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Quantitative aspects of metabolic organization: a discussion of concepts

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Metabolic organization of individual organisms follows simple quantitative rules that can be understood from basic physical chemical principles. Dynamic energy budget (DEB) theory identifies these rules, which quantify how individuals acquire and use energy and nutrients. The theory provides constraints on the metabolic organization of subcellular processes. Together with rules for interaction between individuals, it also provides a basis to understand population and ecosystem dynamics. The theory, therefore, links various levels of biological organization. It applies to all species of organisms and offers explanations for body-size scaling relationships of natural history parameters that are otherwise difficult to understand. A considerable number of popular empirical models turn out to be special cases of the DEB model, or very close numerical approximations. Strong and weak homeostasis and the partitionability of reserve kinetics are cornerstones of the theory and essential for understanding the evolution of metabolic organization.

Keywords: dynamic energy mass budgets; homeostasis; reserves; body-size scaling relationships; evolution

1. INTRODUCTION

Growth curves of organisms as different as amoebas, yeasts, molluscs and birds in constant environments can resemble each other in detail, while we know that their hormonal regulation systems differ substantially. This and other observations suggest that regulation systems are part of the machinery used by organisms to meet the constraints set by general rules for changes in energy and mass budgets. Dynamic energy budget (DEB) theory represents a promising attempt to identify the set of rules quantifying the uptake and use of substrates that all organisms (micro-organisms, plants and animals) seem to have in common. It captures the impressive biodiversity by differences in parameter values. To this end, individuals are considered as dynamic systems that follow predictable patterns during their life cycle. Although many additions are possible to make the theory more detailed, and perhaps more realistic, little room seems to exist to simplify the theory without sacrificing realism.

The basic DEB model for the individual comprises a set of two differential equations that specify the change in amounts of reserve and structure from the assumptions listed in table 1. Inclusion of the ageing process requires a third equation for cumulated damage. Equations for food consumption, faeces and/or nitrogen-waste production can be included for ecological applications. These specifications follow directly from the DEB theory. Given the initial values for all variables, these equations describe how they will vary in time.

The gist of the DEB theory is that it gives a sound theoretical background for a considerable number of popular empirical models for particular details and aspects of processes of substrate uptake and use. The logical link between these models has not yet been revealed because they deal with different biological specializations that have developed almost independently of each other, as will be discussed in § 9. This coherence has been the selection criterion for the material included in this paper. Since the topic is central to biology, a lot of literature has been published about the various aspects, but most of that work is taxon-specific and has very limited scope from a theoretical perspective, with few implications for the relationship between the different levels of biological organization.

This paper discusses the conceptual basis of DEB theory, avoiding mathematical formulations. As the theory is a quantitative one, the discussion has to be sketchy in places. A more rigorous exposition involves the mathematical formulations given by Kooijman (2000). This paper starts by describing the aim of DEB theory in the context of scales in space and time, followed by a discussion of why the behaviour of individuals is hard to deduce from molecular biology. Synthesizing units are then discussed, which will be used as modules in the theory. Two sections on the processes of assimilation and catabolism prepare for the discussion on dynamic budgets. These two processes largely determine the physiological performance of an individual. Sections on body-size scaling relationships and evolutionary aspects discuss the context of the theory and some important implications. The paper concludes with a discussion of consequences for populations and an indication of future challenges.

Technical terms explained in Appendix A are asterisked when they are used for the first time.

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Table 1. *Assumptions that fully specify the basic DEB model as formulated for reproducing multicellular organisms and modified for dividing unicellular organisms*

general: for the specification of mass–energy relationships

1 structural body mass and reserves are the state variables* of the individual; they have a constant composition (strong homeostasis)

2 food is converted into faeces, and assimilates derived from food are added to reserves. Reserves fuel all other metabolic processes, which can be classified into three categories: synthesis of structural body mass, synthesis of gametes, and processes that are not associated with synthesis of biomass. Products that leave the organism may be formed in direct association with these three categories of processes, and with the assimilation process

3 if the individual propagates via reproduction (rather than via division), it starts in the embryonic stage, and initially has a negligibly small structural mass (but a substantial amount of reserves)

specific: for the specification of the powers

3a the reserve density of the hatchling equals that of the mother at egg formation. Foetuses develop in the same way as embryos in eggs, but at a rate unrestricted by energy reserves

4 the transition from embryo to juvenile initiates feeding; that from juvenile to adult initiates reproduction, which is coupled to the cessation of maturation. The transitions occur when the cumulated energy invested in maturation exceeds certain threshold values. Unicellular organisms divide when the cumulated energy invested in maturation exceeds a threshold value

5 somatic and maturity maintenance are proportional to structural body volume, but maturity maintenance does not increase after a given cumulated investment in maturation. Heating costs for endotherms* are proportional to surface area

6 the feeding rate is proportional to the surface area of the organism; the food handling time and the digestion efficiency are independent of food density

7 the reserves must be partitionable, such that the dynamics are not affected; the reserve density *at steady state* does not depend on structural body mass (weak homeostasis)

8 a fixed fraction of energy, used from the reserves, is spent on somatic maintenance plus growth, the rest on maturity maintenance plus maturation or reproduction (the κ-rule)

9 under starvation conditions, individuals always give priority to somatic maintenance and follow one of two possible strategies: they do not change the reserve dynamics (so continue to invest in development or reproduction), or cease energy investment in development and reproduction (thus changing reserves dynamics)

supplementary: for the specification of ageing

1 oxygen causes net DNA damage with a certain efficiency

2 damaged DNA produces ‘wrong’ proteins at constant rate, which accumulate in the body

3 the hazard rate* is proportional to the density of ‘wrong’ proteins

2. SPACE–TIME SCALES

While moving up in space–time scales, new processes become dominant role, while others fade out. Each process has its own range of operation in the space–time domain. Many processes that are important in small space–time scales hardly contribute directly in large ones, but they may contribute indirectly. This is not typical for biology, but applies generally. Knowledge about technical details of engines in automobiles is extremely valuable for optimizing design and reducing air pollution, but is of little help in minimizing traffic jams. Similar relationships hold between molecular biology and ecology; these specializations focus on different space–time scales and deal with different processes that partially overlap.

It is our task as scientists to find out how the process of fading in and out works, while moving up and down scales, and how to avoid the situation in which a large number of variables and parameters* contributes to the processes under study. Complex models contribute little to our understanding and are therefore less useful. This implies that accessible scales in space and time are linked and, for example, that a large spatial scale combines poorly with a small time-scale. Simplifying models, while preserving essential features, is a difficult task requiring careful scientific and numerical judgement. An aim of the DEB theory is to keep the postulates as simple as possible, with a strong focus on major patterns.

A quantitative theory reduces to the form of a model for a particular situation. It differs from a model by being more general, leading to other models for other situations. It can do so because it is based on a set of assumptions from which the model can be derived in combination with situation-specific assumptions. The set of assumptions contains the scientific insight, which (hopefully) evolves. The DEB theory aims at such an axiomatic approach for individuals, to penetrate the lower and higher organization levels in a consistent way.

3. A WEIRD WORLD AT SMALL SCALES

The standard approach to the problem of understanding complex systems is to start from the low organizational level, collect all possible information and use it to explain processes at higher organizational levels. Although spectacular progress has been recorded in the

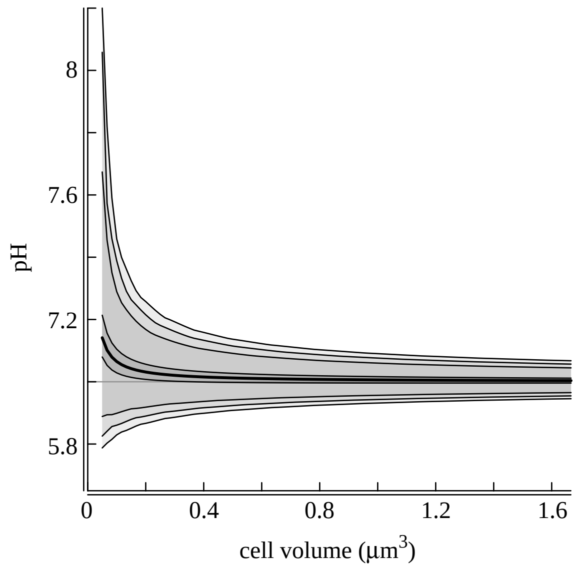

Figure 1. The 95%, 90%, 80% and 60% confidence intervals of pH in cells of pure water at pH 7 as a function of the cell size. They increase dramatically for decreasing cell sizes for cells (or cell compartments) less than 0.5 µm^3. The thick curve represents the mean pH, which goes up sharply for very small cell sizes.

last few decades on qualitative aspects of molecular biology, little is known about quantitative ones, which are substantially more difficult to tackle. Chemical kinetics and enzyme kinetics are developed for (large) reactors, not for small cells. The kinetics are based on the assumptions of homogeneous mixing, large numbers of participating molecules and a constant reactor volume. None of these requirements applies to the cells' interior. Basic to chemical kinetics is the law of mass action*: transformation rates are proportional to meeting frequencies, which are taken to be proportional to the product of concentrations of substrate. This depends on transport by diffusion or convection. A few observations might help to reveal that the application of classic chemical kinetics in cellular metabolism is problematic. This even holds for the concept 'concentration' of a compound inside cells.

Consider, for example, a typical bacterial cell of volume 0.25 µm^3 and an internal pH of 7 (the intracellular compartments of eukaryotic cells are about the same size). It must have 15 free protons, but random dissociation of water and random association of protons and hydroxyl ions make this number fluctuate wildly (McQuarrie 1967). Figure 1 shows that the (asymptotic) frequency distribution of the number of protons, and so of pH, dramatically increases in variance for decreasing cell volumes below 0.5 µm^3. We have to think in terms of pH *distributions* rather than pH *values*. Many chemical properties of compounds depend on the pH, which makes matters really complex.

A water molecule is created by association of a proton and a hydroxyl ion, and is annihilated by dissociation about twice a day at 25 °C. Brownian motion transports a water molecule *ca.* 3 cm between creation and annihilation, while protons and hydroxyl ions are transported some 3 µm, on average. However, these distances do not fit into a cell (or cell compartment), which must lead to the conclusion that undisturbed diffusion does not occur in cells. These expectations are based on pure water, but a more realistic cytoplasm composition does not eliminate the problem.

Water in very small volumes behaves as a liquid crystal (Brown & Wolken 1979; Ball 1999) rather than as a liquid, and this has substantial consequences for kinetics. Electrical potentials reveal the crystalline properties. They decay exponentially as a function of distance L, so they are proportional to $\exp\{-L/L_D\}$. The Debye distance, L_D, is *ca.* 0.1 µm for water at 25 °C (Weiss 1996), which means that the electrical potential of a proton would be felt through most of the cell, even if it did not move.

Intracellular transport of compounds is not by diffusion, but is more active, involving transport proteins with address labels; the cytomatrix might play a role here (Agutter *et al.* 1995). The transformation processes in the time-scale of the division interval are further complicated by dilution resulting from growth*. Many biochemists try to study the rate of these processes by using a reactor at steady state. Between their observations at the level of the population and the object of study at the molecular level, is the level of the individual (cells) which follows a cell cycle and therefore a cyclic pattern.

Classical enzyme kinetics deals with homogeneous mixtures of enzymes and substrates, but many transformations are mediated by membrane-bound enzymes. If a transformation requires several enzymes, they frequently pass precursors via direct transfer (metabolic channelling; Edwards 1996; Ovadi 1991), rather than via the cytoplasm. A cell can regulate the rate of transformation by binding enzymes from the cytoplasm to membranes. Regulation by enzyme production cannot operate quickly because this is a relatively slow process; however, binding to membranes can be fast. Because substrates are acquired from cytoplasm (volume), and amounts of active enzymes are proportional to that of membranes (surface area), the cell has local information about its size, since the ratio of volume to surface area yields a length measure. Changes in this ratio during the cell cycle affect transformation rates and introduce a morphological argument: shape matters, so do changes in shape during growth.

Bioenergetics studies the processes of ATP* generation and use, because cells use ATP to drive energy requiring transformations. ATP turnover is considered to organize metabolism. The energy charge, i.e. the ratio [ATP]/([AMP] + [ADP] + [ATP]), changes; therefore the free energy* of ATP alters. This complicates the understanding of slow transformations in terms of generation and use of ATP. Slow transformations are controlled by polymers (proteins, lipids and carbohydrates) which, however, do not suffer from fluctuating free energies. They are spatially organized in granules and attacked from the periphery. Their amount, or exposed surface area, would quantify their metabolic significance much better than their concentration (see figure 2). The strong focus on ATP is not justified for slow processes. A typical life span of an ATP molecule is 0.3 s, and the quantity of ATP molecules in a cell can drive synthesis for just 2 s. These time-scales are too short to understand processes that are relevant at the time-scale of the division interval. ATP plays the role of purse money, while bank account dynamics is required to understand the changes in cell economics during the cell cycle.

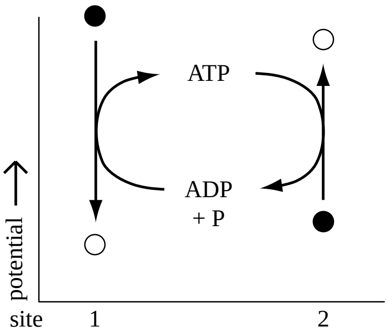

Figure 2. The ATP/ADP shuttle transports energy from a site where an energy producing transformation occurs to a site where an energy requiring transformation occurs. If both transformations occur at the same place and time, and thermodynamics allows, the shuttle is not required. Variations in the free energy of an ATP molecule affect the speed of the shuttle, but not necessarily the transformation rates.

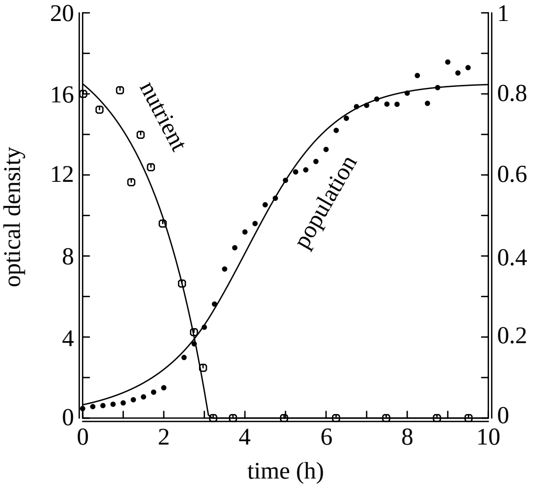

Figure 3. Potassium limited growth of *Escherichia coli* at 30 °C in a batch culture, measured as optical density at 540 nm. Data from Mulder (1988). In the absence of nutrient in the medium the population continues to grow using intracellular reserves. For a small saturation coefficient and small maintenance costs, the DEB theory predicts exponential growth during substrate presence, and logistic* growth during substrate absence. The latter model is a very popular empirical model in population dynamics.

4. INDIVIDUALS

The considerations of § 3 lead to the conclusion that a direct approach to quantify cellular kinetics is demanding. A promising alternative or, rather, a complementary approach is to infer transformation kinetics from the input–output relationships of the integrated dynamic system. These relationships are valuable constraints for modelling intracellular kinetics. This is the approach taken by the DEB theory.

The natural integrated system in which to study metabolic transformations is the individual. Energy and mass balances are relatively easy to measure for individuals. These balances are of great help for the quantitative understanding of the individual as a dynamic system, and constrain the coupling of energy generating and requiring transformations. The individual is also the unit of selection in evolutionary theory, which is essential for understanding their similarities and differences. Individuals come in a great diversity of physiological performances and body sizes (see figure 4). Many individuals are unicellular, which makes the link with cellular metabolism rather direct.

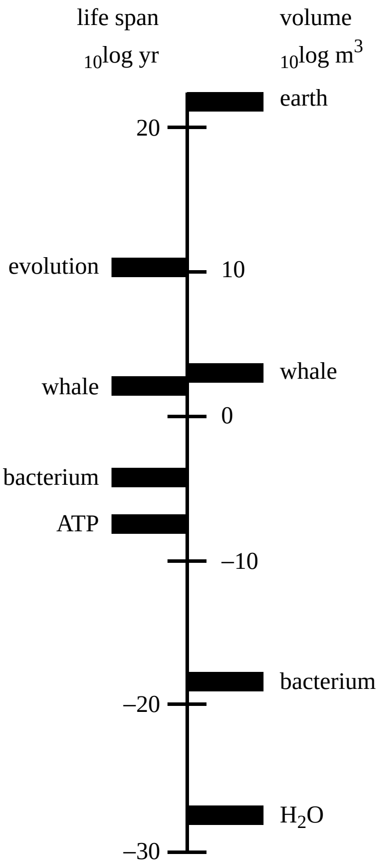

Figure 4. Lifetimes and volumes of individuals span a wide range. The ratio between the volumes of a whale and a bacterium is much larger than that of a bacterium and a water molecule; it is even larger than that of the whole Earth and a whale. The life span of evolution refers to the existence of life on Earth, i.e. 2.8×10^9 yr; that of ATP refers to the mean life span of a molecule in a living cell, i.e. 0.3 s.

DEB theory delineates reserves as separate from structure. Reserves are not meant to be compounds that are set apart for later use, but a pool of compounds synthesized from environmental substrates for metabolism for the purposes of maintenance* (including protein turnover, maintenance of concentration gradients across membranes, activity and other types of work), growth (increase of structural mass), development and reproduction. This creates a rather constant internal chemical environment, with only an indirect coupling with the extra-organismal environment. Reserves as well as structure are taken to be generalized compounds*, i.e. mixtures of a large number of compounds, which do not change in composition. The latter requirement is called the strong homeostasis* assumption. Polymers (carbohydrates, proteins, lipids and sometimes ribosomal RNA*) form the main bulk of reserves and of structure.

Aiming at the simplest realistic characterization of metabolism, one might wonder if reserves are strictly necessary. Most existing models do not deal with reserves, but five compelling reasons exist to include reserves in even the simplest characterizations.

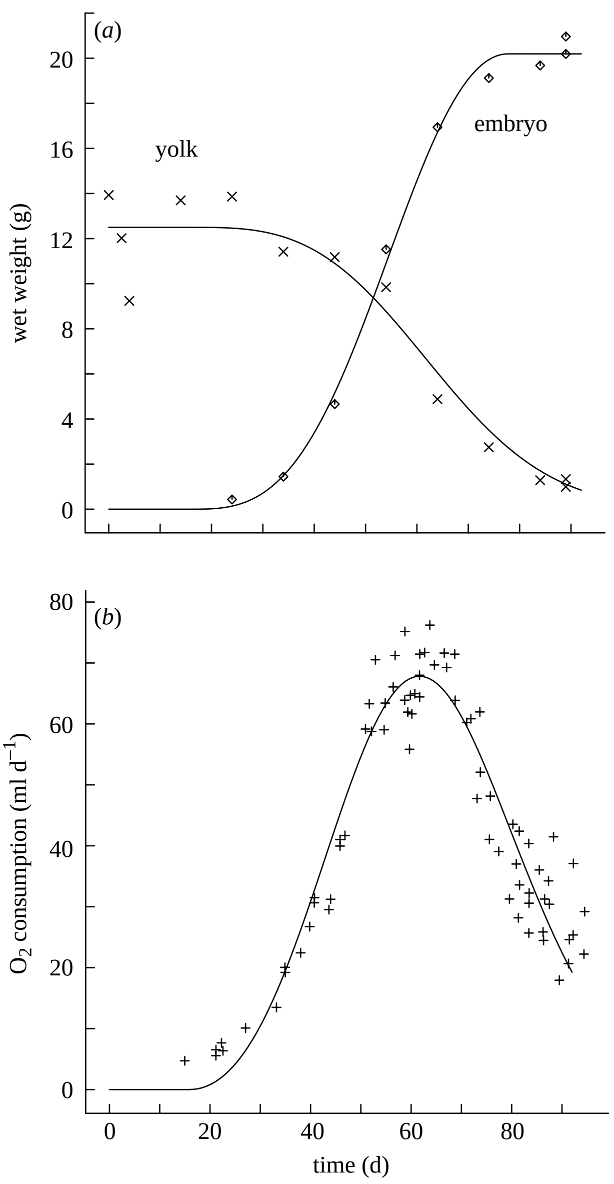

Figure 5. The embryonic development of the New Guinea soft-shelled turtle *Carettochelys insculpta* illustrates that development occurs at the expense of reserves that do not respire; development initiates at abundant reserves and hardly any structure, while respiration is negligibly small. Data from Webb *et al.* (1986). The curves are DEB-based expectations, fitted by C. Zonneveld. The respiration rate decreases because of depletion of reserves. These turtles tend to wait for their brothers and sisters to hatch synchronously.

(i) They account for metabolic memory, which is important during transient states (shifts up and down in substrate availability). Production (growth or reproduction) reacts slowly to changes in feeding condition (see figures 3 and 15); embryo development is fuelled by reserves (see figure 5).

(ii) They explain observed respiration* patterns, which have a close link with the use of energy. Freshly laid eggs hardly respire; the developing embryo respires at an increasing rate, while the total egg-mass decreases (see figure 5). The DEB theory explains this (and other observations) by assuming that structure requires maintenance while reserves do not. Part of maintenance relates to protein turnover of the structure; turnover is already implied in reserves as a result of assimilation and catabolism.

(iii) The composition of biomass depends on growth. This cannot be explained with a single generalized compound. With two components (reserves and structure) particular changes in composition can be captured. More complex changes require several reserves, as is required for autotrophs.

(iv) All mass fluxes turn out to be linear combinations of assimilation*, dissipation and growth. If reserves are omitted, these three processes are mutually dependent and actually provide two degrees of freedom, rather than three. This does not provide enough flexibility to capture product formation.

(v) They allow body-size scaling of life-history parameters. The specific respiration rate decreases with (maximum) body size between species because large-bodied species have relatively more reserves. Many other life-history parameters directly or indirectly relate to respiration.

Although the introduction of reserves is unavoidable, they do complicate the empirical application of the theory. All size measures of the individual (volume, wet or dry weight or total carbon) are combinations of reserves and structure, which complicates the measurement of these components separately. The composition of reserves and of structure can be obtained indirectly only. Reserve density*, i.e. the ratio of the amounts of reserves and structure, increases with the specific growth rate, because the use of reserves increases with the reserves' density and part of the used reserves is allocated to growth. Observations of how the concentration of a particular compound in biomass depends on the growth rate can be used to obtain the (constant) concentration of that compound in the reserves and in the structure. If several reserves are present, this only holds for the growth-limiting reserve; the multivariate extensions of the DEB theory imply that non-limiting reserves can (and usually do) decrease with the growth rate, because they can dam up due to growth limitations set by the limiting reserve.

Reserve compounds can have an active role in metabolism. Figure 6 shows that ribosomal RNA (rRNA) density increases, which means that part of it belongs to the reserves. This has far reaching implications for turnover of ribosomes and elongation rates of peptides, for instance. The latter turns out to be realistic, because the curves in figure 6 are DEB-based expectations. Ribosomes that are part of structure or of reserves will probably have the same turnover rate. The turnover rate of reserve-rRNA is set by the reserve dynamics; part of the (somatic) maintenance requirements are used for the turnover of structure-rRNA. As ribosomes are part of the machinery involved in synthesis, and synthesis rate has to increase with reserve density, it makes sense for the cell to treat part of its ribosomes as reserves. It can extract energy and building blocks from ribosomes during a shift-down in substrate availability. This illustrates the close link between macro-economics and molecular processes.

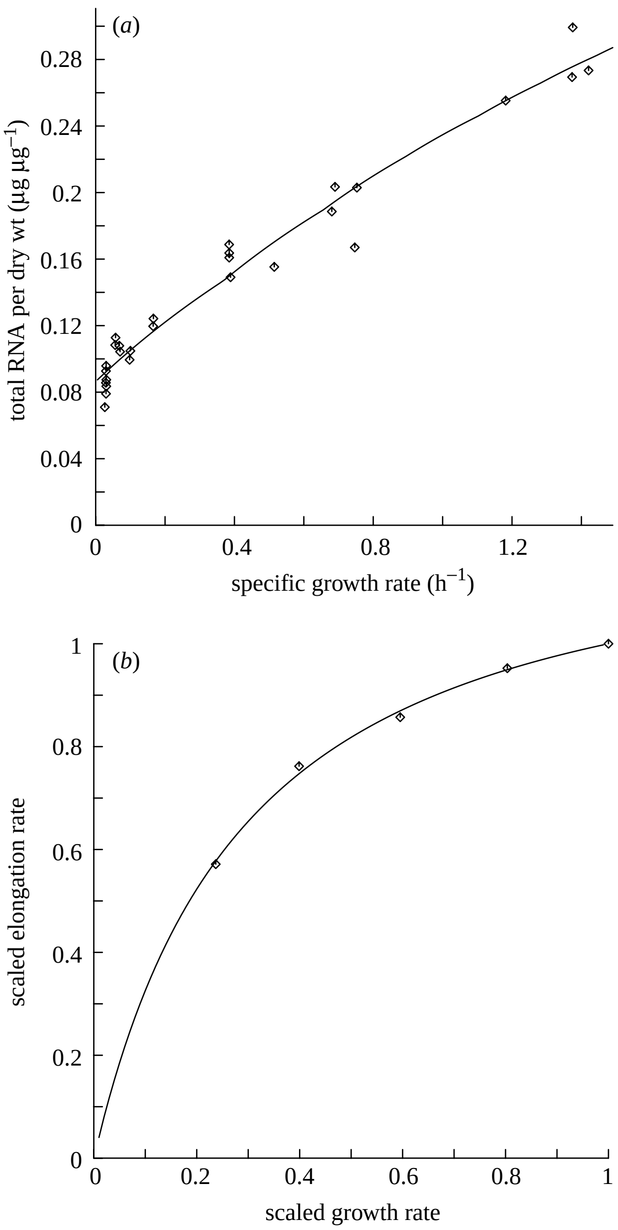

Figure 6. (*a*) Ribosomal RNA density as a function of the specific growth rate in *Escherichia coli*. Data from Koch (1970); the curve is based on a constant density in reserves together with structural mass. (*b*) The elongation rate as a function of the scaled growth rate. Data from Bremer & Dennis (1987). Both elongation rate and growth rate are expressed as fractions of their maximum value of 1.73 h^{-1} with an elongation rate of 21 amino acids per second per ribosome; the curve is based on the ratio of the growth rate to the amount of reserves.

5. FLUX-BASED KINETICS OF SYNTHESIZING UNITS

The problems of application of classical chemical and enzyme kinetics to cellular metabolism, as discussed, lead to the question of finding better alternatives. The construct 'synthesizing unit' (SU)[*] is a promising attempt in this direction. It can be conceived as a (generalized) enzyme that behaves according to the association–dissociation rules prescribed by classical enzyme kinetics, with two modifications: the kinetics is not specified in terms of substrate concentrations, but in terms of arrival fluxes of substrate molecules to the enzyme, and the enzyme–substrate complex does not dissociate (enzymes are only released after transformation of substrate into product, see figure 7). These two modifications have little consequence in simple transformations of a single substrate molecule into a single product molecule in homogeneous environments. Michaelis–Menten kinetics, which takes production rate to be a hyperbolic function of substrate concentration, still belongs to the behavioural repertoire of SUs. The significance of the modifications is revealed in more complex situations where the local environment is not well mixed or the transformations are more complex. If arrival is controlled by diffusion (or convection) in a well-mixed environment, the arrival rate is proportional to the concentration of substrate. Arrival can also be the result of an intracellular allocation process linked to metabolic transformations. Arriving fluxes imply the existence of rejected fluxes that require a destination, which places allocation in a central position in metabolic organization; allocation is basically a partitioning of fluxes to specific destinations.

Liebig's minimum rule has considerable empirical support; it links the production fluxes to the limiting substrate only. The numerical behaviour of an SU closely follows this rule, except for a narrow window of concentrations, where several substrates limit the transformation simultaneously (see figure 8). The transition from one limiting substrate to another at changing substrate concentrations in the environment is smooth, however, which is rather essential in combination with reserves; because of the presence of reserves substrates can be absent from the environment while not (yet) limiting growth. This implies complex rules for limitation in varying environments, which can be avoided with SUs because of the absence of switches.

Another very useful property of SUs is that the stoichiometry of the transformations does not need to be known exactly; an increase in stoichiometric requirement almost cancels out an increase in binding efficiency, which allows the description of transformations of generalized compounds, including overheads that are associated with them.

SUs can be linked into a metabolic network after specification of the handshaking protocols that specify the rules for exchange of metabolites between the SUs (metabolic channelling). The open and closed protocols represent extremes, while mixtures are possible. In an open handshaking protocol, the behaviour of the sending SU is independent of the state of the receiving one, while in a closed protocol, the sending SU only releases its product if the receiving one is free for acceptation. (This is only possible if the SUs are bound on a membrane adjacent to each other.) The type of protocol turns out to hardly affect the production of the end (overall) product, but the production of precursors (intermediate products) is very sensitive to the type of handshaking protocol, as is the amount of substrate accepted by the network. Many biochemical modules, such as the tricarboxylic acid cycle, serve the dual role of energy extraction from a resource and the generation of building blocks (as intermediate products); handshaking protocols balance these functions to the cell's needs.

The application of SU kinetics in the DEB theory is in the assimilation process, which transforms substrates in

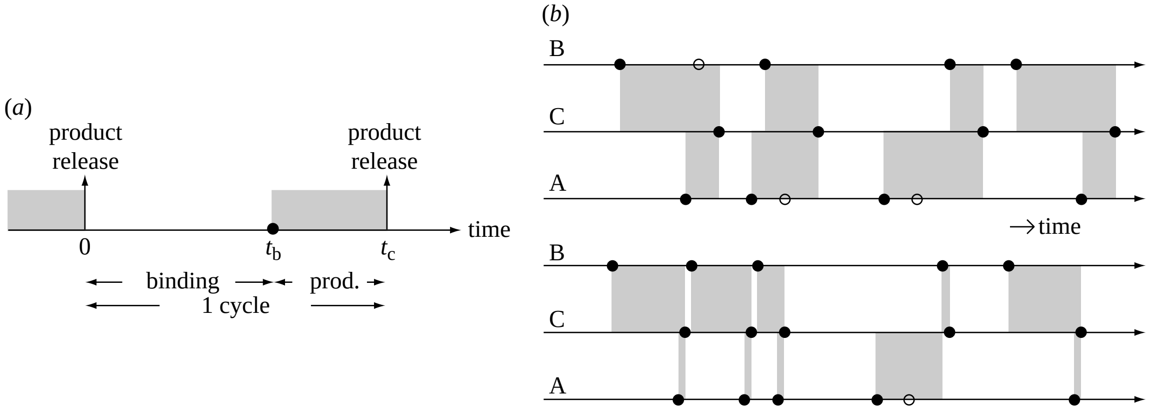

Figure 7. (*a*) This illustrates that the SU goes through a cycle consisting of a binding phase and a production phase. The production rate is inverse to the cycle time. (*b*) The transformation $A + B \rightarrow C$, where substrates A and B are bound at different sites on a SU. The SU switches to the binding phase upon the release of product C. The upper diagram represents a slow SU, the lower diagram a fast one: both experience the same substrate arrival processes. The time axis labelled C shows the production events; the other time axes show arrival events of substrates. Filled dots stand for acceptance, open ones for rejection. The grey areas indicate periods during which the SU cannot bind substrate. Note that the fast SU still has substantial blocked periods due to the stochasticity of the (Poisson) arrival processes.

the environment into reserves, and in the growth process which transforms reserves into structural mass. Each reserve and each structure has a single type of SU; thus the basic DEB model has two types of SU. The flux of substrate that is rejected by the growth-SU is returned to the reserves or excreted into the environment with a fixed fraction. If all rejected substrate were returned, no upper boundary for that reserve would exist. The return fraction quantifies the damming up of non-limiting reserves, with the remarkable consequence that the growth rate increases with limiting reserve density, but decreases with the non-limiting ones. This process has found technical application in, for example, biological phosphate removal. Excretion can be treated implicitly in single-reserve single-structure systems by inclusion into the overheads of assimilation, maintenance and growth; it must be treated explicitly, however, in multivariate cases, because excretion of one reserve depends on the dynamics of the others.

Thinking in terms of fluxes of substrate allows one to treat photons in a similar manner to nutrients*, with fixed stoichiometric requirements. Only photons with wavelengths within a given window are metabolically useful; the extracted energy from a photon is independent of its wavelength and the excess energy dissipates as heat.

6. ASSIMILATION AND BODY SHAPE

The assimilation process is defined as the transformation of substrates in the environment into reserves inside the organism. This involves a transportation process that is linked to a surface area (membranes). The basic DEB formulation takes the relevant surface area proportional to that of the individual, which scales with volume$^{2/3}$ for organisms that do not change in shape during growth. Such organisms are called isomorphs*.

Changes in shape during growth affect assimilation. If surface area is proportional to (structural) volume (a defining property of V1-morphs*), a substantial simplification is possible because maintenance costs are also proportional to volume. Filamentous algae and fungi with a constant diameter, or sheet-like bacterial and algal colonies with a constant small thickness, are examples of V1-morphs. If individuals divide into two parts at a certain volume, V1-morphy is a good numerical approximation for a wide range of changes in shape, because the detailed morphology of the growth curve is less important for such small size ranges. V0-morphs represent another interesting special case, where the surface area that is involved in substrate uptake does not increase during growth. This occurs in special taxa, such as diatoms and dinoflagellates, in biofilms, and small aquatic individuals having a relatively thick stagnant water mantle which limits transportation in the environment. Many structures, such as crusts of lichens, and microbial flocs represent dynamic mixtures between V1- and V0-morphs. This notion helps us to understand that their diameter increases linearly at constant substrate density, whereas the length of an isomorph satiates to an asymptotic value and that of a V1-morph grows exponentially; the ratio of assimilation to maintenance is crucial here.

The functional response* (i.e. the feeding rate as a function of the substrate density) is a hyperbolic function of substrate density in situations of a well-mixed environment; figure 9 illustrates the underlying mechanism. If the mixing rate in the environment is low and an individual depletes its local environment of substrate (food), the feeding rate becomes limited by diffusion (or convection) of substrate through the environment. This affects the shape of the functional response, which shifts from a hyperbolic function to a bilinear one (from Holling type II to Holling type I).

Figure 10 illustrates the various modes of interaction of substrates in the uptake process. An increase in one substrate density leads to a decrease in the uptake rate of other substitutable substrates, but to an increase in other complementary ones. Cats feeding on mice and birds represent an example of sequential uptake of substitutable substrates; bacteria feeding on glucose and fructose represent parallel uptake of substitutable substrates, because they use carriers that can handle either glucose

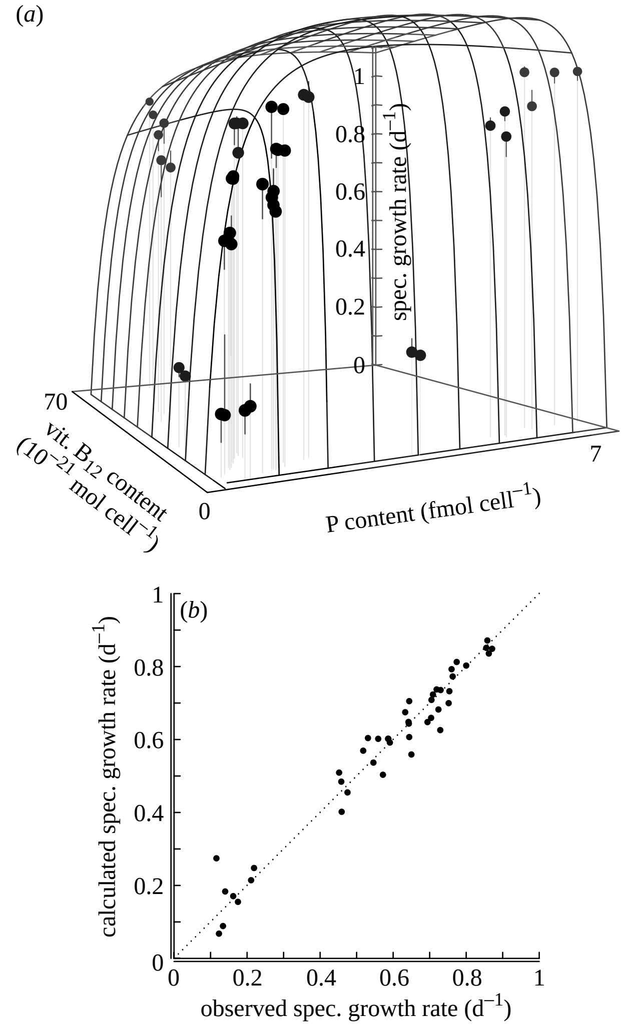

Figure 8. Specific growth rate of the haptophyte *Pavlova lutheri* as a function of intracellular reserves of phosphorus and vitamin B_{12} at 20 °C. Data from Droop (1974). The surface is based on the kinetics of a fast SU for two complementary substrates, where the arrival rates are proportional to the reserve densities (as follows from the reserve kinetics in the absence of maintenance costs). Because the reserve turnover rates are equal, and the phosphorus and vitamin B_{12} concentrations in the structure are known, the surface has three parameters. (*a*) The difference between the data points and the surface is marked with line-segments. (*b*) Calculated values are plotted against observed ones to judge the goodness of fit. The absence of data points for high values of both reserves illustrates the damming up of one reserve due to growth limitation by the other.

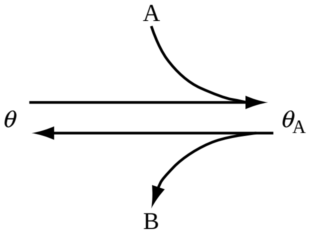

Figure 9. Uptake of a single substrate is well quantified on the basis of a fixed handling time of substrate (prey) by the uptake machinery. The time need not be constant, but it must be independent of substrate density (Metz & Batenburg, 1985*a*,*b*). The handling time not only includes mechanical handling but also metabolic processing. This is why eating prey by predators and transformation rate by enzymes depend in a similar way on substrate (food) density.

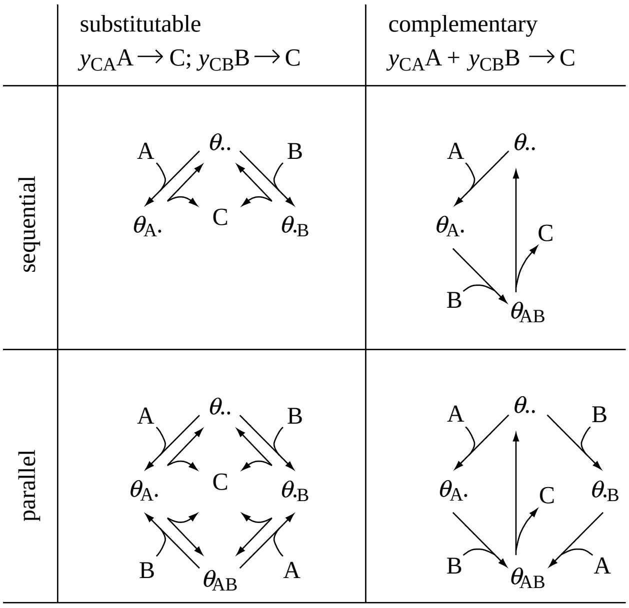

Figure 10. Interaction of substrates A and B in transformations into product C can be understood on the basis of a classification of substrates into substitutable and complementary, and of binding into sequential or parallel. The symbol θ_{*1*2} represents a free SU. The symbol y_{*1*2} denotes a stoichiometric coupling coefficient. The schemes can be generalized to more complex transformations to include co-metabolism and intermediate cases between substitutability and complementarity, without involving new theoretical problems.

or fructose, but not both (while little interaction seems to occur during their processing to reserves).

Photosynthesis, the uptake of photons and carbon dioxide to form carbohydrates, and the use of carbohydrates and nitrogen compounds to form (generalized) reserves represent a nested parallel uptake of complementary processes. The rejected electrons get lost via the Mehler reaction, while the rejected carbohydrates and nitrogen compounds are stored in two specialized reserves. The existence of 'excess' electrons has puzzled plant physiologists; the mechanism of SUs implies their existence, however. Oxygen competes with carbon dioxide for access to ribulose bisphosphate carboxylase, known as photorespiration. This can be included in a simple way into the assimilation SU, while the net carbohydrate production is proportional to the difference in bound carbon dioxide and oxygen.

7. CATABOLISM

The reserve dynamics represents the core of the DEB model because the use of reserves, called the catabolic* rate fuels metabolism. Their specification follows from two requirements: weak homeostasis and partitionability of kinetics. The weak homeostasis assumption states that biomass (i.e. the combination of reserves and structure) does not change in composition during growth at constant substrate density; the partitionability requirement is illustrated in figure 11.

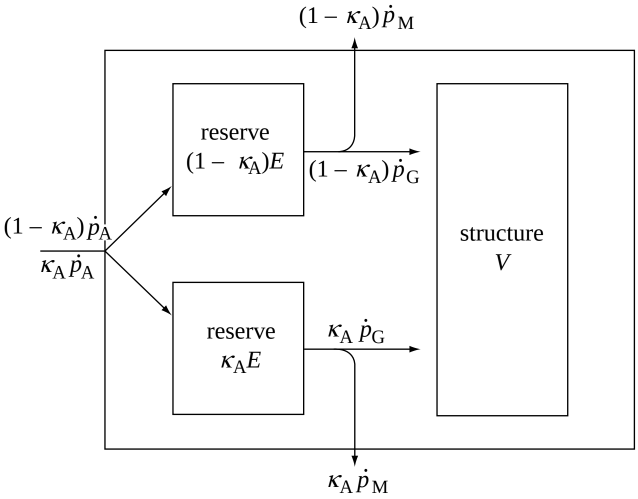

Figure 11. The partionability requirement states that it must be possible to partition reserves into two components (e.g. lipids and non-lipids) such that the kinetics of the reserves are not affected while the two components follow the same kinetics. Multiplication of assimilation $\dot{p}_A$ with an arbitrary partitioning fraction κ_A implies a similar partitioning of the maintenance costs $\dot{p}_M$ and growth costs $\dot{p}_G$, since these costs are paid from both components. This introduces additivity of the kinetics for reserve densities.

The result of these two requirements is that the dynamics of reserve density (i.e. the ratio of the amount of reserves to structure), follows a first-order* process with a turnover rate proportional to the surface area-to-volume ratio. The reserve turnover rate of isomorphs is therefore inversely proportional to their volumetric length*, while that of V1-morphs is constant. These kinetics should not be confused with first-order kinetics in chemistry, because of the problem of 'dilution by growth'.

The partitionability requirement is essential for the smooth stepwise coupling of several reserves, enabling them to behave as a single generalized one. The requirement follows, together with weak homeostasis, from structural homeostasis: this is a set of rules for the membrane kinetics and action that is not part of the DEB theory itself, but a piece of theory at subcellular level that uses the DEB theory as a constraint. These rules provide a mechanistic explanation for the reserve kinetics (Kooijman 2000).

8. BUDGETS AND THE SPECIFICATION OF POWERS

The DEB theory consists of a set of simple, mechanistically inspired rules that fully specify the uptake and use of substrates (nutrients, food) by an individual (see table 1).

The general assumptions relate energy to mass fluxes. The strong homeostasis assumption is vital for the definition of body size, which now reduces to a bivariate measure: the amounts of reserve and structure. If the chemical composition of body components were to vary, any definition of body size would be an arbitrary choice among many alternatives. Strong homeostasis still allows the variation of body composition resulting from varying the ratio of the amounts of reserve and structure.

The wiring of reserves between assimilation and catabolism means that the mineral fluxes of carbon dioxide, water, oxygen and nitrogen waste (frequently ammonia for aquatic organisms) relate linearly to the organic fluxes of food, faeces, reserves and structural mass; these organic fluxes turn out to relate linearly to three powers* (i.e. energy fluxes): assimilation, dissipation and growth. Dissipation includes maintenance (somatic and maturity) and maturation, and in fact all fluxes that are not associated with a net synthesis process.

The powers relate to allocated powers, not to effective powers. Hence the growth power is larger than the flux that is actually fixed in new tissue. The difference is in the overhead costs. These overhead costs might include products that are excreted. Product formation is again a linear combination of the three basic powers.

The energy balance shows that dissipating heat is also a linear combination of the three basic powers, and so of three mineral fluxes: carbon dioxide, oxygen and nitrogen waste. This is because linear functions of linear functions are linear again. The relationship for dissipating heat was discovered empirically by Lavoisier & Laplace (1780) and used for centuries in the method of indirect calorimetry. It is probably the best empirical support for the general assumptions of the DEB theory.

The specific assumptions quantify the powers. Reserve kinetics follows from the two powers assimilation and catabolism, as has already been discussed. The general idea is that the catabolic flux is spent on two 'competing' destinations: somatic maintenance plus somatic growth, and maturity maintenance plus maturity growth (or reproduction in adults) (see figure 12). Maturity growth, or maturation, concerns differentiation and installation of regulation systems; this requires maintenance that cannot be included in somatic maintenance. Maturation relates to a quality rather than a quantity of bulk mass. Somatic growth ceases if all reserves allocated to somatic maintenance plus somatic growth are required for somatic maintenance.

Weak homeostasis and the partitionability of reserve kinetics constrain the allocation of reserves to the various target processes. The fraction allocated to somatic maintenance and growth, rather than to maturity maintenance and maturation of reproduction, should not depend on the reserves. It can still be a function of the amount of structure, but the basic model assumes that it is a constant. This property is called the κ-rule for allocation.

If starvation becomes extreme, growth can become negative, and part of the maintenance is paid from structure, rather than from reserves. The dynamics of reserves and structure are affected by the allocation to development and reproduction during starvation. This allocation can depend on environmental factors, such as the light/dark cycle in pond snails *Lymnaea stagnalis*; the theory specifies two simple strategies (see table 1).

(a) *Ageing and energetics*

Ageing is important in several respects, and provides a mechanism ensuring that no individual stays forever in the population. This sets an upper limit to the amount of memory in population dynamics; the complexity of the dynamics tends to increase with memory. Single cells are either affected or unaffected by ageing; affected cells no longer divide. The occurrence of stringent response in prokaryotes in cases of long interdivision intervals

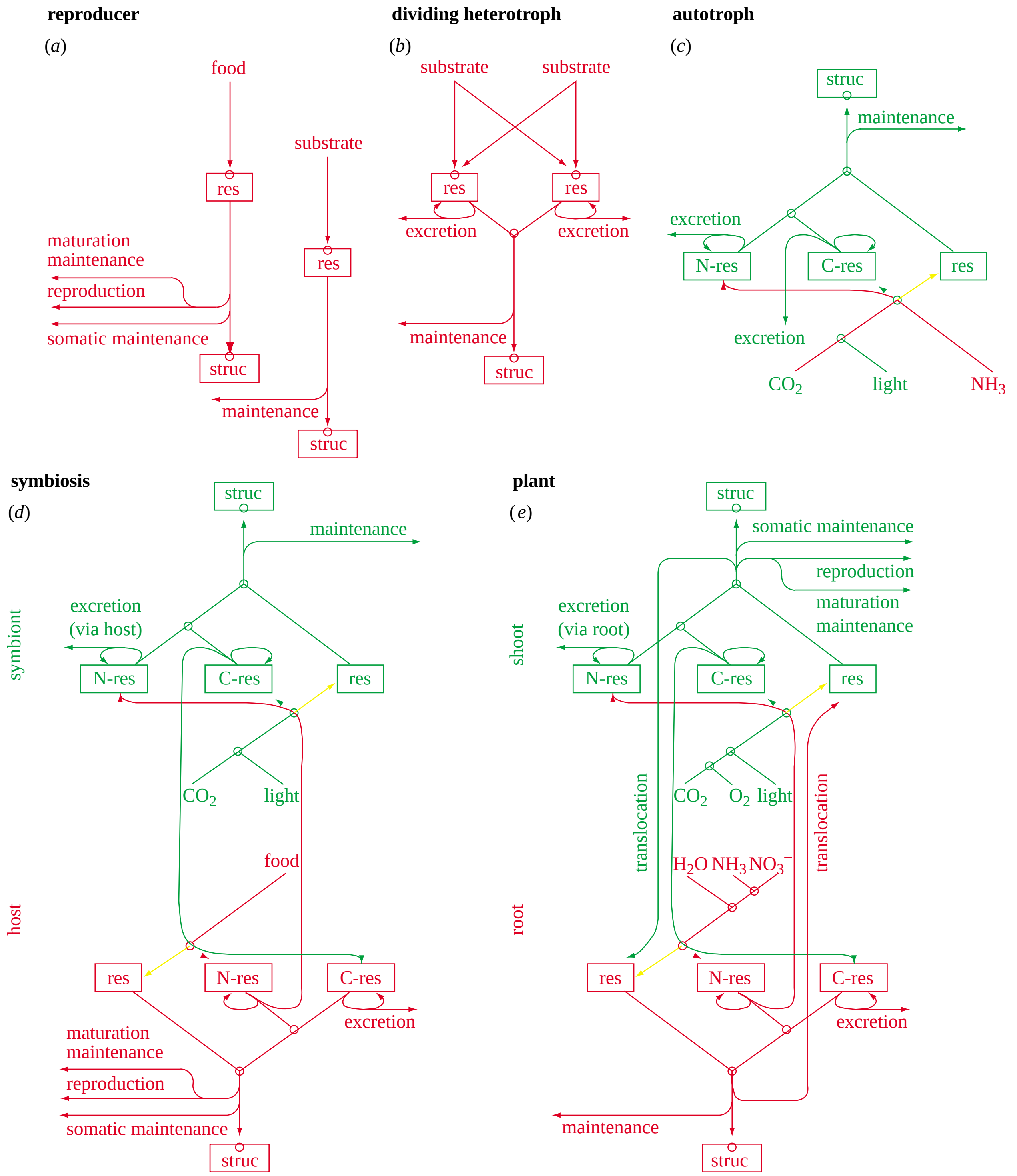

Figure 12. (*a*) The diagram shows fluxes appropriate for a reproducing heterotroph* (such as many animals). A simplification is possible for dividing heterotrophs, which remain in the juvenile phase, by combining allocations to somatic and maturity maintenance, and to somatic and maturity growth (*b*). An extension is required to cope with simultaneous limitation of substrate, which involves excretion. Autotrophs require three reserves generalized, carbon and nitrogen (*c*). Symbiontic partners, e.g. in coral, link excretion fluxes (*d*), while plants have extra translocation processes between root and shoot (*e*).

suggests that this response reflects ageing. Gradual ageing occurs in multicellular organisms, together with irreversible cellular differentiation, which is frequently linked to a limited number of divisions of differentiated cells. Mammals use telomer reduction as a mechanism to stop division in differentiated tissues, which relates to ageing only indirectly.

Linking ageing to respiration, and thus with catabolism and reserve dynamics (table 1), directly couples life span to substrate levels in differentiating multicellular organisms. Kowald & Kirkwood (1994, 1996) followed a very similar line of reasoning and incorporated much more detail. The DEB model reduces to the Weibull model (Weibull 1951) for organisms at constant food density with an expected life span that is large with respect to the growth period. Difference in life spans between sexes (of, for example, waterfleas) could be explained on the basis of difference in energetics, rather than by the ageing process (Kooijman 2000). Mutagenic compounds affect DNA similarly to free radicals and enhance ageing, as is revealed by the hazard profile during lifetime. In the absence of a detailed physiological analysis, experimental results to demonstrate the genetic basis of ageing by selection of old individuals (Rose 1984) can also be understood in terms of a selection of a digestive disorder.

(b) *Multivariate DEB models*

The basic formulation of the DEB model involves a single reserve and a single structural component, as is appropriate for animals that feed on prey. Prey contains all the energy and building blocks required by the consumer. Organisms such as algae, which can be limited in their growth by several types of nutrients (and/or light), require more types of reserves. This allows more degrees of freedom for the composition of their biomass and, because of the interaction of reserves, involves an explicit treatment of excretion: excretion of any reserve depends on the catabolic rates of the other reserves because of stoichiometric constraints on growth (see figure 12). Excretion can be included in overheads for single-reserve systems.

Organisms such as plants, which use different organs for the uptake of different nutrients and light, also require more types of structural biomass. These multivariate extensions of the univariate model for animals follow naturally from the basic set of rules, given the rules for SUs. Each reserve and structure has its own types of SU that specify the interactions between substrates (i.e. nutrients and reserves, respectively) in the assimilation and growth processes (see figure 12). The rules for the behaviour of SUs imply that limitation of assimilation and growth by several substrates simultaneously is confined to a narrow range of substrate arrival rates; the multivariate extensions reduce to the monovariate case for limitations by a single nutrient. Because the multivariate extensions delineate more powers and product formation is a linear combination of these powers, product formation (such as wood production) has considerable metabolic flexibility.

The dynamics of a symbiosis follow from that of the partners (host and symbiont) by mutual linkage of excretion to assimilation fluxes (see figure 12). If a coral polyp has many algal symbionts excreting a lot of carbohydrates, the polyp uses most of its nitrogen waste itself by converting it, together with the carbohydrates, to reserves. The algae then receive little nitrogen, which reduces the algal density. Therefore, this is a self-regulating system that proves to be very stable, and even follows weak homeostasis under certain constraints on parameter values. This type of exchange offers a promising basis for modelling cellular metabolic organization in more detail, where the cell is considered to be a symbiosis consisting of a number of biochemical units (chloroplasts, mitochondria, tricarboxylic acid cycle, etc.). The model can be used to evaluate the effect of calcification on the performance of the symbiosis, on the assumption that it is associated with the uptake of inorganic carbon from the environment. Some 98% of the inorganic carbon in the sea occurs as bicarbonate, which has an electrical charge that is balanced in the uptake of calcium; for each two molecules of bicarbonate that have been taken up, one is excreted as carbonate and the other is converted to carbon dioxide and metabolically processed.

Plants are notoriously difficult to model because of their great ability to adapt to varying environmental conditions. Many of these responses can be captured by considering the plant as a symbiosis between root and shoot (see figure 12). A seed (embryo) initially consists of generalized root reserves; a fixed fraction of its catabolic flux is translocated to the shoot, which behaves in a similar way. The assimilation process is initiated at germination (birth); the root remains in the juvenile phase. The uptake of water by the root is proportional to its (active) surface area; the surface area of the shoot affects the root's saturation constants via evaporation. Nutrient uptake is (partially) coupled to that of water. The surface area-to-volume ratio of plants usually evolves in a predictable pattern; plants tend to develop from a V1-morph, via an isomorph to a V0-morph. The parameter-sparse characterization of the dynamic budgets of plants allows realistic responses to changes in environmental conditions, without making use of any optimization argument: a reduction of light induces a relatively larger investment in shoot growth: a reduction in water or nutrients induces a relatively larger investment in root growth.

(c) *Extensions*

The basic model can be detailed for particular taxa, such as terrestrial endotherms (birds, mammals). The implied water balance can be extended to include evaporation coupled to surface area, and to respiration which defines a heat loss that modifies the heating costs and affects drinking behaviour. The coupling between energy and water budgets with environmental conditions (temperature, moisture) allows a basic understanding of the (potential) geographical distribution of species.

The assumption of three life stages can easily be extended to include more stages, such as the pupal stage that holometabolic insects insert between the juvenile and adult stages; the ontogeny of adults' weight in the pupa is quantified realistically by DEB rules for embryonic development. Dormant periods can also be included. Some specific assumptions can be modified without major consequences for the model structure; examples are other values for the reserve density at birth, and the value of κ depends on the amount of structural mass. Alternatives involve extra parameters, however, which restrict their applicability.

9. MODEL TESTING

Testing against experimental data is an ongoing activity that can never be considered to be complete. Table 2 gives well-known empirical models that are all special cases of the DEB theory. Most of these models can be obtained mathematically from the DEB model by selecting particular parameter values.

The coherence between the models in table 2 is far from obvious at first sight. For instance, Droop (1973, 1974) formulated an empirical relationship between the

Table 2. *Empirical models that are special cases of the DEB theory or very good numerical approximations*

author	year	model
Lavoisier & Laplace	1780	multiple regression of heat against mineral fluxes
Arrhenius	1889	temperature dependence of physiological rates
Huxley	1891	allometric growth of body parts
Henri	1902	Michaelis–Menten kinetics
Blackman	1905	bilinear functional response
Pütter	1920	von Bertalanffy growth of individuals
Pearl	1927	logistic population growth
Fisher & Tippitt	1928	Weibull ageing
Kleiber	1932	respiration scales with body weight$^{3/4}$
Mayneord	1932	cube root growth of tumours
Emerson	1950	cube root growth of bacterial colonies
Huggett & Widdas	1951	foetal growth
Weibull	1951	survival probability for ageing
Best	1955	diffusion limitation of uptake
Smith	1957	embryonic respiration
Leudeking & Piret	1959	microbial product formation
Holling	1959	functional response type I and type II
Marr *et al.*	1962	maintenance in yields of biomass
Pirt	1965	maintenance in yields of biomass
Droop	1973	reserve (cell quota) dynamics
Rahn & Ar	1974	water loss in bird eggs
Hungate	1975	digestion
Beer & Anderson	1997	development of salmonid embryos

Table 3. *Parameters of the DEB model*

(The primary parameters of the basic DEB model classify into intensive parameters, which do not depend on asymptotic body size, and extensive ones which do depend on that size. Ectotherms do not heat, so $\{\dot{p}_\text{T}\} = 0$. Dividers relate size at birth directly to that at puberty, while reproduction overheads (κ_R) do not apply; developmental and somatic allocations can then be combined, which eliminates κ. This gives reductions of one or four parameters. The ageing mechanism amounts to an ageing rate for unicellular organisms, rather than an acceleration. t, time; m, mass; l, length; e, energy.)

symbol	dimension	interpretation
extensive		
X_K	ml^{-3}	saturation constant
$V_\text{b}^{1/3}$	1	length at birth
$V_\text{p}^{1/3}$	1	length at puberty
$\{\dot{J}_\text{Xm}\}$	$ml^{-2}t^{-1}$	maximum specific ingestion rate
$\{\dot{p}_\text{Am}\}$	$el^{-2}t^{-1}$	maximum specific assimilation rate
$[E_\text{m}]$	el^{-3}	maximum specific reserve capacity
intensive		
$[\dot{p}_\text{M}]$	$el^{-3}t^{-1}$	specific maintenance costs
$\{\dot{p}_\text{T}\}$	$el^{-2}t^{-1}$	specific heating costs (endotherms)
$[E_\text{G}]$	el^{-3}	specific growth costs
κ	—	partition coefficient catabolic power
κ_R	—	efficiency reproductive power
h_a	t^{-2}	ageing acceleration

density of intracellular nutrients of algae (called the cell quota) and their population growth rate at steady state. It takes some reconstruction to formulate a model for reserve kinetics resulting in this steady-state relationship (for V1-morphs with negligible maintenance requirements for these nutrients). However, Pütter (1920), formulated a model for the growth of an (isomorphic) animal (with substantial maintenance requirements) that became widely known by von Bertalanffy (1938, 1940). Pütter noted a relationship between the ultimate length and the von Bertalanffy growth rate, but did not make the link to reserves. It turned out that the reserve kinetics as reconstructed from Droop's model just gives Pütter's relationship between the growth rate and the ultimate length. Pütter did realize that uptake is proportional to surface area and maintenance to volume; this notion goes back at least as far as Wallace in 1865, who mentioned it in a note to Poulton (appendix 3 in Finch (1990)). This insight was lost, however. Neither Droop nor Pütter dealt with transient states. Another model, for the substrate limited growth of bacteria (Marr *et al.* 1962; Pirt 1965), is complementary to Droop's model; it is obtained for a V1-morph by setting the maximum reserve capacity equal to zero, while Monod's model (Monod 1942) follows by also setting the maintenance costs equal to zero. Other models, such as 'Kleiber's law' for the body size dependence of respiration (Kleiber 1932) and Huxley's allometric* growth of body parts (Huxley 1932), do not follow mathematically, but behave numerically very similarly to the DEB model.

Because the models in table 2 are classic because of their 'ideal' combination of simplicity and realism, the DEB theory is one of the best tested quantitative theories in biology. Many details of the DEB theory are not covered by the models in this list, however. Additional tests against experimental data are presented by Kooijman (2000), but particular aspects are still in urgent need for tests against reality. The best empirical support for the general assumptions is probably provided by the method of indirect calorimetry, as has been discussed, and for the specific assumptions concerning the body-size scaling relationships, discussed in § 10.

A powerful way to test the model is to measure the realism of reactions to perturbations, such as changes in food density, light cycles, effects of endoparasites and of toxicants with specific modes of action, such as an increase of maintenance, growth or reproductive costs, or mutagenic effects (Kooijman & Bedaux 1996). Effects of toxicants involve a toxicokinetic module, which depends on energetics in several ways. This complicates the testing, but reactions to perturbations have been found to be realistic so far.

The simple κ-rule explains complex responses in growth, reproduction and sizes at birth and reproduction in response to a change in light/dark cycles in the pond snail: the light regime affects the value of κ. The effects of parasites on a host's physiology can also be understood in terms of a change in this value, and an interception of the flux to development plus reproduction.

Not all details of the DEB theory are of importance in all applications, as is obvious from the list of successful simple empirical models in table 2. Part of the value of the DEB theory is the insight it offers about the conditions when particular simplifications are effective, and when they can be expected to fail. Model simplification has direct but complex relationships with model testing.

10. COVARIATION OF PARAMETER VALUES AMONG SPECIES

The power of physics lies in simplification: biology suffers from the problem that the most simple living creature is still extremely complex. Biology also has an advantage, however; comparison between different organisms enables invariants and general rules to be identified. Each individual has a set of fixed parameter values, which vary little among conspecifics but substantially among interspecifics. However, the values do not vary independently, but tend to covary in a very special way. This is because all primary parameters can be classified as intensive or extensive, while ratios of extensive parameters are intensive again (if expressed in the proper units).

All extensive parameters are proportional to the maximum volumetric length that an individual isomorph can reach (after a long exposure to a high food density). This necessity is revealed by considering the expression for this maximum length, which is a simple function of three parameters, $\kappa\{\dot{p}_{\mathrm{Am}}\}/[\dot{p}_{\mathrm{M}}]$: the fraction of energy that is allocated to maintenance plus growth multiplied by the ratio of the surface-area-specific assimilation power, and the volume-specific maintenance costs. (Growth ceases if all energy allocated to maintenance plus growth is consumed by maintenance.) Because the allocation fraction and the volume-specific maintenance costs are intensive parameters, the surface-area-specific assimilation power must be extensive and proportional to maximum volumetric length. The basic difference between intra- and interspecies comparisons is that the first involves differences in state variables, and the second involves differences in parameter values if the comparison concerns fully grown individuals.

The scaling of the primary parameters defines how any quantity that can be written as a function of these parameters depends on maximum length. These quantities include many physiological and life history parameters, such as life span, length of embryonic and juvenile periods, and maximum reproduction rates. Tests against empirical data show that all 35 tested body-size scaling relationships are realistic.

The literature gives most attention to respiration rates. The DEB theory predicts that volume-specific respiration rates decrease with body size because large organisms have relatively more reserves. The respiration is approximately proportional to volume$^{3/4}$, but the coefficient 3/4 varies somewhat in value between the taxa because of the varying contribution of the maintenance costs in respiration (see figure 13). Endotherms (mainly birds and mammals) spend a lot on heating, heat exchange being proportional to surface area. This explains why their scaling parameter is close to 2/3. Volume-specific respiration also decreases for a growing individual, but for a totally different reason: its investment into growth decreases with size.

Figure 14 gives a second example: the von Bertalanffy growth rate is approximately inversely proportional to length. Again, this is because the reserve density increases with body size among species. The scaling relationships help us to understand that feeding rates increase with squared length intraspecifically, but with cubed length interspecifically; reproduction rates increase with body size intraspecifically, but decrease with body size interspecifically.

11. EVOLUTIONARY ASPECTS

Present-day organisms that can be described by a single type of (generalized) reserve probably evolved from organisms with more reserves. The kinetics of these reserves gradually became coupled, resulting in a higher degree of homeostasis which possibly favours the optimization of enzyme properties. The DEB theory has this evolutionary consistency, thanks to the partitionability requirement of reserve dynamics. If all reserves that are rejected by the growth-SU are excreted, all reserve turnover rates set to a common value, and the uptake of all nutrients stoichiometrically coupled, the reserves can be combined mathematically into a single generalized reserve. Tests against experimental data so far have shown that the turnover rates of the reserves hardly differ. This points to a common cellular machinery for the use of reserves which involves among others rRNA. The coupling of reserves is attractive for an organism if the availabilities of nutrients are also coupled. If food consists of animal prey, this coupling is almost perfect, and the conversion is efficient because the composition of prey resembles that of the consumer. If food consists of plant material, the coupling is still considerable, but because the compositions of plant and consumer differ more the conversion is less efficient. This implies more wastes and so an increased return of nutrients to the plant.

The general picture of the evolution of metabolic systems that emerges is more or less as follows. Initially, metabolic systems became increasingly independent of temporary variations in the nutritional quality of the environment by increasing their storage capacity

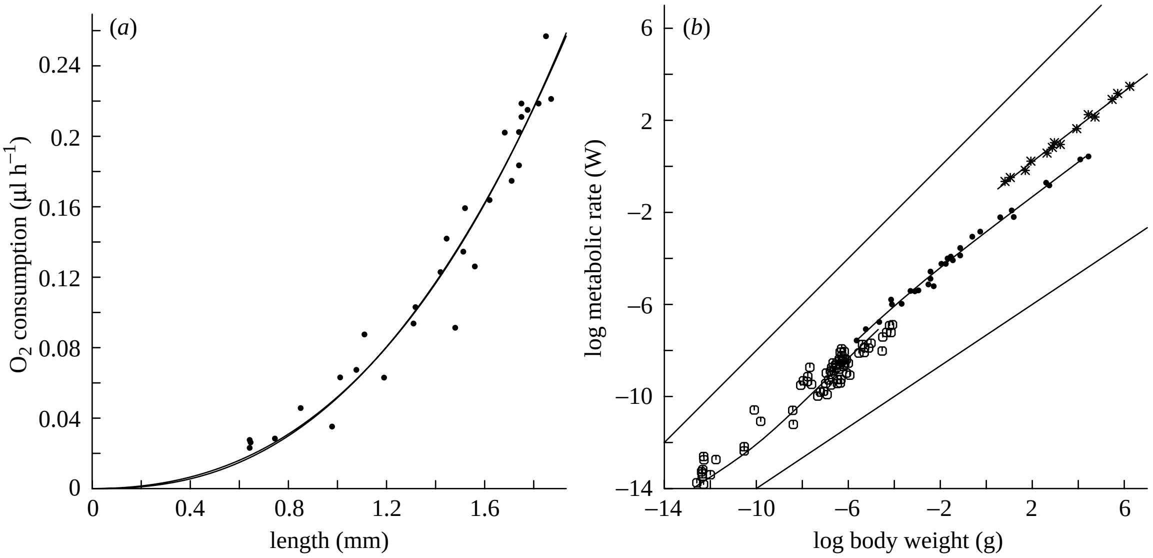

Figure 13. (*a*) The respiration rate of *Daphnia pulex* with few eggs at 20 °C as a function of length. Data from Richman (1958). The DEB-based curve $0.0336L^2 + 0.01845L^3$ and the standard allometric curve $0.0516L^{2.437}$ are plotted on top of each other, but they are so similar that this is hardly visible. If you look hard, you will notice that the line width varies a little. Volume-specific respiration decreases with size because of the decreasing investment in growth. (*b*) The specific respiration also decreases with size among fully grown individuals of different species because of the increasing contribution of reserves in bodyweight. The metabolic rate of unicellular organisms (open circles, at 20 °C), ectotherms (filled circles, at 20 °C) and endotherms (asterisks, at 39 °C) is plotted as a function of body weight. (Modified from Eckert *et al.* 1988; Hemmingsen 1969.) The difference between this figure and the many others of the frequently reproduced data set is that the curves relate to DEB-based expectations, and are not allometric regressions. Nonetheless they appear almost as straight lines. The lower line has a slope of 2/3; the upper one a slope of unity.

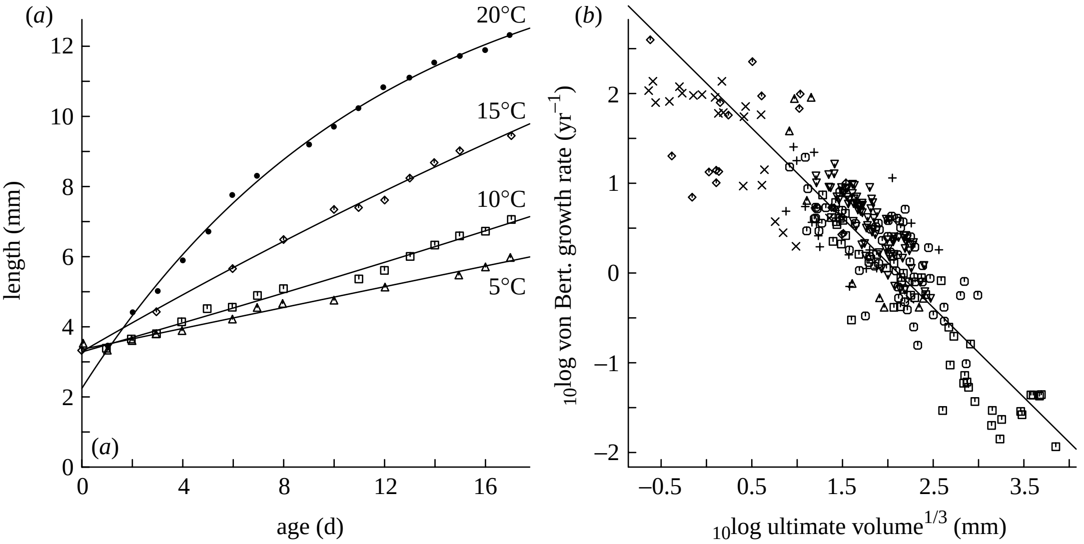

Figure 14. (*a*) Graph to show that *Pleurobrachia pileus* grows according to the von Bertalanffy growth curve at constant food density and temperature, like many other species of animal. Data from Greve (1972). The von Bertalanffy growth rate quantifies the rate at which the asymptotic size is approximated. The DEB theory predicts that an isomorph follows the von Bertalanffy growth curve at abundant food and that the von Bertalanffy growth rate is (approximately) inversely proportional to maximum volumetric length. This is shown for data on 261 widely different species (*b*), corrected to a body temperature of 25 °C using the Arrhenius relationship: open inverted triangles, birds; open squares, mammals; open triangles, reptiles and amphibians; open circles, fishes; crosses, crustaceans; plus signs, molluscs; open diamonds, others.

for the various nutrients; temporary peaks in the availability of a nutrient can only be used if other nutrients are not essential for this uptake, so the number of reserves equals the number of essential nutrients. Then followed a phase where organisms increased their control over the uptake of resources by increasing their taxis and homeostasis abilities at the same time. This allowed them to use specialized enzymes to catalyse particular transformations, so increasing and regulating the rate of these transformations. The proper functioning of these enzymes requires a steady turnover; this involves maintenance costs that are further increased by the taxis activities. These maintenance requirements have tight links with storage capacities in varying environments to ensure the integrity of the metabolic system.

A crucial step in evolution is the optimization of cellular performance, which comes about with a specialization on particular substrates. This took place in the prokaryotic lines: some forms specialized on the products of others, and syntrophy evolved. Reciprocal syntrophy (symbiosis) became advanced in endosymbiosis (Margulis 1993) and resulted in the eukaryotic line (the protists), which radiated and gave rise to animals and plants.

The animal line of development perfected control over uptake by feeding on other organisms: this gives an almost perfect coupling of resources, especially in carnivores. This allowed animals to eliminate many routes of metabolite synthesis, and to couple the use of various reserves such that a single generalized reserve emerged and an almost perfect homeostasis was reached. This specialization came hand in hand with an increase in the taxis abilities, through the development of advanced motor systems and senses that need a nervous system for information processing and muscle control. They also used this nervous system to increase their control over homeostasis and to make the transition from supply to demand systems. (The behaviour of a supply system is controlled by substrate abundance, that of a demand system by its needs; production is 'pre-programmed' and the organism eats what it needs.) This more active lifestyle increased the maintenance costs, which is no problem if it also leads to higher assimilation. The process culminated in the endotherms, which learned to regulate their body temperature to a constant value at substantial maintenance costs.

The plant line of development specialized in increasing the adaptive abilities. Plants became extremely flexible in morphology, with direct links to the control over uptake of the various nutrients and light (roots versus shoots). They developed structural elements (wood, silica) in response to mechanical pressures, and learned to use animals to solve the problem of finding partners in the reproduction process, and of exporting seeds to uncolonized areas while being confined to a particular site. They also learned to use fungi to capture nutrients that are locked in organic compounds, and some use bacteria to make dinitrogen available to them.

The increase in biodiversity allowed a specialization of functions, which enhanced metabolic versatility, by using other species or their activities; compare mixotrophs (the typical metabolic mode for photo-autotrophic protists) with a producer–consumer–decomposer community, for instance. The processes of syntrophy and even more advanced forms of symbiosis developed early in evolution and have been re-established many times since then, resulting in loose and tight links between virtually all organisms.

The main formal differences between the metabolism of a community and a eukaryotic cell conceived as a symbiontic system, are perhaps in spatial aspects (transport) and in the extent to which compounds that are involved in exchange processes can accumulate. The DEB rules for mass exchange between the partners in symbiontic relationships might lead to an understanding of the evolution of homeostasis at the cellular level and the existence of biodiversity.

12. POPULATION AND ECOSYSTEM DYNAMICS

Given rules for interaction between individuals, environmental conditions (supply of nutrient to the system, and/or leakage from the system) and initial conditions, the DEB theory predicts population changes (Nisbet *et al.* 2000). The simplest rule for interaction is competition for the same substrate in a well-mixed environment. Populations in reactors can be forced to approximate these rules, and the resulting predictions are realistic indeed. Figure 15 shows that this even holds for the dynamics of food chains.

Classical (unstructured) population models only count numbers of individuals or total biomass, which implies that individuals are identical. Physiologically structured population theory (Metz & Diekmann 1986; Łomnicki 1988; Ebenman & Persson 1988; DeAngelis & Gross 1992; Tuljapurkar & Caswell 1996; Gurney & Nisbet 1998; Cushing 1998) takes account of differences between individuals such as those specified by the DEB theory, and allows links with evolutionary biology. This is because these models can evaluate how changes in physiological performance of an individual work out in terms of changes in the propagation of its progeny through the population, given rules for heredity of physiological characters as quantified by parameter values. Theory on adaptive dynamics (Dieckmann & Law 1996; Geritz *et al.* 1998; Metz *et al.* 1996) deals with these processes. Within the context of the DEB theory, structured population dynamics reduces to partially structured population dynamics for V1-morphs, where total structural mass and (mean) reserve density are taken into account. Micro-organisms approximate V1-morphs; the individual character is more important in species with a large body size.

Real world population dynamics are very significantly complicated by a large number of factors, such as spatial heterogeneity and social interaction. When two individuals of slightly different size compete for a food item, the bigger one has a slightly better chance of obtaining the item, which enhances the size difference between the individuals. Social interactions have a positive feedback on size difference among individuals that are otherwise similar. The realistic incorporation of such factors frequently involves many parameters and variables, and degrades the generality of the analysis. The main value of models for population dynamics is in broad expectations, rather than detailed analysis.

Many generally accepted concepts in population biology are based on very simple models for the behaviour of individuals. They do not seem to hold for more realistic models, such as those specified by the DEB theory. One example is the competitive exclusion principle, which says that two predator species cannot coexist on a single prey species at steady state. Relatively recent results show that the system can oscillate under very particular conditions, such that two predators can coexist. These results rest, however, on the simplicity of the model that takes the biomass composition of the prey to be constant.

The DEB theory delineates (at least) two components in biomass, which allows coexistence of two predators differing in digestion efficiency of these components. Taking account of faeces production, a microflora living

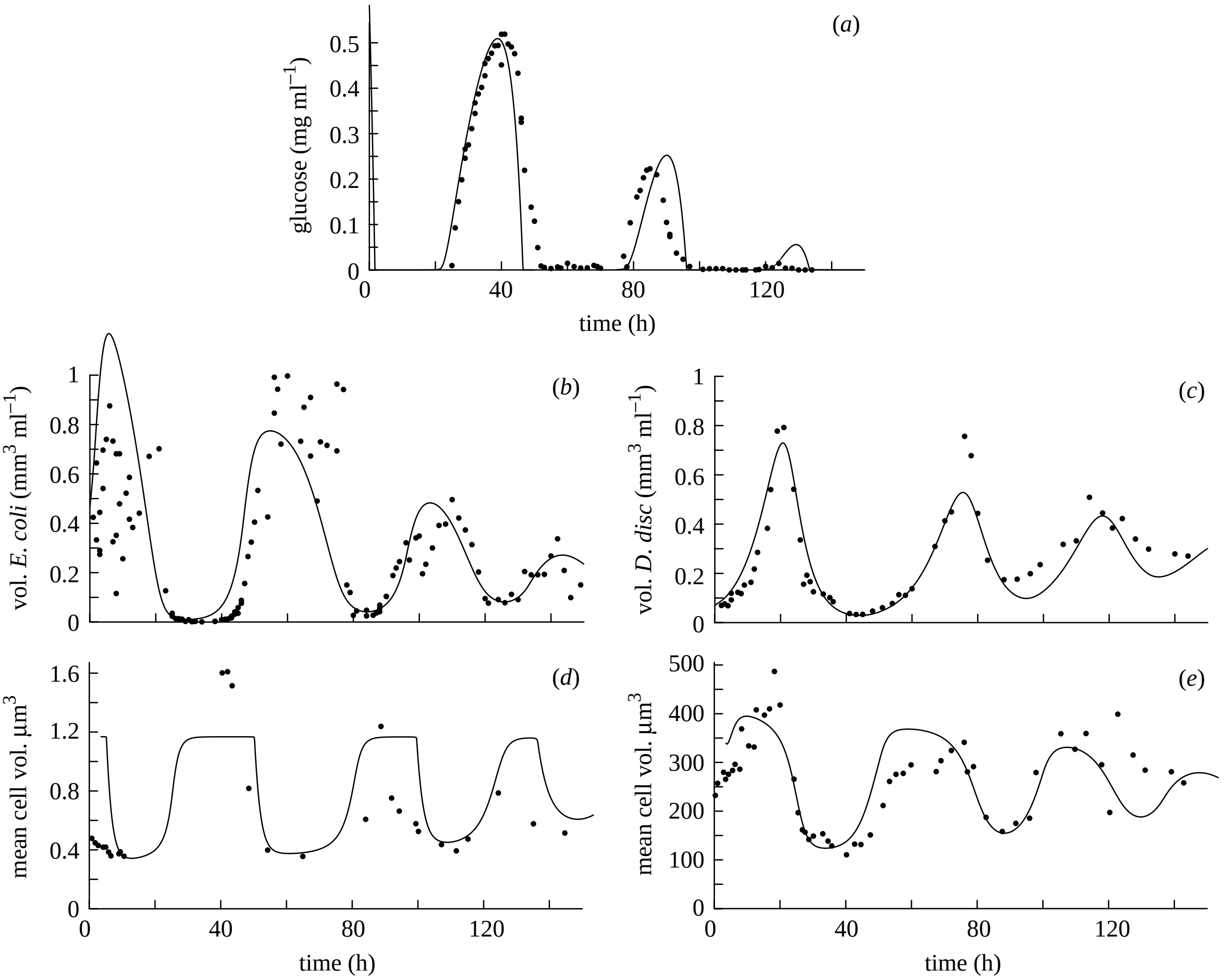

Figure 15. A chemostat with a bitrophic chain of glucose (*a*), the bacterium *Escherichia coli* (*b*,*d*) and the cellular slime mould *Dictyostelium discoideum* (*c*,*e*) at 25 °C, throughput rate 0.064 h^{-1} and 1 mg ml^{-1} glucose in the feed. The mean cell volumes are given in graphs (*d*) and (*e*). Data from Dent *et al.* (1976); the curves were fitted by B. W. Kooi, using the DEB model.

on it that can be eaten by the predators, and of other organic products that can be used by others as nutrients (most photo-autotrophs have heterotrophic capabilities), it is possible to sustain a substantial biodiversity on a single nutrient. This indicates the necessity of thinking in terms of it being community metabolism rather than population dynamics, which links community structure (biomass density over the different biota) and function (nutrient cycling). This has been worked out for a so-called canonical community, i.e. a three-species community consisting of producers, consumers and decomposers (Kooijman & Nisbet 2000).

Another example that illustrates that DEB-based insights can differ from accepted ones in population biology is the stabilizing effect that invading species of competitors and predators can have on an existing community (Kooi & Kooijman 2000). Accepted insight is that an increase in diversity comes with a decrease in stability in spatially homogeneous systems. This no longer seems to hold true for more realistic models.

Predator responses to fluctuations in prey density frequently show a delay, as illustrated in figure 15 where the mean cell sizes of bacteria and slime moulds fluctuate in an opposite way. This has been used to argue that the feeding rate of the slime moulds is a function of prey-to-predator ratios, rather than of prey densities (Saunders 1980). The successful application of DEB theory, however, suggests that the delay is due to reserve dynamics.

The nonlinear decrease of growth rates of individuals can synchronize their life cycles in a population; this can induce oscillatory behaviour of the population size, even under constant environmental conditions.

The asymptotic behaviour of food chains rapidly becomes very complex for increasing chain length. For a length of three species in a chemostat, multiple attractors can occur, some of them of the chaotic type (Kooi *et al.* 1998; Boer *et al.* 1999, 2001). Recent progress has been made in the systematic analysis of possible asymptotic behaviour of food chains (Boer 2000), by reducing their essential features to a one-dimensional map. The next-minimum map for the top predator with chaotic behaviour turns out to approximate a cubic polynomial for DEB-based models (including those of Monod (1942), Marr *et al.* (1962) and Pirt (1965)).

In practice, chaotic behaviour is difficult if not impossible to tell apart from stochastic behaviour. Realistic implementation of stochastic behaviour easily leads to statistical nightmares due to complex statistical interdependence of variables. Computer simulation studies can be of some help here.

13. FUTURE CHALLENGES

Further development of the DEB theory towards the molecular and ecosystem levels can contribute a great deal to our understanding of quantitative aspects of metabolic organization.

The evolution of different forms of homeostasis needs further study at the molecular level. This includes the role of regulation systems, and the nervous system in animals. Extension of the symbiontic argument looks promising, and links up with a modular approach to cellular metabolism (Ainscow & Brand 1995). Though inherently speculative, it is important from a theoretical perspective to delineate the class of models for prebiotic evolution that leads to the DEB kinetics. Reasoning along the lines set out by Dyson (1999) seems very useful to test this extreme form of consistency.

The understanding of ecosystem metabolism could benefit from explicit energy and mass balances, and exclude unrealistic behaviour. The DEB theory provides the rules for constructing these dynamic balances, but considerable effort must still be invested in aggregation methods that allow substantial simplifications. Nutritional links between species span a range between tight (flux-based) and loose, using varying pools in the abiotic environment.

Computer simulation studies seem promising, where parameters that specify food preferences and parameters that tend to covary across species are allowed to follow a random walk across generations. Starting from a single-species community of mixotrophs, recent results show that function segregation can occur under particular environmental conditions, and a canonical community of producers, consumers and decomposers evolves. We are only a few steps away from simulating the spontaneous evolution of food webs and the specialization of decomposers on the various substrates. Many aspects of this spontaneous self-organization of ecosystem metabolism are already implied by the rules of substrate uptake and use by organisms; no additional fitness measures or selection criteria are required. A deeper understanding of these emergent properties still requires substantial research, however. The rapidly growing theory on adaptive dynamics might be of great help here.

Insight into ecosystem metabolism will contribute to our understanding of the role of life in the planetary system, and the coevolution of life and its physical and chemical environment. Although some qualitative effects of biota on global climate are known, quantitative aspects are still elusive.

Practical applications of the DEB theory comprise the optimization of bioproduction and harvesting schemes, conservation biology, ecotoxicology, tumour biology, sewage treatment and biodegradation. Some of these applications have been worked out, but many potential applications still await exploration. Further information about applications, and downloadable software for using the DEB theory, can be found at http://www.bio.vu.nl/thb/deb.

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APPENDIX A. GLOSSARY

allometry: the group of analyses based on a linear relationship between the logarithm of some physiological or ecological variable and the logarithm of the body weight of individuals.

assimilation: generation of reserves from substrates (food, nutrients, light).

ATP: adenosine triphosphate is a chemical compound used by all cells to store or retrieve energy via hydrolysis of one or two phosphate bonds.

catabolism: the collection of biochemical processes involved in the decomposition of compounds for the generation of energy and/or source material for anabolic processes; here used for the use of reserves for metabolism (maintenance and growth).

DEB: dynamic energy budget model or theory. The term 'dynamic' refers to the contrast with the frequently used static energy budget models, where the specifications of the individual do not change explicitly in time.

density: the ratio of two types of masses; these masses are not necessarily homogeneously mixed, contrary to the concept 'concentration'.

endotherm: an animal that usually keeps its body temperature within a narrow range by producing heat. Birds and mammals do this for most of the time that they are active. Some other species (insects, tuna fish) have endothermic tendencies.

first-order process: a process that can be described by a differential equation where the change of a quantity is linear in the quantity itself.

flux: an amount of mass or energy per unit of time. An energy flux is physically known as a power.

free energy: the maximum amount of energy of a system that is potentially available for 'work'. In biological systems, this 'work' usually consists of driving chemical reactions against the direction of their thermodynamic decay.

functional response: the ingestion rate of an organism as a function of food density.

generalized compound: mixture of chemical compounds that does not change in composition—fixed stoichiometries for synthesis (organic substrate, reserves and structural mass are generalized compounds).

growth: increase in structural mass or structural volume.

hazard rate: the probability per time increment that death strikes at a certain age, given survival up to that age.

heterotroph: an organism that uses organic compounds as a source of energy; photo-autotrophs use light.

homeostasis: the ability of most organisms to keep the chemical composition of their body constant, despite changes in the chemical composition of the environment.

isomorph: an organism that does not change its shape during growth, which means that its surface area is proportional to its volume$^{2/3}$.

logistic growth: growth of mass that is proportional to the product of mass and the carrying capacity minus mass. The carrying capacity is a parameter.

maintenance: a rather vague term denoting the collection of energy-demanding processes that life seems to require to keep going, excluding all production processes. I also exclude heat production in endotherms.

mass action law: the law that states that the meeting frequency of two types of particles is proportional to the product of their densities, i.e. number of particles per unit of volume.

morph: an organism with a surface area that is proportional to volume^0 (V0-morph) or to volume^1 (V1-morph); see also 'isomorph'. Only the surface area matters that is involved in uptake of substrate (food) from the environment.

nutrients: inorganic substrates used for the synthesis of reserves; carbon dioxide and ammonia are examples, and light is also included for convenience.

parameter: a quantity in a model that describes the behaviour of state variables. It is usually assumed to be a constant.

power: energy flux. An amount of energy per unit of time.

respiration: the consumption of oxygen, or the production of carbon dioxide. Both fluxes have a relationship with the use of energy.

RNA: ribonucleic acid is a group of compounds that is used by all cells for information storage and retrieval, and for catalysis.

state variable: a variable that determines, together with other state variables, the behaviour of a system. The crux of the concept is that the collection of state variables, together with the input, determines the behaviour of the system completely.

SU: synthesizing unit is a (generalized) enzyme that follows particular rules for binding of substrates and product formation.

volumetric length: the cubic root of the volume of an object. It has the dimension length.

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