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Testing Metabolic Theories

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ABSTRACT: Metabolism is the process by which individual organisms acquire energy and materials from their environment and use them for maintenance, differentiation, growth, and reproduction. There has been a recent push to build an individual-based metabolic underpinning into ecological theory—that is, a metabolic theory of ecology. However, the two main theories of individual metabolism that have been applied in ecology—Kooijman's dynamic energy budget (DEB) theory and the West, Brown, and Enquist (WBE) theory—have fundamentally different assumptions. Surprisingly, the core assumptions of these two theories have not been rigorously compared from an empirical perspective. Before we can build an understanding of ecology on the basis of individual metabolism, we must resolve the differences between these theories and thus set the appropriate foundation. Here we compare the DEB and WBE theories in detail as applied to ontogenetic growth and metabolic scaling, from which we identify circumstances where their predictions diverge most strongly. Promising experimental areas include manipulative studies of tissue regeneration, body shape, body condition, temperature, and oxygen. Much empirical work designed specifically with DEB and WBE theory in mind is required before any consensus can be reached on the appropriate theoretical basis for a metabolic theory of ecology.

Keywords: metabolic rate, scaling, growth model, experimental tests.

Introduction

A fundamental life process is the uptake of energy and material from the environment and its conversion into biomass—that is, metabolism. This process occurs at the level of the individual and is constrained by the laws of thermodynamics. Individual metabolism thus provides a powerful and deep theoretical foundation on which to build an understanding of ecological processes and phenomena from the individual to populations, communities, and ecosystems (Nisbet et al. 2000; Brown et al. 2004), and its study has deep historical roots (Rubner 1883; Kleiber 1932). Among the questions addressed by a metabolic theory are, How should biological rates, such as energy consumption rate, scale with body size? How will environmental changes in food, temperature, and toxicants affect growth, survival, and reproduction? How does energy and matter flux through communities and ecosystems?

The development of a metabolic theory of ecology (MTE) has recently become a major research agenda following influential publications by James Brown and colleagues (e.g., Gillooly et al. 2001; Allen et al. 2002; West et al. 2002; Enquist et al. 2003; Brown et al. 2004). These authors have emphasized the power of a simple description of metabolic rate as a function of body size and temperature to capture variation in ecological patterns ranging from individual growth (West et al. 2001; Hou et al. 2008) and the scaling of metabolic rate to ecosystem-level patterns, such as food-web structure (Brown and Gillooly 2003). It has been claimed that such a theory could underpin and unify much of ecological research (Brown et al. 2004), and it has had an enormous effect in ecology and beyond (Whitfield 2004; Martínez del Rio 2008). Such a foundation will be especially valuable to fields where stronger theoretical underpinnings are particularly sought after, such as community ecology (Brown et al. 2004; McGill et al. 2006).

Two Theories of Individual Metabolism

The basic equation developed by Brown and colleagues to describe whole-organism metabolism is $Q = B_0 M^{3/4} e^{-E/kT}$, where Q is metabolic rate, M is adult mass, E is activation energy, T is body temperature, B_0 is an empirically determined constant, and k is Boltzmann's constant. Such models have been commonplace in physiological ecology for many decades (e.g., Bennett and Dawson's [1976] $Q = M^{0.82} 10^{0.038(T-1.771)}$). The novelty of Brown and colleagues' model is that the body mass scaling exponent is predicted from first principles based on the geometry of the metabolic supply system. The temperature dependence term $e^{-E/kT}$ is derived on the basis of the thermodynamics of simple reactions between particles in the gas phase (but see Clarke 2004; Irlich et al. 2009). West et al. (1997) argued that the mass scaling exponent is mechanistically justified on the basis of the way that fractally branching distribution networks, such as blood vessels of vertebrates

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or branches of trees, scale with body size (from here on, this theory and its derivative models will be referred to as WBE, for West, Brown, and Enquist). Their theory proposes that while the isolated individual cells of a mouse or an elephant require the same amount of energy to function in vitro, the scaling of the supply network is such that it is less able to supply the full demands of in vivo cells as body size increases (West et al. 2002). On this foundation, Brown and colleagues have built models of individual growth, survival, and reproduction and of population and community dynamics (Brown et al. 2004).

The WBE-based models are now being applied in general ecological investigations and have been shown to predict numerous broad-scale physiological and ecological phenomena with remarkable accuracy (e.g., Enquist et al. 2003, 2007*b*; Brown et al. 2004; Savage et al. 2004; Gillooly et al. 2005; Price et al. 2007; Hou et al. 2008). However, vociferous debate has ensued on the universality of the empirically observed scaling (Glazier 2005; White et al. 2007; Makarieva et al. 2008), the empirical and statistical validity of the temperature dependence term (Clarke 2004; Irlich et al. 2009), fundamental principles of the models (Makarieva et al. 2004; Suarez et al. 2004; Kozłowski and Konarzewski 2005; O'Connor et al. 2007; Apol et al. 2008; Banavar et al. 2010), and their predictive capacity (Algar et al. 2007).

One neglected issue in the debates about the WBE theory is that a comprehensive and general theory of individual metabolism had already been proposed more than 10 years prior to the WBE theory, but it was based on very different principles (van der Meer 2006). This second metabolic theory is the dynamic energy budget (DEB) theory of Kooijman (Kooijman 1986, 2000, 2010; Sousa et al. 2008). DEB theory follows a different tack than WBE theory in that it does not take respiration rate and its allometric constraints as a primary focus—that is, it does not begin with an equation for the "basal metabolic rate" as represented by empirical measures of O_2 consumption or CO_2 production. Instead, it aims to abstract the organism into compositionally homogeneous parts ("structure" and "reserve," described in detail below) from which to model ontogenetic growth, differentiation, maintenance, and reproduction, with respiration patterns (including mass-specific basal metabolic rate) subsequently emerging from the stoichiometry of the metabolism. DEB theory is based on generalized surface area (source) and volume (sink) relationships rather than specific details of supply-network constraints and uses the Arrhenius relationship to model the thermal responses of biological rates, but it does not claim a mechanistic basis for this part of the model. The standard DEB model incorporates one food type, one reserve, and one type of (metabolizing) structure and assumes isometric size increase, whereas these model components are altered in variants of the standard model. The standard DEB model predicts scaling exponents of respiration rate with total body mass of between two-thirds and 1, rather than the value of three-fourths as originally emphasized in the WBE theory. Moreover, DEB theory has different explanations for intra- versus interspecific scaling of respiration; the intraspecific (ontogenetic) scaling results from the reducing contribution of growth to respiration as animals grow together with a change in the scaling trajectory at maturity, while the interspecific scaling emerges from the increasing contribution of reserve (which does not contribute to respiration in fasted animals) to weight as species increase in size (Kooijman 2000, 2010).

As with the WBE metabolic theory, it has been argued that the DEB metabolic theory has the power to unite hierarchical levels of ecological organization (Nisbet et al. 2000) and thus can be considered an alternative basis for developing a "metabolic theory of ecology" (van der Meer 2006).

The Domains of DEB and WBE Metabolic Theories and the Need for Experimental Contrasts

The debate in the literature about metabolic theory has mostly focused on the validity of the assumptions and predictive capacity of the higher profile but more recently formulated WBE theory (e.g., Kozłowski and Konarzewski 2004; Algar et al. 2007; Hawkins et al. 2007; Apol et al. 2008; Martínez del Rio 2008; Isaac and Carbone 2010). Surprisingly, the DEB theory has been almost completely overlooked by the proponents and commentators of the WBE-style metabolic theory despite its existence in the literature since the 1980s. Similarly, the implications of WBE theory are not considered at all in the vast majority of DEB studies. A major purpose of this review is therefore to increase awareness among researchers in metabolic theory of the parallel existence of DEB and WBE theories and their similarities and differences as well as to inspire researchers to make empirical, experimental contrasts of the assumptions underlying them.

Both the DEB and the WBE metabolic theories claim to be as simple as possible while capturing the basic elements of whole-organism metabolism. They have the power to inform a robust metabolic theory that unifies ecological patterns with basic physical and chemical principles, providing null expectations for such patterns. However, as the WBE and DEB metabolic theories have fundamentally different underpinning assumptions, one (or both) may be invalid or seriously incomplete. If, as WBE claim, size-related constraints on distribution networks are ubiquitously important in the energetics of individual organisms, the DEB theory is significantly lacking for not

including them. Alternatively, the DEB formulation, whereby surface-area/volume interactions between "reserve" and "structure" constrain metabolism, may be the more biologically realistic, and the network-related supply constraints on individual metabolism may rarely apply.

Part of the challenge in evaluating the WBE and DEB theories is that their predictions are often indistinguishable, in much the same way that Newtonian and Einsteinian physics make very similar predictions for most purposes. In this review, we aim to outline the kinds of circumstances where the two metabolic theories would make different predictions that are likely to be empirically testable. We focus our review specifically on predictions for ontogenetic growth and respiration, leaving aside predictions for reproduction, embryonic development (which are less developed within the WBE framework), and aging. This focus is in part a reflection of where empirical tests of the core assumptions of the metabolic theories are most tractable and in part a reflection of where there has been the most overlap in the application of the two theories. Moreover, our review is mostly written in the context of animals. This in part reflects our own area of expertise and a desire for simplicity (models for plants become more complex for both WBE and DEB). It also reflects the region of greatest taxonomic overlap in applications of the two theories thus far (note that DEB has been more extensively applied than WBE to microorganisms, while WBE has been applied more extensively to plants than has DEB). Our emphasis on a particular subset of metabolic processes—and on animals—does not affect our aim of contrasting the assumptions of these two theories, which are purportedly general across all of life and should thus stand or fall irrespective of which taxonomic group or metabolic process is considered.

The Structure of the WBE and DEB Ontogenetic Growth Models

The literature on metabolic theory has a strong focus on respiration, which reflects dissipated energy use. However, metabolism is more generally about the uptake and allocation of all energy and matter across the life cycle. Thus, we first provide a comparison of the underlying structure of the WBE and DEB theories as illustrated by their growth models for animals, building on the work of van der Meer (2006), including recent elaborations of the WBE approach. This provides a useful comparative perspective on the assumptions of the two theories from which empirical comparative studies can be derived. We subsequently consider the scaling of metabolic rate, as represented by respiration.

The flow and partitioning of assimilated energy through an organism, according to the WBE and DEB models, is presented in figure 1. The revised version of the WBE "ontogenetic growth model" (Hou et al. 2008) begins with the assimilation rate of food, A (J/time), part of which supplies the total metabolic rate B_{tot} (J/time) and part of which is stored as tissue biomass E_{c} (J/g; the energy content of the cellular "building materials"; fig. 1*a*). The total metabolic rate is then partitioned between activity costs B_{act} (J/time) and resting metabolic rate B_{rest} (J/time). The growth model itself involves only resting metabolic rate and its subcomponents of maintenance metabolic rate $B_{\text{maint}} = B_{\text{m}}m$ as well as the overhead cost ("labor") of synthesizing new tissue, $B_{\text{syn}} = E_{\text{m}}dm/dt$:

$$\frac{dm}{dt} = \frac{B_0}{E_{\text{m}}} m^{3/4}\left[1 - \left(\frac{m}{M}\right)^{1/4}\right], \tag{1}$$

where m = mass, M = maximum adult mass, B_0 = a taxon-specific constant reflecting resting metabolic rate and heat from digestion (specific dynamic action; J/mass$^{3/4}$), and B_{m} (J/g) is the mass-specific maintenance rate. Adult mass $M = (B_0/B_{\text{m}})^4 = (B_0 m_{\text{c}}/B_{\text{c}})^4$, where B_{c} is the maintenance cost in vivo per cell and m_{c} is the mass per cell (sensu West et al. 2001). Note that the interpretation of B_0 is therefore as a supply rate (to cells/tissues) rather than a maintenance rate (van der Meer 2006). It is argued that all the parameters are in principle directly calculable from fundamental cellular parameters (West et al. 2001) and therefore that this is a first-principles mechanistic model for growth. The quarter-power scaling terms come directly from the theory of WBE concerning fractal constraints on the supply network (West et al. 1997). Rearranging this formula with the constants $b = B_0$ and $c = B_0/M^{1/4} = B_{\text{m}}$ gives

$$\frac{dm}{dt} = \frac{bm^{3/4} - cm}{E_{\text{m}}}, \tag{2}$$

where it becomes transparent that growth reflects the difference between a rate of supply of energy to the cells (the resting metabolic rate), which scales with $m^{3/4}$, and the maintenance costs of running the cells, which scales with m^1 (see van der Meer 2006). Growth ceases when the supply rate equals the maintenance rate. Thus, according to the Hou et al. formulation, the assimilated energy available for the overheads of growth and cellular maintenance ($bm^{3/4}$) is the total energy assimilated by the organism A minus that which is stored in added tissue $E_{\text{c}}dm/dt$ or used for other, largely unspecified costs (but which include locomotion) $B_{\text{act}} = sB_{\text{rest}}$, where s is the activity scope (fig. 1*b*). The assimilation rate is thus fully determined by the model parameters and is not a simple power law with body mass:

$$A = sB_0m^{3/4} + \gamma B_0 m^{3/4} - \gamma B_0 M^{-1/4} m, \tag{3}$$

where $\gamma = E_{\text{c}}/E_{\text{m}}$.

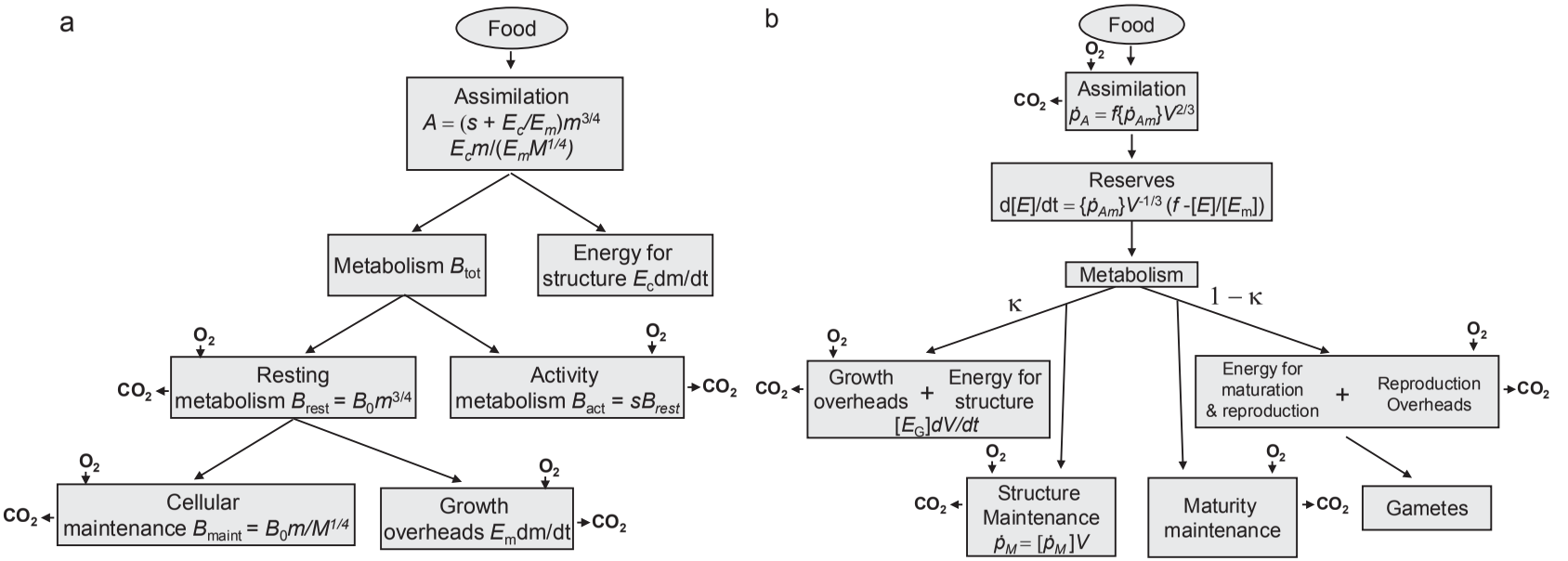

Figure 1: Flow and partitioning of energy assimilated from food throughout ontogeny according to the West, Brown, and Enquist model (*a*; modified from Hou et al. 2008) and the dynamic energy budget model (*b*). Steps influencing the observed fluxes of O_2 and CO_2 are indicated. All terms are described in the text.

In contrast, the DEB growth model (Kooijman 2010; fig. 1*b*) partitions total body mass into "structural volume," V (cm^3), and "reserve," E (J), whereby total wet mass $m = d_V V + E w_E/\mu_E$, where d_V is the density of structure (g/cm^3), w_E is the molar weight of reserve (g/mol), and μ_E is the chemical potential of reserve (J/mol). (Note that we use the DEB notation of Kooijman [2010] here, and his E is not analogous to Hou et al.'s E_c and E_m, so we use E_{max} for Kooijman's E_m to avoid ambiguity; note also that in DEB notation, brackets are used around a symbol to denote that it is volume specific, braces are used around a symbol to denote that is it surface area specific, and primes above symbols denote rates.) Under the DEB theory, energy and matter are assimilated in proportion to the organism's surface area and are directed first to the reserve pool of the organism. The reserve is not necessarily metabolites "set aside for later" but rather is defined by having a common turnover rate and reflects the part of the dry mass of an organism that fluctuates with resource supply. A good analogy is the materials, labor force, and machines used to produce goods in a factory, where there is an upper limit on stock, machines, and staff but their number is up- or downregulated according to the supply of raw materials (S. A. L. M. Kooijman, personal communication). The reserve includes polysaccharides, proteins, RNA, lipids, and wax esters scattered in and among cells across the body, which are constantly used and replenished, and it plays the same role as the supply network in WBE theory in constraining the metabolic rate (but note that it does not assume that resources are constrained by flow from a central source). DEB theory assumes that no maintenance costs are paid for reserves, with the costs for their turnover being covered by overheads in assimilation. The structure is the "permanent" biomass and does require energy for its maintenance (protein turnover and the maintenance of concentration gradients and ionic potentials, etc.) in direct proportion to structural volume.

The total rate of assimilation of energy (and mass) $\dot{p}_A$ is included in the equation for growth and is explicitly related to food density through the functional response curve $\dot{p}_A = f\{\dot{p}_{Am}\}V^{2/3}$, where f is the scaled functional response (ranging from 0 [starved] to 1 [fed ad lib.]) and $\{\dot{p}_{Am}\}$ is the maximum assimilation rate per unit surface area. Assimilation rate is assumed to be proportional to surface area in isomorphs (to which the standard DEB model applies), since food enters the organism through surfaces (feeding apparatus, gut, etc.). The rate of change in structural volume at constant food density is equal to

$$\frac{dV}{dt} = \frac{\kappa f\{\dot{p}_{Am}\}V^{2/3} - [\dot{p}_M]V}{\kappa f[E_{max}] + [E_G]}, \tag{4}$$

where t is time, κ is the (constant) fraction of utilized reserve directed to growth and maintenance (the rest going to maturation, maintenance of maturation state once adult, and reproduction), $[E_{max}]$ is maximum reserve density (which, at constant food, reaches steady state at $f[E_{max}]$), $[\dot{p}_M]$ is maintenance costs per unit structural volume, and $[E_g]$ is the volume-specific cost of structure (tissue energy content plus overheads for synthesis) per unit structural volume (Kooijman 2010). For a constant food density, this equation simplifies to the von Bertalanffy growth curve, albeit with a different mechanistic interpretation (Kooijman 2010). Maximum structural volume $V_{max} = (\kappa\{\dot{p}_{Am}\}/[\dot{p}_M])^3$ and is thus sensitive to reproductive investment (through the term κ). Ultimate structural volume, however, reduces if the assimilation rate drops below the maximum rate because of restricted food availability. Finally, the rate of change in the reserve density (which must be converted to mass and added to the structure to obtain a wet weight) is equal to

$$\frac{d[E]}{dt} = \frac{\{\dot{p}_{Am}\}}{V^{1/3}}\left(f - \frac{[E]}{[E_{max}]}\right). \tag{5}$$

While the Hou et al. model does not explicitly account for the process of reproduction, the original description of the WBE growth model incorporated reproduction through an increase in the maintenance term B_m after the point of reproductive maturity (West et al. 2001). Charnov et al. (2001) expanded on this, whereby a quantity km is subtracted from the energy available for growth after the age of maturity, α, is reached, reflecting the mass-specific investment in reproduction and thereby reducing the asymptotic size (and requiring two extra parameters). Thus, once $m > m_\alpha$,

$$\frac{dm}{dt} = \frac{bm^{3/4} - cm}{E_m} - km. \tag{6}$$

However, in the original WBE growth model E_m was tied to the energy content of new tissue rather than the overhead costs to build it (West et al. 2001). To be consistent with the Hou et al. (2008) formulation, Charnov et al.'s allocation parameter k must therefore reflect the allocation of energy associated with the overhead costs of reproduction and not the allocation of energy to be stored as reproductive tissue.

Very recently, Hou et al. (2011) modified the Hou et al. (2008) formulation to allow asymptotic size to change with food restriction. The new parameter β was introduced, which is the fraction by which food is restricted relative to an ad lib. diet. Thus, β is equivalent to the DEB functional response parameter f. The value of β then acts to reduce the "normalization constant" B_0, given that maximum size $M = (B_0/B_m)^4$ and that reduced food intake should not alter maintenance energy requirements B_m.

Thus, the asymptotic mass under food restriction $M_{FR} = M\beta^{4/3} = \beta^{4/3}(B_0/B_m)^4$ (Hou et al. 2011). This modification brings the Hou et al. (2008) model closer to the DEB model in its level of generality, by allowing environmental changes in food levels to affect growth.

Intraspecific Implications: Growth, Metabolic Scaling, and Ultimate Size

Metabolic theories assume that an animal of a given genotype has constant parameter values during ontogeny, although a real population might consist of many different genotypes at loci affecting the parameter values. In the following discussion, we are concerned with metabolism through ontogeny for a given genotype.

The WBE and DEB growth models provide very good fits to observed growth curves, as does the less mechanistic but frequently applied von Bertalanffy growth curve (Banavar et al. 2002). This is partly because the parameters of the DEB and WBE models, which in theory could be estimated from first principles, are in practice often combined into compound parameters and estimated from data to obtain a good fit (Ricklefs 2003). Moses et al. (2008) recognized this, emphasizing the need for further empirical studies to estimate the growth model parameters in different species and at different ontogenetic stages. The key challenge is to design experiments with careful parameter estimation where the predictions of the two models diverge sufficiently to be detectable. From equations (2) and (4), it can be seen that in both models growth rate depends on the difference between a "supply" term and a "maintenance" term, divided by a cost for growth. In addition, ultimate size depends on the ratio of supply and maintenance rates. Yet the models differ fundamentally in the nature and scaling of the term describing resource supply to the cells, with different implications for intraspecific growth rate, ultimate size, and metabolism.

In the WBE/Hou model, the supply of resources to the cells for growth is independent of the overall rate of assimilation of food (and the environmental availability of food) and instead is limited by the supply network whose delivery capacity scales with mass$^{3/4}$ (fig. 1). While the WBE model is often discussed in the context of metabolic transport in general, Hou et al. (2008) imply that the supply of "building materials" to growing tissue is not constrained by the vascular network per se, since the assimilation rate does not scale with mass$^{3/4}$. The WBE model therefore must assume that building materials can be stored "on site" after delivery to the cells and that the supply constraints limiting metabolism pertain specifically to those metabolites that cannot be stored—that is, to oxygen. As emphasized by West et al. (2002), the WBE model implies that cells in vivo become progressively undersupplied with oxygen as size increases, hence reducing their mass-specific metabolic (supply) rates and constraining whole-organism growth rates. In contrast, these same cells in vitro are predicted to have high metabolic rates that are independent of the mass of the source animal because they are no longer constrained by the network (see fig. 2 in West et al. 2002).

Under the standard DEB model, in contrast, the delivery of oxygen through the circulatory system is not considered to limit metabolism. Instead, the biochemical pathways within the cells are supplied with energy and materials directly from the reserve buffer within or near the cells, and it is assumed that there is normally a sufficient O_2 supply for ATP formation to fuel the labor. The rate of mobilization of the reserve depends on reserve density—that is, the surface area/volume interface between reserve and structure. The reserve density, in turn, depends on the assimilation rate. The maximum rate of assimilation relative to the maximum reserve capacity, $\{\dot{p}_{Am}\}/[E_{max}]$, is referred to as the "energy conductance" $\dot{v}$ (cm/t) and is the primary parameter controlling the reserve mobilization rate.

Thus, in the standard DEB model food assimilation in general (labor and materials) and its effect on reserve density rather than oxygen delivery (to supply labor) is the overall constraint on the supply of resources for growth. This supply rate is thus explicitly tied to intake and so to food availability, and it is surface area limited. DEB assumes that gut surface area scales with (structural) volume$^{2/3}$ in most organisms. This assumption is consistent with data for nonvolant mammals, for which at least the nominal (smooth bore tube) surface area of the small intestine scales with body mass$^{\sim 2/3}$ (fig. 2). The scaling exponents of small intestine nominal surface area for volant birds and nonvolant mammals are not significantly dif-

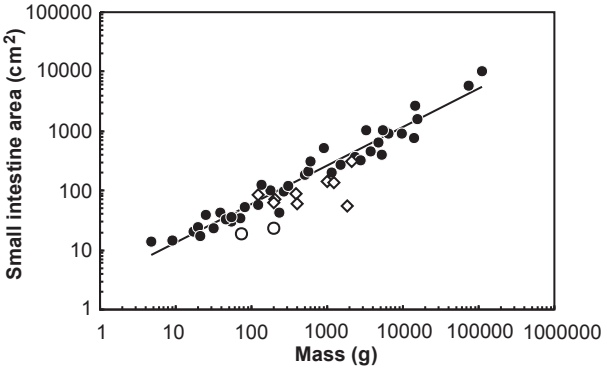

Figure 2: Relationship between body mass (M, g) and small intestine nominal surface area (SI NSA, cm^2) of mammals (filled circles), reptiles (open circles), and fish (open diamonds). Data are from Karasov (1987) and Caviedes-Vidal et al. (2007). Equation of the regression lines: mammals, SI NSA = $3.05M^{0.65}$.

ferent, but the pooled mass scaling exponent of small intestine nominal surface area for both groups is higher (0.73), and the exponent for volant birds is higher still (Caviedes-Vidal et al. 2007). However, small flying birds and mammals have relative higher rates of paracellular absorption than nonflying ones, which may compensate for their reduced small intestines (Cavides-Vidal et al. 2007). Thus, the best data presently available suggest that the DEB assumption of two-thirds-power scaling of uptake is reasonable, although detailed measurements of the scaling of total gut surface area—especially of uptake rates—would be valuable, since the actual surface areas of exchange and uptake surface areas do not always scale with the two-thirds power of volume (e.g., Gehr et al. 1981; Sernetz et al. 1985; White and Seymour 2011).

From these considerations we can summarize that the WBE and DEB theories differ most fundamentally in assuming that vascular oxygen supply (WBE) versus surface-area-specific assimilation rate and reserve density (DEB) constrain patterns of ontogenetic growth and respiration. We next describe and suggest ways that these assumptions could be experimentally tested. We have summarized all tests that we propose in table 1.

Testing Growth Models I: Changing Environments

Growth rate and ultimate body size are sensitive to the environmental conditions of oxygen, temperature, and food. Experimental manipulation of these environmental conditions should therefore provide powerful means for testing the assumptions and performance of metabolic theories. For example, under the WBE model animals can grow larger in a given environment only by reducing their tissue-specific maintenance costs B_m (van der Meer 2006; Hou et al. 2008), because B_0 is assumed to be controlled by universal constraints on the network geometry (fig. 3*a*). However, manipulated environmental oxygen levels could potentially modify this constraint on B_0 if they translate to different oxygen concentrations in the blood and hence act to increase or decrease ultimate size (fig. 3*c*). In contrast, oxygen delivery constraints do not "lead the dance" in the DEB growth model, so growth and ultimate size are not expected to be sensitive to environmental oxygen except at extreme levels (and such levels could be predicted explicitly under DEB using the mass balance elements of the theory). Thus, in comparing the two metabolic theories within this context, one would expect a continuous response of growth rate and ultimate size to decreased oxygen under the WBE model and a threshold response under DEB (table 1, test 1).

What do the available experimental data have to say about these predictions? Some experimental studies of growth under manipulated O_2 are consistent with the WBE expectations; exposure to chronic hypoxia results in a decrease in adult size in some insects (Klok and Harrison 2009; Harrison et al. 2010) as well as reduced growth rates in fish (Wang et al. 2009), American alligators *Alligator mississippiensis* (Owerkowicz et al. 2009), and embryonic mammals and birds (de Grauw et al. 1986; Giussani et al. 2007). For many species, processes like growth respond to hypoxia in a threshold manner and only under extreme conditions (Chabot and Dutil 1999; McNatt and Rice 2004). This may reflect an excess capacity of the supply surfaces and network in most environments, in which case oxygen supply would not limit size and growth, consistent with the assumptions of DEB theory.

Examination of the relationship between oxygen availability and rate of oxygen uptake is also informative. Species for which oxygen uptake is more or less constant over a wide range of ambient oxygen levels are known as oxyregulators, while those for which oxygen uptake is dependent on the level of ambient oxygen are known as oxyconformers. Oxyregulators generally exhibit a threshold response to ambient oxygen, such that oxygen uptake declines only at levels of ambient oxygen below a critical partial pressure (P_c), and they are therefore not constrained at higher levels of ambient oxygen. Data on the relationship between oxygen uptake and ambient oxygen are most numerous for fish and reveal that most species are oxyregulators (e.g., Pörtner and Grieshaber 1993; Pörtner 2010; Urbina et al. 2012). These data therefore support the DEB assumption that oxygen is not usually limiting, but at least one species of fish is clearly an oxyconformer (Urbina et al. 2012), a finding that supports the WBE expectation regarding the importance of oxygen. Moreover, P_c generally increases with temperature for ectotherms (e.g., Schurmann and Steffensen 1997; Barnes et al. 2011), and oxygen delivery can constrain growth performance at high temperatures (Pörtner and Knust 2007). Thus, the extent to which oxygen availability constrains oxygen uptake is temperature dependent, and the nature of the metabolic response to hypoxia (continuous or threshold) varies among species.

Hyperoxia might be expected to increase growth and ultimate size under the WBE model, assuming that blood or other components of the chain of aerobic metabolism are not saturated with O_2 under normoxia. Consistent with this is the correlation between large body sizes and atmospheric hyperoxia in a range of groups of arthropods and vertebrates during the late Palaeozoic (Graham et al. 1997; Dudley 1998; Falkowski et al. 2005; Berner et al. 2007). Nonetheless, such a pattern may arise as a consequence of delivery of oxygen to crucial tissues rather than throughout the oxygen transport system (e.g., Kaiser et al. 2007), and the extent to which other factors have been ruled out as explanations for gigantism has been questioned (Chown

Table 1: Possible tests and associated predictions to contrast the assumptions of metabolic constraints under the dynamic energy budget (DEB) theory and the supply network theory of West, Brown, and Enquist (WBE)

Test	Description	Metabolic process	DEB prediction	WBE prediction
1	Growth under manipulated oxygen levels	Growth	Ultimate size and growth rate independent of blood O_2 levels (or varies in a threshold manner at extremes)	Ultimate size and growth rate continuously and positively related to blood O_2 levels
2	Aerobic respiration under manipulated temperature levels	Respiration	Thermal tolerance zone for aerobic respiration not related to body size	Thermal tolerance zone for aerobic respiration inversely related to body size
3	Size under manipulated temperature where thermal response curves permit two different maintenance rates at the same assimilation rate	Growth	Ultimate size should be smaller under the higher maintenance metabolic rate	No effect on ultimate size
4	Limb regeneration	Growth	Regeneration rate up to threefold faster than ontogenetic growth rate	Regeneration rate equal to ontogenetic growth rate
5	Scaling of respiration in two-dimensional organisms	Respiration	Mass exponent varies from 0.5 (slow growers) to 1.0 (fast growers)	Mass exponent is 0.67 (based on dimensionality) or 0.86 (based on a plane-filling network and area-increasing branching)
6	Temperature dependence of metabolic scaling in size-manipulated colonial organisms	Respiration	Scaling exponent of metabolic rate negatively related to temperature in encrusting species; scaling exponent of metabolic rate independent of temperature in arborescent species	Scaling exponent of metabolic rate independent of temperature in both encrusting and arborescent species
7	Capillary density under manipulated body condition	Supply network design	Capillary density would remain constant with interspecific variation in body size when reserve (and hence body condition) is low	Capillary density scales interspecifically with $M^{3/4}$ irrespective of body condition
8	Metabolic scaling following endurance training (assuming that capillary density increases relatively more with training in small animals [fig. 6])	Supply network design	Scaling exponent of metabolic rate independent of training if reserve unchanged; scaling exponent of metabolic rate increases if reserve decreases with training	Scaling exponent of metabolic decreases with training
9	Respiration rates of cells of isomorphic, nongrowing, nonreproducing animals	Respiration	In vivo and in vitro rates of respiration proportional to L^3 of cell donor, especially in lean individuals (and thus little reserve is stored in cell)	In vivo respiration rates of cells proportional to $M^{3/4}$ of cell donor, and in vitro rates proportional to M^1 of cell donor
10	Interspecific scaling of adult respiration under manipulated body condition	Respiration	Mass exponent of respiration approaches 1 with decreasing body condition near asymptotic size	Mass exponent of respiration independent of body condition

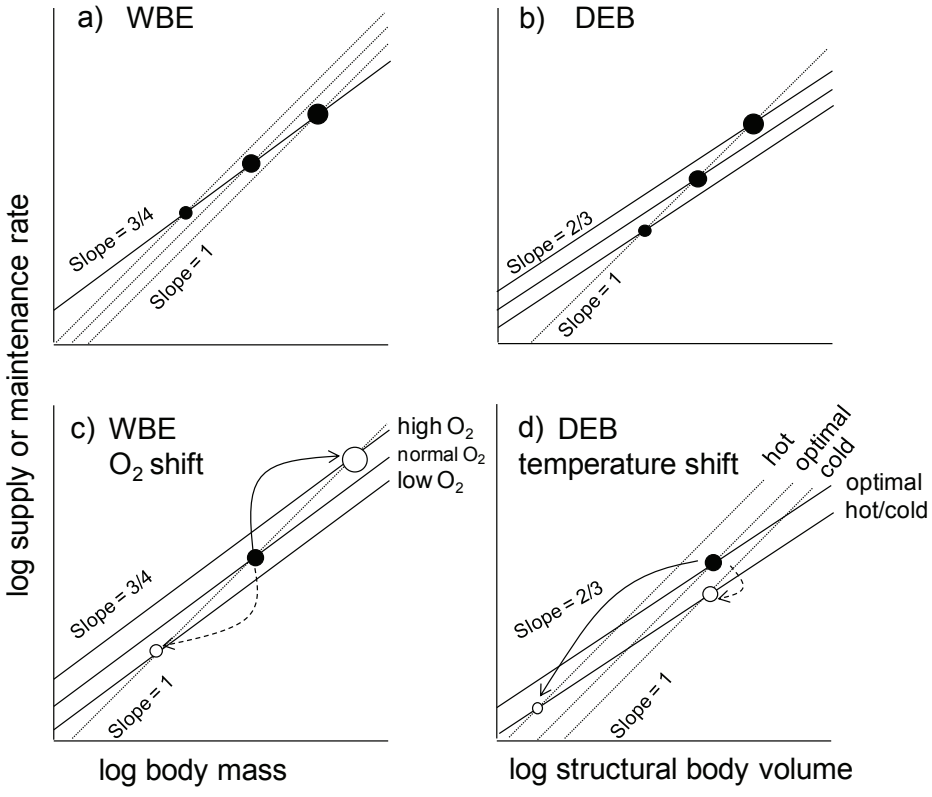

Figure 3: Log-transformed supply rate (solid lines) and maintenance rate (dotted lines) versus body size (modified from van der Meer 2006). Species differing in maximum size have different maintenance costs under the West, Brown, and Enquist (WBE) model (*a*) and different supply curves under the dynamic energy budget (DEB) model (*b*). Manipulations of environmental oxygen should alter supply in WBE, producing different body sizes (*c*), while thermal manipulations of maintenance costs at temperatures producing identical supply rates (see fig. 4) should alter maximum size under DEB (*d*).

and Gaston 2010). Oxygen availability has also been shown to correlate with the spatial distribution of body sizes of extant amphipods (Chapelle and Peck 1999; Peck and Chapelle 2003), gastropods (McClain and Rex 2001), and diving beetles (Vamosi et al. 2007), although some of the evidence for this pattern is controversial (Spicer and Gaston 1999). Recent work has supported a role for oxygen in structuring body size distributions and diversity patterns and emphasizes the role of both oxygen solubility and partial pressure in determining these patterns, thereby emphasizing the interaction between temperature, oxygen availability, and oxygen demand (metabolic rate; Boardman et al. 2011; Verberk and Bilton 2011; Verberk et al. 2011). It is not clear, however, that these effects apply generally across all of life or rather reflect phylogenetic constraints of particular network designs (e.g., open vs. closed circulatory systems).

Many of the patterns described above with respect to oxygen fluctuations may be confounded by interactions with temperature and food availability. Environmental temperature affects environmental oxygen levels in aquatic environments, and there is intense research into the implications of global warming on organisms via hypoxia (Vaquer-Sunyer and Duarte 2008). Temperature may also influence the aerobic capacities of organisms even under stable environmental oxygen levels by affecting the performance of the respiratory system. In this context, Pörtner and Knust (2007) have developed a model for thermal tolerance based on thermal influences on oxygen demand and supply; extreme body temperatures incapacitate the mitochondria either through inactivation of membranes and enzymes (low temperature) or by increasing proton leakage and causing oxygen demand to outstrip supply (high temperature). Pörtner's model therefore suggests that network-related constraints on oxygen delivery, if not generally limiting metabolism, should come to the fore as organisms move out of their "thermal windows" into stressfully high or low body temperatures. In this context, the WBE model predicts that oxygen-supply-related thermal sensitivity should be greater for larger organisms be-

cause oxygen supply to the cells is already significantly constrained by the architecture of the supply network (table 1, test 2). In contrast, the DEB model predicts thermal sensitivity to be independent of body size (but see the discussion of the temperature size rule below).

Body temperature may also affect growth and ultimate size through different sensitivities of supply and demand. Under DEB theory, volume-specific maintenance costs $[\dot{p}_m]$ (as well as κ) are assumed to be independent of size when comparing closely related species (e.g., within a genus; Kooijman 2010, p. 191). Instead, ultimate size is constrained by the maximum assimilation rate $\{\dot{p}_{Am}\}$ (fig. 3*b*). However, the assimilation rate may have a different thermal sensitivity to temperature than the maintenance metabolic rate (Huey 1982). If the assimilation rate starts to drop sooner with increasing temperature than does the maintenance metabolic rate (fig. 4), it is possible to manipulate maintenance costs through temperature while holding the assimilation rate constant. Under DEB theory, this leads to the hypothesis that, at temperatures that produce identical assimilation rates, body size will reduce more markedly at high than at low temperatures (fig. 3*d*; table 1, test 3). One would not expect such a manipulation of temperature to affect size under WBE, since size is not primarily constrained by the food assimilation rate but rather by the delivery rate of metabolites to the cells via the supply network.

Related to this are the implications of the temperature size rule, whereby size at a given stage of development is observed to decrease as rearing temperature increases within thermally benign limits (Atkinson 1994; Angilletta 2009). Van der Have and de Jong (1996) proposed that this was due to differing thermal dependencies of differentiation (or "development") and growth (i.e., biomass accumulation). Until recently, energy allocated to differentiation was not explicit in the WBE-based growth models but was apparently implicit in the flux to the overhead costs of growth, with a fixed proportion of that flux contributing to differentiation through time. Under such a formulation, maturity and maximum size are reached simultaneously. Zuo et al. (2011) recently included the potential for different temperature dependencies for differentiation and growth in the WBE growth model, such that the relative amounts of energy allocated to these processes could vary with temperature. Differentiation and growth then compete. Depending on which process is more sensitive to temperature, either maturation occurs before maximum size, resulting in a smaller body size at maturity, or the animal never matures.

Under DEB theory, maturity equates to differentiation and is an explicit state variable; however, there has been little exploration as to how the temperature-size rule could emerge from DEB theory. We propose a thermally dependent κ as one potential mechanism, whereby κ decreases (and hence energy allocated to maturation increases) with temperature. This would result in a decreased time to birth, maturity, and asymptotic size as well as decreases in size at all of these stages, with an increased mass-specific reproduction rate at maturity. It is not clear how reproduction would be affected under the Zuo et al. formulation. Further empirical and theoretical work on the temperature-size rule in relation to metabolic theory could prove very insightful.

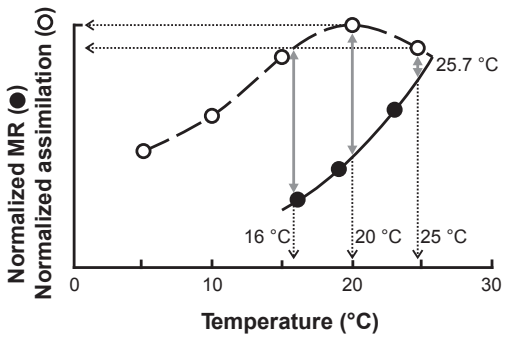

Figure 4: Metabolic rate (MR) and assimilation rate as a function of temperature in *Daphnia* (Kibby 1971; Wiggins and Frappell 2000). Dotted lines show the temperature of maximum assimilation and two temperatures of lower but equivalent assimilation rate. Gray lines show "profit."

Testing Growth Models II: Changing Shapes and Dimensions

The structure of both the WBE and the DEB models is sensitive to the shape of the organism. While the DEB theory predicts assimilation and hence supply rate to scale with surface area, it specifically applies to the surface area involved in uptake. This surface area typically scales with volume$^{2/3}$ in animals that do not change shape as they grow (isomorphs), but the DEB equations of state—and therefore patterns of growth—change significantly for nonisomorphs. In some organisms, for instance, the surface area involved in uptake remains constant as the animal grows (V0-morphs in DEB terminology). These include biofilms, diatoms, and dinoflagellates (Kooijman 2010). Plants, under DEB theory, change shape dramatically during ontogeny. Just after germination they are assumed to grow as if assimilation surface area were proportional to volume (V1-morph), then as isomorphs as self-shading becomes important, and finally as V0-morphs as contact is made with other plants, such that mass increases no longer translate into increases in surface areas involved in light or nutrient uptake (Kooijman 2010, fig. 4.14).

An interesting situation with regard to shape changes

is tissue or organ regeneration—for example, in lizards regrowing autotomized tails, fish regenerating fins, or amphibians regenerating limbs. In such situations, if the gut and other surface areas involved in assimilation remain constant, the regenerating body part should grow as a V0-morph under the DEB theory. This could result in up to threefold faster regrowth than the equivalent rate of ontogenetic growth because the assimilation rate remains constant at the value immediately prior to tail loss rather than scaling with structure$^{2/3}$ (table 1, test 4). In contrast, under the WBE theory the growth trajectory might be expected to be less rapid, following that of ontogenetic growth, and scaling with tail/fin/limb mass$^{3/4}$ because of constraints imposed by the scaling of the circulatory system forming within the regenerating tissue. These predictions are of course sensitive to the composition and maintenance/growth costs of the regenerating limb relative to the rest of the body.

In the extreme, organism shape can approach different dimensions. West et al. (1997) argued that the scaling exponent of the network should change with dimension (D) as $\beta = D/(D + 1)$. Thus, WBE theory predicts a very different mass-dependent scaling (two-thirds rather than three-fourths) of oxygen supply and hence ontogenetic growth and metabolism in nearly two-dimensional animals, such as bryozoans and flatworms, as well as in functionally two-dimensional plants (Koontz et al. 2009). Recent modifications of the fractal geometry model predict values that encompass the range 0.5 to 1.0 (e.g., Price et al. 2007), with the exact value dependent on the assumed structure of the branching nutrient distribution network (Enquist et al. 2007*b*; Savage et al. 2008; Banavar et al. 2010; Kolokotrones et al. 2010). The predicted scaling exponent for an organism with a plane-filling network and area-increasing branching is 0.86 (Price et al. 2007), so the fractal model predicts an exponent of either 0.67 (West et al. 1997, 1999) or 0.86 (Price et al. 2007) for two-dimensional animals, depending on the assumptions used (table 1, test 5). In contrast, DEB theory predictions for ontogenetic scaling of metabolism with dimension vary according to growth rate. For example, in colonial two-dimensional organisms like bryozoans, DEB predicts that the energy expenditure of colony edges differs from that of colony centers, such that respiration rate will scale with a mass exponent of 0.5 if growth is fast and 1 if growth is slow.

Empirically determined metabolic scaling exponents for two-dimensional colonial animals fall between 0.4 and 1.2 (fig. 5) and cluster around the value of 0.86 predicted by Price et al. (2007). However, most of the values close to 0.86 are for colonial ascidians (Nakaya et al. 2003, 2005), and it is not clear whether the nutrient distribution systems of these colonies conform to the network geometry required to predict an exponent of 0.86. In contrast, White et al. (2011) experimentally manipulated size in a colonial bryozoan *Hippoporina indica* and found a scaling exponent for respiration of 0.5, consistent with the DEB prediction for rapidly growing colonies. Moreover, they showed that the DEB formulation could explain the variation in the exponent seen in other bryozoan taxa according to the observed growth rates and growth forms. For example, for slower growing Antarctic species, the model correctly predicted isometric scaling (i.e., $\beta = 1$; fig. 4; Peck and Barnes 2004).

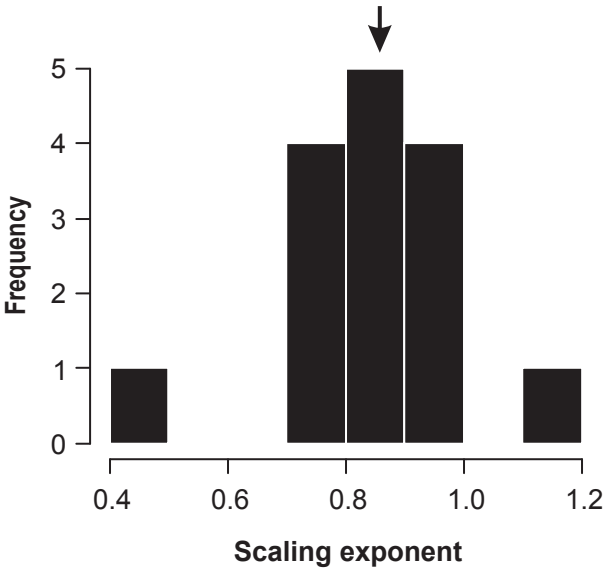

Figure 5: Frequency histogram of scaling exponents for two-dimensional colonial marine invertebrates. Data are from published sources (Hughes and Hughes 1986; Muñoz and Cancino 1989; Hunter et al. 1996; Nakaya et al. 2003, 2005; Peck and Barnes 2004; Barnes and Peck 2005; White et al. 2011). The arrow shows the predicted scaling exponent for a colony with a plane-filling network and area-increasing branching (Price et al. 2007).

The DEB formulation for two-dimensional colonial organisms can be tested by varying temperature or food availability because both influence growth rate (White et al. 2011). For arborescent colonies, on the other hand, the scaling exponent is predicted to be independent of growth rate because a bifurcating tree with n tips has $n - 1$ nodes and a mass proportional to $2n - 1$ if branches retain the same sizes, so for colonies with more than around three levels of bifurcation the number of growing tips scales nearly isometrically with mass. Thus, the DEB formulation makes two explicit predictions that are amenable to testing and comparison with WBE. For the DEB model, the scaling exponent of whole-colony metabolic rate for encrusting (two-dimensional) colonial species is predicted to be negatively associated with thermally induced manipula-

tions of growth rate, but the scaling exponent of metabolic rate for arborescent colonies is predicted to be independent of such manipulations. For the WBE model, the scaling exponent of whole-colony metabolic rate is predicted to be independent of thermally manipulated growth rate for both encrusting and arborescent species because the geometry of distribution networks is independent of temperature (table 1, test 6).

Consequences for Interspecific Allometric Scaling of Metabolic Rate

Biologists have long sought an explanation for the interspecific scaling of metabolic rate with body size—the famous Kleiber's law whereby respiration scales with body mass$^{3/4}$ (Kleiber 1932). A comprehensive metabolic theory should provide an explanation for metabolic scaling patterns, and the WBE and DEB models provide fundamentally different ones. An explanation of Kleiber's law was the original inspiration and emphasis of the WBE theory. It is based on three key assumptions (West et al. 1997). First, if a supply network is to feed the demands of the whole volume of an organism, it must have a space-filling, fractal-like branching configuration. Second, the tips of the network (e.g., capillaries) are of the same diameter, irrespective of the size of the organism. Third, evolution acts such that the energy required to transport the resources to the cells (i.e., the hydrodynamic resistance) is minimized. From these assumptions, it was argued that the number of network tips (capillaries, or "service volumes") scales with body mass$^{3/4}$ and therefore that the whole-organism metabolic rate scales this way as well. Thus, under the WBE theory the explanation for interspecific scaling of metabolic rate with size is the same as the ontogenetic explanation (see above): the metabolic rates of in vivo cells are increasingly constrained as size increases because of the scaling of the supply network. The assumption that the network tips are invariant with size has been argued to be inconsistent with the space-filling assumption, although the branching network model still predicts three-fourths power scaling if blood flow velocity increases with $M^{1/12}$ (Banavar et al. 2010). Given that cardiac output of mammals scales as the product of heart rate $\propto M^{-0.23}$(Seymour and Blaylock 2000) and stroke volume $\propto M^{1.03}$ (Seymour and Blaylock 2000) and that aorta diameter scales with $M^{0.36}$ (Holt et al. 1981), flow velocity through the aorta should scale as $M^{0.08}$, which is very close to $M^{1/12}$.

WBE argued that, if cells were the terminal units of the circulatory system and not capillaries, metabolically active tissue density would have to vary with mass to the one-fourth to match the observed mass-specific scaling of metabolic rate (West and Brown 2005). This is precisely the contention of DEB theory. It assumes that when metabolic rates of fully grown individuals of species of different maximum size are compared, respiration should be directly proportional to structural volume. Wet weight comprises both structure and reserves in the DEB theory, and the reserves, which do not require maintenance (as evident in freshly laid eggs, which are almost entirely reserve and therefore hardly respire), are predicted to scale nonlinearly with structural volume, such that they occupy an increasing proportion of the total mass (Kooijman 1986, 2010). Thus the mass-specific metabolic rate reduces with size with the same exponent of three-fourths, but for a different reason: the increasing contribution of reserves to wet weight.

Implications for Capillary Densities, Cellular Metabolic Rate, and Cell Volume

The first place one might look to test these ideas is the density of capillaries in tissue sections of different-sized organisms. Schmidt-Nielsen and Pennycuik (1961) measured capillary density in muscle from bats (9 g) compared with pigs (454 kg) and found that capillary density did indeed decrease with mass, as later predicted by WBE. However, the muscle fiber density also decreased with mass, such that the number of capillaries per muscle fiber remained constant or increased with body size. Under DEB, capillary density should remain constant per structure but should vary with reserve density. Thus, comparative analysis of capillary densities in species with different reserve levels or under manipulation of reserve density may prove to be a fruitful direction of research (table 1, test 7). Specifically, one would expect that capillary density would remain constant with interspecific variation in body size when reserve (and hence body condition) is low under DEB but that it would scale with $M^{-1/4}$ under WBE.

Further progress could be made by manipulating capillary density, which increases with endurance training (fig. 6), cold acclimation (Sillau et al. 1980), and chronic exposure to hypoxia (Bigard et al. 1991; Hoppeler and Vogt 2001). Although differences between studies in the species and muscles selected for sampling, the method by which capillary density is quantified, and the duration and intensity of endurance training make generalizations difficult, it nonetheless seems that the increase in capillary density that arises as a consequence of endurance training is relatively higher in small species than in large ones (fig. 6). If this observation holds up to detailed scrutiny, then under the WBE model the scaling exponent of metabolic rate should decrease with exercise training because small species, once endurance trained, show greater angiogenesis than large ones. The metabolic rate of small species following endurance training should therefore be relatively

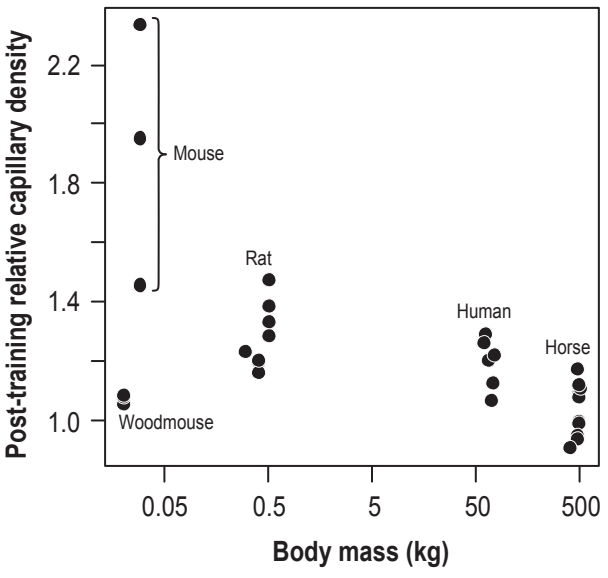

Figure 6: Relationship between post-endurance-training capillary density, expressed relative to capillary density of untrained individuals, and body mass. Data are from published studies, including a range of training durations, methods, and intensities (Hermansen and Wachtlova 1971; Andersen and Henriksson 1977; Ingjer 1979; Adolfsson et al. 1981; Klausen et al. 1981; Hoppeler et al. 1984, 1985; Essén-Gustavsson et al. 1989; Bigard et al. 1991; Rivero et al. 1995; McCall et al. 1996; Serrano et al. 2000; Amaral et al. 2001; Waters et al. 2004; Chinsomboon et al. 2009; Geng et al. 2010).

more free of constraints imposed by the distribution network. Alternatively, under DEB theory the interspecific scaling exponent of metabolic rate is driven by changes in reserve density with size. Thus, if endurance training decreases reserve density, then the scaling exponent of metabolic rate should be higher in endurance-trained animals relative to sedentary ones, because sedentary animals have relatively more reserve. Again, uniform data with which to evaluate these predictions are scarce. However, the scaling exponent of BMR has been shown to differ between wild-caught and captive birds: for wild-caught birds the exponent is close to three-fourths, whereas that for captive birds is close to two-thrids (McKechnie et al. 2006). This finding supports DEB theory if wild-caught birds can be considered more endurance trained than captive ones, although studies that apply uniform endurance-training protocols and measure metabolic rate, capillary density, and body composition in the same individuals would be valuable.

The second place one might look in testing these predictions is the scaling of metabolic rates of individual cells in vitro that have been taken from animals of different size (table 1, test 9). West and colleagues (West et al. 2002; West and Brown 2005) predicted that the metabolic rate of such cells should scale similarly to the mass-specific metabolic rate immediately upon removal from the body because they will have modified their mitochondrial densities to match the supply from the network. Indeed, their theory states that the only way an organism can be bigger is to have the plasticity to reduce its in vivo mass-specific metabolic rate B_m—that is, $M = (B_0/B_m)^4$. Ultimately, however, they expect such cells to converge to a common value when cultured in vitro in the case of mammals converging on the mass-specific metabolic rate of the smallest species (shrews, ~1 g) and therefore to scale independently of mass ("after several generations in tissue culture, the number of mitochondria in a cell derived from a mammal of any body size should converge to a single invariant value"; West et al. 2002, p. 2476).

The DEB prediction is more nuanced because of the abstraction of mass into structure and reserve. Specifically, DEB assumes maintenance costs per "structure" do not vary with size, but a cell from a large organism may have a higher reserve density than that of a smaller organism depending on how reserve is distributed within and among cells. Thus, in lean organisms DEB would predict no allometric scaling of the maintenance rate of individual cells among species of different body size, except in the case of surface-area-related maintenance costs (e.g., heating; Kooijman 2010).

A number of studies have compared the scaling of metabolic rate of individual cells of endotherms (Porter 2001; West et al. 2002). Some of the best data available come from Porter and colleagues (Porter and Brand 1995*a*, 1995*b*; Porter 2001) and Brown et al. (2007), although the results of these two studies are totally opposed. Porter and Brand measured the metabolic rates of isolated liver cells taken from mammals ranging in size from 20-g mice to 200-kg horses and found that they do indeed scale with mass$^{-0.18}$ (Porter and Brand 1995*b*), close to the WBE expectation of mass$^{-0.25}$ for freshly isolated cells. This is in contrast to Brown and colleagues, who found no relationship across body sizes of 5 g to 600 kg in mammalian skin cells (Brown et al. 2007). Porter and Brand found that much of their observed mass-specific decline in metabolic rate could be explained by a reduction in proton leakage with size (Porter and Brand 1995*a*), which in turn may relate to requirements for endothermic heat production. To really test these ideas, we need equivalent data on cellular metabolic scaling in ectotherms, where the potentially confounding effects of heating requirements are absent, together with experimental manipulations of body condition, as discussed below (table 1, test 10).

Savage et al. (2007) proposed an alternative implication of mass-specific declines in metabolic rates whereby cell volume increases while cellular energy consumption remains constant. They provided data from mammalian

studies suggesting a mixture of responses of cell volume to body size. Since DEB theory predicts an increase in the relative amount of reserve with interspecific shifts in size, one might also expect some cell volumes to increase to house this reserve, as discussed above. This leaves open the intriguing possibility that the network design predicted by WBE to minimize transport costs also happens to align with the scaling of reserve and its impact on the energy requirements of service volumes.

Metabolic Scaling and Reserve Manipulation

At the whole-organism level, a conceptually simple test of the two explanations is to measure the scaling of metabolic rate and reserve density with size within a group of species of wide-ranging body size both before and after manipulating reserve density (table 1, test 10). According to DEB theory, reserve density at steady state should scale interspecifically approximately with mass$^{4/3}$, and the resting metabolic rate of fully grown adults should scale with mass^{1} if reserve density is 0 but with mass$^{<1}$ if reserve density is maximal (somewhere between two-thirds and 1; Kooijman 1986). The WBE theory, which does not explicitly include reserves, thus implies that reserves scale proportionately with mass and that the metabolic rate should scale with mass$^{3/4}$ irrespective of how lean organisms are, as dictated by scaling constraints imposed on the supply network.

The difficulty with undertaking empirical studies of the role of reserves is hampered by the abstract nature of the reserve concept. Kooijman (2010) argued that (part of the) ribosomal RNA belongs to the reserve. However, whole-body ribosomal RNA was found to scale interspecifically with mass$^{-1/4}$ by Gillooly et al. (2005), which is inconsistent with the notion of proportional increases in reserve with size predicted by DEB. In contrast, body fat scales interspecifically as $M^{1.19}$ in mammals (Pitts and Bullard 1968; Calder 1984) and has a very low mass-specific metabolic rate (Elia 1992). If the scaling exponent of metabolic rate is calculated on the basis of fat-free mass rather than whole-body mass, it tends to be higher (Wang et al. 2000; Blanc et al. 2003), and in some cases metabolic rate does appear to scale isometrically with fat-free mass (e.g., in humans; Vanderburgh and Katch 1996). While these studies are insightful, manipulative experiments specifically targeting the relationship between body composition and metabolic rate would be more useful.

Parameter Number and Estimation for the WBE and DEB Models

An important consideration in any modeling endeavour is the number of parameters used and how those parameters are estimated. The DEB and WBE metabolic models share the advantage of being parameter sparse relative to more traditional energy budget studies (e.g., Widdows and Johnson 1988), and the parameters themselves have a mechanistic interpretation with dimensional consistency.

However, a misconception has emerged in the literature that DEB models have many more parameters than WBE models. For example, Zou et al. (2008) erroneously argued (apparently from examining the notation summaries in tables 3 and 4 of Sousa et al. [2008]) that the DEB growth model has 18 parameters and 17 variables while their model (Hou et al. 2008) comprised only five parameters and two variables (mass and time). In reality, the full standard DEB model has 12 parameters and three state variables (structure, reserve, and maturity) to cover the processes of feeding, assimilation, growth, maintenance, maturation, reproduction, and aging across the life cycle from egg to juvenile to adult stages under fluctuating food conditions (Sousa et al. 2010). Hou et al.'s (2008) model, in contrast, applies only to assimilation, growth, and maintenance for juveniles growing under conditions of abundant food. If the DEB model is pared down to cover the same number of processes as Hou et al.'s model, it requires only four parameters ($\{\dot{p}_{Am}\}$, $[\dot{p}_M]$, $[E_m]$, and $[E_g]$) and three variables (V, E, and t), while WBE uses five parameters (B_0, B_m, E_m, E_c, and f) and two variables (m and t; see eqq. [1]–[5] and fig. 1). Thus, the principal differences between the two models lie in the inclusion of a single directly measurable state variable (mass) in WBE, compared with the inclusion of two state variables (structure and reserve) that are only indirectly measurable in DEB, and in the WBE model splitting the costs of growth into two components (E_c and E_m), with DEB combining them in one parameter $[E_g]$. As a case in point, Hou and colleagues recently increased the number of parameters of their model to account for food restriction and its effect on maximum size and growth—that is, to bring the number of processes it considers closer to that of the DEB model (as discussed above). It should be noted, however, that DEB models require additional parameters, called "auxiliary parameters," to convert the state variables to operationally measurable values, such as wet mass—that is, $V + ([E]Vw_E)/(d_E\mu_E)$, where μ_E is the molar Gibbs energy of the reserve (J/mol), d_E is the density of the reserve (g/cm^3), and W_E is the molar weight of the reserve (mol/g).

Ideally, comparisons of the WBE and DEB theories would be undertaken using data for a range of species for which parameter estimates for both theories can be obtained. Moreover, the practical utility of the theories is reflected in the "accessibility" of parameter variables from empirical data. The complexity of the WBE and DEB models is similar for some metabolic processes (such as growth), as just discussed. However, the DEB theory is

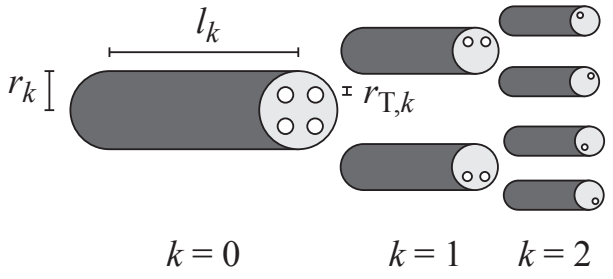

Figure 7: An idealized hierarchical branching network from level 0 to level N (redrawn from Price et al. 2007). Each branching generation is described by its length (l_k), its branch radius (r_k), and its internal tube radius ($r_{T,k}$). In this example, there are two generations beyond the initial branch, so $N = 2$, and there are two daughter branches per parent branch, so $n = 2$. At any level k, there are n^k branches.

less tractable in the sense that the state variables—and hence the parameters—are not directly observable and thus are more difficult to measure than those of the WBE-based models. Recent advances have been made for estimating DEB parameters from empirical data with the use of inverse-fitting approaches (Kooijman 2010; Lika et al. 2011), however, and DEB researchers are developing an online compendium of DEB parameter sets in the form of a resource called "add my pet" (http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/add_my_pet.pdf).

Parameter accessibility is not necessarily straightforward under WBE either. The mass dependence of metabolic rate is estimated according to the optimized network model of West et al. (1997, 1999; i.e., MR $\propto M^{3/4}$), which strictly holds only in the limit of infinitely sized networks (Savage et al. 2008). Recently, it has been shown that models of resource transport through distribution networks can predict mass dependencies of metabolic rate ranging from $M^{0.5}$ to M^{1} (e.g., Enquist et al. 2007*a*; Savage et al. 2008; Banavar et al. 2010; Dodds 2010; Kolokotrones et al. 2010). These revisions invoke separation of the theory into "core" and "secondary" assumptions (e.g., Enquist et al. 2007*a*), relaxation of some assumptions (e.g., Kolokotrones et al. 2010), or modification of assumptions that are internally inconsistent (e.g., Banavar et al. 2010). Such modifications are valuable because they serve to reconcile the WBE theory with the wide range of empirically determined scaling exponents. For example, in the case of a vessel-bundle vascular system composed of diverging vessel elements (fig. 7), scaling exponents ranging from 0.5 to 1 can be predicted with the addition of only two parameters (a and b) that describe the geometry of the network (Price et al. 2007):

$$\gamma_k \equiv \frac{l_{k+1}}{l_k} \equiv n^{-b}, \tag{7}$$

$$\beta_k \equiv \frac{r_{k+1}}{r_k} \equiv n^{-a}, \tag{8}$$

$$\overline{\beta}_k \equiv \frac{r_{T,k+1}}{r_{T,k}} \equiv n^{-\tilde{a}/2}. \tag{9}$$

The scaling exponent is then predicted as $1/(2a + b)$, leading to values of 0.5 and 1.0 for the range of biologically likely values of $b \to 1$ and $a = 1/2$ and of $b = 1/3$ and $a = 1/3$, respectively (fig. 8). The canonical scaling exponent of three-fourths is predicted by the optimized (for transport costs) model (West et al. 1999) when a and b take specific values, $a = 1/2$ and $b = 1/3$. The fundamental equation of WBE can therefore be revised to include two parameters that describe the geometry of the network (a and b):

$$\text{MR} = B_0 M^{1/(2a+b)} e^{-E/kT}. \tag{10}$$

This modification is noteworthy, because for the range of biologically likely values of a and b the range of predicted scaling exponents encompasses almost the entire range of documented scaling exponents (e.g., Glazier 2005; Moses et al. 2008), again with only a single free parameter. Estimation of MR on the basis of equation (10), however, requires knowledge of vascular architecture to determine

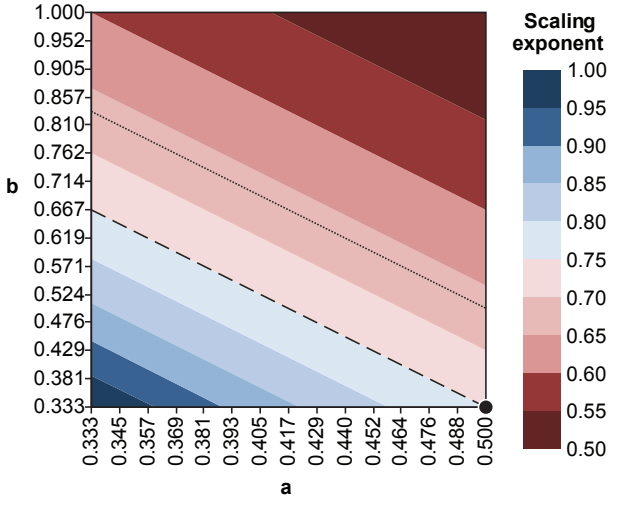

Figure 8: Scaling exponents predicted by the network geometry model with the addition of two extra parameters (a and b) that describe the geometry of the network (Price et al. 2007). The value of three-fourths predicted by the optimized model of West et al. (1997, 1999) arises when a and b take values of 0.5 and one-third, respectively (filled circle). Values of a and b that predict commonly reported scaling exponents of two-thirds and three-fourths are shown as dotted and dashed lines, respectively. See the text for further details.

the values of a and b. To our knowledge, appropriate information is not currently available for any species of animal. For plants, Price et al. (2007) use the parameters a and b to predict and test successfully for covariation of morphology and mass. At present, however, it is not possible to estimate the WBE parameters for the prediction of non-quarter-power scaling of MR for animals because sufficient data for network geometry are not available.

Summary and Conclusions

The DEB and WBE metabolic theories share the common aim of a general and formal model of the processes of assimilation, maintenance, growth, reproduction, and their scaling, grounded in basic principles of physics and chemistry. While there have been different emphases in how the DEB and WBE metabolic theories have been applied to date, their intended domains are in fact the same (Nisbet et al. 2000; Brown et al. 2004). Predictions from a metabolic theory lose their validity as mechanistic ones at whatever scale if the theory's core assumptions prove to be incorrect. As we have shown, the core assumptions of DEB and WBE theory differ substantially, and it is thus critical to assess their relative merits.

We have suggested some ways in which the core assumptions of DEB and WBE could be tested, through experiments that manipulate food (and hence reserve) density, temperature, and oxygen availability, ideally using animals for which parameters have been estimated for the different models. In this way, one could examine the constraints imposed by these variables on growth rates and ultimate size and determine under what conditions each of the theories succeed. It is possible that models deriving from these two theories will be correct for nonoverlapping sets of environmental conditions, which would suggest how one theory could be improved by incorporating aspects of the other.

As has recently been emphasized by Brown and colleagues, the processes of individual-level energetics and stoichiometry have penetrance at higher hierarchical levels in ecology (Brown et al. 2004). The potential clearly exists for metabolic theory to revolutionize ecological research by providing a sound theoretic basis for understanding energy and mass transfer across different biological scales (Nisbet et al. 2000; Brown et al. 2004; Kearney et al. 2010; Sousa et al. 2010). In this context, it is surprising how little the WBE and DEB metabolic theories have been compared and that researchers developing or applying the theories frequently appear to be aware of only one theory and not the other. We hope that this review encourages further discourse and comparative experimental study of the two theories, which in turn would increase the already considerable power and utility of metabolic theories in ecology.

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