem to be equal.

Like in one-reserve systems, maintenance is subtracted from each mobilised reserve before allocation to growth. The maintenance requirement can be reserve-specific; zero maintenance is one of the possibilities. The growth-SUs now receive several, typically complementary, fluxes originating from the various reserves. Although there is, mathematically and conceptually, not a single reserve (flux) that limits growth, numerically there typically is.

Some part of all arriving reserve fluxes is rejected by the growth-SUs; a fixed fraction κ_E of the rejected fluxes is fed-back to the originating reserve, the rest is excreted. If fraction κ_E is zero for a particular reserve, the reserve density will covary with the growth rate, like the most limiting reserve. If it is close to one, however, the reverse situation happens: the non-limiting reserve density becomes high at small growth rates and the reserve dams up.

5.3 Several structural masses

The κ -rule for allocation can be extended statically or dynamically to deal with several structures, rather than a single one. In the static generalisation the κ -fraction of mobilised reserve is further sub-divided, each sub-fraction is allocated to the synthesis of a sub-structure, such as an organ or body part, after subtraction of the somatic maintenance cost of that sub-structure. This allows of a near-allometric growth of sub-structures, relative to the rest of the body, while avoiding Huxley's problem that sums of allometrically growing body parts cannot add up to the whole. This ability of the κ -rule to capture the empirically observed allometric growth of body parts supports the structure of the DEB model.

In the dynamic generalisation the fraction that is allocated to a sub-structure is no longer constant, but relates to the workload of the sub-structure relative to its maximum. This involves a new modelling step, namely to link the function of a sub-structure to its work. I detail the reasoning for two examples.
In the case of a tumour as sub-structure, its function can be the consumption of somatic maintenance, and it does so proportional to its volume. This simple model gives realistic predictions for the growth of tumours during caloric restriction, and helps to understand why the growth of tumours in young (small) individuals differs very much from that in old (large) ones.

In the case of the gut and the velum of bivalve larvae as sub-structures of the assimilatory machinery, their functions are food digestion and filtering respectively, and they do so proportional to their volume again. It turns out that the relative workload of the gut is one minus that of the velum. Given that the allocation to the assimilation machinery is a constant fraction of mobilised reserve, these relative workloads define the competition between gut and velum for access to this resource. The result is that the relative organ size adapts to the existing food level rather rapidly to a constant relative size; a situation that strongly resembles weak homeostasis for body composition. Another nice side-result is that the functional response of adapted individuals deviates from the Holling type II, to become a Hill's functional response.

Plant structure can be subdivided in root (for the uptake of nutrients from the soil) and shoot (for the uptake of photons and carbon dioxide form the atmosphere). By giving these sub-structures their own reserves, a situation is created that strongly resembles a symbiotic interaction between root and shoot on the basis of reciprocal syntrophy: they exchange excreted reserves, which were rejected by their growth-SUs. The mass-communication between these sub-structures can be enhanced by translocation: a fixed fraction of mobilised reserve is allocated to the partner. A nice side-result of this construct is the implied compensatory behaviour. If light is reduced, growth of the root is more reduced than that of the shoot; the reverse happens in case of nutrient reduction. By considering a starting seed as generalised root reserve only, a situation can occur where the generalised shoot reserve density peaks after birth (germination). This behaviour depends on parameter values, and strongly resembles the cotyls of mono- and dicotyls. 6

Effects of compounds on budgets

Organisms can be viewed as physical-chemical machines that manage to survive in physically and chemically varying environments. So far, the discussion was confined to the uptake and use of substrates, but many other chemicals exist that affect the operation of these machines. Some of these chemicals directly relate to the use of substrates, or to the activities of other organisms (including humans). This chapter discusses how these chemicals affect organisms, starting with a special groups of compounds that are naturally formed as by-products metabolism and causes ageing.

6.1 Ageing: Effects of ROS

A natural first question is: why do we need another model for ageing? I see several arguments for such a module in DEB theory

- It links ageing to energetics, which opens the door to body-size scaling relationships (Chapter 8) and for how feeding modifies ageing. This involves the organisation level of the individual, while most modern work on ageing is done at the molecular level.
- It links ageing to responses to chemical compounds in general, placing ageing in a more general context.
- It is simple (2 parameters) and mechanistic and has both the Gompertz and the Weibull models as special cases. These two empirical models are still very popular and describe different data very well, making it hard to choose. Now the choice is no longer necessary, which is quite remarkable from a mathematical point of view, because all three have 2 parameters.
- It explains why mean life span increases with maximum body weight among endotherms, but not among ectotherms. To my knowledge nobody came up with an explanation for this. The mean life span of a mouse is less than that of an elephant, but extremely small wasps exist than can grow very old.

• We need at least on death process to determine population dynamics, so avoiding the topic is not an option. Death is essential for life, so ageing is too important to neglect.

In the context of DEB theory, death by ageing cannot be seen as metabolic switch linked to maturity, because if food levels are low enough, such a threshold is never reached. DEB theory here follows the main-stream in age-research, by linking ageing to damage by reactive oxygen species (ROS), as a side-product of respiration. For this purpose we exclude contributions from assimilation to respiration (assuming that this use of dioxygen is localised near the digestive system only, and for simplicity's sake), and use mobilised reserve flux as a proxy for the use of dioxygen.

The effects are captured in 4 steps:

- damage-inducing compounds (modified nuclear and mitochondrial DNA) are generated at a rate that is proportional to the mobilisation rate
- damage-inducing compounds induce themselves at a rate that is proportional to the mobilisation rate
- damaged-inducing compounds generate damage compounds ('wrong' proteins) at constant rate, which cumulate in the body
- the hazard rate is proportional to the density of damage compounds

These components involve two new state variables (which can be written as the hazard rate and an acceleration with dimension per squared time) and two parameters: the Weibull ageing acceleration and the Gompertz stress coefficient. If the growth period is short relative to the life span (as far as ageing is concerned), and the Gompertz stress coefficient is small, the famous Weibull model for ageing results, with shape-parameter 3. If the Weibull ageing acceleration is small, however, the (general) Gompertz model results. The literature argues which of these two famous models fit ageing data best. Contrary to these models, the DEB module now also specifies the effects of caloric restriction and how ageing depends on energy parameters. The differences in survival curves for male and female daphnids can now be understood to result from energetics only; these sexes seems to have the same ageing parameters.

A relatively large Gompertz stress coefficient, combined with a small Weibull ageing acceleration, gives a high survival provability till some age, followed be a rapid decline of the surviving fraction. This survival pattern is typical for endotherms (demand systems). The fact that this age typically coincides with puberty suggests a functionality of ageing: organisms use ROS to create genetic diversity in their functional gametes.

Gradual ageing only occurs in multicellulars with irreversible tissue differentiation. If unicellulars are hit by ageing, they stop division; stringent response, as observed in bacteria, might be related to ageing. The literature describes this deviating physiological behaviour as response to low substrate levels. At low substrate levels, interdivision intervals become large, giving ageing time to hit. In chemostat cultures, the fraction of the population that is expected to be affected by ageing switches sharply as function of the specific growth rate.

Sublethal effects of ageing in multicellulars follow the pattern that is described more generally for all chemical compounds in the body, where parameter values are linked to the concentration outside the 'enough'-range, as has been done for the hazard rate. The only special aspect of the hazard rate it that the 'too-little'- and the 'enough'-ranges are assumed to be very small. The section on reserve dynamics discussed that the turnover time of reserve increases with growth, which might directly be associated with loss of metabolic performance that has nothing to do with ageing.

6.2 Toxins and toxicants

ROS are examples of toxic compounds that are created inside the organism. They originate from dioxygen, which is synthesized by organisms, and was doubtlessly very toxic to most species when it started to build up in the atmosphere some 500 Ma ago. This and similar considerations illustrate that effects of chemical compounds are basic to the performance of organisms. In many cases, compounds are taken up from the environment, which gives a need to study their kinetics.

6.3 One-compartment kinetics is the standard

Transport rates of compounds between media (water and fish, for instance) depend on the binding strength between the compound with its medium (independent of the other medium). This idea, called fugacity, has a thermodynamic underpinning and makes the escape rate to the other compartment proportional to the concentration in the compartment, if it is well-mixed. This results in a 1,1-compartment model for the kinetics of the compound. If one compartment is very large and the concentration in it is a given function of time (constant being one of the possibilities), the model for the kinetics of the compound from and to the small compartment (e.g. fish) is called a 1-compartment model. This kinetics implies the existence of the partition coefficient (or concentration factor), defined as the ratio of equilibrium concentrations in the compartments. While the elimination rate has dimension 'per time' and can be obtained from effect data, the uptake rate has dimension 'volume of environment per volume of tissue per time' and cannot be obtained from effect data. Many elaborations of this canonical transport model have been developed, and some are discussed in this chapter.

Many compounds have ionic forms, and the kinetics of the ionic and molecular forms differ. The (varying) pH in the environment controls their relative abundances, while the pH in the body is typically constant. Assuming that both ions and molecules follow one-compartment kinetics, ionisation affects kinetics is a very special way.

Film models represent another extension, by considering non-mixed layers (films) at each side of the interface (body surface) where diffusive transport occurs. Transport through the film is generally supposed to occur in pseudo-steady state, with particular concentration gradients in the films and a concentration jump at the interface. I give a derivation of this jump, because it differs from what I found in the literature; the jump only equals the partition coefficient in absence of (net) transport.

More-compartment models represent yet another extension, where the compartments typically stand for organs in the pharmaco-kinetical literature. We will discuss some other extensions in connection to energetics.

6.4 Energetics affects toxicokinetics

Uptake can be directly from the environment and/or via food; the uptake rate is proportional to the surface area of the individual in all cases, which explains how kinetics depends on body size.

Body growth strongly affects the shape of accumulation-elimination curves, even if it is slow. This should be included by accounting for dilution by growth; DEB theory specifies its rate.

Many vertebrates have lipid-rich reserves, and the lipid content affects the kinetics of lipophyllic (as well as hydrophyllic) compounds substantially. Strong homeostasis implies that fixed fractions of reserve and structure consists of lipid, so DEB theory specifies how lipid content varies in time.

Reproduction can represent an important elimination route for compounds, especially if compounds accumulate in reserve (so also in the reproduction buffer). Again DEB theory specifies the quantitative aspects.

Many compounds are metabolically transformed in the organism at a rate linked to the metabolic activity of the organism. The reserve mobilisation rate is a useful proxy for the activity, and can be used to quantify the transformation. Lipophyllic compounds are typically transformed into more hydrophyllic compounds, with the effect that they eliminate faster (but they are frequently also more toxic).

Elimination is frequently metabolically activated, and the elimination rate is a satiating function of the internal concentration, rather than being proportional to it, just like food intake.

6.5 Toxicants affect energetics

In terms of effects of compounds, DEB theory delineates three (internal) concentration ranges: too little, enough and too much. The definition of the enough-range is that changes of internal concentrations within this range hardly affect metabolism, due to compensation of effects at individual level. Compounds differ in their mode of action, which is defined here as the DEB parameter that is most sensitively affected: the target parameter. DEB theory, therefore, specifies the possible modes of action of compounds. For increasing internal concentrations more and more parameters are effected, but our focus is at low concentrations. Effects are modelled by taking the target parameter proportional to the internal concentration minus the internal no-effect concentration (NEC), but don't allow it to become negative. The inverse proportionality constant is called the tolerance concentration (which increases in value when toxic effects diminish). Because internal concentrations are rarely known, we divide all three concentrations (the internal concentration, the internal NEC and the tolerance concentration) by the bioconcentration factor to convert to environmental concentrations. This amounts to the multiplication of the target parameter by a factor (1 + the stress value). At zero stress the parameter is at its blank value. The motivation is that we are only interested in small changes that can be approximated this way. Small changes in parameter values not necessarily translate in small changes in some endpoint, such as the cumulative amount of offspring over some exposure period.

Sometimes hormesis is observed, especially for reproduction: a stimulation of reproduction at low concentrations and a reduction at high ones. The understanding of this phenomenon is still poor, however. I make the observation that if the only effect of a compound is a small increase of the costs of structure, allocation to reproduction is reduced, as well as the cost per egg (because offspring hit their maturity threshold at birth at a smaller size), such that hormesis results. So hormesis is not necessarily 'beneficial'. The generality of this explanation still has to be explored.

Mutagenic effects can be modelled as an acceleration of ageing, by adding the influx to that of ROS. The hazard rate is the target parameter for lethal effects; this construct is similar to the ageing module. The hazard rate is thus proportional to the internal concentration minus the internal NEC; the proportionality factor is called the killing rate (again after conversion to environmental concentrations). Other parameters are targets for sublethal effects, and the DEB structure specifies the sometimes complex interactions between traits. For instance, if the maximum specific assimilation rate is affected, then reserve synthesis and molibisation are reduced. This reduces growth and assimilation, because food intake is linked to size, which further reduces growth, but also reproduction. If a compound increases maintenance costs, little will be noticed in standard toxicity tests, where test animals are well-fed. Maintenance then only represents a small fraction of the budget. Very unlike the situation of populations at carrying capacity, where maintenance dominates the budget and small affects on maintenance directly affect population size. The population implications of properties of individuals will be discussed in more detail in Chapter 9.

Toxic effects sometimes seem to depend not only on the current internal concentration, but also on the exposure history, including adaptations to the presence of the toxicant (resistance). This can be modelled with receptor kinetics, although the detailed nature of these receptors is typically unknown. Receptors are supposed to be present in functional and disfunctional form; functional receptors become disfunctional at a specific rate that is proportional to the internal concentration, and receptors can recover at a constant specific rate. The stress value now equals the fraction of disfunctional receptors. This extension of the effect module involves a single extra parameter that accounts for the importance of the exposure history.

The effects of mixtures of compounds follow naturally in the DEB framework. If compounds affect the same target parameter, they compete for filling the compensation capacity of the individual (which directly links to the NEC concept) and they can interact in a way that can be captured well following the strategy of the analysis of variance. This latter interaction is typically of minor importance. If the compounds affect different target parameters, however, the DEB structure specifies their interaction. 7

Extensions of DEB models

The uni- and multivariate DEB models should sometimes have more detail, especially if shorter space and time scales need to be included. Some of the possibilities are discussed in this chapter of illustrative purposes.

7.1 Handshaking protocols for SUs

The functionality of SUs can be linked by handshaking protocols, especially if they make physical contact.

I first consider the interaction between a set of carriers, i.e. specialised membrane-bound SUs that take substrates from the environment and pass their products to a set of intracellular SUs, using different protocols. In the open handshaking protocol, the products are released independently of the binding state of the SUs, and in closed handshaking, they are only released if the SUs are in the binding state. The difference in product formation (rejected products by the SU) is substantial, while the binding of substrate is hardly affected.

Next I consider a chain of SUs passing their products to each other in a given sequence; intermediate metabolites are rejected by receiving SU that are in the bounded state; these rejected metabolites escape further processing by the chain. Closed handshaking is here derived such that no intermediate metabolites escape and the whole chain acts as if it was a single super-SU. Mixtures between open and closed handshaking are constructed by a weighted addition of the changes in binding fractions. The performance of the chain is evaluated using a pseudo-steady state argument for the bounded fractions of SUs. Such a situation can be realistic for the TCA cycle, where the enzymes are organised in a metabolon, and the cell needs both the end product and intermediate metabolites in particular relative amounts; see Section 7.6.

7.2 Feeding

The feeding behaviour is for many animals complex in detail. Not all the details are of importance for the broad picture; it is in fact amazing how robust the basic formulation is. Many species collect extra-organismal reserves of food for the bleak season; others defend territories during the mating season to secure access to food when it matters. If organisms meet substrate after starvation they typically show hyperphagia: they eat more than expected on the basis of the Holling type II functional response. Some of this behaviour can be modelled by including a digestive systems explicitly, but excess nutrient uptake by algae, for instance cannot.

Hyperphagia can be modelled with a variant of Morel's model (the Morel model itself is inconsistent with DEB theory). The general idea is to make specific uptake dependent on reserve density, such that this extra uptake drops out when reserve is at pseudo-steady state. This extension involves one extra parameter.

The Holling model forces us to classify all behaviour as either food searching or food handling. This idea can by extended in many ways, for instance in partitioning the handling period in a first part (e.g. mechanical handling) that must be sequential to food searching and a second part (e.g. metabolic handling, including digestion) that can be parallel to food searching. SU-dynamics teaches how to include this single-parameter extension. This idea can be further extended to partitioning food handling in more parts, and play with binding probabilities of arriving prey to the SU to model satiation. Together with an increase of searching efficiency, this an attractive way to model the transition from supply to demand systems.

Social interaction costs time, and thus affects feeding. If the amount of time is taken to be proportional to the intra- or inter-specific meeting frequency using the law of mass action, the effect of feeding can be evaluated on the assumption that searching can only start if social interaction is completed. Most interesting is the case where social interaction also starts during food handling; we need SU-dynamics to evaluate the result. If social interaction can only start during searching, hardly any social interaction occurs if food is abundant. The significance of the result is that this module allows the stable coexistence of many species of competing predators on a single prey species in homogeneous environments. A realistic feature that is otherwise difficult to include in simple models.

Small bodies in water, such as bacteria in free suspension, are typically wrapped by a stagnant water mantle in which diffusive transport occurs that might limit substrate uptake, especially for slowly diffusing substrates with big molecules. Depending on the diffusion rate relative to the thickness of the water mantle, this deviation can modify the Holling type II functional response in that of Blackman. This is a special case of a much wider class of related deviations where uptake induces concentration gradients, and functional surface areas differ from physical ones in ways that depend on environmental factors, such as water turbulence or other transport phenomena.

Bacteria cannot exhibit phagocytosis and need to excrete enzymes in the environment to convert substrates into smaller and more mobile metabolites that can pass through the membrane; only part of these metabolites find their way into the cell membrane. I evaluate the dynamics on the basis of diffusive transport and conclude that a single bacterium has a hard time to get its metabolites, compared to social digestion, and even more compared to phagocytotic uptake.

7.3 Digestion in guts

The digestive system of most animals consists of a stomach that more or less behaves as a well-stirred reactor, and a gut that behaves as a plug-flow reactor. These reactor types have opposite properties in terms of residence times of particles and smoothing capacities of varying influxes for instance, which explains why the combination of these types is popular among animals. I discuss the dynamics, which is left out of the standard DEB model because it is typically fast relative to the reserve dynamics, but should be included if even faster pools must be considered, such as blood sugar dynamics.

I discuss on the basis of a more detailed model for digestion to what extent digestion efficiency can be taken independent of feeding rate, as is done in the standard DEB model.

7.4 Division

Bacteria like $E. \ coli$ can have an interdivision interval as short as 20 min under optimal conditions, while the duplication of their DNA takes three times as long. This can happen because they start duplicating their DNA before earlier rounds are completed. Meanwhile they continue to grow as a rod, which makes the mean cell size dependent on the growth rate. Large cells have a relatively small surface area, which affects how the steady state growth rates depends on substrate density. The details are worked out.

7.5 Cell wall and membrane synthesis

When a cell divides, the sum of the volumes of the daughter cells typically equals that of the mother, but the total amount of membranes and cell wall must be more. This synthesis takes time and, therefore, affects the growth rate in ways that can be expressed in terms of energy costs for this synthesis. Such delays at cell division occur in many taxa.

7.6 Organelle-cytosol interactions and dual functions of compounds

The TCA cycle and the respiratory chain are typically housed in mitochondria in eukaryotic cells. The cells needs a particular mixture of ATP and intermediary metabolites for maintenance, and another mixture for growth. The investment in maintenance relative to growth varies all the time, depending on nutritional conditions. I asked myself the question: how do the enzymes in the mitochrondria match the mix of their products to the needs of the cell. Can this happen without intensive real-time regulation at the level of enzymes? The answer is 'yes' for a proper but constant choice of mix of open and closed handshaking protocols for the enzymes. With the latter I mean that fix proportions of reserve and structure consists of these enzymes, and these proportions much be chosen appropriately. In that case the output of mitochondria to the cell can be matched dynamically to the

varying needs of the cell using the size of the substrate flux to the mitochondria as only information and regulation carrier. A remarkable result, in my opinion.

7.7 Mother-foetus system

In mammals, we see that food uptake is up-regulated during pregnancy (and lactation). Suppose that the surface area of the mother is added to that of the placenta, which is taken proportional to that of the foetus, for the quantification of food intake. What scenario would result if the only extra next modelling step is that allocation of mobilised reserve to the foetus gets top-priority? The result is that the mother builds-up more reserve during this period, because the intake is increased, but the energy conductance is not. Just prior to birth, the allocation to maintenance of the mother might not be completely sufficient. This might explain why mothers typically reduce activity (and so maintenance needs) prior to birth.

7.8 Extra life stages

Holometabolic insects have a pupa stage between the juvenile and adult stages that strongly resembles an embryo stage (no assimilation and hardly any structure, only reserve), while the adult (imago) stage can't grow, but does reproduce. The weight-development of the pupa indeed matches the predictions very well. Reproductive output depends on temperature and food intake, which can be experimentally manipulated. Together with maintenance, conversion of food to offspring determines dioxygen consumption, and so ageing. The resulting survival curves match data on cohorts under different regimes very well, which further supports that ROS controls ageing.

Quite a few species of neonate fish first increase only along the main body axis, then, after metamorphosis, also along other directions. Moreover embryonic development seems slow relative to that after metamorphosis. Assuming that the embryo and postmetamorphosis stages behave as isomorphs, but that the first juvenile stage behaves as a V1-morph, this pattern can be captured accurately with a single new parameter: the maturity level at metamorphosis. The acceleration of development after birth is implied because the energy conductance increases with length during the V1-morphic stage.

7.9 Changing parameter values

Parameter values are usually constant, by definition, but environmental and internal factors might make them vary in time: temperature affects rates; toxicants and ageing affect target parameters; parasites and diurnal cycles affect κ ; changes in shapes affect parameters that depend on surface area; metabolic needs affect diet choices; pregnancy affects maximum food intake; prolonged starvation can induce a variety of changes. The specific food uptake rate and κ can change at puberty. Organisms can specialise on particular substrates; micro-organisms do this by controlling the substrate-specific carrier abundance in their outer membranes. This process can be captured by letting the gene-expression for the various carriers depend on the relative workload of these carriers, in a way that strongly resembles the dynamic generalisation of the κ -rule, as discussed in Chapter 5. By letting the expression of one carrier inhibit the expression of other carriers, we can capture the phenomena of diauxic growth and metabolic learning. This can be done in parameter-sparse, but realistic, ways as demonstrated. 8

Covariation of parameter values

Previous chapters were about the structure of metabolic organisation and about chemical transport; this chapter is about parameter values of individuals that follow their life cycles and of compounds that enter and leave organisms. A powerful property of the standard DEB model and of the one-compartment model is that their structure allows us to predict the covariation of parameter values across individuals and compounds without using any empirical argument or new assumption. For the standard DEB model, this is due to three conditions:

- 1 thanks to their clear links with chemical and physical phenomena, the parameters can be classified into two classes: intensive parameters that only depend on the very local physico-chemical suborganismal conditions and design parameters that depend on the size of the individual.
- 2 simple functions of design parameters (typically ratios) are intensive.
- 3 maximum length is a function parameters, of which only one is a design parameter.

Arguments 1 and 2 show that the standard DEB model can be called a mechanistic model, argument 3 shows that we don't need any extra assumption to arrive at theory for the co-variation of parameter values. If we would allow the allocation fraction κ to depend on the amount of structure, for instance, it would become a design parameter, argument 3 no longer applies and we need an additional assumption to determine the co-variation of parameters. For the one-compartment model, the partition coefficient depends on two design parameters, but the fugacity argument offers us skew symmetry, which removes one degree of freedom. The standard DEB and the one-compartment model share a common feature: the equilibrium state (ultimate length of individuals and partition coefficient of compounds) is a ratio of two rates (assimilation and maintenance for organisms and uptake and elimination for compounds). This feature might be key to the possibility to derive rules for co-variation of parameter values.

The covariation of parameter values just concerns a tendency that is based on physicochemical principles. Species-specific deviations from the mean pattern reflect speciesspecific adaptations. The better we can characterise this mean pattern, the more we can appreciate the deviations from it and recognise what properties make a particular species special. I first discuss body size scaling relationships, then quantitative structure-activity relationships and finally the interaction between these relationships. Although these relationships consider variables of interest as function of body size and partition coefficients, respectively, both body size and partition coefficients result from underlying processes. It is the covariation of parameters of the underlying processes that matter.

8.1 Intra-specific parameter variations

Since DEB theory is biologically implicit, all differences between organisms are captured in parameter values. To allow for evolutionary change, parameter values must be individualspecific. Parameter values are partly under genetic control. Adaptations concern changes in parameter values as implications of this approach; I illustrate this with the example of geographical size variations as adaptations to food availability at the growing season. Towards the poles of the Earth, seasonality becomes more important, where the bleak season thins populations, and so reduces competition during the breeding season. Predictable food levels can to some extend be fixed in parameter values within one species in ways that are more clearly demonstrated inter-specifically. The main difference between intraand inter-specific parameter variations is the amount of variation.

Notice that structure, reserve and the reproduction buffers are state variables, not parameters, and they vary during the life of an individual, even if its parameter remain constant. Although we can compare an old (large) individual of a small bodied species with a young (small) individual of a bigger bodied species, the result can be complex. Even if they have the same size (i.e. length or weight), their metabolism will differ. For simplicity's sake we confine inter-species comparisons to fully-grown individuals. Reeding rate increases with squared length intra-specifically, but with cubed length inter-specifically. Reproduction rate increases with size intra-specifically, but decreases with size inter-specifically.

8.2 Inter-specific parameter variations

Implied by the theory, particular parameters tend to co-vary across species, because of plain physics: the *basic co-variation rules*. The primary parameters, as listed in Table 8.1 can be classified in two categories: parameters that depend on the size of the individual and parameters that do not. The latter parameters depend on the local (chemical) conditions, and are referred to as intensive parameters. Only three parameters in this list depend on size: the surface area-specific assimilation rate, and the maturity levels at birth and puberty. If ageing is included, we should also add the Weibull ageing acceleration (not the Gompertz stress coefficient). In the expression for maximum length we can see that the specific assimilation rate must be proportional to maximum length, because the allocation fraction to soma and the specific somatic maintenance costs are intensive parameters. Maturity density is intensive, which is most easy to see when the maturity and somatic maintenance coefficients are equal and the maturity density remains constant during growth, so it must be independent of size. Maturity thresholds must, therefore, be proportional to cubed maximum length. The application of these simple relationships is in writing a physiological quantity of interest, such as body weight, or respiration rate, as a function of the primary parameters. We now know how each of the primary parameters depend on maximum length, so we know how this function depends on maximum length. To facilitate comparisons I introduce a zoom factor, which has value 1 for an individual of maximum structural length of 1 cm with 'mean' values of each of its parameters.

I give many examples, which all give realistic predictions, and now mention the respiration rate only, because the literature is full of unsuccessful attempts to explain why respiration scales approximately with weight to the power 3/4, an empirical observation known as Kleibers' law. Unlike the literature, DEB theory makes a sharp distinction for how we compare, intra- or inter-specifically. The fact that both comparisons work out similarly for respiration, from a numerical point of view, has probably been the reason of the omission to make the distinction and let some workers assume wrongly that respiration drives metabolism. Having contributions from quite a few underlying processes, respiration itself cannot have a central explanatory position.

Intra-specifically, when we follow a growing individual at constant food, body weight is proportional to the amount of structure (weak homeostasis), but the allocation to growth declines with size. The overhead costs of growth contribute to respiration. Reproduction contributes little to respiration directly because it hardly involves a chemical transformation (reserve of the mother becomes reserve of the offspring). Inter-specifically, when we compare fully grown adults and growth plays no role, respiration is dominated by maintenance. If specific assimilation increases, reserve density increases as well as maximum length. Maintenance is only paid for structure, and maintenance dominates respiration in fully grown individuals, so weight-specific respiration decreases for increasing maximum weight. If, on the other hand, specific somatic maintenance increases, reserve density is unaffected, but maximum length is reduced. The implication is again that weight-specific respiration decreases for increasing maximum weight. Both intra- and inter-specifically, respiration in a DEB context scales somewhere between a surface area and a volume, depending on parameter values. This explains the variation between taxa, which is a hot item in the discussion in the literature on this topic.

Apart from co-variation of parameters on the basis of plain physics, a number of patterns exist because of evolutionary and ecological adaptation of parameters. Species with larval stages frequently have small eggs which develop slowly, but at hatch they *accelerate metabolism* by changing morphology: the part of surface area that is involved in assimilation is proportional to structural volume during some time till metamorphosis, which might or might coincide with a rather sudden change in shape, after which they remain isomorphic again. The effect is that both specific assimilation and energy conductance increase with length during the acceleration period. This might help dispersal in areas of low food density; resource needs are little if metabolism is slow.

The concept supply-stress is very important and is defined as the maturity maintenance times the squared somatic one, divided by the cubed assimilation. It does not depend on size and can take values between 0 and $2^2/3^3$. It quantifies where species are in the *supply-demand spectrum*, where, among animals, insects are near the supply-end and birds and mammals near the demand-end of the spectrum. Supply-species are metabolically very flexible and eat what is available. Demand-species have a programmed growth and reproduction and eat according to their needs and evolved advanced forms of parental care, probably to learn offspring these complex forms of food acquisition. Many many properties of species link to the position in the supply-demand spectrum, such as the ratio between peak and standard metabolism and the possession of a closed circulatory system and the mode of development of eggs.

The *altricial-precocial* spectrum ranks species that are born from early to late in their development. It is typically applied to birds, which evolved from precocial to altricial, and mammals, which evolved from altricial to prococial. The reason for this striking difference is probably the invention of expensive, poorly-soluble, hardly-toxic, slurry-forming nitrogenwaste compounds, rather than the cheap, but toxic, ammonia used by aquatic species, when the dinosaurs evolved from amphibians as adaptation to fully terrestrial life. This allowed them to lay large eggs, in which the nitrogen waste accumulates, which hatch into prococial neonates. It also facilitated flight in dinosaur subgroups, pterosaurs and birds, to take air, since they need much less water to drain the nitrogen waste, so less weight to carry. Birds evolved the altricial condition as adaptation to life in trees. Evolved in parallel to the dinosaurs, mammals, on the contrary, used urea, which is only mildy less toxic than ammonia, and forms crystals in high concentration that might damage soft tissues. During 90% of their existence in geo-time they also laid eggs, but these eggs had to remain small, so the neonates were altricial. Only after the invention of foetal development, some 30 Ma ago, they could evolve precocial neonates, probably to reduce on parental care. The altricial condition in birds and mammals is linked to extra parental care, because of their demandcondition. The ratio of maturity at birth and puberty quantifies the altricial-precocial spectrum for all species.

The specific somatic maintenance turned out to have a very large ranges of values in animals. Comparison of species with low and high values revealed the ecological explanation for this: the *waste-to-hurry phenomenon*. Species increase their specific somatic maintenance, together with specific assimilation, to boost growth and reproduction. The κ -rule implies that they remain small this way, and can profit from temporarily abundant resources via offspring, such as seasonal algal blooms, or rain in arid environments, or fire. They need to combine this strategy with torpor stages or migration to survive periods when resources are poor.

Large reserve capacities smooth out fluctuations in food intake rates much more effectively than small ones, as reflected in the turnover rate. This determines a typical temporal scale of living, but scales in space and time only matter if space and time are heterogeneous. They always are in reality, but frequently not in simplistic models. By 'choosing' for a reserve density proportional to maximum structural length, big-bodied species not only solve the problem of preserving the integrity of their reserve relative to their structure, but also increase the time they can survive starvation in proportion to maximum structural length as an aspect of the smoothing capacity of a large reserve. The (walking, swimming, flying) speed and the diameter of the home range also increase with maximum body length, which combines nicely with the feeding rate scaling with cubed length inter-specifically (with squared length intra-specifically). When there is no food, the combination of speed and starvation time scaling with length nicely combines with the fraction of the home range that is searched for food being independent of maximum body length. This demonstrates the natural coupling of scales in space and time, and of behavioural, physiological and even molecular traits.

DEB theory links assimilation to surface area and somatic maintenance to structural volume, but it has no assumptions for how surface area links to volume. The standard DEB model assumes isomorphy, with the implication that surface area is proportional to volume to the power 2/3. Quite a few taxa, especially those with larval stages, take surface area, i.e. the part that is involved in assimilation, proportional to volume during some period after birth. During this period, called *metabolic acceleration*, specific assimilation and energy conductance increase with (structural) length. Before and after this period, these two parameters remain constant (if temperature remains constant). Since energy conductance controls reserve mobilization, species can combine a slow embryonic development, with a much faster post-acceleration development. This might assist dispersal for species with planktontic stages.

8.3 Quantitative structure-activity relationships

Transport of a compound between two media in the one-compartment model rests on a fugacity argument; the partition coefficient being the ratio between uptake and elimination. The role of the media can formally be interchanged, so a skew-symmetry argument applies. This is the summary of a more detailed derivation that I present, which leads to the conclusion that the uptake rate must be proportional to the cubic root of the partition coefficient and the elimination rate inversely proportional to this root.

Because diffusion in the films of film models concerns transport within one medium only, while transport between the media is the one-compartment model again, the previous argument applies with the result that at low partition coefficients the elimination rate does not depend on the partition coefficient, while the uptake rate is proportional to it, and at high partition coefficients the elimination rate is inversely proportional to the partition coefficient, while the uptake is independent of it.

Like in the case of body size scaling, the application of these relationships is writing a quantity of interest as a function of the parameters, and we know how this function depends on the partition coefficient. An example of application is the bioconcentration factor, i.e. the ratio of the concentration in the organism and in the environment, where we now decompose the organism in reserve and structure with different lipid contents, as function of (maximum) body weight. Apart from the argument that big-bodied species are frequently at the top of food chains, we should expect an increase of the bioconcentration factor for lipophyllic compounds because the density of lipid-rich reserve increases with (maximum) body weight.

Even more interesting is how effects depend on the partition coefficients on the assumption that effects fully depend on transport, i.e. the toxicity of a single molecule does not depend on the partition coefficient. The ecotoxicological literature typically characterises toxicity for compounds on a weight per volume basis, strange enough, and lipophyllicity, and so the partition coefficient, typically increases with molecular weights; theory development is weak in ecotoxicity. One of three effect parameters, the elimination rate, was already considered. The NEC and the tolerance concentration turn out to be inversely proportional to the partition coefficient, while the killing rate in fact corresponds with an inverse tolerance concentration for lethal effects. The literature frequently works with the LC50 for a standardised exposure time, i.e. the concentration at which the survival probability is half that in the blank. This can also be written as a function of DEB parameters, and so it follows how it depends on the partition coefficient and the maximum weight of organisms. All these predictions were tested against data and found to be very realistic.

8.4 Interactions between QSARs and body size scaling relationships

For each of the many quantities that were considered in the body size scaling parameters, it is now possible to evaluate how effects on these quantities depend, not only as functions of the zoom factor, but also as functions of the partition coefficient. This makes predictions possible in rather complex situations, which pertain to interactions between species. 9

Living together

9.1 Trophic interactions

Trophic interactions are typically rather complex and more difficult to classify then is generally assumed. Competition typically works out to destabilise coexistence, while syntrophy, and especially reciprocal syntrophy, stabilises. Syntrophy did not get the central role in evolution that it deserves. I demonstrate that syntrophic interactions can result in weak homeostasis and point to this result as a possible mechanism for its origin. The role of predators and preys in the huge literature on their dynamics is, from a DEB perspective, more complex then generally recognised. A predator not only eats prey, but by its selection for the weak, and therefore typically non-reproducing, individuals also stimulates production by reducing competition experienced by the productive form the non-productive individuals. Predators also protect the productive ones for infection by pathogens, which also prefer weak individuals. Once pathogens manage to settle in weak individuals, infection of the strong ones becomes much more likely. On top of this, there is a stimulation of prey-production via nutrient recycling, which directly or indirectly boosts food production for the prev. A proper understanding of the trophic relationships between organisms cannot be achieved without a full analysis of nutrient recycling in an ecosystem setting.

9.2 Population dynamics

The step from individuals to populations in simple homogeneous (i.e. artificial) environments is, in the first place, a purely mathematical one, once the rules of how individuals interact with their local environment are known (as e.g. specified in DEB theory) and the (trophic) interactions between individuals specified. It amounts to advanced bookkeeping, which is substantially easier for unstructured, compared to structured populations where individuals can differ in one of more traits. This is why V1-morphs play an important conceptual role and can make the link between DEB-structured population dynamics and popular existing models. The sequence of models Lotka-Volterra, Monod, Marr-Pirt, Droop and DEB shows a decreasing tendency to oscillate, and a decrease of the ratio of the biomass and substrate concentrations at equilibrium.

I show how the popular logistic growth models can be understood in a DEB context in (at least) two very different situations in a batch culture; the half saturation coefficient can be small, but the reserve capacity large and vice versa. The first limit produces logistic growth mathematically, the second one looks very different, but behaves numerically very similar.

I also show that the behaviour of unstructured population dynamics is dominated by an unrealistic feature: neonates that directly produce new neonates. I make this observation to demonstrate that unrealistic features can be removed, but this comes with a cost in terms of a step-up in the level of model complexity. The characteristic equation plays an important conceptual role, by linking the steady-state specific population growth rate to the age-dependent survival and reproduction.

The dynamics of structure populations can be set-up with the McKendrick-von Foerster equations. DEB-structured populations have a rather strong tendency of self-synchronisation of individuals in the populations, where the new generation out-competes the old one. Variation between individuals reduces the problem. Stochastic formulations suffer much less from this, can generally show a richer and smoother behaviour compared to deterministic ones; the feeding behaviour is intrinsically stochastic.

9.3 Food chains and webs

Using a well-known data set that frequently has been used to demonstrate density-dependent feeding, I show that DEB theory captures the dynamics very well without using density-dependent feeding, just simple DEB rules. The key observation is that the cycling predator grows fast when prey is at minimum. The literature sees this as a demonstration that the amount prey per predator matters, while the DEB interpretation is that the prey and predator cycles run out of phase because of the dynamics of the reserve. Contrary to the analyses in the literature, this application of DEB theory also includes the nutrient of the prey, and the dynamics of the full system over the whole experimental period. The illustrative value of this example is that we need theory-based models to understand population (and ecosystem) data.

Food chains and webs can easily have very complex (asymptotic) behaviour, and some 'details' can have important effects. Maintaining biodiversity in simple food web models has always been a challenge; the inclusion of DEB rules in food webs generally contributes to its stability.

Adaptive dynamics aims to understand changes of individual properties while the individual is interacting with its environment at an evolutionary time scale. The calls for a holistic approach. When various traits of individuals are coupled, and models for the behaviour of individuals are realistic, theoretical prediction becomes really complex, but computer simulation studies can still help. We demonstrated that a single-species ecosystem of mixotrophs can hardly specialise in auto- and heterotrophs in homogeneous space, but if space has a light gradient, for instance, this specialisation is very easy, even in coexistence with the mixotrophic ancestors.

9.4 Canonical community

The simplest non-degenerated community should have three biotic groups: producers, consumers and decomposers. Together they determine nutrient recycling, which is key to understand the behaviour of ecosystems. In the simplest (theoretical) formulation each can consist of a single species (of V1-morphs); more advanced formulations can replace this by sets of competing populations with different properties and replace the consumer population by food webs etc. This approach allows a cost(complexity)-benefit(insight) analysis, where the effectiveness of particular 'details' can be evaluated.

The step to more realistic ecosystem models should introduce spatial structure and transport phenomena in the first place, followed by (geo)chemical aspects. The book gives little attention to these topics, not because they would be less important, but because the book is about DEB theory, not ecosystem modelling. It is still an open question to what extent general theory for realistic ecosystem dynamics can be developed.

10

Evolution

Variation of inheritable properties and selection are still the key processes of evolution, as Darwin pointed out. Initially evolutionary change was slow and mainly based on mutations, creating metabolic diversity, especially among prokaryotes. While this process is still continuing, DNA reshuffling, and syntrophic interactions speeded up evolutionary change by orders of magnitude. Organisational change characterises eukaryotic evolution. I here consider the evolution of metabolic organisation at the various levels in the context of DEB theory.

10.1 Before the first cells

The very beginning of life is still speculative, but useful suggestions start to accumulate in the literature on its chemical nature. Pertinent to DEB theory in these ideas is the development of individuals as organisational units, and the role of membranes (surface area-volume relations) in this. Plausible scenarios place these aspects at a very early stage indeed.

10.2 Early substrates and taxa

A scenario for the evolution of the 4 modules of central metabolism lets the original modules all run in the opposite direction as they typically do at present, and has a crucial syntrophic step: the mergeing of the inverse TCA cycle plus inverse glycolysis of chemotrophic archeal origin with the inverse pentose phosphate cycle plus inverse respiratory chain of phototrophic eubacterial origin. The archeal modules reversed direction at merging, which must have had syntrophic preparation steps to become successful. That the modules can run in opposite directions is not hypothetical, because some contemporary taxa actually do this, but the evolutionary scenario is. My primary purpose of this scenario is to point to the importance of reciprocal syntrophy as a force that shaped evolution, fuelled by the excretion fluxes that are implies by multiple reserve DEB systems (see Chapter 5).

Although archea sport some phototrophy, using machinery that differs substantially from that of the masters of the art: eubacteria. The pinnacle of phototrophy is the oxygenic one sported by cyanobacteria, which boosted the energy supply to biota substantially and allowed for the development of eukaryotes. The key for success was the use of water as electron acceptor (which generates dioxygen) in combination with the inverse application of photon-capture machinery in the respiratory chain to oxidise the reduced co-enzymes what are formed in the TCA cycle.

After a long and slow process of mutational changes that created primary diversity, metabolic modules began to recombine progressively, as already illustrated in the evolution of central metabolism, which boosted the rate of evolutionary change by orders of magnitude, further emphasising the role of syntrophy, even in times before the eukaryotes.

10.3 Evolution of individual as dynamic system

Increasing control over the chemical composition of the individual's structure induces stoichiometric constraints on growth. Since the concentrations of the various complementary substrates fluctuates wildly in the local environment of ancestors of eukaryotes, a prokaryotic cell, it needs to store substrates in reserves to smooth out these fluctuations. The evolution of strong homeostasis might well have been via weak homeostasis; by delineating more and more pools, strong homeostasis becomes less constraining, and if the pools consist of a single chemical compound, the concept strong homeostasis evaporates completely. Weak homeostasis easily evolves in sub-systems that exhibit reciprocal syntrophy using SU-rules (Section 9.1) and in isomorphs it can be induced by minimising variance of composition through control of SU-abundance (Section 2.3), so through regulation of gene expression.

When uptake became more efficient by using proteins that require turnover, the need to increase the reserve capacity increased; the continuous need of resources otherwise combines poorly with a temporary absence of substrates. While homeostasis creates the need for reserves, maintenance enhances it.

Reserves could originally be built up by delaying the processing of internalised substrates, but the need to increase reserve capacity came with the need to temporarily store them in a form that does not create osmotic problems if otherwise they start to interfere with metabolism. To ensure continuity of the fuelling of maintenance, the payment of maintenance cost internalised from fluctuating external substrates to much more constant mobilised reserve.

Size control, i.e. the resetting of cell size by division and the control of surface areavolume ratios, boosted population growth, but came with the need to install a maturity program. These steps were already taken before the eukaryotes evolved.

The origin of eukaryotes is possibly a unique event where an eubacterium entered an archea, that did not have phagocytosis (like all prokaryotes). This important step in evolution is again based on syntrophic interactions; the next section details some metabolic aspects of this integration of once independent systems. The earliest eukaryote was a heterotroph, which explains why pure autotrophy is rare, or even absent, in eukaryotes (including plants). Phagocytosis facilitated modular recombination substantially, including the incorporation of cyanobacteria as plastids, or of individuals that already encapsu-

lated them, or of individuals that encapsulated individuals with plastids. Many symbioses evolved on the basis of reciprocal syntrophy, ranging from loose to tight forms of integration.

After the invention of phagocytosis by eukaryotes, feeding on other living creatures became popular in one line of development, which coupled the uptake of the various complementary substrates and induced a covariation of reserve densities. This encouraged the animal line of development, where homeostatic needs finally reduced the number of independent reserves to one, and the juvenile stage evolved an embryo and adult stage simultaneously with the invention of reproduction. The pattern came with the evolution of mobility, sensors and a neuronal system to allow for fast information exchange between otherwise rather isolated cells.

Another line of development did not start to feed on living creatures and kept their reserves independent, but evolved an increased capacity to cope with changes in the local environment: the plant line of development. They partitioned their structure in a root and a shoot and invented the use of products (wood) to adapt their shape during growth. They became masters of the art of torpor to escape bleak periods, and evolved a much more open (but slow) mass communication between cells. They invented the embryo/adult stages independently.

10.4 Merging of individuals in steps

Knowing that the merging of individuals is more of a rule than an exception, and realising that DEB theory is biologically implicit, I asked myself the question: is it possible to merge two individuals of different species, using only smooth changes of parameter values, such that each of the species as well as the merged system follow the same rules? All biologically implicit models should pass this test but this property constrains the structure of biologically implicit models very much. This is when I discovered the partitionability and mergeability concepts and realised that they should apply to all biologically implicit models. The answer is 'yes' for the standard DEB model and a scenario is as follows.

Two independently living species (the future host and symbiont) first develop reciprocal syntrophy on the basis of excreted reserves that are implied by multiple reserve systems. Initially the products of the partner are substitutable to their original ones, but stepwise they become complementary. This is possible in a smooth way because in both cases the change in binding fractions of the feeding SUs are weighted sums of the binding fractions. After a stage of spatial clustering, the epibiontic sub-population of symbionts outcompetes the free-living one, and the internalised sub-population outcompetes the epibiontic one. These steps reduce losses of products in the environment. Then the structures of host and symbiont start to merge, via weak homeostasis, to strong homeostasis. By coupling assimilation pathways, finally the reserves merge, again via weak to strong homeostasis. Via specialisation on substrates the merged system can partition again, or the symbiont is anhilated, which gives endosymbiosis a cyclic character.

10.5 Multicellularity and body size

Although multicellularity evolved already a few times among prokaryotes, it became much more popular among eukaryotes. When the number of participating cells increased and specialisation of cell tasks got shape, the need develops to install organisation levels between that of the cell and the individual to keep regulation simple and manageable. Organs and tissues developed with specific tasks. Mass exchange between cells in prokaryotes is via gap junctions; the animal line of development kept this limited system, basically, and evolved circulatory and neuronal systems for rapid transport all over the body. The plant line of development opened their cell (walls and) membranes (pores) and evolved a vascular system for slow transport all over the body. Quite a few groups, including the fungi, completely removed the membrane boundaries between cells.

Multicellularity also induced the evolution of the embryo/adult stages, to allow gradual building-up of the infra-structure of the (multicellular) offspring and its metabolic organisation. This contributed to the importance of maturation (metabolic learning). By increasing their size, a whole suit of characters changed in concert (see Chapter 8), including the increase of home range, life span, and starvation times, combined with a reduction of reproduction rates. This opened up new niches for big-bodied species.

Specially animals developed an active life style by specialising on feeding on other creatures, and developing sensors, brains to process this information and use it for feeding and social behaviour. These and other developments further increased maintenance costs and so the use of dioxygen. This comes with the need to protect themselves against ageing; the protection of the neuronal system against ageing occurs during the sleep, which reduces time available for feeding in ways that depend on body size. In some animals feeding behaviour developed to advanced levels, and growth and reproduction evolved from a supply to a demand oriented organisation, which couples size to age (see Section 7.2).

10.6 Control over local conditions

Homeostasis not only occurs inside the body, but organisms also evolved capabilities to control the local physical-chemical environment, by shading, leaf littering and evaporation stimulation by trees in forests, for instance, of by controlling the pH in peats by mosses, or by binding carbon dioxide from the atmosphere and bring it down to the deep ocean by algae, or by soil development and changing soil properties by adding persistent organic compounds.

I hypothesised that the great reef development during the Silurean and Carbonian is directly linked to the terrestrial invasion by plants via enhancing nutrient input to the ocean system. The formation of Pangea changed this, and the ocean system started to flourish again after its breaking up and the resumption of nutrient input into the ocean system.

I mention this to point to the importance of the planetary level of metabolic organisation, which we are only beginning to understand.

10.7 Control over global conditions

Existing biogeochemical climate models are still weak in including biotic activity. I briefly discuss how biota affect water, carbon dioxide, methane, dioxygen and albedo at a plane-tary scale, which should be taken into account by such models.

The essential point is that for climate models we need to understand the carbon cycle, but it depends on the water and nutrient cycles. Biota play an important but complex role in these cycles, which are linked via stoichiometric constraints.

10.8 Effects of climate on life

Better known than how biota affect climate, is how climate affects biota, mainly via temperature and water housekeeping. Both can work out in rather complex ways.

11 Evaluation

The purpose of this chapter is to place DEB theory in the context of research on energetics. I start to review experimental research that is consistent with DEB theory, followed by theoretical work that follows deviating lines of thought.

11.1 Empirical models that are special cases of DEB theory

A natural first question is: What are the criteria for a general explanatory model of this type? Although it is hard to be exhaustive, Table 11.1 is an attempt to present these criteria explicitly.

Models implied by DEB theory meet all 6 criteria for being general and explanatory.

- ad 1 The theory consists of a list of coherent and consistent assumptions, as summarised in Table 2.4 for the standard DEB model. Practical applications require the derivation of specific mathematical models from these assumptions. Originally I thought that these assumptions could easily be replaced by others in the process of testing the implications against experimental data. Later it turned out difficult, if not impossible, to replace any of them without creating inconsistencies. This points to the possible existence of a smaller set of deeper assumptions, from which these assumptions follow. Many parts of the theory were originally more complex. As is typical in science, simplicity does not come naturally, but must be acquired with hard work.
- ad 2 DEB theory has an explanation for each of the empirical stylised facts, Table 11.2. Table 11.1 gives an overview of the many empirical models that turn out to be special cases of DEB models, or very good numerical approximations; the list continued to grow over the years. Many of them are quite old and together they concern very different aspects of life; none of the original authors could be aware of the coherence of these empirical models. This in itself is for me already a most rewarding sideresult of DEB theory. DEB theory reveals how they all follow from simple physical and chemical phenomena; this helps to understand under what conditions these models will probably not work that well. Each of these models was created because it

Table 11.1: Criteria for general explanatory models for the energetics of individuals

- 1 The models must be based on explicit assumptions that are consistent with physics and (geo)chemistry
- 2 The assumptions should be consistent in terms of logic, but also with empirical patterns; see Table 11.2.
- **3** The taxa to which the model applies should be delineated by explicit criteria.
- 4 Different models for the various taxa should be consistent with an explicit evolutionary scenario.
- **5** The assumptions should cover the full life cycle of the individual, from initiation of development to death, and quantify all possible uses of substrates (to allow mass and energy balancing).
- 6 The predictions should be testable in practice, which typically constraints its maximum complexity substantially (quantified in terms of number of variables and parameters).

described experimental data well. Using all this evidence, and the results of some 200 man-year of research by the group working on DEB theory, I dare to state that, at present, DEB theory is the best tested quantitative theory in biology.

- ad 3 DEB theory deals with all organisms, i.e. micro-organisms, animals and plants. It is not only biologically but also chemically implicit; species and compounds only receive names in applications DEB theory meets the objective restriction criterium by including all taxa. The standard DEB model, which deals with isomorphs with one reserve and one structure feeding on one type of food, is supposed to apply to animals, i.e. organisms that feed on other organisms; micro-algae need several reserves, plants also need two structures (roots and shoots).
- ad 4 An explicit evolutionary scenario has been worked out for the models of DEB theory. The applicability to all species restricts the possible structure of DEB theory substantially, because we know that most organisms evolved from the merging of ancestors. Think for instance of mitochondria and chloroplasts that once had an independent existence, and of the many symbioses (e.g. corals) that exist. The constraint that two taxa follow some set of energetic rules, and the merged taxon again follows the same set of rules restricts how this set of rules can potentially look like; see the discussion on partitionability and mergebility of reserve dynamics.
- ad 5 DEB theory specifies the fluxes of all chemical compounds, using conservation laws for chemical elements (and their isotopes). It also exploits the conservation of energy and time and uses the state variable maturity to trigger qualitative changes in metabolism, and reserve to explain why embryos can grow (i.e. increase structure) without feeding.
- ad 6 The core theory deals with the logic of quantitative aspects of metabolic organisation; the set-up has not been constrained by the necessity to test against experimental data. It turned out that quantities that play key roles in DEB theory (maturity,

Table 11.2: Stylised and empirical facts, modified from Sousa et al 2008.

Feeding

- **F1** Many species (almost all animals and plants) have an embryo stage that does not feed
- F2 During starvation, organisms are able to reproduce, grow and survive for some time
- F3 At abundant food, the feeding rate is at some maximum, independent of food density

Growth

- **G1** Growth of isomorphic organisms at abundant food is well described by the von Bertalanffy; at constant food density, no substantial shrinking occurs independent of ageing
- G2 The inverse von Bertalanffy growth rate increases linearly with ultimate length both intraspecifically (or different constant food levels) and inter-specifically
- G3 Foetuses increase in weight approximately proportional to cubed time

Reproduction

- **R1** Many species (almost all animals and plants) have a juvenile stage that does not reproduce
- **R2** Reproduction increases with size intra-specifically, but decreases with size inter-specifically
- R3 A range of constant low food levels exists at which an individual can survive, but not reproduce
- $\mathbf{R4}$ Growth can be simultaneous with reproduction, but growth can also cease long before reproduction is initiated
- R5 Allocation to reproduction can continue during starvation

Respiration

- O1 Animal eggs and plant seeds initially hardly use dioxygen
- O2 The use of dioxygen increases with decreasing mass in embryos and increases with mass in juveniles and adults
- ${\bf O3}\,$ The use of dioxygen by isomorphs scales approximately with body weight raised to a power close to $0.75\,$
- O4 Animals show a transient increase in metabolic rate after ingesting food (heat increment of feeding)

Stoichiometry

- **S1** The chemical composition of organisms depends on the nutritional status (starved vs well-fed)
- S2 The chemical composition of organisms at constant food density becomes constant during growth

Energy

- E1 Some energy always dissipates, also in absence of dioxygen
- E2 Dissipating heat for heterotrophic isomorphs under aerobic conditions is a weighted sum of three mass flows: carbon dioxide, dioxygen and nitrogenous waste

Ageing

- A1 Mean life span typically increases inter-specifically with maximum body length in endotherms, but hardly depends on body length in ectotherms
- A2 Survivor curves for life span terminated by ageing are typically well described by the Weibull and Gompertz models

reserve(s), structure(s)) cannot be measured directly, only indirectly. This calls for elaborate auxiliary theory to relate DEB quantities to quantities that can be measured (lengths, weights, composition, performance in various situations). This auxiliary theory relates sets of different types of measurements to sets of several DEB quantities.

The list of empirical models of Table 11.1 that turn out to be special cases of DEB theory (of very good numerical approximations to it) shows that DEB theory provides a new theoretical umbrella of the large amount of research on energetics. The new element is that we now understand these models, and why and how they are interrelated. The support for these empirical models is also support for DEB theory.

11.2 A weird world at small scales

Many attempts are going on to understand the behaviour of cells in terms of that of specific metabolites. These attempts rest on complex applications of enzyme kinetics, typically with little attention of spatial structure, including membrane-cytosol interactions, allocation, active transport, movement of organelles and cytoplasm. Apart of the fundamental reason that complex models (in terms of number of state variables and parameters) cannot expected to contribute to insight, I review reasons for why I think these attempts are bound to fail and diffusive transport, on which enzyme kinetics is based, does not occur in cells.

11.3 Static Energy Budgets

The current standard in animal energetics, static energy budgets SEBs, focuses on short time scales, where the size of the individual is treated as given. It is basically a bookkeeping approach for food intake where costs are subtracted from income to evaluate the scope for growth. By refraining from the dynamic aspect, this bookkeeping scheme has difficulties with the interpretation of respiration (with its many contributions, including that from overheads of production processes), and therefore with quantifying overhead costs; what is allocated to growth is more than what is fixed in new tissue.

11.4 Net production models

Net production models are based on time-dependent SEBs, frequently supplemented with ideas on how reproduction competes with growth for allocation of substrates using optimisation arguments. They typically have many more parameters, compared to the standard DEB model, and they have difficulties with the inclusion of the embryonic stage, because they don't give reserve the position to smooth out fluctuations in assimilation; most don't consider reserve at all. They can't include changes in body composition or generate appropriate body size scaling relationships and aren't consistent with the rule of indirect calorimetry. They inherit the problems of the interpretation of respiration from SEBs, and typically try to include Kleibers law directly by working with allometric functions for food intake and maintenance. This typically comes with a lot of dimensional problems and hampers the understanding of the underlying processes in terms of physical and chemical principles.


Empirical cycle



From: Basic methods in theoretical biology

This document gives a summary of concepts of the book on Dynamic Energy Budget (DEB) theory.

It follows the sections of the book and aims to explain why the material is discussed in the way it is, avoiding technical discussions, mathematical formulations, derivations, tests against realism and references to the literature.